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Cell Death Induction by Targeting Tumor Metabolism

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Abstract

Over the last century, a broader interest in the topic of tumor metabolism has emerged. From the 1920s onward, when Otto Warburg proposed increased aerobic glycolysis of tumor cells, a deeper understanding has established that tumor cells have an altered metabolism which is directly linked to cancer progression. It was soon discovered that not only do environmental changes lead to alterations in metabolism but that oncogenes have a profound influence in these alterations. They not only induce nutrient uptake and synthesis of proteins and DNA but can lead to a switch toward glycolysis, which identifies them as a major player in tumor metabolism. These observations have raised the interest to target metabolic pathways for cancer therapy and, interestingly, some of the first discovered chemotherapeutics target metabolic pathways and are still in clinic. Concerns that these targets will also affect normal cells has intensified research to understand how changes in tumor metabolism promote tumor growth and which enzymes and signaling pathways are involved. These observations led to the discovery of new targets and drugs that specifically affect tumor metabolism and can exploit the dependence of tumor cells on the metabolic changes.

Keywords: Tumor metabolism, glycolysis, AMPK, lipid metabolism, p53

1. Introduction

Already in the 1920s, Otto Warburg described that tumor cells typically use aerobic glycolysis for energy production rather than oxidative phosphorylation (OXPHOS) despite sufficient oxygen and the lesser yield of ATP. By now, altered tumor metabolism is recognized as a hallmark of cancer cells, which allows them to escape from the typical regulatory constraints that prevent normal cells from uncontrolled growth and proliferation. A number of theories have been proposed to explain this phenomenon, amongst them independence of oxygen especially in hypoxic areas, which often occur in tumors. Furthermore, tumor cells often have mutations in mitochondria, which could explain a shift toward glycolysis. Glucose and

glutamine uptake also have the advantage that they can not only be used as ATP source but also as building blocks for essential metabolites required for uncontrolled growth (e.g., amino acids, nucleotide triphosphates, NADPH) [1, 2]. Glucose is used for the formation of nucleic acids via the pentose phosphate pathway and glycolytic intermediates are used for fatty acid biosynthesis. Therefore, highly proliferating tumor cells need to change different aspects of their metabolism to meet the high demand of energy in the form of ATP and secure the supply of the major classes of macromolecules: carbohydrates, proteins, lipids, and nucleic acids (Figure 1).

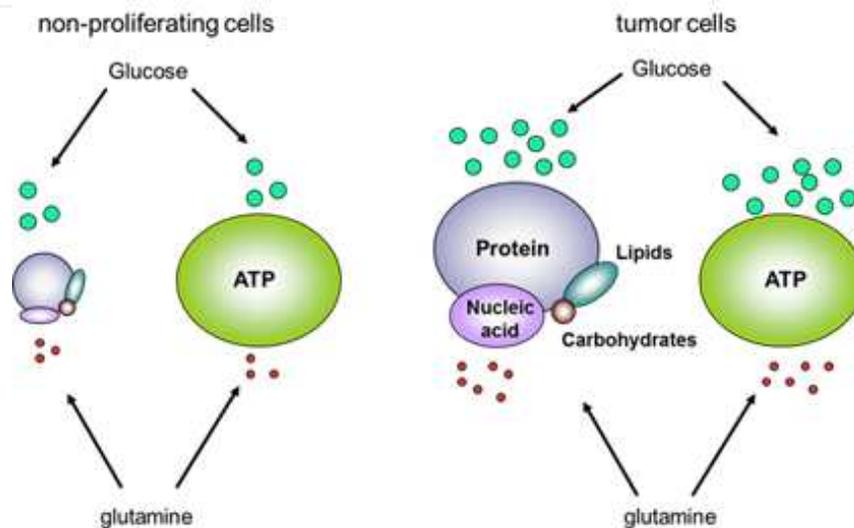


Figure 1. Tumor cells use glucose and glutamine not only for ATP production but also as source of essential metabolites. To ensure uncontrolled growth and proliferation, they have a much higher demand of nutrients, which is provided by aerobic glycolysis.

In recent years, it has also been revealed that the metabolic reprogramming is not necessarily induced by different metabolic requirements but also regulated by oncogenes and tumor suppressors. The PI3K/Akt pathway is often deregulated in tumor cells. Akt is one of the most important proteins in cells which have elevated glycolysis as it promotes the increased expression and membrane localization of the glucose receptor-1 (GLUT-1) and stimulates phosphofruktokinase activity. It thus promotes augmented glucose uptake and increased glycolytic activity. The tumor suppressor p53, on the other hand, can shift tumor cells from glycolysis to oxidative phosphorylation [3, 4]. The major inducer and regulator of glycolysis is the hypoxia-inducible factor-1 α (HIF1 α), a transcription factor that is often found in highly metastatic and neoplastic tumor cells and strongly promotes glycolysis.

Alterations in tumor metabolism are quite heterogeneous, which impedes the finding of a generalized target. The changes depend on the availability of nutrients, oxygen, and the pH, and in turn depend on the different tumor vasculature. Proliferation requires nutrient uptake, metabolite and DNA synthesis, and energy production. Thus, genetic alterations in signaling pathways that drive the cells to proliferate are often involved in changes of the tumor metabolism. But drugs that target, e.g., DNA synthesis to inhibit proliferation are also directed

against normal proliferating cells and therefore lack selectivity. Therefore, the systematic characterization of the metabolic pathways that differ in cancer cells is an ongoing challenge which must lead to the discovery of drugs that specifically target proteins or enzymes altered in tumors. Targeting tumor metabolism has become a promising field in cancer therapy but requires an in-depth understanding of the metabolic regulation and signaling pathways involved, which will be reviewed here.

2. Targeting tumor metabolism

Understanding the metabolic pathways altered in tumor cells targeting the resources or specific pathways that fuel deregulated tumor metabolism has shown to be an attractive strategy for cancer therapeutics. Hexokinase inhibitors like 2-deoxy-D-glucose have already shown promising results in preclinical studies but also dichloroacetate, which targets pyruvate dehydrogenase kinase (PDK1); gemcitabine, which inhibits nucleic acid synthesis; or metformin, which induces the AMP-activated protein kinase (AMPK) are interesting compounds for targeting tumor metabolism. Interestingly, tumors with high glucose uptake detected by the 2-[¹⁸F]fluoro-2-deoxy-D-glucose–positron emission tomography (FDG–PET) scan show a worsened outcome [5], confirming the importance for drugs targeting tumor metabolism.

The complexity and tight regulation of the tumor metabolism raises the possibility to target multiple pathways, enzymes, and proteins, some of the most important ones of which will be addressed here.

2.1. Glycolysis

2.1.1. Hexokinase

Hexokinase catalyzes the first and rate-limiting step in glycolysis (see Figure 2) by the ATP-dependent phosphorylation of glucose to yield glucose-6-phosphate (G6P). There are four different hexokinase isoforms (HK1-4), which show different tissue distribution and enzyme activity. The high affinity kinases HK1 and HK2 are inhibited by excess G6P, are associated with the mitochondria, and implicated in cell survival [6]. Hexokinase-2 was shown to be highly expressed in tumor cells but only in a limited number of normal tissues and is partly responsible for the increased glycolytic activity of tumor cells. Hexokinase level could also be correlated with tumor stage and patient survival [7].

Due to the importance of hexokinase for the glycolytic flux, there exist a variety of inhibitors such as 2-deoxy-D-glucose (2DG), 3-bromopyruvate, and lonidamine, which showed promising effects in preclinical studies [8].

2DG is a glucose analogue that is phosphorylated by hexokinase to form 2DG phosphate, which cannot be further metabolized. Treatment of tumor cells with 2DG inhibits glycolysis, leading to ATP depletion, cell growth inhibition, and apoptosis [9]. 2DG has extensive metabolic effects and not only affects glycolysis but also OXPHOS. In normoxia, it can interfere with N-linked glycosylation and induce the unfolded protein response [10].

Despite promising activities *in vitro*, the effects of 2DG as a single agent *in vivo* and in clinical trials were a disappointment. This might be due to off-target effects like the activation of the PI3K pathway or the induction of pro-survival autophagy [11]. Furthermore, the dose needed for complete inhibition of glycolysis in patients showed severe side effects. In combination therapy, however, lower doses of 2DG, which are better tolerated, could improve the efficiency [12].

2.1.2. Hypoxia-inducible factor-1 α

Tumor growth is associated with intratumoral hypoxia due to the lack of sufficient vascularization. The physiological consequence for the tumor to survive in this hostile environment is to increase angiogenesis to achieve adequate oxygen delivery, to adapt the metabolism by increasing glucose uptake, and switch to glycolysis for energy supply. All these actions are initiated by the transcription factor hypoxia-inducible factor-1 α (HIF1 α), which is the major regulator of glycolysis and induces more than a hundred genes involved in metabolism [13]. Increased activity of HIF1 α is known to correlate with poor patient outcome and, interestingly, some highly neoplastic tumors like renal cell carcinoma frequently carry a mutation that leads to a constitutive active HIF1 α . The best known action of HIF1 α is the induction of VEGF (vasculature endothelial growth factor), which is required for angiogenesis [14, 15]. It also induces the expression of the glucose transporters GLUT-1 and -3, and activates a number of glycolytic enzymes like aldolase, phosphofructokinase, enolase, and lactate dehydrogenase. It furthermore activates pyruvate dehydrogenase-1 (PDK1) and, thereby, reduces the flow of pyruvate used by the TCA cycle, decreasing OXPHOS and further increasing glycolysis, as shown in Figure 2.

HIF1 is a heterodimer composed of the constitutive expressed HIF1 β and the oxygen-sensitive HIF1 α . In the presence of oxygen, HIF1 α is hydroxylated by prolyl hydroxylases on prolyl-residues, which are required for the binding of the ubiquitin E3 ligase Von-Hippel-Lindau (VHL). Binding of VHL results in the degradation of HIF1 α by the proteasome. Under some circumstances like overexpression of the PI3K/Akt pathway or the induction of reactive oxygen and nitrogen species, HIF can be stabilized even under normoxia [16].

Due to the importance of HIF1 α in tumor growth and survival and the poor outcome of patients with high levels of HIF1 α , targeting this protein seemed to be a promising option in tumor therapy. Interestingly, until now no specific inhibitors of HIF1 α exist in the clinic, although there have been various efforts, and some experimental drugs exist, which inhibit transcription, translation, or DNA binding of HIF1 α [17]. The most promising drugs inhibit HIF1 α as a side effect; like rapamycin, which inhibits mTOR activity. The PI3K/Akt/mTOR pathway plays an important role for HIF1 α translation and rapamycin as well as the PI3K inhibitor LY294002 have shown to be able to reduce HIF protein expression as well as expression of its target genes [18, 19].

The proteasome inhibitor bortezomib is another drug that was reported to inhibit adaption to hypoxia of tumors and to functionally inhibit HIF1 α . Bortezomib is approved for the treatment of multiple myeloma and, therefore, an interesting candidate for analyzing effects on HIF. It

inhibits the expression of the HIF target genes VEGF and erythropoietin and the recruitment of p300 coactivator [20].

2.2. Signaling proteins and growth control elements

2.2.1. PI3K/Akt

The PI3K pathway is one of the most common altered signaling pathways in cancer. The main actor is the serine/threonine kinase Akt, which is able to activate many downstream targets involved in cell growth, survival, and cell cycle progression. A constitutive activation of Akt not only leads to strong pro-survival signals but also has a strong impact on tumor metabolism. Increased Akt signaling has been shown to directly correlate with increased glucose metabolism in a variety of tumor cells [21]. It supports aerobic glycolysis even in untransformed cells when overexpressed. Akt increases glucose uptake through promoting the translocation of the glucose receptor GLUT1 to the plasma membrane and also induces several glycolytic enzymes like hexokinase and phosphofructokinase. Furthermore, it mediates the increase of a variety of fatty acid- and cholesterol-synthesis enzymes. It promotes binding of the hexokinase to the mitochondrial membrane and thereby, like Bcl-2 or Bcl-X, increases mitochondrial membrane integrity and inhibits apoptosis [22]. Finally, Akt strongly induces signaling via mTOR by phosphorylating and inhibiting its negative regulator tuberous sclerosis complex 2 (TSC2). mTOR is a key metabolic checkpoint which leads to induction of protein and lipid biosynthesis when activated and, therefore, promotes cell growth. The importance of Akt in glucose metabolism is also supported by the fact that targeting the PI3K pathway in animal models and the use of kinase inhibitors in patients lead to a decrease in glucose uptake as measured by FDG-PET uptake [23, 24].

These observations lead to the assumption that inhibition of Akt would lead to inhibition of glycolysis and especially kill tumor cells that are dependent on a permanent supply of glucose. Using Akt inhibitors on tumor cells with aberrant PI3K signaling would, on the one hand, sensitize them to glucose-starvation-induced apoptosis as shown by Elstrom *et al.* (2004) [21] and, on the other hand, would open a new therapeutic window for PI3K inhibitors in cells dependent on aerobic glycolysis.

2.2.2. AMPK

The AMP-activated protein kinase (AMPK) belongs to a family of serine/threonine kinases and is highly conserved from yeast to mammals. It consists of a catalytic α subunit and regulatory β and γ subunits. It is the most important energy-sensing protein in the cell and activated by a number of metabolic or oncogenic stresses like glucose and nutrient deprivation or hypoxia. AMPK senses the levels of AMP and ATP and is activated by an increased AMP/ATP ratio. Upon activation, it increases catabolic processes that generate ATP-like fatty acid oxidation and glycolysis and inhibits anabolic processes that consume ATP-like protein and lipid synthesis [25] (shown in Figure 3). The dominant upstream kinase that regulates AMPK activity is the liver kinase B1 (LKB1), which is a known tumor suppressor. Loss-of-function mutations of this kinase were first discovered in Peutz-Jeghers syndrome, an autosomal

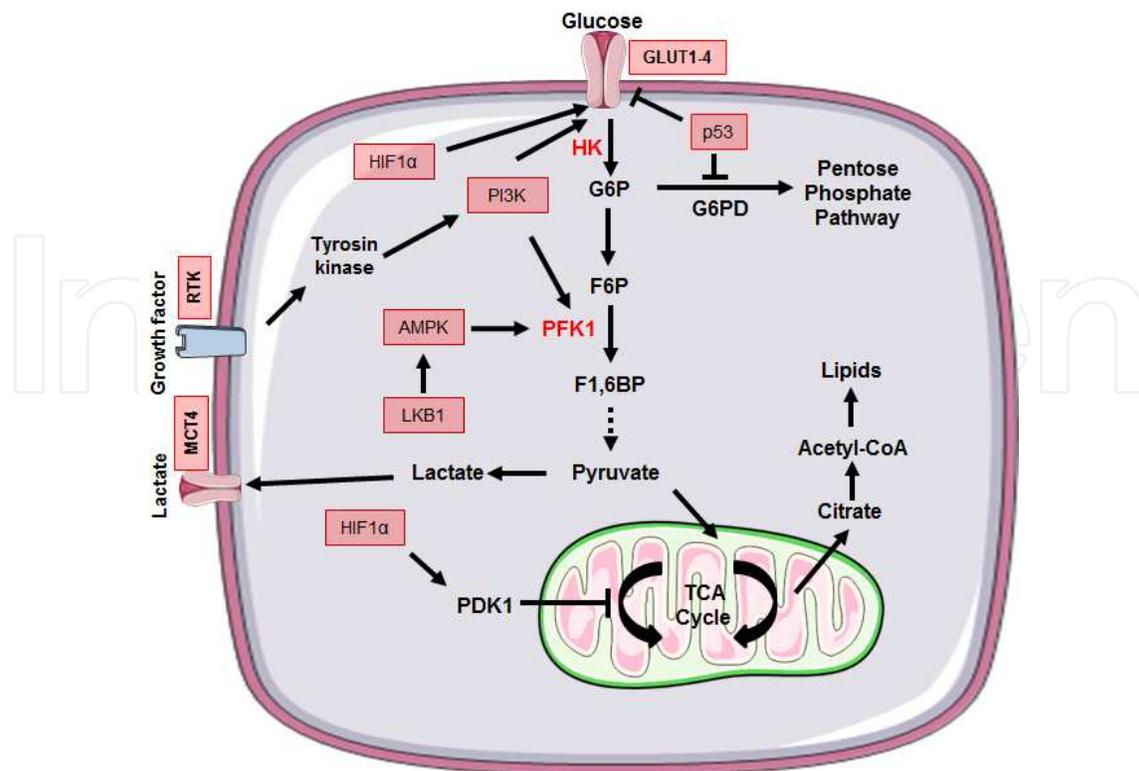


Figure 2. Simplified schema of tumor metabolism showing glycolysis, TCA cycle, lipid metabolism, and pentose phosphate pathway. Shown are proteins that are overexpressed or mutated to guarantee an increased glycolysis and decreased oxidative phosphorylation (HIF1 α , PI3K/Akt, p53). HK (hexokinase), PFK1 (phosphofructokinase-1), GLUT (glucose receptor), G6P (glucose-6-phosphate), PDK1 (pyruvate dehydrogenase kinase-1), TCA cycle (tricarboxylic acid cycle), RTK (receptor tyrosine kinase), MCT4 (monocarboxylate transporter-4).

dominant genetic disorder characterized amongst others by an increased risk of gastrointestinal adenocarcinoma [26]. Mutations of LKB1 were also found in several tumors like sporadic lung adenocarcinoma or cervical carcinoma. One of the major downstream targets of AMPK is mTOR, which induces protein synthesis. During energy stress, AMPK leads to an inhibition of mTOR on the level of TSC2 and consequently to an inhibition of protein synthesis. Interestingly, ablation of LKB1 abolishes this inhibitory effect [27].

Although decreased activity of AMPK was shown to promote tumor growth, in recent years, it has become evident that AMPK activation can also prevent tumor formation, especially under conditions of hypoxia and glucose deprivation. Deletion of AMPK α 1 synergizes with myc to promote lymphangiogenesis of B cell lymphoma, which supports the role as a tumor suppressor. In breast carcinoma, reduced AMPK phosphorylation inversely correlated with histological grade and axillary node metastasis [28]. Furthermore, AMPK was shown to inhibit cell proliferation by stabilization of p53 or regulation of cyclin-dependent kinase (CDK) inhibitors p21 and p27. But although activated AMPK can inhibit tumor cell proliferation, loss of AMPK is not sufficient to allow proliferation in the absence of nutrients. There are even reports that tumor cells lacking AMPK undergo apoptosis during metabolic stress and are

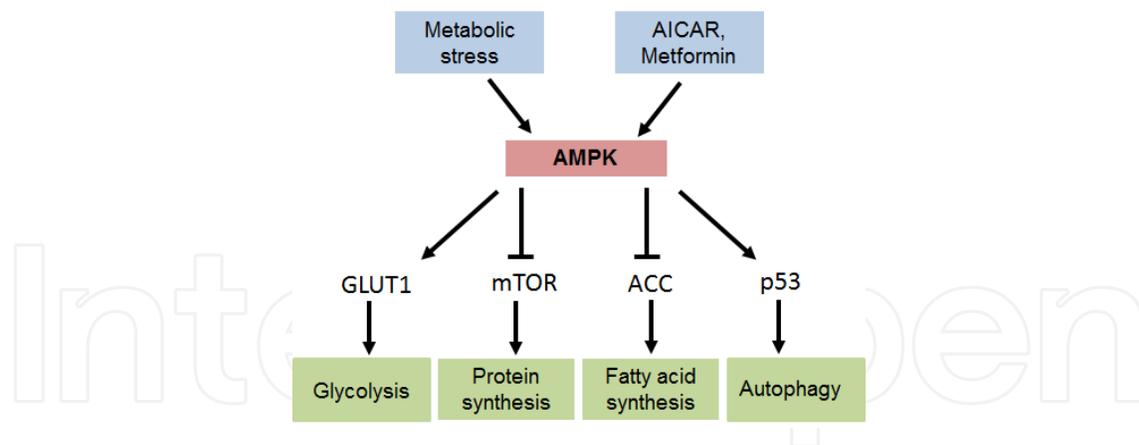


Figure 3. Major functions of AMPK activation. AMPK activation leads to an induction of glycolysis, fatty acid oxidation, and autophagy, and to an inhibition of protein synthesis.

resistant to oncogenic transformation [29]. On the other hand, pharmacological activators of AMPK like metformin, AICAR, or A769662 inhibited or delayed tumor formation in animals [30]. Faubert *et al.* (2013) could show that silencing AMPK promotes a metabolic shift to the Warburg effect with increased glucose uptake, glycolytic flux, and flow of carbon to the tricarboxylic cycle to fuel pathways of ATP production and biosynthesis [31]. The proapoptotic mechanisms of AMPK might be mainly associated with the inhibition mTOR. In line with this assumption, it was shown that AMPK activation by metformin or the AMP analogue AICAR correlated with mTOR inhibition in renal cell carcinoma [32].

Pro-survival mechanisms of AMPK can be partly explained by autophagy induction, which is mediated by p53, and maintenance of proliferative quiescence [33]. Therefore, the role of AMPK as an oncogene or tumor suppressor seems to be dependent on the degree of AMPK activation and duration of nutrient deprivation. Despite the promotion of AMPK agonists like metformin as anticancer treatment, there needs to be a deeper understanding of AMPK regulation in tumorigenesis to implement AMPK-targeting drugs into clinic.

2.2.3. p53

The tumor suppressor protein p53 is the most frequent mutated gene in cancer. It is well known for its role in DNA damage repair and apoptosis induction after cellular stress. Depending on the stress signals, it either leads to growth arrest by inducing p21, Gadd45 or p48 or in response to severe stresses it induces genes involved in apoptosis (Puma, Bax, Fas) or senescence (p21). But it is emerging that it also plays a pivotal role in tumor metabolism by inhibiting glycolysis and switching tumor metabolism to OXPHOS and thus inducing apoptosis.

p53 has multiple functions in tumor metabolism and considering its role as tumor suppressor, it is not surprising that p53 counteracts metabolic changes associated with cancer growth: it transcriptionally represses the expression of the glucose transporters GLUT1 and GLUT4 and indirectly represses expression of GLUT3 via NF κ B inhibition and, therefore, inhibits the first rate-limiting step in glycolysis [34]. At the third step of glycolysis, phosphofruktokinase-1

(PFK1) is inhibited by various metabolites that indicate sufficient supply of energy like ATP, citrate, and lactate. AMP and fructose-2,6-bisphosphate (F26B), which indicate low energy, activate PFK1 and, thus, increase glycolytic flux. TP53-inducible glycolysis and apoptosis regulator (TIGAR) is an important protein activated by p53. It acts as a phosphatase and degrades F26B decreasing the activity of PFK1 and consequently lowers the glycolytic rate [4, 35]. There are also some other glycolytic players that are inhibited by p53-like expression of pyruvate dehydrogenase kinase 2 (PDK2), which leads to a decreased conversion from pyruvate to lactate. Inhibition of the glycolytic pathway on multiple levels would assume that glucose would be shuttled into the pentose phosphate pathway (PPP) but it was recently shown that p53 also inhibits PPP by binding and inhibiting glucose-6-phosphate dehydrogenase (G6PD), the enzyme that catalyzes the first step of the PPP [36]. Therefore, p53 can reduce the production of NADPH and ribose-5-phosphate, which are important for reactive oxygen defense and DNA synthesis.

p53 not only inhibits glycolysis but also enhances OXPHOS: it transcriptionally activates cytochrome c oxidase 2 (SCO2), which is required for the assembly of the cytochrome c oxidase complex (complex IV in the mitochondrial electron transport chain) [37] and induces the expression of AIF (apoptosis-inducing factor), which is important for OXPHOS, most likely by ensuring the proper assembly of mitochondrial respiratory complex I. Furthermore, p53 regulates mitochondrial DNA copy number and mitochondrial quality control by removing damaged mitochondria [38]. By these actions, p53 directly counteracts the Warburg effect and therefore opposes metabolic changes that are essential for malignant transformation.

The metabolic functions of p53 are emerging as critical for tumor suppression and apoptosis induction via targeting tumor metabolism. Nevertheless, the many different stress signals that activate different p53 responses are still not fully understood. Therefore, future studies to understand the molecular mechanisms that activate p53 and mediate the responses to metabolic stress need to be performed to finally exploit and manipulate them for tumor therapy.

2.3. Pathways

2.3.1. *Pentose phosphate pathway*

Glucose is the main energy source not only for tumor cells but for all organisms. It enters the cell via glucose transporters and is then phosphorylated by hexokinase to form glucose-6-phosphate (G6P). From this point, G6P can either enter glycolysis to produce energy in form of ATP or it is shuttled to the pentose phosphate pathway (PPP). There, it is either hydrogenated by G6PD (glucose-6-phosphate dehydrogenase) and further converted to yield ribulose 5-phosphate in the oxidative branch. Or it enters the nonoxidative branch, which is catalyzed by transketolase, and generates ribose-5-phosphate, which is a precursor of biomolecules like nucleotides and, therefore, important for DNA synthesis (see Figure 4). During the oxidative phase, NADP⁺ acts as electron acceptor during the oxidative reactions and 2 molecules of NADPH are yielded. NADPH plays an important role in the protection of the cell from oxidative stress. Therefore, the PPP plays a pivotal role in reductive biosynthesis like lipid and

nucleotide synthesis and is essential for antioxidant defense. It is strongly connected with glycolysis and glucose is shuttled to the pathway where it is needed most. Historically, most attention was paid to glycolysis as it provides the energy for biosynthesis, but highly proliferating cells also need large amounts of lipids as energy storage and building blocks for membranes and nucleotides for DNA replication [39]. These needs are fulfilled by the PPP. In recent years, more attention was paid to changes in the pathway and effort is taken to find ways to target the PPP for tumor therapy.

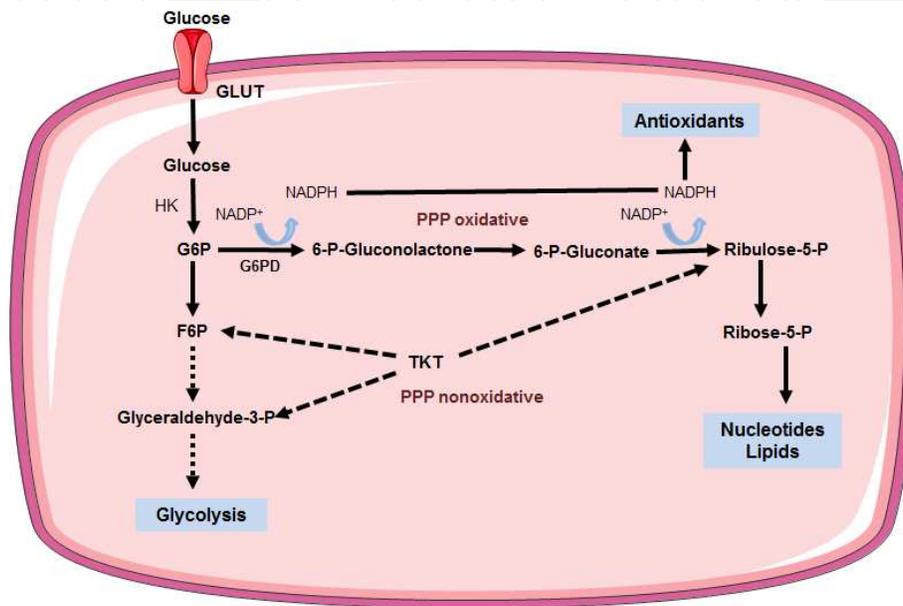


Figure 4. Schematic representation of the PPP and its connection with glycolysis. The oxidative branch of the PPP yields NADPH, which can be used for antioxidant defense and biosynthetic reactions. The nonoxidative branch produces ribulose-5-phosphate and glycolytic intermediates. G6PD – glucose-6-phosphate dehydrogenase; TKT – transketolase.

Considering its importance for proliferation, it is not astonishing that increased PPP flux and overexpression of G6PD have been found in several tumors like large B-cell lymphoma or lung adenocarcinoma. It was also shown that it plays a role in promoting malignant cell growth and inducing anchorage-independent growth in NIH3T3 (mouse embryonic fibroblast cell line) cells overexpressing G6PD [40, 41]. These findings suggest G6PD as an oncogene and interesting target for tumor therapy, which is also supported by the fact that the tumor suppressor p53 can bind to G6PD and inhibit its dimerization leading to a decreased G6PD activity, reduced glucose consumption and NADPH production, and, therefore, decreased tumor cell growth [36]. Furthermore, combinations with G6PD inhibitors like DHEA or 6-aminonicotinamide (6AN) with chemotherapeutics like 2-deoxy-D-glucose or oxythiamine, a TKT inhibitor, are shown to increase the inhibition of cancer growth and enhance the radiosensitivity of human gliomas and squamous carcinoma cell lines [42, 43]. Also, many chemotherapeutics like 5-fluoracil or gemcitabine led to the induction of ROS via DNA damage, which could be enhanced by the use of a G6PD inhibitor, and strengthened the importance of the PPP as a target for chemotherapy.

2.3.2. Lipid metabolism

Not only glucose metabolism is altered in cancer cells but also lipid biosynthesis is enhanced to meet the requirements of fast-proliferating cells. Normal cells obtain lipids via the uptake of free fatty acids (FAs) or low density lipoproteins (LDL) from the bloodstream. New synthesis of fatty acids and cholesterol is restricted to a few specialized tissues like the liver, adipose tissue, or the lactating breast. In cancer cells, however, these restrictions are interrupted and new synthesis of lipids is observed. Lipids are needed for the new synthesis of phospholipid membranes, lipid modified signaling molecules, and as energy storage to survive times of nutrient deprivation. FAs consist of a terminal carboxyl group and a hydrocarbon chain, mostly occurring in even numbers of carbons that can be either saturated or unsaturated. The acetyl groups needed for fatty acid biosynthesis are mostly provided by citrate, which is generated in the TCA cycle. The rate-limiting step of lipid synthesis is the conversion of acetyl-CoA to malonyl-CoA by the acetyl-CoA-carboxylase (ACC), which is then further processed by fatty acid synthase (FAS) to yield saturated and unsaturated fatty acids as shown in Figure 5. Several enzymes in fatty acid biosynthesis have been explored as potential targets for tumor therapy. Especially FAS and ACC are in focus as their inhibition by siRNA or chemical inhibitors led to growth arrest of tumor cells [44].

FAS is a prominent target as inhibition preferentially kills tumor cells and many tumors show increased FAS expression and dependence on *de novo* fatty acid synthesis, whereas nontumor cells rely on exogenous FAs. Inhibition of FAS, for example, leads to apoptosis induction of cells derived from lymph node metastasis of prostate carcinoma LNCaP cells and to an inhibition of HER2 expression in breast cancer cells [45, 46].

ACC is the most regulated enzyme in FA-synthesis. It is positively regulated by citrate and glutamate and inactivated by AMPK. Inhibition of ACC by siRNA or the chemical inhibitor soraphen A induced apoptosis in breast cancer and prostate cancer cells [47, 48]. Another ACC inhibitor, TOFA, showed growth inhibition of ovarian cancer cells and ovarian tumor mouse xenografts [49].

Apart from fatty acids, cholesterol plays a critical role in tumor growth and survival. Cholesterol is either obtained by uptake of LDL from the extracellular environment by the LDL receptor (LDLR) or it is newly synthesized in the mevalonate pathway (Figure 5). The first steps of the mevalonate pathway include the condensation of acetyl-CoA with acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The reduction of HMG-CoA to mevalonate by the HMG-CoA reductase is the rate-limiting step in cholesterol synthesis. Cholesterol is an important component of biological membranes as it modulates fluidity of the lipid bilayer and is part of the detergent-resistant lipid rafts that are membrane parts with high lipid content, which coordinate activation of a variety of signal-transduction pathways. Intermediates of the mevalonate pathway like geranylgeranyl or farnesyl are responsible for isoprenylation of small GTPases like Ras or Rho. Small GTPases play an important role in various cellular events (e.g., intracellular signal transduction, proliferation) and are anchored in the membrane. Accumulation of cholesterol has been associated especially with prostate cancer and an aberrant mevalonate pathway has been linked to several cancers [50].

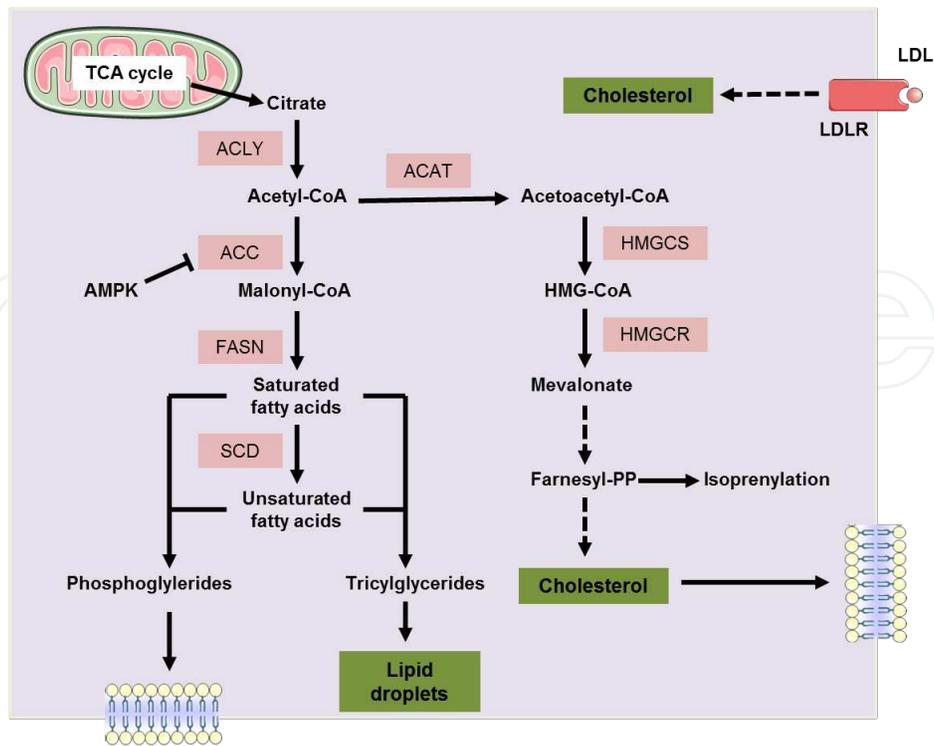


Figure 5. Schematic overview of the lipid and cholesterol metabolism. Phosphoglycerides, which are needed for membrane components, and triglycerides, which are stored in lipid droplets, are generated via multiple steps from fatty acids. Cholesterol is produced either by uptake of LDL or by synthesis via the mevalonate pathway and is an important component of the plasma membrane. Intermediates are used for isoprenylation of small GTPases. ACLY – ATP citrate lyase; ACC – acetyl-CoA-carboxylase; FASN – fatty acid synthase; SCD – stearyl CoA desaturase; ACAT – acetyl-CoA-acetyltransferase, LDLR – low density lipoprotein receptor; HMGCS – 3-hydroxy-3-methylglutaryl-CoA synthase; HMGCR – HMG-CoA reductase.

The HMG-CoA reductase is the target for a group of cholesterol lowering drugs, the statins. Interestingly, there are some reports that statins reduce the risk for some cancers like colorectal cancer or hepatocellular cancer. But other studies found no connections. The effect seems highly dependent on the tumor type. Statins show antiproliferative activities and apoptosis induction in a variety of cancers. The cytostatic properties of statins are mainly due to, on the one hand, inhibition of cholesterol synthesis and, on the other hand, inhibiting the formation of isoprenoids. This could be confirmed by the addition of mevalonate or geranylgeranyl pyrophosphate, which could overcome the antiproliferative effects of statins [51]. Blocking the activity of small GTPases like the oncogene Ras leads to inhibition of pro-survival signaling cascades.

The induction of apoptosis by statins has been widely studied and several mechanisms of action have been revealed. In breast cancer cells, it was shown that apoptosis is induced by the activation of the c-Jun terminal kinase JNK and by downregulation of Bcl-2. In chronic myeloid leukemia, simvastatin induces apoptosis by inhibiting the NF κ B pathway [52, 53].

These promising preclinical data lead to several phase I and II studies with the result that success of statins in tumor therapy is very tumor-specific and dependent on how important the mevalonate pathway is for the tumor.

2.4. pH regulation

2.4.1. Extra-and intracellular pH

The intracellular and extracellular pH of cells is one of the major factors that influence molecular processes involved in cell cycle progression and proliferation. Therefore, tumor formation and response of tumors to chemotherapeutics is also highly affected by the environmental acidity. Fast-growing tumors require a complementary vasculature to fulfill the extensive need of nutrients and oxygen. But solid tumors often develop faster than the blood supply, which results in a hypoxic environment. Due to the high glycolytic rate, tumor cells produce increased amounts of H^+ (lactate, carbonic acids) and therefore create a hostile microenvironment. The resulting acidic environment is toxic for normal cells and establishes an advantage for growth of tumors, which evolve adaptive mechanisms that allow them to survive [54]. By these adaptive mechanisms, tumor cells transport protons and lactic acid into the extracellular space via acid-base regulators like monocarboxylate transporters and Na^+/H^+ exchangers, leading to an acidic microenvironment and to a slightly alkaline cytosol. These pH conditions have shown to be beneficial for metastasis, apoptosis resistance, and increased proliferation. An acidic extracellular space promotes the activation of certain proteases (cathepsins, metalloproteinases) that degrade components of the basement membrane and extracellular matrix (ECM) and, therefore, create the prerequisite for metastasis by enabling tumor cells to get to the bloodstream. Cathepsin family members, especially cathepsin B and K, are overexpressed in metastatic tumors and silencing of these cathepsins was shown to reduce tumor cell invasion [55, 56].

Matrix metalloproteinases (MMP) are also more active at acidic pH. They have been considered as prognostic biomarkers for some metastatic cancers and a number of MMP inhibitors have been developed and tested in clinical trials [57].

The slightly alkaline intracellular pH created by tumor cells was shown to promote proliferation and inhibit apoptosis. Caspases, for example, need an acidic pH to be activated and it was shown in a variety of tumor cells that chemotherapeutics that induce apoptosis lead to an acidification of the cytosol. An alkaline pH_i also plays a role in the uptake of chemotherapeutics as the most common drugs are weak bases with intracellular targets that are protonated at lower pH and neutral at higher pH. Therefore, permeation through the plasma membrane and accumulation in the cell is impaired [58].

The importance of pH regulation for tumor cells drove the development of drugs that disrupt tumor pH-regulating systems. Na^+/H^+ exchangers like NHE1 are the predominant regulators of pH_i. Alkalization of the cytosol triggered by NHE1 is linked to malignant transformation. NHE1 inhibition leads to apoptosis induction of different tumor cell lines and xenograft models and showed a decrease in tumor formation with oncogene-transformed fibroblast lacking NHE1 or KRAS-transformed tumor xenografts. But so far there have not been promising effects for NHE1 inhibitors in monotherapy, especially due to immense toxic side effects [54].

The increased glycolytic flux of tumor cells requires a system to export lactic acid from the cell, which is done by monocarboxylate transporters (MCTs). Among the 14 family members, MCT1

and 4 are specialized for the cotransport of lactate and H⁺, and a high expression of especially MCT4 was found in rapidly growing tumors such as triple-negative breast cancer [59]. Owing to their pivotal role in pH_i regulation by securing a slight alkaline cytosolic pH despite high production of lactate, there was ongoing research for the development of small molecule inhibitors. And indeed, MCT inhibition impaired glioblastoma cell proliferation, migration, and survival [60]. Treatment with metformin could sensitize tumor cells to MCT inhibition, which indicates the possibility that combination with other metabolic or pH regulating drugs could target tumors dependent on glycolysis.

2.4.2. V-ATPase

The vacuolar H⁺-ATPase (V-ATPase) is a multisubunit enzyme, which is located in the membranes of almost all eukaryotes. It is responsible for acidifying intracellular organelles like endosomes, lysosomes, golgi-derived vesicles, or secretory vesicles and is therefore responsible for the pH homeostasis in the cell. Inhibition of the V-ATPase leads to apoptosis induction and inhibition of migration in a variety of tumor cells and has therefore become an interesting target in tumor therapy [61, 62]. As reported above, regulation of pH is important for tumor cell survival and metastasis as an acidic extracellular microenvironment facilitates metastasis of tumor cells by activating proteases like MMPs. The V-ATPase is expressed on the plasma membrane of metastatic and chemoresistant tumor cells and pumps protons across the membrane creating an acidic microenvironment [63].

Apart from regulating pH we could show that V-ATPase inhibition leads to an increased transcription of genes involved in glycolysis, fatty acid, and cholesterol synthesis. Furthermore, V-ATPase inhibition leads to an increased glucose consumption and especially a strong induction of the hypoxia-inducible factor-1 α (HIF1 α) occurs [64]. As described above, HIF1 α is the major inducer of glycolysis and metabolic stress and induction by the V-ATPase inhibitor archazolid strongly indicates that archazolid leads to changes in the tumor metabolism, which could be exploited for cancer therapy.

3. Conclusion

Although in recent years much work has been done in understanding the regulation of tumor metabolism and the discovery of drugs that specifically target tumor-related pathways, the complex interplay between oncogenic signaling pathways and tumor metabolism demands further research. Metabolic reprogramming may render cancer cells highly dependent on specific enzymes or processes that could be exploited for cancer therapy and induce apoptosis. But the search for relevant targets may be complicated due to the high diversity of tumor metabolism and the possibility of compensatory mechanisms. Another challenge is to identify those metabolic pathways that are essential targets and understanding the mechanism of apoptosis induction is inevitable in tumor therapy. It is important to understand that there is not one single tumor-specific metabolism but several programs that differ from tumor to tumor and are adjusted to the special requirements of different tumors in different tissues. Therefore,

targeting different enzymes in glycolysis might be beneficial in some tumors but may have no effect in others that still have the ability to use oxidative phosphorylation for energy production. Also, normal cells need to produce energy, nucleotides, or other metabolites, as well and it is important to investigate why there exists a therapeutic window that does not harm normal cells. The complexity of the tumor metabolism furthermore demands *in vivo* models to study the effect of drugs on a whole organism.

Only by further understanding the regulation of tumor metabolism, we will be able to translate this knowledge into the discovery of drugs that will lead to tumor growth inhibition and a better clinical outcome of cancer patients.

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