DOCTORAL THESIS

Metabolic Alterations in Colorectal Polyps and their Role in Carcinogenesis

Egle Rebane-Klemm

TALLINNA TEHNIKAÜLIKOOL
TALLINN UNIVERSITY OF TECHNOLOGY
TALLINN 2023

TALLINN UNIVERSITY OF TECHNOLOGY
DOCTORAL THESIS
27/2023

Metabolic Alterations in Colorectal Polyps and their Role in Carcinogenesis

EGLE REBANE-KLEMM

TAL TECH PRESS

TALLINN UNIVERSITY OF TECHNOLOGY
School of Science
Department of Chemistry and Biotechnology

NATIONAL INSTITUTE OF CHEMICAL PHYSICS AND BIOPHYSIS
Laboratory of Chemical Biology

This dissertation was accepted for the defence of the degree of Doctor of Philosophy in Gene Technology on 30/06/2023

Supervisor: Dr. Tuuli Käämbre, Ph.D.
Laboratory of Chemical Biology
National Institute of Chemical Physics and Biophysics
Tallinn, Estonia

Opponents: Prof. Vilmantė Borutaitė, Ph.D.
Laboratory of Biochemistry
Neuroscience Institute
Lithuania University of Health Science
Kaunas, Lithuania

Dr. Kairit Joost, MD., Ph.D.
East Tallinn Central Hospital, Internal Medicine Clinic
Tallinn, Estonia

Defence of the thesis: 22/08/2023, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Egle Rebane-Klemm

---
signature

European Union
European Regional
Development Fund

Investing
in your future

ISSN 2585-6898 (publication)
ISBN 978-9916-80-018-8 (publication)
ISSN 2585-6901 (PDF)
ISBN 978-9916-80-019-5 (PDF)
Printed by Koopia Niini & Rauam

TALLINNA TEHNIKAÜLIKOOL
DOKTORITÖÖ
27/2023

Metaboolsed muutused jämesoole polüüpides ja nende roll vähi tekkes

EGLE REBANE-KLEMM

TAL TECH
KIRJASTUS

Contents

List of Publications

The list of author's publications, based on which the thesis has been prepared:

I **Rebane-Klemm E**, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, Timohhina N, Tepp K, Bogovskaja J, Afanasjev V, Suurmaa K, Valvere V, Kaambre T. Mitochondrial Respiration in *KRAS* and *BRAF* Mutated Colorectal Tumors and Polyps. Cancers (Basel). 2020 Mar 28;12(4):815. doi: 10.3390/cancers12040815.

II **Rebane-Klemm E**, Reinsalu L, Puurand M, Shevchuk I, Bogovskaja J, Suurmaa K, Valvere V, Moreno-Sanchez R, Kaambre T. Colorectal polyps increase glycolytic activity. Front Oncol. 2023 May 23; doi: 10.3389/fonc.2023.1171887.

III Klepinin A, Zhang S, Klepinina L, **Rebane-Klemm E**, Terzic A, Kaambre T, Dzeja P. Adenylate Kinase and Metabolic Signaling in Cancer Cells. Front Oncol. 2020 May 19;10:660. doi: 10.3389/fonc.2020.00660. eCollection 2020.

IV Reinsalu L, Puurand M, Chekulayev V, Miller S, Shevchuk I, Tepp K, **Rebane-Klemm E**, Timohhina N, Terasmaa A, Kaambre T. Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis. Front. Oncol. 2021 July 26; 11:698951. doi: 10.3389/fonc.2021.698951.

Author's Contribution to the Publications

Contribution to the papers in this thesis are:

I The author and colleagues contributed to the study's design, conducted the experiments (high-resolution respirometry and mutation analyses), performed the data analysis, and wrote the manuscript.

II The author contributed to the study's design, together with colleagues, conducted the experiments (high-resolution respirometry and mutation analyses), participated in data interpretation, and wrote the manuscript.

III The author participated in interpreting data and performed a manuscript review.

IV The author participated in manuscript review and editing.

Introduction

Colorectal cancer (CRC) is a major public health concern worldwide, with significant morbidity and mortality rates. The pathogenesis of CRC involves genetic mutations that disrupt the normal regulation of cell growth and division and the inactivation of tumor suppressor genes. These changes result in cells' malignant transformation and CRC development. One hallmark of cancer is the ability of cancer cells to reprogram metabolic pathways involved in energy production and biosynthesis, allowing them to adapt and thrive under varying oxygen levels. This metabolic plasticity grants cancer cells a competitive advantage in survival and proliferation. Despite the significant progress made in diagnosing and treating CRC, the metabolic changes that occur in the early stages of this disease remain largely unknown.

While many studies have characterized the metabolic phenotype of CRC cell lines, it is crucial to understand the metabolic reprogramming in the clinical material. High-resolution respirometry, analyzing oxidative phosphorylation (OXPHOS), is a valuable tool for understanding the bioenergetic mechanisms involved. By assessing the ADP-dependent respiration rate in permeabilized tissue samples, two fundamental characteristics for OXPHOS can be found: a maximal exogenous ADP-activated respiration rate (V_{max}), and an apparent affinity of mitochondria for exogenous ADP, expressed as apparent Michaelis-Menten constant K_m (K_m(ADP)). Therefore, this study aimed to investigate and describe metabolic reprogramming in colorectal polyps and CRC by analyzing the mitochondrial respiratory rates and gene expression of specific metabolic markers using post-operative clinical material.

The findings of this study hold the potential to provide valuable insights into the metabolic changes occurring in the early stages of CRC. This knowledge can contribute to developing improved strategies for early detection and prevention of CRC. Analyzing metabolic reprogramming in clinical material could have clinical implications regarding personalized medicine and developing more effective treatments for CRC. Overall, the study provides scientific evidence for the importance of understanding the metabolic changes in colorectal polyps and their role in the development of CRC.

Abbreviations

ADP	Adenosine diphosphate
AK	Adenylate kinase
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
CIMP	CpG island methylator phenotype
CK	Creatine kinase
CRC	Colorectal cancer
EGFR	Epidermal growth factor receptor
ETC	Electron transport chain
$FADH_2$	Flavin adenine dinucleotide
FAO	Fatty acid oxidation
G3P	Glyceraldehyde-3-phosphate
G6P	Glucose-6-phosphate
G6PD	Glucose-6-phosphate dehydrogenase
GDP	Guanosine diphosphate
GI	Gastrointestinal tract
GLUT	Glucose transporter
GTP	Guanosine triphosphate
HIF1	Hypoxia-inducible factor 1
HK	Hexokinase
IEC	Intestinal epithelial cells
IMM	Inner mitochondrial inner membrane
IMS	Intermembrane space
K_m	Michaelis-Menten constant
K_m(ADP)	Apparent affinity of mitochondria foe exogenous ADP
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MCT	Monocarboxylate transporter
MMR	Mismatch repair
MPC	Mitochondrial pyruvate carrier
MSI	Microsatellite instability
MSS	Microsatellite stable
NAD^+	Adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
OMM	Outer mitochondrial outer membrane
OXPHOS	Oxidative phosphorylation
PDH	Pyruvate dehydrogenase
PFK1	Phosphofructokinase-1

PICK3CA	Phosphatodylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PK	Pyruvate kinase
R5P	Ribose-5-phosphate
SCNA	Somatic copy number alteration
SMAD4	Small mother against decapentaplegic 4
TCA	Tricarboxylic acid
TKT	Transketolase
TP53	Tumor protein 53
VDAC	Voltage-dependent channel
V_{max}	Maximal-ADP-activated respiration rate

Explanations of abbreviations used in the thesis—the table.

1 REVIEW OF THE LITERATURE

1.1 Colorectal cancer carcinogenesis

Colorectal cancer (CRC) is a prevalent and deadly cancer worldwide. Despite considerable advances in the molecular genetics of CRC, there remains a pressing need for an improved understanding of the underlying mechanisms that drive tumor initiation and progression.

In the "classic" colorectal cancer formation model, the vast majority of cancers arise from a polyp beginning with an aberrant crypt, which then evolves into an early adenoma (<1 cm in size, with tubular or tubulovillous histology). The adenoma then progresses to an advanced adenoma (>1cm in size and/or with villous histology) before finally becoming colorectal cancer. The adenoma-carcinoma-metastasis model relies on the accumulation of genetic events of "APC-KRAS-TP53," also known as the Vogelstein model (Fearon & Vogelstein, 1990) **(Figure 1).** According to classical theory, three genes – Adenomatous polyposis coli (*APC*), Kristen rat sarcoma (*KRAS*), and Tumor protein 53 (*TP53*) – play a crucial role in the development of most CRC. These genes activate oncogenic signaling pathways, stimulating downstream transcriptional factors, ultimately leading to the formation of highly proliferative cancerous cells (J. Li et al., 2021). Loss of genomic and epigenomic stability has been observed in the majority of early neoplastic lesions in the colon, which is likely a central molecular and pathophysiological event in the initiation and formation of the CRC (Colussi et al., 2013; Grady & Carethers, 2008). Therefore, the development of CRC is driven by the accumulation of mutations and epigenetic alterations in tumor suppressor genes and oncogenes and might take 10–20 years to occur. In specific settings, for example, some hereditary cancer syndrome, it can progress more rapidly (Hossain et al., 2022; Jones et al., 2008).

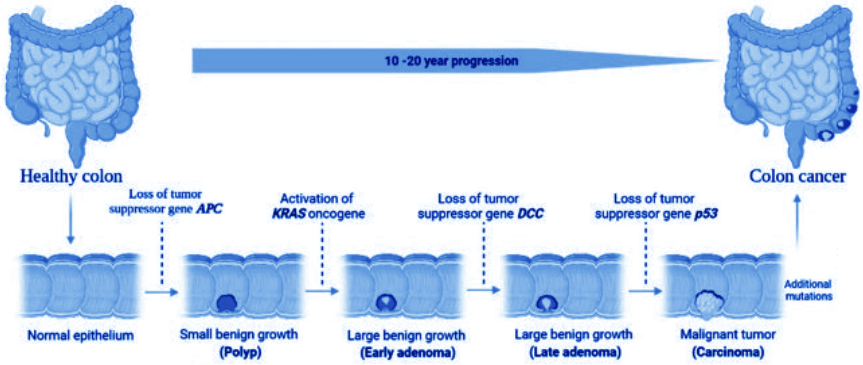

Figure 1. A genetic model of colorectal carcinogenesis*. Figure modified from (Hossain et al., 2022).*

The histology of conventional tubular adenomas is homogenous, but the molecular characteristics of these polyps are heterogenous, which might explain why only ~10% of polyps progress to colorectal cancer (Luo et al., 2014; van Engeland et al., 2011). About ten years ago, tubular and tubulovillous adenomatous polyps were thought to be the only lesions capable of progressing to the cancer (Kuipers et al., 2015). However, some colorectal cancers have been shown to arise from a subset of polyps called sessile serrated polyps, which constitute 5–10% of all polyps (Kuipers et al., 2015). These serrated polyps

arise from molecular and histological events that are distinct from tubular adenomas and are classified into three categories: hyperplastic polyps, sessile serrated adenomas, and traditional serrated adenomas (Bettington et al., 2013; Goldstein, 2006; Jass, 2004; Rex et al., 2012) **(Figure 2)**.

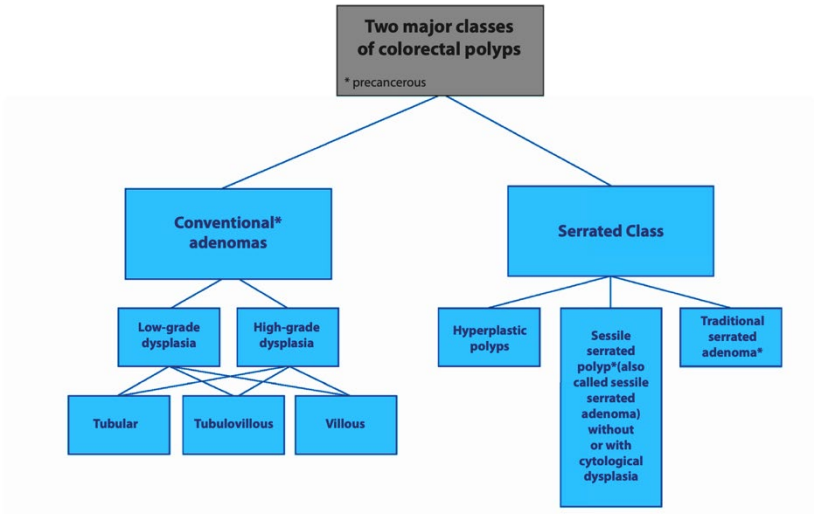

Figure 2. The two major classes of colorectal polyps.

Sessile serrated polyps have the potential to transform into CRC through the following sequence: hyperplastic polyp to sessile serrated polyp to adenocarcinoma (Goldstein, 2006; Kambara et al., 2004). Furthermore, serrated polyps in the right colon commonly show microsatellite instability (MSI) and a form of epigenetic instability characterized by CpG island methylator phenotype (CIMP). Polyps that arise in the left colon are typically microsatellite stable (MSS) but frequently carry mutations in *KRAS,* and a subset of these polyps have an attenuated form of the CIMP (Jass, 2004; Noffsinger, 2009; Rex et al., 2012).

1.1.1 Molecular pathways of colorectal cancer

Colorectal cancer is a multistep process in which several genetic events drive the initiation and progression of colorectal tumors. Over the last few decades, understanding of the diverse molecular events in the pathogenesis of CRC has improved significantly. Two molecular pathological classifications for CRC are described ("Comprehensive molecular characterization of human colon and rectal cancer," 2012; Guinney et al., 2015).

The first classification is based on the genetic heterogeneity of CRC and divides tumors into two broad categories: hypermutated (more than 12 mutations per 10^6 bases) or non-hypermutated (fewer than 8.24 mutations per 10^6 bases) ("Comprehensive molecular characterization of human colon and rectal cancer," 2012). Hypermutated tumors, which account for approximately 16% of CRC cases, exhibit microsatellite instability (MSI) due to defective mismatch repair (MMR) ("Comprehensive molecular characterization of human colon and rectal cancer," 2012). In contrast, non-hypermutated tumors (~84%) are microsatellite stable (MSS) and exhibit high levels of somatic copy number alterations (SCNAs) and dysregulated Wnt signaling. Non-hypermutated tumors also frequently harbor mutations in genes such *APC*, *KRAS*, *PICK3CA* (Phosphatidylinositol-4,5-bisphosphate

3-kinase catalytic subunit alpha), *SMAD4* (Small mothers against decapentaplegic 4), and *TP53* (Tumor protein 53) ("Comprehensive molecular characterization of human colon and rectal cancer," 2012).

The second classification system is based on gene expression profiles and divides CRC into four consensus molecular subtypes (CM1, CMS2, CMS3, and CMS4) based on distinctive molecular and biological features (Guinney et al., 2015) **(Table 1)**.

Table 1. Consensus molecular subtypes of CRC

CMS subtype	CMS1 MSI Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
Frequency	14%	37%	13%	23%
Pathological characteristics	Hypermutation, MSI-H and CIMP-H	Wnt/B-catenin and MYC signaling pathway activation	Metabolic dysregulation of carbohydrate and fatty acid oxidation pathways	Epithelial mesenchymal transformation with prominent stromal infiltration, angiogenesis, and TGF-B upregulation
SCNA	Low	High	Low-moderate	High
Immunological profile	Immune activated	Immune ignorant	Immune ignorant	Immune tolerant inflamed
Associated gene mutations	BRAF	TP53, EGFR	KRAS, PIK3CA and IGFBP2	NOTCH3/VEGFR
Site	Right sided colon	Left sided colon and rectal	Relatively more common in right sided tumors	Relatively more common in left sided and rectal tumors

The CMS classification was developed using data from predominantly early-stage CRC and was shown to be prognostic in these cohorts (Guinney et al., 2015). The CMS classification holds clinical potential for predicting prognosis and response to systemic therapy (ten Hoorn et al., 2021).

1.1.2 *KRAS* and *BRAF* oncogenes

Mutations in *KRAS* or *BRAF* appear to play an essential role in the carcinogenesis of multiple cancers, including CRC, lung, and pancreas tumors (Prior et al., 2020). These proteins play critical roles in the EGFR (Epidermal growth factor receptor) signaling pathway, and oncogenic mutations in *KRAS* or *BRAF* can drive downstream activation of this pathway even in the absence of upstream EGFR activation (Cantwell-Dorris et al., 2011; Lavoie & Therrien, 2015; Schubbert et al., 2007; Wellbrock et al., 2004). The *KRAS* is one of the most frequently mutated oncogenes in CRC, with approximately 40% of CRC patients harboring activating missense mutations in the *KRAS* (Zhu et al., 2021). CRC-bearing *KRAS* mutations are associated with advanced disease status, poor tumor differentiation, distant metastasis, and inferior survival in patients (Dienstmann et al., 2017; Karapetis et al., 2008). Also, aberrant activation of the KRAS pathway results in resistance to receptor tyrosine kinase inhibitors, such as monoclonal antibodies against EGFR (cetuximab and panitumumab). Treatment of *KRAS*-mutant CRC remains challenging because of the lack of an ideal small molecular binding pocket in the KRAS protein and its high affinity towards abundant guanosine triphosphate (GTP) (Zhu et al., 2021).

The *KRAS* gene encodes a GTP/guanosine diphosphate (GDP)-binding protein that belongs to the guanosine triphosphatase (GTPase) RAS family. The KRAS protein has a molecular weight of 21 kDa and comprises six beta strands and five alpha helices, forming two major domains: the G-domain and the C-terminal (Bourne et al., 1991). The G domain is highly conserved and contains switch I and II loops responsible for GDP-GTP exchange (Vögler et al., 2008). The KRAS protein acts as a "switch" that cycles between a GDP-bound inactive state and a GTP-bound active state (Simanshu et al., 2017). GTP binding to KRAS facilitates the binding of effectors to trigger several downstream pathways, which promote cell growth and survival. In contrast, GDP-bound KRAS loses activity and prevents its persistent signal transduction activation.

KRAS mutation causes the protein to become constitutively active and promote the signaling through growth and survival pathways – the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascade (Kerk et al., 2021). In CRC, *KRAS* mutations are most associated with the right-sided colon, and approximately 85% of mutations occur in one of three major hotspots (codons 12, 13, and 61) (Haigis, 2017). The specific mutations of *KRAS* influence its activity and differ between the tissues of the origin (S. Li et al., 2018; Serebriiskii et al., 2019; Zafra et al., 2020). For example, non-small cell lung cancer (NSCLC) more frequently harbors the tobacco smoking-associated *KRAS* G12C mutation, while the *KRAS* G12D mutation is predominant in pancreatic ductal adenocarcinoma (PDA) (Prior et al., 2020). Colorectal tumors at the primary site display heterogeneity in *KRAS* status, as not all transformed cells harbor *KRAS* mutations. Therefore, it is reasonable to assume that remarkable metabolic heterogeneity exists within a colorectal tumor. In addition, tumors arising in different tissues may have unique metabolic programs and associated dependencies (Kerk et al., 2021).

BRAF belongs to the RAF family of kinases, including ARAF and CRAF (Davies et al., 2002). The *BRAF* oncogene codes for a serine/threonine kinase that acts downstream of *KRAS* in the MAPK pathway. *BRAF* has three highly conserved domains – CR1, CR2, and CR3. CR1 and CR2 are regulatory regions located toward the N-terminal of the protein. CR1 encompasses the RAS-binding domain, and CR2 is a serine/threonine-rich domain. CR3 has a kinase domain on the C-terminal and is regulated via the phosphorylation (Roskoski, 2010). The *BRAF* kinase activation segment is evolutionarily conserved among many species. Mutations of *BRAF* are found in many forms of cancers and are classified into three subtypes according to their activation pathways (Śmiech et al., 2020). The *BRAF* gene is mutated in ~7% of human cancers, including colorectal, melanoma, papillary thyroid, and non-small cell lung cancer (Cantwell-Dorris et al., 2011). There are three classes of *BRAF* mutations: class I includes *BRAF* V600E mutations and allows the *BRAF* to act as a constitutively active monomer; class II mutations allow for constitutively active dimers; and class III either has impaired kinase activity or are inactive (Lin et al., 2019; Yao et al., 2017). Mutation *BRAF* V600E accounts for more than 90% of cases of cancer ("Comprehensive molecular characterization of human colon and rectal cancer," 2012). *BRAF* V600E is activated approximately 500-fold, and it induces constitutive ERK signaling through hyperactivation of the RAS-MEK-ERK pathway and constitutive nuclear factor kappa-B signaling in response to this hyperactivation. Although the mechanism of how the mutations cause malignancy differs in their interaction with the RAS pathway and partners to form a dimer, they all are known to activate ERK phosphorylation (Śmiech et al., 2020).

In a recent meta-analysis, *BRAF* V600E mutated tumors more commonly arise from serrated adenomas, mainly in the right colon, with a higher incidence in women and

elderly patients (age >60 years old). Moreover, CRCs with *BRAF* V600E mutation are often poorly differentiated, presenting mucinous histology, and are characterized by a dismal prognosis and resistance to standard therapies (Sinicrope et al., 2015; Wang et al., 2019). Interestingly, a mutually exclusive relationship exists between *KRAS* mutation and *BRAF* V600E (only 0.56% were KRAS and BRAF mutated) (Caputo et al., 2019).

1.2 Cellular energetics

1.2.1 Mitochondria

Mitochondria are bioenergetic and biosynthetic organelles that take up substrates from the cytoplasm and use them to drive fatty acid oxidation (FAO), the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC) and respiration, and to synthesize amino acid, lipids, nucleotides as well as nicotinamide adenine dinucleotide phosphate (NADPH) for their antioxidant defense (Wallace, 2012). Mitochondria are dynamic organelles organized in networks physically and functionally interacting with other cellular compartments, such as the endoplasmic reticulum and peroxisomes. Mitochondria possess their own genome, a circular double-stranded mtDNA (~16 kb) housed inside the matrix. The mitochondrial genome comprises hundreds to thousands of mtDNA copies per cell, depending on the tissue (O'Hara et al., 2019). Compelling evidence indicates that the quantity of mitochondria and copies of mtDNA is linked to cellular energy needs, as higher energy demands generally necessitate a more significant number of mitochondria and an increased abundance of mtDNA compared to situations with lower energy demand (Capps et al., 2003).

Mitochondria are organelles that are delimited by two phospholipid bilayers: an inner mitochondrial membrane (IMM) and an outer mitochondrial membrane (OMM), separated by an intermembrane space (IMS). The proteins within these membranes enable mitochondria to carry out essential functions, including energy production, calcium homeostasis, protein import, and signaling (Sinicrope et al., 2015). The OMM contains gate porins that permit the peptides, metabolites, and ions to pass. The voltage-dependent anion channel (VDAC) is an example of a gated porin and is recognized as the OMM key metabolite pathway and regulator (Zahedi et al., 2006). VDAC is not just a significant conduit for the fluxes of most water-soluble metabolites and ions that need to enter mitochondria and fuel oxidative phosphorylation (OXPHOS); it also controls these fluxes (Rostovtseva et al., 2021; Varughese et al., 2021). Examples of water-soluble metabolites and ions regulated by VDAC include calcium, ATP, ADP, NADH/NAD+, nucleotides, and citrate (Hodge & Colombini, 1997). VDAC has two states (open or closed) that allow for the selective permeability of metabolites and ions. The open state is primarily permeable to anions and metabolites, the most important of which is ATP (Varughese et al., 2021). While VDAC can exist in a closed state at high membrane potential, VDAC permeability can also be modulated via binding partners like hexokinase, tubulin, or alpha-synuclein, which bind VDAC and block the pore, leading to a reversible closed state (Maldonado et al., 2013; Sheldon et al., 2011).

Mitochondria are the cell's powerhouse that provides energy in the form of ATP. This energy is produced through OXPHOS, which consists of the oxidation of the redox cofactors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) coupled with the phosphorylation of ADP into ATP. The main principle of OXPHOS is proton transport across IMM against the proton gradient, coupled with thermodynamically favorable reactions. NADH and $FADH_2$ are obtained through catabolic

pathways such as fatty acid β-oxidation via the Lynen helix or carbohydrate catabolism, which includes glycolysis, or through amino acid catabolism. Those pathways give rise to acetyl-CoA that enters the TCA, also known as the Krebs cycle, in which each oxidative decarboxylation generates NADH. During their oxidation, NADH and $FADH_2$ transfer their electrons, initiating the electron flux through the ETC, also known as the respiratory chain. This chain is made of 5 enzymatic complexes 1) NADH dehydrogenase, Complex I; 2) succinate dehydrogenase, Complex II; 3) ubiquinol cytochrome c oxidoreductase, Complex III; 4) cytochrome c oxidase, Complex IV; and 5) ATP synthase, Complex V (Bellance et al., 2009) **(Figure 3)**.

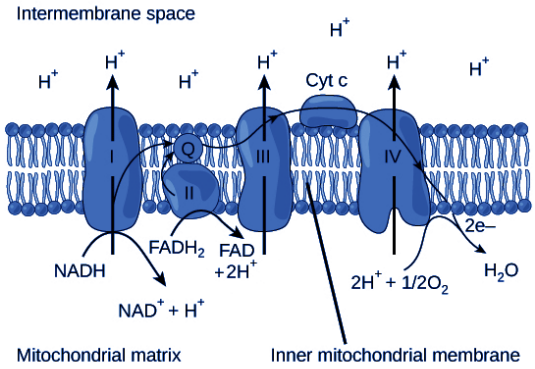

Figure 3. Electron Transport Chain.

Electron transfer through ETC complexes is accompanied by proton pumping from the mitochondrial matrix toward IMS, generating electrical and chemical gradients. Those gradients, in turn, drive the transport of protons from IMS to the matrix through ATP synthase and constitute a proton conducting force that drives the rotation of ATP synthase subunits. This mechanical energy finally allows the chemical synthesis of ATP from the condensation of inorganic phosphate and ADP.

1.2.2 Phosphotransfer network

Well-operating cellular bioenergetics system requires that energy-rich phosphoryl groups are produced and delivered to energy-consuming sites at the rate corresponding to the ATPase velocity (Dzeja & Terzic, 2003). Intracellular energy transfer processes occur within the tightly folded structures of IMM known as cristae. The arrangement of cristae significantly enhances the capacity of mitochondrial ATP production, increasing it several-fold. Still, it creates difficulties in ATP export from IMM, as diffusional flux requires a significant concentration gradient (Dzeja & Terzic, 2003). One of the options to overcome this limitation is to place in the intracristal space near-equilibrium phosphotransfer system. This view is supported by the observation that the presence of creatine kinase, adenylate kinase, and nucleoside diphosphate kinase in the intermembrane space facilitates ATP/ADP exchange between mitochondria and cytosol (Laterveer et al., 1997; Roberts et al., 1997; Saks et al., 1994). Spatially arranged intracellular enzymatic networks for energy transport are catalyzed by creatine kinase (CK), adenylate kinase (AK), and glycolytic enzymes (Joubert et al., 2002; Saks et al., 1994; Theo Wallimann et al., 1992).

Creatine kinase is a major phosphotransfer system in cells with high-energy demand, and it acts together with other enzymatic systems to facilitate an intracellular energetic communication (Dzeja & Terzic, 2003). The enzyme catalyzes the phosphorylation of creatine to form phosphocreatine (*Creatine* + ATP ↔Phosphocreatine + ADP), which is used as an energy storage system in cells (T. Wallimann et al., 1992). There is two genes for cytosolic CK subunit isoforms forming three types of dimers (CKMM, CKBB, and CKMB) and two mitochondrial creatine kinase (mtCK) isoenzymes (the ubiquitous form – gene CKMT1 and the sarcomeric form – gene CKMT2) (Schlattner et al., 2006). The interplay between cytosolic and mitochondrial CK isoenzymes depends on a large intracellular pool of phosphocreatine and prevents a rapid fall in global ATP concentrations (Schlattner et al., 2006). In CK-deficient muscles, phosphotransfer catalyzed by AK as glycolytic enzymes provide the primary route for intracellular high energy phosphoryl transfer (Dzeja & Terzic, 2003; Dzeja et al., 1998). Such alternative high-energy phosphoryl routes may rescue cellular bioenergetics in cells with compromised CK-catalyzed phosphotransfer (Boehm et al., 2000; Dzeja et al., 2000).

Adenylate kinase (AK) catalyzes the nucleotide phosphoryl exchange reaction 2ADP ↔ATP + AMP response to intracellular ATP and ADP fluctuations (Dzeja & Terzic, 2009). The AK system, consisting of multiple isoforms (AK1-AK9), is implicated in cell energetics and metabolic signaling (Dzeja & Terzic, 2009; Fujisawa et al., 2009; Noma, 2005; Panayiotou et al., 2014). The AK network is thought to generate and convey adenine nucleotide signals to metabolic sensors in response to oxygen tension or energy metabolism (Carrasco et al., 2001; Dzeja et al., 2002; Noma, 2005). AK isoforms localize in the different subcellular compartments: AK1, AK7, and AK8 are solely found in the cytosol; AK2, AK3, and AK4 are in the mitochondria; and AK5, AK6, and AK9 are positioned either in the cytosol or nucleus (Panayiotou et al., 2014). Only AK1 and AK6 are expressed in all tissues, whereas AK5 is expressed purely in the brain (Panayiotou et al., 2014). AK2 is critically positioned in the IMS and intra-cristae space to facilitate high-energy phosphoryl exchange between mitochondria and the cytosol (Dzeja & Terzic, 2009). In fact, in energy-demanding organs, such as the heart, characterized by high energy turnover and an elaborate mitochondrial circuit, AK2 appears to account for ~40% of the total AK activity (Dzeja et al., 1999; Pucar et al., 2001). Molecular studies indicate that AK2 participates in cell fate decisions and nuclear energetics (Dzeja et al., 2011; Fujisawa et al., 2009; Oshima et al., 2018). AK3 and AK4 are in the mitochondria matrix and are involved in regulating the Krebs cycle and OXPHOS; AK6 could fulfill the energy needs for the nuclear process.

The spatial extension of the glycolytic pathway indicates that it can comprise a network of phosphotransfer circuits and metabolite shuttles, which facilitates high-energy phosphoryl delivery, lactate/pyruvate, and Pi shuttling to maintain cellular energy and redox balance (Dzeja et al., 2007). Energy-rich phosphoryls from ATP, used to phosphorylate glucose and fructose-6-phosphate at the mitochondria site, traverse the glycolytic pathway and can phosphorylate ADP through the pyruvate kinase-catalyzed reactions at remote ATP utilization sites. (Dzeja & Terzic, 2003). Additional phosphoryls can be transferred through the near-equilibrium reaction system catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (Dzeja & Terzic, 2003). High-energy phosphoryls generated by glycolysis can be preferentially delivered and used to support specific cellular functions, such as maintaining membrane ionic gradients, cell motility, muscle contraction, and nuclear processes (Perez-Terzic et al., 2007).

Coordination between increased cellular energy demand and supply is secured through integrated energetic and metabolic signaling circuits composed of phosphotransfer enzymes, which couple mitochondria with cytoplasmic and nuclear energetic events.

1.3 Intestinal homeostasis

The intestine's primary function is the absorption and processing of nutrients, fluid homeostasis, and removing waste (Taylor & Colgan, 2007). The small intestine is organized into crypt-villus units. At the base of each villus, invagination from the crypt where intestinal stem cells reside. The colon is distally localized along the gastrointestinal tract to the small intestine and comprises only crypts.

Intestinal homeostasis relies on complex interactions between the microbiota, the intestinal epithelium, and the host immune system that allow the intestinal barrier function maintenance. This barrier consists of a monolayer of intestinal epithelial cells (IEC) and a mucus layer that protects the IEC surface. The intestinal barrier acts as a filter in the absorption process in the colon, which requires high amounts of energy (Guerbette et al., 2022). The gastrointestinal tract (GI) is a highly proliferative tissue; the intestinal epithelium constantly renews itself every 3–5 days, requiring great amounts of energy (Barker, 2014). The IEC is crucial for the digestive process and forms a physical and immune barrier for the host defense (Peterson & Artis, 2014). The intestine is a highly unique tissue adjacent to a diverse and dense microbial population – microbiota. The microbiota mainly consists of anaerobes that decrease environmental O_2, and therefore the intestine is highly hypoxic compared with most issues (Singhal & Shah, 2020). A unique oxygen gradient exists within the human intestinal tract. A vertical oxygen gradient has been documented in more distal, colonic regions of the GI tract, from the anaerobic lumen across the epithelium to the richly vascularized subepithelial mucosa (Colgan et al., 2016). At baseline, epithelial cells lining the intestinal mucosa exist in a relatively low partial pressure (pO_2) environment. pO_2 is estimated with EPR oximetry 42–71 mm Hg (7-10%) across the colonic muscle wall to around ~42 mm Hg (~6%) in the vascularized submucosa, 5-10 mm Hg near the crypt-lumen interface, and 11 (~2%) and 3 mm Hg (~0,4%) in the lumen of ascending and sigmoid colon, respectively (He et al., 1999). The pO_2 drops precipitously along the radial axis from the intestinal submucosa to the lumen, home to trillions of anaerobic microbes. The microbiota is critical in establishing the hypoxic intestinal microenvironment.

During low-oxygen conditions, cells adapt to hypoxic stress by inducing the expression of several genes involved in energy metabolism. Many metabolic responses to hypoxia are orchestrated by the Hypoxia-inducible factors (HIFs) (Singhal & Shah, 2020). HIFs fulfill the high cellular metabolic demands for glucose, protein, and lipid in oxygen-starved cells, and this function is conserved in many cell types and tissues. Besides energy metabolism, HIF is the transcriptional regulator of numerous genes essential to erythropoiesis, angiogenesis, and inflammation (Giaccia et al., 2003). The cellular metabolic pathways utilized for growth and function largely depend on the proliferative nature of cell type. The colon contains highly proliferative stem cells, a transit-amplifying progenitor population, and post-mitotic differentiated cells (Rangel-Huerta & Maldonado, 2017). Post-mitotic differentiated cells have a high rate of energy expenditure due to energy-consuming digestive, secretory, and absorptive processes (Van Der Schoor et al., 2002). The colon is more hypoxic than the small intestine and largely relies on commensally derived fuel sources in short-chain fatty acids (den Besten et al., 2013).

1.3.1 Short-chain fatty acids in the human colon

Short-chain fatty acids (SCFAs) produced by the intestinal microbiota through anaerobic fermentation of undigested fiber have multiple roles within the human gut. Energy procurement depends on the metabolism of SCFAs, including butyrate, through β-oxidation, and contributes up to 15% of the host's total daily caloric requirements (Bergman, 1990). Butyrate is considered the primary energy source for healthy colon cells (Roediger, 1982). The luminal butyrate concentration in humans and animals has been estimated at 10–20mM, higher than other SCFA (Cummings et al., 1987; Newmark et al., 1994). More than ninety percent of butyrate in the colon is absorbed by colonocytes, for which it serves as a dominant energy source via β-oxidation and tricarboxylic acid (TCA) (Roediger, 1982). While small intestinal enterocytes can also absorb butyrate, these cells primarily derive energy from glucose and glutamate (Newsholme et al., 2003). Butyrate is important in influencing colonic epithelial cell growth by regulating physiologic hypoxia. Butyrate increases colon epithelial O_2 consumption via driving oxidative phosphorylation and stabilizes HIF (Kelly et al., 2015). In normoxia, HIF-α subunits are degraded in an oxygen-dependent manner. When oxygen is limited, HIF-α is stabilized and forms a heterodimeric complex with HIF-1β in the nucleus to bind hypoxia-responsive elements in the promoter region of hundreds of target genes (Kaelin & Ratcliffe, 2008; Lee et al., 2020).

In contrast to its role in fueling normal colonocytes, recent studies have shown that butyrate exhibits an antitumorigenic function by inhibiting the proliferation or inducing the apoptosis of colorectal cancer cells (Fung, Cosgrove, et al., 2012; Fung, Ooi, et al., 2012). This phenomenon of molecule's opposing effects on healthy versus cancerous colonocytes was named the butyrate paradox (Comalada et al., 2006; Mariadason et al., 2001). Studies have shown that in lung tumor cells (H460), colorectal adenocarcinoma cells (HT29, Caco-2, HCT116), and breast cancer cells (MCF-7, T47-D, MDA-MB231), butyrate can increase oxidative pathway and/or decrease glycolytic metabolism. These metabolic alterations have been associated with reduced proliferation and/or induced differentiation of cancer cells (Alcarraz-Vizán et al., 2010; Amoêdo et al., 2011; Blouin et al., 2011; Q. Li et al., 2018; Rodrigues et al., 2015).

1.3.2 Intestinal transport of butyrate

Butyrate is a weak acid (pKa=4.8), and more than 90% exists in the ionized form under physiological conditions in the colon (pH 5.5–6.7), thus requiring a transporter for absorption (Bergman, 1990; Cummings et al., 1987). Butyrate is preferentially absorbed in the proximal part of the colon, where the highest luminal concentration occurs (Gonçalves & Martel, 2016). Several different mechanisms for butyrate uptake in colonocytes have been proposed, including counter-transport with bicarbonate (BT/HCO_3^- exchanger) and transport by monocarboxylate transporters (Hadjiagapiou et al., 2000; Kawamata et al., 2007; McNeil et al., 1979; Thangaraju et al., 2008).

Butyrate plays a key role in colonic epithelium homeostasis by having multiple regulatory functions. The monocarboxylate transporter (MCT) family comprises 14 members encoded by the SLC16 gene family (Halestrap & Meredith, 2004). MCT1 is a protein consisting of 500 amino acids that is highly conserved and expressed widely in various tissues(Jackson et al., 1997). The levels of MCT1 protein vary throughout the human digestive tract. MCT1 is expressed at very low levels in the small intestine, but its expression increases in the colon. Its highest levels are found in the distal segment of the colon, specifically in the upper regions of colonic crypts.(Gill et al., 2005; Iwanaga et al.,

2006). The first report on MCT1 protein expression in human tumor samples described a decrease in MCT1 expression in the colonic transition from normality to malignancy (Daly et al., 2005). Studies have shown that when MCT1 is lost or silenced, it is associated with three significant changes: 1) a shift from normal to malignant growth in the colonic epithelium, 2) disruption of genes responsible for differentiation and apoptosis that respond to butyrate, and 3) a crucial metabolic change from butyrate oxidation to glycolysis (Cuff et al., 2005; Lambert et al., 2002). CRC cells show a reduction in butyrate uptake because of reduced MCT1 expression, associated with an increased glucose uptake rate (Macheda et al., 2005; Moreno-Sánchez et al., 2007). MCT1 expression is down-regulated in the early stages of carcinogenesis (Lambert et al., 2002). MCTs are transporters that work in both directions and have a significant impact on intracellular pH by removing lactate along with a proton (Dimmer et al., 2000; Latham et al., 2012). Inhibiting MCT1 results in a drop of pH level inside cells, leading to tumoral cell death. It is possible that certain MCT isoforms may be up-regulated in order to export lactate (Gonçalves & Martel, 2016).

1.4 Metabolic reprogramming in cancer

Deregulation of cellular metabolism has emerged as a key hallmark of the cancer (Hanahan & Weinberg, 2000). Evidence shows that metabolic reprogramming is an active process governed by oncogenes and tumor suppressors, which provide cancer cells with energy, reducing equivalents and biosynthetic precursors (Vander Heiden & DeBerardinis, 2017). The most core metabolic pathways, including glucose, glutamine, amino acids, serine/glycerin, and lipid metabolism, are exploited by cancer cells to sustain their high rate of cell division (Pavlova & Thompson, 2016).

Metabolism is broadly defined as “the sum of biochemical processes in living organisms that either produce or consume energy” (DeBerardinis & Thompson, 2012). The primary source of cellular energy is glucose, a simple carbohydrate transported into cells by a family of transmembrane glucose transporters (GLUTs). In the cytoplasm, glucose is catabolized to pyruvate, generating two molecules of ATP in a process known as glycolysis. The conversion of glucose to pyruvate occurs in two stages, the first stage consuming ATP and the second stage generating ATP. In the first stage, hexokinase (HK) phosphorylates glucose into glucose-6-phosphate (G6P), one of the rate-limiting enzymes in glycolysis. Hexokinases have four isoforms, namely HK1, HK2, HK3 and HK4 (Robey & Hay, 2006). HKs sustain cellular glucose levels by regulating the entry and utilization of glucose and influencing the magnitude and direction of glucose flux within cells (Patra et al., 2013). HK1 is the predominant HK isoform in most tissues and is more abundant than HK2. HK2 is the most well-characterized gene whose expression is significantly up-regulated in many cancers, such as prostate cancer, breast cancer, lung cancer, renal cancer, liver cancer, and colorectal cancer (Lee et al., 2019; Nishihashi et al., 2017; Shi et al., 2019; Xu et al., 2018; Xu & Herschman, 2019). Next step, phosphofructokinase-1 (PFK1) catalyzes the conversion fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate. PFK1 phosphorylates F6P into fructose-1,6-bisphosphate, the second irreversible glycolysis reaction. This reaction is a crucial and rate-limiting step in glycolysis. PFK1 is regulated by ATP and F6P substrates (Cabrera et al., 2011). Studies have shown that PFK1 activity is increased in cancer cell lines, and expression of PFK1 is up-regulated in breast and liver cancers (Moon et al., 2011; Park et al., 2013). In the subsequent stage, ATP is generated by substrate-level phosphorylation and glucose metabolism.

Additionally, a metabolic pathway parallel to glycolysis is the pentose phosphate pathway (PPP), which represents the first committed step of glucose metabolism (Ramos-Martinez, 2017). Glycolysis and PPP are metabolically linked for sharing the common intermediate G6P. The third irreversible reaction of glycolysis is phosphoenolpyruvate conversion into pyruvate catalyzed by PK; thus, PK serves an essential role in the control of metabolism in cancer cells. The ratio between the active and inactive forms of PK determines whether glucose is used for OXPHOS or for PPP to support the cell growth (Gui et al., 2013). Low PK activity increases entrance into PPP for biosynthesis, while high PK activity increases OXPHOS and decreases glucose entrance into PPP (Fukuda et al., 2015).

The PPP occurs in the cytosol and comprises two phases: irreversible oxidative and reversible nonoxidative (Jin & Zhou, 2019) (**Figure 4**).

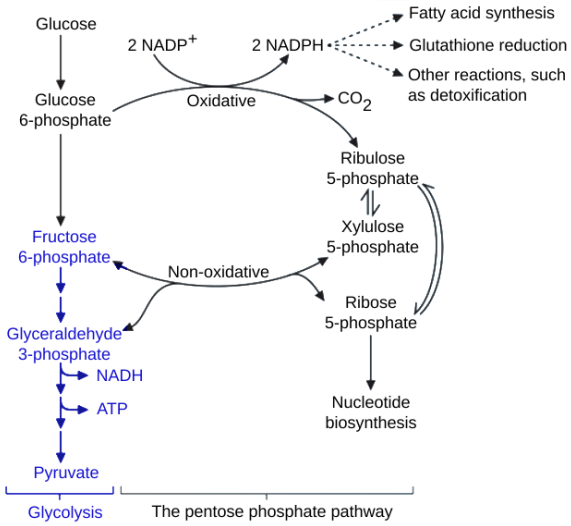

Figure 4. The pentose phosphate pathway.

The oxidative branch converts G6P into ribulose-5-phosphate (Ru5P), CO2, and NADPH (Kruger & von Schaewen, 2003). NADPH is vital to maintain cytosolic reduction-oxidation balance under stress conditions and allows cells to proliferate rapidly (Pavlova & Thompson, 2016). The non-oxidative branch yields the glycolytic intermediates F6P, glyceraldehyde-3-phosphate (G3P), and sedoheptulose sugars, resulting in the production of sugar-phosphate precursors for amino acid synthesis and ribose-5-phosphate (R5P), which is essential for nucleic acid synthesis (Stincone et al., 2015) (**Figure 4**). Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme, catalyzes the first irreversible reaction of PPP. The activity of G6PD directly reflects the flux of oxidative PPP and determines the flux partitioning between glycolysis and the PPP (Jiang et al., 2014). Whereas G6PD acts as a control site in the oxidative branch, transketolase (TKT) and transaldolase are the two key enzymes in the nonoxidative branch. Rapidly proliferating cells like cancer cells usually increase the PPP flux by activating G6PD to meet the increased bioenergetic demands. Although many studies have reported that numerous factors may stimulate it, the mechanism by which G6PD is regulated remains largely unknown. (Jiang et al., 2014).

In the absence of oxygen, normal cells convert pyruvate to lactate, which is secreted from the cell. In contrast, in the presence of oxygen, pyruvate is transported into the mitochondria by the mitochondrial pyruvate carrier (MPC) and then converted to acetyl-CoA by pyruvate dehydrogenase (PDH). Acetyl-CoA, which can also be generated by the catabolism of fatty acids (FAs) and some amino acids, enters the TCA cycle, reducing NAD^+ and FAD to NADH and $FADH_2$. These high-energy electron carriers are then used to create a proton gradient across the inner mitochondrial membrane that drives ATP synthase in the electron transport chain (ETC). This OXPHOS process results in additional 32–36 molecules of ATP.

Cancer cells utilize many nutrients to sustain infinite proliferation and growth, which requires reprogramming energy metabolism. The unique metabolic phenotype of cancer cells was first observed by Otto H. Warburg almost 100 years ago. In normal cells with sufficient oxygen levels, pyruvate could enter the TCA cycle to generate abundant energy, whereas tumor cells exhibit high glycolysis activity regardless of the oxygen levels. Lactate is produced through the activation of lactate dehydrogenase (LDH) and inhibition of pyruvate metabolism in the mitochondria (Lunt & Vander Heiden, 2011). This phenomenon is called the Warburg effect or aerobic glycolysis (Warburg, 1956). Aerobic glycolysis could meet the energy and nutritional demands essential for severe living conditions of tumor cells for cancer progression (Lunt & Vander Heiden, 2011). Metabolic reprogramming is critical for the rapid proliferation of cancer cells and is thus recognized as a hallmark of cancer. Like other cancer, CRC cells undergo rewiring of cellular metabolism via dysregulation of oncogenes and tumor suppressors during carcinogenesis (Brown et al., 2018).

1.4.1 Glycolytic metabolism in cancer

The increased rate of glycolysis is a common metabolic change that can be observed in cancer. Mutations in *KRAS* or *BRAF* lead to the constitutive activation of ERK1/2 cascade, which sustains aerobic glycolysis through the activation of transcriptional factors, including hypoxia-inducible factor 1 (HIF1) and c-myc (Zhong et al., 2022). HIF-1 is a heterodimeric transcription factor composed of a HIF-1α subunit that senses the changed oxygen levels and a HIF-1β subunit constitutively expressed (McGettrick & O'Neill, 2020). Besides the heterogeneous oxygen level inside the tumor, oncogene activation (e.g., *KRAS*, *BRAF*) or tumor suppressor inactivation (e.g., *TP53*, *PTEN*) can enhance the HIF-1α expression (Semenza, 2013). It has been shown that HIF-1α promotes the expression of glycolytic enzymes, including *HK2*, lactate dehydrogenase (*LDH*), and glycolytic transporters such as *MCT4* and *GLUT1* and increases the intracellular level of glycolysis (Nagao et al., 2019; Zhong et al., 2022). In addition, HIF-1α switches on pyruvate dehydrogenase kinase (PDK), which prevents the entry of pyruvate in the TCA cycle by inhibiting pyruvate dehydrogenase (PDH) (Kim et al., 2006). PDH converts pyruvate to acetyl-Co, and PDK-mediated inhibition of PDH leads to a lower consumption of pyruvate in the mitochondria, which results in a higher amount of pyruvate available in the cytosol (Avagliano et al., 2020). The increase of cytosolic pyruvate promotes sustained lactic fermentation and elevated lactate production (Kim et al., 2006). In addition, persistent ERK1/2 activation, induced by mutant *BRAF* or *KRAS*, leads to mitochondrial translocation of phosphoglycerate kinase (PGK1) and pyruvate dehydrogenase kinase 1 (PDK1), which in turn inactivates PDH, contributing to aerobic glycolytic switch in cancer (Li et al., 2016).

Glycolytic flux and glucose uptake are also changed by c-myc, which transcriptionally activates *LDH*, *GLUT-1*, and *HK2* (Stine et al., 2015; Zeller et al., 2003). The transportation of extracellular glucose to the cytoplasm is performed by sodium-glucose transporters (SGLTs) and facilitated diffusion GLUTs (Navale & Paranjape, 2016). GLUTs belong to a homologous family of fourteen uniporter transporter proteins. Among these, GLUTs 1–4 have been comprehensively studied and observed to be up-regulated in the cancer (Barron et al., 2016). Further, glucose uptake correlates with *KRAS* and *BRAF* mutations and *GLUT1* overexpression in CRCs measured by FDG-PET (Chen et al., 2014; Kawada et al., 2012). It is understood that KRAS signaling confers a competitive advantage to cancer cells through increased glucose uptake and elevated flux through glycolysis, concurrently fueling numerous branching biosynthetic pathways (Gaglio et al., 2011; Hutton et al., 2016; Ying et al., 2012; Yun et al., 2009). Cancer cells with *KRAS* or *BRAF* mutations depend on glycolysis for survival and growth, and that pyruvate is a major carbon source for the mitochondrial TCA cycle (Weinberg & Chandel, 2015). Several glycolytic intermediates are shunted into biosynthetic pathways essential for nucleotide production, amino acid synthesis, or glycosylation reactions, the activity of which is coordinated by KRAS signaling (Ali et al., 2020; Mattaini et al., 2016). *KRAS* also promotes the rapid reduction of glycolysis-derived pyruvate to lactate (Kerk et al., 2021). Lactate transporters are expressed in a KRAS- dependent manner and efficiently export lactate from the cell (Baek et al., 2014; McCleland et al., 2013). This glycolytic phenotype driven by *KRAS* or *BRAF* supports malignant progression and correlates with poorer prognoses in patients with KRAS-driven cancers (Avagliano et al., 2020; Graziano et al., 2017).

2 AIMS OF THE STUDY

The primary objective of this thesis was to investigate and describe the metabolic reprogramming that occurs in colorectal polyps by analyzing both the mitochondrial respiratory rates and gene expression of specific metabolic markers. More specifically, the aims of the study were:

- To compare the mitochondrial respiratory rate in colorectal polyps with those in the healthy colon and cancerous tissue. By measuring the respiratory rates, the study aimed to identify any significant difference that may contribute to the development and progression of colorectal polyps to cancerous tissue.
- To evaluate the effect of clinicopathological characteristics and the mutation status of *KRAS* and *BRAF* genes on mitochondrial respiratory rates in both colorectal polyps and CRC.
- To assess the expression levels of selected metabolic markers involved in glycolysis and intracellular phosphotransfer pathways in colorectal polyps, healthy colon tissue, and CRC. By examining the expression levels, the objective was to identify any dysregulation in these metabolic pathways that may be associated with the development of polyps and CRC.
- To explore the potential correlation between changes in mitochondrial respiratory rates and altered gene expression of metabolic markers in colorectal polyps. This analysis aimed to uncover any relationship between mitochondrial function and metabolic gene expression of colorectal polyps.
- To discuss the role of adenylate kinase in the development of colorectal cancer.
- To discuss the concept of energy metabolic plasticity in the development of colorectal cancer considering gained study results.

3 MATERIALS AND METHODS

The following methods, described in more detail in the respective publications, were used in this study:

- Clinical material – Publication I, II
- Oxygraphic measurement – Publication I, II
- RNA extraction – Publication II
- cDNA synthesis and real-time quantitative polymerase chain reaction – Publication II
- DNA extraction – Publication I, II
- KRAS and BRAF mutation analysis – Publication I, II

4 RESULTS AND DISCUSSION

4.1 Mitochondrial outer membrane permeability for ADP is different in the healthy colon, colon polyps, and colorectal cancer tissue (Publication I, II)

The permeability of the outer mitochondrial membrane to ADP plays a crucial role in regulating cellular energy metabolism, and its dysregulation has been associated with cancer. This process facilitates ADP entry into the mitochondrial matrix through the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane. The VDAC and OXPHOS are intricately connected in their contributions to cellular energy metabolism.

4.1.1 ADP-dependent mitochondria respiration

To identify the changes in OXPHOS activity in CRC carcinogenesis, high-resolution respirometry on permeabilized post-operative tissues – CRC tissue, colon polyps, and healthy colon tissue was applied. The rate of maximal ADP-activated respiration (V_{max}) was determined to estimate the coupling of mitochondrial oxygen consumption to OXPHOS. Additionally, the apparent Michaelis-Menten constant values for exogenously added ADP (K_m(ADP)) to describe the permeability of VDAC for exogenous ADP. Therefore, determining V_{max} and K_m(ADP) could provide relevant information about the activity of OXPHOS key components and the type of metabolism in the three different colon tissue types included in the current thesis.

Significant differences in V_{max} and K_m(ADP) values between colon polyps and tumors suggest that polyps and tumors probably have different bioenergetic profiles (**Figure 5**).

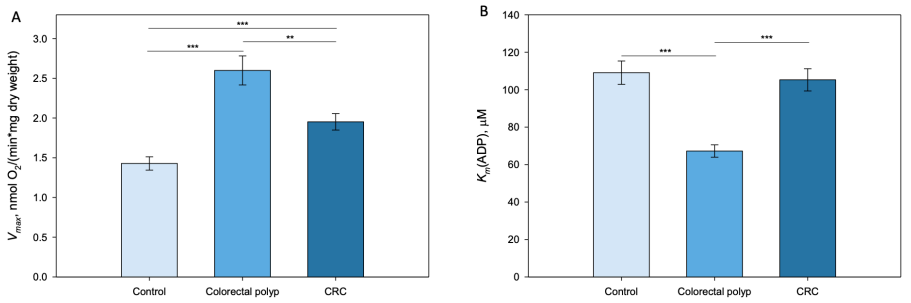

Figure 5. Kinetic parameters of mitochondrial respiration in normal tissue, colorectal polyps, and colorectal cancer tissue. *(A) Comparative analysis of maximal ADP-stimulated respiratory rate (V_{max}); and (B) apparent Michaelis-Menten constant values for ADP (K_m(ADP)) in control tissue (n=46), colorectal polyps (n=42), and colorectal cancer (CRC) tissue (n=57).* $p < 0.05$, *** $p < 0.001$ (t-test). (Publication II).*

The V_{max} for CRC was higher than for healthy colon tissue, and the V_{max} for colon polyps was twice higher for healthy colon tissue (**Figure 5A**). The values of K_m(ADP) exhibited that colon polyps have a significantly lower K_m(ADP) compared to both colorectal cancer and healthy tissue (**Figure 5B**). Orders of magnitude different K_m(ADP) values have been

found between glycolytic and oxidative striated muscles, which have different metabolic features. Tissue-specific K_m(ADP) values are higher in oxidative tissues compared to glycolytic tissues (Kuznetsov et al., 1996; Puurand et al., 2019). Healthy tissue and tumors showed similar K_m(ADP) values, indicating a lower affinity for ADP. The V_{max}/ K_m(ADP) ratio was 0.039 min^{-1} mg^{-1} mL for colon polyps.

In contrast, this ratio was similar and lower for tumor and healthy tissue (0.019 and 0.013, respectively), indicating a more catalytically efficient system in polyps. The mathematical model employed for muscle cells was applied to estimate the percent of mitochondria with highly regulated (oxidative) and unregulated (glycolytic) permeability of IMM to adenine nucleotides. According to the model, the hypothetical percentage of low oxidative capacity mitochondria in tissue is calculated from the K_m(ADP) value as an inverse asymptotic dependence (Saks et al., 1998). Polyps demonstrated a higher percentage of mitochondrion with low control over the movement of adenine nucleotides through OMM (**Table 2**), which indicates the metabolic shift to a glycolytic state.

Table 2. Modelled percentage of low oxidative capacity of mitochondrion in control, colon polyps, and CRC tissue group *(Publication II)*.

Sample group	% of low oxidative capacity of mitochondrion
Control (n=46)	31,2
Colon polyps (n=45)	55,9
CRC (n=68)	32,9

The changes in glycolytic markers have been observed in the early premalignant colorectal mucosal field, and these changes would be expected to promote increased glycolysis. Our findings on the K_m value for ADP for colorectal polyps suggest an early metabolic reprogramming towards glycolysis. Increased affinity for ADP on IMM and the high ADP-induced respiration level suggested a metabolic shift towards more glycolytic metabolism while maintaining OXPHOS functionality in polyps.

Maintaining the functional activity of OXPHOS is crucial in cancer cells, as those with a very low respiration rate are unable to form tumors (Hubackova et al., 2019). A moderate reduction in respiration can benefit the functioning of signaling molecules and the synthesis of anabolic precursors (Lu et al., 2015). The importance of functional OXPHOS varies depending on whether cells are proliferating or non-proliferating, with each situation exhibiting a unique functional manner (Hubackova et al., 2019).

Research, including the study by Koit et al. (2017) using breast cancer tissue and cell lines, has demonstrated that the metabolic profile of cancer cells in culture can vary significantly based on the specific conditions of the culture (Koit et al., 2017). For instance, cells grown in a glucose-free medium lead to the development of hyper-glycolytic cells with reduced respiratory flux (Gnaiger & Kemp, 1990; Gstraunthaler et al., 1999; Swerdlow et al., 2013). This knowledge highlights the impact of culture conditions on the metabolic behavior of cancer cells.

Given the influence of culture conditions on metabolic profiles, utilizing post-operative tissue material is a more suitable approach for studying OXPHOS in human tumors and describing the metabolic changes that occur during carcinogenesis.

4.1.2 Subgroup analysis by clinicopathological characteristics

To unveil respiratory rate kinetic parameters dependence on clinicopathological characteristics, possible relationships were analyzed of V_{max} and K_m(ADP) of polyps, colorectal cancer, and healthy colon tissue with age, gender, location, size, and histological type.

CRC is more frequently observed in the distal side (left colon) than in the proximal side (right colon) (Missiaglia et al., 2014). According to the studies, left and right colon tumors are distinct in their epidemiology, biology, histology, and microbial diversity (Drewes et al., 2016; Missiaglia et al., 2014). In **Publication I and II**, no relationship between V_{max} and K_m(ADP) values of healthy tissue, polyps, and CRC groups with clinicopathological factors was found with an exception. In **Publication I**, comparing all the distal and proximal tumors showed the difference in K_m(ADP) value but not in V_{max} (**Figure 6**).

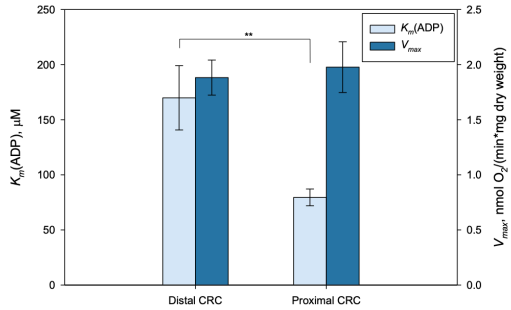

Figure 6. The distal (n=20) and proximal (n=24) tumors showed a difference in K_m(ADP) value but not in V_{max}. *** p<0.01. (Publication I).*

However, there were no differences in K_m(ADP) in Publication II, but a difference was seen in V_{max} values when comparing distal and proximal tumors and polyps. These results require further assessment by using a more extensive study group.

4.1.3 Subgroup analysis by *KRAS* and *BRAF* mutation status in CRC

The current thesis investigated two established and common molecular markers of prognosis in CRC: *KRAS* and *BRAF*. Almost half of the CRC tumors harbor *KRAS* mutations, and this mutation status is associated with poorer survival and response to chemotherapeutics (Phipps et al., 2013). *BRAF* V600E mutation status is also associated with an unfavorable prognosis (Yokota et al., 2011). Additionally, mutations in *KRAS* and *BRAF* genes appear to play a significant role in CRC's transcriptional regulation of the metabolic reprogramming (Zhong et al., 2022). In **Publication I**, V_{max} in *KRAS* or *BRAF* mutated tumors was significantly lower than in the wild-type tumor (**Figure 7**). This suggests an involvement of oncogenic *KRAS* and *BRAF* in metabolic reprogramming in CRC and supports the role of shifting CRC metabolism to a more glycolytic type.

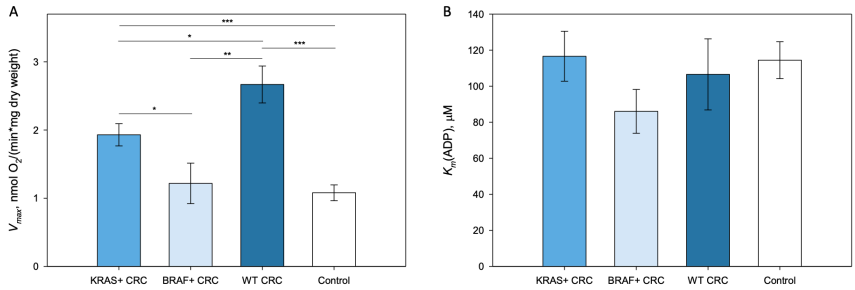

Figure 7. Kinetic parameters of mitochondrial respiration in KRAS and BRAF mutated and wild-type CRC. *(A) Comparative analysis of maximal ADP-activated respiratory rate (V_{max}); and (B) apparent Michaelis-Menten constant values for ADP (K_m(ADP)) in KRAS mutated CRC (n=13), BRAF mutated CRC (n=6), and wild-type polyps (n=14) * p < 0.05, ** p < 0.01 (t-test). (Publication I).*

Comparison of *KRAS* mutated, *BRAF* mutated, and WT tumors value for K_m(ADP) showed no differences. Interestingly, all CRC groups had similar K_m(ADP) values for tumor and control tissue. The lack of difference in K_m(ADP) in studied subgroups could be attributed to various factors. Aside from *KRAS* and *BRAF* mutations, other factors could contribute to the observed similarities in K_m(ADP). Metabolic adaptations and environmental influence might play a role in maintaining consistent K_m(ADP) values across different subgroups. Also, it is possible that *KRAS* and *BRAF* mutations do not directly affect the K_m(ADP) values in cancer. Small sample sizes and cancer tissue heterogeneity could mask any potential difference in K_m(ADP) values between the subgroups. Further research, employing a larger sample size and exploring additional metabolic and molecular parameters, would be valuable in elucidating the reasons behind the observed similarities in K_m(ADP) values among different CRC groups.

4.1.4 Subgroup analysis by *KRAS* and *BRAF* mutation status in colon polyp

The transformation of colon epithelium into a malignant state involves significant changes in energy production and biosynthesis pathways, supporting cancer development. The potential effect of *KRAS* and *BRAF* mutations on mitochondrial respiration was investigated in the colorectal polyp group (***Figure 8***). Polyps with *KRAS* mutation showed higher V_{max} values than those with *BRAF* mutation **(*Figure 8*A**). Additionally, mitochondria in *KRAS-mutated* polyps exhibited a reduced affinity for exogenous ADP compared to the *BRAF*-mutated polyp group (***Figure 8*B**). There were no significant differences in V_{max} and K_m(ADP) nor V_{max}/K_m(ADP) ratios between *KRAS* or *BRAF* mutated and wild-type polyps. However, due to the considerably lower V_{max} observed in both polyps and tumors with *BRAF* mutation, it is plausible to assume that cells carrying this mutation required sustained activation of active glycolysis while down-regulating OXPHOS.

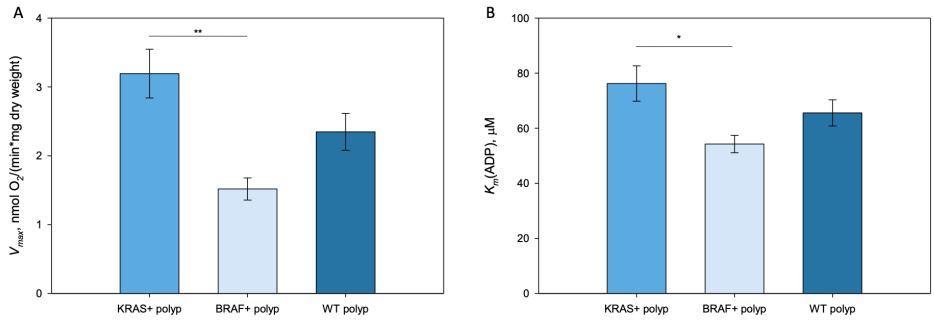

Figure 8. Kinetic parameters of mitochondrial respiration in KRAS, BRAF mutated, and wild-type polyps. *(A) Comparative analysis of maximal ADP-activated respiratory rate (V_{max}); and (B) apparent Michaelis-Menten constant values for ADP (K_m(ADP)) in KRAS mutated polyps (n=14), BRAF mutated polyps (n=6), and wild-type polyps (n=21).*
** p < 0.05, ** p < 0.01 (t-test). (Publication II).*

4.2 Different gene expression levels show changes in energy metabolism during CRC tumorigenesis (Publication II, III, I)

The precise mechanisms by which VDAC contributes to the development and progression of CRC still need to be fully understood. However, it is believed that VDAC plays a role in regulating glucose uptake in colorectal cancer cells by interacting with various glucose transporters and metabolic enzymes. Several studies have reported increased expression of genes involved in glucose uptake and metabolism in cancer, supporting the notion of VDAC's involvement in the metabolic reprogramming (Azuma et al., 2007; Graziano et al., 2017; Leclerc et al., 2017; N. N. Wang et al., 2021).

In the current thesis, RT-qPCR was performed to detect expression levels of genes encoding key enzymes involved in glycolysis control, including *GLUT1, HK1, and HK2.* Additionally, the expression levels of essential, non-controlling enzymes such as *MCT1, MCT2, MCT4,* and *LDHA* were analyzed to assess their potential contribution to metabolic reprogramming in colon polyps. The complete absence of essential genes or proteins leads to the complete cessation of the cellular process. Conversely, partial removal or inhibition of controlling steps results in a corresponding reduction in the analyzed cellular function.

4.2.1 Higher expression levels of genes important in glycose indicate increased glycolytic activity in polyps

During glycolysis, glucose is transported into the cell's cytoplasm via glucose transport proteins (GLUTs). GLUTs belong to the family of 14 glucose transporters found in various tissues throughout the body. In CRC, *GLUT*1 expression is often up-regulated, which allows cancer cells to take up more glucose and use it for energy (Barron et al., 2016). High expression of *GLUT* is associated with a poorer prognosis and a higher risk of recurrence for CRC (Ancey et al., 2018).

In **Publication II**, there was lower *GLUT1* expression in healthy colon tissue compared to the polyp and CRC group, which is aligned with the fact that glucose provides a small fraction of the energy requirements for the healthy colonic epithelium **(Figure 9A)**. The expression of *GLUT1* in the colorectal polyp group was significantly higher than in healthy tissue, suggesting an increased demand for glucose in polyps. The CRC group also

showed an increased level of *GLUT1* expression compared to the control group. The polyp tended toward a lower expression of *GLUT1* than the CRC group, but there was no statistically significant difference (p=0.136). A higher level of *GLUT1* expression in colon polyps compared to healthy tissue is aligned with the understanding that changes in energy metabolism can occur in the very early stage of carcinogenesis.

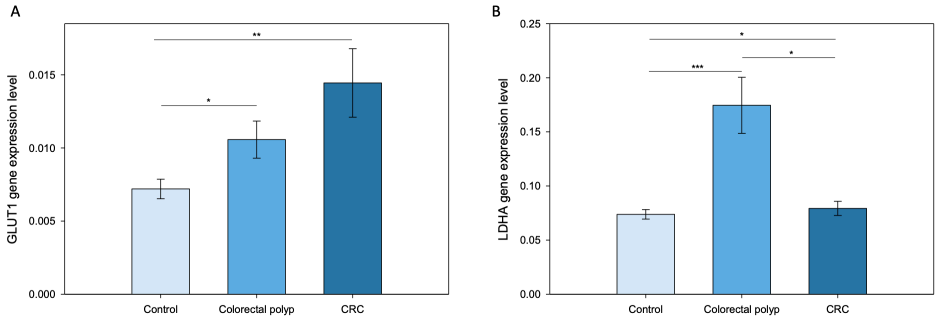

Figure 9. (A) GLUT1 expression levels in healthy colon tissue (n=16), colorectal polyps (n=8), and CRC (n=9) and (B) LDHA expression levels in healthy colon tissue (n=16), colorectal polyps (n=8), and CRC (n=9). ****p<0.001 **p<0.01, *p<0.05 (t-test). (Publication II).*

Lactate dehydrogenase A (LDHA) is an enzyme that plays a key role in converting pyruvate to lactate during glycolysis, primarily occurring in the absence of oxygen. Several studies have reported increased *LDHA* expression in various cancers such as liver, breast, and colon cancer, and it is closely related to the malignant process (Guddeti et al., 2019; Guo et al., 2019; Y. Wang et al., 2021). Although *LDHA* expression may also be increased in colon polyps, its role in developing colorectal polyps remains incompletely understood.

Compared to healthy colon tissue, elevated levels of *LDHA* expression in polyps suggest increased lactate production (**Figure 9B**). While LDHA is not a rate-limiting step in glycolysis, it maintains significant regulatory control over the equilibrium between pyruvate and lactate, affecting the cytosolic redox balance. The balance between pyruvate and lactate is tightly correlated with the NADH/NAD+ ratio, which can be modulated by an up-regulation of LDHA activity. Increased LDHA activity catalyzes the conversion of pyruvate to lactate, with a concomitant increase in the production of H^+ ions. This process contributes to malignancy progression, reducing the extracellular pH and potentially mitigating the host immune response (Kim et al., 2007). In addition to its role in ATP production, the LDHA-facilitated aerobic glycolysis pathway generates key metabolic intermediates for the biosynthesis of essential macromolecules, such as nucleotides, amino acids, and lipids (Vander Heiden et al., 2009). These precursors are crucial for sustaining neoplastic growth's rapid cell division characteristics.

Comparing *LDHA* expression levels in CRC and healthy tissue samples revealed no significant difference. This suggests that CRC cells might not augment lactate production due to a predominant reliance on OXPHOS for their energy requirements. These findings provide insight into the metabolic changes that occur during the development of colorectal polyps and CRC, highlighting the importance of glycolytic metabolism in early-stage polyps and the potential therapeutic implications of targeting glycolysis in these precancerous lesions.

Hexokinase (HK) is an enzyme that plays a crucial role in the first step of glucose metabolism. Abnormal expression of hexokinase has been observed in several types of

cancers, including colorectal cancer. One proposed mechanism by which hexokinase contributes to cancer growth is its ability to enhance the production of ATP (Patra et al., 2013). In the current thesis, the expression levels of *HK1* and *HK2* were investigated across healthy colon tissue, colorectal polyp, and CRC groups. HK1 expression level in the polyp group was twice as high as in healthy tissue and CRC groups, while the polyp group's HK2 expression was similar to the healthy tissue group (**Figure 10A**). Several studies have shown that overexpression of HK2 is commonly observed in colorectal cancer and higher HK2 expression is related to more aggressive tumor behavior and poorer prognosis (Ho & Coomber, 2016; Mathupala et al., 2006; N. N. Wang et al., 2021). However, current expression analyses of *HK2* did not reveal overexpression in the CRC group. It was significantly lower than healthy tissue ($p<0.001$). Typically, overexpression of *HK2* correlates with elevated glycolytic activity, permitting cancer cells to generate ATP and other vital metabolites that fuel their rapid growth and proliferation.

Decreased *HK2* expression in CRC, as was observed in **Publication II**, might be associated with reduced glycolytic activity and a shift towards alternative metabolic pathways. The outcomes elucidated in **Publication II** implied a heightened reliance of colorectal polyp cells on the glycolytic pathway, as substantiated by their low K_m(ADP) value and increased hexokinase expression levels. It was supported by research activities reviewed in **Publication IV**. Prof. Pedersen and his colleagues discovered the binding of HK2 to VDAC, concluding that this phenomenon could play a pivotal role in the Warburg effect. In such a setting, mitochondrial ATP is preferentially directed to glycolysis and produced ADP is channeled back to OXPHOS.

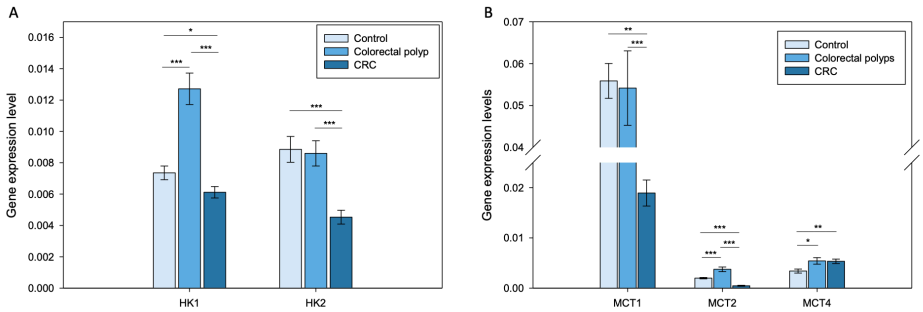

Figure 10. HK1 and HK2 expression levels in the healthy colon (n=27), colorectal polyps (n=9), and CRC (n=27). (B) MCT1 and MCT2 expression levels in healthy colon tissue (n=16), colorectal polyps (n=8), and CRC (n=9), and expression levels of MCT4 in healthy colon tissue (n=24), colorectal polyps (n=8) and CRC (n=22). ***p<0.001 **p<0.01, *p<0.05 (t-test) (Publication II).*

Monocarboxylate transporters (MCTs) are critical in transporting short-chain fatty acids, such as butyrate, across the luminal membrane of colonic epithelial cells. (Donohoe et al., 2011). Aberrant expression of *MCT1*, *MCT2,* and *MCT4* has been demonstrated in various cancers, including colon cancer; however, there is no data on the colorectal polyps (Baltazar et al., 2014; Fisel et al., 2013). In the current thesis, *MCT1*, *MCT2*, and *MCT4* expression levels were evaluated in healthy colon tissue, polyp, and CRC group.

Notably, healthy colon tissue exhibited higher expression levels of *MCT1* than the CRC tissue (**Figure 10B**). This observation aligns with the understanding that butyrate is the primary energy source for colonic epithelial cells. Lower expression of *MCT1* in cancerous tissue could imply that cancer cells possess an enhanced degree of metabolic plasticity

and are less reliant on butyrate as a nutrient. Such a metabolic shift during carcinogenesis enables cancer cells to use alternative energy sources, such as glucose and glutamine, to sustain their growth and proliferation. Comparable expression levels of *MCT1* in healthy tissue and polyp group suggested that colon polyps maintain their usage of short-chain fatty acids, such as butyrate. Low levels of butyrate in the colon may contribute to the development of colorectal cancer, emphasizing the importance of a fiber-rich diet, which can increase butyrate production and promote a healthy colon (Hamer et al., 2008).

The increased expression of *MCT2* has been observed in various cancer types, including breast, prostate, and colorectal cancer. Studies suggest that the increased expression of *MCT2* in cancer cells is related to the altered metabolic pathways of these cells, which rely on the glycolysis (Baltazar et al., 2014; Pinheiro et al., 2010). MTC2 is responsible for transporting lactate, produced during glycolysis, out of the cells, thereby maintaining a favorable metabolic environment for cancer cell survival and proliferation (Pinheiro et al., 2010). Findings in **Publication II** showed that the polyp group exhibited increased expression of *MCT2* compared to healthy colon tissue, consistent with our previous results demonstrating increased glycolytic phenotype. In contrast to previously mentioned studies, there was decreased expression level of *MCT2* in CRC tissue compared to healthy colon tissue and polyp group. This finding is consistent with the hypothesis that CRC cells may not rely on the typical Warburg effect, where glucose is preferentially metabolized via glycolysis, leading to lactate accumulation and subsequent upregulation of *MCT2* expression.

Polyps also exhibit elevated expression levels of *MCT4* compared to healthy tissue, indicating an increase in glycolytic activity. MCT 4 plays a vital role in exporting lactate out of the cells, preventing intracellular acidification. Also, *MCT4* is one of the target genes of hypoxia-inducible factor 1alpha (HIF-1*aplha*), and its expression is up-regulated in response to the hypoxia (Ullah et al., 2006). The overexpression of *MCT4* in colon polyps suggests an increase in glycolytic activity, possibly as an adaption to elevated lactate and the low-oxygen environment found in the colon (Giles et al., 2006).

4.2.2 Intracellular phosphotransfer pathways are up-regulated in colon polyps

Phosphotransfer circuits are biochemical pathways involved in the transfer of phosphate groups between molecules, typically involving protein kinases. These circuits play a crucial role in cellular signaling, allowing cells to respond to various stimuli and regulate important processes such as metabolism, growth, and differentiation. Two well-known phosphotransfer pathways are the adenylate kinase (AK) circuit and creatine kinase (CK) circuits. These circuits involve separate enzymatic systems that participate in different biochemical processes. AK is involved in maintaining the balance of ATP, ADP, and AMP in the cell. When energy consumption is high, and ATP levels decrease, the relative concentration of ADP increases. AK can catalyze the reaction of 2 ADP to form 1 ATP and 1 AMP. AK acts as a sensor and regulator of energy balance in cells, indirectly influencing the rate of OXPHOS by affecting the availability of ADP, which is a substrate for the ATP synthase complex. CK, particularly the mitochondrial isoform (mtCK), is tightly associated with the energy-producing process of OXPHOS. CKMT helps convert ATP into phosphocreatine (PCr), a form of energy storage that can be quickly mobilized to generate ATP when needed.

Dysregulation of intracellular phosphotransfer pathways involving adenylate and creatine kinase has been linked to cancer development and progression. Studies have shown that alterations in *AK* and *CK* expression and activity are common in cancer cells

and can contribute to tumor growth and metastasis (Balasubramani et al., 2006; Jan et al., 2019; M. Li et al., 2021; Xin et al., 2019; Xu et al., 2021). In **Publication II**, *AK1, AK2, AK4, AK6, CKBB, CKMT1,* and *CKMT2* expression level was assessed in healthy colon tissue, polyp, and CRC group.

AK1, which is highly expressed in the brain, heart, skeletal muscles, and erythrocytes, has been suggested as a negative regulator of the CRC development (Kaldma et al., 2014; Panayiotou et al., 2014). In the current study, the expression level of *AK1* in polyp groups was similar to that in healthy tissue and significantly higher than in the CRC group **(Figure 11A)**.

AK2, localized in the mitochondrial intermembrane space, regulates the cytosol and mitochondrial matrix's ATP/ADP transference rate. Previous studies have shown changes in the regulation of *AK2* in several human cancers. Its overexpression has been observed in lung adenocarcinoma, triple-negative breast cancer cells, and neuroblastoma cell lines, which may be related to the aggressive nature of these cancer types (Klepinin et al., 2022; Klepinin et al., 2016; Liu et al., 2019). In **Publication II**, the expression of *AK2* was up-regulated in the polyp group compared to CRC and healthy tissue groups. The CRC group showed significantly lower *AK2* expression than healthy colon tissue, suggesting fundamental rearrangement in the mitochondrial energy-related communication networks between cytosol and mitochondria during cancer progression.

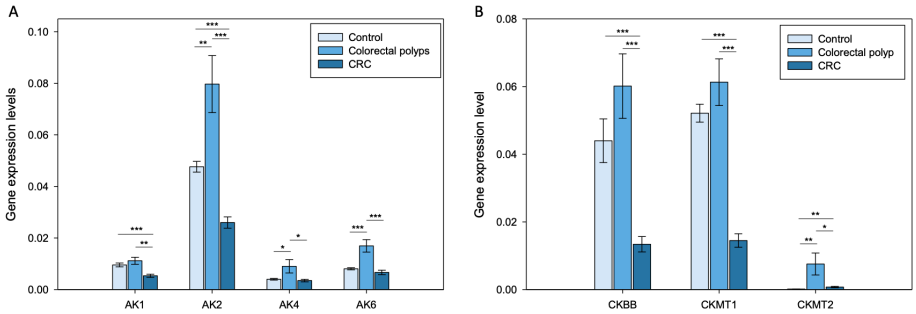

Figure 11. (A) Expression levels of adenylate kinases AK1, AK2, AK4, and AK6 (B) and creatine kinases CKBB, CKMT1, and CKMT2 in the healthy colon (n=19), colorectal polyps (n=9), and CRC (n=14). ****p<0.001 **p<0.01, *p<0.05 (t-test). (Publication II).*

AK4, expressed in the mitochondria matrix, may indirectly modulate the mitochondrial membrane permeability via its interactions with the ADP/ATP translocase (ANT). In this study, the expression level of *AK4* was significantly higher in the polyp group than in the healthy tissue group. Research has shown that AK4 can promote a shift toward the glycolysis (Jan et al., 2019), consistent with this thesis's observation of glycolytic phenotype in the polyp group.

AK6 is localized in the nucleus and cytosol and is ubiquitously expressed in different tissue and cell types (Ren et al., 2005; Xu et al., 2021). AK6 is a glycolysis regulator via phosphorylation of LDHA and a modulator of invasion and metastatic activity of cancer stem cells (Ji et al., 2017). Surprisingly, *AK6* was up-regulated at the polyp stage, suggesting it could support glycolytic activity in benign tumors. Higher LDHA expression levels in polyps than in CRC (**Figure 9C**) correlate with the same outcome in AK6 expression. Overall, these findings provide some insight into the potential roles of *AK1*, *AK2*, *AK4*, and *AK6* in glycolytic phenotypes of polyps.

CK provides a shuttle system for ATP between the mitochondria and cytosol, allowing for rapid ATP production in the cytosol during glycolysis. It is thought that isoforms are up- and down-regulated in tumors depending on the nature of carcinogenesis down-regulated (Yan, 2016). In **Publication II**, significantly lower expression of *CKBB* was observed in the CRC group compared to healthy colon tissue and polyp groups **(Figure 11B)**. Additionally, the expression of *CKMT1* was significantly higher in the polyp group compared to the CRC group. Together, these findings support the proposal that polyps have a higher glycolytic rate than cancerous tissue. The changes in intracellular energy transfer that occur in CRC and how AK and CK energy shuttles may be affected to prevent polyps from becoming malignant remain to be investigated.

4.2.3 The paradoxical role of adenylate kinase in cancer and polyps

Cancer is a complex and diverse disease that involves specific tissues and different stages of growth. Enzymatic changes can vary between the initial and advanced stages of the tumor growth (Boissan et al., 2018; González et al., 2020), additionally to the type of cancer. This variation in enzymatic changes may explain some conflicting findings regarding the expression of *AK* in cancer **(Table 3)**. In **Publication II**, no increased expression level of *AK* isoforms was observed in colorectal tissue samples.

Table 3. Adenylate kinase isoforms in colon and breast cancer. *Combined data from Publication II and III.*

Enzyme	Type of cancer	Status in tumor	Experimental model	References
AK	Colon cancer	↑	Tissue samples	(Chekulayev et al., 2015; Kaldma et al., 2014)
AK1	**Colon cancer**	**↓**	**Tissue samples**	**Publication II**
AK2	**Colon cancer**	**↓**	**Tissue samples**	**Publication II**
AK2	Breast cancer	↑	Tissue samples	(Speers et al., 2009)
AK2	Breast cancer	↑	Cancer stem cells	(Lamb et al., 2015)
AK2	Breast cancer	↑	Tissue samples and cell lines	(Klepinin et al., 2016)
AK2	Breast cancer	↓	Tissue samples and cell lines	(Kim et al., 2014)
AK4	**Colon cancer**	**No difference**	**Tissue samples**	**Publication II**
AK6	Breast and colon cancer	↑	Tissue samples	(Bai et al., 2016)
AK6	Colon cancer	↑	Cancer stem cells	(Ji et al., 2017)
AK6	**Colon cancer**	**No difference**	**Tissue samples**	**Publication II**

To date, the expression levels of *AK* in colorectal polyps have yet to be previously thoroughly investigated. However, **Publication II** found that all investigated AK isoforms were significantly up-regulated in polyps compared to colorectal tissue. Also, it was observed that the expression level of *AK* isoforms was significantly higher in polyps compared to healthy colon tissue except for AK1, which showed no significant difference in expression levels. This finding suggests that dysregulation of AK may have an important role in energy metabolism in colorectal polyps.

AK regulates the AMP-activated protein kinase (AMPK) pathway (Dzeja & Terzic, 2009; Hardie, 2011). AMPK is known to be activated in response to cellular stress, such as low energy levels, and it plays an essential role in regulating cellular energy homeostasis. Under normal conditions, the concentration of AMP in cells is very low due to the high ATP: ADP ratio, which drives the AK reaction (ATP+AMP $\leftrightarrow$ 2ADP) toward ADP synthesis. However, when cells are subjected to energetic stress and the ADP: ATP ratio rises, the AK reaction is partially displaced toward the synthesis of AMP, resulting in a significant increase in the cellular concentration of AMP. Increases in ADP and AMP are thus signals that cells are under energetic stress (D. Grahame Hardie et al., 2012). AMPK switches on catabolic processes that provide alternative pathways to generate ATP while switching off anabolic pathways and other processes consuming ATP. AK2 has a high affinity for AMP, and it has been proposed that AK2's primary function is to regulate intracellular AMP levels and to guard the cellular adenine pool (Dzeja & Terzic, 2009). Interestingly, in **Publication II**, a significant increase in *AK2* expression was observed in the polyp group compared to healthy tissue and CRC.

However, as reviewed in **Publication IV**, the role of AMPK in cancer is a subject of ongoing debate and investigation. While some studies suggest that AMPK acts as a tumor suppressor in certain cancers, others have proposed that it may have a contextual oncogenic role (Faubert et al., 2013; D. G. Hardie et al., 2012; Huang et al., 2016; Khan & Frigo, 2017; Park et al., 2009; Rehman et al., 2014; Wang & Guan, 2009). Further research is needed to fully elucidate the mechanisms underlying these effects and identify potential therapeutic targets for treating CRC.

4.3 Energy metabolic plasticity in colorectal cancer (Publications I, II, IV)

Metabolic plasticity is a phenomenon that allows cells to alter the configuration of their metabolic pathways in response to environmental changes. This process is regulated by key metabolic enzymes and transcription factors and is recognized as a hallmark of cancer. In CRC, the Warburg effect is commonly observed, where cancer cells primarily rely on aerobic glycolysis for energy, resulting in increased glucose consumption and lactate production (Altenberg & Greulich, 2004; Iwamoto et al., 2014; Kawada et al., 2015; Rubie et al., 2005; Shonk et al., 1965). However, studies have shown that both anaerobic and aerobic glycolysis operates in cancer cells simultaneously, and tumor cells often exhibit high rates of OXPHOS (Kaldma et al., 2014; Koit et al., 2017; Moreno-Sánchez et al., 2014). Three distinct metabolic phenotypes are observed in cancer cells: glycolytic, OXPHOS, and hybrid (a combination of glycolysis and OXPHOS). In contrast, normal cells only exhibit glycolytic and OXPHOS states and lack the hybrid state (Paudel & Quaranta, 2019; Yu et al., 2017).

Most of the molecular mechanisms of metabolic reprogramming in CRC have been studied in cell culture, which have limitations due to variations in cell culture conditions that can significantly affect the cells' metabolic profile (Gnaiger & Kemp, 1990; Gstraunthaler et al., 1999; Sherr & DePinho, 2000; Swerdlow et al., 2013). Previous studies

from the author's lab on clinical material from cancer patients have revealed unchanged glycolytic activity and up-regulation of OXPHOS in CRC compared to surrounding healthy tissue, which contrasts with cell culture data (Koit et al., 2017; Ounpuu et al., 2018). The **Publication I** and **II** outcomes suggested similar glycolytic activity in CRC compared to healthy colon tissue. Analysis of respirometry data (as described in **Publication I** and **II**) enables rapid functional profiling of metabolic plasticity. The dependence of mitochondrial O_2 consumption on ADP concentration follows Michaelis-Menten kinetics, which allows the evaluation of the apparent Michaelis-Menten constant for ADP K_m(ADP) in different tissues, cancers, and cell cultures (**Figure 12**). Determined in permeabilized cells and tissues, K_m(ADP) is the affinity of the mitochondria for exogenous ADP and characterizes the permeability of OMM for adenine nucleotides and VDAC permeability.

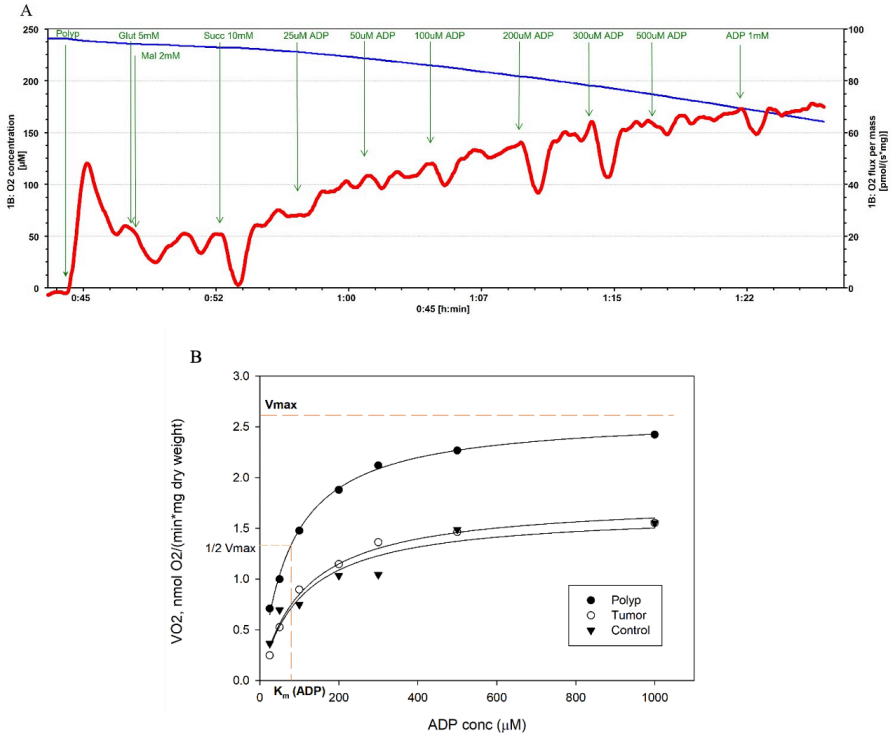

Figure 12. Recording of the original traces of O_2 consumption by permeabilized tissue upon the addition of increasing concentrations of ADP in polyp (A). *The red line shows the O_2 flux per sample mass, and the blue line indicates the O_2 consumption.* ***(B) The measured rates of respiration for samples were plotted against respective ADP concentrations****. The apparent K_m(ADP) and V_{max} were calculated by nonlinear regression using the Michaelis-Menten equation. The visual representation of how K_m(ADP) and V_{max} values are determined is only shown for the polyp. The upper dashed line shows V_{max} for the polyp sample, and K_m(ADP) is the ADP concentration at which the velocity of the reaction is half of V_{max}. (Publication II).*

The specific structural and functional organization of energy metabolism likely causes the cell-specific difference in K_m(ADP). For example, cells with low K_m(ADP) value (~10 µM), like a glycolytic muscle, possess less structural and functional restrictions for ADP/ATP movement through OMM as compared to the oxidative muscles (Km(ADP) ~300uM). Thus, relatively low K_m(ADP) for colorectal polyps seen in **Publication I** and **II**

indicates a metabolic reprogramming toward the glycolytic phenotype with functional OXPHOS (as in glycolytic muscles). Meanwhile, CRC tumors express high oxidative capacity calculated by the proposed model from Saks V. et al.

In addition to K_m(ADP), maximal ADP-dependent oxygen consumption (V_{max}) is a defining characteristic of metabolic plasticity and is correlated to mitochondrial content in the tissue. V_{max} values are higher in CRC than healthy colon tissue (**Publication I** and **II**), indicating vigorous metabolic activity. Moreover, V_{max} values in biopsy material from patients who succumbed to colon cancer were significantly higher than in patients in remission. However, the extent to which high V_{max} values correlate with tumor aggressiveness must be confirmed in further studies.

4.4 Concluding remarks

The primary objective of this investigation was to unravel the complex metabolic reprogramming events that transpire during the progression of colorectal polyps to cancerous tissue. The research methodology encompassed the utilization of post-operative clinical samples, allowing for the detailed examination of mitochondrial oxidative phosphorylation processes, along with the transcriptional modulation of key genes involved in the glycolysis pathway.

Previously, a considerable number of studies have depicted colorectal cancer as a disease heavily reliant on glycolysis for its metabolic needs. Contrary to this perception, the present research echoes recent scientific discourse, suggesting that colorectal cancer tissues are not purely glycolytic.

A particularly noteworthy discovery from our investigation was identifying unique metabolic patterns in colorectal polyps, displaying distinct differences from healthy and colorectal cancerous tissues. Intriguingly, the colorectal polyps exhibited a stronger inclination towards a glycolytic phenotype than cancer tissues. Furthermore, the study explored the relationship between the glycolytic phenotype and established colorectal biomarkers, specifically *KRAS* and *BRAF* mutations. The findings indicated that BRAF mutations in both polyps and tumors are associated with an increase in glycolysis and a simultaneous decrease in oxidative phosphorylation.

Significantly, these observations illuminate that metabolic reprogramming is not a late-stage phenomenon in cancer development but rather an early-stage process occurring even before the malignant transformation. Acquiring a glycolytic phenotype in colorectal polyps might act as a defense mechanism, regulating energy production and potentially inhibiting the transition from benign polyps to malignant tumors.

Collectively, this research offers invaluable insights into the elaborate metabolic shifts that occur during colorectal cancer progression. It enhances our comprehensive understanding of the molecular dynamics driving colorectal cancer development by reaffirming prior findings and drawing attention to the discrepancies between polyp and cancerous tissue bioenergetics.

Conclusions

- The results of this study revealed a significant difference in the bioenergetics profile of colorectal polyps compared to colorectal cancer, determined by ADP-dependent mitochondria respiration:
    - Colorectal polyps exhibited a metabolic shift towards a glycolytic state, indicating a preference for glycolysis as an energy source.
    - This metabolic alteration was observed irrespective of clinicopathological characteristics.
    - *BRAF* mutation in both colorectal polyps and cancer suggested more active glycolysis.
- Increased expression levels of genes involved in glycolysis were observed in colorectal polyps:
    - *GLUT1*, *LDHA*, *HK1, MCT2, and MCT4* expression levels were higher in colorectal polyps than in healthy tissue.
    - *LDHA, HK1, HK2, MCT1, and MCT2 expression levels were higher in colorectal polyps than in CRC*
- Intracellular phosphotransfer pathways were up-regulated in colorectal polyps:
    - The expression levels of *AK1, AK2, AK4, AK6, CKBB, CKMT1*, and *CKMT2* were significantly higher in the colorectal polyp group compared to CRC. These findings suggest activating intracellular phosphotransfer pathways in colorectal polyps, potentially contributing to their altered metabolic profile.

List of Figures

List of Tables

References

Alcarraz-Vizán, G., Boren, J., Lee, W. N., & Cascante, M. (2010). Histone deacetylase inhibition results in a common metabolic profile associated with HT29 differentiation. *Metabolomics*, *6*(2), 229-237. https://doi.org/10.1007/s11306-009-0192-0

Ali, E. S., Sahu, U., Villa, E., O'Hara, B. P., Gao, P., Beaudet, C., Wood, A. W., Asara, J. M., & Ben-Sahra, I. (2020). ERK2 Phosphorylates PFAS to Mediate Posttranslational Control of De Novo Purine Synthesis. *Mol Cell*, *78*(6), 1178-1191.e1176. https://doi.org/10.1016/j.molcel.2020.05.001

Altenberg, B., & Greulich, K. O. (2004). Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics*, *84*(6), 1014-1020. https://doi.org/10.1016/j.ygeno.2004.08.010

Amoêdo, N. D., Rodrigues, M. F., Pezzuto, P., Galina, A., da Costa, R. M., de Almeida, F. C., El-Bacha, T., & Rumjanek, F. D. (2011). Energy metabolism in H460 lung cancer cells: effects of histone deacetylase inhibitors. *Plos one*, *6*(7), e22264. https://doi.org/10.1371/journal.pone.0022264

Ancey, P. B., Contat, C., & Meylan, E. (2018). Glucose transporters in cancer - from tumor cells to the tumor microenvironment. *Febs j*, *285*(16), 2926-2943. https://doi.org/10.1111/febs.14577

Avagliano, A., Fiume, G., Pelagalli, A., Sanità, G., Ruocco, M. R., Montagnani, S., & Arcucci, A. (2020). Metabolic Plasticity of Melanoma Cells and Their Crosstalk With Tumor Microenvironment. *Front Oncol*, *10*, 722. https://doi.org/10.3389/fonc.2020.00722

Azuma, M., Shi, M., Danenberg, K. D., Gardner, H., Barrett, C., Jacques, C. J., Sherod, A., Iqbal, S., El-Khoueiry, A., Yang, D., Zhang, W., Danenberg, P. V., & Lenz, H. J. (2007). Serum lactate dehydrogenase levels and glycolysis significantly correlate with tumor VEGFA and VEGFR expression in metastatic CRC patients. *Pharmacogenomics*, *8*(12), 1705-1713. https://doi.org/10.2217/14622416.8.12.1705

Baek, G., Tse, Y. F., Hu, Z., Cox, D., Buboltz, N., McCue, P., Yeo, C. J., White, M. A., DeBerardinis, R. J., Knudsen, E. S., & Witkiewicz, A. K. (2014). MCT4 defines a glycolytic subtype of pancreatic cancer with poor prognosis and unique metabolic dependencies. *Cell Rep*, *9*(6), 2233-2249. https://doi.org/10.1016/j.celrep.2014.11.025

Bai, D., Zhang, J., Li, T., Hang, R., Liu, Y., Tian, Y., Huang, D., Qu, L., Cao, X., Ji, J., & Zheng, X. (2016). The ATPase hCINAP regulates 18S rRNA processing and is essential for embryogenesis and tumour growth. *Nat Commun*, *7*, 12310. https://doi.org/10.1038/ncomms12310

Balasubramani, M., Day, B. W., Schoen, R. E., & Getzenberg, R. H. (2006). Altered expression and localization of creatine kinase B, heterogeneous nuclear ribonucleoprotein F, and high mobility group box 1 protein in the nuclear matrix associated with colon cancer. *Cancer Res*, *66*(2), 763-769. https://doi.org/10.1158/0008-5472.Can-05-3771

Baltazar, F., Pinheiro, C., Morais-Santos, F., Azevedo-Silva, J., Queirós, O., Preto, A., & Casal, M. (2014). Monocarboxylate transporters as targets and mediators in cancer therapy response. *Histol Histopathol*, *29*(12), 1511-1524. https://doi.org/10.14670/hh-29.1511

Barker, N. (2014). Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat Rev Mol Cell Biol*, *15*(1), 19-33. https://doi.org/10.1038/nrm3721

Barron, C. C., Bilan, P. J., Tsakiridis, T., & Tsiani, E. (2016). Facilitative glucose transporters: Implications for cancer detection, prognosis and treatment. *Metabolism*, *65*(2), 124-139. https://doi.org/10.1016/j.metabol.2015.10.007

Bellance, N., Lestienne, P., & Rossignol, R. (2009). Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis. *Front Biosci (Landmark Ed)*, *14*(11), 4015-4034. https://doi.org/10.2741/3509

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev*, *70*(2), 567-590. https://doi.org/10.1152/physrev.1990.70.2.567

Bettington, M., Walker, N., Clouston, A., Brown, I., Leggett, B., & Whitehall, V. (2013). The serrated pathway to colorectal carcinoma: current concepts and challenges. *Histopathology*, *62*(3), 367-386. https://doi.org/10.1111/his.12055

Blouin, J. M., Penot, G., Collinet, M., Nacfer, M., Forest, C., Laurent-Puig, P., Coumoul, X., Barouki, R., Benelli, C., & Bortoli, S. (2011). Butyrate elicits a metabolic switch in human colon cancer cells by targeting the pyruvate dehydrogenase complex. *Int J Cancer*, *128*(11), 2591-2601. https://doi.org/10.1002/ijc.25599

Boissan, M., Schlattner, U., & Lacombe, M. L. (2018). The NDPK/NME superfamily: state of the art. *Lab Invest*, *98*(2), 164-174. https://doi.org/10.1038/labinvest.2017.137

Bourne, H. R., Sanders, D. A., & McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. *Nature*, *349*(6305), 117-127. https://doi.org/10.1038/349117a0

Brown, R. E., Short, S. P., & Williams, C. S. (2018). Colorectal Cancer and Metabolism. *Curr Colorectal Cancer Rep*, *14*(6), 226-241. https://doi.org/10.1007/s11888-018-0420-y

Cabrera, R., Baez, M., Pereira, H. M., Caniuguir, A., Garratt, R. C., & Babul, J. (2011). The crystal complex of phosphofructokinase-2 of Escherichia coli with fructose-6-phosphate: kinetic and structural analysis of the allosteric ATP inhibition. *J Biol Chem*, *286*(7), 5774-5783. https://doi.org/10.1074/jbc.M110.163162

Cantwell-Dorris, E. R., O'Leary, J. J., & Sheils, O. M. (2011). BRAFV600E: implications for carcinogenesis and molecular therapy. *Mol Cancer Ther*, *10*(3), 385-394. https://doi.org/10.1158/1535-7163.Mct-10-0799

Capps, G. J., Samuels, D. C., & Chinnery, P. F. (2003). A model of the nuclear control of mitochondrial DNA replication. *J Theor Biol*, *221*(4), 565-583. https://doi.org/10.1006/jtbi.2003.3207

Caputo, F., Santini, C., Bardasi, C., Cerma, K., Casadei-Gardini, A., Spallanzani, A., Andrikou, K., Cascinu, S., & Gelsomino, F. (2019). BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. *Int J Mol Sci*, *20*(21). https://doi.org/10.3390/ijms20215369

Carrasco, A. J., Dzeja, P. P., Alekseev, A. E., Pucar, D., Zingman, L. V., Abraham, M. R., Hodgson, D., Bienengraeber, M., Puceat, M., Janssen, E., Wieringa, B., & Terzic, A. (2001). Adenylate kinase phosphotransfer communicates cellular energetic signals to ATP-sensitive potassium channels. *Proc Natl Acad Sci U S A*, *98*(13), 7623-7628. https://doi.org/10.1073/pnas.121038198

Chekulayev, V., Mado, K., Shevchuk, I., Koit, A., Kaldma, A., Klepinin, A., Timohhina, N., Tepp, K., Kandashvili, M., Ounpuu, L., Heck, K., Truu, L., Planken, A., Valvere, V., & Kaambre, T. (2015). Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes. *Biochem Biophys Rep*, *4*, 111-125. https://doi.org/10.1016/j.bbrep.2015.08.020

Chen, S. W., Chiang, H. C., Chen, W. T., Hsieh, T. C., Yen, K. Y., Chiang, S. F., & Kao, C. H. (2014). Correlation between PET/CT parameters and KRAS expression in colorectal cancer. *Clin Nucl Med*, *39*(8), 685-689. https://doi.org/10.1097/rlu.0000000000000481

Colgan, S. P., Campbell, E. L., & Kominsky, D. J. (2016). Hypoxia and Mucosal Inflammation. *Annu Rev Pathol*, *11*, 77-100. https://doi.org/10.1146/annurev-pathol-012615-044231

Colussi, D., Brandi, G., Bazzoli, F., & Ricciardiello, L. (2013). Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci*, *14*(8), 16365-16385. https://doi.org/10.3390/ijms140816365

Comalada, M., Bailón, E., de Haro, O., Lara-Villoslada, F., Xaus, J., Zarzuelo, A., & Gálvez, J. (2006). The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. *J Cancer Res Clin Oncol*, *132*(8), 487-497. https://doi.org/10.1007/s00432-006-0092-x

Comprehensive molecular characterization of human colon and rectal cancer. (2012). *Nature*, *487*(7407), 330-337. https://doi.org/10.1038/nature11252

Cuff, M., Dyer, J., Jones, M., & Shirazi-Beechey, S. (2005). The human colonic monocarboxylate transporter Isoform 1: its potential importance to colonic tissue homeostasis. *Gastroenterology*, *128*(3), 676-686. https://doi.org/10.1053/j.gastro.2004.12.003

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, *28*(10), 1221-1227. https://doi.org/10.1136/gut.28.10.1221

Daly, K., Cuff, M. A., Fung, F., & Shirazi-Beechey, S. P. (2005). The importance of colonic butyrate transport to the regulation of genes associated with colonic tissue homoeostasis. *Biochem Soc Trans*, *33*(Pt 4), 733-735. https://doi.org/10.1042/bst0330733

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., . . . Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. *Nature*, *417*(6892), 949-954. https://doi.org/10.1038/nature00766

DeBerardinis, R. J., & Thompson, C. B. (2012). Cellular metabolism and disease: what do metabolic outliers teach us? *Cell*, *148*(6), 1132-1144. https://doi.org/10.1016/j.cell.2012.02.032

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*, *54*(9), 2325-2340. https://doi.org/10.1194/jlr.R036012

Dienstmann, R., Mason, M. J., Sinicrope, F. A., Phipps, A. I., Tejpar, S., Nesbakken, A., Danielsen, S. A., Sveen, A., Buchanan, D. D., Clendenning, M., Rosty, C., Bot, B., Alberts, S. R., Milburn Jessup, J., Lothe, R. A., Delorenzi, M., Newcomb, P. A., Sargent, D., & Guinney, J. (2017). Prediction of overall survival in stage II and III colon cancer beyond TNM system: a retrospective, pooled biomarker study. *Ann Oncol*, *28*(5), 1023-1031. https://doi.org/10.1093/annonc/mdx052

Dimmer, K. S., Friedrich, B., Lang, F., Deitmer, J. W., & Bröer, S. (2000). The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochem J*, *350 Pt 1*(Pt 1), 219-227.

Donohoe, D. R., Garge, N., Zhang, X., Sun, W., O'Connell, T. M., Bunger, M. K., & Bultman, S. J. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab*, *13*(5), 517-526. https://doi.org/10.1016/j.cmet.2011.02.018

Drewes, J. L., Housseau, F., & Sears, C. L. (2016). Sporadic colorectal cancer: microbial contributors to disease prevention, development and therapy. *Br J Cancer*, *115*(3), 273-280. https://doi.org/10.1038/bjc.2016.189

Dzeja, P., Chung, S., & Terzic, A. (2007). Molecular System Bioenergetics: Energy for Life.

Dzeja, P., & Terzic, A. (2009). Adenylate Kinase and AMP Signaling Networks: Metabolic Monitoring, Signal Communication and Body Energy Sensing. *International Journal of Molecular Sciences*, *10*(4), 1729-1772. https://www.mdpi.com/1422-0067/10/4/1729

Dzeja, P. P., Bortolon, R., Perez-Terzic, C., Holmuhamedov, E. L., & Terzic, A. (2002). Energetic communication between mitochondria and nucleus directed by catalyzed phosphotransfer. *Proc Natl Acad Sci U S A*, *99*(15), 10156-10161. https://doi.org/10.1073/pnas.152259999

Dzeja, P. P., Chung, S., Faustino, R. S., Behfar, A., & Terzic, A. (2011). Developmental enhancement of adenylate kinase-AMPK metabolic signaling axis supports stem cell cardiac differentiation. *Plos one*, *6*(4), e19300. https://doi.org/10.1371/journal.pone.0019300

Dzeja, P. P., & Terzic, A. (2003). Phosphotransfer networks and cellular energetics. *Journal of Experimental Biology*, *206*(12), 2039-2047.

Dzeja, P. P., Vitkevicius, K. T., Redfield, M. M., Burnett, J. C., & Terzic, A. (1999). Adenylate kinase-catalyzed phosphotransfer in the myocardium : increased contribution in heart failure. *Circ Res*, *84*(10), 1137-1143. https://doi.org/10.1161/01.res.84.10.1137

Dzeja, P. P., Zeleznikar, R. J., & Goldberg, N. D. (1998). Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes. *Molecular and cellular biochemistry*, *184*(1), 169-182.

Faubert, B., Boily, G., Izreig, S., Griss, T., Samborska, B., Dong, Z., Dupuy, F., Chambers, C., Fuerth, B. J., Viollet, B., Mamer, O. A., Avizonis, D., DeBerardinis, R. J., Siegel, P. M., & Jones, R. G. (2013). AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. *Cell Metab*, *17*(1), 113-124. https://doi.org/10.1016/j.cmet.2012.12.001

Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell*, *61*(5), 759-767. https://doi.org/10.1016/0092-8674(90)90186-i

Fisel, P., Kruck, S., Winter, S., Bedke, J., Hennenlotter, J., Nies, A. T., Scharpf, M., Fend, F., Stenzl, A., Schwab, M., & Schaeffeler, E. (2013). DNA methylation of the SLC16A3 promoter regulates expression of the human lactate transporter MCT4 in renal cancer with consequences for clinical outcome. *Clin Cancer Res*, *19*(18), 5170-5181. https://doi.org/10.1158/1078-0432.Ccr-13-1180

Fujisawa, K., Murakami, R., Horiguchi, T., & Noma, T. (2009). Adenylate kinase isozyme 2 is essential for growth and development of Drosophila melanogaster. *Comp Biochem Physiol B Biochem Mol Biol*, *153*(1), 29-38. https://doi.org/10.1016/j.cbpb.2009.01.006

Fukuda, S., Miyata, H., Miyazaki, Y., Makino, T., Takahashi, T., Kurokawa, Y., Yamasaki, M., Nakajima, K., Takiguchi, S., Mori, M., & Doki, Y. (2015). Pyruvate Kinase M2 Modulates Esophageal Squamous Cell Carcinoma Chemotherapy Response by Regulating the Pentose Phosphate Pathway. *Ann Surg Oncol*, *22 Suppl 3*, S1461-1468. https://doi.org/10.1245/s10434-015-4522-3

Fung, K. Y., Cosgrove, L., Lockett, T., Head, R., & Topping, D. L. (2012). A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br J Nutr*, *108*(5), 820-831. https://doi.org/10.1017/s0007114512001948

Fung, K. Y., Ooi, C. C., Lewanowitsch, T., Tan, S., Tan, H. T., Lim, T. K., Lin, Q., Williams, D. B., Lockett, T. J., Cosgrove, L. J., Chung, M. C., & Head, R. J. (2012). Identification of potential pathways involved in induction of apoptosis by butyrate and 4-benzoylbutyrate in HT29 colorectal cancer cells. *J Proteome Res*, *11*(12), 6019-6029. https://doi.org/10.1021/pr3007107

Gaglio, D., Metallo, C. M., Gameiro, P. A., Hiller, K., Danna, L. S., Balestrieri, C., Alberghina, L., Stephanopoulos, G., & Chiaradonna, F. (2011). Oncogenic K-Ras decouples glucose and glutamine metabolism to support cancer cell growth. *Mol Syst Biol*, *7*, 523. https://doi.org/10.1038/msb.2011.56

Giaccia, A., Siim, B. G., & Johnson, R. S. (2003). HIF-1 as a target for drug development. *Nat Rev Drug Discov*, *2*(10), 803-811. https://doi.org/10.1038/nrd1199

Giles, R. H., Lolkema, M. P., Snijckers, C. M., Belderbos, M., van der Groep, P., Mans, D. A., van Beest, M., van Noort, M., Goldschmeding, R., van Diest, P. J., Clevers, H., & Voest, E. E. (2006). Interplay between VHL/HIF1alpha and Wnt/beta-catenin pathways during colorectal tumorigenesis. *Oncogene*, *25*(21), 3065-3070. https://doi.org/10.1038/sj.onc.1209330

Gill, R. K., Saksena, S., Alrefai, W. A., Sarwar, Z., Goldstein, J. L., Carroll, R. E., Ramaswamy, K., & Dudeja, P. K. (2005). Expression and membrane localization of MCT isoforms along the length of the human intestine. *Am J Physiol Cell Physiol*, *289*(4), C846-852. https://doi.org/10.1152/ajpcell.00112.2005

Gnaiger, E., & Kemp, R. B. (1990). Anaerobic metabolism in aerobic mammalian cells: information from the ratio of calorimetric heat flux and respirometric oxygen flux. *Biochim Biophys Acta*, *1016*(3), 328-332. https://doi.org/10.1016/0005-2728(90)90164-y

Goldstein, N. S. (2006). Serrated pathway and APC (conventional)-type colorectal polyps: molecular-morphologic correlations, genetic pathways, and implications for classification. *Am J Clin Pathol*, *125*(1), 146-153.

Gonçalves, P., & Martel, F. (2016). Regulation of colonic epithelial butyrate transport: Focus on colorectal cancer. *Porto Biomed J*, *1*(3), 83-91. https://doi.org/10.1016/j.pbj.2016.04.004

González, A., Hall, M. N., Lin, S. C., & Hardie, D. G. (2020). AMPK and TOR: The Yin and Yang of Cellular Nutrient Sensing and Growth Control. *Cell Metab*, *31*(3), 472-492. https://doi.org/10.1016/j.cmet.2020.01.015

Grady, W. M., & Carethers, J. M. (2008). Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology*, *135*(4), 1079-1099. https://doi.org/10.1053/j.gastro.2008.07.076

Graziano, F., Ruzzo, A., Giacomini, E., Ricciardi, T., Aprile, G., Loupakis, F., Lorenzini, P., Ongaro, E., Zoratto, F., Catalano, V., Sarti, D., Rulli, E., Cremolini, C., De Nictolis, M., De Maglio, G., Falcone, A., Fiorentini, G., & Magnani, M. (2017). Glycolysis gene expression analysis and selective metabolic advantage in the clinical progression of colorectal cancer. *Pharmacogenomics J*, *17*(3), 258-264. https://doi.org/10.1038/tpj.2016.13

Gstraunthaler, G., Seppi, T., & Pfaller, W. (1999). Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? *Cell Physiol Biochem*, *9*(3), 150-172. https://doi.org/10.1159/000016312

Guddeti, R. K., Bali, P., Karyala, P., & Pakala, S. B. (2019). MTA1 coregulator regulates LDHA expression and function in breast cancer. *Biochem Biophys Res Commun*, *520*(1), 54-59. https://doi.org/10.1016/j.bbrc.2019.09.078

Guerbette, T., Boudry, G., & Lan, A. (2022). Mitochondrial function in intestinal epithelium homeostasis and modulation in diet-induced obesity. *Mol Metab*, *63*, 101546. https://doi.org/10.1016/j.molmet.2022.101546

Gui, D. Y., Lewis, C. A., & Vander Heiden, M. G. (2013). Allosteric regulation of PKM2 allows cellular adaptation to different physiological states. *Sci Signal*, *6*(263), pe7. https://doi.org/10.1126/scisignal.2003925

Guinney, J., Dienstmann, R., Wang, X., de Reyniès, A., Schlicker, A., Soneson, C., Marisa, L., Roepman, P., Nyamundanda, G., Angelino, P., Bot, B. M., Morris, J. S., Simon, I. M., Gerster, S., Fessler, E., De Sousa E Melo, F., Missiaglia, E., Ramay, H., Barras, D., . . . Tejpar, S. (2015). The consensus molecular subtypes of colorectal cancer. *Nature Medicine*, *21*(11), 1350-1356. https://doi.org/10.1038/nm.3967

Guo, Y., Li, X., Sun, X., Wang, J., Yang, X., Zhou, X., Liu, X., Liu, W., Yuan, J., Yao, L., Li, X., & Shen, L. (2019). Combined Aberrant Expression of NDRG2 and LDHA Predicts Hepatocellular Carcinoma Prognosis and Mediates the Anti-tumor Effect of Gemcitabine. *Int J Biol Sci*, *15*(9), 1771-1786. https://doi.org/10.7150/ijbs.35094

Hadjiagapiou, C., Schmidt, L., Dudeja, P. K., Layden, T. J., & Ramaswamy, K. (2000). Mechanism(s) of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. *Am J Physiol Gastrointest Liver Physiol*, *279*(4), G775-780. https://doi.org/10.1152/ajpgi.2000.279.4.G775

Haigis, K. M. (2017). KRAS Alleles: The Devil Is in the Detail. *Trends Cancer*, *3*(10), 686-697. https://doi.org/10.1016/j.trecan.2017.08.006

Halestrap, A. P., & Meredith, D. (2004). The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch*, *447*(5), 619-628. https://doi.org/10.1007/s00424-003-1067-2

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2008). Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther*, *27*(2), 104-119. https://doi.org/10.1111/j.1365-2036.2007.03562.x

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, *100*(1), 57-70. https://doi.org/10.1016/s0092-8674(00)81683-9

Hardie, D. G. (2011). AMP-activated protein kinase: a cellular energy sensor with a key role in metabolic disorders and in cancer. *Biochem Soc Trans*, *39*(1), 1-13. https://doi.org/10.1042/bst0390001

Hardie, D. G., Ross, F. A., & Hawley, S. A. (2012). AMP-activated protein kinase: a target for drugs both ancient and modern. *Chem Biol*, *19*(10), 1222-1236. https://doi.org/10.1016/j.chembiol.2012.08.019

He, G., Shankar, R. A., Chzhan, M., Samouilov, A., Kuppusamy, P., & Zweier, J. L. (1999). Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proc Natl Acad Sci U S A*, *96*(8), 4586-4591. https://doi.org/10.1073/pnas.96.8.4586

Ho, N., & Coomber, B. L. (2016). Hexokinase II expression is correlated with colorectal cancer prognosis. *Cancer Treatment Communications*, *6*, 11-16.

Hodge, T., & Colombini, M. (1997). Regulation of metabolite flux through voltage-gating of VDAC channels. *J Membr Biol*, *157*(3), 271-279. https://doi.org/10.1007/s002329900235

Hossain, M. S., Karuniawati, H., Jairoun, A. A., Urbi, Z., Ooi, J., John, A., Lim, Y. C., Kibria, K. M. K., Mohiuddin, A. K. M., Ming, L. C., Goh, K. W., & Hadi, M. A. (2022). Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. *Cancers (Basel)*, *14*(7). https://doi.org/10.3390/cancers14071732

Huang, X., Li, X., Xie, X., Ye, F., Chen, B., Song, C., Tang, H., & Xie, X. (2016). High expressions of LDHA and AMPK as prognostic biomarkers for breast cancer. *Breast*, *30*, 39-46. https://doi.org/10.1016/j.breast.2016.08.014

Hubackova, S., Magalhaes Novais, S., Davidova, E., Neuzil, J., & Rohlena, J. (2019). Mitochondria-driven elimination of cancer and senescent cells. *Biol Chem*, *400*(2), 141-148. https://doi.org/10.1515/hsz-2018-0256

Hutton, J. E., Wang, X., Zimmerman, L. J., Slebos, R. J., Trenary, I. A., Young, J. D., Li, M., & Liebler, D. C. (2016). Oncogenic KRAS and BRAF Drive Metabolic Reprogramming in Colorectal Cancer. *Mol Cell Proteomics*, *15*(9), 2924-2938. https://doi.org/10.1074/mcp.M116.058925

Iwamoto, M., Kawada, K., Nakamoto, Y., Itatani, Y., Inamoto, S., Toda, K., Kimura, H., Sasazuki, T., Shirasawa, S., Okuyama, H., Inoue, M., Hasegawa, S., Togashi, K., & Sakai, Y. (2014). Regulation of 18F-FDG accumulation in colorectal cancer cells with mutated KRAS. *J Nucl Med*, *55*(12), 2038-2044. https://doi.org/10.2967/jnumed.114.142927

Iwanaga, T., Takebe, K., Kato, I., Karaki, S., & Kuwahara, A. (2006). Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8. *Biomed Res*, *27*(5), 243-254. https://doi.org/10.2220/biomedres.27.243

Jackson, V. N., Price, N. T., Carpenter, L., & Halestrap, A. P. (1997). Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-transcriptional regulation. *Biochem J*, *324 (Pt 2)*(Pt 2), 447-453. https://doi.org/10.1042/bj3240447

Jan, Y.-H., Lai, T.-C., Yang, C.-J., Lin, Y.-F., Huang, M.-S., & Hsiao, M. (2019). Adenylate kinase 4 modulates oxidative stress and stabilizes HIF-1α to drive lung adenocarcinoma metastasis. *Journal of Hematology & Oncology*, *12*(1), 12. https://doi.org/10.1186/s13045-019-0698-5

Jass, J. R. (2004). Hyperplastic polyps and colorectal cancer: is there a link? *Clin Gastroenterol Hepatol*, *2*(1), 1-8. https://doi.org/10.1016/s1542-3565(03)00284-2

Ji, Y., Yang, C., Tang, Z., Yang, Y., Tian, Y., Yao, H., Zhu, X., Zhang, Z., Ji, J., & Zheng, X. (2017). Adenylate kinase hCINAP determines self-renewal of colorectal cancer stem cells by facilitating LDHA phosphorylation. *Nat Commun*, *8*, 15308. https://doi.org/10.1038/ncomms15308

Jiang, P., Du, W., & Wu, M. (2014). Regulation of the pentose phosphate pathway in cancer. *Protein Cell*, *5*(8), 592-602. https://doi.org/10.1007/s13238-014-0082-8

Jin, L., & Zhou, Y. (2019). Crucial role of the pentose phosphate pathway in malignant tumors. *Oncol Lett*, *17*(5), 4213-4221. https://doi.org/10.3892/ol.2019.10112

Jones, S., Chen, W. D., Parmigiani, G., Diehl, F., Beerenwinkel, N., Antal, T., Traulsen, A., Nowak, M. A., Siegel, C., Velculescu, V. E., Kinzler, K. W., Vogelstein, B., Willis, J., & Markowitz, S. D. (2008). Comparative lesion sequencing provides insights into tumor evolution. *Proc Natl Acad Sci U S A*, *105*(11), 4283-4288. https://doi.org/10.1073/pnas.0712345105

Joubert, F., Mazet, J. L., Mateo, P., & Hoerter, J. A. (2002). 31P NMR detection of subcellular creatine kinase fluxes in the perfused rat heart: contractility modifies energy transfer pathways. *J Biol Chem*, *277*(21), 18469-18476. https://doi.org/10.1074/jbc.M200792200

Kaelin, W. G., Jr., & Ratcliffe, P. J. (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell*, *30*(4), 393-402. https://doi.org/10.1016/j.molcel.2008.04.009

Kaldma, A., Klepinin, A., Chekulayev, V., Mado, K., Shevchuk, I., Timohhina, N., Tepp, K., Kandashvili, M., Varikmaa, M., Koit, A., Planken, M., Heck, K., Truu, L., Planken, A., Valvere, V., Rebane, E., & Kaambre, T. (2014). An in situ study of bioenergetic properties of human colorectal cancer: the regulation of mitochondrial respiration and distribution of flux control among the components of ATP synthasome. *Int J Biochem Cell Biol*, *55*, 171-186. https://doi.org/10.1016/j.biocel.2014.09.004

Kambara, T., Simms, L. A., Whitehall, V. L., Spring, K. J., Wynter, C. V., Walsh, M. D., Barker, M. A., Arnold, S., McGivern, A., Matsubara, N., Tanaka, N., Higuchi, T., Young, J., Jass, J. R., & Leggett, B. A. (2004). BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut*, *53*(8), 1137-1144. https://doi.org/10.1136/gut.2003.037671

Karapetis, C. S., Khambata-Ford, S., Jonker, D. J., O'Callaghan, C. J., Tu, D., Tebbutt, N. C., Simes, R. J., Chalchal, H., Shapiro, J. D., Robitaille, S., Price, T. J., Shepherd, L., Au, H. J., Langer, C., Moore, M. J., & Zalcberg, J. R. (2008). K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*, *359*(17), 1757-1765. https://doi.org/10.1056/NEJMoa0804385

Kawada, K., Nakamoto, Y., Kawada, M., Hida, K., Matsumoto, T., Murakami, T., Hasegawa, S., Togashi, K., & Sakai, Y. (2012). Relationship between 18F-fluorodeoxyglucose accumulation and KRAS/BRAF mutations in colorectal cancer. *Clin Cancer Res*, *18*(6), 1696-1703. https://doi.org/10.1158/1078-0432.Ccr-11-1909

Kawada, K., Toda, K., Nakamoto, Y., Iwamoto, M., Hatano, E., Chen, F., Hasegawa, S., Togashi, K., Date, H., Uemoto, S., & Sakai, Y. (2015). Relationship Between 18F-FDG PET/CT Scans and KRAS Mutations in Metastatic Colorectal Cancer. *J Nucl Med*, *56*(9), 1322-1327. https://doi.org/10.2967/jnumed.115.160614

Kawamata, K., Hayashi, H., & Suzuki, Y. (2007). Propionate absorption associated with bicarbonate secretion in vitro in the mouse cecum. *Pflugers Arch*, *454*(2), 253-262. https://doi.org/10.1007/s00424-006-0200-4

Kelly, C. J., Zheng, L., Campbell, E. L., Saeedi, B., Scholz, C. C., Bayless, A. J., Wilson, K. E., Glover, L. E., Kominsky, D. J., Magnuson, A., Weir, T. L., Ehrentraut, S. F., Pickel, C., Kuhn, K. A., Lanis, J. M., Nguyen, V., Taylor, C. T., & Colgan, S. P. (2015). Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe*, *17*(5), 662-671. https://doi.org/10.1016/j.chom.2015.03.005

Kerk, S. A., Papagiannakopoulos, T., Shah, Y. M., & Lyssiotis, C. A. (2021). Metabolic networks in mutant KRAS-driven tumours: tissue specificities and the microenvironment. *Nat Rev Cancer*, *21*(8), 510-525. https://doi.org/10.1038/s41568-021-00375-9

Khan, A. S., & Frigo, D. E. (2017). A spatiotemporal hypothesis for the regulation, role, and targeting of AMPK in prostate cancer. *Nat Rev Urol*, *14*(3), 164-180. https://doi.org/10.1038/nrurol.2016.272

Kim, H., Lee, H. J., Oh, Y., Choi, S. G., Hong, S. H., Kim, H. J., Lee, S. Y., Choi, J. W., Su Hwang, D., Kim, K. S., Kim, H. J., Zhang, J., Youn, H. J., Noh, D. Y., & Jung, Y. K. (2014). The DUSP26 phosphatase activator adenylate kinase 2 regulates FADD phosphorylation and cell growth. *Nat Commun*, *5*, 3351. https://doi.org/10.1038/ncomms4351

Kim, J. W., Tchernyshyov, I., Semenza, G. L., & Dang, C. V. (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*, *3*(3), 177-185. https://doi.org/10.1016/j.cmet.2006.02.002

Kim, R., Emi, M., & Tanabe, K. (2007). Cancer immunoediting from immune surveillance to immune escape. *Immunology*, *121*(1), 1-14. https://doi.org/10.1111/j.1365-2567.2007.02587.x

Klepinin, A., Miller, S., Reile, I., Puurand, M., Rebane-Klemm, E., Klepinina, L., Vija, H., Zhang, S., Terzic, A., Dzeja, P., & Kaambre, T. (2022). Stable Isotope Tracing Uncovers Reduced γ/β-ATP Turnover and Metabolic Flux Through Mitochondrial-Linked Phosphotransfer Circuits in Aggressive Breast Cancer Cells. *Front Oncol*, *12*, 892195. https://doi.org/10.3389/fonc.2022.892195

Klepinin, A., Ounpuu, L., Guzun, R., Chekulayev, V., Timohhina, N., Tepp, K., Shevchuk, I., Schlattner, U., & Kaambre, T. (2016). Simple oxygraphic analysis for the presence of adenylate kinase 1 and 2 in normal and tumor cells. *J Bioenerg Biomembr*, *48*(5), 531-548. https://doi.org/10.1007/s10863-016-9687-3

Koit, A., Shevchuk, I., Ounpuu, L., Klepinin, A., Chekulayev, V., Timohhina, N., Tepp, K., Puurand, M., Truu, L., Heck, K., Valvere, V., Guzun, R., & Kaambre, T. (2017). Mitochondrial Respiration in Human Colorectal and Breast Cancer Clinical Material Is Regulated Differently. *Oxid Med Cell Longev*, *2017*, 1372640. https://doi.org/10.1155/2017/1372640

Kruger, N. J., & von Schaewen, A. (2003). The oxidative pentose phosphate pathway: structure and organisation. *Curr Opin Plant Biol*, *6*(3), 236-246. https://doi.org/10.1016/s1369-5266(03)00039-6

Kuipers, E. J., Grady, W. M., Lieberman, D., Seufferlein, T., Sung, J. J., Boelens, P. G., van de Velde, C. J., & Watanabe, T. (2015). Colorectal cancer. *Nat Rev Dis Primers*, *1*, 15065. https://doi.org/10.1038/nrdp.2015.65

Kuznetsov, A. V., Tiivel, T., Sikk, P., Kaambre, T., Kay, L., Daneshrad, Z., Rossi, A., Kadaja, L., Peet, N., Seppet, E., & Saks, V. A. (1996). Striking differences between the kinetics of regulation of respiration by ADP in slow-twitch and fast-twitch muscles in vivo. *Eur J Biochem*, *241*(3), 909-915. https://doi.org/10.1111/j.1432-1033.1996.00909.x

Lamb, R., Bonuccelli, G., Ozsvári, B., Peiris-Pagès, M., Fiorillo, M., Smith, D. L., Bevilacqua, G., Mazzanti, C. M., McDonnell, L. A., Naccarato, A. G., Chiu, M., Wynne, L., Martinez-Outschoorn, U. E., Sotgia, F., & Lisanti, M. P. (2015). Mitochondrial mass, a new metabolic biomarker for stem-like cancer cells: Understanding WNT/FGF-driven anabolic signaling. *Oncotarget*, *6*(31), 30453-30471. https://doi.org/10.18632/oncotarget.5852

Lambert, D. W., Wood, I. S., Ellis, A., & Shirazi-Beechey, S. P. (2002). Molecular changes in the expression of human colonic nutrient transporters during the transition from normality to malignancy. *British Journal of Cancer*, *86*(8), 1262-1269. https://doi.org/10.1038/sj.bjc.6600264

Laterveer, F. D., Nicolay, K., & Gellerich, F. N. (1997). Experimental evidence for dynamic compartmentation of ADP at the mitochondrial periphery: Coupling of mitochondrial adenylate kinase and mitochondrial hexokinase with oxidative phosphorylation under conditions mimicking the intracellular colloid osmotic pressure. *Molecular and cellular biochemistry*, *174*(1), 43-51. https://doi.org/10.1023/A:1006899621926

Latham, T., Mackay, L., Sproul, D., Karim, M., Culley, J., Harrison, D. J., Hayward, L., Langridge-Smith, P., Gilbert, N., & Ramsahoye, B. H. (2012). Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res*, *40*(11), 4794-4803. https://doi.org/10.1093/nar/gks066

Lavoie, H., & Therrien, M. (2015). Regulation of RAF protein kinases in ERK signalling. *Nat Rev Mol Cell Biol*, *16*(5), 281-298. https://doi.org/10.1038/nrm3979

Leclerc, D., Pham, D. N., Lévesque, N., Truongcao, M., Foulkes, W. D., Sapienza, C., & Rozen, R. (2017). Oncogenic role of PDK4 in human colon cancer cells. *Br J Cancer*, *116*(7), 930-936. https://doi.org/10.1038/bjc.2017.38

Lee, H.-J., Li, C.-F., Ruan, D., He, J., Montal, E. D., Lorenz, S., Girnun, G. D., & Chan, C.-H. (2019). Non-proteolytic ubiquitination of Hexokinase 2 by HectH9 controls tumor metabolism and cancer stem cell expansion. *Nature Communications*, *10*(1), 2625. https://doi.org/10.1038/s41467-019-10374-y

Lee, P., Chandel, N. S., & Simon, M. C. (2020). Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat Rev Mol Cell Biol*, *21*(5), 268-283. https://doi.org/10.1038/s41580-020-0227-y

Li, J., Ma, X., Chakravarti, D., Shalapour, S., & DePinho, R. A. (2021). Genetic and biological hallmarks of colorectal cancer. *Genes Dev*, *35*(11-12), 787-820. https://doi.org/10.1101/gad.348226.120

Li, M., Liu, H., Li, J., Guo, S., & Lv, Y. (2021). Mitochondrial creatine kinase 1 in non-small cell lung cancer progression and hypoxia adaptation. *Respiratory Research*, *22*(1), 1-8.

Li, Q., Cao, L., Tian, Y., Zhang, P., Ding, C., Lu, W., Jia, C., Shao, C., Liu, W., Wang, D., Ye, H., & Hao, H. (2018). Butyrate Suppresses the Proliferation of Colorectal Cancer Cells via Targeting Pyruvate Kinase M2 and Metabolic Reprogramming. *Mol Cell Proteomics*, *17*(8), 1531-1545. https://doi.org/10.1074/mcp.RA118.000752

Li, S., Balmain, A., & Counter, C. M. (2018). A model for RAS mutation patterns in cancers: finding the sweet spot. *Nat Rev Cancer*, *18*(12), 767-777. https://doi.org/10.1038/s41568-018-0076-6

Li, X., Jiang, Y., Meisenhelder, J., Yang, W., Hawke, D. H., Zheng, Y., Xia, Y., Aldape, K., He, J., Hunter, T., Wang, L., & Lu, Z. (2016). Mitochondria-Translocated PGK1 Functions as a Protein Kinase to Coordinate Glycolysis and the TCA Cycle in Tumorigenesis. *Mol Cell*, *61*(5), 705-719. https://doi.org/10.1016/j.molcel.2016.02.009

Lin, Q., Zhang, H., Ding, H., Qian, J., Lizaso, A., Lin, J., Han-Zhang, H., Xiang, J., Li, Y., & Zhu, H. (2019). The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients. *J Transl Med*, *17*(1), 298. https://doi.org/10.1186/s12967-019-2036-7

Liu, H., Pu, Y., Amina, Q., Wang, Q., Zhang, M., Song, J., Guo, J., & Mardan, M. (2019). Prognostic and therapeutic potential of Adenylate kinase 2 in lung adenocarcinoma. *Sci Rep*, *9*(1), 17757. https://doi.org/10.1038/s41598-019-53594-4

Lu, J., Tan, M., & Cai, Q. (2015). The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett*, *356* (2 Pt A), 156-164. https://doi.org/10.1016/j.canlet.2014.04.001

Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol*, *27*, 441-464. https://doi.org/10.1146/annurev-cellbio-092910-154237

Luo, Y., Wong, C. J., Kaz, A. M., Dzieciatkowski, S., Carter, K. T., Morris, S. M., Wang, J., Willis, J. E., Makar, K. W., Ulrich, C. M., Lutterbaugh, J. D., Shrubsole, M. J., Zheng, W., Markowitz, S. D., & Grady, W. M. (2014). Differences in DNA methylation signatures reveal multiple pathways of progression from adenoma to colorectal cancer. *Gastroenterology*, *147*(2), 418-429.e418. https://doi.org/10.1053/j.gastro.2014.04.039

Macheda, M. L., Rogers, S., & Best, J. D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol*, *202*(3), 654-662. https://doi.org/10.1002/jcp.20166

Maldonado, E. N., Sheldon, K. L., DeHart, D. N., Patnaik, J., Manevich, Y., Townsend, D. M., Bezrukov, S. M., Rostovtseva, T. K., & Lemasters, J. J. (2013). Voltage-dependent anion channels modulate mitochondrial metabolism in cancer cells: regulation by free tubulin and erastin. *J Biol Chem*, *288*(17), 11920-11929. https://doi.org/10.1074/jbc.M112.433847

Mariadason, J. M., Velcich, A., Wilson, A. J., Augenlicht, L. H., & Gibson, P. R. (2001). Resistance to butyrate-induced cell differentiation and apoptosis during spontaneous Caco-2 cell differentiation. *Gastroenterology*, *120*(4), 889-899. https://doi.org/10.1053/gast.2001.22472

Mathupala, S. P., Ko, Y. H., & Pedersen, P. L. (2006). Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene*, *25*(34), 4777-4786. https://doi.org/10.1038/sj.onc.1209603

Mattaini, K. R., Sullivan, M. R., & Vander Heiden, M. G. (2016). The importance of serine metabolism in cancer. *J Cell Biol*, *214*(3), 249-257. https://doi.org/10.1083/jcb.201604085

McCleland, M. L., Adler, A. S., Deming, L., Cosino, E., Lee, L., Blackwood, E. M., Solon, M., Tao, J., Li, L., Shames, D., Jackson, E., Forrest, W. F., & Firestein, R. (2013). Lactate dehydrogenase B is required for the growth of KRAS-dependent lung adenocarcinomas. *Clin Cancer Res*, *19*(4), 773-784. https://doi.org/10.1158/1078-0432.Ccr-12-2638

McGettrick, A. F., & O'Neill, L. A. J. (2020). The Role of HIF in Immunity and Inflammation. *Cell Metab*, *32*(4), 524-536. https://doi.org/10.1016/j.cmet.2020.08.002

McNeil, N. I., Cummings, J. H., & James, W. P. (1979). Rectal absorption of short chain fatty acids in the absence of chloride. *Gut*, *20*(5), 400-403. https://doi.org/10.1136/gut.20.5.400

Missiaglia, E., Jacobs, B., D'Ario, G., Di Narzo, A. F., Soneson, C., Budinska, E., Popovici, V., Vecchione, L., Gerster, S., Yan, P., Roth, A. D., Klingbiel, D., Bosman, F. T., Delorenzi, M., & Tejpar, S. (2014). Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol*, *25*(10), 1995-2001. https://doi.org/10.1093/annonc/mdu275

Moon, J. S., Kim, H. E., Koh, E., Park, S. H., Jin, W. J., Park, B. W., Park, S. W., & Kim, K. S. (2011). Krüppel-like factor 4 (KLF4) activates the transcription of the gene for the platelet isoform of phosphofructokinase (PFKP) in breast cancer. *J Biol Chem*, *286*(27), 23808-23816. https://doi.org/10.1074/jbc.M111.236737

Moreno-Sánchez, R., Marín-Hernández, A., Saavedra, E., Pardo, J. P., Ralph, S. J., & Rodríguez-Enríquez, S. (2014). Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism. *Int J Biochem Cell Biol*, *50*, 10-23. https://doi.org/10.1016/j.biocel.2014.01.025

Moreno-Sánchez, R., Rodríguez-Enríquez, S., Marín-Hernández, A., & Saavedra, E. (2007). Energy metabolism in tumor cells. *Febs j*, *274*(6), 1393-1418. https://doi.org/10.1111/j.1742-4658.2007.05686.x

Nagao, A., Kobayashi, M., Koyasu, S., Chow, C. C. T., & Harada, H. (2019). HIF-1-Dependent Reprogramming of Glucose Metabolic Pathway of Cancer Cells and Its Therapeutic Significance. *Int J Mol Sci*, *20*(2). https://doi.org/10.3390/ijms20020238

Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. *Biophys Rev*, *8*(1), 5-9. https://doi.org/10.1007/s12551-015-0186-2

Newmark, H. L., Lupton, J. R., & Young, C. W. (1994). Butyrate as a differentiating agent: pharmacokinetics, analogues and current status. *Cancer Lett*, *78*(1-3), 1-5. https://doi.org/10.1016/0304-3835(94)90023-x

Newsholme, P., Procopio, J., Lima, M. M., Pithon-Curi, T. C., & Curi, R. (2003). Glutamine and glutamate--their central role in cell metabolism and function. *Cell Biochem Funct*, *21*(1), 1-9. https://doi.org/10.1002/cbf.1003

Nishihashi, K., Kawashima, K., Nomura, T., Urakami-Takebayashi, Y., Miyazaki, M., Takano, M., & Nagai, J. (2017). Cobalt Chloride Induces Expression and Function of Breast Cancer Resistance Protein (BCRP/ABCG2) in Human Renal Proximal Tubular Epithelial Cell Line HK-2. *Biol Pharm Bull*, *40*(1), 82-87. https://doi.org/10.1248/bpb.b16-00684

Noffsinger, A. E. (2009). Serrated polyps and colorectal cancer: new pathway to malignancy. *Annu Rev Pathol*, *4*, 343-364. https://doi.org/10.1146/annurev.pathol.4.110807.092317

Noma, T. (2005). Dynamics of nucleotide metabolism as a supporter of life phenomena. *J Med Invest*, *52*(3-4), 127-136. https://doi.org/10.2152/jmi.52.127

O'Hara, R., Tedone, E., Ludlow, A., Huang, E., Arosio, B., Mari, D., & Shay, J. W. (2019). Quantitative mitochondrial DNA copy number determination using droplet digital PCR with single-cell resolution. *Genome Res*, *29*(11), 1878-1888. https://doi.org/10.1101/gr.250480.119

Oshima, K., Saiki, N., Tanaka, M., Imamura, H., Niwa, A., Tanimura, A., Nagahashi, A., Hirayama, A., Okita, K., Hotta, A., Kitayama, S., Osawa, M., Kaneko, S., Watanabe, A., Asaka, I., Fujibuchi, W., Imai, K., Yabe, H., Kamachi, Y., . . . Saito, M. K. (2018). Human AK2 links intracellular bioenergetic redistribution to the fate of hematopoietic progenitors. *Biochem Biophys Res Commun*, *497*(2), 719-725. https://doi.org/10.1016/j.bbrc.2018.02.139

Ounpuu, L., Truu, L., Shevchuk, I., Chekulayev, V., Klepinin, A., Koit, A., Tepp, K., Puurand, M., Rebane-Klemm, E., & Käämbre, T. (2018). Comparative analysis of the bioenergetics of human adenocarcinoma Caco-2 cell line and postoperative tissue samples from colorectal cancer patients. *Biochem Cell Biol*, 1-10. https://doi.org/10.1139/bcb-2018-0076

Panayiotou, C., Solaroli, N., & Karlsson, A. (2014). The many isoforms of human adenylate kinases. *Int J Biochem Cell Biol*, *49*, 75-83. https://doi.org/10.1016/j.biocel.2014.01.014

Park, H. U., Suy, S., Danner, M., Dailey, V., Zhang, Y., Li, H., Hyduke, D. R., Collins, B. T., Gagnon, G., Kallakury, B., Kumar, D., Brown, M. L., Fornace, A., Dritschilo, A., & Collins, S. P. (2009). AMP-activated protein kinase promotes human prostate cancer cell growth and survival. *Mol Cancer Ther*, *8*(4), 733-741. https://doi.org/10.1158/1535-7163.Mct-08-0631

Park, Y. Y., Kim, S. B., Han, H. D., Sohn, B. H., Kim, J. H., Liang, J., Lu, Y., Rodriguez-Aguayo, C., Lopez-Berestein, G., Mills, G. B., Sood, A. K., & Lee, J. S. (2013). Tat-activating regulatory DNA-binding protein regulates glycolysis in hepatocellular carcinoma by regulating the platelet isoform of phosphofructokinase through microRNA 520. *Hepatology*, *58*(1), 182-191. https://doi.org/10.1002/hep.26310

Patra, K. C., Wang, Q., Bhaskar, P. T., Miller, L., Wang, Z., Wheaton, W., Chandel, N., Laakso, M., Muller, W. J., Allen, E. L., Jha, A. K., Smolen, G. A., Clasquin, M. F., Robey, B., & Hay, N. (2013). Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell*, *24*(2), 213-228. https://doi.org/10.1016/j.ccr.2013.06.014

Paudel, B. B., & Quaranta, V. (2019). Metabolic plasticity meets gene regulation. *Proc Natl Acad Sci U S A*, *116*(9), 3370-3372. https://doi.org/10.1073/pnas.1900169116

Pavlova, N. N., & Thompson, C. B. (2016). The Emerging Hallmarks of Cancer Metabolism. *Cell Metab*, *23*(1), 27-47. https://doi.org/10.1016/j.cmet.2015.12.006

Perez-Terzic, C., Faustino, R. S., Boorsma, B. J., Arrell, D. K., Niederländer, N. J., Behfar, A., & Terzic, A. (2007). Stem cells transform into a cardiac phenotype with remodeling of the nuclear transport machinery. *Nat Clin Pract Cardiovasc Med*, *4 Suppl 1*, S68-76. https://doi.org/10.1038/ncpcardio0763

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol*, *14*(3), 141-153. https://doi.org/10.1038/nri3608

Phipps, A. I., Buchanan, D. D., Makar, K. W., Win, A. K., Baron, J. A., Lindor, N. M., Potter, J. D., & Newcomb, P. A. (2013). KRAS-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers. *Br J Cancer*, *108*(8), 1757-1764. https://doi.org/10.1038/bjc.2013.118

Pinheiro, C., Reis, R. M., Ricardo, S., Longatto-Filho, A., Schmitt, F., & Baltazar, F. (2010). Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44. *J Biomed Biotechnol*, *2010*, 427694. https://doi.org/10.1155/2010/427694

Prior, I. A., Hood, F. E., & Hartley, J. L. (2020). The Frequency of Ras Mutations in Cancer. *Cancer Res*, *80*(14), 2969-2974. https://doi.org/10.1158/0008-5472.Can-19-3682

Pucar, D., Dzeja, P. P., Bast, P., Juranic, N., Macura, S., & Terzic, A. (2001). Cellular energetics in the preconditioned state: protective role for phosphotransfer reactions captured by 18O-assisted 31P NMR. *J Biol Chem*, *276*(48), 44812-44819. https://doi.org/10.1074/jbc.M104425200

Puurand, M., Tepp, K., Timohhina, N., Aid, J., Shevchuk, I., Chekulayev, V., & Kaambre, T. (2019). Tubulin βII and βIII Isoforms as the Regulators of VDAC Channel Permeability in Health and Disease. *Cells*, *8*(3). https://doi.org/10.3390/cells8030239

Ramos-Martinez, J. I. (2017). The regulation of the pentose phosphate pathway: Remember Krebs. *Arch Biochem Biophys*, *614*, 50-52. https://doi.org/10.1016/j.abb.2016.12.012

Rangel-Huerta, E., & Maldonado, E. (2017). Transit-Amplifying Cells in the Fast Lane from Stem Cells towards Differentiation. *Stem Cells Int*, *2017*, 7602951. https://doi.org/10.1155/2017/7602951

Rehman, G., Shehzad, A., Khan, A. L., & Hamayun, M. (2014). Role of AMP-activated protein kinase in cancer therapy. *Arch Pharm (Weinheim)*, *347*(7), 457-468. https://doi.org/10.1002/ardp.201300402

Ren, H., Wang, L., Bennett, M., Liang, Y., Zheng, X., Lu, F., Li, L., Nan, J., Luo, M., Eriksson, S., Zhang, C., & Su, X. D. (2005). The crystal structure of human adenylate kinase 6: An adenylate kinase localized to the cell nucleus. *Proc Natl Acad Sci U S A*, *102*(2), 303-308. https://doi.org/10.1073/pnas.0407459102

Rex, D. K., Ahnen, D. J., Baron, J. A., Batts, K. P., Burke, C. A., Burt, R. W., Goldblum, J. R., Guillem, J. G., Kahi, C. J., Kalady, M. F., O'Brien, M. J., Odze, R. D., Ogino, S., Parry, S., Snover, D. C., Torlakovic, E. E., Wise, P. E., Young, J., & Church, J. (2012). Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol*, *107*(9), 1315-1329; quiz 1314, 1330. https://doi.org/10.1038/ajg.2012.161

Roberts, J., Aubert, S., Gout, E., Bligny, R., & Douce, R. (1997). Cooperation and Competition between Adenylate Kinase, Nucleoside Diphosphokinase, Electron Transport, and ATP Synthase in Plant Mitochondria Studied by 31P-Nuclear Magnetic Resonance. *Plant Physiol*, *113*(1), 191-199. https://doi.org/10.1104/pp.113.1.191

Robey, R. B., & Hay, N. (2006). Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. *Oncogene*, *25*(34), 4683-4696. https://doi.org/10.1038/sj.onc.1209595

Rodrigues, M. F., Carvalho, É., Pezzuto, P., Rumjanek, F. D., & Amoêdo, N. D. (2015). Reciprocal modulation of histone deacetylase inhibitors sodium butyrate and trichostatin A on the energy metabolism of breast cancer cells. *J Cell Biochem*, *116*(5), 797-808. https://doi.org/10.1002/jcb.25036

Roediger, W. E. (1982). Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology*, *83*(2), 424-429.

Roskoski, R., Jr. (2010). RAF protein-serine/threonine kinases: structure and regulation. *Biochem Biophys Res Commun*, *399*(3), 313-317. https://doi.org/10.1016/j.bbrc.2010.07.092

Rostovtseva, T. K., Bezrukov, S. M., & Hoogerheide, D. P. (2021). Regulation of Mitochondrial Respiration by VDAC Is Enhanced by Membrane-Bound Inhibitors with Disordered Polyanionic C-Terminal Domains. *Int J Mol Sci*, *22*(14). https://doi.org/10.3390/ijms22147358

Rubie, C., Kempf, K., Hans, J., Su, T., Tilton, B., Georg, T., Brittner, B., Ludwig, B., & Schilling, M. (2005). Housekeeping gene variability in normal and cancerous colorectal, pancreatic, esophageal, gastric and hepatic tissues. *Mol Cell Probes*, *19*(2), 101-109. https://doi.org/10.1016/j.mcp.2004.10.001

Saks, V. A., Khuchua, Z. A., Vasilyeva, E. V., Belikova, O., & Kuznetsov, A. V. (1994). Metabolic compartmentation and substrate channelling in muscle cells. Role of coupled creatine kinases in in vivo regulation of cellular respiration--a synthesis. *Mol Cell Biochem*, *133-134*, 155-192. https://doi.org/10.1007/bf01267954

Saks, V. A., Veksler, V. I., Kuznetsov, A. V., Kay, L., Sikk, P., Tiivel, T., Tranqui, L., Olivares, J., Winkler, K., Wiedemann, F., & Kunz, W. S. (1998). Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. *Mol Cell Biochem*, *184*(1-2), 81-100.

Schlattner, U., Tokarska-Schlattner, M., & Wallimann, T. (2006). Mitochondrial creatine kinase in human health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1762*(2), 164-180. https://doi.org/https://doi.org/10.1016/j.bbadis.2005.09.004

Schubbert, S., Shannon, K., & Bollag, G. (2007). Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer*, *7*(4), 295-308. https://doi.org/10.1038/nrc2109

Semenza, G. L. (2013). HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J Clin Invest*, *123*(9), 3664-3671. https://doi.org/10.1172/jci67230

Serebriiskii, I. G., Connelly, C., Frampton, G., Newberg, J., Cooke, M., Miller, V., Ali, S., Ross, J. S., Handorf, E., Arora, S., Lieu, C., Golemis, E. A., & Meyer, J. E. (2019). Comprehensive characterization of RAS mutations in colon and rectal cancers in old and young patients. *Nat Commun*, *10*(1), 3722. https://doi.org/10.1038/s41467-019-11530-0

Sheldon, K. L., Maldonado, E. N., Lemasters, J. J., Rostovtseva, T. K., & Bezrukov, S. M. (2011). Phosphorylation of voltage-dependent anion channel by serine/threonine kinases governs its interaction with tubulin. *Plos one*, *6*(10), e25539. https://doi.org/10.1371/journal.pone.0025539

Sherr, C. J., & DePinho, R. A. (2000). Cellular senescence: mitotic clock or culture shock? *Cell*, *102*(4), 407-410. https://doi.org/10.1016/s0092-8674(00)00046-5

Shi, T., Ma, Y., Cao, L., Zhan, S., Xu, Y., Fu, F., Liu, C., Zhang, G., Wang, Z., Wang, R., Lu, H., Lu, B., Chen, W., & Zhang, X. (2019). B7-H3 promotes aerobic glycolysis and chemoresistance in colorectal cancer cells by regulating HK2. *Cell Death & Disease*, *10*(4), 308. https://doi.org/10.1038/s41419-019-1549-6

Shonk, C. E., Arison, R. N., Koven, B. J., Majima, H., & Boxer, G. E. (1965). ENZYME PATTERNS IN HUMAN TISSUES. 3. GLYCOLYTIC ENZYMES IN NORMAL AND MALIGNANT TISSUES OF THE COLON AND RECTUM. *Cancer Res*, *25*, 206-213.

Simanshu, D. K., Nissley, D. V., & McCormick, F. (2017). RAS Proteins and Their Regulators in Human Disease. *Cell*, *170*(1), 17-33. https://doi.org/10.1016/j.cell.2017.06.009

Singhal, R., & Shah, Y. M. (2020). Oxygen battle in the gut: Hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine. *J Biol Chem*, *295*(30), 10493-10505. https://doi.org/10.1074/jbc.REV120.011188

Sinicrope, F. A., Shi, Q., Smyrk, T. C., Thibodeau, S. N., Dienstmann, R., Guinney, J., Bot, B. M., Tejpar, S., Delorenzi, M., Goldberg, R. M., Mahoney, M., Sargent, D. J., & Alberts, S. R. (2015). Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology*, *148*(1), 88-99. https://doi.org/10.1053/j.gastro.2014.09.041

Śmiech, M., Leszczyński, P., Kono, H., Wardell, C. P., & Taniguchi, H. (2020). Emerging BRAF Mutations in Cancer Progression and Their Possible Effects on Transcriptional Networks. *Genes*, *11*.

Speers, C., Tsimelzon, A., Sexton, K., Herrick, A. M., Gutierrez, C., Culhane, A., Quackenbush, J., Hilsenbeck, S., Chang, J., & Brown, P. (2009). Identification of novel kinase targets for the treatment of estrogen receptor-negative breast cancer. *Clin Cancer Res*, *15*(20), 6327-6340. https://doi.org/10.1158/1078-0432.Ccr-09-1107

Stincone, A., Prigione, A., Cramer, T., Wamelink, M. M., Campbell, K., Cheung, E., Olin-Sandoval, V., Grüning, N. M., Krüger, A., Tauqeer Alam, M., Keller, M. A., Breitenbach, M., Brindle, K. M., Rabinowitz, J. D., & Ralser, M. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biol Rev Camb Philos Soc*, *90*(3), 927-963. https://doi.org/10.1111/brv.12140

Stine, Z. E., Walton, Z. E., Altman, B. J., Hsieh, A. L., & Dang, C. V. (2015). MYC, Metabolism, and Cancer. *Cancer Discov*, *5*(10), 1024-1039. https://doi.org/10.1158/2159-8290.Cd-15-0507

Swerdlow, R. H., E, L., Aires, D., & Lu, J. (2013). Glycolysis-respiration relationships in a neuroblastoma cell line. *Biochim Biophys Acta*, *1830*(4), 2891-2898. https://doi.org/10.1016/j.bbagen.2013.01.002

Taylor, C. T., & Colgan, S. P. (2007). Hypoxia and gastrointestinal disease. *Journal of Molecular Medicine*, *85*(12), 1295-1300. https://doi.org/10.1007/s00109-007-0277-z

Ten Hoorn, S., de Back, T. R., Sommeijer, D. W., & Vermeulen, L. (2021). Clinical Value of Consensus Molecular Subtypes in Colorectal Cancer: A Systematic Review and Meta-Analysis. *JNCI: Journal of the National Cancer Institute*, *114*(4), 503-516. https://doi.org/10.1093/jnci/djab106

Thangaraju, M., Cresci, G., Itagaki, S., Mellinger, J., Browning, D. D., Berger, F. G., Prasad, P. D., & Ganapathy, V. (2008). Sodium-coupled transport of the short chain fatty acid butyrate by SLC5A8 and its relevance to colon cancer. *J Gastrointest Surg*, *12*(10), 1773-1781; discussion 1781-1772. https://doi.org/10.1007/s11605-008-0573-0

Ullah, M. S., Davies, A. J., & Halestrap, A. P. (2006). The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *J Biol Chem*, *281*(14), 9030-9037. https://doi.org/10.1074/jbc.M511397200

Van Der Schoor, S. R., Reeds, P. J., Stoll, B., Henry, J. F., Rosenberger, J. R., Burrin, D. G., & Van Goudoever, J. B. (2002). The high metabolic cost of a functional gut. *Gastroenterology*, *123*(6), 1931-1940. https://doi.org/10.1053/gast.2002.37062

van Engeland, M., Derks, S., Smits, K. M., Meijer, G. A., & Herman, J. G. (2011). Colorectal cancer epigenetics: complex simplicity. *J Clin Oncol*, *29*(10), 1382-1391. https://doi.org/10.1200/jco.2010.28.2319

Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, *324*(5930), 1029-1033. https://doi.org/10.1126/science.1160809

Vander Heiden, M. G., & DeBerardinis, R. J. (2017). Understanding the Intersections between Metabolism and Cancer Biology. *Cell*, *168*(4), 657-669. https://doi.org/10.1016/j.cell.2016.12.039

Varughese, J. T., Buchanan, S. K., & Pitt, A. S. (2021). The Role of Voltage-Dependent Anion Channel in Mitochondrial Dysfunction and Human Disease. *Cells*, *10*(7). https://doi.org/10.3390/cells10071737

Vögler, O., Barceló, J. M., Ribas, C., & Escribá, P. V. (2008). Membrane interactions of G proteins and other related proteins. *Biochim Biophys Acta*, *1778*(7-8), 1640-1652. https://doi.org/10.1016/j.bbamem.2008.03.008

Wallace, D. C. (2012). Mitochondria and cancer. *Nat Rev Cancer*, *12*(10), 685-698. https://doi.org/10.1038/nrc3365

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., & Eppenberger, H. M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *The Biochemical journal*, *281 (Pt 1)*, 21-40.

Wang, J., Shen, J., Huang, C., Cao, M., & Shen, L. (2019). Clinicopathological Significance of BRAF(V600E) Mutation in Colorectal Cancer: An Updated Meta-Analysis. *J Cancer*, *10*(10), 2332-2341. https://doi.org/10.7150/jca.30789

Wang, N. N., Zhang, P. Z., Zhang, J., Wang, H. N., Li, L., Ren, F., Dai, P. F., Li, H., & Lv, X. F. (2021). Penfluridol triggers mitochondrial-mediated apoptosis and suppresses glycolysis in colorectal cancer cells through down-regulating hexokinase-2. *Anat Rec (Hoboken)*, *304*(3), 520-530. https://doi.org/10.1002/ar.24464

Wang, W., & Guan, K. L. (2009). AMP-activated protein kinase and cancer. *Acta Physiol (Oxf)*, *196*(1), 55-63. https://doi.org/10.1111/j.1748-1716.2009.01980.x

Wang, Y., Nie, H., Liao, Z., He, X., Xu, Z., Zhou, J., & Ou, C. (2021). Expression and Clinical Significance of Lactate Dehydrogenase A in Colon Adenocarcinoma [Original Research]. *Frontiers in Oncology*, *11*. https://doi.org/10.3389/fonc.2021.700795

Warburg, O. (1956). On the origin of cancer cells. *Science*, *123*(3191), 309-314. https://doi.org/10.1126/science.123.3191.309

Weinberg, S. E., & Chandel, N. S. (2015). Targeting mitochondria metabolism for cancer therapy. *Nat Chem Biol*, *11*(1), 9-15. https://doi.org/10.1038/nchembio.1712

Wellbrock, C., Karasarides, M., & Marais, R. (2004). The RAF proteins take centre stage. *Nat Rev Mol Cell Biol*, *5*(11), 875-885. https://doi.org/10.1038/nrm1498

Xin, F., Yao, D. W., Fan, L., Liu, J. H., & Liu, X. D. (2019). Adenylate kinase 4 promotes bladder cancer cell proliferation and invasion. *Clin Exp Med*, *19*(4), 525-534. https://doi.org/10.1007/s10238-019-00576-5

Xu, R., Yang, Y., & Zheng, X. (2021). Unique structural features of the adenylate kinase hCINAP/AK6 and its multifaceted functions in carcinogenesis and tumor progression. *FEBS Lett*, *595*(16), 2071-2084. https://doi.org/10.1002/1873-3468.14158

Xu, S., Catapang, A., Braas, D., Stiles, L., Doh, H. M., Lee, J. T., Graeber, T. G., Damoiseaux, R., Shirihai, O., & Herschman, H. R. (2018). A precision therapeutic strategy for hexokinase 1-null, hexokinase 2-positive cancers. *Cancer Metab*, *6*, 7. https://doi.org/10.1186/s40170-018-0181-8

Xu, S., & Herschman, H. R. (2019). A Tumor Agnostic Therapeutic Strategy for Hexokinase 1-Null/Hexokinase 2-Positive Cancers. *Cancer Res*, *79*(23), 5907-5914. https://doi.org/10.1158/0008-5472.Can-19-1789

Yan, Y.-B. (2016). Creatine kinase in cell cycle regulation and cancer. *Amino Acids*, *48*(8), 1775-1784. https://doi.org/10.1007/s00726-016-2217-0

Yao, Z., Yaeger, R., Rodrik-Outmezguine, V. S., Tao, A., Torres, N. M., Chang, M. T., Drosten, M., Zhao, H., Cecchi, F., Hembrough, T., Michels, J., Baumert, H., Miles, L., Campbell, N. M., de Stanchina, E., Solit, D. B., Barbacid, M., Taylor, B. S., & Rosen, N. (2017). Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*, *548*(7666), 234-238. https://doi.org/10.1038/nature23291

Ying, H., Kimmelman, A. C., Lyssiotis, C. A., Hua, S., Chu, G. C., Fletcher-Sananikone, E., Locasale, J. W., Son, J., Zhang, H., Coloff, J. L., Yan, H., Wang, W., Chen, S., Viale, A., Zheng, H., Paik, J. H., Lim, C., Guimaraes, A. R., Martin, E. S., . . . DePinho, R. A. (2012). Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell*, *149*(3), 656-670. https://doi.org/10.1016/j.cell.2012.01.058

Yokota, T., Ura, T., Shibata, N., Takahari, D., Shitara, K., Nomura, M., Kondo, C., Mizota, A., Utsunomiya, S., Muro, K., & Yatabe, Y. (2011). BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br J Cancer*, *104*(5), 856-862. https://doi.org/10.1038/bjc.2011.19

Yu, L., Lu, M., Jia, D., Ma, J., Ben-Jacob, E., Levine, H., Kaipparettu, B. A., & Onuchic, J. N. (2017). Modeling the Genetic Regulation of Cancer Metabolism: Interplay between Glycolysis and Oxidative Phosphorylation. *Cancer Res*, *77*(7), 1564-1574. https://doi.org/10.1158/0008-5472.Can-16-2074

Yun, J., Rago, C., Cheong, I., Pagliarini, R., Angenendt, P., Rajagopalan, H., Schmidt, K., Willson, J. K., Markowitz, S., Zhou, S., Diaz, L. A., Jr., Velculescu, V. E., Lengauer, C., Kinzler, K. W., Vogelstein, B., & Papadopoulos, N. (2009). Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science*, *325*(5947), 1555-1559. https://doi.org/10.1126/science.1174229

Zafra, M. P., Parsons, M. J., Kim, J., Alonso-Curbelo, D., Goswami, S., Schatoff, E. M., Han, T., Katti, A., Fernandez, M. T. C., Wilkinson, J. E., Piskounova, E., & Dow, L. E. (2020). An In Vivo Kras Allelic Series Reveals Distinct Phenotypes of Common Oncogenic Variants. *Cancer Discov*, *10*(11), 1654-1671. https://doi.org/10.1158/2159-8290.Cd-20-0442

Zahedi, R. P., Sickmann, A., Boehm, A. M., Winkler, C., Zufall, N., Schönfisch, B., Guiard, B., Pfanner, N., & Meisinger, C. (2006). Proteomic analysis of the yeast mitochondrial outer membrane reveals accumulation of a subclass of preproteins. *Mol Biol Cell*, *17*(3), 1436-1450. https://doi.org/10.1091/mbc.e05-08-0740

Zeller, K. I., Jegga, A. G., Aronow, B. J., O'Donnell, K. A., & Dang, C. V. (2003). An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. *Genome Biol*, *4*(10), R69. https://doi.org/10.1186/gb-2003-4-10-r69

Zhong, X., He, X., Wang, Y., Hu, Z., Huang, H., Zhao, S., Wei, P., & Li, D. (2022). Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. *Journal of hematology & oncology*, *15*(1), 160. Retrieved 2022/11//, from http://europepmc.org/abstract/MED/36319992

Zhu, G., Pei, L., Xia, H., Tang, Q., & Bi, F. (2021). Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. *Mol Cancer*, *20*(1), 143. https://doi.org/10.1186/s12943-021-01441-4

Acknowledgments

I want to express my heartfelt appreciation to the following individuals who have played a significant role in the completion of my Ph.D. thesis:

I am deeply grateful to my supervisor, Tuuli Käämbre, for her guidance, expertise, and support throughout my research. Her invaluable insights and mentorship have played a pivotal role in shaping the direction of my work, and I am genuinely thankful for her dedication.

I also sincerely thank my lab colleagues at the Laboratory of Chemical Biology for their collaboration, assistance, and fruitful discussions. Their contributions and shared knowledge have significantly enhanced the quality of my research outcomes. Special recognition goes to Leenu Reinsalu and Marju Puurand, whose support has been remarkable.

I am profoundly grateful to my family for their unconditional love, support, and understanding during the entire period. I am incredibly thankful to my 2-year-old daughter Leilani, whose presence has bought immense joy and inspiration to my life. Her innocent laughter and boundless curiosity serve as constant reminders of the importance of maintaining balance and cherishing each amidst the rigors of work and research.

To my husband, Sten, I owe a deep sense of gratitude. His willingness to care for Leilani and understanding of my frequent absence allowed me the necessary time and mental space to focus on my research. His unwavering support throughout this journey has been invaluable, and without him, this journey would have been far more challenging.

To all those mentioned above and others who have supported me, I sincerely appreciate your contributions to my Ph.D. journey. The knowledge and skills I have gained during my Ph.D. will serve me well in future pursuits, and this would not have been possible without your support.

Abstract
Metabolic Alterations in Colorectal Polyps and their Role in Carcinogenesis

Colorectal cancer (CRC) presents a significant public health burden worldwide due to its high morbidity and mortality rates. CRC arises from various genetic and epigenetic alterations accumulating in cells, leading to tumor development and progression. One notable characteristic of cancer cells is their ability to modify metabolic pathways related to energy production and biosynthesis. However, this aspect remains underexplored in the early stages of carcinogenesis. Hence, the objective of this doctoral research was to elucidate the metabolic alterations that take place during the premalignant stage of colorectal polyps.

Clinical samples were collected for this study, and mitochondrial oxygen consumption was measured. Based on this, the Michaelis-Menten constant for ADP (K_m(ADP)) and the maximum ADP-induced respiration (V_{max}) was calculated. The results yielded a statistically significant difference between the V_{max} and K_m(ADP) values of polyps and CRC, indicating distinct bioenergetic alterations between the premalignant and malignant stages.

Interestingly, when comparing polyps and tumor tissue, there was a noticeable metabolic shift toward glycolysis in polyps. Moreover, the impact of two colorectal biomarkers, KRAS and BRAF, on mitochondrial respiration was investigated. The presence of the *BRAF* mutation was observed to promote glycolysis in both polyps and tumors. This finding indicates the potential role of the *BRAF* mutation in metabolic reprogramming, even in the early stage of tumor development.

Elevated glycolytic activity in polyps was supported by an increase in the expression levels of *GLUT1, LDHA, HK1*, and *HK2*, indicating increased glucose demand and lactate production. Increased LDHA activity leads to lactate accumulation and acidification of the tissue microenvironment, which may potentially promote the formation of tumors.

Butyrate, a short-chain fatty acid, serves as the primary energy source for colonic epithelial cells, with MCTs mediating its transportation across the luminal membrane. This study shows that there was an increase in the expression of *MCT2* and MCT4 in polyps compared to healthy colon tissue. The overexpression of *MCT4* in colon polyps suggests an increase in glycolytic activity, possibly as an adaption to elevated lactate and the low-oxygen environment found in the colon. The *MCT1* expression remained consistent in both healthy tissue and polyps, indicating a continued reliance on butyrate. However, in cases of CRC, there was a noticeable decrease in *MCT1* and *MCT2* expression, revealing a significant metabolic shift. This reprogramming could be associated with the metabolic plasticity of tumor cells, allowing them to use alternative sources of energy.

Adenylate kinase (AK) and creatine kinase (CK) are important for maintaining cellular energy balance and regulating energy transport pathways. Alterations in phosphotransfer pathways have been linked to tumor progression and metastasis. This doctoral thesis found that the levels of all AK and CK isoforms studied were higher in polyps than in tumor tissue. In this study, it was discovered that the AK1 isoform, which is known to have a negative effect on colorectal cancer (CRC), had similar levels of expression in both polyps and healthy tissue. The *AK2* gene, responsible for transferring ATP/ADP between the cytoplasm and mitochondria, was found to be overexpressed in polyps but not in tumors or healthy tissue. The expression level of AK4 was also elevated in polyps

compared to the tumor and healthy tissue, indicating, based on previous studies, a shift in metabolism towards glycolysis. The expression levels of CK isoforms were found to be higher in polyps than in tumor tissue. Similarly, the results for AK and CK suggest that the intracellular phosphate transfer pathways are activated in colorectal polyps, which could be linked to the changes observed in the metabolic profile during mitochondrial respiration analyses.

According to the findings of the doctoral thesis, it has been observed that the glycolytic activity is significantly higher in colorectal polyps when compared to both healthy and tumor tissues. This interesting discovery has led to further speculation regarding whether this glycolytic phenotype in polyps could possibly be a defensive mechanism employed by the body. This could hypothetically explain why it takes anywhere from 10 to 20 years for a tumor to develop from a polyp. Further investigation and research are required in order to fully understand the relationship between glycolytic activity and the development of colorectal cancer.

Lühikokkuvõte
Metaboolsed muutused jämesoole polüüpides ja nende roll vähi tekkes

Kolorektaalvähk ehk jämesoolevähk kujutab oma kõrge haigestumuse ja suremuse tõttu märkimisväärset rahvatervise koormust kogu maailmas. Jämesoolevähk tekib erinevate geneetiliste ja epigeneetiliste muutuste kuhjumisest rakkudes, mis viib kasvaja arengule ja progresseerumisele. Vähirakkude oluliseks tunnuseks on võime muuta energiatootmise ja biosünteesiga seotud metaboolseid radu, mida on seni kasvaja varajases staadiumis vähe uuritud. Käesoleva doktoritöö eesmärk oli kirjeldada metaboolseid muutuseid, mis esinevad premaliigsetes jämesoole polüüpides.

Kogutud kliinilisel materjalil mõõdeti mitokondriaalset hapnikutarbimist ja selle põhjal arvutati Michaelis-Menteni konstant ADP suhtes (K_m(ADP)) ning maksimaalne ADP poolt indutseeritud hingamine (V_{max}). Saadud tulemused näitasid statistiliselt olulist erinevust polüüpide ja jämesoolevähi V_{max} ning K_m(ADP)) väärtustes, mis viitab erinevatele bioenergeetilistele muutustele. Jämesoole polüüpides, võrreldes kasvaja koega, oli metabolism nihutatud glükolüüsi suunas. Lisaks uuriti kahe soolevähi biomarkeri, KRAS ja BRAF, mõju mitokondriaalsele hingamisele. Leiti, et *BRAF* mutatsioon soodustab glükolüüsi nii polüüpides kui kasvajates, viidates mutatsiooni võimalikule rollile metaboolses reprogrammeerimises juba varajases kasvaja staadiumis.

Polüüpide kõrgenenud glükolüütilist aktiivsust toetasid *GLUT1*, *LDHA*, *HK1* ja *HK2* ekspressioonitaseme tõus, näidates suurenenud glükoosinõudlust ja laktaadi tootmist. Suurenenud LDHA aktiivsus viib laktaadi kuhjumiseni ja koe mikrokeskkonna hapestumiseni, mis võib potentsiaalselt soodustada kasvaja teket.

Butüraat on peamine energiaallikas jämesoole epiteelrakkudele, mille transporti läbi membraani vahendavad MCTd. Käesolevas töös näidati *MCT2* ja *MCT4* ekspressiooni märkimisväärset suurenemist polüüpides võrreldes terve koega. *MCT4* üleekspressioon polüüpide viitab glükolüütilise aktiivsuse suurenemisele, mis võib olla kohastumine kõrgenenud laktaadi taseme ja madala hapnikusisaldusega keskkonnas. *MCT1* ekspressioon püsis nii terves koes kui polüüpides muutumatuna, näidates jätkuvat sõltuvust butüraadist kui energiaallikast. Olulisel määral vähenes kasvaja koe *MCT1* ja *MCT2* ekspressioon, peegeldades polüüpide ja kasvaja koe vahelisi märkimisväärseid metaboolseid erinevusi. See ümberkorraldus võib olla seotud kasvajarakkude metaboolse plastilisusega, võimaldades neil kasutada alternatiivseid energiaallikaid.

Energiatranspordi radade regulatsioonis on oluline osas adenülaatkinaasil (AK) ja kreatiinkinaasil (CK), mis tagavad rakus energia homöostaasi. Muutused fosfaadi ülekanderadades on seotud kasvaja progresseerumise ja metastaasidega. Antud doktoritöös näidati, et polüüpides on kõigi uuritud *AK* ja *CK* isovormide ekspressioonitase tõusnud võrreldes kasvaja koega. AK1 isovormi peetakse jämesoolevähi negatiivseks regulaatoriks ja käesolevas töös leiti AK1 ekspressioonitase sama nii polüüpides kui ka terves koes. AK2, mis soodustab ATP/ADP ülekandmist tsütoplasma ja mitokondrite vahel, oli polüüpides üleekspresseeritud võrreldes kasvaja ja terve koega. *AK4* ekspressioonitase oli samuti polüüpides tõusnud võrreldes kasvaja ja terve koega, mis viitab eelnevate uuringute põhjal metabolismi nihkumisele glükolüüsi suunas. CK isovormide puhul täheldati polüüpides kõrgenenud ekspressioonitasemeid võrreldes kasvaja koega. *AK* ja *CK* ekspressioonitaseme tulemused viitavad rakusiseste fosforiülekande radade aktiveerimisele jämesoole polüüpides, mis võivad olla seotud muutustega metaboolses profiilis, mida kinnitasid mitokondriaalse hingamise analüüsid.

Käesolevas doktoritöös näidati jämesoole polüüpide suurenenud glükolüütilist aktiivsust võrreldes terve ja kasvaja koega. Järgmine uurimissuund võiks olla, kas glükolüütiline fenotüüp polüüpides võib olla organismi kaitsemehhanism, mis selgitaks, miks võib polüübist kasvaja tekkeni võtta 10–20 aastat.

Appendix 1

Publication I

Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, Timohhina N, Tepp K, Bogovskaja J, Afanasjev V, Suurmaa K, Valvere V, Kaambre T. Mitochondrial Respiration in *KRAS* and *BRAF* Mutated Colorectal Tumors and Polyps. Cancers (Basel). 2020 Mar 28;12(4):815. doi: 10.3390/cancers12040815

cancers

MDPI

Article

Mitochondrial Respiration in *KRAS* and *BRAF* Mutated Colorectal Tumors and Polyps

Egle Rebane-Klemm [1,2,†,*], Laura Truu [1,2,†], Leenu Reinsalu [1,2], Marju Puurand [1], Igor Shevchuk [1], Vladimir Chekulayev [1], Natalja Timohhina [1], Kersti Tepp [1], Jelena Bogovskaja [3], Vladimir Afanasjev [4], Külliki Suurmaa [5], Vahur Valvere [6] and Tuuli Kaambre [1]

1 Laboratory of Chemical Biology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia; laura.truu@gmail.com (L.T.); leenu.reinsalu@gmail.com (L.R.); marju.puurand@kbfi.ee (M.P.); igor@chemnet.ee (I.S.); vladimir@chemnet.ee (V.C.); natalja.timohhina@kbfi.ee (N.T.); kersti.tepp@kbfi.ee (K.T.); tuuli.kaambre@kbfi.ee (T.K.)
2 Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Ehitajate tee 5, 12618 Tallinn, Estonia
3 Clinic of Diagnostics at the North Estonia Medical Centre, J. Sütiste tee 19, 13419 Tallinn, Estonia; jelena.bogovskaja@regionaalhaigla.ee
4 Clinic of Surgery at the North Estonia Medical Centre, J. Sütiste tee 19, 13419 Tallinn, Estonia; Vladimir.Afanasjev2@regionaalhaigla.ee
5 Department of Gastroenterology, the West Tallinn Central Hospital, Paldiski mnt 68, 10617 Tallinn, Estonia; kulliki.suurmaa@keskhaigla.ee
6 Oncology and Haematology Clinic at the North Estonia Medical Centre, J. Sütiste tee 19, 13419 Tallinn, Estonia; vahur.valvere@regionaalhaigla.ee
* Correspondence: egle.rebane@gmail.com
† These authors contributed equally to this work.

Received: 14 February 2020; Accepted: 27 March 2020; Published: 28 March 2020

Abstract: This study aimed to characterize the ATP-synthesis by oxidative phosphorylation in colorectal cancer (CRC) and premalignant colon polyps in relation to molecular biomarkers KRAS and BRAF. This prospective study included 48 patients. Resected colorectal polyps and postoperative CRC tissue with adjacent normal tissue (control) were collected. Patients with polyps and CRC were divided into three molecular groups: *KRAS* mutated, *BRAF* mutated and *KRAS/BRAF* wild-type. Mitochondrial respiration in permeabilized tissue samples was observed using high resolution respirometry. ADP-activated respiration rate (V_{max}) and an apparent affinity of mitochondria to ADP, which is related to mitochondrial outer membrane (MOM) permeability, were determined. Clear differences were present between molecular groups. *KRAS* mutated CRC group had lower V_{max} values compared to wild-type; however, the V_{max} value was higher than in the control group, while MOM permeability did not change. This suggests that *KRAS* mutation status might be involved in acquiring oxidative phenotype. *KRAS* mutated polyps had higher V_{max} values and elevated MOM permeability as compared to the control. *BRAF* mutated CRC and polyps had reduced respiration and altered MOM permeability, indicating a glycolytic phenotype. To conclude, prognostic biomarkers KRAS and BRAF are likely related to the metabolic phenotype in CRC and polyps. Assessment of the tumor mitochondrial ATP synthesis could be a potential component of patient risk stratification.

Keywords: energy metabolism; colorectal cancer; colorectal polyps; mitochondria; oxidative phosphorylation; *KRAS*; *BRAF*

1. Introduction

Colorectal cancer (CRC) is the leading cause of premature cancer death worldwide, prompting the urgent need to develop more effective treatment strategies. CRC is a heterogeneous disease and presents distinct subtypes with different molecular and pathological features. The majority of sporadic CRC typically develops progressively from premalignant precursor lesions, known as polyps, to malignant tumors. Most colorectal polyps are harmless, but some can develop (by not fully understood mechanisms) into malignant invasive adenocarcinomas. According to modern concepts, CRC is triggered by various molecular events in several proto-oncogenes (such as the *PIK3CA*, *p53*, *KRAS*, *BRAF* and *c-MYC* genes) and tumor suppressor genes (such as the APC, PTEN, SMAD4 genes) [1–3]. The malignant transformation of cells, including colon epithelium, is accompanied by strong alterations (reprogramming) of metabolic pathways involved in energy production and biosynthesis that promote tumor growth and metastasis [4–6]. A better understanding of the pathogenesis of CRC, the metabolic heterogeneity of emerging polyps and potential drivers is very important to develop new prognostic markers and successful agents for the prevention and treatment of this disease.

Transcriptome-based classification has been used in CRC as it can better describe the behavior of the tumors. The international CRC Subtyping Consortium classifies CRC into four consensus molecular subtypes (CMSs), each with distinct features: CMS1 (hypermutated, microsatellite instability (MSI), *BRAF* mutation, and immune infiltration and activation); CMS2 (epithelial, WNT and MYC signaling pathway activation); CMS3 (metabolic dysregulation, *KRAS* mutations); and CMS4 (transforming growth factor beta activation, stromal invasion, TGFβ activation, and angiogenesis) [7]. Although transcriptome profiles are not associated with specific mutations, the frequency of *KRAS* mutation varies among the CRC subtypes (23% in CMS1, 28% in CMS2, 68% in CMS3, and 38% in CMS4), these data suggest mutations may drive distinct programs of metabolism gene expression [7]. Mutations in *KRAS* or *BRAF* genes appear to play an important role in the regulation of metabolic reprogramming in multiple cancers, including CRC [8–11]. In this study, two established and common prognostic biomarkers in CRC were investigated: *KRAS* and *BRAF* mutation status. Mutation in *BRAF* codon 600 of exon 15 (V600E) is associated with unfavorable prognosis [12]. Activating *KRAS* mutations in codon 12 and 13 of exon 2, which is common in CRC (30–50% of tumors), are associated with poorer survival and response to chemotherapeutics [13,14]. Our study aims to contribute to understanding how prognostic biomarkers KRAS and BRAF are correlating to cellular metabolic phenotypes in the course of CRC carcinogenesis.

The metabolism of cancer cells is specially adapted to meet their needs to survive and proliferate in both well oxygenated and hypoxic microenvironments. To date, transcriptomics and metabolomics studies have shown the coexistence of three distinct cellular metabolic phenotypes that exist in cancer cells, which are characterized by the following predominant states: glycolytic (aerobic glycolysis, so called Warburg phenotype [15]), oxidative (energy production relying mainly on oxidative phosphorylation, OXPHOS), and hybrid (both OXPHOS and glycolysis can be active simultaneously). Normal cells exhibit only glycolytic and oxidative states [16–18]. Premalignant polyps and arising adenocarcinomas are still regarded as highly glycolytic tumors of the Warburg phenotype [19–21]. Previous studies indicate that although polyps have higher inclination to aerobic glycolysis, the metastatic carcinomas maintain high rates of O_2 consumption (much more than adjacent normal tissues) and exhibit obvious signs of stimulated mitochondrial biogenesis [6,22–24]. In this regard, we assume that upon malignant transformation, there is a selection of specific cell clones that have stimulated mitochondrial biogenesis and, as a result, have elevated aggressiveness. Among patients with CRC, a high level of mitochondrial respiration of tumor samples have been found to be associated with reduced survival [25].

As part of cancer bioenergetic studies, analysis of OXPHOS with high-resolution respirometry can be applied to study the mechanisms of this key element in cellular bioenergetics. Investigating the dependency of adenosine diphosphate (ADP)-dependent respiration rate on ADP concentration in tissue samples can provide two fundamental characteristics for OXPHOS: a maximal ADP-activated

respiration rate (V_{max}), and an apparent affinity of mitochondria for exogenous ADP expressed as apparent Michaelis–Menten constant Km (K_m(ADP)). Our previous experiments showed that the V_{max} value for CRC cells is significantly higher than in cells in healthy colorectal control tissue showing more active ATP-synthesis by OXPHOS. This finding corresponds well with differences in the content of mitochondria in these cells (the number of mitochondria in CRC is almost two times higher than in healthy tissue) [6,25]. The changes in K_m(ADP) show changes in tissue-specific intracellular complexity in terms of energy transport and regulation of mitochondrial outer membrane (MOM) permeability. For the operation of OXPHOS, the flux of respiratory substrates, ATP, ADP and Pi through MOM is regulated by the voltage-dependent anion channel (VDAC) permeability control. In the closed state, VDAC is impermeable to adenine nucleotides [26,27]. Several studies have shown that during carcinogenesis the VDAC permeability for ADP is altered [22,28–30]. The cell-specific differences in K_m(ADP) are likely due to specific structural and functional organization of energy metabolism. For example, cells with a low K_m(ADP) value (~10 μM) like glycolytic muscle, possess less structural and functional obstacles for movement ADP/ATP though MOM as compared to the oxidative muscles (~300 μM) [31]. Known K_m(ADP) values for CRC measured for tumor tissue are about 100 μM [22,25], implying existence of some restrictions for ADP passing VDAC. The sensitivity of the mitochondrial respiration for exogenous ADP in cell cultures is very high (low K_m(ADP) values) and is similar to isolated mitochondria [25,28,32–34], which suggests the need to investigate cancer energy metabolism directly in fresh clinical material. To our knowledge, there is no data on the rate of OXPHOS and its regulation in colon polyps. Assessment of OXPHOS status of this pathology enhances our understanding of colon carcinogenesis.

Thus, the main goal of our study was to characterize the functional activity of mitochondrial OXPHOS among premalignant polyps and CRC, taking into account their *KRAS* and *BRAF* mutation status. To date, it has been shown that *KRAS* and *BRAF* mutations increase the glycolytic capacity of tumor cells and their glutaminolysis [8,35]. In our work, the function of the OXPHOS system was analyzed by means of high-resolution respirometry using freshly prepared postoperative tissue samples.

2. Results and Discussion

Cancer metabolism profoundly differs from normal cellular metabolism, and interrelated connections between cancer mitochondrial respiration and oncogenic driver genes like *KRAS* and *BRAF* are relatively unexplored. Somatic mutations involving the GTP-ase RAS protein family and its downstream serine/threonine-protein kinase BRAF lead to loss of cell cycle regulation at key checkpoints and are the main driver mutations for colorectal carcinogenesis [36]. *KRAS* mutations are detected in approximately 40% of all CRC patients, suggesting the importance of *KRAS* in tumor development [37]. The *KRAS* mutation is an early event in CRC and most *KRAS* mutations are located in codons 12 and 13. However, at least 5–10% of CRCs are believed to initiate via acquiring activating mutations in the *BRAF* oncogene [38]. Mutations of *KRAS* and *BRAF* are usually mutually exclusive. Although the existence of intertumoral heterogeneity in CRC is well established and illustrated by molecular subtyping [7], pure genome or transcriptome data are not sufficient to describe the final in situ modifications and the final outcomes of pathways or cellular processes [25]. The purpose of this study was to determine the activity of ATP production by OXPHOS in human tissues during the development of CRC from normal colon tissue to polyps and cancer, depending on the status of *BRAF* and *KRAS* mutations.

To characterize ATP-synthesis by OXPHOS during CRC carcinogenesis we used high resolution respirometry to measure the rate of maximal ADP-activated respiration (V_{max}). We also used apparent K_m values for exogenously added ADP (K_m(ADP)) using permeabilized postoperative tissue (CRC, colon polyps and normal colon tissue). Our previous studies showed that OXPHOS can be a significant supplier of ATP in CRC because its V_{max} values (corresponding to the number of mitochondria) were almost two times higher than in surrounding normal tissues [6,39,40]. Among all the studied groups, the wild-type tumor showed the highest V_{max}, while these values measured for *BRAF* or *KRAS* mutated

tumors were significantly lower (Figure 1A, Tables S1 and S2). This reveals involvement of oncogenic *KRAS* and *BRAF* in metabolic reprogramming of colon mucosa and confirms their role in shifting CRC metabolism to a more glycolytic type. Furthermore, in contrast to the results from an in vitro study conducted by Yun et al.—done with CRC cell cultures where oxygen consumption in cells with mutant *KRAS* or *BRAF* alleles was similar to that in cells with wild type alleles of these genes [41]—we saw a difference in V_{max} values between *BRAF* mutated and *KRAS* mutated tumors (Figure 1A, Tables S1 and S2). Interestingly, the V_{max} of *BRAF* mutated tumors was similar to that in control tissues. These results suggest a distinct role of mutated *KRAS* and *BRAF* in affecting mitochondrial biogenesis and likely tissue differentiation as well.

In colorectal polyps, the V_{max} pattern largely followed that of the respective tumors. The respiration rates in polyps in *KRAS* mutated and wild-type molecular groups showed remarkably higher V_{max} values than the control tissue (V_{max} values 2.19 ± 0.19 and 1.95 ± 0.28 for *KRAS* mutated and wild-type group, respectively, $p < 0.001$ and $p = 0.004$ as compared to the control group (Tables S1 and S2). Polyps that had acquired the *BRAF* mutation showed a tendency to have lower OXPHOS rates (V_{max} 1.41 ± 0.27) than in mutated *KRAS* and wild-type groups. Similar to the *BRAF* tumor group, polyps with mutated *BRAF* did not show a difference with the control tissue (Figure 1, Tables S1 and S2). This suggests that alterations in mitochondrial biogenesis is a very early event and already happens in the pre-malignant stage.

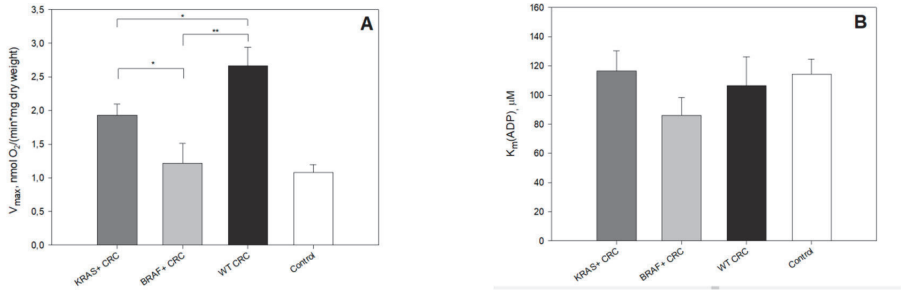

Figure 1. Regulation of mitochondrial respiration in *KRAS*+, *BRAF*+ and wild-type tumors and control. (**A**) Comparative analysis of maximal ADP-activated respiratory rate (V_{max}) and (**B**) the apparent Michaelis–Menten constant (K_m(ADP)) values for ADP. *KRAS*+: *KRAS* mutated; *BRAF*+: *BRAF* mutated; WT: wild type; CRC: colorectal cancer; Control: control tissue. * $p < 0.05$; ** $p < 0.01$.

Maintaining high functional activity of OXPHOS may be necessary because cancer cells with a very low respiration rate cannot form tumors [42]. At the same time, a certain reduction in respiration may be useful for the functioning of signaling molecules, the synthesis of anabolic precursors and other typical aspects of cancer phenotypes [43]. Thus, functional OXPHOS is important in both proliferating and non-proliferating cells, but each situation will emphasize its unique functional aspects [42]. It has been shown that the metabolic profile of cancer cells in culture can have significant variations as a consequence of the culture conditions [25]. In general, cells growing in a glucose-free medium display relatively high rates of oxygen consumption, whereas cultivation in a high-glucose medium results in hyperglycolytic cells together with declined respiratory flux [44–48]. Therefore, for the study of OXPHOS in human tumors, the use of postoperative tissue material is likely to be a more suitable approach.

To investigate possible regulatory alterations affecting OXPHOS during carcinogenesis, we estimated apparent affinity mitochondria for ADP. In all CRC and polyp groups, the corresponding K_m(ADP) value was determined and the measured values (Figure 1B, Tables S1 and S2) were found to be 4 to 8 times higher than in isolated mitochondria (15 µM, measured by Chance and Williams [49,50]). This finding points to the existence of restrictions for the movement of ADP through mitochondrial membranes. The OXPHOS system is located in the inner mitochondrial membrane

and the ADP/ATP carrier has the function of crossing the adenine nucleotides through the membrane into the mitochondrial matrix. In our previous study, we applied metabolic control analysis on ATP-synthasome which consisted of the respiratory system, ATP-synthase, ATP/ADP carrier and Pi transporter, all in CRC tissue. In the framework of metabolic control analysis and by using specific inhibitors, the rate of effect each enzyme has in a pathway (flux control coefficients) can be determined. This analysis showed that the main control over ATP-synthesis by OXPHOS (the highest flux control coefficients) in CRC relied on respiratory complexes I and III and Pi transporter. Inhibition of the ADP/ATP carrier had no major rate-limiting effect on ATP synthesis by OXPHOS [26]. Thus, we assumed that the considerable control over ability of exogenous ADP to influence respiration was mainly dependent on ADP passage through MOM in CRC. The comparison of K_m(ADP) values for *KRAS* mutated, *BRAF* mutated and wild-type tumors did not reveal any substantial differences. In all CRC groups the Km(ADP) values for tumor and control tissue were similar. Our previous study showed that we can distinguish two different populations of mitochondria in control tissue—what we believe could be a mucosal population with lower K_m(ADP) (75 ± 4 μM), and the smooth muscle population with a much higher K_m(ADP) value (362 ± 60 μM) [25]. This is in good agreement with our preliminary results obtained from separately measured colon smooth muscle and mucosa (259 ± 35 μM and 118 ± 11 μM, respectively). To estimate the percentage of mitochondria with highly regulated (oxidative) and unregulated (glycolytic) MOM permeability, we applied the mathematical model used for muscle cells and adapted it to tissues studied by us. According to the model proposed earlier [51], the hypothetical percentage of low oxidative capacity mitochondria in tissue is calculated from the K_m(ADP) value as an inverse asymptotic dependence. Percent of low oxidative capacity of mitochondrion demonstrates the metabolic shift to glycolytic state in all colon polyps, but not in *KRAS* mutated and wild-type tumors compared to control tissue (Table 1, Tables S1 and S2). The changes in glycolytic markers have been observed in the early premalignant colorectal mucosal field and these changes would be expected to promote increased glycolysis [19]. The K_m(ADP) values in polyp molecular groups were 55.3 ± 7.4 μM, 52.5 ± 4.7 μM and 60.1 ± 6.3 μM for *KRAS* mutated, *BRAF* mutated and wild-type group, respectively. These were lower than in control tissue (Tables S1 and S2), which indicates significant changes in regulation MOM permeability. Interestingly, despite the similar V_{max} values in *KRAS* mutated polyp and CRC groups, the difference in K_m(ADP) between these groups was significant, $p = 0.014$ (Tables S1, S2 and Figure S1). Our findings of the relatively low K_m value for ADP for colorectal polyps suggest an early metabolic reprogramming towards the glycolytic phenotype with functional OXPHOS.

Table 1. Modelled percentage of low oxidative capacity of mitochondrion in KRAS+, BRAF+ and wild-type tumors and controls.

Sample	% of Low Oxidative Capacity of Mitochondrion
KRAS tumors	28.1
KRAS polyps	65.9
BRAF tumors	43.0
BRAF polyps	68.6
Wild-type tumors	32.4
Wild-type polyps	61.7
All controls	29.0

The results of the current study confirm our previous findings, indicating that in cancer tissues, the regulation of MOM permeability to adenine nucleotides is different from that in normal cells [25,28,29]. Proteins that could regulate the VDAC permeability for adenine nucleotides in colonocytes and corresponding cancer cells are still unknown. There are two possible mechanisms proposed for this regulation. According to the first model, cancer cells due to overexpression of

mitochondrially-bound hexokinase 2 support high permeability of the VDAC to adenine nucleotides and direct the ATP formed in mitochondria to the glycolytic pathway. As a consequence, the aerobic glycolysis is facilitated and malignant metabolic reprogramming occurs [52,53]. The second model involves the inhibition of VDAC by free tubulin to limit mitochondrial metabolism in cancer cells [30,54]. The possible candidates are βIII–tubulin and γ-tubulin. βIII–tubulin acts as a marker of cancer aggressiveness, and γ-tubulin formed meshwork has been shown to be associated with mitochondrial membranes [29,55,56]. However, the regulation of energy metabolism through control over metabolites and energy fluxes that pass through the MOM is only one aspect of the possible role of VDAC influencing carcinogenesis. VDAC1—the major mitochondrial protein expressed in mammals and functions in metabolism, Ca^{2+} homeostasis, apoptosis and other activities—is regulated via its interaction with many proteins associated with cell survival and cellular death pathways. VDAC1 is overexpressed in many cancers and represents a promising cancer drug target (reviewed in [57,58]). The mechanistic understanding behind the changes in K_m(ADP) during CRC carcinogenesis observed in the current study and connections with other functions of VDAC require further investigation.

Further, we analyzed whether the observed changes in V_{max} and K_m(ADP) values are related to tumor location. CRC is more frequently observed in the distal colon (left colon, from splenic flexure to rectum) than in the proximal side (right colon, from the cecum to transverse colon [59]). In the current study, the distal and proximal tumors were presented almost equally—20 and 24 samples, respectively. Studies have shown that tumors arising from the left and right colon are distinct in their epidemiology, biology, histology and microbial diversity [59,60]. In the current study, comparing all the distal and proximal tumors showed differences in K_m(ADP) but not in V_{max} values (Figure 2A). A study including 57,847 patients showed proximal patients had better outcomes than those with distal CRC in several subgroups including stage II disease, patients aged >70 years and mucinous adenocarcinoma [61]. Inside the *KRAS* mutated group, proximal and distal tumors were compared to see the potential effect of cancer location on metabolic changes. No statistically significant difference between V_{max} and K_m(ADP) values comparing proximal and distal tumors in the *KRAS* mutated group (Figure 2B) was seen. The location of a tumor did not have an effect on the mitochondrial respiration in the *KRAS* mutated group and all observed alterations were related to the *KRAS* status of the tumor. All *BRAF* mutated tumors were located in the proximal side.

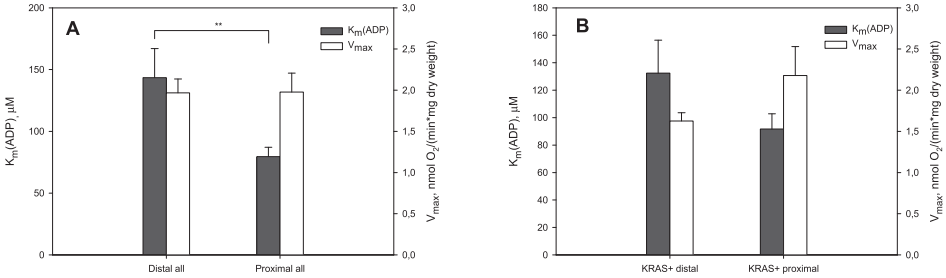

Figure 2. (**A**) In the current study, a comparison of all distal and proximal tumors showed a difference in K_m(ADP) values, but not in V_{max}. (**B**) V_{max} and K_m(ADP) values comparing proximal and distal tumors in the *KRAS* mutated group. ** Significant difference, $p < 0.01$.

All together, we found that colon polyps and colon tumors had higher rates of maximal ADP-activated respiration (a marker of mitochondrial mass) than normal colon tissue (Figure 1A, Tables S1 and S2). *BRAF* mutant tumors and polyps exhibited lower V_{max} values than *KRAS* mutated lesions and they had a relatively high percentage of mitochondria with low control over the movement adenine nucleotides through MOM (Table 1). Therefore, it is most likely that lesions with *BRAF* mutations have higher glycolytic activity, which is confirmed by some published data [62]. In contrast to the *BRAF* mutated lesions, *KRAS* mutated polyps showed signs of stimulated mitochondrial

biogenesis and upon progression could give highly metastatic malignant tumors (i.e., polyps with this energetic phenotype can be more prone to tumor formation). This was unexpected, since the transformed cells carrying the *KRAS* gene mutations were characterized by an increased glycolytic flow associated with the over-expression of glucose transporter 1 (GLUT1) and hexokinase 2 and reduced oxygen consumption due to mitochondrial dysfunction in cell cultures [41,63,64]. Our previous studies demonstrated that the oxygen consumption in vitro significantly differed compared to what occurred in vivo [25]. Moreover, the rate of oxidative ATP production of the tumor seems to be a prognostic marker for cancer survival and metastatic potential [22]. The estimation of *KRAS* or *BRAF* mutation status in colorectal pre- and neoplastic lesions could be a predictor of their response to drugs affecting the OXPHOS. Recently, a new class of anticancer drugs called "mitocans" was proposed. These affect different mitochondrial-associated activities including ATP/ADP carrier, hexokinase, electron transport/respiratory chain inhibitors, and others [65].

3. Materials and Methods

3.1. Reagents

Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich Chemical Com. (St. Louis, MO, USA) and were used directly without further purification.

3.2. Clinical Material

All tumor patients examined (n = 33 with ages ranging from 38 to 91 years) had local or locally advanced disease (T2-4 N0-1, M0-1). The patients in the study had not received prior radiation or chemotherapy (Table 2). All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Medical Research Ethics Committee (National Institute for Health Development, Tallinn, Estonia) of nr.1728.

Table 2. Clinicopathological patient characteristics of the colon cancer and polyps cohort.

Characteristics	*n*
Total patients	48
Females	19
Males	29
Age at diagnosis	
Mean	72
Median	74
Range	38–91
Stage of tumor	
I-II	15
III-IV	9
Unknown	9
Molecular subtype of tumor	
KRAS mutated	13
BRAF mutated	6
KRAS and *BRAF* wild-type	14
Molecular subtypes of polyps	
KRAS mutated	4
BRAF mutated	2
KRAS and *BRAF* wild-type	9

CRC post operational and normal tissue samples (0.1–0.5 g) were provided by the Oncology and Hematologic Clinic at the North Estonia Medical Centre (NEMC, Tallinn, Estonia). Pathology reports were obtained by the NEMC for each tissue sample. Only primary tumor samples were examined. All investigations were approved by the Medical Research Ethics Committee (National Institute for Health Development, Tallinn, Estonia) and were in accordance with Helsinki Declaration and Convention of the Council of Europe on Human Rights and Biomedicine.

Normal tissue samples were taken from the same location at sites distant from the tumor and they were evaluated for presence of malignant cells. The adjacent control tissues consisted of colonocytes and smooth muscle cells.

Patients with colorectal polyps (n = 15) (Table 2) were consecutive patients undergoing a colonoscopy for resection of the polyps at the West Tallinn Central Hospital. After removal, tissue samples were immediately placed in medium B, which consisted of the following: 0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 3 mM KH_2PO_4, 110 mM sucrose, 0.5 mM dithiothreitol, 20 mM HEPES, 5 µM leupeptin, 2 mg/mL fatty acids free bovine serum albumin (BSA), pH 7.1. All polyps were analyzed immediately after the colonoscopy with quick cancer tests. Only part of the cancer negative polyps was subjected to further analysis for OXPHOS. Due to the limited amount of fresh tissue, *KRAS* and *BRAF* mutation analyses were performed using Formalin-Fixed Paraffin-Embedded (FFPE) samples.

3.3. Preparation of Skinned Tumor Fibers and Permeabilization Procedure

Immediately after the surgery, the tissue samples were placed into pre-cooled (4 °C) medium A, which consisted of 20 mM imidazole, 3 mM KH_2PO_4, 0.5 mM dithiothreitol, 20 mM taurine, 4 mM $MgCl_2$, 100 mM 2-morpholinoethanesulfonic acid, 2.74 mM K_2Ca-EGTA, 4.72 mM K_2-EGTA, 5 µM leupeptin and 2 mg/mL BSA [39]. The samples were dissected into small fiber bundles (10–20 mg) and permeabilized in the same medium with 50 µg/mL of saponin. They were mildly stirred for 30 min at 4 °C [39,66]. The obtained permeabilized (skinned) fibers were then washed three times for 5 min in pre-cooled medium B (without leupeptin). After that, samples were kept in medium B at 4 °C until use. The typical dimension of skinned fibers was about 2 × 2 × 2 mm, and one of these pieces was used in oxygraphic experiments.

3.4. Oxygraphic Measurements

Mitochondrial respiration of permeabilized tissue samples was measured at 25 °C in medium B supplemented with 5 mM glutamate, 2 mM malate and 10 mM succinate, with respiratory substrates using a high-resolution respirometer Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) as described previously [66,67]. The solubility of oxygen at 25 °C was taken as 240 nmol/mL [68]. All respiration rates were normalized per mg dry weight of tissue. To determine the apparent affinity of mitochondria to exogenous ADP (K_m(ADP)), the dependence of respiration rate on exogenous ADP was measured (Figure 3A). The obtained data were plotted as rates of O_2 consumption (the basal respiration rate of respiration was subtracted) versus ADP concentration and K_m(ADP) and V_{max} values were calculated from these plots by nonlinear regression using Michaelis–Menten equation [69,70] (Figure 3B). Additionally, plotting the data to double reciprocal plot gives information about presence of different mitochondrial populations with differently regulated MOM.

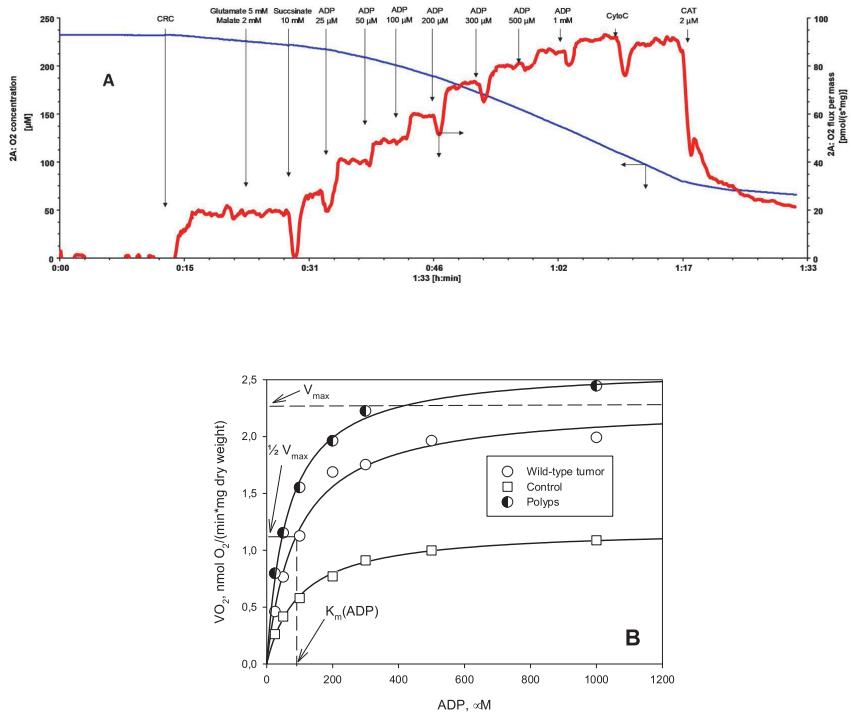

Figure 3. Different kinetics of regulation of mitochondrial respiration by exogenous ADP in colon tissue. (**A**) Recording of original traces of O_2 consumption by permeabilized colorectal cancer (CRC) tissue upon additions of increasing concentrations of ADP. CAT stands for carboxyatractyloside; CyoC stands for cytochrome C. **(B)** The measured respiration rates were plotted vs ADP concentrations, and from this plot corresponding V_{max} and K_m(ADP) values were calculated by nonlinear regression using Michaelis–Menten equation. There was a marked difference in ADP kinetics between wild-type CRC, colon polyps and normal colon tissue (control).

3.5. DNA Extraction

DNA from formalin-fixed paraffin-embedded tissue (FFPE) samples was extracted using ZYMO Quick-DNA™ FFPE Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. DNA concentrations and quality were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

3.6. KRAS and BRAF Mutation Analysis

Mutations in *BRAF* codon 600 of exon 15 (V600E) and *KRAS* codon 12 and 13 of exon 2 were screened using High-Resolution Melt (HRM) analysis. Briefly, a 10 µl reaction mix contained 1x HOT FIREPol® EvaGreen® HRM Mix (Solis BioDyne, Estonia), 250 nM of sense and antisense primers (*KRAS*-antisense, 5′- AAATGACTGAATATAAACTTGTGGTAGT-3′; *KRAS*-sense, 5′- TGAATTAGCTGTATCGTCAAGGCACT-3′; *BRAF*-antisense wild-type, 5′-cgccgcgcgccAAAATAG GTGATTTTGGTCT-3′; *BRAF*-antisense mutation, 5′-TAAAAATAGGTGATTTTGGTCTAGCTACA-3′; *BRAF*-sense, 5′- CCACAAAATGGATCCAGAC AACTG 3′) and 100× dilution of PCR amplification product. PCR amplification and HRM analysis were performed with Rotor-Gene 6000 (QIAGEN) and consisted of an initial 15 min denaturation step at 95 °C, followed by 45 cycles at 95 °C for 10 s, 54 °C for 10 s and 72 °C for 15 s, with a final extension at 72 °C for 3 min. The resulting PCR products were heated at 95 °C for 1 min and cooled to 40 °C to facilitate heteroduplex formation. HRM analysis was

performed from 62 °C to 92 °C with a 0.1 °C step. The results were analyzed using Rotor-Gene 6000 software and unknown samples were compared to control samples with known genotypes.

3.7. Data Analysis

Data in the text, tables and figures are presented as mean ± standard error (SEM). Results were analyzed by Student's *t*-test and *p*-values < 0.05 were considered statistically significant. Apparent K_m values for ADP were measured by fitting experimental data to a non-linear regression (according to a Michaelis–Menten model equation, as shown in Figure 3).

4. Conclusions

While many studies have characterized the metabolic phenotype of CRC cell lines, it is important to understand the metabolic reprogramming in clinical material. Our findings confirm that early changes in mitochondria respiration occur in CRC carcinogenesis and precede the development of pre-cancerous lesions. Mitochondrial respiration differs in *KRAS*, *BRAF* mutated and wild-type tumor groups, confirming that oncogenes may affect the metabolic requirements of cancer cells. In common polyps, it still remains unclear whether the specific metabolic requirement of tumor cells is dictated by oncogenes or if they change dynamically during tumor evolution. Mitochondrial biogenesis, involved in mitochondrial respiration rate, may be developed to be the prognostic marker for cancer prognosis. As there are profound differences in mitochondrial respiration, the assessment of the metabolic profile of CRC polyps and tumors has the potential to become a component of patient risk stratification.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6694/12/4/815/s1, Figure S1: Regulation of mitochondrial respiration in KRAS+, BRAF+ and wild-type tumors and controls, Table S1: The maximal ADP-activated respiration rates (Vmax) comparison by molecular groups. Respiration rates are given in nmol O2/(min×mg dry weight), Table S2: Km comparison by molecular groups.

Author Contributions: Conceptualization, E.R.-K., L.T. and T.K.; data curation, I.S.; formal analysis, E.R.-K., L.T., LR. and I.S.; funding acquisition, T.K.; investigation, E.R.-K., L.T., L.R., M.P., V.C., N.T. and K.T.; methodology, E.R.-K., L.T. and T.K.; project administration, K.S., V.V. and T.K.; resources, J.B., V.A., K.S. and V.V.; supervision, T.K.; visualization, L.R. and I.S.; writing—original draft, E.R.-K., L.T. and M.P.; writing—review & editing, I.S., V.C. and T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by institutional research funding IUT23-1 of the Estonian Ministry of Education and Research.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

ADP	adenosine diphosphate
CMS	consensus molecular subtype
CRC	colorectal cancer
K_m	Michaelis–Menten constant
K_m(ADP)	apparent affinity of mitochondria for exogenous ADP
OXPHOS	oxidative phosphorylation
MOM	outer mitochondrial membrane
VDAC	voltage-dependent anion channel
V_{max}	maximal-ADP-activated respiration rate

References

1. Vogelstein, B.; Kinzler, K.W. Cancer genes and the pathways they control. *Nat. Med.* **2004**, *10*, 789–799. [CrossRef]
2. Boutin, A.T.; Liao, W.T.; Wang, M.; Hwang, S.S.; Karpinets, T.V.; Cheung, H.; Chu, G.C.; Jiang, S.; Hu, J.; Chang, K.; et al. Oncogenic kras drives invasion and maintains metastases in colorectal cancer. *Genes Dev.* **2017**, *31*, 370–382. [CrossRef]

3. Hao, Y.; Samuels, Y.; Li, Q.; Krokowski, D.; Guan, B.J.; Wang, C.; Jin, Z.; Dong, B.; Cao, B.; Feng, X.; et al. Oncogenic pik3ca mutations reprogram glutamine metabolism in colorectal cancer. *Nat. Commun.* **2016**, *7*, 11971. [CrossRef]
4. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [CrossRef]
5. Schwitalla, S. Tumor cell plasticity: The challenge to catch a moving target. *J. Gastroenterol.* **2014**, *49*, 618–627. [CrossRef]
6. Kaldma, A.; Klepinin, A.; Chekulayev, V.; Mado, K.; Shevchuk, I.; Timohhina, N.; Tepp, K.; Kandashvili, M.; Varikmaa, M.; Koit, A.; et al. An in situ study of bioenergetic properties of human colorectal cancer: The regulation of mitochondrial respiration and distribution of flux control among the components of atp synthasome. *Int. J. Biochem. Cell Biol.* **2014**, *55*, 171–186. [CrossRef]
7. Guinney, J.; Dienstmann, R.; Wang, X.; de Reynies, A.; Schlicker, A.; Soneson, C.; Marisa, L.; Roepman, P.; Nyamundanda, G.; Angelino, P.; et al. The consensus molecular subtypes of colorectal cancer. *Nat. Med.* **2015**, *21*, 1350–1356. [CrossRef]
8. Hutton, J.E.; Wang, X.; Zimmerman, L.J.; Slebos, R.J.; Trenary, I.A.; Young, J.D.; Li, M.; Liebler, D.C. Oncogenic kras and braf drive metabolic reprogramming in colorectal cancer. *Mol. Cell. Proteom.* **2016**, *15*, 2924–2938. [CrossRef]
9. Corazao-Rozas, P.; Guerreschi, P.; Andre, F.; Gabert, P.E.; Lancel, S.; Dekiouk, S.; Fontaine, D.; Tardivel, M.; Savina, A.; Quesnel, B.; et al. Mitochondrial oxidative phosphorylation controls cancer cell's life and death decisions upon exposure to mapk inhibitors. *Oncotarget* **2016**, *7*, 39473–39485. [CrossRef]
10. Ruocco, M.R.; Avagliano, A.; Granato, G.; Vigliar, E.; Masone, S.; Montagnani, S.; Arcucci, A. Metabolic flexibility in melanoma: A potential therapeutic target. *Semin. Cancer Biol.* **2019**, *59*, 187–207. [CrossRef]
11. Pupo, E.; Avanzato, D.; Middonti, E.; Bussolino, F.; Lanzetti, L. Kras-driven metabolic rewiring reveals novel actionable targets in cancer. *Front. Oncol.* **2019**, *9*, 848. [CrossRef] [PubMed]
12. Yokota, T.; Ura, T.; Shibata, N.; Takahari, D.; Shitara, K.; Nomura, M.; Kondo, C.; Mizota, A.; Utsunomiya, S.; Muro, K.; et al. Braf mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br. J. Cancer* **2011**, *104*, 856–862. [CrossRef] [PubMed]
13. Phipps, A.I.; Buchanan, D.D.; Makar, K.W.; Win, A.K.; Baron, J.A.; Lindor, N.M.; Potter, J.D.; Newcomb, P.A. Kras-mutation status in relation to colorectal cancer survival: The joint impact of correlated tumour markers. *Br. J. Cancer* **2013**, *108*, 1757–1764. [CrossRef]
14. De Roock, W.; Claes, B.; Bernasconi, D.; De Schutter, J.; Biesmans, B.; Fountzilas, G.; Kalogeras, K.T.; Kotoula, V.; Papamichael, D.; Laurent-Puig, P.; et al. Effects of kras, braf, nras, and pik3ca mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. *Lancet Oncol.* **2010**, *11*, 753–762. [CrossRef]
15. Warburg, O. On the origin of cancer cells. *Science* **1956**, *123*, 309–314. [CrossRef] [PubMed]
16. Paudel, B.B.; Quaranta, V. Metabolic plasticity meets gene regulation. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 3370–3372. [CrossRef]
17. Jia, D.; Lu, M.; Jung, K.H.; Park, J.H.; Yu, L.; Onuchic, J.N.; Kaipparettu, B.A.; Levine, H. Elucidating cancer metabolic plasticity by coupling gene regulation with metabolic pathways. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 3909–3918. [CrossRef]
18. Yu, L.; Lu, M.; Jia, D.; Ma, J.; Ben-Jacob, E.; Levine, H.; Kaipparettu, B.A.; Onuchic, J.N. Modeling the genetic regulation of cancer metabolism: Interplay between glycolysis and oxidative phosphorylation. *Cancer Res.* **2017**, *77*, 1564–1574. [CrossRef]
19. Cruz, M.D.; Ledbetter, S.; Chowdhury, S.; Tiwari, A.K.; Momi, N.; Wali, R.K.; Bliss, C.; Huang, C.; Lichtenstein, D.; Bhattacharya, S.; et al. Metabolic reprogramming of the premalignant colonic mucosa is an early event in carcinogenesis. *Oncotarget* **2017**, *8*, 20543–20557. [CrossRef]
20. Grassetto, G.; Capirci, C.; Marzola, M.C.; Rampin, L.; Chondrogiannis, S.; Musto, A.; Crepaldi, G.; Minicozzi, A.M.; Massaro, A.; Rubello, D. Colorectal cancer: Prognostic role of 18f-fdg-pet/ct. *Abdom. Imaging* **2012**, *37*, 575–579. [CrossRef]
21. Graziano, F.; Ruzzo, A.; Giacomini, E.; Ricciardi, T.; Aprile, G.; Loupakis, F.; Lorenzini, P.; Ongaro, E.; Zoratto, F.; Catalano, V.; et al. Glycolysis gene expression analysis and selective metabolic advantage in the clinical progression of colorectal cancer. *Pharm. J.* **2017**, *17*, 258–264. [CrossRef] [PubMed]

22. Chekulayev, V.; Mado, K.; Shevchuk, I.; Koit, A.; Kaldma, A.; Klepinin, A.; Timohhina, N.; Tepp, K.; Kandashvili, M.; Ounpuu, L.; et al. Metabolic remodeling in human colorectal cancer and surrounding tissues: Alterations in regulation of mitochondrial respiration and metabolic fluxes. *Biochem. Biophys. Rep.* **2015**, *4*, 111–125. [CrossRef]
23. Ou, J.; Miao, H.; Ma, Y.; Guo, F.; Deng, J.; Wei, X.; Zhou, J.; Xie, G.; Shi, H.; Xue, B.; et al. Loss of abhd5 promotes colorectal tumor development and progression by inducing aerobic glycolysis and epithelial-mesenchymal transition. *Cell Rep.* **2014**, *9*, 1798–1811. [CrossRef] [PubMed]
24. Ounpuu, L.; Truu, L.; Shevchuk, I.; Chekulayev, V.; Klepinin, A.; Koit, A.; Tepp, K.; Puurand, M.; Rebane-Klemm, E.; Kaambre, T. Comparative analysis of the bioenergetics of human adenocarcinoma caco-2 cell line and postoperative tissue samples from colorectal cancer patients. *Biochim. Biol. Cell.* **2018**, *96*, 808–817. [CrossRef] [PubMed]
25. Koit, A.; Shevchuk, I.; Ounpuu, L.; Klepinin, A.; Chekulayev, V.; Timohhina, N.; Tepp, K.; Puurand, M.; Truu, L.; Heck, K.; et al. Mitochondrial respiration in human colorectal and breast cancer clinical material is regulated differently. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 1372640. [CrossRef]
26. Colombini, M. The vdac channel: Molecular basis for selectivity. *Biochim. Biophys. Acta (BBA) Mol. Cell Res.* **2016**, *1863*, 2498–2502. [CrossRef]
27. Noskov, S.Y.; Rostovtseva, T.K.; Chamberlin, A.C.; Teijido, O.; Jiang, W.; Bezrukov, S.M. Current state of theoretical and experimental studies of the voltage-dependent anion channel (vdac). *Biochim. Biophys. Acta* **2016**, *1858*, 1778–1790. [CrossRef]
28. Klepinin, A.; Ounpuu, L.; Mado, K.; Truu, L.; Chekulayev, V.; Puurand, M.; Shevchuk, I.; Tepp, K.; Planken, A.; Kaambre, T. The complexity of mitochondrial outer membrane permeability and vdac regulation by associated proteins. *J. Bioenerg. Biomembr.* **2018**, *50*, 339–354. [CrossRef]
29. Puurand, M.; Tepp, K.; Timohhina, N.; Aid, J.; Shevchuk, I.; Chekulayev, V.; Kaambre, T. Tubulin betaii and betaiii isoforms as the regulators of vdac channel permeability in health and disease. *Cells* **2019**, *8*, 239. [CrossRef]
30. Maldonado, E.N.; Sheldon, K.L.; DeHart, D.N.; Patnaik, J.; Manevich, Y.; Townsend, D.M.; Bezrukov, S.M.; Rostovtseva, T.K.; Lemasters, J.J. Voltage-dependent anion channels modulate mitochondrial metabolism in cancer cells: Regulation by free tubulin and erastin. *J. Biol. Chem.* **2013**, *288*, 11920–11929. [CrossRef]
31. Varikmaa, M.; Bagur, R.; Kaambre, T.; Grichine, A.; Timohhina, N.; Tepp, K.; Shevchuk, I.; Chekulayev, V.; Metsis, M.; Boucher, F.; et al. Role of mitochondria-cytoskeleton interactions in respiration regulation and mitochondrial organization in striated muscles. *Biochim. Biophys. Acta* **2014**, *1837*, 232–245. [CrossRef] [PubMed]
32. Anmann, T.; Guzun, R.; Beraud, N.; Pelloux, S.; Kuznetsov, A.V.; Kogerman, L.; Kaambre, T.; Sikk, P.; Paju, K.; Peet, N.; et al. Different kinetics of the regulation of respiration in permeabilized cardiomyocytes and in hl-1 cardiac cells. Importance of cell structure/organization for respiration regulation. *Biochim. Biophys. Acta* **2006**, *1757*, 1597–1606. [CrossRef] [PubMed]
33. Monge, C.; Beraud, N.; Tepp, K.; Pelloux, S.; Chahboun, S.; Kaambre, T.; Kadaja, L.; Roosimaa, M.; Piirsoo, A.; Tourneur, Y.; et al. Comparative analysis of the bioenergetics of adult cardiomyocytes and nonbeating hl-1 cells: Respiratory chain activities, glycolytic enzyme profiles, and metabolic fluxes. *Can. J. Physiol. Pharmacol.* **2009**, *87*, 318–326. [CrossRef] [PubMed]
34. Klepinin, A.; Chekulayev, V.; Timohhina, N.; Shevchuk, I.; Tepp, K.; Kaldma, A.; Koit, A.; Saks, V.; Kaambre, T. Comparative analysis of some aspects of mitochondrial metabolism in differentiated and undifferentiated neuroblastoma cells. *J. Bioenerg. Biomembr.* **2014**, *46*, 17–31. [CrossRef]
35. Kawada, K.; Toda, K.; Sakai, Y. Targeting metabolic reprogramming in kras-driven cancers. *Int. J. Clin. Oncol.* **2017**, *22*, 651–659. [CrossRef]
36. Oikonomou, E.; Koustas, E.; Goulielmaki, M.; Pintzas, A. Braf vs ras oncogenes: Are mutations of the same pathway equal? Differential signalling and therapeutic implications. *Oncotarget* **2014**, *5*, 11752–11777. [CrossRef]
37. Vaughn, C.P.; Zobell, S.D.; Furtado, L.V.; Baker, C.L.; Samowitz, W.S. Frequency of kras, braf, and nras mutations in colorectal cancer. *Genes Chromosomes Cancer* **2011**, *50*, 307–312. [CrossRef]
38. Lai, E.; Pretta, A.; Impera, V.; Mariani, S.; Giampieri, R.; Casula, L.; Pusceddu, V.; Coni, P.; Fanni, D.; Puzzoni, M.; et al. Braf-mutant colorectal cancer, a different breed evolving. *Expert Rev. Mol. Diagn.* **2018**, *18*, 499–512. [CrossRef]

39. Kaambre, T.; Chekulayev, V.; Shevchuk, I.; Karu-Varikmaa, M.; Timohhina, N.; Tepp, K.; Bogovskaja, J.; Kutner, R.; Valvere, V.; Saks, V. Metabolic control analysis of cellular respiration in situ in intraoperational samples of human breast cancer. *J. Bioenerg. Biomembr.* **2012**, *44*, 539–558. [CrossRef]
40. Kaambre, T.; Chekulayev, V.; Shevchuk, I.; Tepp, K.; Timohhina, N.; Varikmaa, M.; Bagur, R.; Klepinin, A.; Anmann, T.; Koit, A.; et al. Metabolic control analysis of respiration in human cancer tissue. *Front. Physiol.* **2013**, *4*, 151. [CrossRef]
41. Yun, J.; Rago, C.; Cheong, I.; Pagliarini, R.; Angenendt, P.; Rajagopalan, H.; Schmidt, K.; Willson, J.K.; Markowitz, S.; Zhou, S.; et al. Glucose deprivation contributes to the development of kras pathway mutations in tumor cells. *Science* **2009**, *325*, 1555–1559. [CrossRef]
42. Hubackova, S.; Magalhaes Novais, S.; Davidova, E.; Neuzil, J.; Rohlena, J. Mitochondria-driven elimination of cancer and senescent cells. *Biol. Chem.* **2019**, *400*, 141–148. [CrossRef]
43. Lu, J.; Tan, M.; Cai, Q. The warburg effect in tumor progression: Mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett.* **2015**, *356*, 156–164. [CrossRef]
44. Gnaiger, E.; Kemp, R.B. Anaerobic metabolism in aerobic mammalian cells: Information from the ratio of calorimetric heat flux and respirometric oxygen flux. *Biochim. Biophys. Acta* **1990**, *1016*, 328–332. [CrossRef]
45. Gstraunthaler, G.; Seppi, T.; Pfaller, W. Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? *Cell Physiol. Biochem.* **1999**, *9*, 150–172. [CrossRef]
46. Sherr, C.J.; DePinho, R.A. Cellular senescence: Mitotic clock or culture shock? *Cell* **2000**, *102*, 407–410. [CrossRef]
47. Jose, C.; Rossignol, R. Rationale for mitochondria-targeting strategies in cancer bioenergetic therapies. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 123–129. [CrossRef]
48. Swerdlow, R.H.; Lezi, E.; Aires, D.; Lu, J. Glycolysis-respiration relationships in a neuroblastoma cell line. *Biochim. Biophys. Acta* **2013**, *1830*, 2891–2898. [CrossRef]
49. Chance, B.; Williams, G.R. Respiratory enzymes in oxidative phosphorylation. Ii. Difference spectra. *J. Biol. Chem.* **1955**, *217*, 395–407.
50. Chance, B.; Williams, G.R. Respiratory enzymes in oxidative phosphorylation. Vi. The effects of adenosine diphosphate on azide-treated mitochondria. *J. Biol. Chem.* **1956**, *221*, 477–489.
51. Saks, V.A.; Veksler, V.I.; Kuznetsov, A.V.; Kay, L.; Sikk, P.; Tiivel, T.; Tranqui, L.; Olivares, J.; Winkler, K.; Wiedemann, F.; et al. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. *Mol. Cell. Biochem.* **1998**, *184*, 81–100. [CrossRef]
52. Mathupala, S.P.; Ko, Y.H.; Pedersen, P.L. Hexokinase-2 bound to mitochondria: Cancer's stygian link to the "warburg effect" and a pivotal target for effective therapy. *Semin. Cancer Biol.* **2009**, *19*, 17–24. [CrossRef]
53. Pedersen, P.L. Warburg, me and hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "warburg effect", i.E., elevated glycolysis in the presence of oxygen. *J. Bioenerg. Biomembr.* **2007**, *39*, 211–222. [CrossRef]
54. Maldonado, E.N.; Patnaik, J.; Mullins, M.R.; Lemasters, J.J. Free tubulin modulates mitochondrial membrane potential in cancer cells. *Cancer Res.* **2010**, *70*, 10192–10201. [CrossRef]
55. Draberova, E.; Sulimenko, V.; Vinopal, S.; Sulimenko, T.; Sladkova, V.; D'Agostino, L.; Sobol, M.; Hozak, P.; Kren, L.; Katsetos, C.D.; et al. Differential expression of human gamma-tubulin isotypes during neuronal development and oxidative stress points to a gamma-tubulin-2 prosurvival function. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2017**, *31*, 1828–1846. [CrossRef]
56. Lindstrom, L.; Li, T.; Malycheva, D.; Kancharla, A.; Nilsson, H.; Vishnu, N.; Mulder, H.; Johansson, M.; Rossello, C.A.; Alvarado-Kristensson, M. The gtpase domain of gamma-tubulin is required for normal mitochondrial function and spatial organization. *Commun. Biol.* **2018**, *1*, 37. [CrossRef]
57. Shoshan-Barmatz, V.; Krelin, Y.; Shteinfer-Kuzmine, A.; Arif, T. Voltage-dependent anion channel 1 as an emerging drug target for novel anti-cancer therapeutics. *Front. Oncol.* **2017**, *7*, 154. [CrossRef]
58. Shoshan-Barmatz, V.; Maldonado, E.N.; Krelin, Y. Vdac1 at the crossroads of cell metabolism, apoptosis and cell stress. *Cell Stress* **2017**, *1*, 11–36. [CrossRef]
59. Missiaglia, E.; Jacobs, B.; D'Ario, G.; Di Narzo, A.F.; Soneson, C.; Budinska, E.; Popovici, V.; Vecchione, L.; Gerster, S.; Yan, P.; et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann. Oncol.* **2014**, *25*, 1995–2001. [CrossRef]

60. Drewes, J.L.; Housseau, F.; Sears, C.L. Sporadic colorectal cancer: Microbial contributors to disease prevention, development and therapy. *Br. J. Cancer* **2016**, *115*, 273–280. [CrossRef]
61. De Palma, F.D.E.; D'Argenio, V.; Pol, J.; Kroemer, G.; Maiuri, M.C.; Salvatore, F. The molecular hallmarks of the serrated pathway in colorectal cancer. *Cancers (Basel)* **2019**, *11*, 1017. [CrossRef]
62. Fang, S.; Fang, X. Advances in glucose metabolism research in colorectal cancer. *Biomed. Rep.* **2016**, *5*, 289–295. [CrossRef]
63. Iwamoto, M.; Kawada, K.; Nakamoto, Y.; Itatani, Y.; Inamoto, S.; Toda, K.; Kimura, H.; Sasazuki, T.; Shirasawa, S.; Okuyama, H.; et al. Regulation of 18f-fdg accumulation in colorectal cancer cells with mutated kras. *J. Nucl. Med.* **2014**, *55*, 2038–2044. [CrossRef]
64. Wang, P.; Song, M.; Zeng, Z.L.; Zhu, C.F.; Lu, W.H.; Yang, J.; Ma, M.Z.; Huang, A.M.; Hu, Y.; Huang, P. Identification of ndufaf1 in mediating k-ras induced mitochondrial dysfunction by a proteomic screening approach. *Oncotarget* **2015**, *6*, 3947–3962. [CrossRef]
65. Ralph, S.J.; Low, P.; Dong, L.; Lawen, A.; Neuzil, J. Mitocans: Mitochondrial targeted anti-cancer drugs as improved therapies and related patent documents. *Recent Pat. Anticancer Drug Discov.* **2006**, *1*, 327–346. [CrossRef]
66. Kuznetsov, A.V.; Veksler, V.; Gellerich, F.N.; Saks, V.; Margreiter, R.; Kunz, W.S. Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. *Nat. Protoc.* **2008**, *3*, 965–976. [CrossRef]
67. Kuznetsov, A.V.; Tiivel, T.; Sikk, P.; Kaambre, T.; Kay, L.; Daneshrad, Z.; Rossi, A.; Kadaja, L.; Peet, N.; Seppet, E.; et al. Striking differences between the kinetics of regulation of respiration by adp in slow-twitch and fast-twitch muscles in vivo. *Eur. J. Biochem.* **1996**, *241*, 909–915. [CrossRef]
68. Gnaiger, E. Oxygen solubility in experimental media. *OROBOROS Bioenerg. News* **2001**, *6*, 1–6.
69. Puurand, M.; Tepp, K.; Klepinin, A.; Klepinina, L.; Shevchuk, I.; Kaambre, T. Intracellular energy-transfer networks and high-resolution respirometry: A convenient approach for studying their function. *Int. J. Mol. Sci.* **2018**, *19*, 2933. [CrossRef]
70. Timohhina, N.; Guzun, R.; Tepp, K.; Monge, C.; Varikmaa, M.; Vija, H.; Sikk, P.; Kaambre, T.; Sackett, D.; Saks, V. Direct measurement of energy fluxes from mitochondria into cytoplasm in permeabilized cardiac cells in situ: Some evidence for mitochondrial interactosome. *J. Bioenerg. Biomembr.* **2009**, *41*, 259–275. [CrossRef]

Appendix 2

Publication II

Rebane-Klemm E, Reinsalu L, Puurand M, Shevchuk I, Bogovskaja J, Suurmaa K, Valvere V, Moreno-Sanchez R, Kaambre T. Colorectal polyps increase glycolytic activity. Front Oncol. 2023 May 23; doi: 10.3389/fonc.2023.1171887.

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 05 June 2023
DOI 10.3389/fonc.2023.1171887

OPEN ACCESS

EDITED BY
Egidio Iorio,
National Institute of Health (ISS), Italy

REVIEWED BY
Elena Sacco,
University of Milano-Bicocca, Italy
Anna La Salvia,
National Institute of Health (ISS), Italy

*CORRESPONDENCE
Leenu Reinsalu
leenu.reinsalu@kbfi.ee

†These authors have contributed equally to this work and share first authorship

RECEIVED 22 February 2023
ACCEPTED 23 May 2023
PUBLISHED 05 June 2023

CITATION
Rebane-Klemm E, Reinsalu L, Puurand M, Shevchuk I, Bogovskaja J, Suurmaa K, Valvere V, Moreno-Sanchez R and Kaambre T (2023) Colorectal polyps increase the glycolytic activity. *Front. Oncol.* 13:1171887. doi: 10.3389/fonc.2023.1171887

Colorectal polyps increase the glycolytic activity

Egle Rebane-Klemm[1,2†], Leenu Reinsalu[1,2*†], Marju Puurand[1], Igor Shevchuk[1], Jelena Bogovskaja[3], Kulliki Suurmaa[4], Vahur Valvere[5], Rafael Moreno-Sanchez[6] and Tuuli Kaambre[1]

[1]Laboratory of Chemical Biology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, [2]Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Tallinn, Estonia, [3]Clinic of Diagnostics, North Estonia Medical Centre, Tallinn, Estonia, [4]Department of Gastroenterology, West Tallinn Central Hospital, Tallinn, Estonia, [5]Oncology and Hematology Clinic, North Estonia Medical Centre, Tallinn, Estonia, [6]Laboratorio de Control Metabólico, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Los Reyes Iztacala, Barrio de los Árboles/Barrio de los Héroes, Tlalnepantla, Mexico

In colorectal cancer (CRC) energy metabolism research, the precancerous stage of polyp has remained rather unexplored. By now, it has been shown that CRC has not fully obtained the glycolytic phenotype proposed by O. Warburg and rather depends on mitochondrial respiration. However, the pattern of metabolic adaptations during tumorigenesis is still unknown. Understanding the interplay between genetic and metabolic changes that initiate tumor development could provide biomarkers for diagnosing cancer early and targets for new cancer therapeutics. We used human CRC and polyp tissue material and performed high-resolution respirometry and qRT-PCR to detect changes on molecular and functional level with the goal of generally describing metabolic reprogramming during CRC development. Colon polyps were found to have a more glycolytic bioenergetic phenotype than tumors and normal tissues. This was supported by a greater *GLUT1*, *HK*, *LDHA*, and *MCT* expression. Despite the increased glycolytic activity, cells in polyps were still able to maintain a highly functional OXPHOS system. The mechanisms of OXPHOS regulation and the preferred substrates are currently unclear and would require further investigation. During polyp formation, intracellular energy transfer pathways become rearranged mainly by increasing the expression of mitochondrial adenylate kinase (*AK*) and creatine kinase (*CK*) isoforms. Decreased glycolysis and maintenance of OXPHOS activity, together with the downregulation of the CK system and the most common AK isoforms (*AK1* and *AK2*), seem to play a relevant role in CRC development.

KEYWORDS

metabolic phenotype, energy metabolism, colorectal cancer, colonic adenoma, OXPHOS, Warburg effect

1 Introduction

Colorectal cancer (CRC) is a multifactorial and heterogeneous disease that mostly arises from precursor lesions known as polyps. Two major classes of colorectal polyps are conventional adenomas (tubular, tubulovillous, or villous adenoma) and serrated polyps (hyperplastic polyps, sessile serrated adenoma/polyps, and traditional serrated adenomas) (1), which are believed to arise from distinct etiologic pathways. The current understanding of CRC development suggests that the progressive accumulation of oncogenic changes begins with abnormal growth of colon epithelial cells. Sequence alterations in specific genes, including *APC* and *KRAS*, contribute to the development of early precancerous lesions (2) and metabolic reprogramming towards a glycolytic phenotype. Over time, adenomas develop increasingly dysplastic features and eventually acquire malignant potential. However, most adenomas stabilize their growth progression or even regress (3). Although genetic events in colonic polyps are quite well characterized (4, 5), the reprogramming of metabolic pathways has not been widely investigated.

Metabolic reprogramming is one of the hallmarks of cancer (6). However, metabolic alterations in the precancerous stage and colorectal carcinogenesis are not well understood. Almost 100 years ago, Otto Warburg first described that cancer cells metabolize glucose directly to lactic acid even in the presence of high oxygen. This modified glucose metabolism is known as the "Warburg effect" (7). Warburg proposed that the increased rate of aerobic glycolysis was due to irreversible injury of mitochondrial oxidative phosphorylation (OXPHOS), the main pathway providing energy for eukaryotic cells, and generating more adenosine triphosphate (ATP) than glycolysis. Nowadays, it has become clear that glycolysis is upregulated in many tumors without mitochondrial dysfunction.

Several *in vivo* studies have demonstrated up-regulation of the components of the OXPHOS system in certain types of cancer cells (8), which is accompanied by increased mitochondrial respiration and OXPHOS flux. The OXPHOS machinery in most cancer cells seems to be fully functional. Moreover, cells can switch between OXPHOS and aerobic glycolysis or even perform them simultaneously, depending on the availability of substrates (including oxygen) (9). This metabolic plasticity is defined as the ability of cancer cells to reprogram their metabolic pathways to fulfill energetic and anabolic needs in a changing extracellular microenvironment during the various steps of disease progression.

Another important aspect of energy metabolism and metabolic plasticity is the interplay between energy transfer pathways in cancer cells. The isoenzymes of hexokinase (HK), adenylate kinase (AK), and creatine kinase (CK) support specific cellular processes ranging from muscle contraction and cell motility to mitochondrial/nuclear energetics (10). Indeed, it has been proposed that some AK and HK isoenzymes may be targets for antitumor therapy (11, 12). A complete spectrum of HK, AK, and CK isoforms in clinically well-defined patient groups may inform us about the changes in the maintenance of energy homeostasis of tumor cells. Several high-resolution respirometry studies performed on different permeabilized tissues and cells show that there is specificity on how adenosine diphosphate (ADP) may regulate OXPHOS at the level of the mitochondrial outer membrane (MOM). The basis of this last premise is the structurally different intracellular arrangement of functional units; such complexity of the intracellular environment determines the need for the use of energy transfer pathways. Moreover, the differences between cancer and non-cancer cells in the composition of cytoskeleton proteins and their interaction with mitochondria are related to the prevalent type of metabolism, facilitating metabolic plasticity (13, 14).

The present study was aimed to identify and characterize metabolic reprogramming in colon polyps by assessing mitochondrial respiratory rates and gene expression of selected metabolic markers. The precise contribution of different metabolic pathways to the adenoma-carcinoma sequence is not known yet. Understanding the relationship between genetic and metabolic changes, as well as the role of these interactions in tumor initiation, is essential for designing efficient therapeutic approaches targeting the metabolism of tumors.

2 Materials and methods

2.1 Clinical material

All experiments were performed with human tissue samples. The present research protocol was approved by the Medical Research Ethics Committee (National Institute for Health Development, Tallinn, Estonia) by decisions number KK557 and KK558, and was following the Helsinki Declaration and Convention of the Council of Europe on Human Rights and Biomedicine. Research subjects were fully informed about the study and gave their consent.

Tumor and control tissue samples were obtained from the North Estonia Medical Centre. All patients (n=56 with ages ranging from 38 to 101) showed local or locally advanced disease (T2-4, N0-2), and only primary tumors were used. Normal tissue samples were taken from the same location at sites distant from the tumor and were checked for malignancies. Colorectal polyps were resected from patients (n=28, with ages ranging from 50 to 84) undergoing a colonoscopy at the West Tallinn Central Hospital. Only non-cancerous polyps were used. To maintain physiological conditions during geographical displacement, samples were placed immediately after removal into medium B (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 3 mM KH_2PO_4, 110 mM sucrose, 0.5 mM dithiothreitol, 20 mM HEPES, 5 µM leupeptin, 2 mg/mL fatty acids free bovine serum albumin, pH 7.1). Additionally, a small amount of tissue was transported in RNALater Stabilization Solution (Qiagen).

2.2 Preparation of skinned tumor samples and permeabilization procedure

Upon arriving, samples were placed into pre-cooled (4°C) medium A consisting of 3 mM KH_2PO_4, 20 mM taurine, 5.7 mM ATP, 15 mM PCr, 9.5 mM $MgCl_2$, 49 mM MES, 7.23 mM K_2EGTA, and 2.77 mM $K_2CaEGTA$, pH 7.1). Fat and blood vessels were removed from the tissue samples, which were then dissected into

small samples (5-15 mg). These were permeabilized in medium A containing 50 µg/mL of saponin for 30 min at 4°C. The permeabilized samples were then washed three times for 5 min in pre-cooled medium B without leupeptin and kept at 4°C until use in oxygraphic analysis.

2.3 Oxygraphic measurements

The mitochondrial respiration of permeabilized tissue samples was measured in medium B at 25°C using a high-resolution respirometer Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria). The medium was supplemented with 5 mM glutamate, 2 mM malate, and 10 mM succinate to fully activate respiratory chain complexes 1 and 2 (15). ADP was added in increasing concentrations to measure the dependence of respiration rate on exogenous ADP (Supplementary S1) and then calculate the apparent affinity of mitochondria to exogenous ADP (K_m(ADP)). The obtained data were plotted as rates of O_2 consumption (the basal respiration rate of respiration was subtracted) *versus* ADP concentration and K_m(ADP) and V_{max} values were calculated from these plots by nonlinear regression using Michaelis–Menten equation.

2.3.1 Calibration of ADP stock solutions

To calibrate the concentration of ADP stock solution, the absorbance of NADH was determined using spectrophotometry. The reaction mixture contained a high K^+ concentration medium (120 mM KCl, 20 mM MOPS, 1 mM EGTA, pH 7.2), 5 mM $MgCl_2$, 1 mM phosphoenolpyruvate, 2,5 IU/mL lactate dehydrogenase, 3.75 IU/mL pyruvate kinase, and 0.15 mM NADH. The reaction was initiated by adding 1 µL of ADP stock and the concentration of ADP stock was defined as the decrease of NADH concentration. The extinction coefficient for NADH (6.22×10^3 M^{-1} cm^{-1}) was used to convert its absorbance to molar concentration. K_m(ADP) values were corrected accordingly.

2.4 RNA extraction

Tissue samples from patients were transported in RNALater solution (Qiagen) to protect cellular RNA until it was frozen in liquid nitrogen and stored at -80°C. The frozen tissue samples were homogenized by using the TRIzol reagent (Ambion). For RNA isolation, the RNeasy Mini Kit (Qiagen) was used by following the protocol by Untergasser (16). Genomic DNA was removed by using RNase-free DNase I solution (Qiagen). RNA was eluted in 30 µL of RNase-free water and the total concentration of RNA was measured by a BioSpec-Nano spectrophotometer (Shimadzu). Isolated RNA was stored at -80°C.

2.5 cDNA synthesis and real-time quantitative polymerase chain reaction

For cDNA synthesis and qRT-PCR, all reagents used were by Applied Biosynthesis. cDNA was synthesized from 2 µg of RNA by using a High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor following the manufacturer's instructions. Reverse transcription was performed with Eppendorf® 5332 Mastercycler thermocycler.

qRT-PCR was performed with LightCycler 480 II (Roche) and by using the TaqMan Gene Expression Master Mix (Thermo Fisher Scientific). To detect gene expression levels, FAM-labeled TaqMan probes were used: actin-β (Hs01060665_g1), AK1 (Hs00176119_m1), AK2 (Hs01123132_g1), AK4 (Hs03405743_g1), AK6 (Hs00360444_g1), CK-BB (Hs00176483_m1), CK-MT1 (Hs00179727_m1), CK-MT2 (Hs00176502_m1), HK1 (Hs00175976_m1), HK2 (Hs00606086_m1), GLUT1 (Hs00892681_m1), LDHA (Hs03405707_g1), MCT1 (Hs00161826_m1), MCT2 (Hs04332706_m1), and MCT4 (Hs00358829_m1). MQ was used as a negative control.

2.6 DNA extraction

DNA was extracted from tissue samples using Invitrogen™ PureLink™ Genomic DNA Mini Kit following the instructions provided by the manufacturer. DNA concentrations and quality were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.7 *KRAS* and *BRAF* mutation analysis

High-Resolution Melt (HRM) analysis was performed to detect the mutations in *KRAS* codon 12 and 13 of exon 2 and *BRAF* codon 600 of exon 15 (V600E). The reaction mix contained 1x HOT FirePol® EvaGreen® HRM Mix (Solis BioDyne, Estonia), 250 nM of sense and antisense primers (Supplementary Table S2), and 100x dilution of PCR amplification product. PCR amplification and HRM analysis were carried out with Rotor-Gene 6000 (QIAGEN) and consisted of an initial 15 min denaturation step at 95°C, followed by 45 cycles at 95°C for 10 s, 54°C for 10 s, and 72°C for 15 s, with a final extension at 72°C for 3 min. The obtained PCR products were heated at 95°C for 1 min and cooled down to 40°C to facilitate the formation of heteroduplex. HRM analysis was performed from 62°C to 92°C with a 0.1°C step. The results were analyzed using Rotor-Gene 6000 software and unknown samples were compared to control samples with known genotypes.

2.8 Data analysis

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials. Data in text, figures, and tables are presented as mean ± standard error (SEM). Bar charts with individual data points were made by using SigmaPlot 11.0. The results from oxygraphic analysis and qRT-PCR were analyzed by Student's *t*-test and p-values <0.05 were considered statistically significant. Apparent K_m(ADP) values were measured by fitting experimental data to non-linear regression.

10.3389/fonc.2023.1171887

3 Results and discussion

3.1 Mitochondrial outer membrane permeability for ADP is different in healthy colon, polyps, and cancer tissue

To identify the changes in OXPHOS activity during the development of CRC, we applied high-resolution respirometry on permeabilized postoperative tissues (CRC, colon polyps, and healthy colon tissue). We determined the rate of maximal ADP-activated respiration (V_{max}) and calculated the apparent Michaelis-Menten constant values for exogenously added ADP (K_m(ADP)), to estimate the coupling of mitochondrial oxygen consumption to OXPHOS and the permeability of voltage-dependent anion channel (VDAC) for exogenous ADP, respectively.

Tissue or cell-specific tuning of OXPHOS activity through regulation of creatine, creatine-phosphate and adenine nucleotides movement *via* VDAC resulting in a certain K_m(ADP) value could be a suitable indicator of the specific complexity of the intracellular organization, which is dealt with *ad hoc* isoforms of CK and AK for catalysis and intracellular energy transfer. In this regard, orders of magnitude different K_m(ADP) values have been found between glycolytic and oxidative striated muscles (tissue-specific K_m (ADP) values are higher in oxidative tissues), which have different metabolic features (17, 18). Thus, the determination of V_{max} and K_m (ADP) values for cellular oxygen consumption rates could provide relevant information about the activity of OXPHOS key components, the type of metabolism, and the complexity of the internal organization of the cells in the three different tissues included in the present study.

There were significant differences in oxygen consumption V_{max} and K_m(ADP) values between polyps and tumors, suggesting that polyps and tumors have different bioenergetic profiles and demands for energy (Figure 1). The observation that V_{max} for tumors was higher than for healthy colon tissue (Figure 1A; Supplementary Table S3) was in agreement with our previous studies (19–21). Interestingly, V_{max} for colon polyps exceeded that in tumors and was two times higher than V_{max} for healthy tissue. Determination of K_m(ADP) values revealed that colon polyps have a significantly lower K_m(ADP) compared to both cancerous and healthy tissue (Figure 1B). At the same time, healthy tissue and tumors showed similar K_m(ADP) values, indicating a lower affinity for ADP than in polyps. The V_{max}/K_m(ADP) ratio was 0.039 min^{-1} mg^{-1} mL for colon polyps, whereas this ratio was similar and lower (0.019 and 0.013, respectively) for tumor and healthy tissue, indicating a more catalytically efficient system in polyps. Additionally, by calculating the % of mitochondrion with low oxidative capacity using the model developed by Saks and colleagues (22), polyps were characterized by a higher % of mitochondrion with low control over the movement of adenine nucleotides through MOM (Supplementary Table S4) compared to both healthy tissue and malignant tumors. This suggests that polyps have higher glycolytic capacity. These observations suggested a metabolic shift towards a more glycolytic type of metabolism while maintaining OXPHOS functionality in polyps, which was indicated respectively by the increased affinity for ADP on MIM and the high ADP-induced respiration level.

3.2 Polyps with *BRAF* mutation demonstrate a higher glycolytic activity together with some down-regulation of OXHOS

The malignant transformation of cells, including colon epithelium, is accompanied by metabolic reprogramming of energy production and biosynthesis pathways that promote tumor growth and metastasis (23). Mutations in *KRAS* or *BRAF* genes appear to play a significant role in the transcriptional regulation of metabolic reprogramming in multiple cancers, including CRC (21, 24–27). The potential effect of *KRAS* and *BRAF* mutations on mitochondrial respiration was investigated in the colorectal polyp group (Figure 2).

Polyps with *KRAS* mutation showed higher V_{max} values compared to those of polyps with *BRAF* mutation (Figure 2A; Supplementary Table S5). This pattern was similar to that obtained when comparing *BRAF* and *KRAS* mutations in CRC, with the difference that the V_{max} of non-mutated CRC was higher than that in the tissue with *KRAS* and *BRAF* mutations (21). Mitochondria in *KRAS* mutated polyps showed lower affinity for exogenous ADP compared to that of *BRAF* mutated polyp group (Figure 2B). There

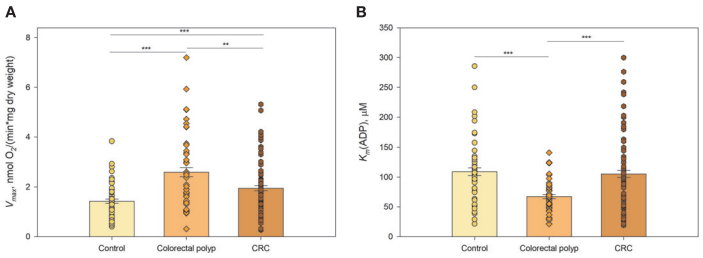

FIGURE 1
Kinetic parameters of mitochondrial respiration in normal tissue, colorectal polyps, and colorectal cancer tissue **(A)** Comparative analysis of maximal ADP-stimulated respiratory rate (V_{max}); and **(B)** apparent Michaelis-Menten constant values for ADP (K_m(ADP)) in control tissue (n=46), colorectal polyps (n=42), and colorectal cancer (CRC) tissue (n=57) ** p < 0,01, *** p < 0,001 (t-test).

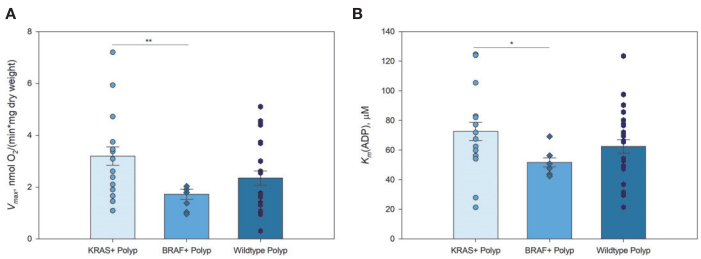

FIGURE 2
Kinetic parameters of mitochondrial respiration in *KRAS* and *BRAF* mutated, and wild-type polyps **(A)** Comparative analysis of maximal ADP-activated respiratory rate (V_{max}); and **(B)** apparent Michaelis-Menten constant values for ADP (K_m(ADP)) in *KRAS* mutated polyps (n=14), *BRAF* mutated polyps (n=6), and wild-type polyps (n=21) * p < 0,05, ** p < 0,01 (t-test).

were no significant differences in V_{max} and K_m(ADP), nor V_{max}/K_m (ADP) ratios, between *KRAS* or *BRAF* mutated and wild-type polyps. However, due to the significantly lower V_{max} in both polyps and tumors with *BRAF* mutation, it may be assumed that cells with this mutation display a more active glycolysis with a parallel moderate down-regulation of OXPHOS. This metabolic profile of *KRAS* and *BRAF* mutated polyps suggested that energy metabolism during the transformation of polyps to colorectal cancer remain relatively unchanged. These results clearly need further assessment by using a larger study group.

To unveil respiratory rate kinetic parameters dependence on clinicopathological characteristics, possible relationships were analyzed of V_{max} and K_m(ADP) of polyps, tumors, and healthy colon tissue with age, gender, location, size, histological type, and molecular group (Supplementary Table S5). No relationship of V_{max} and K_m(ADP) values of healthy tissue, polyps, and CRC groups with clinicopathological factors was found. One exception was the location of polyps and tumors, which rendered different V_{max} values. However, sample sizes among groups were unequal as CRC is more frequently observed in the distal than in the proximal area (28) and genetic architectures of proximal and distal CRC are partly distinct (29). Again, these results clearly require further assessment by using a larger study group.

3.3 Different gene expression levels show changes in energy metabolism during CRC tumorigenesis

Numerous genes and proteins essential for glucose uptake and glycolysis are upregulated in CRC and colon polyps (30–33). In the present study, RT-qPCR was performed to detect mRNA of genes coding for the glycolysis-controlling steps *GLUT1*, *HK1*, and *HK2* (8), as well as for the essential but not controlling steps *MCT1*, *MCT2*, *MCT4*, and *LDHA* and analyze their involvement in metabolic reprogramming in colon polyps. Absence of essential genes or proteins completely stops the functioning of the cellular process/function whereas fractional removal or inhibition of a controlling step brings about a corresponding decrease in the analyzed cellular process/function.

3.3.1 Higher expression levels of genes coding for flux-controlling steps of glucose metabolism indicate an increased glycolytic activity in polyps

The first step in glucose metabolism is the entrance of glucose into the cell, which relies on glucose transport proteins (GLUTs). GLUTs belong to a homologous family of fourteen uniporter transporter proteins. Among these, GLUTs 1-4 have been extensively studied and shown to be upregulated in cancers (34). There is an increasing number of studies identifying GLUT1 (glucose low affinity isoform) and GLUT3 (glucose high affinity isoform) as preeminent actors in accelerated glucose metabolism. High expression of *GLUT1* is associated with poor survival in most cancer types, including colorectal cancer (35). The lower *GLUT1* expression levels in control colon tissue (Figure 3A) compared to diseased states were consistent with the notion that glucose provides a smaller fraction of the energy requirements for the healthy colonic epithelium. The expression of GLUT1 in colorectal polyps was significantly higher than in normal tissue, suggesting an increased demand for glucose. The CRC group also showed an increased level of *GLUT1* expression compared to the healthy colon tissue. The polyp group showed a tendency towards a lower expression of *GLUT1* than the CRC group but there was no significant difference (p=0.136). An increase in glucose uptake may indicate significant changes in energy metabolism as well as in anabolic precursors demand such as glucose-6-phosphate for pentose phosphate pathway, dihydroacetonephosphate for triacylglyceride and phospholipid syntheses, and 3-phosphoglycerate for serine, cysteine and glycine syntheses occurring in the tissue at early events of carcinogenesis.

Hexokinase (HK) is a flux-controlling step of glycolysis catalyzing ATP-dependent phosphorylation of glucose into glucose-6-phosphate (8). Four major HK isoforms, encoded by separated genes, are expressed in human tissues – HK1-4 (36). HKs help to sustain cellular glucose levels by regulating the entry and utilization of glucose and influencing the magnitude and the direction of glucose flux within cells (37). HK1 is the predominant HK isoform in most tissues, is a glucose high affinity isoform and is more abundant than HK2. HK2 is a glucose low affinity isoform and the main isoenzyme in insulin-sensitive organs such as heart, skeletal muscle, and adipose tissue, and in a wide range of tumors.

HK1 and HK2 can also dock to mitochondria through an N-terminal motif absent in the other isoforms. When bound to

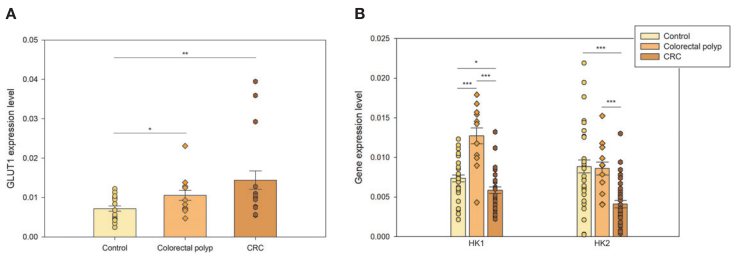

FIGURE 3
The expression levels of genes coding for flux-controlling steps in glycolysis **(A)** GLUT 1 expression levels in healthy colon (n=16), colorectal polyps (n=8), and colorectal tumors (n=9) **(B)** Expression levels of *hexokinase 1* (*HK1*) and *hexokinase 2* (*HK2*) in healthy tissue (n=27), colorectal polyps (n=9), and colorectal cancer (CRC) tissue (n=27). * p < 0,05, ** p < 0,01, *** p < 0,001.

mitochondria, HK1 and HK2 exert cytoprotective effects in healthy and neoplastic cells and increase their efficiency in glucose usage (38). Pedersen proposed that HK2 promotes the Warburg effect by binding to VDAC (11). This interaction leads VDAC to redirect mitochondrial ATP to HK2 to be used in glycolysis. Thus, HK has been proposed to regulate the MOM permeability in glycolytic cancer cells (14, 39). The high expression level and activity of *HK2* together with that of GLUTs in glycolytic cancers are indirectly revealed by ^{18}FDG-PET imaging (38).

Expression of *HK1* and *HK2* was analyzed in healthy colon tissue, colorectal polyp, and CRC groups. *HK1* expression level in polyps was twice as high as in healthy tissue and CRC group, while their *HK2* expression was similar to that of healthy tissue group (Figure 3B). Distinct differences in the regulation of mitochondrial respiration in polyps (Figure 1), specifically lower K_m(ADP) values, suggested that polyps have a more glycolytic type of regulation of energy metabolism than CRC and healthy tissue. *HK2* overexpression has been previously shown in CRC cells, in comparison to normal cells (33, 40). However, our data did not reveal *HK2* overexpression in the CRC group. In fact, it was significantly lower than that of healthy tissue ($p<0.001$). Then, the low *HK2* expression levels in the CRC group suggested that energy metabolism in CRC cells was not entirely glycolytic and that OXPHOS system was an important energy provider.

In turn, higher expression levels of *HK1* and *HK2* in polyps compared to the CRC group suggested that glycolytic metabolism played a more essential role in polyps than in CRC. Considering the moderate *GLUT1* expression levels and the ensuing moderate glucose uptake in polyps (Figure 3A), then *HK* overexpression seemed counterproductive. We speculate that there was no need to remarkably increase the glucose uptake mediated by GLUT1 in polyps, because highly expressed *HK*s, and perhaps GLUT3, were able to drive an enhanced glycolytic flux.

3.3.2 Expression levels of genes coding for essential but non-controlling steps support the conclusion that polyps increase glycolytic activity

Lactate dehydrogenase A (LDHA) is an essential enzyme in the glycolytic pathway that catalyzes the conversion of pyruvate to lactic acid using NADH and recycling NAD+. Elevated levels of this protein have been found in several cancer types (41, 42), supporting cancer cell proliferation and survival. The low K_m(ADP) value (Figure 1B) and high expression levels of *HK*s (Figure 3B) suggested that cells in colon polyps developed an increased dependence on the glycolytic pathway. This was further supported by the elevated levels of *LDHA* expression in polyps compared to the healthy colon tissue (Figure 4A), suggesting a rise in lactate production. Although LDH is not a flux-controlling step of glycolysis since it is one of the fastest pathway steps, it exerts full control on the pyruvate and lactate levels and hence on the cytosolic redox balance (the Pyr/Lac ratio is tightly linked to the NADH/NAD+ ratio by the overexpressed high LDH activity).

Moreover, increased production of lactate and its further release together with H^+ promotes malignant progression by lowering extracellular pH, which helps cancer cells to overcome host immune response (43). In addition, aerobic glycolysis does not only supply ATP, but also yields metabolic precursors for nucleotides, amino acids, and lipids biosynthesis for cell proliferation (44). Therefore, the high expression of *LDHA* in polyps helps promoting the disease progression to malignancy. There was no significant difference between the expression levels of *LDHA* between the CRC and the healthy tissue group, suggesting that CRC cells do not need to increase lactate production and the ensuing external acidification further because they mainly increase OXPHOS for energy supply and expression of glycolytic controlling steps for anabolic precursors and pyruvate provision.

Healthy colonocytes derive 60-70% of their energy supply from short-chain carboxylic acids, particularly butyrate. Butyrate is transported across the luminal membrane of the colonic epithelium *via* a monocarboxylate transporter (MCT1) (45). MCT1 is a member of the monocarboxylate transporter family, of which 14 isoforms have been identified. In the present study, the expression of *MCT1*, *MCT2*, and *MCT4* was analyzed. Healthy colon tissue showed higher expression level of *MCT1* compared to the tumor (Figure 4B), which is aligned with the fact that butyrate is the main source of energy for colonic epithelial cells (46). Decreased *MCT1* expression in cancer tissue could indicate that cancer cells use less butyrate, displaying metabolic plasticity and making them less dependent on this nutrient.

MCT1 has a high affinity for extracellular lactate and has been shown to transport lactate to sustain energy production in malignant cells (47). Therefore, its low expression level in tumors suggested that

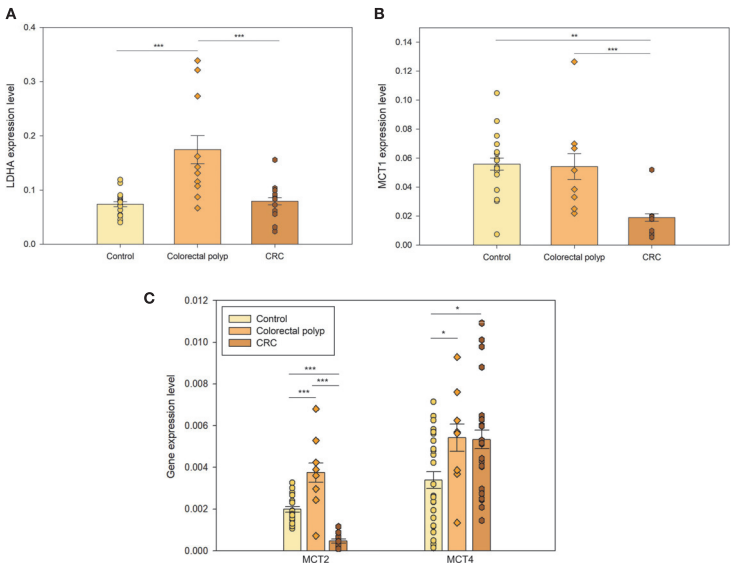

FIGURE 4
The expression levels of essential but non-controlling steps in glycolysis **(A)** The expression levels of *lactate dehydrogenase A* (*LDHA*) in healthy tissue (n=16), colorectal polyps (n=8), and colorectal cancer (CRC) tissue (n=9). **(B)** The xpression levels of monocarboxylate transporters *MCT1* and **(C)** *MCT2* in healthy tissue (n=16), colorectal polyps (n=8) and colorectal cancer (CRC) tissue (n=9); and expression levels of *MCT4* in healthy tissue (n=24), colorectal polyps (n=8) and CRC tissue (n=22) * p < 0,05, ** p < 0,01, *** p < 0,001 (t-test).

CRC cells did not depend on lactate as a metabolic fuel. *MCT1* was expressed in colon polyps similarly to healthy tissue but the expression levels of isoforms *MCT2* and *MCT4* were increased (Figure 4C). In highly glycolytic cancer cells, MCT2 has been shown to localize mainly in the cytosol (48). Decreased expression of *MCT2* in the CRC group compared to the healthy colon tissue group again supported the idea that CRC did not acquire a typical Warburg effect. MCT4 has a low affinity for extracellular lactate and high affinity for intracellular lactate, as well as very high activity for lactate transport and a very low affinity for pyruvate (48), meaning that pyruvate is rather converted to lactate than transported out of the cell whereas internal lactate can be actively expelled.

Similar expression level of *MCT1* (Figure 4B) in control and polyp groups suggested that colon polyps kept using short-chain carboxylic acids, and perhaps other substrates (*e.g.* glutamine). However, polyps exhibited higher *MCT4* expression (Figure 4C) than control indicating that they simultaneously increased glycolytic activity. *MCT4* is upregulated by hypoxia and hypoxia-inducible factor 1alpha (HIF-1*alpha*) (49). It has been shown that *HIF-1alpha* levels are increased in colon polyps and CRC (50). There is a steep oxygen gradient from the anaerobic lumen of the intestine across the epithelium into the highly vascularized sub-epithelium. Epithelial cells lining the mucosa are exposed to a relatively low O_2 tension environment that has been described as "physiological hypoxia (51, 52)." From this perspective, it is perhaps not surprising to see overexpression of *MCT4* in colon polyps as energy demand increases while there is still a low level of oxygenation.

3.3.3 Intracellular phosphotransfer pathways are upregulated in colon polyps

Adenylate kinase (AK) and creatine kinase (CK) play an important role in adjusting mitochondrial ATP synthesis to cellular ATP consumption by forming phosphotransfer circuits, which connect sites of ATP production (glycolysis and OXPHOS) with subcellular sites of ATP utilization (ATPases) to support robust metabolic homeostasis (53–55).

AKs catalyze the reversible interconversion of adenine nucleotides (AMP, ADP, ATP), and they represent the main mediator of intracellular nucleotide exchange and AMP metabolic signaling (56). Suppression of AK phosphotransference and AMP generation in cancer cells, and consequently signaling through AMPK, might be a triggering factor in the initiation of malignant transformation, unleashing uncontrolled cell cycle turnover and proliferation (57). Nine different adenylate kinase isoenzymes (AK1-9) have been identified and characterized so far in human tissues, displaying different organ and subcellular distributions.

In the present study, the gene expression level of *AK1*, *AK2*, *AK4*, and *AK6* was analyzed. AK1 is expressed in the cytosol at high levels in brain, heart, skeletal muscles, and erythrocytes (58). Previous studies have shown that AK activity in CRC tumor tissue is higher than in normal mucosa (59). AK1 has been proposed to be a negative regulator of colorectal cancer development. Its expression level in the polyp group was like that found in healthy tissue and significantly higher compared to the CRC group (Figure 5A).

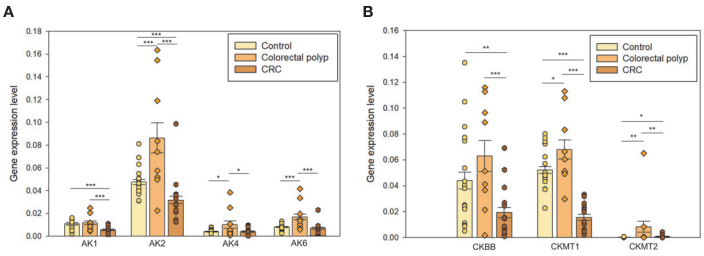

FIGURE 5
The expression levels of **(A)** adenylate kinases *AK1, AK2, AK4,* and *AK6;* and creatine kinases **(B)** *CKBB, CKMT1, and CKMT2* in the healthy colon (n=19), colorectal polyps (n=9), and colorectal cancer tissue (n=15) * p < 0,05, ** p < 0,01, *** p < 0,001 (t-test).

AK2 isoform is localized in the mitochondrial intermembrane space and regulates the ATP/ADP transference rate between the cytosol and mitochondrial matrix (56). Changes in the regulation of AK2 have been observed in several human cancers. *AK2* overexpression has been observed in lung adenocarcinoma, triple-negative breast cancer cells, and neuroblastoma cell lines and it could be related to the aggressive nature of these cancer types (60–62). The expression of AK2 was upregulated in the polyp group compared to CRC and healthy tissue groups (Figure 5A). The CRC group showed a significantly lower *AK2* expression than the control group suggesting that fundamental rearrangements in the energy-related communication networks between cytosol and mitochondria take place during progression to cancer. Although the potential role of AK2 in tumorigenesis has been reported for a long time already, its underlying mechanism is still unclear.

AK4 is expressed in the mitochondrial matrix and may indirectly modulate the mitochondrial membrane permeability *via* its interactions with the ADP/ATP translocase (ANT) (58). Previous studies have demonstrated the involvement of AK4 in the progression of different cancer types, as well as in the resistance to radiation therapy and multiple chemotherapeutic agents (63–65). Indeed, the expression level of *AK4* was significantly higher in the polyp group compared to the healthy tissue group and CRC. AK4 has been demonstrated to promote a glycolytic shift (66), which is aligned with our observation of the glycolytic phenotype in the polyp group.

AK6, renamed as human coilin interacting nuclear ATPase protein (HCINAP), is localized in nucleus and cytosol, and is ubiquitously expressed in different tissues and cell types (67, 68). *AK6* expression was higher in polyps than in control tissue and CRC groups (Figure 5A). AK6 is a glycolysis regulator *via* phosphorylation of LDHA and a modulator of invasion and metastatic activity of cancer stem cells (69). However, *AK6* became upregulated already at the polyp stage and it may support the glycolytic activity in benign tumors. Higher *LDHA* expression level in polyps than CRC (Figure 4A) correlates with a similar pattern in *AK6* expression. It can be hypothesized that AK6 is required to support cell division and that in polyps with active anaerobic glycolysis AK6 could be preferentially located in the cytosol.

Moreover, AKs may regulate intracellular AMP levels and thus directly affect AMPK metabolic signaling. The elevated levels of AK expression in polyps could support AMPK activation and OXPHOS in conditions of intense competition for cytosolic ADP (glycolysis has a high affinity for ADP). In this regard, a model has been developed where the bioenergetics signatures combine the metabolic networks of AMPK and HIF-1 alpha activity (70). The activity of these two regulators defines the metabolic states as follows: a glycolytic state is established by high HIF-1 and low AMPK, an oxidative state is characterized by low HIF-1 and high AMPK, and in a hybrid state both regulators are active (70). As both proteins are important determinants of cell metabolism and fate, understanding the interplay between different AKs in their various locations and their regulation might uncover new targets for cancer treatments or biomarkers for cancer occurrence and prognosis.

Creatine kinase (CK) has a crucial role in cell bioenergetics to efficiently regenerate ATP from phosphocreatine and is over-expressed in cells with high energy requirements such as skeletal, cardiac and smooth muscle, kidney, and brain (71). There are two genes for cytosolic CK subunit isoforms forming three types of dimers (CKMM, CKBB, and CKMB) and two mitochondrial creatine kinase (mtCK) isoenzymes (the *ubiquitous* form – gene *CKMT1* and the *sarcomeric* form – gene *CKMT2* (72). The interplay between cytosolic and mitochondrial CK isoenzymes depends on a large intracellular pool of creatine/phosphocreatine and prevents a rapid fall in global ATP concentrations (72). This ATP buffering system is known as the phosphocreatine (PCr)-creatine kinase (CK) shuttle, or PCr-CK circuit (53).

Mitochondrial CKs catalyze the interconversion of ATP into PCr at the main ATP-producing sites to store the energy in the form of PCr and facilitate its intracellular diffusion across the different subcellular organelles, whereas cytosolic CKs regenerate *in situ* ATP from the PCr pool at ATP-consuming sites (73, 74). CKs are expressed in colon epithelial cells and are coordinately regulated by HIFs. Such regulation is critical for their barrier function (75). Attenuated expression of CK enzymes in inflammatory bowel disease tissue (75), downregulation of CK-BB functional activity and low expression of *MTCK1* in colon cancer (which is a different feature from other cancer types) (59, 76) suggest that intestinal creatine metabolism and PCr/CK circuit may be compromised in colon polyps as well.

Here, the expression level of *CKBB*, *CKMT1*, and *CKMT2* was assessed. Downregulation of *CKBB* in CRC was observed (Figure 5B). CK isoforms may be up- and down-regulated in tumors depending on the nature of the carcinogenesis (77). Mitochondrial CK transcribed

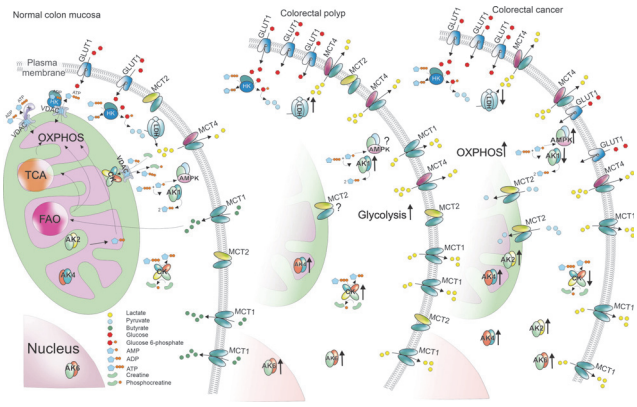

FIGURE 6
Changes of gene expression levels in colorectal polyps and tumors compared to normal colon mucosa. ATP production in normal colon mucosa is presented in the left. Glucose enters the cell through glucose transporters (GLUT1) and is then phosphorylated by hexokinase (HK). The glycolytic product pyruvate enters mitochondria through voltage dependent anion channel (VDAC) in mitochondrial outer membrane and is used to produce ATP through TCA cyle and oxidative phosphorylation (OXPHOS). Some of the pyruvate is converted into lactate by lactate dehydrogenase A (LDHA) and lactate is transported out of the cell using monocarboxylate transporter (MCT) 4. Additionally, colon cells uptake short fatty acids like butyrate using MCT1 and use them in fatty acid oxidation (FAO). To transport ATP and ADP between energy production and energy consumption sites, cells use creatine kinases (CKs) and adenylate kinases (AKs). In colorectal polyps, the energy metabolism is reprogrammed by increasing the glycolytic activity. This phenomenon could be facilitated by higher *GLUT1, HK* and *LDHA* expression boosting lactate production. The excessive amounts of lactate are transported out of the cell using MCT1 and MCT4 that were upregulated in polyps. Polyps also displayed higher levels of both CKs and AKs. However, colorectal cancer cells (right) seemed to depend more on OXPHOS suggested by the decreased expression levels of *HK*s and *LDHA*. Poor *MCT* levels indicate that the need for lactate transport is lower. Opposite to polyps, energy transport pathways in cancer cells showed signs of downregulation.

from *CKMT1*, also known as U-MtCK is localized in the inner membrane of mitochondria. CKMT1 may participate in the development of human cancers because of its involvement in several cellular processes such as cell proliferation, migration, and apoptosis (78, 79). Expression of *CKMT1* was significantly lower in the CRC group compared to healthy tissue and polyps (Figure 5B). In this regard, it has been suggested that *MtCK* expression is regulated by the metabolic energy cell status and their expression may represent a mechanism to compensate for a low energy state (72). Thus, high expression of *CKBB* and *CKMT1* in control and polyp, and overexpression of *CKMT2* in polyps is consistent with the observation that polyps are highly glycolytic compared to healthy and cancerous tissue. Whether the changes in intracellular energy transfer are the cause or consequence of CRC and hence how AK and CK energy shuttles may be affected to prevent polyps from becoming malignant remains to be investigated.

4 Conclusions

Although our knowledge on cancer metabolism has increased, the whole process of metabolic reprogramming during tumorigenesis is still rather unexplored. Here we showed that changes in energy production already occur in benign colorectal tumors and the alterations continue throughout the development of colorectal cancer. Colon polyps seem to increase glycolytic activity by overexpressing glucose transporter 1 and hexokinases. The low K_m (ADP) value determined in polyps by high-resolution respirometry as well as their LDHA overexpression added support to the proposal of a glycolytic phenotype for polyps. The higher glycolytic activity may drive cell proliferation in the diseased state (80). On the other hand, while cancer cells seem to upregulate the glycolytic pathway, they still depend highly on mitochondrial respiration. Besides glycolysis, colon polyps upregulate the activity of energy transfer pathways like adenylate kinase and creatine kinase systems. The observations of metabolic reprogramming described in the results are presented in Figure 6. The significant changes in gene expression levels could be used as biomarkers to detect benign tumors in early stages.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Research Ethics Committee of the National Institute for Health Development. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ER-K, LR and MP wrote the main manuscript text. ER-K and LR performed the experiments and analyzed the data. LR and IS prepared

the figures. JB, KS and VV provided the samples. IS prepared the graphical abstract. IS, RM-S and TK reviewed and edited the text. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by institutional research funding.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1171887/full#supplementary-material

References

1. Rex DK, Hassan C, Bourke MJ. The colonoscopist's guide to the vocabulary of colorectal neoplasia: histology, morphology, and management. *Gastrointest Endosc* (2017) 86(2):253–63. doi: 10.1016/j.gie.2017.03.1546

2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* (1990) 61(5):759–67. doi: 10.1016/0092-8674(90)90186-I

3. Risio M. The natural history of colorectal adenomas and early cancer. *Pathologe* (2012) 33 Suppl 2:206–10. doi: 10.1007/s00292-012-1640-6

4. Rashid A, Houlihan PS, Booker S, Petersen GM, Giardiello FM, Hamilton SR. Phenotypic and molecular characteristics of hyperplastic polyposis. *Gastroenterology* (2000) 119(2):323–32. doi: 10.1053/gast.2000.9361

5. Miller Q, Saeed O, Mesa H. Clinical, pathologic, and molecular-genetic aspects of colorectal polyps. *Gastrointest Endosc Clin N Am* (2022) 32(2):313–28. doi: 10.1016/j.giec.2021.12.007

6. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* (2000) 100(1):57–70. doi: 10.1016/S0092-8674(00)81683-9

7. Warburg O. On the origin of cancer cells. *Science* (1956) 123(3191):309–14. doi: 10.1126/science.123.3191.309

8. Moreno-Sanchez R, Marin-Hernandez A, Saavedra E, Pardo JP, Ralph SJ, Rodriguez-Enriquez S. Who controls the ATP supply in cancer cells? biochemistry lessons to understand cancer energy metabolism. *Int J Biochem Cell Biol* (2014) 50:10–23. doi: 10.1016/j.biocel.2014.01.025

9. Schiliro C, Firestein BL. Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells* (2021) 10(5). doi: 10.3390/cells10051056

10. Zhang S, Nemutlu E, Terzic A, Dzeja P. Adenylate kinase isoform network: a major hub in cell energetics and metabolic signaling. In: Aon MA, Saks V, Schlattner U, editors. *Systems biology of metabolic and signaling networks*. Heidelberg, Berlin: Springer (2014). p. 145–62.

11. Pedersen PL. Warburg, me and hexokinase 2: multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg effect", i.e., elevated glycolysis in the presence of oxygen. *J Bioenerg Biomembr* (2007) 39(3):211–22. doi: 10.1007/s10863-007-9094-x

12. Fujisawa K, Terai S, Takami T, Yamamoto N, Yamasaki T, Matsumoto T, et al. Modulation of anti-cancer drug sensitivity through the regulation of mitochondrial activity by adenylate kinase 4. *J Exp Clin Cancer Res* (2016) 35(1):48. doi: 10.1186/s13046-016-0322-2

13. Maldonado EN, Lemasters JJ. Warburg revisited: regulation of mitochondrial metabolism by voltage-dependent anion channels in cancer cells. *J Pharmacol Exp Ther* (2012) 342(3):637–41. doi: 10.1124/jpet.112.192153

14. Puurand M, Tepp K, Timohhina N, Aid J, Shevchuk I, Chekulayev V, et al. Tubulin betaII and betaIII isoforms as the regulators of VDAC channel permeability in health and disease. *Cells* (2019) 8(3). doi: 10.3390/cells8030239

15. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. In: Palmeira CM, Moreno AJ, editors. *Mitochondrial bioenergetics: methods and protocols*. Totowa, NJ: Humana Press (2012). p. 25–58.

16. Untergasser A. *RNAprep - trizol combined with columns* (2008). Available at: http://www.untergasser.de/lab/protocols/rna_prep_comb_trizol_v1_0.htm.

17. Kuznetsov AV, Tiivel T, Sikk P, Kaambre T, Kay L, Daneshrad Z, et al. Striking differences between the kinetics of regulation of respiration by ADP in slow-twitch and fast-twitch muscles in vivo. *Eur J Biochem* (1996) 241(3):909–15. doi: 10.1111/j.1432-1033.1996.00909.x

18. Puurand M, Tepp K, Klepinin A, Klepinina L, Shevchuk I, Kaambre T. Intracellular energy-transfer networks and high-resolution respirometry: a convenient approach for studying their function. *Int J Mol Sci* (2018) 19(10). doi: 10.3390/ijms19102933

19. Chekulayev V, Mado K, Shevchuk I, Koit A, Kaldma A, Klepinin A, et al. Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes. *Biochem Biophysics Rep* (2015) 4:111–25. doi: 10.1016/j.bbrep.2015.08.020

20. Koit A, Shevchuk I, Ounpuu L, Klepinin A, Chekulayev V, Timohhina N, et al. Mitochondrial respiration in human colorectal and breast cancer clinical material is regulated differently. *Oxid Med Cell Longev 2017* (2017) p:1372640. doi: 10.1155/2017/1372640

21. Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, et al. Mitochondrial respiration in KRAS and BRAF mutated colorectal tumors and polyps. *Cancers (Basel)* (2020) 12(4). doi: 10.3390/cancers12040815

22. Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, Tranqui L, et al. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. *Mol Cell Biochem* (1998) 184:81–100. doi: 10.1023/A:1006834912257

23. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013

24. Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, et al. Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science* (2009) 325(5947):1555–9. doi: 10.1126/science.1174229

25. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* (2013) 496(7443):101–5. doi: 10.1038/nature12040

26. Nagarajah J, Ho AL, Tuttle RM, Weber WA, Grewal RK. Correlation of BRAFV600E mutation and glucose metabolism in thyroid cancer patients: an ^{18}F-FDG PET study. *J Nucl Med* (2015) 56(5):662–7. doi: 10.2967/jnumed.114.150607

27. Faubert B, Solmonson A, DeBerardinis RJ. Metabolic reprogramming and cancer progression. *Science* (2020) 368(6487):eaaw5473. doi: 10.1126/science.aaw5473

28. Missiaglia E, Jacobs B, D'Ario G, Di Narzo AF, Soneson C, Budinska E, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol* (2014) 25(10):1995–2001. doi: 10.1093/annonc/mdu275

29. Huyghe JR, Harrison TA, Bien SA, Hampel H, Figueiredo JC, Schmit SL, et al. Genetic architectures of proximal and distal colorectal cancer are partly distinct. *Gut* (2021) 70(7):1325–34. doi: 10.1136/gutjnl-2020-321534

30. Leclerc D, Pham DN, Lévesque N, Truongcao M, Foulkes WD, Sapienza C, et al. Oncogenic role of PDK4 in human colon cancer cells. *Br J Cancer* (2017) 116(7):930–6. doi: 10.1038/bjc.2017.38

31. Azuma M, Shi M, Danenberg KD, Gardner H, Barrett C, Jacques CJ, et al. Serum lactate dehydrogenase levels and glycolysis significantly correlate with tumor VEGFA and VEGFR expression in metastatic CRC patients. *Pharmacogenomics* (2007) 8(12):1705–13. doi: 10.2217/14622416.8.12.1705

32. Graziano F, Ruzzo A, Giacomini E, Ricciardi T, Aprile G, Loupakis F, et al. Glycolysis gene expression analysis and selective metabolic advantage in the clinical

progression of colorectal cancer. *Pharmacogenomics J* (2017) 17(3):258–64. doi: 10.1038/tpj.2016.13

33. Wang NN, Zhang PZ, Zhang J, Wang HN, Li L, Ren F, et al. Penfluridol triggers mitochondrial-mediated apoptosis and suppresses glycolysis in colorectal cancer cells through down-regulating hexokinase-2. *Anat Rec (Hoboken)* (2021) 304(3):520–30. doi: 10.1002/ar.24464

34. Barron CC, Bilan PJ, Tsakiridis T, Tsiani E. Facilitative glucose transporters: implications for cancer detection, prognosis and treatment. *Metabolism* (2016) 65 (2):124–39. doi: 10.1016/j.metabol.2015.10.007

35. Ancey PB, Contat C, Meylan E. Glucose transporters in cancer - from tumor cells to the tumor microenvironment. *FEBS J* (2018) 285(16):2926–43. doi: 10.1111/febs.14577

36. Robey RB, Hay N. Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and akt. *Oncogene* (2006) 25:4683–96. doi: 10.1038/sj.onc.1209595

37. Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, et al. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell* (2013) 24(2):213–28. doi: 10.1016/j.ccr.2013.06.014

38. Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* (2006) 25(34):4777–86. doi: 10.1038/sj.onc.1209603

39. Klepinin A, Ounpuu L, Mado K, Truu L, Chekulayev V, Puurand M, et al. The complexity of mitochondrial outer membrane permeability and VDAC regulation by associated proteins. *J Bioenerg Biomembr* (2018) 50(5):339–54. doi: 10.1007/s10863-018-9765-9

40. Ho N, Coomber BL. Hexokinase II expression is correlated with colorectal cancer prognosis. *Cancer Treat Commun* (2016) 6:11–6. doi: 10.1016/j.ctrc.2016.02.008

41. Cai H, Li J, Zhang Y, Liao Y, Zhu Y, Wang C, et al. LDHA promotes oral squamous cell carcinoma progression through facilitating glycolysis and epithelial–mesenchymal transition. *Front Oncol* (2019) 9. doi: 10.3389/fonc.2019.01446

42. Sun X, Sun Z, Zhu Z, Guan H, Zhang J, Zhang Y, et al. Clinicopathological significance and prognostic value of lactate dehydrogenase a expression in gastric cancer patients. *PloS One* (2014) 9(3):e91068. doi: 10.1371/journal.pone.0091068

43. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology* (2007) 121(1):1–14. doi: 10.1016/B978-012372551-6/50066-3

44. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324(5930):1029–33. doi: 10.1126/science.1160809

45. Lambert DW, Wood IS, Ellis A, Shirazi-Beechey SP. Molecular changes in the expression of human colonic nutrient transporters during the transition from normality to malignancy. *Br J Cancer* (2002) 86(8):1262–9. doi: 10.1038/sj.bjc.6600264

46. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* (1987) 28(10):1221–7. doi: 10.1136/gut.28.10.1221

47. Sonveaux P, Végran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest* (2008) 118(12):3930–42. doi: 10.1172/JCI36843

48. Pinheiro C, Reis RM, Ricardo S, Longatto-Filho A, Schmitt F, Baltazar F. Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44. *J Biomedicine Biotechnol* (2010) 2010:427694. doi: 10.1155/2010/427694

49. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1 α-dependent mechanism *. *J Biol Chem* (2006) 281(14):9030–7. doi: 10.1074/jbc.M511397200

50. Giles RH, Lolkema MP, Snijckers CM, Belderbos M, van der Groep P, Mans DA, et al. Interplay between VHL/HIF1alpha and wnt/beta-catenin pathways during colorectal tumorigenesis. *Oncogene* (2006) 25(21):3065–70. doi: 10.1038/sj.onc.1209330

51. Colgan SP, Taylor CT. Hypoxia: an alarm signal during intestinal inflammation. *Nat Rev Gastroenterol Hepatol* (2010) 7(5):281–7. doi: 10.1038/nrgastro.2010.39

52. Furuta GT, Turner JR, Taylor CT, Hershberg RM, Comerford K, Narravula S, et al. Hypoxia-inducible factor 1-dependent induction of intestinal trefoil factor protects barrier function during hypoxia. *J Exp Med* (2001) 193(9):1027–34. doi: 10.1084/jem.193.9.1027

53. Wallimann T, Tokarska-Schlattner M, Schlattner U. The creatine kinase system and pleiotropic effects of creatine. *Amino Acids* (2011) 40(5):1271–96. doi: 10.1007/s00726-011-0877-3

54. Dzeja PP, Terzic A. Phosphotransfer networks and cellular energetics. *J Exp Biol* (2003) 206(12):2039–47. doi: 10.1242/jeb.00426

55. Dzeja PP, Zeleznikar RJ, Goldberg ND. Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes. *Mol Cell Biochem* (1998) 184(1):169–82. doi: 10.1023/A:1006859632730

56. Dzeja P, Terzic A. Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing. *Int J Mol Sci* (2009) 10 (4):1729–72. doi: 10.3390/ijms10041729

57. Klepinin A, Zhang S, Klepinina L, Rebane-Klemm E, Terzic A, Kaambre T, et al. Adenylate kinase and metabolic signaling in cancer cells. *Front Oncol* (2020) 10. doi: 10.3389/fonc.2020.00660

58. Panayiotou C, Solaroli N, Karlsson A. The many isoforms of human adenylate kinases. *Int J Biochem Cell Biol* (2014) 49:75–83. doi: 10.1016/j.biocel.2014.01.014

59. Kaldma A, Klepinin A, Chekulayev V, Mado K, Shevchuk I, Timohhina N, et al. An in situ study of bioenergetic properties of human colorectal cancer: the regulation of mitochondrial respiration and distribution of flux control among the components of ATP synthasome. *Int J Biochem Cell Biol* (2014) 55:171–86. doi: 10.1016/j.biocel.2014.09.004

60. Liu H, Pu Y, Amina Q, Wang Q, Zhang M, Song J, et al. Prognostic and therapeutic potential of adenylate kinase 2 in lung adenocarcinoma. *Sci Rep* (2019) 9 (1):17757. doi: 10.1038/s41598-019-53594-4

61. Klepinin A, Ounpuu L, Guzun R, Chekulayev V, Timohhina N, Tepp K, et al. Simple oxygraphic analysis for the presence of adenylate kinase 1 and 2 in normal and tumor cells. *J Bioenerg Biomembr* (2016) 48(5):531–48. doi: 10.1007/s10863-016-9687-3

62. Klepinin A, Miller S, Reile I, Puurand M, Rebane-Klemm E, Klepinina L, et al. Stable isotope tracing uncovers reduced gamma/beta-ATP turnover and metabolic flux through mitochondrial-linked phosphotransfer circuits in aggressive breast cancer cells. *Front Oncol* (2022) 12:892195. doi: 10.3389/fonc.2022.892195

63. Jan YH, Tsai HY, Yang CJ, Huang MS, Yang YF, Lai TC, et al. Adenylate kinase-4 is a marker of poor clinical outcomes that promotes metastasis of lung cancer by downregulating the transcription factor ATF3. *Cancer Res* (2012) 72(19):5119–29. doi: 10.1158/0008-5472.CAN-12-1842

64. Xin F, Yao DW, Fan L, Liu JH, Liu XD. Adenylate kinase 4 promotes bladder cancer cell proliferation and invasion. *Clin Exp Med* (2019) 19(4):525–34. doi: 10.1007/s10238-019-00576-5

65. Lei W, Yan C, Ya J, Yong D, Yujun B, Kai L. MiR-199a-3p affects the multi-chemoresistance of osteosarcoma through targeting AK4. *BMC Cancer* (2018) 18 (1):631. doi: 10.1186/s12885-018-4460-0

66. Jan Y-H, Lai T-C, Yang C-J, Lin Y-F, Huang M-S, Hsiao M. Adenylate kinase 4 modulates oxidative stress and stabilizes HIF-1α to drive lung adenocarcinoma metastasis. *J Hematol Oncol* (2019) 12(1):12. doi: 10.1186/s13045-019-0698-5

67. Ren H, Wang L, Bennett M, Liang Y, Zheng X, Lu F, et al. The crystal structure of human adenylate kinase 6: an adenylate kinase localized to the cell nucleus. *Proc Natl Acad Sci USA* (2005) 102(2):303–8. doi: 10.1073/pnas.0407459102

68. Xu R, Yang Y, Zheng X. Unique structural features of the adenylate kinase hCINAP/AK6 and its multifaceted functions in carcinogenesis and tumor progression. *FEBS Lett* (2021) 595(16):2071–84. doi: 10.1002/1873-3468.14158

69. Ji Y, Yang C, Tang Z, Yang Y, Tian Y, Yao H, et al. Adenylate kinase hCINAP determines self-renewal of colorectal cancer stem cells by facilitating LDHA phosphorylation. *Nat Commun* (2017) 8:15308. doi: 10.1038/ncomms15308

70. Paudel BB, Quaranta V. Metabolic plasticity meets gene regulation. *Proc Natl Acad Sci USA* (2019) 116(9):3370–2. doi: 10.1073/pnas.1900169116

71. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem J* (1992) 281(Pt 1):21–40. doi: 10.1042/bj2810021

72. Schlattner U, Tokarska-Schlattner M, Wallimann T. Mitochondrial creatine kinase in human health and disease. *Biochim Biophys Acta (BBA) - Mol Basis Dis* (2006) 1762(2):164–80. doi: 10.1016/j.bbadis.2005.09.004

73. Saks V, Kaambre T, Guzun R, Anmann T, Sikk P, Schlattner U, et al. The creatine kinase phosphotransfer network: thermodynamic and kinetic considerations, the impact of the mitochondrial outer membrane and modelling approaches. *Subcell Biochem* (2007) 46:27–65. doi: 10.1007/978-1-4020-6486-9_3

74. Sahlin K, Harris RC. The creatine kinase reaction: a simple reaction with functional complexity. *Amino Acids* (2011) 40(5):1363–7. doi: 10.1007/s00726-011-0856-8

75. Glover LE, Bowers BE, Saeedi B, Ehrentraut SF, Campbell EL, Bayless AJ, et al. Control of creatine metabolism by HIF is an endogenous mechanism of barrier regulation in colitis. *Proc Natl Acad Sci USA* (2013) 110(49):19820–5. doi: 10.1073/pnas.1302840110

76. Balasubramani M, Day BW, Schoen RE, Getzenberg RH. Altered expression and localization of creatine kinase b, heterogeneous nuclear ribonucleoprotein f, and high mobility group box 1 protein in the nuclear matrix associated with colon cancer. *Cancer Res* (2006) 66(2):763–9. doi: 10.1158/0008-5472.CAN-05-3771

77. Yan Y-B. Creatine kinase in cell cycle regulation and cancer. *Amino Acids* (2016) 48(8):1775–84. doi: 10.1007/s00726-016-2217-0

78. Li M, Liu H, Li J, Guo S, Lv Y. Mitochondrial creatine kinase 1 in non-small cell lung cancer progression and hypoxia adaptation. *Respir Res* (2021) 22(1):1–8. doi: 10.1186/s12931-021-01765-1

79. Amamoto R, Uchiumi T, Yagi M, Monji K, Song Y, Oda Y, et al. The expression of ubiquitous mitochondrial creatine kinase is downregulated as prostate cancer progression. *J Cancer* (2016) 7(1):50–9. doi: 10.7150/jca.13207

80. Hsu PP, Sabatini DM. Cancer cell metabolism: warburg and beyond. *Cell* (2008) 134(5):703–7. doi: 10.1016/j.cell.2008.08.021

Appendix 3

Publication III

Klepinin A, Zhang S, Klepinina L, Rebane-Klemm E, Terzic A, Kaambre T, Dzeja P. Adenylate Kinase and Metabolic Signaling in Cancer Cells. Front Oncol. 2020 May 19;10:660. doi: 10.3389/fonc.2020.00660. eCollection 2020

frontiers in Oncology

MINI REVIEW
published: 19 May 2020
doi: 10.3389/fonc.2020.00660

Adenylate Kinase and Metabolic Signaling in Cancer Cells

Aleksandr Klepinin[1], Song Zhang[2], Ljudmila Klepinina[1], Egle Rebane-Klemm[1], Andre Terzic[2], Tuuli Kaambre[1] and Petras Dzeja[2]**

[1] *Laboratory of Chemical Biology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia,* [2] *Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, United States*

A hallmark of cancer cells is the ability to rewire their bioenergetics and metabolic signaling circuits to fuel their uncontrolled proliferation and metastasis. Adenylate kinase (AK) is the critical enzyme in the metabolic monitoring of cellular adenine nucleotide homeostasis. It also directs AK→ AMP→ AMPK signaling controlling cell cycle and proliferation, and ATP energy transfer from mitochondria to distribute energy among cellular processes. The significance of AK isoform network in the regulation of a variety of cellular processes, which include cell differentiation and motility, is rapidly growing. Adenylate kinase 2 (AK2) isoform, localized in intermembrane and intra-cristae space, is vital for mitochondria nucleotide exchange and ATP export. AK2 deficiency disrupts cell energetics, causes severe human diseases, and is embryonically lethal in mice, signifying the importance of catalyzed phosphotransfer in cellular energetics. Suppression of AK phosphotransfer and AMP generation in cancer cells and consequently signaling through AMPK could be an important factor in the initiation of cancerous transformation, unleashing uncontrolled cell cycle and growth. Evidence also builds up that shift in AK isoforms is used later by cancer cells for rewiring energy metabolism to support their high proliferation activity and tumor progression. As cell motility is an energy-consuming process, positioning of AK isoforms to increased energy consumption sites could be an essential factor to incline cancer cells to metastases. In this review, we summarize recent advances in studies of the significance of AK isoforms involved in cancer cell metabolism, metabolic signaling, metastatic potential, and a therapeutic target.

Keywords: adenylate kinase, energy metabolism, phosphotransfer, mitochondria, cancer

OPEN ACCESS

Edited by:
Stefano Falone,
University of L'Aquila, Italy

Reviewed by:
Daria Capece,
Imperial College London, United Kingdom
Paul Dent,
Virginia Commonwealth University, United States
Elisabetta Benedetti,
University of L'Aquila, Italy

****Correspondence:***
Aleksandr Klepinin
aleksandr.klepinin@kbfi.ee
Petras Dzeja
dzeja.petras@mayo.edu

Specialty section:
This article was submitted to Cancer Metabolism, a section of the journal Frontiers in Oncology

Received: *15 February 2020*
Accepted: *08 April 2020*
Published: *19 May 2020*

Citation:
Klepinin A, Zhang S, Klepinina L, Rebane-Klemm E, Terzic A, Kaambre T and Dzeja P (2020) Adenylate Kinase and Metabolic Signaling in Cancer Cells. Front. Oncol. 10:660. doi: 10.3389/fonc.2020.00660

INTRODUCTION

The significance of metabolism and metabolic signaling in human diseases is rapidly growing. New features and molecular players that are vital for cell homeostasis and function are being uncovered. Well-organized high-energy phosphoryl transfer systems are required to mediate intracellular communication between ATP-consuming and ATP-producing cellular compartments and thus to maintain normal growth and development of the cell (1–5). The main components of the cellular phosphotransfer system are AK, creatine kinase (CK), and glycolytic networks (1, 2). The significance of organized phosphotransfer was demonstrated by genetic manipulations in animal models, cellular systems, and alterations or mutations in separate phosphotransfer enzymes, which are associated with human diseases (6–16). Studies on *Drosophila* and mice model demonstrate that deletion of adenylate kinase 2 (AK2) is embryonically lethal, signifying

the importance of AK phosphotransfer network in cell homeostasis (13, 17–19). In humans, mutations in the mitochondrial AK2 gene are associated with reticular dysgenesis characterized by immunodeficiency and sensorineural deafness, where processes of nucleotide signaling, cell differentiation, and motility are affected (15, 16, 20). So far, nine isoforms of adenylate kinase (AK1–AK9) and several subforms have been found and well characterized in mammalian cells (21). AK, which catalyzes reaction 2ADP↔AMP+ATP, is a recognized facilitator of AMP metabolic signaling, optimizing intracellular energetic communication, and local ATP supply (5, 22). Historically, the function of AK has been ascribed to *de novo* adenine nucleotide synthesis and cell energy economy through regulation of nucleotide ratios in different intracellular compartments and AMP-sensitive metabolic enzymes (14, 23, 24). The unique properties of AK lie on its ability to deliver γ- and β-phosphoryl groups of ATP, thereby doubling the ATP energetic potential. Moreover, the AK network provides an efficient mechanism for high-energy phosphoryl transport from mitochondria to ATP utilization sites (2). Evolutionary AK isoforms have been positioned to different subcellular compartments (21, 25). AK1, AK7, and AK8 are solely found in the cytosol; AK2, AK3, and AK4 are located in the mitochondria; and AK5 and AK9 can be found in either the cytosol or nucleus. Only AK1 and AK6 are known to be expressed in all tissues, whereas AK5 is expressed only in the brain (21). In the cytosol, the main isoform is AK1, which is predominantly expressed in high energy demand tissues such as the brain, heart, and skeletal muscles. AK2 is strategically located in the mitochondrial intermembrane and cristae space to facilitate high-energy phosphoryl exchange between mitochondria and cytosol (22). Two other AK isoforms, AK3 and AK4, are located in the mitochondrial matrix and are involved in the regulation of mitochondrial Krebs cycle and oxidative phosphorylation (OXPHOS), whereas AK5 and AK6 isoforms that are localized in the nucleus could serve to fulfill the energy needs of nuclear processes. In general, distinct intracellular localization and kinetic properties of AK isoforms favor energy support of specific cellular processes ranging from muscle contraction, electrical activity, cell motility, unfolded protein response, and mitochondrial/nuclear energetics (22). Importantly, reprogramming of energy metabolism has been proposed as one of the hallmarks of cancer (26), which is required to drive biosynthesis pathways necessary for rapid cell replication and proliferation. Cancer cells are believed to have a greater reliance on glycolytic phosphotransfer (27, 28). However, during the last decade, it was found that some tumors contain numerous mitochondria producing ATP predominantly *via* OXPHOS (29–31). The observed shift in hexokinase (HK) isoforms, upregulation of HK2 in cancer cells (32), indicates a closer integration of mitochondria with glycolytic phosphotransfer (see **Figure 1**). The association of HK2 with mitochondria and expression of pyruvate kinase PKM2 could promote effective yet uncontrolled energy distribution in cancer cells (27, 33, 34). Phosphotransfer enzymes such as CK and AK have been implicated in cancer cell proliferation (35, 36). However, it is not clear whether the redistribution of phosphotransfer enzymes, especially those which are localized in mitochondria, occurs during cancer formation. In this review, we focus on the significance of AK isoforms in the rewiring of cancer cell energy metabolism and AMP signaling. Specifically, we will overview how AK isoforms, localized in mitochondria (AK2 and AK4), and their main communication partners cytosolic AK (AK1 and AK6) are involved in cancer formation and metastasis.

Abbreviations: AK, adenylate kinase; CK, creatine kinase; CKmit, mitochondrial creatine kinase; CKB, brain-type creatine kinase; MOM, mitochondrial outer membrane; ANT, adenine nucleotide translocase; AMPK, AMP-activated protein kinase; CSCs, cancer stem cells; NB, neuroblastoma; VDAC, voltage-dependent anion channel; ABC, ATP-binding cassette; FADD, Fas-associated protein with death domain; HIF, hypoxia-inducible factor; hCINAP, human coilin-interacting nuclear ATPase protein; DUSP26, dual-specificity phosphatase 26; OXPHOS, oxidative phosphorylation; AMPD, AMP-deaminase; 5′-NT, 5′-nucleotidase; LDHA, lactate dehydrogenase A; HK, hexokinase.

ADENYLATE KINASE 2 AND MITOCHONDRIAL CREATINE KINASE INTERPLAY IN MALIGNANT TRANSFORMATION

AK2 and mitochondrial CK (CKmit) are major phosphotransfer enzymes located in the intermembrane/cristae space in mitochondria (3, 14, 22). AK2 and CKmit provide nucleotide exchange and metabolic signaling capacity, allowing mitochondria to export ATP and reception of cytosolic feedback signals such as ADP, AMP, and creatine (22, 23, 37). Phosphotransfer enzymes CK and AK have been implicated in cancer cell proliferation (35, 36). In general, CK is involved in cancerous transformation, as CKB (brain-type CK) is upregulated in a variety of cancers to support growing energy needs (38). The elevation of creatine metabolites was noted in drug-resistant cancer cells (39). However, in other cancer types, the downregulation of CKB and rewiring of metabolism may play an important role in colon cancer progression (40). Moreover, several studies have demonstrated that in colorectal cancer (41), breast cancer (42), neuroblastoma (35), prostate cancer (43), and sarcoma (36, 44), the CKmit was downregulated. The reduction of CKmit in cancer cells was associated with the upregulation of adenylate kinase AK2 isoform in intermembrane space (36, 41, 42, 45, 46) (see **Table 1**). There is evidence that the expression of AK2 on the cell surface could facilitate nucleotide signaling and metastatic potential (60). It was found that Ak2 gene expression is upregulated in the metastatic pancreatic endocrine neoplasms (60), indicating the significance of nucleotide metabolic signaling in cancer invasion (61). Moreover, increased expression of the Ak2 on the surface of the metastatic F9DR murine terato-carcinoma cells compared with the nonmetastatic F9B9 cell line has been demonstrated (53). Furthermore, a recent study showed that AK2 has prognostic and therapeutic potential in lung adenocarcinoma (55). The knockdown of AK2 suppressed proliferation, migration, and invasion, as well as induced apoptosis and autophagy in human lung adenocarcinoma cells. In this regard, the AK2-FADD (Fas-associated protein with death domain) mediated apoptosis pathway was found to be defective in some tumor cells,

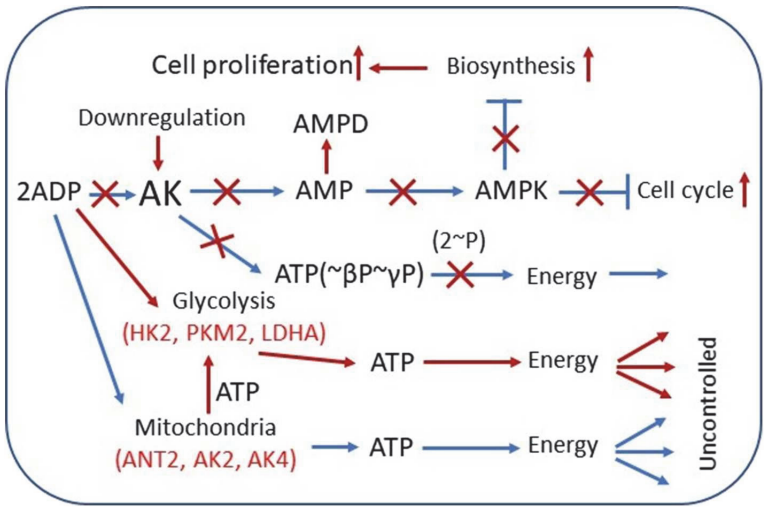

FIGURE 1 | Overview of adenylate kinase (AK) isoform involvement in the rewiring of cancer cell metabolic signaling and energetic circuits. Increased competition for cytosolic ADP downregulates AK-mediated AMP signaling, reducing control over cell cycle and proliferation. AK expression is downregulated in several tumors. AMP can be consumed by AMPD and by 5′-NT, also overexpressed in some cancer cells. Augmented glycolytic metabolism, owing to higher affinity, scavenges cytosolic ADP, and uses mitochondrial ATP to drive glucose conversion to lactate. Overexpression of glycolytic HK2, PKM2, and LDHA and mitochondrial ANT2, AK2, AK4, and other genes in cancer cells promotes rewiring of energetic circuits resulting in unrestrained energy distribution. The result of these metabolic transformations is deficient AMP signaling and AMPK-mediated control of cellular katabolic and anabolic processes. Red color indicates the augmented pathways and gene expression in cancer cells. AMPD, AMP-deaminase; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; ANT2, adenine nucleotide translocase 2; AMPK, AMP-activated protein kinase.

which may contribute to tumor development by preventing apoptosis (62). A recent study indicates that AK2 and FADD are crucial for caspase-10 activation upon metabolic stress, and this activation is independent of death receptors and extrinsic pathway of apoptosis (63). Moreover, the deletion of the Ak2 gene or exit AK2 from mitochondria during apoptosis disrupts nucleotide exchange between mitochondria and cytosol, causing hyperpolarization of mitochondria and reactive oxygen species (ROS) production (20). It was found that the presence of AK2 in mitochondrial cristae nanochannels is critical for ATP export (2, 22). There are evidence that the AK2 upregulation could be used by cancer cells to support energy supply to biosynthetic processes and cellular growth (18, 64). These results, as well as studies on CK and AK knockout mice, demonstrate remarkable plasticity of cellular energetics and phosphotransfer systems, which could be used in cancer cells to promote uncontrolled cell growth (9, 17, 22, 65).

ADENYLATE KINASE MODULATE TUMOR CELL RESPONSE TO SURVIVE UNDER OXIDATIVE STRESS

The ability to conduct metabolic signaling and rewire metabolism is critical for cell survival. The AK4 isoform increased expression has been associated with a poor clinical outcome marker for lung cancer (56) as well as for glioma patients (57) (see **Table 1**). It was found that AK4 expression is under tight control of noncoding RNA. The AK4 is negatively regulated by microRNA miR-556-3p and positively by circular RNA of ATP-binding cassette (ABC) subfamily B member 10, circ-ABCB10 (66). In same study was demonstrated that downregulation of AK4 restrained lung cancer progression and sensitized lung cancer cells to cisplatin (66). Moreover, new data indicate that AK4 was shown to be involved in the radioresistance of esophageal cancer cells (67) and in chemoresistance of other cancers (68, 69). Previously, it was suggested that overexpression of AK4 could protect cells against oxidative stress (70). Other studies on HeLa (68) and HEK293 cells (71) demonstrated that tumor cells respond to a hypoxic condition by upregulating the AK4. However, in HepG2 cells (71), it was found that under oxidative stress, AK4 oppositely was downregulated. Although AK4 might be downregulated, it can still regulate OXPHOS because it retains the nucleotide-binding capability, and it can interact with the mitochondrial adenine nucleotide translocase (ANT) (70). It was found that knockout of AK4 increased cellular ATP through raised OXPHOS activity as well as mitochondrial number (68). Fujisawa and colleagues in 2016 have proposed that there are two mechanisms how AK4 regulates mitochondrial respiration in cancer cells (68). First, in cancer cells, AK4 interacts with ANT, which forms with voltage-dependent anion channel (VDAC) and HK transmembrane complex AK4-ANT-VADC-HK (see **Figure 1**). Under the hypoxic conditions, the

TABLE 1 | Adenylate kinase isoforms in cancer.

Enzyme	Type of cancer	Status in tumor	Localization	Function/therapeutic target	Experimental model	References
AK	Lung cancer	↓	-	Negative regulator of cancer	Tissue samples	(47)
AK	Hepatomas	↓	-	Decreased during de-differentiation of cancer cells	Rat liver and hepatomas	(48)
AK	Colon cancer	↑	-	Metabolic regulator. Energy distribution shifts from CK toward AK	Tissue samples	(41, 49)
AK1	Transformed embryonic fibroblasts	↓	Cytosol	Negative regulator of tumor malignant	ras^{V12}/E1A-transformed primary mouse embryonic fibroblasts	(50)
AK2	Breast cancer	↑	Mitochondria intermembrane space	Prognostic and therapeutic target	Estrogen receptor-negative breast cancer tissue samples	(51)
AK2	Breast cancer	↑	Mitochondria intermembrane space	Oncotarget of the breast CSC	Breast CSC	(52)
AK2	Breast cancer and neuroblastoma	↑	Mitochondria intermembrane space	Oncotarget of the poorly differentiated cancer cells	Tissue samples and cancer cell lines	(46)
AK2	Embryonic carcinoma	↑	Mitochondria intermembrane space	Metabolic regulator. Energy distribution shifts from CK toward AK	Cell lines	(36)
AK2	Teratocarcinoma	↑	Plasma membrane	Overexpressed on the plasma membrane in metastatic cells	Cell lines	(53)
AK2	Breast cancer	↓	Nuclear	Negative regulator of tumor cell growth via DUSP26/FADD signaling	Breast cancer cell lines and tissue samples	(54)
AK2	Lung cancer	↑	Mitochondria intermembrane space	Associated with poor survival of patients. Prognostic and therapeutic potential	Tissue samples	(55)
AK4	Lung cancer	↑	Mitochondrial matrix	Associated with poor survival of patients. Prognostic and therapeutic potential	Tissue samples and various cell lines	(56)
AK4	Glioma	↑	Mitochondrial matrix	A key regulator of intracellular ATP level. Prognostic and therapeutic potential	Tissue samples and cancer cell lines	(57)
AK6	Breast cancer Colon cancer	↑	Nuclear	Promote cancer cell growth. Prognostic and therapeutic potential	Colon adenocarcinoma and breast cancer tissues	(58)
AK6	Colon cancer	↑	Cytosol	Glycolysis regulator via phosphorylation LDHA. Modulator of CSC invasion and metastasis activity	CSC from tissues	(59)

complex supports the high glycolytic activity of cancer cells. It allows efficient ADP recycling between mitochondrial ATP synthesis and glucose phosphorylation by HK, which interacts with the mitochondrial outer membrane (MOM) (68, 72). In addition, in hepatoma cells (73), it was found that up to 50% of ATP is provided by intramembrane space located AK2 through VDAC binding to HK. Thus, AK2 may also be a member of the metabolic circuit channeling ADP–ATP in and out of mitochondria. The second mechanism is related to the fact that AK4 and AK3 have highly homologous sequences; therefore, they compete with each other for their substrates (68). According to this mechanism, AK4 interferes with AK3 action in supplying of the GDP required for the conversion of succinyl-CoA to succinate. That is why overexpression of AK4 in HeLa cells induces a decrease in Krebs cycle metabolites such as succinate, fumarate, and malate while glutamine and glutamate are increased. In several tumors, it was shown that the predominate substrate for mitochondria is glutamine (74). However, further studies are needed to confirm the role of AK4 in mitochondria and Krebs cycle substrate metabolism.

ADENYLATE KINASE NETWORK ROLE IN CANCER STEM CELLS

Traditional therapies against cancer, such as chemotherapy and radiotherapy, have many limitations. The limitation is due to systematic and local toxicity as well as drug resistance of small populations of tumor cells that have self-renewal properties. This small population of cells is called cancer stem cells (CSCs) (75). Previously, studies on CSC have shown that the cancer resistance for chemotherapy is related to increased OXPHOS in CSC. That is why a new generation of cancer

chemotherapy could be targeted against pathways that interact with OXPHOS, such as the phosphotransfer system. Lamb and colleagues have shown on the breast cancer model that mitochondrial mass is a new biomarker of CSC, which have increased AK2 expression level (see **Table 1**) (52). In our previous study on neuroblastoma (NB) (46), which contains numerous CSC (76), and embryonal carcinoma cells (36), we also found that those cells have a high activity of AK2 (see **Table 1**). Moreover, another feature of CSC is that mitochondria are localized around the cell nucleus (77). There is evidence that AK2 can play an important role in communication between mitochondria and the nucleus (78). In another study using proteomic analysis of mouse teratocarcinoma cells (53), it was demonstrated that metastatic cancer cells have increased AK2 levels than have nonmetastatic cancer cells (see **Table 1**). As metastasis is related to cell motility, positioning of phosphotransfer enzymes to sites of increased energy consumption could be an important factor of tumor formation (59). The AK4 has been identified as a biomarker of metastasis in lung cancer (56, 79, 80). Overexpression of AK4 promoted lung cancer metastasis by enhancing hypoxia-inducible factor HIF-1 stability and epithelial-to-mesenchymal transition under hypoxia (79). Moreover, it was found that aferin-A could suppress AK4-HIF-1a signaling and may serve as a novel anti-metastatic agent in lung cancer (79). The AK4 was also implicated in breast and bladder cancers, where it promoted cell proliferation and invasion (81, 82). Furthermore, it was demonstrated that another AK isoform AK6 could affect colorectal cancer migration and invasion (59). Although significant progress has been made, at this time, the complete role of the AK system in cancer metastasis is still unclear. Moreover, the other reason why CSCs are drug resistant relates to the increased expression of ABC transporters in those cells (83, 84). The model for ABC transporters was proposed (85), which is based on ^{31}P solid-state NMR spectroscopy, suggesting that intrinsic ATPase is coupled with AK activity where AK participates in ATP exchange. It is known that cytosolic and membrane-associated AK can regulate the activity of another ABC protein—K-ATP channel (86, 87). Nevertheless, the exact role of AK in supporting adenylate charge and function of ABC transporters in CSC remains unknown yet. In this respect, the ABC transporters are not unique proteins that possess both ATPase and AK activities; there are other proteins like AK6 (58), also known as transcription factor TAF9, human coilin interacting nuclear ATPase protein (hCINAP), and highly conserved DNA repair complex Rad50 (88).

PARADOXES REGARDING THE ROLE OF ADENYLATE KINASE IN TUMOR FORMATION

Cancer is a very complex and diverse phenomenon, including tissue specificity and different phases. Enzymatic changes can be different in the initial and advanced stages of tumor growth (89, 90). There are some contradictory studies where it was found that in lung cancer and hepatoma, AK was downregulated compared with that in normal tissue (47, 48) (see **Table 1**), whereas a recent study has shown that high expression of AK2 correlates with a worse prognosis for lung cancer patients (55) (see **Table 1**). In mouse embryonic fibroblasts, it was demonstrated that during their transformation into tumor cells, a significant reduction of AK1 expression occurs (50). More recently, the existence of AK1 additional gene product AK1β has been reported, and it is known that the AK1β expression level is regulated by p53 (91). In some cancers, p53 is mutated or suppressed. In this context, experiments on mouse embryonic fibroblast (50) have shown that during their transformation into tumor cells, augmentation of AK1 might be related to the downregulation of AK1β (see **Table 1**). Also, Kim et al. have postulated that AK2 is a negative regulator of tumor growth (54) (see **Table 1**). They demonstrated that in some cells, the AK2 localized not only in mitochondria but also in the nucleus, where it interacted with dual-specificity phosphatase 26 (DUSP26). This protein complex can dephosphorylate FADD leading to suppressed cell growth. They also suggested that AK2 downregulation was associated with breast cancer formation. In contrast, Speers and colleagues have found that AK2 is overexpressed in ER-negative breast cancer (51) (see **Table 1**). They proposed that AK2 should be a novel target for the treatment of ER-negative breast cancer. Indeed, a diterpene lactone neoandrographolide from extracts of the traditional medicinal herb *Andrographis paniculata* has been suggested to inhibit AK2 and have strong anticancer properties (92). Nevertheless, studies on human breast cancer and colorectal cancer demonstrated another AK isoform AK6 was overexpressed during cancer formation (58) (see **Table 1**). These data correlate with our previous studies on colorectal and breast cancers (41, 46) (see **Table 1**). It was also shown, that in both colon and breast tissues, AK6 is located not only in nuclear but also in the cytosol. However, only in cytosolic compartmentalized AK6 did expression level increase during tumorogenesis of breast and colorectal cancer cells (58) (see **Table 1**). They have found that AK6's main function is to regulate ribosome assembly and, consequently, protein expression and cancer cell growth. Recently, it was demonstrated that hCINAP or AK6 is a potent modulator of metabolic reprogramming by phosphorylating LDHA, a key player in cancer glycolysis (59) (see **Table 1**). Thus, AK isoform role can be different depending on cancer cell type and development stage.

ADENYLATE KINASE-MEDIATED AMP METABOLIC SIGNALING IN CANCER CELLS

In recent years, AK-mediated AMP signaling is emerging as one of the most versatile systems in the regulation of diverse cellular processes (5, 22, 93). Particularly, AMP signaling to AMP-activated protein kinase (AMPK) plays a critical role in adjusting ATP-producing and ATP-consuming processes (90, 94) (**Figure 1**). In several cancers, it has been demonstrated

that AMPK, a master regulator of cellular energy homeostasis, possesses tumor suppressor function (95–97). In cells, AMPK activation/suppression is regulated via changes in cellular AMP levels. The principal activator of AMPK is the AK-catalyzed pathway, where it monitors cellular ATP–ADP balance and signals to AMPK by increased AMP cellular level. A recent study indicates that AK and AMPK cooperate to maintain cellular ATP levels (98). On the other end, AMP-deaminase (AMPD) and 5′-nucleotidase (5′-NT) suppress AMPK via decreasing AMP cellular levels (22, 99, 100). Moreover, the product of AMPD and 5'-NT reactions is adenosine, an immunosuppressive metabolite. At a high level in tumors, adenosine can promote cell growth, invasion, metastasis of cancer cells, and tumor immune evasion (101). Our previous work has demonstrated that in NB and heart adenocarcinoma cells HL-1, their mitochondrial permeability for AMP was increased than in healthy cells (46). It is known that AK2, which has unique localization in mitochondrial space, has a high affinity for AMP among AMP metabolizing enzymes. Therefore, it has been proposed that the AK2's primary function is to regulate intracellular AMP levels and to guard the cellular adenine nucleotide pool (22). Our study also suggested that cancer cells have a high level of AK2 (46) (**Figure 1**). Altogether, in cancer cells, most cellular AMP transport occurs via MOM where it is converted immediately to ADP and channeled into, maintaining a low cytosolic AMP concentration. Recent direct measurements of AK-mediated metabolic flux indicate that cancer cells have suppressed ATP β-phosphoryl energetics and AMP signaling, as indicated from ^{18}O-labeling experiments demonstrating that highly aggressive breast cancer cells MDAMB231 have lower β-ATP[^{18}O] turnover (AMP phosphorylation) than have the control MCF10A cells (Klepinin et al., in preparation). This could be due to the rewiring of energy metabolism and glycolytic takeover. Activated glycolysis usually suppresses AK metabolic flux apparently by scavenging ADP (102) (**Figure 1**). Suppression of AK phosphotransfer, AMP generation, and consequent signaling through AMPK could be the biggest culprit of a cancerous transformation of a cell (**Figure 1**). There is also evidence that other AMP removal pathway enzymes like AMPD2 as well as 5′-NT are upregulated in colorectal cancer (103, 104). In this regard, the 5′-NT expression in breast cancer depends on tumor estrogen receptor status, suggesting a coordinated network (105). Our previous work has shown that in several tumors, MOM permeability has also increased for ADP, which may be related with keeping an intracellular ADP level low (41, 42, 49, 106) (see **Figure 1**). It was found that not only AMP but also ADP can regulate the activity of AMPK (107). Further studies are needed to elucidate detailed mechanisms: (1) how increased MOM permeability for ADP and AMP and (2) raised expression of AMP metabolizing enzymes can regulate intracellular nucleotide levels and the activity of AMPK and (3) what the significance is of AMP metabolic signaling in cancer progression.

CONCLUSIONS

The present review is a snapshot from recent AK studies that focused on the significance of AK network in energetics and metabolic signaling in cancer cells. Of the nine AK isoforms (AK1–AK9), four of them (AK1, AK2, AK4, and AK6) are involved in the progression of malignant transformation. Studies indicate that AK isoforms (AK1, AK2, AK4, and AK6) have an important role in the regulation of cancer cell metabolism, metabolic signaling, and cell migration and invasion. Moreover, at the initial stage, suppression of AK phosphotransfer and AMP generation and consequently signaling through AMPK by a variety of factors could be the biggest culprit of the cancerous transformation of a cell. Downregulation of AK→ AMP→ AMPK signaling can lead to the loss of control of cell cycle, growth, and proliferation. In the later stages, as emerging data suggest, cancer cells may use the shift in AK isoforms and other phosphotransfer enzymes to rewire their energy supply circuits to support proliferation and metastasis. Knockdown of overexpressed AK2 in human lung adenocarcinoma cells suppressed proliferation, migration, and invasion as well as induced apoptosis and autophagy. In this regard, a diterpene lactone neoandrographolide from extracts of the traditional medicinal herb *Andrographis paniculata* has been suggested to inhibit AK2 and has strong anticancer properties. Further studies that involve all AK isoforms have the potential to bring new understanding and novel therapeutic strategies targeting the AK isoform network to suppress growth and metastasis of cancer cells.

AUTHOR CONTRIBUTIONS

AK and SZ performed the study design, development of methodology, data analysis and interpretation, drafting of the manuscript, and critical revision. LK and ER-K analyzed and interpreted data and performed manuscript review. AT, TK, and PD performed the study conception, design, writing, and reviewing of the manuscript.

FUNDING

This work was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute (HL 85744-09) and U24DK100469 (Mayo Clinic Metabolomics Resource Core); Marriott Foundation and Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, USA; Institutional Research Funding IUT23-1 of the Estonian Ministry of Education and Research; and Estonia national scholarship program Kristjan Jaak, which is funded and managed by Archimedes Foundation in collaboration with the Estonian Ministry of Education and Research.

REFERENCES

1. Dzeja P, Chung S, Terzic A. Integration of adenylate kinase, glycolytic and glycogenolytic circuits in cellular energetics. In: Saks V, editor. *Molecular System Bioenergetics: Energy for Life*. Weinheim: Wiley-VCH. (2007). p. 265–301. doi: 10.1002/9783527621095.ch8
2. Dzeja PP, Terzic A. Phosphotransfer networks and cellular energetics. *J Exp Biol.* (2003) 206:2039–47. doi: 10.1242/jeb.00426
3. Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A, Wallimann T. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. *J Physiol.* (2006) 571:253–73. doi: 10.1113/jphysiol.2005.101444
4. Saks V, Monge C, Anmann T, Dzeja P. Integrated and organized cellular energetic systems: theories of cell energetics, compartmentation and metabolic channeling. In: Saks V, editor. *Molecular System Bioenergetics: Energy for Life*. Weinheim: Wiley-VCH. (2007). p. 59–109. doi: 10.1002/9783527621095.ch3
5. Zhang S, Nemutlu E, Terzic A, Dzeja P. Adenylate kinase isoform network: a major hub in cell energetics and metabolic signaling. system biology of metabolic and signaling networks. *Springer Ser Biophys.* (2014) 16:145–62. doi: 10.1007/978-3-642-38505-6_6
6. Dzeja PP, Bast P, Pucar D, Wieringa B, Terzic A. Defective metabolic signaling in adenylate kinase AK1 gene knock-out hearts compromises post-ischemic coronary reflow. *J Biol Chem.* (2007) 282:31366–72. doi: 10.1074/jbc.M705268200
7. Dzeja PP, Terzic A, Wieringa B. Phosphotransfer dynamics in skeletal muscle from creatine kinase gene-deleted mice. *Mol Cell Biochem.* (2004) 256-257:13–27. doi: 10.1023/B:MCBI.0000009856.23646.38
8. Janssen E, de Groof A, Wijers M, Fransen J, Dzeja PP, Terzic A, et al. Adenylate kinase 1 deficiency induces molecular and structural adaptations to support muscle energy metabolism. *J Biol Chem.* (2003) 278:12937–45. doi: 10.1074/jbc.M211465200
9. Janssen E, Terzic A, Wieringa B, Dzeja PP. Impaired intracellular energetic communication in muscles from creatine kinase and adenylate kinase (M-CK/AK1) double knock-out mice. *J Biol Chem.* (2003) 278:30441–9. doi: 10.1074/jbc.M303150200
10. Steeghs K, Benders A, Oerlemans F, de Haan A, Heerschap A, Ruitenbeek W, et al. Altered Ca2+ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. *Cell.* (1997) 89:93–103. doi: 10.1016/S0092-8674(00)80186-5
11. van Deursen J, Heerschap A, Oerlemans F, Ruitenbeek W, Jap P, ter Laak H, et al. Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. *Cell.* (1993) 74:621–31. doi: 10.1016/0092-8674(93)90510-W
12. van Horssen R, Janssen E, Peters W, van de Pasch L, Lindert MM, van Dommelen MM, et al. Modulation of cell motility by spatial repositioning of enzymatic ATP/ADP exchange capacity. *J Biol Chem.* (2009) 284:1620–7. doi: 10.1074/jbc.M806974200
13. Horiguchi T, Fuka M, Fujisawa K, Tanimura A, Miyoshi K, Murakami R, et al. Adenylate kinase 2 deficiency limits survival and regulates various genes during larval stages of Drosophila melanogaster. *J Med Invest.* (2014) 61:137–50. doi: 10.2152/jmi.61.137
14. Noma T. Dynamics of nucleotide metabolism as a supporter of life phenomena. *J Med Invest.* (2005) 52:127–36. doi: 10.2152/jmi.52.127
15. Lagresle-Peyrou C, Six EM, Picard C, Rieux-Laucat F, Michel V, Ditadi A, et al. Human adenylate kinase 2 deficiency causes a profound hematopoietic defect associated with sensorineural deafness. *Nat Genet.* (2009) 41:106–11. doi: 10.1038/ng.278
16. Pannicke U, Honig M, Hess I, Friesen C, Holzmann K, Rump EM, et al. Reticular dysgenesis (aleukocytosis) is caused by mutations in the gene encoding mitochondrial adenylate kinase 2. *Nat Genet.* (2009) 41:101–5. doi: 10.1038/ng.265
17. Dzeja PP, Chung S, Faustino RS, Behfar A, Terzic A. Developmental enhancement of adenylate kinase-AMPK metabolic signaling axis supports stem cell cardiac differentiation. *PLoS ONE.* (2011) 6:e19300. doi: 10.1371/journal.pone.0019300
18. Fujisawa K, Murakami R, Horiguchi T, Noma T. Adenylate kinase isozyme 2 is essential for growth and development of Drosophila melanogaster. *Comp Biochem Physiol.* (2009) 153:29–38. doi: 10.1016/j.cbpb.2009.01.006
19. Zhang S, Nemutlu E, Dzeja P. Metabolomic profiling of adenylate kinase AK1-/- and AK2+/- transgenic mice: effect of physical stress. *Circulation.* (2010) 122:A20435.
20. Ghaloul-Gonzalez L, Mohsen AW, Karunanidhi A, Seminotti B, Chong H, Madan-Khetarpal S, et al. Reticular dysgenesis and mitochondriopathy induced by adenylate kinase 2 deficiency with atypical presentation. *Sci Rep.* (2019) 9:15739. doi: 10.1038/s41598-019-51922-2
21. Panayiotou C, Solaroli N, Karlsson A. The many isoforms of human adenylate kinases. *Int J Biochem Cell Biol.* (2014) 49:75–83. doi: 10.1016/j.biocel.2014.01.014
22. Dzeja P, Terzic A. Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing. *Int J Mol Sci.* (2009) 10:1729–72. doi: 10.3390/ijms10041729
23. Dzeja PP, Zeleznikar RJ, Goldberg ND. Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes. *Int J Mol Sci.* (1998) 184:169–82. doi: 10.1007/978-1-4615-5653-4_13
24. Noda L. Adenylate kinase. In: Boyer P, editor. *The Enzymes, 3rd edition*. New York, NY: Academic Press. (1973). p. 279-305. doi: 10.1016/S1874-6047(08)60068-2
25. Van Rompay AR, Johansson M, Karlsson A. Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. *Pharmacol Ther.* (2000) 87:189–98. doi: 10.1016/S0163-7258(00)00048-6
26. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
27. Chung S, Arrell DK, Faustino RS, Terzic A, Dzeja PP. Glycolytic network restructuring integral to the energetics of embryonic stem cell cardiac differentiation. *J Mol Cell Cardiol.* (2010) 48:725–34. doi: 10.1016/j.yjmcc.2009.12.014
28. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer cell.* (2006) 9:425–34. doi: 10.1016/j.ccr.2006.04.023
29. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? *Biochim Biophys Acta.* (2011) 1807:552–61. doi: 10.1016/j.bbabio.2010.10.012
30. Moreno-Sanchez R, Rodriguez-Enriquez S, Marin-Hernandez A, Saavedra E. Energy metabolism in tumor cells. *FEBS J.* (2007) 274:1393–418. doi: 10.1111/j.1742-4658.2007.05686.x
31. Moreno-Sanchez R, Rodriguez-Enriquez S, Saavedra E, Marin-Hernandez A, Gallardo-Perez JC. The bioenergetics of cancer: is glycolysis the main ATP supplier in all tumor cells? *Biofactors.* (2009) 35:209–25. doi: 10.1002/biof.31
32. Han CY, Patten DA, Lee SG, Parks RJ, Chan DW, Harper ME, et al. p53 Promotes chemoresponsiveness by regulating hexokinase II gene transcription and metabolic reprogramming in epithelial ovarian cancer. *Mol Carcinog.* (2019) 58:2161–74. doi: 10.1002/mc.23106
33. Masters C. Cellular differentiation and the microcompartmentation of glycolysis. *Mech Ag Dev.* (1991) 61:11–22. doi: 10.1016/0047-6374(91)90003-I
34. Zahra K, Dey T, Ashish, Mishra SP, Pandey U. Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. *Front Oncol.* (2020) 10:159. doi: 10.3389/fonc.2020.00159
35. Klepinin A, Chekulayev V, Timohhina N, Shevchuk I, Tepp K, Kaldma A, et al. Comparative analysis of some aspects of mitochondrial metabolism in differentiated and undifferentiated neuroblastoma cells. *J Bioenerg Biomembr.* (2014) 46:17–31. doi: 10.1007/s10863-013-9529-5
36. Ounpuu L, Klepinin A, Pook M, Teino I, Peet N, Paju K, et al. 2102Ep embryonal carcinoma cells have compromised respiration and shifted bioenergetic profile distinct from H9 human embryonic stem cells. *Biochim Biophys Acta Gen Subj.* (2017) 1861:2146–54. doi: 10.1016/j.bbagen.2017.05.020
37. Gellerich FN, Gizatullina Z, Trumbeckaite S, Nguyen HP, Pallas T, Arandarcikaite O, et al. The regulation of OXPHOS by extramitochondrial calcium. *Biochim Biophys Acta.* (2010) 1797:1018–27. doi: 10.1016/j.bbabio.2010.02.005
38. Li XH, Chen XJ, Ou WB, Zhang Q, Lv ZR, Zhan Y, et al. Knockdown of creatine kinase B inhibits ovarian cancer progression by decreasing glycolysis. *Int J Biochem Cell Biol.* (2013) 45:979–86. doi: 10.1016/j.biocel.2013.02.003

39. Dewar BJ, Keshari K, Jeffries R, Dzeja P, Graves LM, Macdonald JM. Metabolic assessment of a novel chronic myelogenous leukemic cell line and an imatinib resistant subline by H NMR spectroscopy. *Metabolomics.* (2010) 6:439–50. doi: 10.1007/s11306-010-0204-0
40. Mooney SM, Rajagopalan K, Williams BH, Zeng Y, Christudass CS, Li Y, et al. Creatine kinase brain overexpression protects colorectal cells from various metabolic and non-metabolic stresses. *J Cell Biochem.* (2011) 112:1066–75. doi: 10.1002/jcb.23020
41. Kaldma A, Klepinin A, Chekulayev V, Mado K, Shevchuk I, Timohhina N, et al. An in situ study of bioenergetic properties of human colorectal cancer: the regulation of mitochondrial respiration and distribution of flux control among the components of ATP synthasome. *Int J Biochem Cell Biol.* (2014) 55:171–86. doi: 10.1016/j.biocel.2014.09.004
42. Kaambre T, Chekulayev V, Shevchuk I, Karu-Varikmaa M, Timohhina N, Tepp K, et al. Metabolic control analysis of cellular respiration in situ in intraoperational samples of human breast cancer. *J Bioenerg Biomembr.* (2012) 44:539–58. doi: 10.1007/s10863-012-9457-9
43. Amamoto R, Uchiumi T, Yagi M, Monji K, Song Y, Oda Y, et al. The expression of ubiquitous mitochondrial creatine kinase is downregulated as prostate cancer progression. *J Cancer.* (2016) 7:50–9. doi: 10.7150/jca.13207
44. Patra S, Bera S, SinhaRoy S, Ghoshal S, Ray S, Basu A, et al. Progressive decrease of phosphocreatine, creatine and creatine kinase in skeletal muscle upon transformation to sarcoma. *Febs Journal.* (2008) 275:3236–47. doi: 10.1111/j.1742-4658.2008.06475.x
45. Lam YW, Yuan Y, Isaac J, Babu CV, Meller J, Ho SM. Comprehensive identification and modified-site mapping of S-nitrosylated targets in prostate epithelial cells. *PLoS ONE.* (2010) 5:e9075. doi: 10.1371/journal.pone.0009075
46. Klepinin A, Ounpuu L, Guzun R, Chekulayev V, Timohhina N, Tepp K, et al. Simple oxygraphic analysis for the presence of adenylate kinase 1 and 2 in normal and tumor cells. *J Bioenerg Biomembr.* (2016) 48:531–48. doi: 10.1007/s10863-016-9687-3
47. Balinsky D, Greengard O, Cayanis E, Head JF. Enzyme activities and isozyme patterns in human lung tumors. *Cancer Res.* (1984) 44:1058–62.
48. Criss WE, Litwack G, Morris HP, Weinhouse S. Adenosine triphosphate: adenosine monophosphate phosphotransferase isozymes in rat liver and hepatomas. *Cancer Res.* (1970) 30:370–5.
49. Chekulayev V, Mado K, Shevchuk I, Koit A, Kaldma A, Klepinin A, et al. Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes. *Biochem Biophys Rep.* (2015) 4:111–25. doi: 10.1016/j.bbrep.2015.08.020
50. Vasseur S, Malicet C, Calvo EL, Dagorn JC, Iovanna JL. Gene expression profiling of tumours derived from ras(V12)/EIA-transformed mouse embryonic fibroblasts to identify genes required for tumour development. *Mol Cancer.* (2005) 4:4. doi: 10.1186/1476-4598-4-4
51. Speers C, Tsimelzon A, Sexton K, Herrick AM, Gutierrez C, Culhane A, et al. Identification of novel kinase targets for the treatment of estrogen receptor-negative breast cancer. *Clin Cancer Res.* (2009) 15:6327–40. doi: 10.1158/1078-0432.CCR-09-1107
52. Lamb R, Bonuccelli G, Ozsvari B, Peiris-Pages M, Fiorillo M, Smith DL, et al. Mitochondrial mass, a new metabolic biomarker for stem-like cancer cells: Understanding WNT/FGF-driven anabolic signaling. *Oncotarget.* (2015) 6:30453–71. doi: 10.18632/oncotarget.5852
53. Roesli C, Borgia B, Schhemann C, Gunthert M, Wunderli-Allenspach H, Giavazzi R, et al. Comparative analysis of the membrane proteome of closely related metastatic and nonmetastatic tumor cells. *Cancer Res.* (2009) 69:5406–14. doi: 10.1158/0008-5472.CAN-08-0999
54. Kim H, Lee HJ, Oh Y, Choi SG, Hong SH, Kim HJ, et al. The DUSP26 phosphatase activator adenylate kinase 2 regulates FADD phosphorylation and cell growth. *Nat Commun.* (2014) 5:4351. doi: 10.1038/ncomms4351
55. Liu H, Pu Y, Amina Q, Wang Q, Zhang M, Song J, et al. Prognostic and therapeutic potential of Adenylate kinase 2 in lung adenocarcinoma. *Sci Rep.* (2019) 9:17757. doi: 10.1038/s41598-019-53594-4
56. Jan YH, Tsai HY, Yang CJ, Huang MS, Yang YF, Lai TC, et al. Adenylate kinase-4 is a marker of poor clinical outcomes that promotes metastasis of lung cancer by downregulating the transcription factor ATF3. *Cancer Res.* (2012) 72:5119–29. doi: 10.1158/0008-5472.CAN-12-1842
57. Lanning NJ, Looyenga BD, Kauffman AL, Niemi NM, Sudderth J, DeBerardinis RJ, et al. A mitochondrial RNAi screen defines cellular bioenergetic determinants and identifies an adenylate kinase as a key regulator of ATP levels. *Cell Rep.* (2014) 7:907–17. doi: 10.1016/j.celrep.2014.03.065
58. Bai DM, Zhang JF, Li TT, Hang RL, Liu Y, Tian YL, et al. The ATPase hCINAP regulates 18S rRNA processing and is essential for embryogenesis and tumour growth. *Nat Commun.* (2016) 7:12310. doi: 10.1038/ncomms12310
59. Ji Y, Yang C, Tang Z, Yang Y, Tian Y, Yao H, et al. Adenylate kinase hCINAP determines self-renewal of colorectal cancer stem cells by facilitating LDHA phosphorylation. *Nat Commun.* (2017) 8:15308. doi: 10.1038/ncomms15308
60. Hansel DE, Rahman A, House M, Ashfaq R, Berg K, Yeo CJ, et al. Met proto-oncogene and insulin-like growth factor binding protein 3 overexpression correlates with metastatic ability in well-differentiated pancreatic endocrine neoplasms. *Clin Cancer Res.* (2004) 10(18 Pt 1):6152–8. doi: 10.1158/1078-0432.CCR-04-0285
61. Karhemo PR, Hyvonen M, Laakkonen P. Metastasis-associated cell surface oncoproteomics. *Front Pharmacol.* (2012) 3:192. doi: 10.3389/fphar.2012.00192
62. Lee HJ, Pyo JO, Oh Y, Kim HJ, Hong SH, Jeon YJ, et al. AK2 activates a novel apoptotic pathway through formation of a complex with FADD and caspase-10. *Nat Cell Biol.* (2007) 9:1303–10. doi: 10.1038/ncb1650
63. Kumari R, Deshmukh RS, Das S. Caspase-10 inhibits ATP-citrate lyase-mediated metabolic and epigenetic reprogramming to suppress tumorigenesis. *Nat Commun.* (2019) 10:4255. doi: 10.1038/s41467-019-12194-6
64. Oshima K, Saiki N, Tanaka M, Imamura H, Niwa A, Tanimura A, et al. Human AK2 links intracellular bioenergetic redistribution to the fate of hematopoietic progenitors. *Biochem Biophys Res Commun.* (2018) 497:719–25. doi: 10.1016/j.bbrc.2018.02.139
65. Dzeja PP, Hoyer K, Tian R, Zhang S, Nemutlu E, Spindler M, et al. Rearrangement of energetic and substrate utilization networks compensate for chronic myocardial creatine kinase deficiency. *J Physiol.* (2011) 589:5193–211. doi: 10.1113/jphysiol.2011.212829
66. Wu Z, Gong Q, Yu Y, Zhu J, Li W. Knockdown of circ-ABCB10 promotes sensitivity of lung cancer cells to cisplatin via miR-556-3p/AK4 axis. *BMC Pulm Med.* (2020) 20:10. doi: 10.1186/s12890-019-1035-z
67. Zang C, Zhao F, Hua L, Pu Y. The miR-199a-3p regulates the radioresistance of esophageal cancer cells via targeting the AK4 gene. *Cancer Cell Int.* (2018) 18:186. doi: 10.1186/s12935-018-0689-6
68. Fujisawa K, Terai S, Takami T, Yamamoto N, Yamasaki T, Matsumoto T, et al. Modulation of anti-cancer drug sensitivity through the regulation of mitochondrial activity by adenylate kinase 4. *J Exp Clin Cancer Res.* (2016) 35:48. doi: 10.1186/s13046-016-0322-2
69. Lei W, Yan C, Ya J, Yong D, Yujun B, Kai L. MiR-199a-3p affects the multi-chemoresistance of osteosarcoma through targeting AK4. *BMC Cancer.* (2018) 18:631. doi: 10.1186/s12885-018-4460-0
70. Liu RJ, Strom AL, Zhai JJ, Gal J, Bao SL, Gong WM, et al. Enzymatically inactive adenylate kinase 4 interacts with mitochondrial ADP/ATP translocase. *Int J Biochem Cell B.* (2009) 41:1371–80. doi: 10.1016/j.biocel.2008.12.002
71. Kong F, Binas B, Moon JH, Kang SS, Kim HJ. Differential expression of adenylate kinase 4 in the context of disparate stress response strategies of HEK293 and HepG2 cells. *Arch Biochem Biophys.* (2013) 533:11–7. doi: 10.1016/j.abb.2013.02.014
72. Pedersen PL. Warburg, me and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. *J Bioenerg Biomembr.* (2007) 39:211–22. doi: 10.1007/s10863-007-9094-x
73. Nelson BD, Kabir F. Adenylate Kinase Is a Source of Atp for Tumor Mitochondrial Hexokinase. *Biochimica Et Biophysica Acta.* (1985) 841:195–200. doi: 10.1016/0304-4165(85)90021-2
74. Choi YK, Park KG. Targeting Glutamine Metabolism for Cancer Treatment. *Biomol Ther (Seoul).* (2018) 26:19–28. doi: 10.4062/biomolther.2017.178
75. Deshmukh A, Deshpande K, Arfuso F, Newsholme P, Dharmarajan A. Cancer stem cell metabolism: a potential target for cancer therapy. *Mol Cancer.* (2016) 15:555. doi: 10.1186/s12943-016-0555-x

76. Ross RA, Spengler BA. Human neuroblastoma stem cells. *Semin Cancer Biol.* (2007) 17:241–7. doi: 10.1016/j.semcancer.2006.04.006
77. Song IS, Jeong JY, Jeong SH, Kim HK, Ko KS, Rhee BD, et al. Mitochondria as therapeutic targets for cancer stem cells. *World J Stem Cells.* (2015) 7:418–27. doi: 10.4252/wjsc.v7.i2.418
78. Dzeja PP, Bortolon R, Perez-Terzic C, Holmuhamedov EL, Terzic A. Energetic communication between mitochondria and nucleus directed by catalyzed phosphotransfer. *Proc Natl Acad Sci USA.* (2002) 99:10156–61. doi: 10.1073/pnas.152259999
79. Jan YH, Lai TC, Yang CJ, Lin YF, Huang MS, Hsiao M. Adenylate kinase 4 modulates oxidative stress and stabilizes HIF-1alpha to drive lung adenocarcinoma metastasis. *J Hematol Oncol.* (2019) 12:12. doi: 10.1186/s13045-019-0698-5
80. Jan YH, Lai TC, Yang CJ, Huang MS, Hsiao M. A co-expressed gene status of adenylate kinase 1/4 reveals prognostic gene signature associated with prognosis and sensitivity to EGFR targeted therapy in lung adenocarcinoma. *Sci Rep.* (2019) 9:12329. doi: 10.1038/s41598-019-48243-9
81. Xin F, Yao DW, Fan L, Liu JH, Liu XD. Adenylate kinase 4 promotes bladder cancer cell proliferation and invasion. *Clin Exp Med.* (2019) 19:525–34. doi: 10.1007/s10238-019-00576-5
82. Zhang J, Yin YT, Wu CH, Qiu RL, Jiang WJ, Deng XG, et al. AK4 promotes the progression of HER2-positive breast cancer by facilitating cell proliferation and invasion. *Dis Markers.* (2019) 2019:8186091. doi: 10.1155/2019/8186091
83. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. *Nature.* (2008) 451:345–U11. doi: 10.1038/nature06489
84. Adams JM, Strasser A. Is tumor growth sustained by rare cancer stem cells or dominant clones? *Cancer Res.* (2008) 68:4018–21. doi: 10.1158/0008-5472.CAN-07-6334
85. Kaur H, Lakatos-Karoly A, Vogel R, Noll A, Tampe R, Glaubitz C. Coupled ATPase-adenylate kinase activity in ABC transporters. *Nat Commun.* (2016) 7:13864. doi: 10.1038/ncomms13864
86. Dzeja PP, Terzic A. Phosphotransfer reactions in the regulation of ATP-sensitive K+ channels. *Faseb J.* (1998) 12:523–9. doi: 10.1096/fasebj.12.7.523
87. Carrasco AJ, Dzeja PP, Alekseev AE, Pucar D, Zingman LV, Abraham MR, et al. Adenylate kinase phosphotransfer communicates cellular energetic signals to ATP-sensitive potassium channels. *Proc Natl Acad Sci USA.* (2001) 98:7623–8. doi: 10.1073/pnas.121038198
88. Bhaskara V, Dupre A, Lengsfeld B, Hopkins BB, Chan A, Lee JH, et al. Rad50 adenylate kinase activity regulates DNA tethering by Mre11/Rad50 complexes. *Mol Cell.* (2007) 25:647–61. doi: 10.1016/j.molcel.2007.01.028
89. Boissan M, Schlattner U, Lacombe ML. The NDPK/NME superfamily: state of the art. *Lab Invest.* (2018) 98:164–74. doi: 10.1038/labinvest.2017.137
90. Gonzalez A, Hall MN, Lin SC, Hardie DG. AMPK and TOR: the yin and yang of cellular nutrient sensing and growth control. *Cell Metab.* (2020) 31:472–92. doi: 10.1016/j.cmet.2020.01.015
91. Collavin L, Lazarevic D, Utrera R, Marzinotto S, Monte M, Schneider C. wt p53 dependent expression of a membrane-associated isoform of adenylate kinase. *Oncogene.* (1999) 18:5879–88. doi: 10.1038/sj.onc.1202970
92. Sholihah MM, Indarto D, Pramana TY. The inhibitory effect ofAndrographis paniculataextract on proliferation of breast cancer cell line. *Mat Sci Eng.* (2019) 546:062029. doi: 10.1088/1757-899X/546/6/062029
93. Hardie DG. AMP-activated protein kinase: a cellular energy sensor with a key role in metabolic disorders and in cancer. *Biochem Soc Trans.* (2011) 39:1–13. doi: 10.1042/BST0390001
94. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev.* (2007) 8:774–85. doi: 10.1038/nrm2249
95. Dasgupta B, Chhipa RR. Evolving Lessons on the Complex Role of AMPK in Normal Physiology and Cancer. *Trends Pharmacol Sci.* (2016) 37:192–206. doi: 10.1016/j.tips.2015.11.007
96. Hardie DG, Alessi DR. LKB1 and AMPK and the cancer-metabolism link - ten years after. *BMC Biol.* (2013) 11:36. doi: 10.1186/1741-7007-11-36
97. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer.* (2009) 9:563–75. doi: 10.1038/nrc2676
98. Takaine M, Imamura H, Yoshida S. AMP-activated protein kinase and adenylate kinase prevent the ATP catastrophe and cytotoxic protein aggregation. *bioRxiv.* (2019) 801738. doi: 10.1101/801738
99. Plaideau C, Liu JM, Hartleib-Geschwindner J, Bastin-Coyette L, Bontemps F, Oscarsson J, et al. Overexpression of AMP-metabolizing enzymes controls adenine nucleotide levels and AMPK activation in HEK293T cells. *Faseb Journal.* (2012) 26:2685–94. doi: 10.1096/fj.11-198168
100. Plaideau C, Lai YC, Kviklyte S, Zanou N, Lofgren L, Andersen H, et al. Effects of pharmacological amp deaminase inhibition and ampd1 deletion on nucleotide levels and AMPK activation in contracting skeletal muscle. *Chem Biol.* (2014) 21:1497–510. doi: 10.1016/j.chembiol.2014.09.013
101. Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. *J Immunother Cancer.* (2018) 6:57. doi: 10.1186/s40425-018-0360-8
102. Olson LK, Schroeder W, Robertson RP, Goldberg ND, Walseth TF. Suppression of adenylate kinase catalyzed phosphotransfer precedes and is associated with glucose-induced insulin secretion in intact HIT-T15 cells. *J Biol Chem.* (1996) 271:16544–52. doi: 10.1074/jbc.271.28.16544
103. Wang F, Liu Y, Jiang J, Qin Y, Ge X, Li J, et al. High expression of AMPD2 and obesity are associated with poor prognosis in colorectal cancer. *Int J Clin Exp Pathol.* (2018) 11:216–23.
104. Parr C, Jiang WG. Quantitative analysis of lymphangiogenic markers in human colorectal cancer. *Int J Oncol.* (2003) 23:533–9. doi: 10.3892/ijo.23.2.533
105. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5'-nucleotidase and adenosine in breast cancer. *Clin Cancer Res.* (2004) 10:708–17. doi: 10.1158/1078-0432.CCR-0811-03
106. Klepinin A, Ounpuu L, Mado K, Truu L, Chekulayev V, Puurand M, et al. The complexity of mitochondrial outer membrane permeability and VDAC regulation by associated proteins. *J Bioenerg Biomembr.* (2018) 50:339–54. doi: 10.1007/s10863-018-9765-9
107. Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D, et al. Structure of mammalian AMPK and its regulation by ADP. *Nature.* (2011) 472:230–3. doi: 10.1038/nature09932

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix 4

Publication IV

Reinsalu L, Puurand M, Chekulayev V, Miller S, Shevchuk I, Tepp K, Rebane-Klemm E, Timohhina N, Terasmaa A, Kaambre T. Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis. Front. Oncol. 2021 July 26; 11:698951. doi: 10.3389/fonc.2021.698951.

frontiers in Oncology

REVIEW
published: 26 July 2021
doi: 10.3389/fonc.2021.698951

Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis

*Leenu Reinsalu[1,2], Marju Puurand[1], Vladimir Chekulayev[1], Sten Miller[1,2], Igor Shevchuk[1], Kersti Tepp[1], Egle Rebane-Klemm[1,2], Natalja Timohhina[1], Anton Terasmaa[1] and Tuuli Kaambre[1]**

[1] Laboratory of Chemical Biology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, [2] Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Tallinn, Estonia

OPEN ACCESS

Edited by:
Miriam Martini,
University of Turin, Italy

Reviewed by:
Tatiana Rostovtseva,
National Institutes of Health (NIH), United States
Wael Rabeh,
New York University Abu Dhabi, United Arab Emirates

***Correspondence:**
Tuuli Kaambre
tuuli.kaambre@kbfi.ee

Specialty section:
This article was submitted to Cancer Metabolism, a section of the journal Frontiers in Oncology

Received: 22 April 2021
Accepted: 08 July 2021
Published: 26 July 2021

Citation:
Reinsalu L, Puurand M, Chekulayev V, Miller S, Shevchuk I, Tepp K, Rebane-Klemm E, Timohhina N, Terasmaa A and Kaambre T (2021) Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis. Front. Oncol. 11:698951. doi: 10.3389/fonc.2021.698951

Metabolic plasticity is the ability of the cell to adjust its metabolism to changes in environmental conditions. Increased metabolic plasticity is a defining characteristic of cancer cells, which gives them the advantage of survival and a higher proliferative capacity. Here we review some functional features of metabolic plasticity of colorectal cancer cells (CRC). Metabolic plasticity is characterized by changes in adenine nucleotide transport across the outer mitochondrial membrane. Voltage-dependent anion channel (VDAC) is the main protein involved in the transport of adenine nucleotides, and its regulation is impaired in CRC cells. Apparent affinity for ADP is a functional parameter that characterizes VDAC permeability and provides an integrated assessment of cell metabolic state. VDAC permeability can be adjusted *via* its interactions with other proteins, such as hexokinase and tubulin. Also, the redox conditions inside a cancer cell may alter VDAC function, resulting in enhanced metabolic plasticity. In addition, a cancer cell shows reprogrammed energy transfer circuits such as adenylate kinase (AK) and creatine kinase (CK) pathway. Knowledge of the mechanism of metabolic plasticity will improve our understanding of colorectal carcinogenesis.

Keywords: tumor energy metabolism, aerobic glycolysis, oxidative phosphorylation, VDAC, creatine kinase, adenylate kinase, mitochondria

INTRODUCTION

Analysis of mitochondrial function is central to the study of intracellular energy metabolism and pathophysiological mechanisms of various human diseases, including cancer. The metabolism of cancer cells is adapted to meet their needs to survive and proliferate in a hypoxic and also in a well-oxygenated microenvironment and thus must acquire metabolic flexibility. At the molecular level,

Abbreviations: ADP, adenosine diphosphate; AMPK, adenosine 5'-monophosphate-activated protein kinase; AK, adenylate kinase; ANT, adenine nucleotide translocator; CK, creatine kinase; CRC, colorectal cancer; HK, hexokinase; HIF, hypoxia-inducible factor; ISC, iron-sulfur clusters; OMM, outer mitochondrial membrane; TCA, tricarboxylic acid; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel.

metabolic flexibility relies on the configuration of metabolic pathways, which are regulated by key metabolic enzymes and transcription factors. Reprogramming of cellular energetics is recognized as a distinctive hallmark of cancer (1). The first theory on the peculiarities of cancer metabolism was formulated by Otto Warburg in the early 20th century. He concluded that tumors, unlike normal cells, obtain their energy mainly from aerobic glycolysis, while normal cells usually favor oxidative phosphorylation (OXPHOS), which is much more efficient in terms of ATP gain. This observation is coined as the Warburg effect (2, 3) and became the central model for oncobioenergetics for most of the 20th century. The glycolytic part of the Warburg hypothesis was firmly and thoroughly confirmed for many cancer types, in contrast to the OXPHOS part, which was and still is a matter of intense research and controversy. Verified evidence indicates that in reality, both anaerobic (glucose to lactate) and aerobic (glucose to pyruvate) glycolysis operate in cancer cells simultaneously like in normal cells, although at higher rates than in non-tumor cells (4). In addition, tumor cells often exhibit high rates of OXPHOS (5, 6). Transcriptomics and end-product metabolites analyses of complex molecular pathways converge into a three-node minimum regulatory network consisting of hypoxia-inducible factor 1 (HIF-1), adenosine monophosphate-activated protein kinase (AMPK), and reactive oxygen species (ROS). Therefore, the coexistence of three distinct cellular metabolic phenotypes is revealed in cancer cells: 1) glycolytic, characterized by high activity of HIF-1α and high activity of the glycolytic pathway; 2) OXPHOS state, characterized by high activity of AMPK and high activity of OXPHOS pathways such as glucose oxidation and fatty acid oxidation; 3) hybrid metabolic state, characterized by high activity of AMPK and HIF-1α and concomitant functioning of glycolysis and OXPHOS pathways. In contrast, normal cells exhibit only two metabolic states, namely, glycolytic and OXPHOS, and lack the hybrid state (7, 8). In this regulatory network, HIF-1 and AMPK are the master regulators of glycolysis and OXPHOS, respectively (9), and both cytosolic and mitochondrial ROS mediate the complex interplay between AMPK and HIF-1. Accordingly, the hybrid metabolic state in cancer cells can be promoted by the stabilization of HIF-1α and elevated production of mitochondrial ROS. Hypoxia activates glycolysis *via* stabilization of HIF-1α and HIF-2α, which in turn upregulates the activity of several members of the glycolytic pathway and increases glucose uptake (10, 11). In addition, the elevation of HIF-1α levels could be induced by high concentrations of succinate (pseudohypoxia) (12). A striking feature of cancer cells is their ability to switch their metabolic phenotypes to glycolysis or OXPHOS in response to changes in their microenvironment or inhibition of one of these pathways, giving survival advantage during tumor progression (8, 13). This metabolic plasticity is promoted by the hybrid phenotype of cancer cells and is linked with metastasis and chemoresistance (14). However, it is still largely unknown how cancer cells regulate gene expression to maintain their hybrid metabolic state and metabolic plasticity.

Implementation of the hybrid metabolism paradigm may reveal new therapeutic targets and opportunities for the treatment of cancer. It was previously shown that administration of glycolytic inhibitors alone may be ineffective to eradicate tumors, and targeting the hybrid state to eliminate metabolic plasticity could be a new therapeutic strategy to eliminate cancer aggressiveness (15, 16). We review the changes in OMM permeability and intracellular energy transfer pathways in connection with the metabolic plasticity of CRC cells.

METABOLIC REPROGRAMMING OF COLORECTAL CANCER

Colorectal cancer has been regarded as a purely hypoxic tumor of the Warburg phenotype for many years. This was confirmed by increased expression of several glycolytic enzymes, pentose phosphate pathway, and glucose transporters associated with elevated rates of glucose consumption and lactate production as compared with normal surrounding tissues (17–25). Normal colonocytes use the OXPHOS system as the primary energy source (26, 27). Short-chain fatty acids undergo β-oxidation to form acetyl-CoA, which enters into the tricarboxylic acid (TCA) cycle to yield citrate, NADH, and finally ATP. But, unlike normal colonocytes, colorectal carcinomas cannot utilize butyrate as an energy source and carbon donor (26, 28), implying the truncated TCA cycle in CRC. Importantly, some metabolites of the TCA cycle, such as succinate, fumarate, and α-ketoglutarate, act as "oncometabolites" that support tumor growth *via* oncogenic signaling, inter alia *via* upregulation and stabilization of HIF-1α (29).

Metabolic reprogramming during large intestine carcinogenesis is largely mediated by (a) altered expression of several oncogenes and a loss of tumor suppressor genes, encoding usually various transcriptional factors and protein kinases (30, 31), (b) adaptation to nutrient and oxygen availability in the local tumor microenvironment (metabolic plasticity) (32), and (c) metabolic cross-talk with stromal, adipose tissue and immune cells (31, 33–37).

Data on molecular mechanisms of the metabolic reprogramming of CRC are mostly obtained from studies using cell culture models, while the number of functional studies using clinical material is limited. Moreover, cell culture conditions have variations that could significantly affect the metabolic profile of the cells. For example, cells grown in glucose-free medium display a relatively high rate of oxygen consumption, while cultivation of cells in a high-glucose medium results in hyperglycolytic profile and declined respiratory flux (38–42). Our recent studies revealed remarkable differences in the regulation of outer mitochondrial membrane (OMM) permeability between cultured tumor cells and clinical material from cancer patients (5, 43). Comparative analysis of the biopsy or surgical cancer material and surrounding healthy tissue showed almost unchanged glycolytic activity and upregulation of OXPHOS in CRC, which is inconsistent with the data obtained by using cell culture (43–47). In addition, two widely

used breast cancer cell lines MCF7 and MCF-MDA-231 failed to replicate mitochondrial function in respect to metabolic activity and OXPHOS as seen in respective human samples (43, 46).

Why the CRC cells shift their metabolism in favor of OXPHOS? Perhaps, under normal conditions, the amount of ATP produced through aerobic glycolysis is insufficient to support cell proliferation and migration. There is a growing body of evidence that CRC is characterized by stimulated mitochondrial biogenesis expressed as an increase in mitochondrial DNA copy number (48) and elevated ADP-dependent oxygen consumption in CRC tissue (5, 6, 43–45). Activated mitochondrial biogenesis can be an adaptive response of tumor cells to overcome the chronic energy crisis caused by glucose starvation or defects in the function of their respiratory enzymes due to pathogenic nuclear or mtDNA mutations (49–51). The elevated lactate level may act as a signaling molecule to affect genes and proteins known to be involved in mitochondrial biogenesis (52), *via* upregulation of AMPK- and SIRT1-associated PGC-1α activation (53). Nuclear Respiratory Factor 1 (NRF1) (54) and some cytokines, IL-6/8 (55, 56), activate the AMPK signaling pathway as well as apoptotic resistance of cancer cells (56–58). Some types of tumor cells support their high rates of OXPHOS and drug resistance by transferring mtDNA or even the entire mitochondria from surrounding healthy tissues; this intercellular mitochondrial transfer may occur through exosomes or tunnel nanotubes (59, 60). The signaling pathways responsible for the stimulation of mitochondrial biogenesis can have both intracellular and external origins.

THE ROLE OF VDAC AND THE REGULATION OF OUTER MITOCHONDRIAL MEMBRANE PERMEABILITY IN METABOLIC PLASTICITY

The flux of water-soluble metabolites into and out of the mitochondria occurs through a variety of inner mitochondrial membrane (IMM) carriers, but the flux of ATP, ADP, and Pi across the OMM occurs through a single pathway, the VDAC, and therefore the regulation of OXPHOS is largely mediated by the VDAC permeability control (61). Based on studies of muscle permeabilized fibers, cellular respiration and associated ATP synthesis are regulated by a protein complex called Mitochondrial Interactosome (MI), which is located at the junction of mitochondrial membranes (62, 63). Restrictions for adenine nucleotides in VDAC are evident by measuring an apparent affinity of mitochondria for exogenous ADP [Km (ADP)] in permeabilized cells and tissues by using high-resolution respirometry (64, 65). These barriers appear only in permeabilized cells and not in isolated mitochondria and disappear during mild proteolytic treatment with trypsin (66). Therefore, the metabolic plasticity of cancer cells is associated with the protein-mediated control of VDAC permeability towards ADP.

Cancer Metabolic Plasticity Is Functionally Defined by Changes in ADP Dependent Oxygen Consumption

Analysis of respirometry data provides instant functional profiling of metabolic plasticity. Dependence of mitochondrial O_2 consumption upon ADP concentration follows Michaelis-Menten kinetics and allows evaluation of apparent Michaelis-Menten constant for ADP Km(ADP) in different tissues, cancers, and cell cultures (**Figure 1**). Determined in permeabilized cells and tissues, Km(ADP) is the affinity of the mitochondria for exogenous ADP and characterizes permeability of OMM for adenine nucleotides and, thus, VDAC permeability. Measured Km(ADP) values for human colon mucosa is ~110 μM (47), ~100 μM for CRC (5, 44, 47), ~60 μM for colon polyps (47), and ~40 μM for Caco2 CRC cell line (43), indicating the alteration of control mechanisms over VDAC permeability and OXPHOS during the progression of CRC. Thus, the regulation of OMM permeability to adenine nucleotides in cancer tissues is different from that in normal cells (5, 67, 68). Notably, Km(ADP) values measured in cell cultures are much lower than in tissue biopsies and are similar to Km(ADP) values for isolated mitochondria (69). This illustrates the shortcomings of cell culture studies and highlights the importance of using clinical material for the evaluation of the mechanism of cancer metabolic plasticity.

The cell-specific differences in Km(ADP) are likely caused by the specific structural and functional organization of energy metabolism. For example, cells with a low Km(ADP) value (~10 μM), like glycolytic muscle, possess less structural and functional restrictions for ADP/ATP movement through OMM as compared to the oxidative muscles (Km(ADP) ~300 μM) (64). Thus, relatively low Km(ADP) for colorectal polyps indicates a metabolic reprogramming towards the glycolytic phenotype with functional OXPHOS (as in glycolytic muscle), and an increase in Km values in the CRC reflects a shift to OXPHOS phenotype with increased intracellular complexity (analogy with oxidative muscle). Hence, Km(ADP) value is an important parameter describing metabolic plasticity. According to the model proposed by Saks V. et al, the proportion of mitochondria with low oxidative capacity in the tissue can be inferred from the Km (ADP) value (70). For example, the proportion of mitochondria with high oxidative capacity is 67% in CRC tumors and only 38% in colorectal polyps (47).

In addition to Km(ADP), the maximal ADP-dependent oxygen consumption (V_{max}) is a defining characteristic of metabolic plasticity and is correlated to mitochondrial content (density) in the tissue. V_{max} values are higher in CRC than in normal colon tissue (5, 6, 47), indicating a vigorous metabolic activity. Moreover, V_{max} values in biopsy material from patients that succumbed to colon cancer were significantly higher than in patients staying in remission (5). However, the extent to which high V_{max} values correlate with tumor aggressiveness needs to be confirmed in further studies.

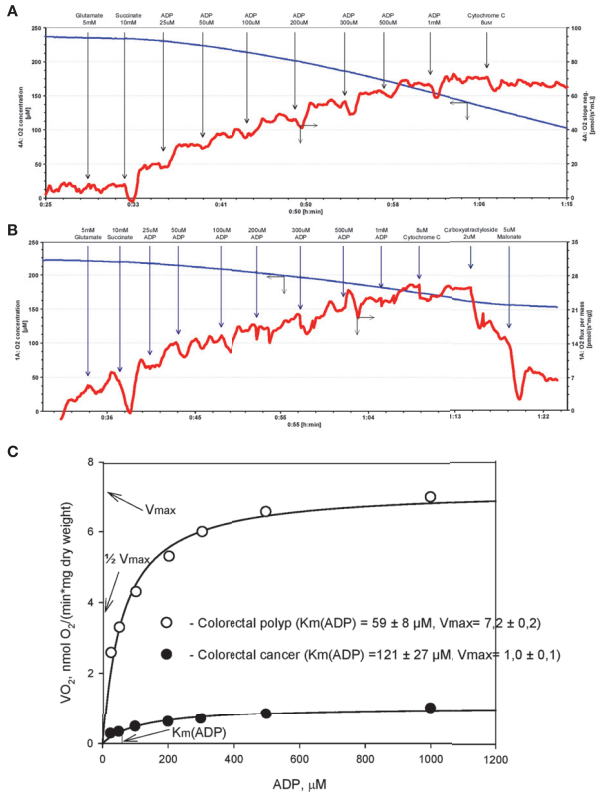

FIGURE 1 | Michaels-Menten kinetics of ADP-dependent respiration of human colorectal cancer and polyp biopsy material. Representative tracing of adenosine diphosphate (ADP)-activated oxygen consumptions rates in human permeabilized tissue of **(A)** colorectal polyp and **(B)** and colorectal cancer. **(C)** Corresponding Km (ADP) and V_{max} values were calculated by non-linear regression using the Michaelis-Menten equation.

The Possible Mechanisms of VDAC Permeability Regulation

Several studies show that VDAC isoform 1 (VDAC1) is the dominant isoform in most malignant tumors including CRC (44, 71, 72). VDAC1 is crucial in communication between the mitochondria and the cytosol. Cancer cells display high levels of metabolic flexibility combined with apoptosis resistance, which provides a survival advantage for these cells. VDAC1 is well recognized as a metabolic checkpoint at the crossroad of these two processes (72, 73). VDAC mediates and regulates the transport of metabolites, ions, and ROS across OMM. Thus, VDAC1 plays a major role in the control of mitochondrial function. Transport of ADP through OMM is mediated *via* VDAC1 and through the inner membrane *via* ANT. Metabolic control analysis of the OXPHOS system of CRC revealed that ANT does not exert exclusive control over the mitochondrial ADP-dependent oxygen consumption (5, 43). Therefore, the rate-limiting step of ADP transport into the mitochondria appears to be VDAC. Therefore, the alteration of Km(ADP) value depends on the changes in interactions of VDAC1 with other proteins or on the modification of VDAC1 itself.

As the name implies, VDAC is regulated by a change of membrane potential. Studies of isolated VDAC1 reconstituted into planar lipid bilayers reveal sharp and symmetrical voltage dependence of VDAC1 permeability (72, 74, 75). At membrane potentials close to zero (between −20 to +20 mV), VDAC1 is open and displays low anionic selectivity. At more positive or more negative membrane potentials (+30.+60 mV or −30.−60 mV), VDAC1 shows diminished permeability to large anions and becomes more selective to small cations (72). However, it is unknown whether the voltage dependence of VDAC1 is relevant in physiological conditions, as the value of membrane potential across OMM is unknown. It is generally believed that any membrane potential generated at OMM will be offset by a relatively undisturbed movement of small ions across OMM. However, there is a theoretical possibility that OMM can be polarized to potentials large enough to alter the permeability of VDAC1 (2, 3). Although the role of OMM potential in the

regulation of VDAC1 permeability is unlikely, it remains to be investigated whether potential across OMM changes in CRC and whether such change can alter Km(ADP).

Hexokinase-VDAC Interaction Regulates the Permeability of VDAC to Adenine Nucleotides

Although the VDAC-hexokinase (HK) binding was demonstrated by several groups using different experimental approaches, it still remains somewhat speculative, and there are different hypothesis on its functional consequences. Research activities of Prof. Pedersen and his colleagues resulted in the discovery of the binding of HK-II to VDAC with the conclusion that this phenomenon could play a pivotal role in the "Warburg Effect" (76–80). Review paper of V. Shoshan-Barmatz et al. proposed the hypothesis that HK-II binds to VDAC and promotes VDAC closing (81). Neumann et al. demonstrated the binding of the cytosolic protein HK-I to VDAC by two-color STED microscopy (82). Our group showed the colocalization of VDAC1 and hexokinase II in cell cultures and clinical cancer samples by confocal microscopy imaging (6, 67). Based on these studies, two models of VDAC permeability control have been proposed. The model proposed by Pedersen et al. states that the binding of HK-II to VDAC plays a pivotal role in maintaining the Warburg phenotype in cancer cells (77, 83). In such a setting, mitochondrial ATP is preferentially directed to glycolysis (HK reaction) and the produced ADP is channeled back to the OXPHOS (**Figure 2**). At the same time, VDAC is assumed to be in an open state and mitochondria have free access to exogenous ADP (84, 85), thus low Km(ADP) values are expected. Glucose-stimulated increase of mitochondrial respiration shows the amount of ADP released in the HK reaction that passes through VDAC and is utilized in mitochondrial ATP synthesis (86). Such glucose effect comprises a fraction of total ADP-stimulated respiration and is higher in cancer cells as compared to normal cells. Accordingly, the glucose effect is about 20% for CRC tissue, about 12% for normal colon tissue samples (6), and about 48% for Caco-2 CRC cell line (43). These results show that the lower affinity of mitochondria for ADP could be related to the weaker ability for glucose to stimulate respiration. CRC displays elevated levels of VDAC1 as compared with surrounding healthy tissues (43), and this is in good agreement with the fact that V_{max} for ADP-dependent respiration is higher in CRC (44). The total HK activity and expression levels of HK1 and HK2 in CRC do not differ from that of normal tissue (6, 44). In both the normal mucosa and the CRC, HK2 is colocalized with VDAC (6, 43). The interaction of HK1 or HK2 with VDAC1 gives numerous advantages to cancer cells: (1) it mediates the increased permeability of the OMM to adenine nucleotides; (2) it increases the rate of aerobic glycolysis and thereby allows the cells to adapt to hypoxic conditions; (3) it mediates elevated resistance to apoptosis and protection from oxidative stress as VDAC1-bound HK acts as an anti-apoptotic protein (73, 87–89). VDAC-HK interaction is reversed with inhibitors of HK2 (e.g., 3-bromopyruvate), and agents that disrupt the VDAC-HK interaction have been tested as anticancer drugs (73, 90–93). It was also reported that silencing of VDAC1 expression by siRNA inhibited the proliferation of several cancer cell lines (including CRC) (94).

Free Beta-Tubulins Controlling VDAC Permeability in CRC

According to the free-tubulin model, the binding of free tubulin blocks VDAC and thereby regulates respiration (95). The

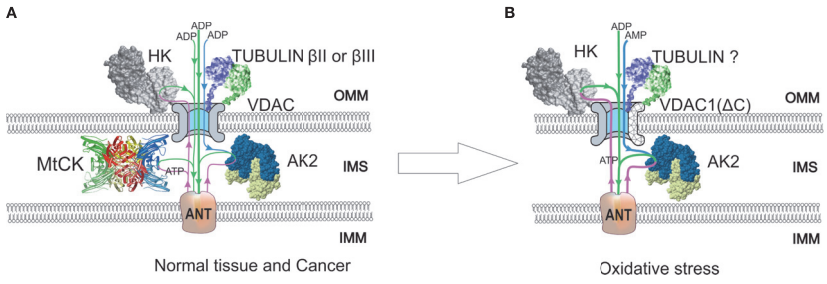

FIGURE 2 | A model of regulation of outer mitochondrial membrane (OMM) permeability for adenine nucleotides in normal and colorectal cancer (CRC) cells. Voltage-dependent anion channel (VDAC) is the pore through which adenine nucleotides move into and out of the mitochondria. **(A)** In normal and possibly some cancer cells, a minor amount of hexokinase (HK) is bound to VDAC and utilizes mitochondrial ATP to initiate glycolysis. Produced ADP is channeled back to the mitochondrial matrix *via* VDAC and adenine nucleotide translocase (ANT) for use in oxidative phosphorylation (OXPHOS). VDAC permeability is also regulated by tubulin binding. As a result of beta-tubulin-VDAC interaction, the VDAC is less permeable to adenine nucleotides. This in turn promotes cells to use creatine kinase (CK) and adenylate kinase (AK) energy transfer networks for intracellular distribution of high-energy phosphates. Mitochondrial intermembrane space (IMS)-residing mitochondrial CK (MtCK) is functionally coupled to ANT, turning OXPHOS to be dependent on ADP originating from MtCK reaction. Mitochondrial AK isoform AK2 uses AMP passing through VDAC and ATP passing through ANT to produce ADP, which stimulates OXPHOS. These energy transport systems provide feedback between ATP consumption and synthesis. **(B)** Redox stress may induce an increased amount of HK bound to VDAC. In addition, VDAC can be truncated at C-terminus by proteases activated in response to oxidative stress. The role of tubulin in the regulation of VDAC permeability remains unclear, as the interaction of truncated VDAC with tubulin might be impaired. The AK2 activity in cancer cells is increased, resulting in enhanced utilization of extra-mitochondrial AMP to OXPHOS. IMM, inner mitochondrial membrane; IMS, intermembrane space.

rationale behind this model is the observation that proliferating cancer cells have high levels of free tubulin for mitotic spindle formation. Free tubulin dimers bound to VDAC induce a closed state of VDAC (**Figure 2**) and cause a suppression of mitochondrial metabolism; thus, aerobic glycolysis will become the main source of energy. Maldonado and Lemasters's group shows at HepG2, A549, and UM-SCC-1 cells that tubulin binding closes the VDAC channel (95). It sounds like the hypothesis in this review contradicts Maldonado's publications (95, 96). However, in fact, the results of both works are in agreement. The amount of dimeric and polymerized tubulin in cells is nearly constant, but the ratio could change significantly. In both cases it is dimeric tubulin, which affects VDAC permeability, but this effect depends on the polymerization state. Also, it should be definitely noted that the regulation of VDAC permeability is tissue specific. Unlike striated muscles, where the main regulator of VDAC is beta-II tubulin (97), in CRC the VDAC and beta-II tubulin colocalization is absent (6). Instead, beta-III tubulin (TUBB3) could be the partner of VDAC in CRC cells. Beta-III tubulin overexpression has been reported in several intestinal cancers like carcinoids of the small intestine and rectal carcinoids (98), gastric cancer (99), colon neoplasias like polyps, and CRC (6, 100). *TUBB3* expression has been associated with the resistance to drugs perturbing the microtubule dynamics (e.g., paclitaxel) and studied as a prognostic biomarker in various cancers (101, 102). It has been demonstrated that in non-small-cell lung cancer, the expression of beta-III tubulin decreases the dependence of cells on glycolysis and thus improves the tumor's ability to cope with the changing nutrient supply in the microenvironment (103). From a functional analysis of the network of proteins forming disulfide bonds with beta-III tubulin, it appears that some of them are involved in oxidative stress and glucose deprivation response (104). It was shown that hypoxia *via* HIF-1α can induce the expression of *TUBB3* (105). Beta-III tubulin is likely part of a complex pathway induced by hypoxia and shortage of nutrients (101). However, our recent study revealed that microtubule destabilizing (colchicine) and stabilizing (taxol) agents do not affect the Km(ADP) in glioblastoma and sarcoma cells (67). Hence, the actual role of beta-tubulins in cancer metabolism and mitochondrial respiratory control needs further investigation.

Regulation of VDAC1 by Protein-Protein Interactions and Redox Stress

In addition to the two previous models, the modifications of VDAC1 protein induced by oxidative stress could be responsible for alterations of apparent value of Km(ADP). Tumor cells are well adapted to a hypoxic environment, and VDAC1 is regulated by oxygen tension in HIF-1α-dependent manner at the levels of transcription and protein modification. Transcription of the *VDAC1* gene is regulated by HIF-1α and NRF-1 (nuclear respiratory factor 1), which leads to increased levels of VDAC1 in response to hypoxia or nutrient deprivation of the cells (106). Along with VDAC1 expression regulation, HIF-1α is also involved in the cleavage of VDAC1, resulting in a truncated form of VDAC1 (107). In normoxic conditions, VDAC1 is expressed as a full-length protein of molecular weight of approximately 30 kDa, while in response to hypoxia, there is a larger proportion of a shorter VDAC1 variant lacking C-terminal part (VDAC1-ΔC) with a molecular weight of approximately 25 kDa (107). The shorter variant is a product of the cleavage of VDAC1 at asparagine 214 by the asparagine endopeptidase Legumain (LGMN), which in turn is activated in a HIF-1α-dependent way upon hypoxia (107). The electrophysiological properties of VDAC1-ΔC are similar to full-length protein; however, its permeability is slightly reduced (107). Levels of VDAC1-ΔC were higher in late-stage lung tumors (107), and it was suggested that HIF-1α mediated induction of VDAC1-ΔC provides protection from apoptosis and enhances cell survival in hypoxia (107, 108). Hypoxia-induced VDAC1-ΔC lacks a phosphorylation site at serine 215, and therefore its interaction with tubulin is impaired (108). Notably, *HIF-1α* overexpression was significantly associated with higher CRC-specific mortality in a cohort of 731 patients (109). Consequently, inhibition of HIF-1α is proposed as a possible treatment strategy for CRC (110). Moreover, the expression of endopeptidase LGMN is elevated in CRC and is associated with a poor prognosis (111). Furthermore, a meta-analysis revealed the overexpression of *LGMN* to be correlated with the aggressiveness of different cancer types, with higher levels of LGMN in late-stage tumors (112).

It is currently unknown whether VDAC1-ΔC is present in CRC cells and whether truncation-induced impairment of VDAC1 interaction with tubulin affects apparent affinity for ADP (**Figure 2**). Given the role of tubulin in the regulation of VDAC1 and the discovery of VDAC1-ΔC in lung cancer, VDAC1 truncation may also play a role in metabolic alterations of CRC. Future studies should reveal whether the truncated form of VDAC1 plays a role in metabolic adaptations of CRC.

Recent studies indicate a link between iron-sulfur cluster (ISC) synthesis and regulation of VDAC1. Biogenesis of ISC is an ancient process, and ISCs are important redox-sensitive cofactors for many enzymes involved in energy homeostasis. Synthesis of ISC starts within the mitochondrial matrix, and depletion of proteins involved in mitochondrial ISC assembly leads to accumulation of VDAC1-ΔC in normoxic conditions independent of HIF-1α (113). Depletion of the iron-sulfur cluster containing protein CISD2 also resulted in the accumulation of truncated VDAC1-ΔC (113). Therefore, mitochondria-associated membrane-localized Fe-S protein CISD2 acts as a link between ISC machinery and accumulation of VDAC1-ΔC (113).

Another iron-sulfur cluster protein, mitoNEET, was found to interact with VDAC1 in a redox-sensitive way (114). MitoNEET harbors [2Fe-2S] cluster and binds to VDAC1 when its cluster is oxidized, thus inhibiting VDAC1 conductivity. Such interaction does not occur when mitoNEET-bound ISC cluster is reduced (114). Therefore, mitoNEET governs VDAC1 permeability in a redox-sensitive way, inhibiting VDAC1 in high redox stress conditions. Oxidative stress is increased in CRC (115); thus, the interaction of mitoNEET with VDAC1 can be altered in CRC.

It remains to be investigated whether such redox-sensitive mitoNEET-VDAC1 interaction can alter the apparent Km (ADP) value and is involved in the metabolic plasticity of CRC.

There is a large number of proteins that were found to interact with VDAC1 and are therefore potentially able to modulate VDAC permeability. Interacting partners of VDAC1 are involved in the regulation of apoptosis (Bax, Bcl2, Bak, etc.), energy metabolism (HK1, HK2, ACSL, CPT1, ANT, etc.), cytoskeletal organization (Tubulin, actin, dynein, etc.), and other cellular functions [Parkin, alpha-synuclein, APP, gamma-secretase) [reviewed in (116)]. However, the role of these interactions in the modulation of cellular respiration needs to be further investigated.

ENERGY TRANSPORT PATHWAYS IN CRC CELLS—THE PARTICIPANTS IN THE METABOLIC PLASTICITY

In addition to the altered transport of adenine nucleotides through OMM alterations of energy transport circuits formed from creatine kinase (CK) and adenylate kinase (AK) isoenzymes are also involved in the development of metabolic plasticity. Cancer cells have uncontrolled cell division, which is accompanied by a high energy need for anabolic processes and large cell structure rearrangements. Therefore, it is hypothesized that energy transport pathways are also reprogrammed in cancer cells to meet these demands. Previous data show downregulation of the CK pathway and mitochondrial CK (MtCK) in CRC cells, which results in functional uncoupling between the CK circuit and OXPHOS (6, 44). In contrast, total AK activity is higher in CRC than in normal intestinal tissue, and it also reflects enhanced coupling between AK and OXPHOS (i.e., AMP can affect the rate of oxygen consumption) (**Figure 2**) (6, 44). This is in agreement with the observation that expression of AK mitochondrial isoform AK2 is increased in several cancers including lung adenocarcinoma (117) and breast cancer (118, 119). Also, there is evidence that another mitochondrial isoform, AK4, is involved in the regulation of mitochondrial metabolism in cancer cells. In HeLa cells, AK4 forms complexes with ANT, VDAC, and HK2 for the efficient recycling of ADP (120). Further, AK4 expression is induced by hypoxia, and protein complex AK4-ANT-VDAC-HK2 complex supports the high glycolytic activity of cancer cells (120). Intestinal cells are able to switch off the CK circuit and turn on the AK pathway to establish metabolic plasticity. Such flexibility of phosphotransfer networks in Caco2 CRC cell lines depends on the availability of key metabolic substrates and is associated with the cell differentiation state (121). The abovementioned data indicate a possible role of the phosphotransfer networks related to the regulation of VDAC permeability for adenine nucleotides and metabolic plasticity.

The function of energy transfer pathways is well characterized in striated muscle cells where its role is to overcome the diffusion restrictions for ATP and ADP, thereby directing the energy-rich phosphate groups to the CK, AK, and glycolytic energy transfer circuits. This way of energy transfer allows the formation of micro-compartments at energy consumption sites where high ATP/ADP levels are maintained for maximal performance. Similarly, in the compartment where energy is produced (e.g., mitochondrial membranes), favorable levels of ADP are maintained to ensure efficient ATP synthesis [reviewed in (65, 122)]. In the case of CRC, downregulation of MtCK leads to the inability to produce phosphocreatine and a loss of functional coupling between the VDAC-MtCK-ANT complex, accompanied by the formation of other regulating combinations like VDAC-HK-ANT. In this aspect, more studies are required to determine the profile of HK, AK, ANT, and VDAC isoform expression in human CRC.

In addition to their role in energy transfer among cellular processes, AKs are an integral part of intracellular energy sensing and metabolic signaling (123, 124). Due to its catalytic reaction ($2ADP \leftrightarrow AMP + ATP$), it can amplify a small change in the ATP/ADP ratio into relatively large changes in AMP concentration. This relates AKs to the activation of cellular AMP-sensitive components like AMPK. In general, activation of AMPK switches on catabolic pathways that generate ATP, while switching off biosynthetic pathways and cell-cycle progress (125). The role of AMPK in cancer is controversial; it has been recognized as a tumor suppressor in some cancers (126–129) and in some cases described as a contextual oncogene, as the AMPK activation promotes tumor progression and chemoresistance (130–132). Downregulation of AK→ AMP→ AMPK signaling could lead to loss of control over the cell cycle, growth, and proliferation (124). A recent in-depth review about AKs and metabolic signaling in cancer cells by Klepinin et al. (124) highlights the role of suppression of AK phosphotransfer and signaling through AMPK as a potential target for cancer metabolism. How different AK isoforms are distributed in CRC cells and how their activities affect AMPK activation and metabolic plasticity need further investigation.

Adenylate kinases network promotes cancer growth and metastasis through participating in AMPK metabolic signaling and regulating mitochondrial adenine nucleotide exchange.

CONCLUSION AND PROSPECTS

Metabolic plasticity is a defining characteristic of the cancer cells that allow undisturbed proliferation in changing environment. At the functional level, different metabolic states of the cancer cells can be identified and characterized by measuring the dependence of mitochondrial respiration upon ADP concentration using the classical Michaelis-Menten kinetic model. The apparent affinity of ADP provides an integrated assessment of cell metabolic state, which is functionally determined by the permeability of VDAC1. Regulation of VDAC1 involves many protein-protein interactions, as well as hypoxia- and redox-sensitive mechanisms. The regulation of OMM permeability for adenine nucleotides is presumably more complex than the binding between the VDAC1 channel and some single type of protein molecule. Unraveling the molecular mechanisms of metabolic plasticity will reveal new therapeutic targets for the development of novel cancer treatments. This knowledge combined with relatively simple

functional evaluation of cancer metabolism in biopsy material can form a new prospect for personalized medicine.

AUTHOR CONTRIBUTIONS

Conceptualization, LT, MP, AT, and TK. Funding acquisition, TK. Project administration, AT and TK. Visualization, LR and IS. Writing—original draft, MP, AT, VC, and TK. Writing—review and editing, LR, SM, ER-K, NT, KT, IS, and TK. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Estonian Research Council grant PRG1035 and NICPB institutional Development Fund grant.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013
2. Lemeshko SV, Lemeshko VV. Metabolically Derived Potential on the Outer Membrane of Mitochondria: A Computational Model. *Biophys J* (2000) 79 (6):2785–800. doi: 10.1016/S0006-3495(00)76518-0
3. Lemeshko VV. Model of the Outer Membrane Potential Generation by the Inner Membrane of Mitochondria. *Biophys J* (2002) 82(2):684–92. doi: 10.1016/S0006-3495(02)75431-3
4. Moreno-Sanchez R, Marin-Hernandez A, Saavedra E, Pardo JP, Ralph SJ, Rodriguez-Enriquez S. Who Controls the ATP Supply in Cancer Cells? Biochemistry Lessons to Understand Cancer Energy Metabolism. *Int J Biochem Cell Biol* (2014) 50:10–23. doi: 10.1016/j.biocel.2014.01.025
5. Koit A, Shevchuk I, Ounpuu L, Klepinin A, Chekulayev V, Timohhina N, et al. Mitochondrial Respiration in Human Colorectal and Breast Cancer Clinical Material Is Regulated Differently. *Oxid Med Cell Longev* (2017) 2017:1372640. doi: 10.1155/2017/1372640
6. Kaldma A, Klepinin A, Chekulayev V, Mado K, Shevchuk I, Timohhina N, et al. An *in Situ* Study of Bioenergetic Properties of Human Colorectal Cancer: The Regulation of Mitochondrial Respiration and Distribution of Flux Control Among the Components of ATP Synthasome. *Int J Biochem Cell Biol* (2014) 55:171–86. doi: 10.1016/j.biocel.2014.09.004
7. Yu L, Lu M, Jia D, Ma J, Ben-Jacob E, Levine H, et al. Modeling the Genetic Regulation of Cancer Metabolism: Interplay Between Glycolysis and Oxidative Phosphorylation. *Cancer Res* (2017) 77(7):1564–74. doi: 10.1158/0008-5472.CAN-16-2074
8. Paudel BB, Quaranta V. Metabolic Plasticity Meets Gene Regulation. *Proc Natl Acad Sci USA* (2019) 116(9):3370–2. doi: 10.1073/pnas.1900169116
9. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science* (2009) 324(5930):1029–33. doi: 10.1126/science.1160809
10. Libby CJ, McConathy J, Darley-Usmar V, Hjelmeland AB. The Role of Metabolic Plasticity in Blood and Brain Stem Cell Pathophysiology. *Cancer Res* (2020) 80(1):5–16. doi: 10.1158/0008-5472.CAN-19-1169
11. Berridge MV, Herst PM, Tan AS. Metabolic Flexibility and Cell Hierarchy in Metastatic Cancer. *Mitochondrion* (2010) 10(6):584–8. doi: 10.1016/j.mito.2010.08.002
12. Garrigue P, Bodin-Hullin A, Balasse L, Fernandez S, Essamet W, Dignat-George F, et al. The Evolving Role of Succinate in Tumor Metabolism: An (18)F-FDG-Based Study. *J Nucl Med* (2017) 58(11):1749–55. doi: 10.2967/jnumed.117.192674
13. Lin Y, Ma C, Bezabeh T, Wang Z, Liang J, Huang Y, et al. 1h NMR-Based Metabolomics Reveal Overlapping Discriminatory Metabolites and Metabolic Pathway Disturbances Between Colorectal Tumor Tissues and Fecal Samples. *Int J Cancer* (2019) 145(6):1679–89. doi: 10.1002/ijc.32190
14. Jia D, Park JH, Jung KH, Levine H, Kaipparettu BA. Elucidating the Metabolic Plasticity of Cancer: Mitochondrial Reprogramming and Hybrid Metabolic States. *Cells* (2018) 7(3):21. doi: 10.3390/cells7030021
15. Jia D, Lu M, Jung KH, Park JH, Yu L, Onuchic JN, et al. Elucidating Cancer Metabolic Plasticity by Coupling Gene Regulation With Metabolic Pathways. *Proc Natl Acad Sci* (2019) 116(9):3909–18. doi: 10.1073/pnas.1816391116
16. Sotgia F, Ozsvari B, Fiorillo M, De Francesco EM, Bonuccelli G, Lisanti MP. A Mitochondrial Based Oncology Platform for Targeting Cancer Stem Cells (CSCs): MITO-ONC-Rx. *Cell Cycle* (2018) 17(17):2091–100. doi: 10.1080/15384101.2018.1515551
17. Altenberg B, Greulich KO. Genes of Glycolysis Are Ubiquitously Overexpressed in 24 Cancer Classes. *Genomics* (2004) 84(6):1014–20. doi: 10.1016/j.ygeno.2004.08.010
18. Kawada K, Toda K, Nakamoto Y, Iwamoto M, Hatano E, Chen FS, et al. Relationship Between F-18-FDG PET/CT Scans and KRAS Mutations in Metastatic Colorectal Cancer. *J Nucl Med* (2015) 56(9):1322–7. doi: 10.2967/jnumed.115.160614
19. Iwamoto M, Kawada K, Nakamoto Y, Itatani Y, Inamoto S, Toda K, et al. Regulation of 18F-FDG Accumulation in Colorectal Cancer Cells With Mutated KRAS. *J Nucl Med* (2014) 55(12):2038–44. doi: 10.2967/jnumed.114.142927
20. Rubie C, Kempf K, Hans J, Su T, Tilton B, Georg T, et al. Housekeeping Gene Variability in Normal and Cancerous Colorectal, Pancreatic, Esophageal, Gastric and Hepatic Tissues. *Mol Cell Probes* (2005) 19(2):101–9. doi: 10.1016/j.mcp.2004.10.001
21. Shonk CE, Arison RN, Koven BJ, Majima H, Boxer GE. Enzyme Patterns in Human Tissues. 3. Glycolytic Enzymes in Normal and Malignant Tissues of the Colon and Rectum. *Cancer Res* (1965) 25:206–13.
22. Hennipman A, Smits J, van Oirschot B, van Houwelingen JC, Rijksen G, Neyt JP, et al. Glycolytic Enzymes in Breast Cancer, Benign Breast Disease and Normal Breast Tissue. *Tumor Biol* (1987) 8(5):251–63. doi: 10.1159/000217529
23. Hennipman A, van Oirschot BA, Smits J, Rijksen G, Staal GEJ. Glycolytic Enzyme Activities in Breast Cancer Metastases. *Tumor Biol* (1988) 9(5):241–8. doi: 10.1159/000217568
24. Groheux D, Cochet A, Humbert O, Alberini J-L, Hindié E, Mankoff D. 18f-FDG PET/CT for Staging and Restaging of Breast Cancer. *J Nucl Med* (2016) 57(Supplement 1):17S–26S. doi: 10.2967/jnumed.115.157859
25. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, et al. Quantitative Metabolome Profiling of Colon and Stomach Cancer Microenvironment by Capillary Electrophoresis Time-of-Flight Mass Spectrometry. *Cancer Res* (2009) 69(11):4918–25. doi: 10.1158/0008-5472.CAN-08-4806
26. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The Microbiome and Butyrate Regulate Energy Metabolism and Autophagy in the Mammalian Colon. *Cell Metab* (2011) 13(5):517–26. doi: 10.1016/j.cmet.2011.02.018
27. Fleming SE, Fitch MD, DeVries S, Liu ML, Kight C. Nutrient Utilization by Cells Isolated From Rat Jejunum, Cecum and Colon. *J Nutr* (1991) 121 (6):869–78. doi: 10.1093/jn/121.6.869
28. Donohoe DR, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. : The Warburg Effect Dictates the Mechanism of Butyrate-Mediated Histone Acetylation and Cell Proliferation. *Mol Cell* (2012) 48(4):612–26. doi: 10.1016/j.molcel.2012.08.033
29. Martinez-Reyes I, Chandel NS. Mitochondrial TCA Cycle Metabolites Control Physiology and Disease. *Nat Commun* (2020) 11(1):102. doi: 10.1038/s41467-019-13668-3
30. Rodriguez-Enriquez S, Marin-Hernandez A, Gallardo-Perez JC, Pacheco-Velazquez SC, Belmont-Diaz JA, Robledo-Cadena DX, et al. Transcriptional Regulation of Energy Metabolism in Cancer Cells. *Cells* (2019) 8(10):1225. doi: 10.3390/cells8101225

31. Neitzel C, Demuth P, Wittmann S, Fahrer J. Targeting Altered Energy Metabolism in Colorectal Cancer: Oncogenic Reprogramming, the Central Role of the TCA Cycle and Therapeutic Opportunities. *Cancers (Basel)* (2020) 12(7):1731. doi: 10.3390/cancers12071731
32. Epstein T, Gatenby RA, Brown JS. The Warburg Effect as an Adaptation of Cancer Cells to Rapid Fluctuations in Energy Demand. *PloS One* (2017) 12 (9):e0185085–e0185085. doi: 10.1371/journal.pone.0185085
33. Brown RE, Short SP, Williams CS. Colorectal Cancer and Metabolism. *Curr Colorectal Cancer Rep* (2018) 14(6):226–41. doi: 10.1007/s11888-018-0420-y
34. Gandhi N, Das MG. Metabolic Reprogramming in Breast Cancer and Its Therapeutic Implications. *Cells* (2019) 8(2):89. doi: 10.3390/cells8020089
35. Dai C, Arceo J, Arnold J, Sreekumar A, Dovichi NJ, Li J, et al. Metabolomics of Oncogene-Specific Metabolic Reprogramming During Breast Cancer. *Cancer Metab* (2018) 6:5. doi: 10.1186/s40170-018-0175-6
36. D'Esposito V, Ambrosio MR, Giuliano M, Cabaro S, Miele C, Beguinot F, et al. Mammary Adipose Tissue Control of Breast Cancer Progression: Impact of Obesity and Diabetes. *Front Oncol* (2020) 10(1554):1554. doi: 10.3389/fonc.2020.01554
37. Tian W, Zhang W, Zhang Y, Zhu T, Hua Y, Li H, et al. FABP4 Promotes Invasion and Metastasis of Colon Cancer by Regulating Fatty Acid Transport. *Cancer Cell Int* (2020) 20(1):512. doi: 10.1186/s12935-020-01582-4
38. Swerdlow RH, L E, Aires D, Lu J. Glycolysis-Respiration Relationships in a Neuroblastoma Cell Line. *Biochim Biophys Acta* (2013) 1830(4):2891–8. doi: 10.1016/j.bbagen.2013.01.002
39. Jose C, Rossignol R. Rationale for Mitochondria-Targeting Strategies in Cancer Bioenergetic Therapies. *Int J Biochem Cell Biol* (2013) 45(1):123–9. doi: 10.1016/j.biocel.2012.07.005
40. Gnaiger E, Kemp RB. Anaerobic Metabolism in Aerobic Mammalian Cells: Information From the Ratio of Calorimetric Heat Flux and Respirometric Oxygen Flux. *Biochim Biophys Acta* (1990) 1016(3):328–32. doi: 10.1016/0005-2728(90)90164-y
41. Gstraunthaler G, Seppi T, Pfaller W. Impact of Culture Conditions, Culture Media Volumes, and Glucose Content on Metabolic Properties of Renal Epithelial Cell Cultures. Are Renal Cells in Tissue Culture Hypoxic? *Cell Physiol Biochem* (1999) 9(3):150–72. doi: 10.1159/000016312
42. Sherr CJ, DePinho RA. Cellular Senescence: Mitotic Clock or Culture Shock? *Cell* (2000) 102(4):407–10. doi: 10.1016/s0092-8674(00)00046-5
43. Ounpuu L, Truu L, Shevchuk I, Chekulayev V, Klepinin A, Koit A, et al. Comparative Analysis of the Bioenergetics of Human Adenocarcinoma Caco-2 Cell Line and Postoperative Tissue Samples From Colorectal Cancer Patients. *Biochem Cell Biol* (2018) 96(6):808–17. doi: 10.1139/bcb-2018-0076
44. Chekulayev V, Mado K, Shevchuk I, Koit A, Kaldma A, Klepinin A, et al. Metabolic Remodeling in Human Colorectal Cancer and Surrounding Tissues: Alterations in Regulation of Mitochondrial Respiration and Metabolic Fluxes. *Biochem Biophys Rep* (2015) 4:111–25. doi: 10.1016/j.bbrep.2015.08.020
45. Kaambre T, Chekulayev V, Shevchuk I, Karu-Varikmaa M, Timohhina N, Tepp K, et al. Metabolic Control Analysis of Cellular Respiration *in Situ* in Intraoperational Samples of Human Breast Cancer. *J Bioenerg Biomembr* (2012) 44(5):539–58. doi: 10.1007/s10863-012-9457-9
46. Koit A, Timohhina N, Truu L, Chekulayev V, Gudlawar S, Shevchuk I, et al. Metabolic and OXPHOS Activities Quantified by Temporal Ex Vivo Analysis Display Patient-Specific Metabolic Vulnerabilities in Human Breast Cancers. *Front Oncol* (2020) 10:1053. doi: 10.3389/fonc.2020.01053
47. Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, et al. Mitochondrial Respiration in KRAS and BRAF Mutated Colorectal Tumors and Polyps. *Cancers (Basel)* (2020) 12(4):815. doi: 10.3390/cancers12040815
48. Feng S, Xiong LL, Ji ZN, Cheng W, Yang HJ. Correlation Between Increased Copy Number of Mitochondrial DNA and Clinicopathological Stage in Colorectal Cancer. *Oncol Lett* (2011) 2(5):899–903. doi: 10.3892/ol.2011.322
49. Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, et al. Oxidants in Mitochondria: From Physiology to Diseases. *Biochim Biophys Acta* (1995) 1271(1):67–74. doi: 10.1016/0925-4439(95)00012-S
50. Lee HC, Yin PH, Chi CW, Wei YH. Increase in Mitochondrial Mass in Human Fibroblasts Under Oxidative Stress and During Replicative Cell Senescence. *J BioMed Sci* (2002) 9(6 Pt 1):517–26. doi: 10.1007/BF02254978
51. Lee HC, Yin PH, Lu CY, Chi CW, Wei YH. Increase of Mitochondria and Mitochondrial DNA in Response to Oxidative Stress in Human Cells. *Biochem J* (2000) 348 Pt 2:425–32. doi: 10.1042/bj3480425
52. Genders AJ, Martin SD, McGee SL, Bishop DJ. A Physiological Drop in pH Decreases Mitochondrial Respiration, and HDAC and Akt Signaling, in L6 Myocytes. *Am J Physiol Cell Physiol* (2019) 316(3):C404–14. doi: 10.1152/ajpcell.00214.2018
53. Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an Energy Sensing Network That Controls Energy Expenditure. *Curr Opin Lipidol* (2009) 20 (2):98–105. doi: 10.1097/MOL.0b013e328328d0a4
54. Das KJ, Felty Q, Poppiti R, Jackson MR, Roy D. Nuclear Respiratory Factor 1 Acting as an Oncoprotein Drives Estrogen-Induced Breast Carcinogenesis. *Cells* (2018) 7(12):234. doi: 10.3390/cells7120234
55. Wang G, Wang Q, Huang Q, Chen Y, Sun X, He L, et al. Upregulation of mtSSB by Interleukin-6 Promotes Cell Growth Through Mitochondrial Biogenesis-Mediated Telomerase Activation in Colorectal Cancer. *Int J Cancer* (2018) 144:2516–28. doi: 10.1002/ijc.31978
56. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of Interleukin-6 in Cancer Progression and Therapeutic Resistance. *Tumor Biol* (2016) 37 (9):11553–72. doi: 10.1007/s13277-016-5098-7
57. Kumari N, Das A, Bhatt AN. Interleukin-6 Confers Radio-Resistance by Inducing Akt-Mediated Glycolysis and Reducing Mitochondrial Damage in Cells. *J Biochem* (2019) 167(3):303–14. doi: 10.1093/jb/mvz091
58. Ham I-H, Oh HJ, Jin H, Bae CA, Jeon S-M, Choi KS, et al. Targeting Interleukin-6 as a Strategy to Overcome Stroma-Induced Resistance to Chemotherapy in Gastric Cancer. *Mol Cancer* (2019) 18(1):68. doi: 10.1186/s12943-019-0972-8
59. Berridge MV, McConnell MJ, Grasso C, Bajzikova M, Kovarova J, Neuzil J. Horizontal Transfer of Mitochondria Between Mammalian Cells: Beyond Co-Culture Approaches. *Curr Opin Genet Dev* (2016) 38:75–82. doi: 10.1016/j.gde.2016.04.003
60. Sahinbegovic H, Jelinek T, Hrdinka M, Bago JR, Turi M, Sevcikova T, et al. Intercellular Mitochondrial Transfer in the Tumor Microenvironment. *Cancers (Basel)* (2020) 12(7):1787. doi: 10.3390/cancers12071787
61. Lemasters JJ, Holmuhamedov E. Voltage-Dependent Anion Channel (VDAC) as Mitochondrial Governator–Thinking Outside the Box. *Biochim Biophys Acta* (2006) 1762(2):181–90. doi: 10.1016/j.bbadis.2005.10.006
62. Guzun R, Kaambre T, Bagur R, Grichine A, Usson Y, Varikmaa M, et al. Modular Organization of Cardiac Energy Metabolism: Energy Conversion, Transfer and Feedback Regulation. *Acta Physiol (Oxf)* (2015) 213(1):84–106. doi: 10.1111/apha.12287
63. Saks V, Guzun R, Timohhina N, Tepp K, Varikmaa M, Monge C, et al. Structure-Function Relationships in Feedback Regulation of Energy Fluxes *In Vivo* in Health and Disease: Mitochondrial Interactosome. *Biochim Biophys Acta* (2010) 1797(6-7):678–97. doi: 10.1016/j.bbabio.2010.01.011
64. Kuznetsov AV, Tiivel T, Sikk P, Kaambre T, Kay L, Daneshrad Z, et al. Striking Differences Between the Kinetics of Regulation of Respiration by ADP in Slow-Twitch and Fast-Twitch Muscles *In Vivo*. *Eur J Biochem* (1996) 241(3):909–15. doi: 10.1111/j.1432-1033.1996.00909.x
65. Puurand M, Tepp K, Klepinin A, Klepinina L, Shevchuk I, Kaambre T. Intracellular Energy-Transfer Networks and High-Resolution Respirometry: A Convenient Approach for Studying Their Function. *Int J Mol Sci* (2018) 19 (10):2933. doi: 10.3390/ijms19102933
66. Kay L, Li Z, Mericskay M, Olivares J, Tranqui L, Fontaine E, et al. Study of Regulation of Mitochondrial Respiration *In Vivo*. An Analysis of Influence of ADP Diffusion and Possible Role of Cytoskeleton. *Biochim Biophys Acta* (1997) 1322(1):41–59. doi: 10.1016/s0005-2728(97)00071-6
67. Klepinin A, Ounpuu L, Mado K, Truu L, Chekulayev V, Puurand M, et al. The Complexity of Mitochondrial Outer Membrane Permeability and VDAC Regulation by Associated Proteins. *J Bioenerg Biomembr* (2018) 50 (5):339–54. doi: 10.1007/s10863-018-9765-9
68. Puurand M, Tepp K, Timohhina N, Aid J, Shevchuk I, Chekulayev V, et al. Tubulin betaII and betaIII Isoforms as the Regulators of VDAC Channel Permeability in Health and Disease. *Cells* (2019) 8(3):239. doi: 10.3390/cells8030239

69. Saks VA, Belikova YO, Kuznetsov AV. *In Vivo* Regulation of Mitochondrial Respiration in Cardiomyocytes: Specific Restrictions for Intracellular Diffusion of ADP. *Biochim Biophys Acta* (1991) 1074(2):302–11. doi: 10.1016/0304-4165(91)90168-g
70. Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, et al. Permeabilized Cell and Skinned Fiber Techniques in Studies of Mitochondrial Function *In Vivo*. *Mol Cell Biochem* (1998) 184(1-2):81–100. doi: 10.1023/A:1006834912257
71. Messina A, Reina S, Guarino F, De Pinto V. VDAC Isoforms in Mammals. *Biochim Biophys Acta (BBA) - Biomembr* (2012) 1818(6):1466–76. doi: 10.1016/j.bbamem.2011.10.005
72. Shoshan-Barmatz V, Mizrachi D. VDAC1: From Structure to Cancer Therapy. *Front Oncol* (2012) 2:164. doi: 10.3389/fonc.2012.00164
73. Shoshan-Barmatz V, Ben-Hail D, Admoni L, Krelin Y, Tripathi SS. The Mitochondrial Voltage-Dependent Anion Channel 1 in Tumor Cells. *Biochim Biophys Acta (BBA) - Biomembr* (2015) 1848(10, Part B):2547–75. doi: 10.1016/j.bbamem.2014.10.040
74. Mangan PS, Colombini M. Ultrasteep Voltage Dependence in a Membrane Channel. *Proc Natl Acad Sci* (1987) 84(14):4896. doi: 10.1073/pnas.84.14.4896
75. Colombini M, Mannella CA. VDAC, The Early Days. *Biochim Biophys Acta (BBA) - Biomembr* (2012) 1818(6):1438–43. doi: 10.1016/j.bbamem.2011.11.014
76. Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: Cancer's Double-Edged Sword Acting as Both Facilitator and Gatekeeper of Malignancy When Bound to Mitochondria. *Oncogene* (2006) 25(34):4777–86. doi: 10.1038/sj.onc.1209603
77. Pedersen PL. Warburg, Me and Hexokinase 2: Multiple Discoveries of Key Molecular Events Underlying One of Cancers' Most Common Phenotypes, the "Warburg Effect", I.e., elevated glycolysis in the presence of oxygen. *J Bioenerg Biomembr* (2007) 39(3):211–22. doi: 10.1007/s10863-007-9094-x
78. Pedersen PL. The Cancer Cell's "Power Plants" as Promising Therapeutic Targets: An Overview. *J Bioenerg Biomembr* (2007) 39(1):1–12. doi: 10.1007/s10863-007-9070-5
79. Mathupala SP, Ko YH, Pedersen PL. Hexokinase-2 Bound to Mitochondria: Cancer's Stygian Link to the "Warburg Effect" and a Pivotal Target for Effective Therapy. *Semin Cancer Biol* (2009) 19(1):17–24. doi: 10.1016/j.semcancer.2008.11.006
80. Mathupala SP, Pedersen PL. Voltage Dependent Anion Channel-1 (VDAC-1) as an Anti-Cancer Target. *Cancer Biol Ther* (2010) 9(12):1053–6. doi: 10.4161/cbt.9.12.12451
81. Shoshan-Barmatz V, Maldonado EN, Krelin Y. VDAC1 at the Crossroads of Cell Metabolism, Apoptosis and Cell Stress. *Cell Stress* (2017) 1(1):11–36. doi: 10.15698/cst2017.10.104
82. Neumann D, Buckers J, Kastrup L, Hell SW, Jakobs S. Two-Color STED Microscopy Reveals Different Degrees of Colocalization Between Hexokinase-I and the Three Human VDAC Isoforms. *PMC Biophys* (2010) 3(1):4. doi: 10.1186/1757-5036-3-4
83. Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH. Mitochondrial Bound Type II Hexokinase: A Key Player in the Growth and Survival of Many Cancers and an Ideal Prospect for Therapeutic Intervention. *Biochim Biophys Acta* (2002) 1555(1-3):14–20. doi: 10.1016/S0005-2728(02)00248-7
84. Rostovtseva TK, Tan W, Colombini M. On the Role of VDAC in Apoptosis: Fact and Fiction. *J Bioenerg Biomembr* (2005) 37(3):129–42. doi: 10.1007/s10863-005-6566-8
85. Majewski N, Nogueira V, Bhaskar P, Coy PE, Skeen JE, Gottlob K, et al. Hexokinase-Mitochondria Interaction Mediated by Akt Is Required to Inhibit Apoptosis in the Presence or Absence of Bax and Bak. *Mol Cell* (2004) 16(5):819–30. doi: 10.1016/j.molcel.2004.11.014
86. Eimre M, Paju K, Pelloux S, Beraud N, Roosimaa M, Kadaja L, et al. Distinct Organization of Energy Metabolism in HL-1 Cardiac Cell Line and Cardiomyocytes. *Biochim Biophys Acta* (2008) 1777(6):514–24. doi: 10.1016/j.bbabio.2008.03.019
87. Ahmad A, Ahmad S, Schneider BK, Allen CB, Chang LY, White CW. Elevated Expression of Hexokinase II Protects Human Lung Epithelial-Like A549 Cells Against Oxidative Injury. *Am J Physiol Lung Cell Mol Physiol* (2002) 283(3):L573–84. doi: 10.1152/ajplung.00410.2001
88. Zaid H, Abu-Hamad S, Israelson A, Nathan I, Shoshan-Barmatz V. The Voltage-Dependent Anion Channel-1 Modulates Apoptotic Cell Death. *Cell Death Differ* (2005) 12(7):751–60. doi: 10.1038/sj.cdd.4401599
89. Pastorino J, Hoek J. Regulation of Hexokinase Binding to VDAC. *J Bioenerg Biomembr* (2008) 40(3):171–82. doi: 10.1007/s10863-008-9148-8
90. Wei L, Zhou Y, Dai Q, Qiao C, Zhao L, Hui H, et al. Oroxylin A Induces Dissociation of Hexokinase II From the Mitochondria and Inhibits Glycolysis by SIRT3-Mediated Deacetylation of Cyclophilin D in Breast Carcinoma. *Cell Death Dis* (2013) 4:e601. doi: 10.1038/cddis.2013.131
91. Ikeda S, Abe F, Matsuda Y, Kitadate A, Takahashi N, Tagawa H. Hypoxia-Inducible Hexokinase-2 Enhances Anti-Apoptotic Function via Activating Autophagy in Multiple Myeloma. *Cancer Sci* (2020) 111:4088–101. doi: 10.1111/cas.14614
92. Fan T, Sun G, Sun X, Zhao L, Zhong R, Peng Y. Tumor Energy Metabolism and Potential of 3-Bromopyruvate as an Inhibitor of Aerobic Glycolysis: Implications in Tumor Treatment. *Cancers (Basel)* (2019) 11(3):317. doi: 10.3390/cancers11030317
93. Woldetsadik AD, Vogel MC, Rabeh WM, Magzoub M. Hexokinase II–derived Cell-Penetrating Peptide Targets Mitochondria and Triggers Apoptosis in Cancer Cells. *FASEB J* (2017) 31(5):2168–84. doi: 10.1096/fj.201601173R
94. Arif T, Vasilkovsky L, Refaely Y, Konson A, Shoshan-Barmatz V. Silencing VDAC1 Expression by siRNA Inhibits Cancer Cell Proliferation and Tumor Growth *In Vivo*. *Mol Ther - Nucleic Acids* (2014) 3:e159. doi: 10.1038/mtna.2014.9
95. Maldonado EN, Patnaik J, Mullins MR, Lemasters JJ. Free Tubulin Modulates Mitochondrial Membrane Potential in Cancer Cells. *Cancer Res* (2010) 70(24):10192–201. doi: 10.1158/0008-5472.CAN-10-2429
96. Maldonado EN, Lemasters JJ. ATP/ADP Ratio, the Missed Connection Between Mitochondria and the Warburg Effect. *Mitochondrion* (2014) 19 Pt A:78–84. doi: 10.1016/j.mito.2014.09.002
97. Varikmaa M, Bagur R, Kaambre T, Grichine A, Timohhina N, Tepp K, et al. Role of Mitochondria-Cytoskeleton Interactions in Respiration Regulation and Mitochondrial Organization in Striated Muscles. *Biochim Biophys Acta* (2014) 1837(2):232–45. doi: 10.1016/j.bbabio.2013.10.011
98. Jirasek T, Mandys V, Viklicky V. Expression of Class III Beta-Tubulin in Neuroendocrine Tumours of Gastrointestinal Tract. *Folia Histochem Cytobiol* (2002) 40(3):305–10.
99. Urano N, Fujiwara Y, Doki Y, Kim SJ, Miyoshi Y, Noguchi S, et al. Clinical Significance of Class III Beta-Tubulin Expression and Its Predictive Value for Resistance to Docetaxel-Based Chemotherapy in Gastric Cancer. *Int J Oncol* (2006) 28(2):375–81. doi: 10.3892/ijo.28.2.375
100. Oztop S, Isik A, Guner G, Gurdal H, Karabulut E, Yilmaz E, et al. Class III Beta-Tubulin Expression in Colorectal Neoplasms Is a Potential Predictive Biomarker for Paclitaxel Response. *Anticancer Res* (2019) 39(2):655–62. doi: 10.21873/anticanres.13160
101. Mariani M, Karki R, Spennato M, Pandya D, He S, Andreoli M, et al. Class III Beta-Tubulin in Normal and Cancer Tissues. *Gene* (2015) 563(2):109–14. doi: 10.1016/j.gene.2015.03.061
102. Mariani M, Shahabi S, Sieber S, Scambia G, Ferlini C. Class III Beta-Tubulin (TUBB3): More Than a Biomarker in Solid Tumors? *Curr Mol Med* (2011) 11(9):726–31. doi: 10.2174/156652411798062368
103. Parker AL, Turner N, McCarroll JA, Kavallaris M. betaIII-Tubulin Alters Glucose Metabolism and Stress Response Signaling to Promote Cell Survival and Proliferation in Glucose-Starved Non-Small Cell Lung Cancer Cells. *Carcinogenesis* (2016) 37(8):787–98. doi: 10.1093/carcin/bgw058
104. Cicchillitti L, Penci R, Di Michele M, Filippetti F, Rotilio D, Donati MB, et al. Proteomic Characterization of Cytoskeletal and Mitochondrial Class III Beta-Tubulin. *Mol Cancer Ther* (2008) 7(7):2070–9. doi: 10.1158/1535-7163.MCT-07-2370
105. Raspaglio G, Filippetti F, Prislei S, Penci R, De Maria I, Cicchillitti L, et al. Hypoxia Induces Class III Beta-Tubulin Gene Expression by HIF-1alpha Binding to its 3' Flanking Region. *Gene* (2008) 409(1-2):100–8. doi: 10.1016/j.gene.2007.11.015
106. Guarino F, Zinghirino F, Mela L, Pappalardo XG, Ichas F, De Pinto V, et al. NRF-1 and HIF-1alpha Contribute to Modulation of Human VDAC1 Gene Promoter During Starvation and Hypoxia in HeLa Cells. *Biochim Biophys Acta Bioenerg* (2020) 1861(12):148289. doi: 10.1016/j.bbabio.2020.148289

107. Brahimi-Horn MC, Ben-Hail D, Ilie M, Gounon P, Rouleau M, Hofman V, et al. Expression of a Truncated Active Form of VDAC1 in Lung Cancer Associates With Hypoxic Cell Survival and Correlates With Progression to Chemotherapy Resistance. *Cancer Res* (2012) 72(8):2140–50. doi: 10.1158/0008-5472.CAN-11-3940
108. Meyenberg Cunha-de Padua M, Fabbri L, Dufies M, Lacas-Gervais S, Contenti J, Voyton C, et al. Evidences of a Direct Relationship Between Cellular Fuel Supply and Ciliogenesis Regulated by Hypoxic VDAC1-DeltaC. *Cancers (Basel)* (2020) 12(11):3484. doi: 10.3390/cancers12113484
109. Baba Y, Nosho K, Shima K, Irahara N, Chan AT, Meyerhardt JA, et al. HIF1A Overexpression is Associated With Poor Prognosis in a Cohort of 731 Colorectal Cancers. *Am J Pathol* (2010) 176(5):2292–301. doi: 10.2353/ajpath.2010.090972
110. Ioannou M, Paraskeva E, Baxevanidou K, Simos G, Papamichali R, Papacharalambous C, et al. HIF-1alpha in Colorectal Carcinoma: Review of the Literature. *J BUON* (2015) 20(3):680–9.
111. Haugen MH, Boye K, Nesland JM, Pettersen SJ, Egeland EV, Tamhane T, et al. High Expression of the Cysteine Proteinase Legumain in Colorectal Cancer - Implications for Therapeutic Targeting. *Eur J Cancer* (2015) 51(1):9–17. doi: 10.1016/j.ejca.2014.10.020
112. Zhen Y, Chunlei G, Wenzhi S, Shuangtao Z, Na L, Rongrong W, et al. Clinicopathologic Significance of Legumain Overexpression in Cancer: A Systematic Review and Meta-Analysis. *Sci Rep* (2015) 5:16599. doi: 10.1038/srep16599
113. Ferecatu I, Canal F, Fabbri L, Mazure NM, Bouton C, Golinelli-Cohen MP. Dysfunction in the Mitochondrial Fe-S Assembly Machinery Leads to Formation of the Chemoresistant Truncated VDAC1 Isoform Without HIF-1alpha Activation. *PloS One* (2018) 13(3):e0194782. doi: 10.1371/journal.pone.0194782
114. Lipper CH, Stofleth JT, Bai F, Sohn YS, Roy S, Mittler R, et al. Redox-Dependent Gating of VDAC by mitoNEET. *Proc Natl Acad Sci USA* (2019) 116(40):19924–9. doi: 10.1073/pnas.1908271116
115. Basak D, Uddin MN, Hancock J. The Role of Oxidative Stress and Its Counteractive Utility in Colorectal Cancer (CRC). *Cancers (Basel)* (2020) 12(11):3336. doi: 10.3390/cancers12113336
116. Caterino M, Ruoppolo M, Mandola A, Costanzo M, Orru S, Imperlini E. Protein-Protein Interaction Networks as a New Perspective to Evaluate Distinct Functional Roles of Voltage-Dependent Anion Channel Isoforms. *Mol Biosyst* (2017) 13(12):2466–76. doi: 10.1039/c7mb00434f
117. Liu H, Pu Y, Amina Q, Wang Q, Zhang M, Song J, et al. Prognostic and Therapeutic Potential of Adenylate Kinase 2 in Lung Adenocarcinoma. *Sci Rep* (2019) 9(1):17757. doi: 10.1038/s41598-019-53594-4
118. Klepinin A, Ounpuu L, Guzun R, Chekulayev V, Timohhina N, Tepp K, et al. Simple Oxygraphic Analysis for the Presence of Adenylate Kinase 1 and 2 in Normal and Tumor Cells. *J Bioenerg Biomembr* (2016) 48(5):531–48. doi: 10.1007/s10863-016-9687-3
119. Speers C, Tsimelzon A, Sexton K, Herrick AM, Gutierrez C, Culhane A, et al. Identification of Novel Kinase Targets for the Treatment of Estrogen Receptor-Negative Breast Cancer. *Clin Cancer Res* (2009) 15(20):6327–40. doi: 10.1158/1078-0432.CCR-09-1107
120. Fujisawa K, Terai S, Takami T, Yamamoto N, Yamasaki T, Matsumoto T, et al. Modulation of Anti-Cancer Drug Sensitivity Through the Regulation of Mitochondrial Activity by Adenylate Kinase 4. *J Exp Clin Cancer Res* (2016) 35:48. doi: 10.1186/s13046-016-0322-2
121. Klepinina L, Klepinin A, Truu L, Chekulayev V, Vija H, Kuus K, et al. Colon Cancer Cell Differentiation by Sodium Butyrate Modulates Metabolic Plasticity of Caco-2 Cells *via* Alteration of Phosphotransfer Network. *PloS One* (2021) 16(1):e0245348. doi: 10.1371/journal.pone.0245348
122. Dzeja PP, Terzic A. Phosphotransfer Networks and Cellular Energetics. *J Exp Biol* (2003) 206(Pt 12):2039–47. doi: 10.1242/jeb.00426
123. Dzeja P, Terzic A. Adenylate Kinase and AMP Signaling Networks: Metabolic Monitoring, Signal Communication and Body Energy Sensing. *Int J Mol Sci* (2009) 10(4):1729–72. doi: 10.3390/ijms10041729
124. Klepinin A, Zhang S, Klepinina L, Rebane-Klemm E, Terzic A, Kaambre T, et al. Adenylate Kinase and Metabolic Signaling in Cancer Cells. *Front Oncol* (2020) 10:660. doi: 10.3389/fonc.2020
125. Hardie DG, Ross FA, Hawley SA. AMPK: A Nutrient and Energy Sensor That Maintains Energy Homeostasis. *Nat Rev Mol Cell Biol* (2012) 13(4):251–62. doi: 10.1038/nrm3311
126. Wang W, Guan KL. AMP-Activated Protein Kinase and Cancer. *Acta Physiol* (2009) 196(1):55–63. doi: 10.1111/j.1748-1716.2009.01980.x
127. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, et al. AMPK Is a Negative Regulator of the Warburg Effect and Suppresses Tumor Growth *In Vivo*. *Cell Metab* (2013) 17(1):113–24. doi: 10.1016/j.cmet.2012.12.001
128. Rehman G, Shehzad A, Khan AL, Hamayun M. Role of AMP-Activated Protein Kinase in Cancer Therapy. *Archiv der Pharm* (2014) 347(7):457–68. doi: 10.1002/ardp.201300402
129. Hardie DG, Ross FA, Hawley SA. AMP-Activated Protein Kinase: A Target for Drugs Both Ancient and Modern. *Chem Biol* (2012) 19(10):1222–36. doi: 10.1016/j.chembiol.2012.08.019
130. Park HU, Suy S, Danner M, Dailey V, Zhang Y, Li H, et al. AMP-Activated Protein Kinase Promotes Human Prostate Cancer Cell Growth and Survival. *Mol Cancer Ther* (2009) 8(4):733–41. doi: 10.1158/1535-7163.MCT-08-0631
131. Khan AS, Frigo DE. Regulation, Role and Therapeutic Targeting of AMPK in Prostate Cancer. *Nat Rev Urol* (2017) 14(3):164–80. doi: 10.1038/nrurol.2016.272
132. Huang X, Li X, Xie X, Ye F, Chen B, Song C, et al. High Expressions of LDHA and AMPK as Prognostic Biomarkers for Breast Cancer. *Breast* (2016) 30:39–46. doi: 10.1016/j.breast.2016.08.014

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Curriculum vitae

Personal data

Name:	Egle Rebane-Klemm
Date of birth:	27.10.1983
Place of birth:	Tartu
Citizenship:	Estonia

Contact data

E-mail:	egle.rebane@gmail.com

Education

2017–2023	Tallinn University of Technology, PhD study
2005–2009	Tartu University, MSc, Biomedicine
2002–2005	Tartu University, BSc, Biochemistry
1990–2002	Tartu Tamme Gymnasium

Language competence

Estonia	Native language
English	Fluent
Russian	Basic

Professional employment

2020– …	Swixx Biopharma, Medical Advisor
2017–2020	Sanofi, Baltic Medical Advisor in Rare Disease
2016–2017	Pfizer, Medical Advisor in Oncology
2007–2015	IB Genetics, Head of Laboratory
2006–2015	Competence Centre for Cancer Research, Project Manager

Publications

Reinsalu, Leenu; Puurand, Marju; Chekulayev, Vladimir; Miller, Sten; Shevchuk, Igor; Tepp, Kersti; **Rebane-Klemm, Egle**; Timohhina, Natalja; Terasmaa, Anton; Kaambre, Tuuli (2021). Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis. *Frontiers in Oncology, 11, ARTN 698951.*

Klepinin A, Zhang S, Klepinina L, **Rebane-Klemm E**, Terzic A, Kaambre T, Dzeja P. Adenylate Kinase and Metabolic Signaling in Cancer Cells. *Front Oncol. 2020 May 19;10:660. eCollection 2020.*

Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, Timohhina N, Tepp K, Bogovskaja J, Afanasjev V, Suurmaa K, Valvere V, Kaambre T. Mitochondrial Respiration in *KRAS* and *BRAF* Mutated Colorectal Tumors and Polyps. *Cancers (Basel). 2020 Mar 28;12(4):815*.

Lyudmila Ounpuu,* Laura Truu,* Igor Shevchuk, Vladimir Chekulayev, Aleksandr Klepinin, Andre Koit, Kersti Tepp, Marju Puurand, **Egle Rebane-Klemm**, Tuuli Käämbre. Comparative analysis of the bioenergetics of human adenocarcinoma Caco-2 cell line and postoperative tissue samples from colorectal cancer patients**.** *Biochemistry and Cell Biology, 2018, 96(6): 808-817.*

Andrus Kaldma; Aleksandr Klepinin; Vladimir Chekulayev, Ph.D.; Kati Mado; Igor Shevchuk, Ph.D.; Natalja Timohhina, Ph.D.; Kersti Tepp, Ph.D.; Manana Kandashvili; Minna Varikmaa, Ph.D.; Andre Koit; Margus Planken; Karoliina Heck; Laura Truu; Anu Planken, Ph.D.; Vahur Valvere, Ph.D.; **Egle Rebane**; Tuuli Käämbre, Ph.D. An in situ study of bioenergetic properties of human colorectal cancer: the regulation of mitochondrial respiration and distribution of flux control among the components of ATP synthasome. *Int J Biochem Cell Biol. 2014 Oct;55:171-86.*

D Dymerska, G Kurzawski, J Suchy, H Roomere, K Toome, A Metspalu, R Janavicius, A Irmejs, D Berzina, E Miklasevic, J Garadovskis, **E Rebane** et al. Lynch syndrome mutations shared by the Baltic States and Poland. *Clin Genet 2013*.

Valdur Mikita, Kairit Joost, Piret Laidre, Kadri Toome, Neeme Tõnisson, Jaan Soplepmann, **Egle Rebane**, Olga Kostina, Hanno Roomere. Hereditary colorectal Cancer: Guidelines in clinical practice. *Eesti Arst 2012; 91(5):269-275.*

Mario Plaas, Alar Karis, Jürgen Innos, **Egle Rebane**, Veerle Baekelandt, Annika Vaarmann, Hendrik Luuk, Eero Vasar, Sulev Kõks. Alpha-synuclein a30p point-mutation generates agedependent Nigrostriatal deficiency in mice. *J Physiol Pharmacol. 2008 Jun;59(2):205-216.*

Elulookirjeldus

Isikuandmed

Nimi:	Egle Rebane-Klemm
Sünniaeg:	27.10.1983
Sünnikoht:	Tartu
Kodakondsus:	Eesti

Kontaktandmed

E-post:	egle.rebane@gmail.com

Hariduskäik

2017–2023	Tallinna Tehnikaülikool, doktoriõpe
2005–2009	Tartu Ülikool, magistriõpe, biomeditsiin
2002–2005	Tartu Ülikool, bakalaureuseõpe, biokeemia
1990–2002	Tartu Tamme Gümnaasium

Keelteoskus

Eesti keel	emakeel
Inglise keel	kõrgtase
Vene keel	algtase

Teenistuskäik

2020–…	Swixx Biopharma, meditsiiniala nõunik
2017–2020	Sanofi, Balti meditsiiniala nõunik harvikhaigustes
2016–2017	Pfizer, medistiiniala nõunik onkoloogias
2007–2015	IB Genetics, laborijuhataja
2006–2015	Vähiuuringute Tehnoloogia Arenduskeskus, projektijuht

Publikatsioonid

Reinsalu, Leenu; Puurand, Marju; Chekulayev, Vladimir; Miller, Sten; Shevchuk, Igor; Tepp, Kersti; **Rebane-Klemm, Egle**; Timohhina, Natalja; Terasmaa, Anton; Kaambre, Tuuli (2021). Energy Metabolic Plasticity of Colorectal Cancer Cells as a Determinant of Tumor Growth and Metastasis. *Frontiers in Oncology, 11, ARTN 698951.*

Klepinin A, Zhang S, Klepinina L, **Rebane-Klemm E**, Terzic A, Kaambre T, Dzeja P. Adenylate Kinase and Metabolic Signaling in Cancer Cells. *Front Oncol. 2020 May 19;10:660. eCollection 2020.*

Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, Timohhina N, Tepp K, Bogovskaja J, Afanasjev V, Suurmaa K, Valvere V, Kaambre T. Mitochondrial Respiration in *KRAS* and *BRAF* Mutated Colorectal Tumors and Polyps. *Cancers (Basel). 2020 Mar 28;12(4):815*.

Lyudmila Ounpuu,* Laura Truu,* Igor Shevchuk, Vladimir Chekulayev, Aleksandr Klepinin, Andre Koit, Kersti Tepp, Marju Puurand, **Egle Rebane-Klemm**, Tuuli Käämbre. Comparative analysis of the bioenergetics of human adenocarcinoma Caco-2 cell line and postoperative tissue samples from colorectal cancer patients**.** *Biochemistry and Cell Biology, 2018, 96(6): 808-817.*

Andrus Kaldma; Aleksandr Klepinin; Vladimir Chekulayev, Ph.D.; Kati Mado; Igor Shevchuk, Ph.D.; Natalja Timohhina, Ph.D.; Kersti Tepp, Ph.D.; Manana Kandashvili; Minna Varikmaa, Ph.D.; Andre Koit; Margus Planken; Karoliina Heck; Laura Truu; Anu Planken, Ph.D.; Vahur Valvere, Ph.D.; **Egle Rebane**; Tuuli Käämbre, Ph.D. An in situ study of bioenergetic properties of human colorectal cancer: the regulation of mitochondrial respiration and distribution of flux control among the components of ATP synthasome. *Int J Biochem Cell Biol. 2014 Oct;55:171-86.*

D Dymerska, G Kurzawski, J Suchy, H Roomere, K Toome, A Metspalu, R Janavicius, A Irmejs, D Berzina, E Miklasevic, J Garadovskis, **E Rebane** et al. Lynch syndrome mutations shared by the Baltic States and Poland. *Clin Genet 2013*.

Valdur Mikita, Kairit Joost, Piret Laidre, Kadri Toome, Neeme Tõnisson, Jaan Soplepmann, **Egle Rebane**, Olga Kostina, Hanno Roomere. Hereditary colorectal Cancer: Guidelines in clinical practice. *Eesti Arst 2012; 91(5):269-275.*

Mario Plaas, Alar Karis, Jürgen Innos, **Egle Rebane**, Veerle Baekelandt, Annika Vaarmann, Hendrik Luuk, Eero Vasar, Sulev Kõks. Alpha-synuclein a30p point-mutation generates agedependent Nigrostriatal deficiency in mice. *J Physiol Pharmacol. 2008 Jun;59(2):205-216.*

ISSN 2585-6901 (PDF)
ISBN 978-9916-80-019-5 (PDF)