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Metabolic heterogeneity of cancer cells: an interplay between reprogrammed and oxidative metabolism and roles of HIF-1, GLUTs and AMPK

Nurbubu T. Moldogazieva[1,*], Innokenty M. Mokhosoev[2], Alexander A. Terentiev[2]

[1] I.M. Sechenov First Moscow State Medical University (Sechenov University), 119991, Moscow, Russia; nmoldogazieva@mail.ru (N.T.M)

[2] N.I. Pirogov Russian National Research Medical University, 117997, Moscow, Russia; imokhosoev@mail.ru (I.M.M.); aaterent@inbox.ru (A.A.T.)

*Correspondence: nmoldogazieva@mail.ru

Running title: HIF-1, AMPK, and GLUTs in cancer metabolic heterogeneity

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Abstract: It has been long recognized that under hypoxia conditions cancer cells reprogram their metabolism through shift from oxidative phosphorylation (OXPHOS) to glycolysis to meet elevated requirements in energy and nutrients for proliferation, migration and survival. However, data accumulated over the last years increasingly evidence that cancer cells can revert from glycolysis to OXPHOS and maintain both reprogrammed and oxidative metabolism even in the same tumor. The phenomenon denoted as cancer cell metabolic plasticity or hybrid metabolism depends on a tumor micro-environment, which is highly heterogeneous and influenced by intensity of vasculature and blood flow, oxygen concentration, nutrient and energy supply, and requires regulatory interplay between multiple oncogenes, transcription factors, growth factors, reactive oxygen species (ROS), etc. Hypoxia-inducible factor-1 (HIF-1) and AMP-activated protein kinase (AMPK) represent key modulators of switch between reprogrammed and oxidative metabolism. The present review focuses on cross-talks between HIF-1, GLUTs, and AMPK and other regulatory proteins including oncogenes such as c-Myc, p53 and KRAS, growth factor-initiated PKB/Akt, PI3K and mTOR signaling pathways and tumor suppressors such as LKB1 and TSC1 in controlling cancer cell metabolism. The multiple switches between metabolic pathways can underlie chemo-resistance to conventional anti-cancer therapy and should be taken into account in choosing molecular targets to discovery novel anti-cancer drugs.

Key words: cancer metabolism, OXPHOS, HIF-1, AMPK, GLUTs

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1. Introduction

Cancer cells often suffer from hypoxia, nutrient (glucose and amino acid) and energy deprivation resulted from insufficient vasculature and blood supply [1]. These stress conditions are key factors imposed on proliferating tumor cells to trigger their malignant transformation and to enable them to overcome or escape antitumor immune surveillance and to avoid cellular senescence and apoptosis [2–4]. This is resulted in tumor progression and aggressiveness, genetic instability, development of chemo- and radio-resistance and poor prognosis [5,6].

Under physiological conditions, oxidative phosphorylation (OXPHOS), i.e. coupling of oxidation reactions with mitochondrial electron transportation chain (ETC), is the most efficient way of ATP production, which generates much larger energy as compared to anaerobic glycolysis; however, under hypoxic conditions glycolysis is the only process providing cells with energy [7,8]. In the hypoxic microenvironment cancer growth is maintained by metabolic and bioenergetic reprogramming characterized by adaptive switch from OXPHOS to glycolysis followed by excessive glucose consumption and lactate production. This phenomenon was first discovered by German scientist Otto Warburg in 1927 and was denoted as Warburg effect by Efraim Racker in 1972 [9–11].

Molecular mechanisms underlying cancer cell tolerance to prolonged hypoxia and nutrient/energy starvation are very complex and can work both at transcriptional and post-translational levels. Hypoxia-inducible factor-1 (HIF-1) is a master regulator of cellular oxygen sensing and adaptation to hypoxia and ubiquitous transcriptional activator, which regulates expression of numerous genes at both DNA level and epigenetic, chromatin remodeling/histone modifications, level [12–14]. Modulation of gene expression by HIF-1 causes alterations in mitochondrial oxidative metabolism, glucose uptake and oxidation, energy production and angiogenesis to enable cancer cell proliferation, migration and survival.

However, a large body of data has shown that most tumors grow in the interaction with highly heterogeneous microenvironment with different densities of blood and lymph vessels, amount and types of infiltrating cells, extracellular matrix composition, content of signaling molecules, etc. [15] Moreover, many tumors are not monoclonal despite they originate from a single cell; instead they are composed of multiple distinct clones differed by morphological and phenotypic features, which can vary depending on a cancer type and stage, treatment regimes, etc. [16–18]. This phenomenon denoted as tumor heterogeneity implies that within a definite tumor a heterogeneous population of various cell types with distinct gene expression and metabolic profiles, proliferative, angiogenic and metastatic potential co-exist.

Furthermore, results of experimental, bioinformatics and computer/mathematical modeling approaches are increasingly evidencing that cancer cells do not fully rely on glycolysis, instead

they preserve oxidative metabolism [19,20]. This indicates that cancer cells acquire hybrid or heterogeneous metabolism, which enables them to use both glycolysis and OXPHOS as sources of ATP and that oxidative catabolic pathways including tricarboxylic acid (TCA) cycle (Krebs cycle), oxidative decarboxylation of pyruvate by pyruvate decarboxylase complex (PDC), glutaminolysis and fatty acids β-oxidation (FAO) can remain functional as sources of reducing equivalents (NADH and $FADH_2$), and carbon and nitrogen [20]. Moreover, multiple switches between the metabolic pathways can exist depending on nutrient and energy availability, micro-environmental factors, and clinico-pathological characteristics such as tumor stage, histological type, differentiation grade, lymph node involvement, depth of invasion, etc.

To enable cancer cell metabolic plasticity, induction of numerous genes and activation/inhibition of multiple oncogenes, growth factors and tumor suppressors are required [21]. Crucial role in this phenomenon belongs to interplay between HIF-1 and AMP-activated protein kinase (AMPK), energy sensor and master regulator of cellular metabolism and bioenergetics. AMPK is a heterotrimeric serine/threonine kinase, which is activated by decrease in AMP/ATP ratio to provide ATP production through both glycolysis and OXPHOS [22]. In general, AMPK maintains ATP level in cells by switching from anabolic to catabolic metabolism through stimulation of glucose uptake, aerobic glycolysis and mitochondrial oxidative metabolism, mainly, due to fatty acid β-oxidation [23].

Additionally, both hypoxia and nutrient deprivation can cause elevated generation of reactive oxygen species (ROS) by mitochondrial ETC and Nox family NADPH oxidases resulted in oxidative stress followed by alterations in cell signaling pathways [24]. Various ROS types can affect the activities of both HIF-1 and AMPK along with intracellular effectors of cell signaling pathways and transcription factors to trigger cancer progression and metastasis under hypoxia, nutrient/energy and oxidative stress conditions.

This review focuses on the recent advancements in understanding novel mechanisms underlying the ability of cancer cells to maintain hybrid metabolism, both metabolic/bioenergetic reprogramming and oxidative metabolism, for growth, invasion and metastasis. We demonstrate here the importance of consideration of cross-talks between HIF-1 and AMPK and the expression of GLUTs and enzymes involved in glucose and fatty acid metabolism in cancer initiation and progression. Furthermore, we show that growth factor-initiated phosphatydyl-3-kinase (PI3K), protein kinase B (PKB)/Akt, and mammalian target of rapamycin (mTOR) cell signaling pathways along with oncogenes and transcription factors such as KRAS, c-Myc and p53 interplay with HIF-1 and AMPK, and ROS generation to enable cancer cell metabolic plasticity.

2. Hypoxia-inducible factor-1

Usually, under experimental *in vitro* conditions oxygen concentration up to 20% is used, the condition denoted as normoxia [25]. However, at physiological conditions, oxygen concentration in peripheral tissues can vary from 3% to 7.4% with an average value of about 5% (38 mmHg), the condition denoted physioxia [26]. Additionally, oxygen concentration can transiently decrease under physiological conditions *in vivo* due to various processes such vasodilation and increase in the blood flow [27]. Moreover, oxygen consumption rates can considerably vary between cell types depending on mitochondrial content and metabolic activity [25].

The master regulator of oxygen homeostasis, HIF-1, belongs to evolutionarily conserved transcription factors expressed in all eukaryotes in three isoforms, HIF-1α, HIF-2α, and HIF-3α (reviewed by [28,29]). HIF-1α undergoes heterodimerization with HIF-1β, both containing basic helix-loop-helix (bHLH) domains along with Per-aryl hydrocarbon nuclear translocation (ARNT)-Sim homology (PAS) domain [30]. Under normoxia conditions HIF-1α is destabilized through the continuous degradation by ubiquitin-proteasome system (UPS). However, under acute hypoxia conditions, HIF-1α becomes stabilized due to the dimerization with HIF-1β to translocate into the nucleus and to bind to hypoxia response elements (HREs) on DNA. This leads to the over-expression of key regulatory enzymes of glycolysis and pentose phosphate pathway (PPP), down-regulation or mutations in genes encoding of PDC and TCA cycle enzymes or ETC enzymatic complex I as observed in various cancer types (reviewed by [24, 31–33]).

Two proline residues of HIF α-subunit have been shown to undergo hydroxylation by prolyl-hydroxylase domain proteins (PHDs), which exist in three isoforms, PHD1-PHD3, and have distinct functions [34]. This chemical modification enables recognition and polyubiquitination of HIF1α, HIF2α, and HIF3α for the E3 ubiquitin ligase complex with von Hippel-Lindau protein (pVHL) [35]. Genetic loss in the pVHL tumor suppressor has been shown to cause HIF-1 stabilization and activation even under normoxia conditions followed by tumor cell proliferation and survival [36,37]. Unlike acute hypoxia, over-expression and hyper-activation of PHDs followed by HIFα desensitization to protect cells from necrosis has been observed under chronic hypoxia conditions [38]. Additionally, hydroxylation of asparagine residue by the factor inhibiting HIF (FIH) prevents binding of coactivator p300/CBP followed by the decrease in HIFα transcriptional activity [39].

With the use of tumor metabolism modeling approach, it has been shown that in the hypoxic microenvironment, both intracellular and environmental factors contribute to metabolic reprogramming of cancer cells and various growth factor-initiated cell signaling

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cascades and transcription factors can affect HIF-1 activity [40]. An interplay between HIF-1 and variety of oncogenes such as Ras, c-Myc, p53, AMPK, along with PKB/Akt, PI3K and mTOR signaling pathways has been observed (Figure 1) to control mitochondrial ETC functioning and energy production to maintain cancer cell proliferation and survival [41–48].

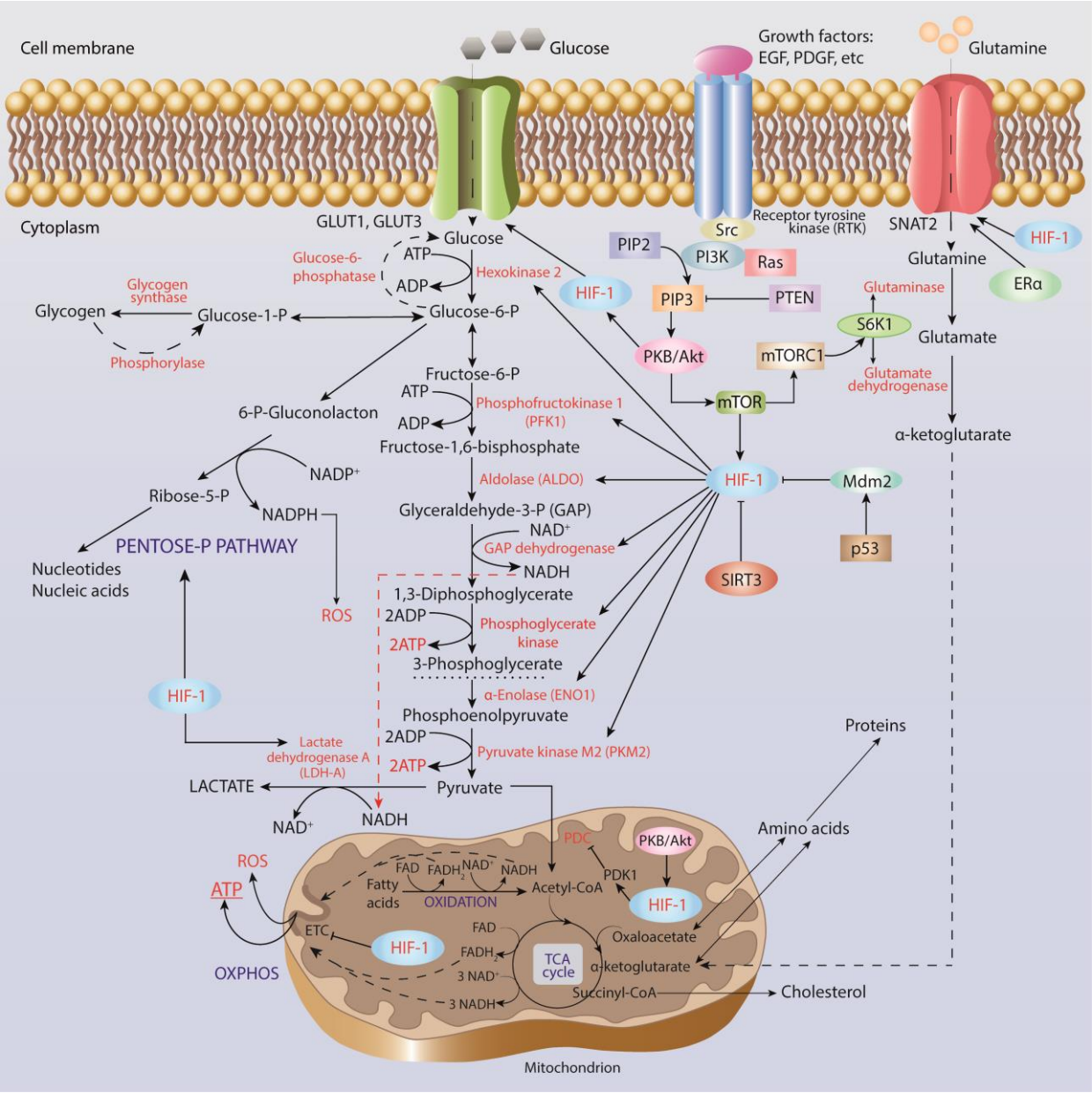

Figure 1. Regulation of HIF-1 and its implication in metabolic reprogramming in cancer cells. HIF-1 induces the expression of genes, which encode glucose transporters, GLUT1 and GLUT3, enzymes of glycolysis and pentose-phosphate pathway, and pyruvate dehydrogenase complex kinase. Activity of HIF-1 is regulated by Ras-PKB/Akt-mTOR axis.

For example, interplay between HIF-1α and p53, two transcription factors regulated by both E3 ubiquitin ligase and murine double minute 2 (Mdm2), in response to hypoxia during carcinogenesis has been observed [49]. The p53 activation by gamma-rays used in cancer

treatment triggers Mdm2-mediated HIF-1α UPS-mediated degradation. This leads to decrease in the peroxisome-proliferator activated receptor gamma co-activator 1β (PGC-1β) inhibition and promoting mitochondrial biogenesis [50]. Additionally, both HIF-1α and HIF-2α can be modulated in cancer cells by oncogenic KRAS leading to decreased OXPHOS and ATP production and increased mitochondrial ROS generation [51]. Moreover, KRAS can enhance ROS generation by NADPH oxidases, for example, in Rac1-Nox4-dependent manner [52].

Under hypoxic conditions, HIF-1α expression can associate with growth factor expression. Indeed, signal transduction pathways initiated by binding of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) to their membrane-bound receptors, receptor tyrosine kinases, RTKs, activate Ras/PI3K/PTEN/Akt and mTOR signaling to induce HIF-1 and c-Myc expression [53,54]. For example, mechanism of the PDC activity inhibition in response to hypoxia may include the accumulation of PKB/Akt in mitochondria and phosphorylation of pyruvate dehydrogenase kinase 1 (PDK1) at Thr-346 to inactivate PDC and to inhibit acetyl-CoA production through oxidative decarboxylation of pyruvate. This triggers glycolysis to maintain tumor cell proliferation antagonizing apoptosis and autophagy [55].

Additionally, in tumor patients HIF-1α expression has been observed to correlate with VEGF over-expression, tumor vascularization, invasion, stage, and metastasis and lymphatic invasion, as shown in many cancer types such as colorectal and hepatocellular carcinomas, and oesophageal squamous cell cancer [56–59]. For example, PI3K/Akt/mTOR pathway-mediated stimulation of HIF-1 mRNA translation through the activation of two downstream targets of mTOR, p70SK6 and 4E-BP1, in breast cancer cells has been observed [37].

Moreover, inhibition of HIF-1α and VEGF expression both at mRNA and protein levels has been reported to suppress tumor growth and targeting mTOR/HIF-1α/VEGF can be considered as promising strategy in anti-cancer therapy [58]. For example, inhibiting human cervical cancer growth and enhancement of the tumor radio-sensitivity can be achieved by down-regulating HIF-1α and VEGF and up-regulating p53 [60]. In animal model of cervical cancer, inhibition of the tumor growth by formononetin and cisplatin has been associated with decrease in HIF-1α and VEGF expression [61]. Additionally, Krüppel-like factor 5 (KLP5) promotes non-small cell lung cancer (NSCLC) cell apoptosis via direct suppression of HIF-1α and glycolysis. Furthermore, KLF5 over-expression promotes cancer cell survival and hypoxia-induced cisplatin resistance through the activation of PI3K/Akt/mTOR pathway [62].

Furthermore, metabolomics and quantitative proteomics approaches have showed that mitochondrial NAD^+-dependent deacetylase family of enzymes, sirtuins (SIRTs), can alter cellular metabolism and revert the Warburg effect in tumor cells [63]. For example, SIRT3 over-expression has been implicated in kidney cancer growth inhibition and maintaining

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mitochondrial homeostasis, as well as modulating ROS production to sensitize biomolecules and cells to oxidative damage [64], while low SIRT3 level associated with poor differentiation and unfavorable prognosis in primary hepatocellular carcinoma (HCC) [65]. Moreover, anti-cancer agent etoposide-induced genotoxic-mediated cancer cell apoptosis has been shown to correlate with both SIRT1 and SIRT3 over-expression [66].

SIRTs can regulate mitochondrial metabolism in response to diverse nutrient signals through the deacetylation of various proteins including TCA cycle enzyme isocitrate dehydrogenase-2, ETC enzymatic complexes and antioxidant enzyme Mn-superoxide dismutase (SOD2). Thus, they promote TCA cycle and ETC functioning, and reduce ROS generation and oxidative stress, while the loss in SIRTs enhances metabolic reprogramming in cancer cells through destabilization of HIF-1α to down-regulate glycolytic genes [67]. For example, in breast cancer, the over-expression of SIRT3 causes decrease in rate of glycolysis and inhibition of cell proliferation followed by tumor suppression, where as the loss of SIRT3 increases ROS production leading to HIF-1α stabilization and upregulation of HIF-1α target genes [68]. Seemingly, SIRT3 can regulate the metabolic reprogramming of cancer cells also through deacetylation of p53 transcription factor at Lys-320 and Lys-382 residues to promote its UPS-mediated degradation as shown in phosphatase and tensin homolog (PTEN)-deficient non-small cell lung cancer cells [69].

3. Interplay between HIF-1 and facilitative glucose transporters

The phenotypic hallmark of more than 90% of primary and metastatic tumors is an increase in glucose uptake from the blood, which at a great extend depends on facilitative glucose transporters (GLUTs) encoded by *SLC2A* family of genes [70]. This family comprises fourteen members, GLUT1-GLUT14, divided into four classes depending on their sequence similarity. GLUTs vary in their affinity to glucose, regulation, and tissue distribution and expression level in both physiological and pathological conditions.

Under physiological conditions, GLUT4 acts as a major insulin-sensitive glucose transporter. TBC1D1, Tre2/Bub2/Cdc15 (TBC) domain family member 1 protein, can regulate insulin-stimulated GLUT4 translocation into mammalian cell membrane, thereby, triggering glucose uptake. TBC1D1 is Rab-GTPase-activating protein and contains *N*-terminal phosphotyrosine-binding (PTB) domains and *C*-terminal Rab-GTPase (GAP) domain. It has been shown to be phosphorylated by Akt and AMPK in response to insulin, however, this did not alter intrinsic Rab-GAP activity [71,72].

The efficacies of glucose uptake by cancer cells depend, mainly, on activities of GLUT1 and GLUT3 and, in less extend, GLUT4 and GLUT10 [73]. The expression level of GLUT1

has been observed to correlate with that of HIF-1α in many cancer types including colorectal and ovarian cancers, and to associate with the tumor clinicopathological characteristics [74,75]. GLUT1 and HIF-1α expression was similar in relation to tumor size, location and patient age and gender however there were differences in intracellular location of these two proteins. Immunoreactivity of GLUT1 was significantly higher in node-positive colorectal cancer compared to node-negative one. Furthermore, GLUT1 was found in membranes of multifocally necrotizing cancer cells and in the cytoplasm of cancer cells with no necrosis, while HIF-1α, mostly, had cytoplasmic location [75].

Additionally, an interplay between GLUTs, HIF-1 and glycolytic enzymes has been observed in many cancer types. For example, HIF-1α expression has been reported to correlate positively with those of LDH-5 and GLUT1 both at mRNA and protein levels in human gastric and ovarian cancers, and this was associated with the tumor size, depth of invasion, distant metastasis, clinical stage and differentiation status [76,77]. Additionally, an existence of correlation between expression of GLUT1, vascular endothelial growth factor (VEGF) and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases-3 and -4 (PFKFB-3 and PFKFB-4) in gastric and pancreatic cancers has been reported. GLUT3 induction also has been shown to correlate with the over-expression of glycolytic enzymes including those encoding HK2 and PKM2 in metastatic cancers, and this was associated with cancer invasiveness and poor prognosis [78].

GLUT1 localization in a cell membrane can be increased by phosphorylation of α-arrestin family protein, thioredoxin-interacting protein (TXNIP). The elevated glucose concentration can induce expression of TXNIP, which inhibits glucose uptake directly by binding to GLUT1 and stimulating its endocytosis via clathrin-coated pits or indirectly by reducing GLUT1 at mRNA level. Furthermore, AMPK has been shown to cause phosphorylation and rapid degradation of TXNIP increasing GLUT1 function [79]. In addition to gene expression regulation at DNA level, histone modifications can contribute to modulation of glucose transporter induction. For example, epigenetic regulation of expression of *SLC2A1* gene encoding GLUT1 due to induction of *HDAC2* gene by beta-hydroxybutyrate, ketone body, to enhance H3K9 acetylation under starvation conditions in brain tissue have been observed [80].

GLUT3 induction during epithelial-mesenchymal transition (EMT) by ZEB1 transcription factor to promote non-small cell lung cancer cell proliferation has been observed [81]. Additionally, in non-small cell lung carcinoma cell culture and *in vivo* model, increased glucose uptake with the involvement of GLUT3 and caveolin 1 (Cav1), an important component of lipid rafts, triggers tumor progression and metastasis. Interestingly, Cav1-GLUT3 signaling can be targeted by atorvastatin, FDA-approved statin, which increases

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cholesterol biosynthesis, and this inhibits EGFR-tyrosine kinase inhibitor (TKI)-resistant tumor growth and increases the overall patient survival [82].

Higher expression of GLUT1 and GLUT3 in papillary carcinoma as compared to follicular carcinoma and non-neoplastic thyroid lesions has also been reported [83]. Additionally, both GLUT1 and GLUT3 have been up-regulated in poorly differentiated endometrial and breast cancers both at mRNA and protein levels [84]. Transactivation of GLUT3 was in Yes-associated protein (YAP)-dependent manner suggesting that this pathway serves as regulator of metabolic reprogramming during cancer progression and can be considered as promising anti-cancer therapeutic target [85].

4. Role of HIF-1 in metabolic reprogramming of cancer cells

4.1. Enhancement of glycolysis

As early as in 1925 C. Cori and G. Cori discovered that glucose content in the axillary veins of hens with Rous sarcoma was 23 mg less whereas content of lactate was 16 mg greater than those in veins of normal tissue [86]. Afterwards, Otto Warburg and co-workers compared glucose and lactate concentrations in tumor veins and arteries to find 69 mg greater lactate in the vein blood than that in the same volume of aorta blood of rats with Jensen sarcoma, while glucose uptake by tumor tissue was 52-70% as compared to 2-18% by normal tissues [9].

Warburg effect has been experimentally confirmed by over-expression of glycolytic enzymes accompanied by deficit in ATP production by OXPHOS in many cancer types in both cultured cell lines and animal models [87,88]. Genes affected by HIF-1 and implicated in carcinogenesis include solute carrier family *SCL2A* of genes and those encoding glycolytic enzymes such as hexokinase II (HK II), phosphofructokinase 1 (PFK1), fructose-bisphosphate aldolase A (ALDOA), α-enolase (ENO1), pyruvate kinase M2 (PKM2), and lactate dehydrogenase A (LDH-A or LDH-5) as well as genes encoding pyruvate dehydrogenase complex kinase (PDK) and enzymes of PPP [89,90].

The first reaction of glycolysis (Figure 1) is catalyzed by key rate-limiting enzyme, hexokinase, which has four isoforms in mammalian cells, among which HK II over-expression at both mRNA and protein levels has been reported for many tumor types including hepatocellular carcinoma, ovarian cancer, etc. [91–93]. Furthermore, correlation of over-expression and co-localization of both HK II and HIF-1α in cancer cells near necrosis regions have been shown.

The second key rate-limiting glycolytic enzyme is PFK, a tetrameric enzyme in mammals that catalyzes the third reaction of glycolysis, i.e. phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (FBP) accompanied by ATP utilization. Interplay between HIF-1α

and Ras and Src oncogenes in tumor microenvironment in regulation of PFK1 and PFK2 isoenzymes has been suggested to contribute to human cancer cell proliferation and survival [94]. PFK1 is an allosteric enzyme activated by fructose-2,6-bisphosphate that is produced from fructose-6-phosphate by bifunctional enzyme, phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK2/FBPase-2 or PFKFB2), which is induced by HIF-1α. Thus, targeting the fructose-2,6-bisphosphate can be considered as a promising therapeutic strategy to combat tumor growth, invasion and metastasis. For example, silencing of *PFKFB2* gene has been shown to significantly inhibit ovarian and breast cancer growth and to enhance paclitaxel sensitivity and patient's survival [95].

In glycolytic pathway, there are two enzymes, which catalyze transfer of phosphoryl group from a substrate to ADP producing, thereby, ATP in reactions of substrate-level phosphorylation and serving as energy source in hypoxia conditions. The first enzyme is phosphoglycerate kinase (PGK) that catalyzes conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate. Several single nucleotide polymorphism variants of PGK1 with decreased catalytic efficiency and thermodynamic stability due to alterations in local protein conformations have been found in carcinoma cells [96]. The second enzyme is pyruvate kinase that catalyzes the last reaction of glycolysis under aerobic conditions, i.e. convertion of phosphoenol-pyruvate (PEP) into pyruvate, being allosterically regulated by fructose-2,6-bisphosphate. Four mammalian PK isoforms differed by regulation and tissue specificity designated as PKM1, PKM2, PKR, and PKL have been described [97]. PKM2 is expressed in embryonic, proliferating and tumor cells to have a role in progression of many cancer types such as ovarian, gastric, lung cancers, etc. [98,99]. PKM2 up-regulation has been shown to occur through mTOR-mediated HIF-1α stabilization and c-Myc-heterogeneous nuclear ribonucleoproteins (hnRNPs)-dependent regulation [100].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the sixth reaction of glycolysis, oxidation of GAP to 1,3-biphosphoglycerate accompanied by reduction of NAD^+ to NADH. Regulation of GAPDH expression in cancer cells is not obvious. For example, it has been shown that GAPDH was not regulated in Hep-1-6 mouse hepatoma, Hep-3-B and HepG2 human hepatocellular carcinoma, A-549 human adenocarcinoma, and HT-29 and HCT-116 colon cancer cell lines [101]. This indicates that GAPDH is not attractive target in anti-cancer therapy and emphasizes importance of proper choice of housekeeping genes for correct interpretation of experimental results.

Indeed, various glycolytic enzymes can be differentially regulated in cancer cells under hypoxia conditions. For example, analysis and interpretation of transcriptomic data with the use of Cytoscape network showed highly over-expressed glycolytic genes including HK2, PFKP,

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ENO2, SLC2A3, SLC16A1, PDK1 in patients with clear cell renal carcinoma [102]. However, according to this study, some other glycolytic enzymes such as ALDOB, PKLR, PFKFB2, G6PC, PCK1, FBP1, and SUCLG1 were highly down-regulated. Proteomics approaches can contribute to explanation of altered metabolic phenotype of cancer cells along with their bioenergetic signature and increased glucose uptake resulted from both the activation of anaerobic glycolysis for cell proliferation and the impairment in mitochondrial functions [103].

Other metabolic pathways can contribute to Warburg effect by producing intermediates, which fuel glycolysis. For example, over-expression of the PPP enzymes associates with HIF-1α stabilization and tumor progression to serve as an indicator of poor prognosis. Since, PPP is linked to glycolysis, inhibiting of PPP enzymes can serve as a promising strategy in anti-cancer therapy. For example, the ability of natural peptide carnosine to decrease activities of PPP enzymes, as well as malate-aspartate and glycerol-3-phosphate shuttle mechanisms, which carry electrons from glycolysis to ETC, has been observed in glioblastoma cell lines [104].

4.2. Tissue acidification and its role in reverse to OXPHOS

Under oxygen deficiency conditions (oxygen concentration less than 2%), the efficacy of mitochondrial ETC decreases to cause the increase in NADH/NAD^+ ratio, which triggers conversion of pyruvate into lactate instead of further oxidation through oxidative decarboxylation catalyzed by pyruvate dehydrogenase complex (PDC). NADH accumulation causes the over-production of lactate due to activity of lactate dehydrogenase, which has 5 isoforms (LDH-1 to LDH-5) differentially expressed in normal tissues and over-expressed during carcinogenesis [105]. Indeed, increased glucose uptake, induction of glycolysis-related genes, excessive lactate production and HIF-1α activation associated with aggressive phenotype and poor prognosis have been observed in patients with HCC and in Ewing sarcoma cells [106,107]. These observations have led to the conclusion that forcing cancer cells into mitochondrial metabolism can efficiently suppress tumor progression, while targeting glycolytic enzymes can be effective strategy to combat cancer growth.

Accumulation of lactate in tissues, up to 40mM, leads to acidosis (pH ≤ 6.8), which is hallmark phenotypic feature of tumor microenvironment affecting tumor progression, invasion and metastasis [108,109]. Normal cells cannot grow in acidic microenvironment [110,111]. However, it is necessary condition to promote cancer cell migration and invasion. Both endogenous and exogenous lactate have been shown to activate some enzymes such as matrix metalloproteinases and to affect the expression of oncogenes (Myc, Ras), transcription factors (HIF-1, E2F1), tumor suppressors (BRCA1, BRCA2) and cell cycle genes [112].

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In addition to lactate, carbon dioxide produced in catabolic pathways such as PPP, contributes to acidification of tumor microenvironment [113]. For example, in hypoxia conditions tumor cells have been shown to produce more HIF-1-induced IX and XII isoforms of carbonic anhydrase, which catalyzes reversible hydration of carbone dioxide into bicarbonate and protons to contribute to intracellular acidification and tumor cell survival [114]. Moreover, in a mouse model of ductal carcinoma *in situ* differences in levels of GLUT1 and carbonic anhydrase IX expression between normal and precancer cells along with heterogeneity in intracellular pH values have been demonstrated [115].

However, data obtained in the last years evidence that tumor cells demonstrate increased proton export due to the up-regulation of proton transporters such as Na^+/H^+ exchanger 1 (NHE1), H^+-lactate co-transporter and monocarboxylate transporters (MCTs) to regulate intracellular pH values [116,117]. Activities of these proton exchange systems represent additional adaptation and selection mechanism, which enable emerging of chemoresistant cell clones and tumor progression and metastasis.

Due to activities of lactate shuttle mechanisms lactate can serve as energy fuel, important gluconeogenic substrate and signaling molecule [118]. For example, lactate can fuel TCA cycle as show in human non-small cell lung cancers [119]. More importantly, lactate accumulation has been shown to be a mechanism underlying the reverse from glycolysis to OXPHOS in ATP production in cancer cells. Quantification of ATP amount produced via glycolysis and OXPHOS in 9 randomly selected cancer cell lines demonstrated that in the lactic acidosis microenvironment (20 mM lactate, pH 6.7) ATP was generated almost twice greater by OXPHOS and almost four times less by glycolysis than that without lactic acidosis [120]. Moreover, glucose consumption was much greater in lactic acidosis environment than that without lactic acidosis in the same tumor cell lines.

5. Oxidative metabolism and OXPHOS in cancer

Warburg wrote that “cancer cells can obtain approximately the same amount of energy from fermentation as from respiration, whereas the normal body cells obtain much more energy from respiration than from fermentation” and that uncoupling of respiration and phosphorylation with no diminishing oxygen consumption causes decrease in ATP production [10].

Currently, it is obvious that molecular oxygen is an indispensable component of mitochondrial ETC and serves as a final acceptor of electrons transferred through ETC enzymatic complexes (I, II, III and IV) localized in the inner mitochondrial membrane (Figure 2). The energy of electrons is used for ATP biosynthesis with the involvement of ATP-synthase

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(complex V) in the process denoted as OXPHOS [121]. Data obtained over the last years evidence that elevated oxidative metabolism with increased uptake of mitochondrial fuels such as lactate, pyruvate, and ketone bodies are characteristic for many cancer types including head and neck cancer, breast cancer, lymphomas, etc. [122–124]. For example, up-regulation of mitochondrial OXPHOS featured by succinate dehydrogenase (complex II) and cytochrome c oxidase (complex IV) activation to allow them producing higher ATP amount has been observed in epithelial cancer cells [125,126]. Cancer stem cells resist glucose deprivation and over-express genes associated with oxidative metabolism including OXPHOS, PPP and FAO along with higher level of ROS generation [127]. Additionally, chemotherapy has been shown to induce shift from glycolysis to OXPHOS mediated by SIRT1 and transcriptional co-activator PGC1 to promote tumor survival during treatment [128].

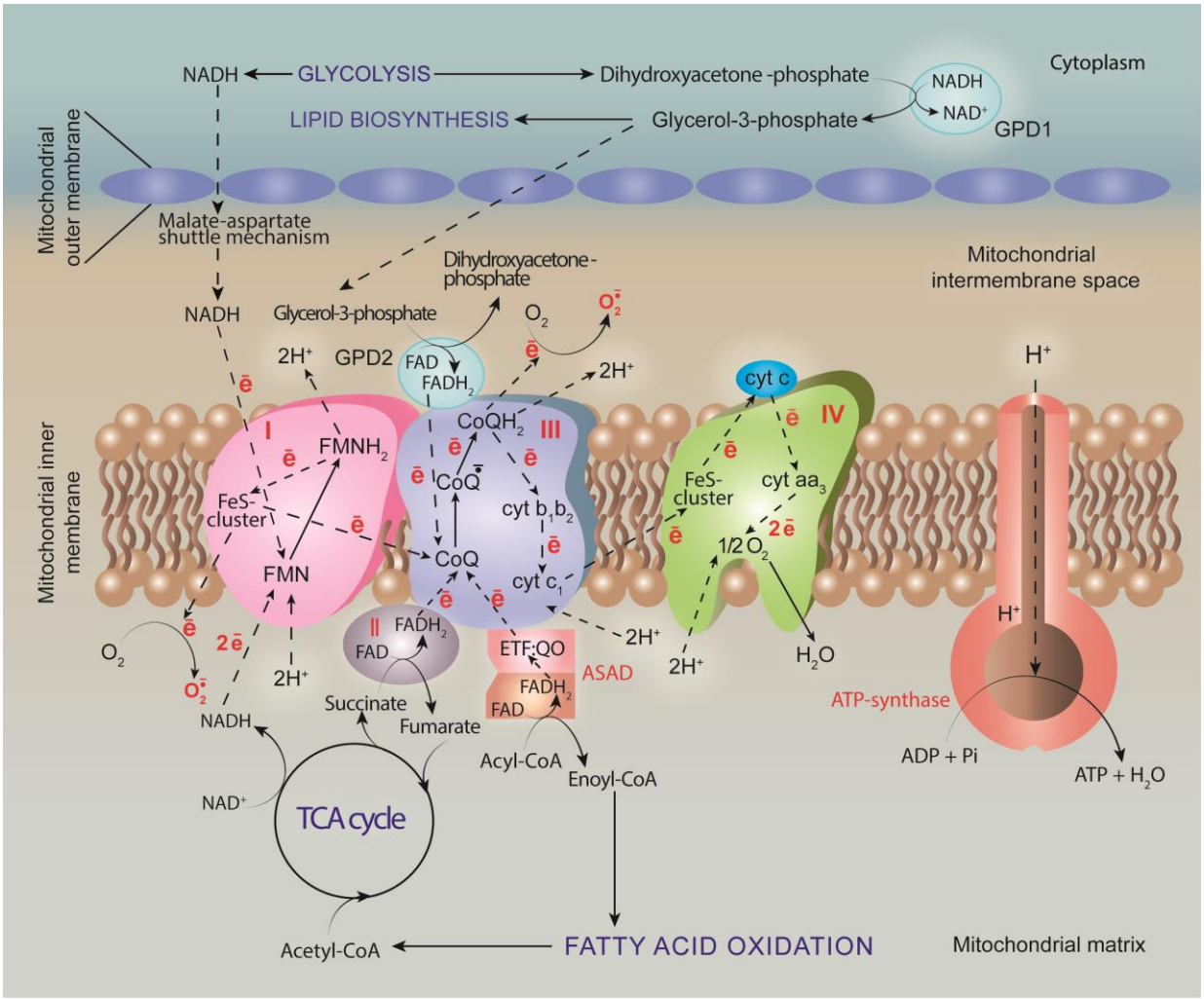

Figure 2. Oxidative metabolism, OXPHOS and ROS generation. NADH is produced, mainly, by glycolysis, pyruvate dehydrogenase complex, fatty acid oxidation and TCA cycle to fuel ETC via Complex I, while FADH2 is produced, predominantly, by fatty acid oxidation and TCA cycle and fuels ETC via Complex III. Glycerol-phosphate and malate-aspartate shuttle mechanisms serve to transfer reducing equivalents through outer mitochondrial membrane from

the cytoplasm to ETC. OXPHOS is ATP biosynthesis by ATP-synthase (Complex V). Superoxide anion radical, a primary type of ROS is produced as a byproduct of ETC.

Two co-enzymes, NADH and $FADH_2$ produced in reactions of oxidation of various biomolecules in the cytoplasm or in mitochondrial matrix, are the main suppliers of high-energy electrons for ETC (reviewed by [3,33]). The major sources of NADH or $FADH_2$ are FAO and oxidative degradation of glucose, which proceeds through the following three sequential metabolic processes: (i) aerobic glycolysis, which occurs in the cytoplasm through 10 enzymatic reactions to give rise to two molecules of pyruvate per one glucose molecule, (ii) oxidative decarboxylation of pyruvate by PDC to form acetyl-CoA, which further enters (iii) TCA cycle, which is a source of not only electrons, but also important intermediates such as α-ketoacids including α-ketoglutarate (α-KG) and oxaloacetate utilized in biosynthesis of amino acids and other biomolecules.

5.1. PDC and TCA cycle

In many cancer types, mutations in Krebs cycle enzymes have been shown to cause accumulation of oncometabolites such as citrate and 2-hydroxyglutarate, which stabilize HIF-1 and Nrf2 transcription factors and ROS generation, along with inhibiting tumor suppressor p53 and PDC enzyme, pyruvate dehydrogenase isoenzyme 3 (PDH3) [129,130]. Cancer-associated mutations have been found in genes encoding three TCA cycle enzymes: succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH), which lead to accumulation of succinate, fumarate and 2-hydroxyglutarate, respectively [131-133].

Indeed, multiple mutations in IDH isoenzymes, IDH1 and IDH2, which normally catalyze oxidative decarboxylation of isocitrate to α-KG, have been shown to occur frequently in gliomas and acute myeloid leukemia [113,134]. They lead to decrease in α-KG content and simultaneous increase in the amount of its antagonist, 2-hydroxyglutarate [135]. 2-Hydroxyglutarate being accumulated in tumor cells serves as a competitive inhibitor of multiple α-KG-dependent dioxygenases including histone demethylases and TET (ten-eleven translocation) family 5-methylcytosine (5mC) hydroxylases. Fumarate and succinate also have been proposed to act as competitive inhibitors of α-KG-dependent oxygenases including the HIFα hydroxylases contributing to HIF stabilization [136,137].

Nevertheless, cancer metabolome analysis has demonstrated that proliferating tumor cells require more diverse and large quantities of nutrients. Despite the earlier opinion that cancer cells bypass TCA cycle, emerging evidences are increasingly demonstrating that many cancer cells rely heavily on this process to meet the requirements in nutrients and energy [138].

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Moreover, most tumor cells can retain functional mitochondria and TCA cycle intermediates, which serve as substrates for nucleotides and nucleic acid, amino acid and fatty acid biosynthesis [139].

Indeed, higher pyruvate uptake and mitochondrial activity associated with increased ATP production have been observed in more invasive ovarian cancer cells as compared to less invasive ones [140]. Additionally, the activation of both PDC and TCA cycle enzymes with the production of about 50% acetyl-CoA from glucose along with synthesis of glutamine and glycine from TCA cycle metabolites have been observed in brain cancers in both humans and animal models [141]. For example, metaboluic complexity, which includes oxidation of glucose to pyruvate and further to acetyl-CoA by PDC followed by TCA cycle as well as glutamine metabolism have been observed in mouse in vivo model of genetically diverse primary human glioblastomas [142].

5.2. Glutaminolysis

It has been recognized that cancer cells demonstrate higher rate of glutamine consumption as compared to normal cells, because glutamine catabolism can accommodate carbon and nitrogen demands for nucleotide and nucleic acid biosynthesis required for cell division and proliferation [143]. This occurs due to glutaminolysis, the catabolic pathway of glutamine degradation to α-KG through the following reactions: (i) deamination of glutamine by glutaminase (GLS) yielding glutamic acid and ammonia followed by (ii) oxidative deamination of glutamate by glutamate dehydrogenase (GDH) or (iii) glutamate transamination with alanine by alanine amino transferase and with aspartate by aspartate amino transferase. α-KG can further enter the TCA cycle, since it is an anaplerotic intermediate of this process and serves as energy fuel for cells [144].

Metabolic profiling studies showed that glycolysis is decoupled from TCA cycle in cancer cells, mainly, through glutaminolysis to feed TCA as an alternative source of carbon (reviewed by [145]). Glutaminolysis yields lactate and pyruvate, the latter can be carboxylated by pyruvate carboxylase (PC) to oxaloacetate, which also anaplerotically fuels TCA cycle for cancer growth and metastasis [146,147]. Thus, anaplerotic replenishment of TCA cycle in cancer cells depends on both glutamine degradation and glucose-derived pyruvate carboxylation to oxaloacetate. However, carbon can travel through TCA in reverse direction to feed fatty acid biosynthesis, while lactate and pyruvate can be used in gluconeogenesis for biosynthesis of non-essential amino acid [145].

Under glutamine deprivation conditions, cancer cells have been shown to undergo oncogenic transcription factor c-Myc-driven up-regulation of GLS and GDH, and cell cycle

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arrest [148]. Moreover, activation of mTOR complex 1 and ribosomal protein S6 kinase-β1 (mTORC1/S6K1)-mediated pathway have been observed to regulate c-Myc to promote uptake of glutamine and to stimulate its catabolism via up-regulation of GLS in pancreatic cancer through modulating phosphorylation of eukaryotic translation initiation factor eIF4B, which is crucial to unwind its 5'-untranslated region (5'UTR) [149]. Activation of GDH proceeds through suppression of mitochondrial sirtuin SIRT4, which is over-expressed in human cancers [150].

Additionally, glutamine transporter SNAT2 (Figure 1) facilitates transportation of glutamine into cancer cells to promote tumor growth. In breast cancer cells, SNAT2 can be induced by both HIF-1α and estrogen receptor-α (ER-α), binding sites for both HIF-1α and ER-α being overlapped in cis-regulatory elements of SNAT2 gene [151]. Up-regulation of SNAT2 can cause complete resistance to anti-estrogen therapy and, partly, to anti-VEGF treatment indicating that developing drugs targeting SNAT2 is promising strategy in endocrine-resistance breast cancer.

5.3. Pentose phosphate pathway

Oxidative glucose metabolism can proceed through PPP, the process occurring in the cytoplasm and composed of two branches: (i) oxidative yielding ribose-5-phosphate used in nucleotide and nucleic acid biosynthesis, and NADPH utilized in fatty acid biosynthesis, and (ii) non-oxidative giving rise to glyceraldehyde-3-phosphate (GAP) and fructose-6-phosphate, the both can enter glycolysis [32]. There are two oxidation reactions in the oxidative branch of PPP, each of which yielding NADPH.

The first reaction is oxidation of glucose-6-phosphate to 6-phosphogluno-δ-lactone catalyzed by key time-limiting PPP enzyme, glucose-6-phosphate dehydrogenase (G6PDH), which is up-regulated in many cancer types and has been considered as a promising target for anti-cancer therapy and to revert cancer cell chemotherapy resistance [152,153]. In human clear renal cell carcinoma, both elevated glucose uptake and consumption and increased activity of G6PDH along with PPP-derived metabolites including NADPH have been observed [154]. The second oxidation reaction is conversion of phosphoglucono-δ-lactone into ribulose-5-phosphate catalyzed by 6-phosphoglucono-δ-lactone dehydrogenase (6PGD), which is also over-expressed in many cancer types including lung and ovarian cancer [155,156]. Up-regulated PPP enzymes such as NADP-dependent G6PDH and thiamine pyrophosphate (TPP)-dependent transketolase family enzymes, TKTL, TKTL1 and TKTL2, in various cancer types including breast, lung, gastric, endometrial, head and neck cancer have been reported [157–161].

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Importantly, NADPH is an essential component of NADPH oxidases, which represent a major source of ROS and produce superoxide anion radical ($O_2^{\bullet -}$) as a primary product [162]. In addition to PPP, there are two NADP-dependent enzymes which produce NADPH (i) IDH, and (ii) decarboxylating malate-dehydrogenase (malic enzyme). Both enzymes are associated with TCA cycle and tumor growth. Over-expression of both ME1 and ME2 isomers of malic enzyme causes reduction in tumor suppressor 53 level, however down-regulation of ME2 causes more prominent increase in ROS generation and phosphorylation/activation of p53 by AMPK followed by senescence as compared to ME1 [163].

5.4. Fatty acid β-oxidation

The most efficient metabolic pathway producing NADH and $FADH_2$ is FAO proceeding in mitochondrial matrix to yield acetyl-CoA, which further enters the TCA cycle (i) to provide a link between glucose and fatty acid metabolism, (ii) to enable generation of larger amount of ATP, and (iii) to produce important intermediates used in other metabolic pathways [164]. Energetically, FAO is more efficient and produces greater amount of ATP per one substrate molecule through OXPHOS as compared to oxidative degradation of glucose. Triacylglycerols and fatty acids of adipose tissue have been shown as potential sources to feed cancer growth. Up-regulation of FAO enzymes and their key roles in aerobic respiration have been observed in many cancer cell lines including human malignant gliomas, HCC and breast, lung and ovarian cancers [165-169].

Indeed, FAO activation was driven by over-expression of c-Myc oncogene in triple-negative breast cancer [165], while AMPK- and liver kinase B1 (LKB1)-mediated acetyl-CoA-carboxylase phosphorylation/activation has been observed to increase intracellular levels of ATP and induction of resistance to glucose deprivation in HCC cells and MCF-7 and MDA-MB-231 breast cancer cell lines [166,170]. Up-regulation of carnitine palmitoyltransferase 1 (CPT1), a rate-limiting enzyme of FAO that catalyzes interaction of long-chain fatty acids-containing acyl-CoA with carnitine to transfer them into the mitochondrial matrix, has been observed in ovarian cancer in mice [169]. Additionally, JNK and p38 MAPK-mediated phosphorylation and activation of FoxO transcription factor correlated with CPT1 inactivation and cell cycle regulator, cyclin-dependent kinase inhibitor p21, activation.

5.5. ROS generation

In addition to ATP production, ETC serves as a primary endogenous source of ROS (Figure 2) and generates superoxide anion radical in large amounts, however, as a byproduct rather than a primary product [171,172]. ROS generation by ETC results from leak of electrons

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and incomplete reduction of molecular oxygen to yield $O_2^{\bullet-}$. In addition to well-recognized ETC sites of ROS generation, enzymatic complexes I and III, mitochondrial FAD-dependent glyrerol-3-phosphate dehydrogenase (GPD2) and a system of electron transfer flavoprotein and electron transfer flavoprotein: ubiquinone oxidoreductase (ETF/ETF:QO system) have been identified. GPD2 is involved in glycerolphosphate shuttle mechanism to carry reducing equivalents produced in glycolysis by cytoplasmic glyrerol-3-phosphate dehydrogenase (GPD1) through outer mitochondrial membrane to ETC [173]. ETF/ETF:QO system is involved in transferring of electrons from 11 different mitochondrial flavoprotein dehydrogenases including FAD-dependent acyl-CoA dehydrogenases (ASADs), which catalyze dehydrogenation of acyl-CoA to enoyl-CoA during β-oxidation of fatty acids [174,175]. In ETC, the both systems transfer electrons from $FADH_2$ to CoQ to yield FAD and $CoQH_2$, respectively, and to give one electron for incomplete reduction of O_2 to $O_2^{\bullet-}$. Thus, oxidative metabolism is associated with generation of ROS, which can both cause alterations in redox homeostasis and underlie redox signaling to regulate cell response to stress stimuli.

Various human cancer types produce much greater amount of ROS as compared to normal tissues (reviewed in [176]). Alteration in signal transduction pathways that control mitochondrial bioenergetics and dynamics has been observed to cause mitochondrial dysfunction and elevated ROS production, which are implicated in determining the cancer cell fate for survival or death [177]. ROS production contributes to tumor microenvironment, which is highly heterogeneous and can affect tumor growth by multiple ways depending on interplay between various intracellular and environmental factors among which a key role belongs to AMPK.

6. Role of AMPK in promoting cancer cell oxidative metabolism

AMPK is an energy and nutrient sensor activated in response to energy starvation to provide the restoration of ATP level in cells by switching from anabolic to catabolic metabolism (reviewed by [178-180]. High AMPK activity associates with variety of metabolic processes including stimulation of glucose uptake by cells and mitochondrial oxidative metabolism, i.e. glucose oxidation, FAO and OXPHOS. Additionally, AMPK activation leads to inhibition of fatty acid and protein biosynthesis, cell cycle progression and cell proliferation in both normal and tumor cells [181].

AMPK is a heterotrimeric serine/threonine protein kinase that is expressed in different tissues and exists in various combinations of catalytic α-subunit and two regulatory β- and γ-subunits to provide diverse roles in regulating cell proliferation, autophagy and metabolism [22]. At low ATP level AMPK is allosterically activated by AMP/ADP binding to enable

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phosphorylation of specific enzymes. Adenine nucleotides bind to four tandemly arranged cystathionine-β-synthase (CBS) domains in the AMPK γ-subunit (Figure 3). Binding of AMP stimulates phosphorylation of Thr172 residue in the kinase domain of α-subunit by upstream kinases such as LKB1, which is activated by formation of a complex with STRAD (STE20-related kinase adapter protein-α) and scaffolding protein MO25 [182,183]. The β-subunit contains glycogen-binding domain and allows AMPK accumulation in the large cytoplasmic inclusions.

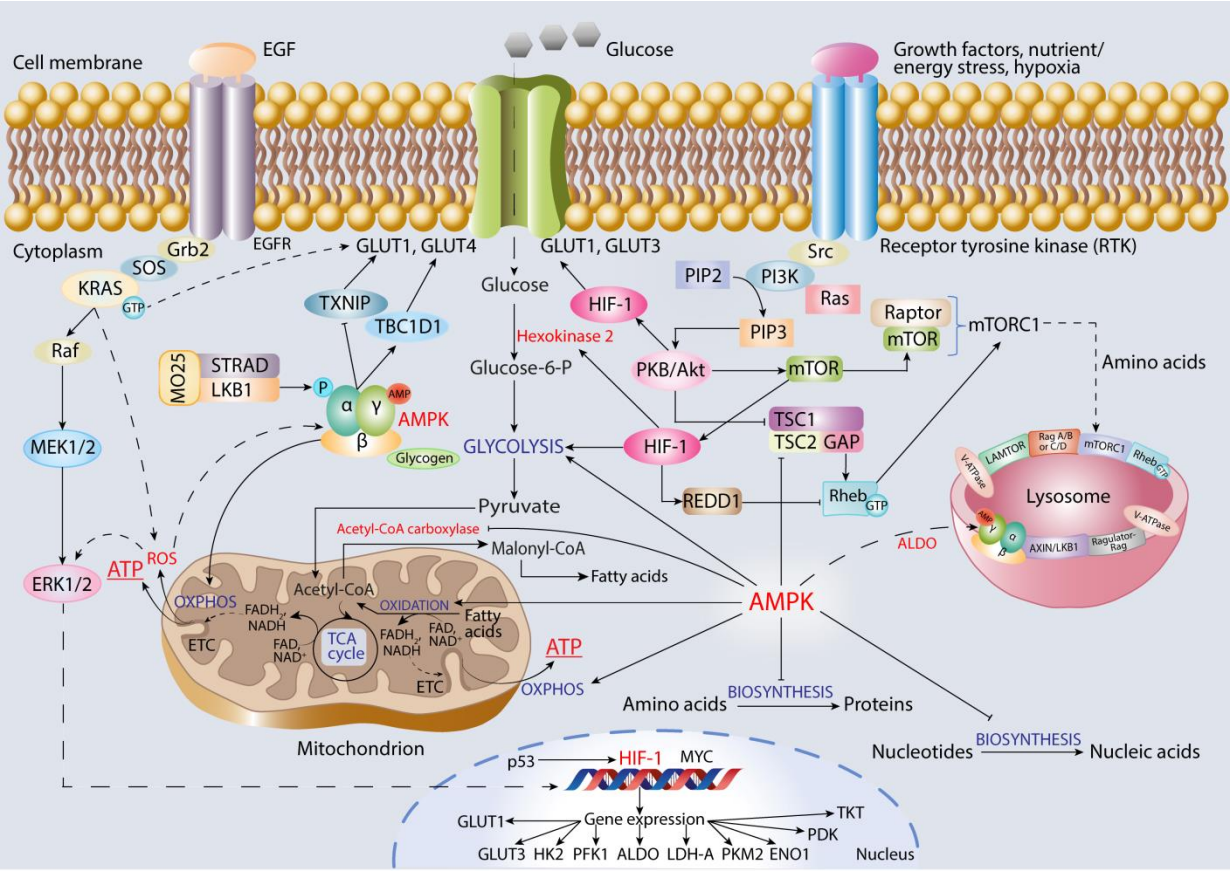

Figure 3. Interplay between AMPK, HIF-1 and ROS-regulated growth factor/nutrient and energy stress/hypoxia-initiated cell signaling pathways in regulation of both glycolysis and OXPHOS to produce ATP for cancer cell proliferation, invasion and migration. The involvement of AMPK in lysosomal complex formation is shown.

In some cell types, AMPK activation can occur through the AMP/ADP-independent mechanism, when an intact AMP-binding site is not required for the AMPK activation. For example, fructose-bisphosphate aldolase, a sensor of glucose availability, when occupied by its substrate, fructose-1,6-bisphosphate, cannot promote AMPK activation, while ALDO free of FBP stimulates formation of a lysosomal complex composed of AMPK, V-ATPase, Ragulator, AXIN and LKB1 kinase, tumor suppressor; this complex is required for AMPK

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phosphorylation and activation by LKB1 [184]. Inhibition of LKB1-AMPK signaling by G6PD activation and ribulose-5-phosphate formation in PPP followed by activation of acetyl-CoA carboxylase to provide a link between PPP, lipid biosynthesis and tumor growth has been observed [185].

With the use of comprehensive proteomics and phosphor-proteomics approaches a large network of AMPK substrate proteins involved in cell migration, adhesion and invasion has been identified [186,187]. One of the key downstream signaling pathways regulated by AMPK is mTOR-mediated signaling, which controls cellular response to environmental stress stimuli through the formation of two distinct complexes, mTORC1 and mTORC2 [188]. mTORC1 is sensitive to changes in cell growth conditions and contains scaffolding protein Raptor and mTOR to trigger anabolic metabolism, i.e. protein, lipid and nucleic acid biosynthesis (Figure 3). It can be regulated by growth factors and changes in cellular energy and nutrient concentrations to control numerous cellular processes at both transcriptional and translational levels. mTOR-mediated signaling functions to integrate it with PKB/Akt, HIF-1 and AMPK signaling pathways to control cell proliferation and survival in nutrient and energy deprivation conditions [189].

AMPK can inhibit mTORC1 through direct phosphorylation of several residues including Ser1387 in tumor suppressor TSC2, which forms heterodimeric complex with TSC1 for activation [190]. TSC1-TSC2 complex relays signals from diverse cellular pathways to properly modulate mTORC1 activity [191,192]. TSC2 contains GTPase-activating protein (GAP) domain, which activates small GTPase Ras homolog enriched in brain (Rheb), which in turn in active, GTP-bound, form can activate mTORC1. Rheb and Rag small GTPases act together to localize mTORC1 to lysosomal membrane and Ragulator complex in response to amino acids to activate mTORC1 and to drive maturation of endosomes into lysosomes [193]. Rag GTPases form A/C and B/D heterodimers, which use unique mechanism to stabilize their active (^{GTP}RagA-Ragc^{GDP}) or inactive (^{GDP}RagA-Ragc^{GTP}) states. Ragulator and lysosomal protein SLC38A9, arginine sensor, are guanine exchange factors (GEFs), which control nucleotide loading state [194].

Warburg effect can be closely associated with interplay between HIF-1 stabilization and decrease in AMPK activity, which underlies cancer cell survival and chemoresistance. In tamoxifen-resistant LCC2 and LCC9 breast cancer cell lines, rate of glycolysis was higher than that in MCF-7S cells and HIF-1 was activated through Akt/mTOR signaling pathway and phosphorylated AMPK was decreased without hypoxic conditions. The specific inhibition of glycolytic enzyme HK2 associates with suppression of Akt/mTOR/HIF-1 axis, and this along with increase in AMPK activity led to reduced lactate accumulation and cell survival [195].

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Using a combination of mathematical modeling, bioinformatics and experimental data, an association between ROS, HIF-1 and AMPK activities in breast cancer cell lines has been shown. Hybrid metabolic phenotype of cancer cells comprising both aerobic glycolysis and OXPHOS to adapt to varying microenvironment has been reported [196]. Three stable steady state depending on levels of HIF-1 and phosphorylated AMPK (pAMPK)–HIF-1^{high}/$pAMPK^{low}$, HIF-1^{low}/$pAMPK^{high}$, HIF-1^{high}/$pAMPK^{high}$, which correspond to glycolytic phenotype, OXPHOS phenotype (oxidative glucose degradation and fatty acid β-oxidation) and hybrid phenotype, respectively, have been described. Analyzing of well-annotated metabolomics and transcriptomics data along with mRNA sequencing data an association of HIF-1/AMPK activities with aggressive metastatic phenotype has been reported [197]. The authors concluded that targeting both glycolysis and OXPHOS is necessary to combat cancer aggressiveness.

Over-expression of AMPK contributes to tumor progression through multiple ways including stimulation of EMT and cell migration and adhesion. Moreover, AMPK signaling can exert opposite effects on tumor growth depending on cancer cell microenvironment including ROS production (reviewed in [198]). For example, an ability of AMPK to inhibit cancer growth through mitochondria-mediated metabolism has been suggested [199].

AMPK is a major controller of fatty acid metabolism. It inhibits acetyl-CoA carboxylase (ACC) by phosphorylation ACC1 at Ser79 and ACC2 at Ser212. ACC catalyzes conversion of acetyl-CoA into malonyl-CoA, a substrate for fatty acid biosynthesis [200]. Additionally, malonyl-CoA inhibits carnitine palmitoyl transferase 1 found in mitochondrial membrane to facilitate entry of fatty acids into mitochondria associated with increased FAO [201]. On the other hand, elevated production of ROS and inhibition of AMPK by isorhamnetin, which triggers cell cycle arrest at G2/M phase due to increase in the expression of cyclin-dependent kinase (Cdk) inhibitor $p21^{WAF1/CIP1}$ have been observed. Additionally, isorhamnetin induced apoptosis associated with down-regulation of Fas/Fas ligand, reduced ratio of B-cell lymphoma 2 (Bcl-2)/Bcl-2 associated X protein (Bax) expression, release of cytochrome *c* from mitochondria, and activation of caspases [202].

The increased mitochondrial ROS generation and AMP/ATP ratio caused AMPK activation, enhanced glycolysis and upregulation of uncoupling protein 2 (UCP2) have been observed in human cholangiocarcinoma associated with poor prognosis [203]. Additionally, gemcitabine has been shown to induce ROS/KRAS/AMPK-mediated metabolic reprogramming, mitochondrial oxidation and aerobic glycolysis to promote stem-like properties of pancreatic cancer cells [204]. Small GTPse KRAS is involved in Ras-MAPK-mediated signal transduction and formation of its active, GTP-bound, form is dramatically increased by

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GAP. It activates c-Raf and can contribute to Warburg effect in cancer cells through up-regulation of GLUT1. Enhanced glucose uptake and glycolysis rate along with increased cell survival associated with GLUT1 up-regulation has been observed in colorectal cancer cell lines with mutations in *KRAS* and *BRAF* genes under glucose deprivation conditions [205].

Smolkova and co-authors hypothesized that during carcinogenesis there may be waves in metabolic changes, which start from alterations in oncogene expression followed by HIF-1 stabilization and metabolic reprogramming characterized by increased glycolysis and suppression of mitochondrial oxidation and OXPHOS [206]. High rate of cell proliferation causes hypoxia, and nutrient and energy deficiency and this stimulate oxidative glutaminolysis and the involvement of LKB1-AMPK-p53, PI3K/Akt-mTOR signaling along with c-Myc dysregulation [207]. This leads to resumption of mitochondrial OXPHOS and each type of neoplasm is characterized by distinct metabolic phenotype according to waves of metabolic changes and oncogenic mutations.

7. Conclusions

Cancer is a complex disorder depending on multiple intracellular and micro-environmental factors influencing its initiation and progression. Tumor cells grow in a highly heterogeneous microenvironment characterized by both hypoxia and physioxia, and this requires the involvement of numerous regulatory proteins to control tumor growth, invasion and metastasis. Under hypoxia conditions, HIF-1α serves as a key oxygen sensor and a major transcriptional regular of numerous genes involved in glucose uptake and metabolism to provide switch in ATP production from OXPHOS to glycolysis. However, in many cancers the reverse from glycolysis to oxidative mitochondrial metabolism has been observed. Therefore, HIF-1α interplays with another master regulator of ATP production, AMPK, which enables switch from anabolic to catabolic metabolism to trigger oxidative degradation of glucose and β-oxidation of fatty acids, the major producers of NADH and $FADH_2$ as sources electrons for OXPHOS. This interplay involves growth factor-initiated signaling pathways, oncogenes and transcription factors, and these multiple cross-talks underlie uncontrolled cancer growth, invasion and metastasis and underlie cancer chemoresistence to conventional anti-tumor drugs. Thus, more investigation are needed to understand cancer complexity and numerous interactions between various signaling pathways, which can cause switch between metabolic pathways to enable cancer cell tolerance to micro-environmental changes for proliferation and migration. This should be also taken into account in discovery of novel molecular targets for anti-cancer agents.

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Abbreviations: HIF-1, hypoxia-inducible factor-1; AMPK, AMP-activated protein kinase; GLUT, glucose transporter; ETC, electron transportation chain; OXPHOS, oxidative phosphorylation; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase complex kinase; PFK, phosphofructokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; TCA, tricarboxylic acid; EGF, epidermal growth factor, VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PKB, protein kinase B; mTOR, mechanistic (mammalian) target of rapamycin; UPS, ubiquitin-proteasome system.

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