Role of Oncogenes and Tumor Suppressors in Metabolic Reprogramming and Cancer Therapeutics: A Review

Authors' Contribution

This work was carried out solely by this author. This author reads and approves the final manuscript.

ABSTRACT

Recently there has been a renewed interest on the signaling pathways and metabolic changes in cancer cells. It is well known that there are several oncogenes and tumor suppressors that affect cancer metabolism and re-engineer it for better growth and survival. The best description of tumor metabolism is the Warburg effect, which shifts from ATP production through oxidative phosphorylation to ATP production through glycolysis, even in the presence of oxygen. The Warburg effect is controlled by oncogenes—c-Myc, Kras, P1K/AKT/mTOR pathway—and tumor suppressors—p53, LKB1/AMPK, PTEN, and RB. Studies on oncogenes and tumor suppressors suggest potential therapeutic strategies. The oncogene Kras promotes increased glucose uptake, glycolytic flux and ribose biogenesis, and mediates reprogramming of glutamine metabolism by changes in gene expression. The tumor suppressor p53 promotes the expression of antioxidant proteins that regulate oxidative stress and glucose metabolism. The LKB1/AMPK agonists have potential to be anticancer drugs, as patients treated by metformin for diabetes had a lower incidence of cancer. Discovering the mechanism by which oncogenes and tumor suppressors regulate metabolism will allow for designing treatment strategies. This review discusses how several oncogenes and tumor suppressors regulate cellular metabolism, and the current therapeutic findings.

Keywords: Cellular metabolism; Tumor Suppressors; Oncogenes; Cancer therapy; Review

1. INTRODUCTION

For many years, cancer research has focused on understanding how cancer cells cope with their metabolic needs in order to survive [1]. Cancer is a disease in which cells lose their normal checks on proliferation and normal survival [2]. In order to meet their need to multiply, tumor cells often show major changes in pathways of energy metabolism and nutrient uptake [2]. One notable change is their preference to metabolize glucose through glycolysis [3].

Contrary to normal cells, proliferating cells have a greater need for glucose and glutamine. Through glycolysis, glucose is metabolized to produce lactate even in the presence of oxygen [3,4]. To enter the TCA cycle, glutamine is first deaminated to glutamate, and then converted to a-ketoglutarate to be used as a substrate in the TCA cycle [5,6]. This conversion of pyruvate to lactate is necessary to regenerate NADP for glycolysis. Glucose

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and amino acids are also used to generate nucleic acids through the pentose phosphate pathway (PPP). TCA cycle intermediates are used to as precursors for building macromolecules such as fatty acids and non-essential amino acids, which are used in biosynthetic pathways that refill carbon to the cycle to maintain the supply of intermediates. Increased glycolysis and lipid synthesis commonly occur in all highly proliferative cells, indicating the need to adapt to new metabolic needs [7,8].

1.1 The Warburg Effect

In order to meet the higher energetic and biosynthetic needs, tumor cells exhibit key changes in their metabolism by taking up much more glucose, producing larger quantities of lactate, and lower use of oxidative phosphorylation (OXPHOS) [9,10]. This preferential use of glycolysis over mitochondrial OXPHOS is called aerobic glycolysis or the 'Warburg Effect,' which meets the demands of proliferating cells by providing substrates for macromolecular synthesis and energy production [2,11,12]. In 1924, Otto Warburg observed that cancer cells break down glucose differently than normal cells [2]. By studying how Louis Pasteur's observations on the possibility of glucose fermenting to ethanol in mammalian tissues, Warburg discovered that cancer cells "ferment" glucose into lactate even when oxygen is present for mitochondrial OXPHOS. In 1962, Warburg showed that glucose was not metabolized the same way in cancer cells versus normal, differentiated cells [13,14]. Even when ample oxygen is present, cancer cell prefer glycolysis instead of the TCA cycle, causing the resulting pyruvate to convert to lactate and be released from the cell [13,14].

Warburg observed that tumor slices and ascites cancer cells tend to take up glucose and yield lactate even with oxygen present (aerobic glycolysis), an observation similar to numerous cancer cells and tumors. This characteristic is also in normal proliferative tissues. Warburg's studies led him to propose that cancer was originated by irreversible damage of mitochondrial respiration and impaired mitochondria [13,14]. He believed that cells were unable to use oxygen efficiently due to permanent damage of oxidative metabolism, thus leading to cancer [15].

Warburg theorized that the metabolic switch from oxidative phosphorylation to glycolysis helped cancer cells proliferate due to use of glycolytic intermediates to produce new cells, such as nucleotides, amino acids, lipid synthesis pathways, and NADPH production to maintain redox balance [8, 16]. As a result, cancer cells display enhanced glucose uptake and produce higher levels of lactate [13] Warburg suggested that this observation exhibits the shortcomings of energy metabolism in the mitochondria, and may be the root cause of cancer [13, 14].

Recently, Warburg's hypothesis has been reevaluated. His original theory that cancer cells have impaired mitochondria, causing a shift in glucose metabolism from OXPHOS to glycolysis even in the presence of oxygen, led to a misconception that cancer cells primarily rely on glycolysis for ATP and yielded significantly less ATP through substrate-level phosphorylation reactions of glycolysis [8,17]. However, it is now clear that a majority of tumor cells possess normal functioning mitochondria and are able to undergo OXPHOS in both cancer cells and normal proliferating cells [2,16,18]. In fact, depleting mitochondrial DNA lowers the tumorigenicity of cancer cell lines in vitro and in vivo. Additionally, conversion of glucose to lactate has been displayed in genetically normal proliferating cells, as well as in virally-infected cells [18,19]. These observations suggest that the Warburg effect is a controlled metabolic state and may also be helpful when there is a need for increased biosynthesis [18].

1.2 Bioenergetics and Biosynthesis in Cancer Cells

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Although Warburg's observation of tumors consuming large amounts of glucose had been validated in many human cancers, many studies showed that most tumor cells are able to produce energy by oxidizing glucose to CO_2 in the TCA cycle, producing ATP via OXPHOS. In addition, lower ATP production through glycolysis via inactive pyruvate kinase does not prevent tumor formation, suggesting that the primary role of glycolysis is not ATP production [20]. Moreover, despite their high glycolytic rates, cancer cells require mitochondrial metabolism to generate high rates of ATP for proliferation [21].

Although Warburg initially only noted higher rates of glycolysis with increased lactate production in tumor ascites, tumor cell metabolism may also be rewired by micro-environmental changes including acidosis, substrate, and oxygen availability. Thus, tumor cells increase glycolysis and glutaminolysis to meet their ATP and NADPH needs [15]. Increased glucose uptake leads to glycolytic intermediates providing secondary pathways to meet metabolic needs of proliferating cells [8]. Fatty acids and amino acids can provide substrates (ex. pyruvate from glycolysis) to the TCA cycle to maintain production of mitochondrial ATP in cancer cells. Fatty acids break down in the mitochondria to produce acetyl-CoA, NADH, and FADH2, which are used to generate mitochondrial ATP [8].

While glucose metabolizing to lactate produces only 2 ATPs per molecule of glucose, OXPHOS produces up to 36 ATPs per glucose molecule. Although normal cells yield increased ATP production from glucose by mitochondrial oxidative phosphorylation, cancer cells generate much less ATP by glycolysis [2]. Although it is a less efficient process to produce ATP, aerobic glycolysis is a more rapid process. This is partly due to enhanced control of glucose transporters (Glut 1, Glut 2, Glut 3, and Glut 4) for higher glucose intake [2]. Therefore, the shift to aerobic glycolysis requires tumor cells to have unusually high rates of glucose intake via glucose transports to meet increased needs of energetics, biosynthesis, and redox [15].

Highly proliferating cancer cells not only need high ATP levels for growth and proliferation, but also require carbon skeletons for macromolecule biosynthesis (fatty acid and nucleotide biosynthesis). While these cells use enhanced aerobic glycolysis for ATP, they also preserve carbon skeletons since CO_2 is not produced in glycolysis [15]. Macromolecular synthesis uses TCA cycle intermediates, which resupply carbon to the cycle to maintain intermediate pools via glutaminolysis and pyruvate carboxylation [8].

Biosynthetic or anabolic pathways are necessary in cancer metabolism since they allow cells to generate macromolecules needed for cell division and tumor proliferation [8]. Two biosynthetic products need to be produced in tumor proliferation, including: (a) fatty acids for lipid biosynthesis and (b) ribose-5-phosphate (R5P) for nucleotide biosynthesis [15]. These anabolic pathways generally need simple nutrients (sugars, essential amino acids, etc.) from the extracellular space, and are converted into biosynthetic intermediates via metabolic pathways like glycolysis, the PPP, the TCA cycle, and finally the formation of more complex molecules via ATP-dependent processes [8]. Tumor cells require a robust nutrient intake to maintain their anabolic metabolism [15].

Biosynthesis of proteins, lipids, and nucleic acids may be under control of the same signaling pathways that control cell growth and are stimulated in cancer via PI3K-mTOR signaling (described below). Protein biosynthesis is highly controlled and needs access to all essential and nonessential amino acids. Both glutamine uptake and glutaminase are activated by mTORC1, which assists in amino acid synthesis [8].

1.3 Oncogenes and Tumor Suppressors Contributing to Warburg Effect

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A number of theories have been proposed to explain 'the Warburg effect.' It is now clear that cancer cells undergo aerobic glycolysis due to activation of oncogenes, loss of tumor suppressors, and that increased glycolytic activity indicates that anabolic pathways are available [18]. Both oncogenes and tumor suppressor gene products influence the switch between aerobic glycolysis and a more extensive use of the TCA cycle to generate more ATP [10]. Many of the well characterized oncogenes—PI3K, AKT, mTOR, c-Myc, and RAS—promote glucose and amino acid uptake and metabolism in order to make new lipids, nucleotides, and proteins. Conversely, tumor suppressors—p53, LKB1/AMPK, PTEN, and RB—tend to inhibit glycolysis and upregulate oxidative phosphorylation [22]. Most oncogenes and tumor suppressor genes encode proteins that promote either cellular proliferation or cell cycle arrest by driving signaling pathways that support core functions like anabolism, catabolism, and redox balance (Fig. 1) [8,14,16,23].

Cancer metabolism has become an area of intense research, and several oncogenes and tumor suppressors are intimately involved in this process. This review will discuss how several oncogenes and tumor suppressors regulate cellular metabolism. Understanding and unraveling the mechanisms by which oncogenes and tumor suppressors regulate metabolism will be key to developing new therapeutic targets.

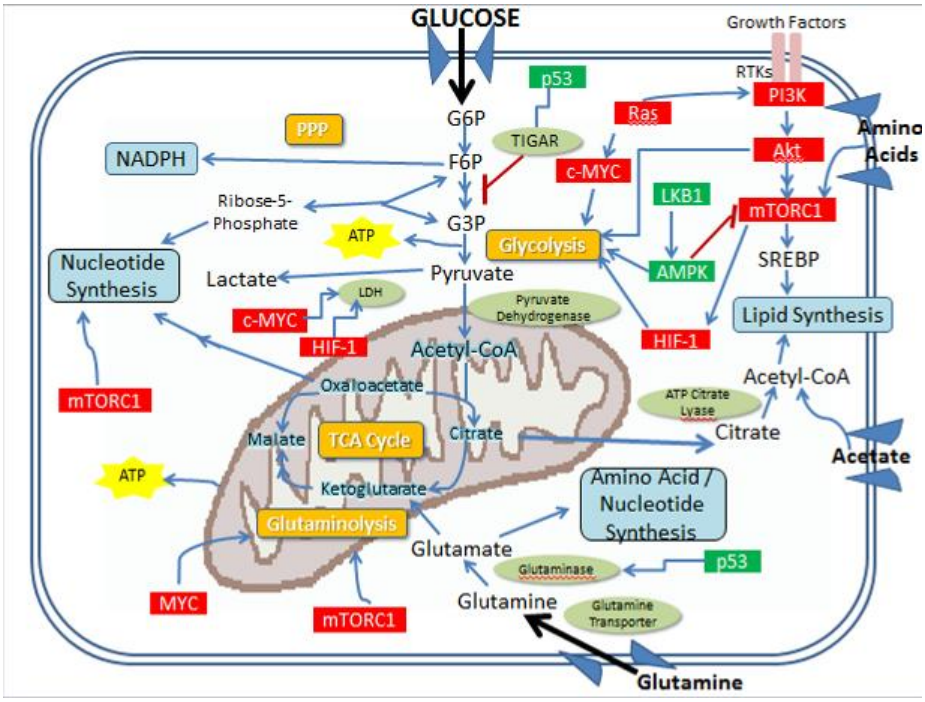

FIGURE 1: Signaling pathways of oncogenes and tumor suppressors contributing to the Warburg Effect

Glycolysis, oxidative phosphorylation, pentose phosphate pathway, and glutamine metabolism are all involved in regulating cancer metabolism. Through growth factor stimulation, receptor tyrosine kinases (RTKs) activate downstream pathways PI3K-Akt-mTORC1 and Ras, causing an anabolic reaction with increased glycolysis and fatty acid production by activating hypoxia-inducible factor–1 (HIF-1) and sterol regulatory element-binding protein (SREBP). RTK also signals oncogenic c-Myc, which increases the expression of many genes to support anabolism, including transporters and enzymes involved in glycolysis, fatty acid synthesis, glutaminolysis, serine metabolism, and mitochondrial metabolism. Oncogenic Kras works with PI3K and MYC pathways to support tumor formation. On the contrary, proto-oncogenes such as LKB1/AMPK signaling and p53 decrease metabolic flux through

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glycolysis in response to cell stress. The p53 transcription factor transactivates enzyme TIGAR and results in increased NADPH production by PPP. Signals impacting levels of hypoxia inducible factor (HIF) can increase expression of enzymes such as LDHA to promote lactate production, and pyruvate dehydrogenase kinase (PDK) to limit pyruvate entering into the Krebs Cycle.

2. ROLE OF ONCOGENES

2.1 HIF-1: Regulates Hypoxic Responses and Growth Factors in Cancer Metabolism

Due to increased oxygen consumption, proliferating cancer cells are in a low oxygen or hypoxic environment. In mammalian cells, the chief inducer of cellular responses to low oxygen is hypoxia-inducible factor 1 (HIF-1), a transcription factor complex whose levels are increased in many human cancers [24]. HIF-1 induces metabolic genes involved in increasing glycolysis, and thus coordinates adaptation to the hypoxic environment [8]. Besides activating cancer cells through aerobic glycolysis, HIF-1 plays a key role in converting glucose to lactate. HIF-1's targets include genes that convert glucose transporters and enzymes such as: PFK-1, phosphofructokinase type 2 (PFK- 2), HK, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) aldolase (ALD), enolase, pyruvate kinase, phosphoglycerate kinase, and LDH-A [25].

Hypoxia inducible factors HIF-1, HIF-2 and HIF-3 are the primary controllers of homeostatic responses to hypoxic conditions [26]. HIF-1 is more commonly expressed than HIF-2/3, and is composed of two subunits: oxygen-dependent HIF-1α and HIF-1β [27]. Activity of HIF is tightly controlled by synthesis cycles and oxygen-dependent proteasomal degradation. Under aerobic conditions, HIF-α subunits (HIF-1α/2α) undergoes posttranslational modification (i.e., hydroxylation on proline residues in the oxygen-dependent degradation domain by prolyl hydroxylase enzymes), leading to ubiquitination and eventual degradation by the tumor suppressor von Hippel–Lindau (VHL) [26,27]. However under hypoxic conditions, pyruvate dehydrogenase activity decreased and further inactivated through ferrous ion oxidation by ROS released from mitochondrial respiration, thus preventing interaction with VHL [26-27]. With VHL protein mutated, HIF-1α can be stabilized, causing inactivation of VHL (Fig. 2) [4,29]. A previous study demonstrated that loss of VHL causes decreased sensitivity of renal cell carcinomas to glutamine deprivation through HIF-induced metabolic reprogramming [30].

Cancer cells frequently undergo oxygen shortage, causing HIF-1 stabilization, which induces stimulation of the HIF-1 complex involved in growth, metabolism, apoptosis, and proliferation [21]. Stable HIFα/β subunits form heterodimers and transfer to the nucleus to bind to hypoxia response element (HRE) in the promoter region of hypoxia-responsive genes to transcriptionally activate cellular adaptation to hypoxia [26].

Recently, a new role for HIF-2 has been discovered in glutamine-dependent lipid formation [31]. Active HIF-2 molecule expression was found to cause a shift of isocitrate dehydrogenase/aconitase (IDH/ACO) towards reductive carboxylation of glutamine to citrate, higher production of lipogenic acetyl-coA, and increased MYC transcription by increased binding of the promotor region. Therefore, both HIF-2 and MYC are associated with activating glutamine-dependent lipogenesis [31].

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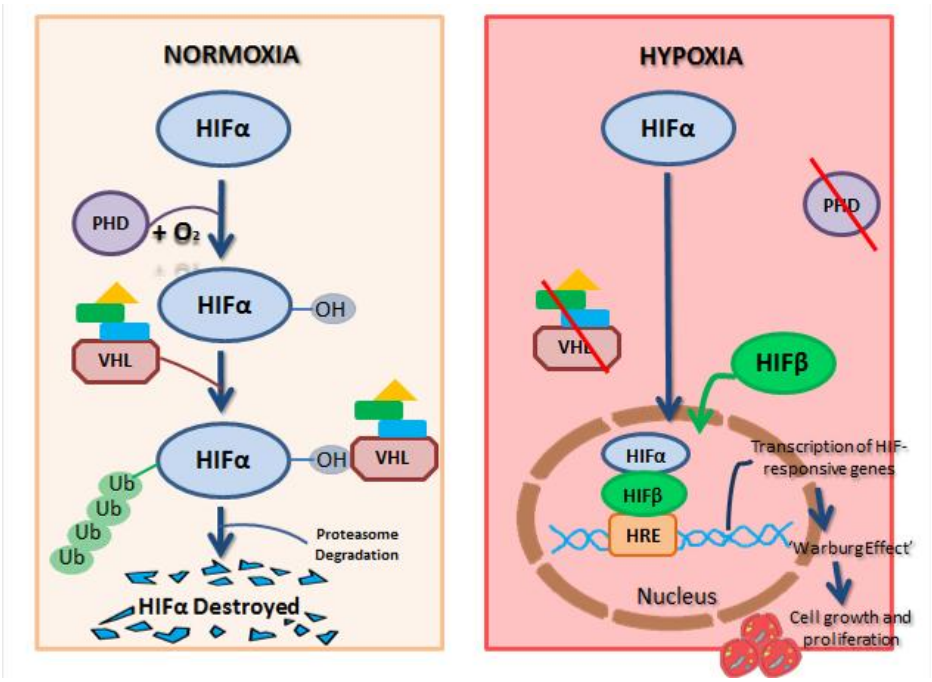

FIGURE 2: HIF Under Normoxic vs. Hypoxic Conditions

HIF-1α is a transcription factor that is activated based upon oxygen availability. Under aerobic conditions, HIF-1α undergoes posttranslational modification, leading to inactivation and eventual degradation. This is done through hydroxylation by prolyl-hydroxylase domain–containing enzymes (PHDs), which allows for binding to the tumor suppressor von Hippel–Lindau (VHL), which ubiquitinates HIF1a for destruction. However under hypoxic conditions, HIF-1α can be stabilized by mutations in the VHL protein, causing inactivation of VHL. Cancer cells frequently undergo oxygen shortage causing HIF-1 stabilization, which induces stimulation of the HIF-1 complex involved in growth, metabolism, apoptosis, and proliferation.

2.2 C-Myc: Master Regulator of Cell Metabolism And Proliferation

The oncogenic transcription factor MYC plays a critical role in many human cancers. From the MYC family of genes, MYC is the only isoform that is universally expressed in a broad range of tissues [26]. It includes a “general” transcription factor, c-MYC (or MYC), which links altered cellular metabolism to cancer formation. MYC has multiple functions, including controlling cell proliferation, cell cycle progression, cell growth, metabolism, apoptosis, differentiation, and stress response by transcriptionally regulating its target genes [26,32]. Elevated levels of c-Myc in tumor cells produce increased gene expression for genes involved in glucose metabolism, nucleotide, lipid, amino acid, and protein synthesis [33,34]. MYC expression is mutated in many human cancers, and expression and stability of MYC protein and MYC mRNA can also be mutated, supporting tumor formation through unregulated cell proliferation, inhibited cell differentiation, metabolic adaptation, blood vessel formation, reduction of cell bonding and genomic instability. MYC protein heterodimerizes with MYC-associated factor X (MAX) to form an activated complex that finds E box sequences (CACGTG) and promotes transcription of its targets genes [26,32,35].

MYC also behaves as a transcriptional repressor by binding to MIZ1 or SP1 transcription factors and blocking their transcriptional activity.26 Several genes repressed by MYC encode negative regulators for cell proliferation including CDKN2B, CDKN2C, CDKN1A, CDKN1B, and CDKN1C [26]. Many glycolytic enzymes are also upregulated in tumors because of elevated c-Myc and HIF-1α transcriptional activity and inadequate p53-mediated

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regulation. These two transcription factors coordinate to promote tumor cell metabolism by expressing key glycolytic enzymes such as hexokinase 2 (HK2), phospho-fructo-kinase (PFK1), TPI1, enolase, Lactate dehydrogenase-A (LDHA), monocarboxylate transporter (MCT1), among others, in tumors [36,37,38]. In fact, most of glycolytic gene promoter regions contain both Myc and HIF-1α binding motifs. C-myc increases the expression of PDK1 and MCT1, which coordinates the outflow of lactate into the extracellular matrix [35]. Other than c-myc, upregulation of MCT1 and PDK1 transcription is coordinated by B-catenin/TCF signaling, and upregulation of LDH-A and PDK1 is facilitated by HIF-a stabilization by hypoxia [39]. While HIF-1α mainly functions in hypoxic environments, c-Myc can promote expression of its glycolytic target genes in normoxic conditions, allowing tumors to constantly drive glycolysis to promote efficient proliferation and biosynthesis [12].

MYC is also a critical regulator of glutamine uptake and utilization in cancer cells (Fig. 3) [40]. Oncogenic levels of Myc are overexpressed in many cancers which causes glutamine addiction, and cells undergo apoptosis when glutamine is reduced [40,41]. Oncogenic Myc, along with HIF-1, stimulates glutamine metabolism both directly and indirectly [40]. It directly activates the expression of glutamine transporters SLC1A5 (a.k.a. ASCT2) and SLC7A5/SLC3A2, increasing protein synthesis and cell mass and thus activating mTORC1.4 mTORC1 downstream effector S6K1 phosphorylates the eukaryotic initiation factor eIF4B, increasing MYC translation and upregulating GLS and glutamate dehydrogenase (GDH) [16,26,41,42,43,44,45]. Myc indirectly promotes glutaminolysis by increasing expression of glutamine-utilizing enzymes glutaminase-1 (GLS-1) at the microRNA level by inhibiting GLS repressors, micro RNAs (miR)-23A/B.38 MYC also promotes another key oncogenic miRNA, miR-9, which is involved in tumor cell formation and proliferation [38,46].

HIF-2 and MYC activation may induce glutamine-dependent lipogenesis. Chromosome 8q24 was critically augmented in renal cell cancer (RCC) specimens, which is the exact position of MYC [47]. Overexpression of MYC in transgenic mouse models of RCC promoted increased control of glutaminases (GLS1-2) and transporters (SLC1A5) and increased glutamate and α- ketoglutarate levels [48]. Positive regulation of glutamine metabolism was also supplemented with excess lipids in RCC tumors [48].

C-myc also coordinates nucleotide formation by positively regulating the expression of various nucleotide biosynthetic enzymes. Along with GLS-1, Myc promote the expression of phosphoribosyl pyrophosphate synthetase (PRPS2), and carbamoyl-phosphate synthetase 2 (CAD), all of which result in increased glutaminase expression and glutamine metabolism [16,21,27,49]. Particularly, PRPS2 catalyzes the initial step of purine formation, and CAD initiates the pyrimidine ring-building cascade [50]. Other enzymes involved in nucleotide formation that c-myc targets include thymidylate synthase (TS), inosine monophosphate dehydrogenase 1(IMPDH1), and 2 (IMPDH2) [18]. Therefore, not only does c-myc coordinate glutamine uptake, but it also aides in using it to form purine and pyrimidine bases. In addition to enhancing glycolysis and glutamine metabolism, MYC has been known to promote mitochondrial genes expression and its reproduction [27].

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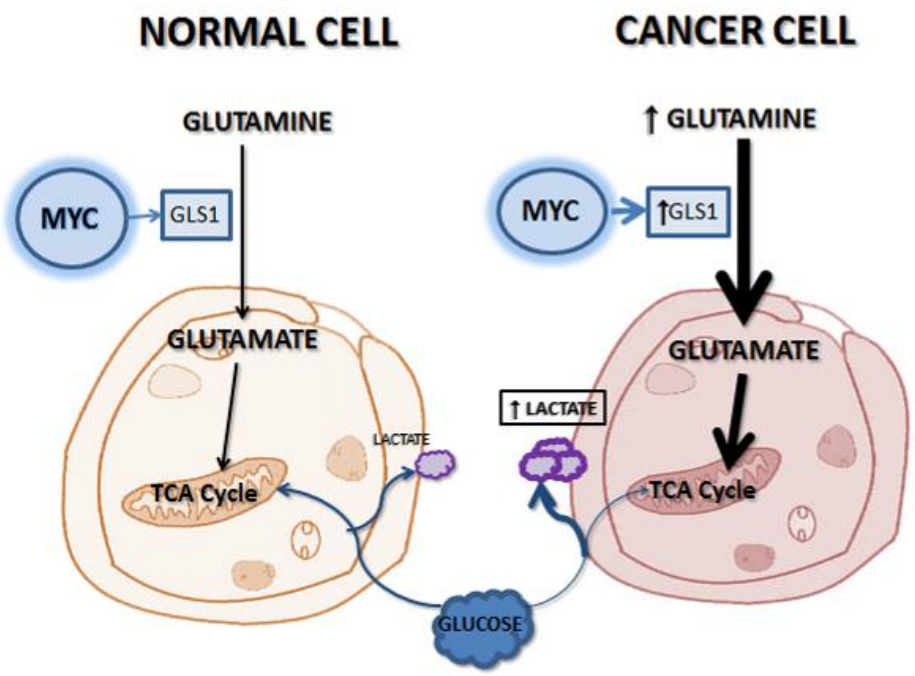

FIGURE 3: c-Myc controls glutamine metabolism using Gls1

MYC has emerged as a critical regulator of glutamine uptake and utilization in cancer cells. Glutamine is converted to glutamate by GLS1, whose expression is increased in c-Myc-dependent tumors. Glutamate then enters the Krebs cycle to produce ATP or glutathione.

2.3 Kras Regulates Metabolic Reprogramming

Like MYC, Ras oncogene controls increased metabolic and proliferative response in tumor cells [27]. The Ras complex involves several small GTPases that transduce proliferation signals, including the metabolic switch [51]. In order to drive uncontrolled proliferation and enhanced survival of cancer cells, Ras proteins are activated away from growth factors or self-activated in tumors, and assist in activating many effector signaling pathways, such as MAP kinases and PI3K/Akt [52]. Thus, Ras' metabolic effects may be facilitated either through the PI3K/AKT/mTOR pathway or through stimulation of Myc.

Additionally, Ras-associated changes in cellular metabolism include increased flow of glucose and glycolysis, dysfunctional mitochondria, increased lactic acid production, and expression of key glycolytic enzymes. These cellular changes are due to increased gene expression of the aerobic glycolytic pathway and lactate dehydrogenase [53]. Like other oncogenes, Ras is linked with formation of new lipids, mainly through directing SREBP-mediated by the MAPK pathway [54]. Loss of Kras causes inhibition of glucose uptake and a decrease in various glycolytic intermediates, including G6P, F6P, and FBP [55].

Pancreatic tumor cells often contain activated Kras mutations, in which Kras transcriptionally regulates several metabolic pathways to stimulate glucose uptake with the help of MAP kinases and MYC [56]. In addition, previous studies have shown that pancreatic ductal adenocarcinomas depend on a glutamine-associated pathway which is stimulated by Kras at the mRNA level. Kras directs cellular metabolism to be used by glutamine as a source of pyruvate and NADPH to preserve the cellular redox balance [57].

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Ras also regulates autophagy and removal of damaged mitochondria. In Ras-driven tumors, loss of essential autophagy genes can cause buildup of abnormal mitochondria which are unable to metabolize lipids [58]. Similarly, tumors stimulated by B-Raf Proto-oncogene (*BRAF*) rely on cell death to preserve mitochondria and glutamine metabolism [21,59].

The RAS/MAPK (mitogen-activated protein kinase) signaling pathway is commonly unregulated in non-small-cell lung cancer, usually by KRAS activating mutations [5,60,61]. One inner mutant Kras allele is enough to cause lung tumorigenesis in mice, but malignant progression requires further genetic variations [6,62,63].

2.4 PI3K/AKT/mTOR1 Drives Anabolism and Tumorigenesis

The PI3K/AKT/mTOR pathway is perhaps the most commonly uncontrolled pathways in human cancers. The phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that link prosurvival signals (i.e., growth factors, cytokines, hormones, other environmental cues) and convert them into intracellular signals to stimulate Akt-dependent/independent downstream signaling pathways [64]. PI3Ks have various biological roles including directing cell growth, metabolism, and cell proliferation. These lipid kinases regulate the levels of phosphorylated phosphatidylinositol (PIP3) at the plasma membrane [14]. The PI3K pathway is activated by several mutations, negative regulators such as PTEN, or enhanced signaling by receptor tyrosine kinases [65]. Once activated, the PI3K pathway provides signals for tumor cell growth and survival, greatly impacts cellular metabolism, and is involved in recruiting and activating downstream effectors such as the serine/threonine kinases Akt and mTOR [66]. PI3K also stimulates uptake of fatty acids and blocks fatty acid oxidation to increase lipogenesis in proliferating cells via control of growth factors [8].

The PI3K/Akt/mTORC1 signaling is the primary controller of aerobic glycolysis and formation, inducing the surface expression of nutrient transporters and increased control of glycolytic enzymes [26]. PI3K/Akt signaling is often over-activated in human cancers for cell proliferation, growth, survival, and metabolic reprogramming [28]. Interestingly, the miR-221/222 gene cluster, an activator of PI3K/AKT, was found to prompt angiogenesis [38]. Contrarily, miR-126 can maintain vascular network and block tumour angiogenesis by controlling VEGF signaling [67].

As the best studied effector downstream of PI3K, AKT (also known as Protein Kinase B, PKB) serine-threonine protein kinase that is regulated through PI3K activation via successive phosphorylation at Thr308 and Ser473 [26,68]. Activated Akt itself can induce glycolysis, glucose uptake, and lactate production and suppress macromolecular degradation in cancer cells. In addition, Akt plays important role in enhanced lipid biosynthesis, and increases the activity of HIF1 [4,14,27].

Activated Akt or introduction of KRAS mutant, with loss or gain of glucose, increases total histone acetylation, promoting increased and broadened gene expression [69]. Analyzing glioblastoma and prostate tumor samples showed that Akt activation levels were closely linked with global histone acetylation status, and expanded the extra-mitochondria pool of acetyl-CoA by activating ACLY, which turns cytosolic citrate into acetyl-CoA [69].

The PI3K/AKT pathway is regulated by many miRNAs, including oncogenic miR-21, miR-337, miR-543, miR-214 and miR-130, via tumour-associated neo-vascularisation directly targeting PTEN and activating PI3K/AKT [70-73]. Cancer cells are known to have high expression of miR-181a through a metabolic shift by blocking PTEN expression, causing higher Akt phosphorylation [74]. In addition, miR-26a has metastasis and angiogenic

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potential, since it directly regulates PTEN, and loss of PTEN has been linked with uncontrolled Akt activity [38].

AKT also stimulates mammalian target of rapamycin kinase (mTOR), a conserved cytoplasmic serine-threonine protein kinase. The mTOR pathway is an integrative point between growth signals and nutrient availability, which regulates several metabolic pathways including protein synthesis, autophagy, ribosome biogenesis, and mitochondria formation [21,27,59,76].

mTOR is part of two distinct multi-protein complexes, TORC1 and TORC2., mTORC1 growth-factor-independent activation is observed in up to 80% of tumors, and is controlled by growth factors, oxygen and nutrient availability. Through the interaction between mTOR and raptor (regulatory-associated protein of mTOR), mTORC1 controls protein translation through modulation of eukaryotic Initiating Factor 4E Binding Protein 1 (4E-BP1) phosphorylation [26]. mTOR regulates many anabolic pathways such as glycolysis and the oxidative arm of PPP through regulation of HIF1, and lipid synthesis through activation transcription factor sterol regulatory element-binding protein 1/2 (SREBP1/2), which then regulates gene expression for fatty acid, triglyceride, phospholipid and cholesterol formation [26,59,76]. mTORC1 is known to support mitochondria formation and expressing genes of oxidative metabolism, while mTORC2 directly activates Akt by phosphorylating Ser473 residue, leading to mTORC1 activation [26,59,77].

mTORC1 is also activated by amino acids, and activates protein synthesis through its translation and ribosome formation [8]. mTORC1 stimulates both glutamine uptake and glutaminase activity, allocating glutamate for transamination reactions or to maintain the TCA cycle for amino acid synthesis. Moreover, when there is excess intracellular glutamine, it can be transported exported for essential amino acids to activate mTORC1 and protein synthesis [8]. However, since autophagy degrades proteins and provides amino acids, there is no net protein synthesis, and it is most likely suppressed by mTORC1 [78,79]. Inhibiting pathways that degrade proteins may increase rates of net protein synthesis when there are active mTORC1 and extracellular amino acids [8].

mTOR also regulates nucleotide synthesis through regulation of the PPP and by activation of an enzyme of pyrimidine synthesis [80,81]. At the molecular level, mTOR directly stimulates mRNA translation and ribosome synthesis and indirectly causes other metabolic changes by activating transcription factors such as HIF1 even under normoxic conditions [27]. mTOR is also released in metabolic disorders, such as obesity and type 2 diabetes. Hyperactive mTORC1 signaling in the liver of mice show metabolic abnormalities such as defective glucose and lipid homeostasis, thus developing into hepatocellular carcinoma [82].

Activated PI3K/Akt and RAS pathways by growth factors cause Akt- and ERK-facilitated phosphorylation and suppression of heterodimer tuberous sclerosis 1 (TSC1)/TSC2, which is a GTPase-activating protein (GAP) that down-regulates mTORC1 by blocking the RAS homolog enriched in brain (RHEB) GTPase [26]. mTOR responds to growth factors through blocking TSC1/2 via AKT. PI3K also controls mTOR activity by phosphorylating and inhibiting TSC which works with LKB1 to down-regulate mTOR activity. For mTORC1 activation, intracellular amino acids are needed to stimulate the pathways by which mTORC1 is activated by RHEB [83].

The PI3K/AKT pathway involves mTOR kinase in a negative feedback mechanism to actively facilitate cell growth and metabolism. Activated mTOR blocks the PI3K pathway, thus increasing effector Akt activity [38,84]. Thus, miR-144 targets mTOR to block cell growth by prompting cell cycle arrest [38,84]. PI3K/AKT/mTOR kinase pathways also

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controls apoptosis and autophagy using survival signaling. In low energy conditions, PI3K/AKT/mTOR kinase is blocked, leading to apoptosis/autophagy activation [85].

A recent study revealed that blocking mTORC1 lowers glutamine metabolism via SIRT4 expression regulation in order to inhibit GDH activity [86]. GBM cells were found to increase glutamine metabolism with high GLS expression due to mTOR-targeted treatments. After mTOR inhibition treatment, the study found that ammonia, intracellular glutamate, αKG, and ATP levels were the same or higher, which is consistent with high glutamine metabolism. This study proposed a potential mechanism for the resistance to mTOR kinase inhibition in at least some GBM cells [86].

3. ROLE OF TUMOR SUPPRESSOR GENES

3.1 LKB1/AMPK Pathways: Inhibitor Of mTOR Upon Bioenergetic Stress

mTOR is inhibited in conditions of nutritional stress, such as low nutrient conditions and hypoxia, by signaling through the AMP-activated protein kinase (AMPK) [14]. Tumors under these metabolic stress conditions adapt by altering the liver kinase B1 (LKB1)–AMPK pathway. The AMPK is a heterotrimeric serine/threonine protein kinase and an ATP sensor that directs cellular energy homeostasis, aimed at preserving cellular energy and viability. There are seven subunit isoforms of AMPK encoded by separate genes (PRKAA1–2, PRKAB1–2,and PRKAG1–3), two catalyst α subunits (α1–2), two regulatory β subunits (β1–2), and three γ subunits (γ1–3) (Fig. 4). The α-subunit has catalytic activity and is made up of a kinase domain at the N-terminus, led by a regulatory domain with an self-inhibiting sequence and a subunit linking domain that attaches to the β-subunit [87]. For full enzyme activity, AMPK must be phosphorylated on its conserved αThr172 residue in the activation loop.87 The β subunits of AMPK are a support structure to attach the α and γ-subunits to form a functional AMPK heterotrimeric complex [88]. The γ-subunit of AMPK has four tandem cystathionine β synthase (CBS) recurrences, with three of the sites bound to adenine nucleotides.

AMPK is controlled by adenylate levels in the cell (i.e. ATP, ADP and AMP) [87]. AMP is a direct agonist of AMPK, and AMPK activation depends upon AMP:ATP ratio levels and conditions of metabolic stress such as nutrient deprivation or hypoxia, when ATP levels decline and the AMP and ADP levels increase [87,89]. Low glucose causes energetic stress in cells, leading to structure changes that promotes phosphorylation of AMPK at α-subunit Thr172 and suppression of Thr172 de-phosphorylation by phosphatases [87]. Activated AMPK then directly phosphorylates several downstream substrates to impact energy metabolism and growth, stimulating gene expression for extensive changes in metabolic programming, suppressing protein synthesis, and stimulating fatty acid oxidation to replenish ATP [87,90].

To date, three upstream activators of AMPK have been identified, including: the tumor suppressor protein LKB1, calmodulin-dependent protein kinase kinase b (CamKKb), and transforming growth factor-b (TGFb)-activated kinase-1 (TAK1). In the hypothalamus, neurons, and T lymphocytes, AMPK is also regulated by calcium (Ca2+) signals [87]. CaMKKβ appears to be the main kinase that phosphorylates AMPKα on Thr172. AMPK being phosphorylated by additional kinases such as CAMKKb suggests that it can act independently without LKB1 [87].

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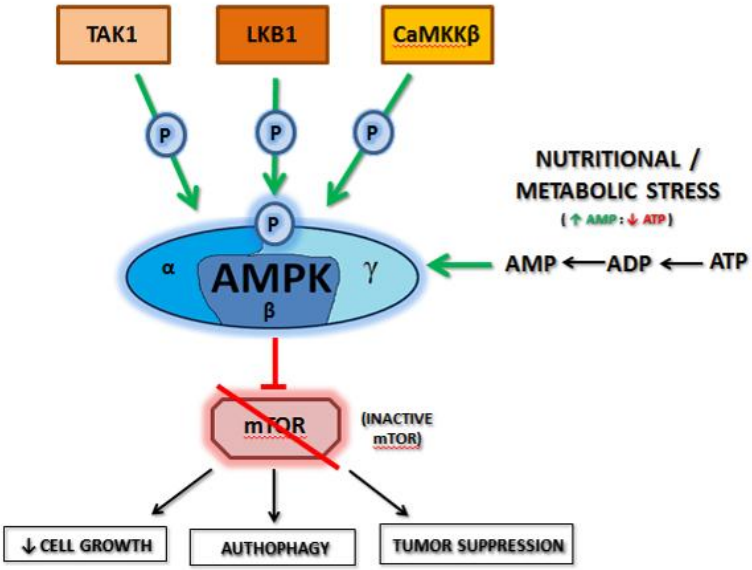

FIGURE 4: AMPK Structure and Function

The AMPK is a heterotrimeric serine/threonine protein kinase that consists of a catalyst α subunit and two regulatory subunits (β and γ). AMPK activation depends upon AMP/ATP ratio levels and conditions of metabolic stress such as nutrient deprivation or hypoxia. When ATP levels decline, AMP and ADP levels increase. AMPK is activated by either three protein kinases: LKB1, CamKKb, and TAK1. Once activated, AMPK can inhibit cell growth, proliferation, and autophagy through regulation of various downstream metabolic pathways such as the mTOR pathway.

AMPK directly phosphorylates peroxisome proliferator activated receptor gamma (PPAR-γ) coactivator-1-α (PGC-1α), a transcriptional co-activator that controls several metabolic genes and mitochondria formation [16]. AMPK may also directly phosphorylate p53 on Ser15, stabilizing p53. Another study suggested AMPK-faciliated p53 stability by suppressing its deacetylation with SIRT1, a NAD-dependent protein deacetylase that silences genes and is the homolog to the yeast Sir2 protein [16].

STK11 encodes LKB1, a master serine/threoninekinase with several roles in cell proliferation, polarity, metabolism, and survival [87,89]. Once activated, AMPK inhibits growth and proliferation, increases oxidative phosphorylation to preserve ATP, and can target various downstream metabolic pathways such as the mTOR pathway [15,89]. AMPK contributes to homeostasis by maintaining NADPH levels and thus redox stress by inhibiting lipid synthesis and promoting lipid oxidation [91]. AMPK-phosphorylated acetyl-CoA carboxylase (ACC) 1 and ACC2 produce NADPH and compensate for PPP shortage under glucose deprivation [92]. As a reducing agent, NADPH has a key role in preventing ROS formation within cells.

During energetic stress, AMPK can inhibit mTORC1 through phosphorylation of either tuberous sclerosis complex TSC2 and Raptor (component of mTOR), which is essential for protein synthesis [9,87]. AMPK triggers tumor suppressor TSC2 activity by directly phosphorylating on its Thr1227 and Ser1345 residues, leading to inactivation of Rheb by converting it to a GDP-bound confirmation [16].

Loss of AMPK signaling increases tumorigenesis and enhances the glycolytic metabolism in cancer cells. This promotes a metabolic shift toward the Warburg effect [93]. However, loss of LKB1 expression in tumor cells reduces the AMPK signaling, making cells more sensitive

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to low nutrient level, and leading to unregulated metabolism and cell growth in energetically stressful conditions [89,94,95,96,97]. This can promote cancer formation, as it leads to elevated glucose and glutamine flow, rising ATP levels, and a metabolic switch to aerobic glycolysis. Thus, LKB1 is a key regulator of tumor-cell metabolism and growth by controlling HIF-1α–dependent metabolic reprogramming [89,98].

Loss of LKB1–AMPK signaling causes metabolic programming to be facilitated by oxygen-sensitive HIF-1a, where high protein levels in AMPKa-deficient cells in aerobic conditions causes HIF-1a-dependent transcriptional program stimulation, which promotes increased glycolysis under normoxia [93]. Thus, HIF-1a is a key mediator of the metabolic transformation with loss of AMPK. Loss of LKB1 induces increased HIF-1a transcription and translation, which are sensitive to mTORC1 repression [87,98].

Several studies suggested that activating AMPK inhibits cell proliferation in both cancer and normal cells. A recent trial has shown that control of pAMPK—a phosphorylated AMP activated protein kinase as an energy sensor) and inhibition of insulin signals proposed a cytostatic metformin's pathway [99]. Inactive or defective LKB1-AMPK pathways lead to high metabolic changes in pre-cancerous cell [100].

Furthermore, AMPK was recently shown to also be activated by various oncogenic signals via proto-oncogene stimulation or inhibition of tumor suppressor genes [101,102]. Recently a mechanism of LBK1 activating AMPK in energetically stressful conditions was proposed, reporting that AMP has higher control of AMPK than ADP since it is significantly more potent than ADP in blocking T172 dephosphorylation, and it can increase LKB1-induced AMPK phosphorylation compared to ADP [103].

Amino-acid transporters—L-type amino acid transporter 1 (LAT1; SLC7A5) and glutamine/amino acid transporter (ASCT2; SLC1A5)—control mTOR, which is why AMPK-mTOR axis behaves like a sensor of energetic change in nutrients or growth factor environment [104]. Specifically, amino acid transporter LAT1 takes up leucine to stimulate the mTOR signal pathway [104,105]. Thus, the LKB1-AMPK-mTOR axis is controlled by amino-acid concentration in the tumor microenvironment, and this pathway supports metabolic reprogramming of cancer cells due to energetic changes in the microenvironment [41].

3.2 The PI3K–AKT–PTEN Pathway Regulates Metabolism

The PI3K/AKT signaling pathway can be inhibited by the tumor suppressor gene phosphatase and tensin homologue (PTEN). PTEN dephosphorylates phosphatidyl inositol tri-phosphate (PIP-3), which is formed by PI3K activation and primarily activates AKT, thus blocking activation of the PI3K–AKT–mTOR pathway. PTEN has key tumor-suppressor abilities since it regulates cell growth, metabolism, and survival [106].

PTEN exhibits remarkable effects on metabolism homeostasis since it must remain at fixed levels; even the slightest decrease or change in PTEN gene expression is enough to stimulate cancer [107]. Mutation or loss of PTEN function induces glycolysis and cancer formation, which is essential for cancer cells since they are dependent on increased glycolytic flux [108]. PTEN negatively regulates the insulin pathway, and thus has negative effects on lipogenesis, which is another characteristic of cancer cells. Loss of PTEN through increased PI3K/Akt/mTOR signaling leads to HIF activation and thus the Warburg effect [109].

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Conversely, elevated PTEN levels can switch the cancer metabolic reprogramming from glycolysis to oxidative phosphorylation [110]. For example, transgenic mice with additional copies of PTEN have lower chances of developing cancer. Increase of PTEN resulted in mice with healthier metabolism, increased oxygen and energy usage, increased mitochondrial ATP generation, reduced body fat buildup, reduced glucose and glutamine uptake in cells, increased mitochondrial oxidative phosphorylation, and resistance to cancer formation [110]. On the contrary, mouse cells with loss of PTEN displayed downregulation of the TCA cycle and oxidative phosphorylation, defective mitochondria, and decreased respiration [111].

3.3 Retinoblastoma (Rb): Suppressing Tumorigenesis and Anabolism

The Retinoblastoma Susceptibility gene, RB, was the first tumor suppressor to be discovered and characterized. Retinoblastoma is an uncommon hereditary or non-hereditary childhood eye tumor. In about 25% of all retinoblastoma cases, tumors formed in both eyes, while the remaining cases had only one affected eye [112]. RB encodes a nuclear phosphoprotein, RB or pRb, which is either missing or defective in retinoblastoma, osteosarcoma, breast cancer, and small-cell lung carcinoma [112].

RB is now known to be a ubiquitous cell cycle controller, mainly regulating the pathway of cells through the G1 phase and the restriction point (R point), which is unregulated in most cancer cells [19]. In normoxic conditions, RB is phosphorylated by cyclin DCDK4/6 and cyclin E-CDK2 complexes upon triggering of mitosis [112]. Cyclin-CDK complexes are negatively controlled by CDK inhibitors that primarily counteracts CDK4/6, and three remaining CDK inhibitors. Phosphatase 1α (PP1α) dephosphorylates RB at the end of the M phase, and is known to have competed with CDKs for a common binding site on RB [112].

Un-phosphorylated or hypo-phosphorylated Rb binds to and separates the transcriptional activator, E2F, to block target gene transcription using chromatin remodeling complexes and Histone Deacetylases (HDACs). However, hyper-phosphorylated RB detaches from the E2Fs, allowing E2F/DP to bind with histone acetylase to activate transcription [112]. RB tumor suppression focuses on negatively controlling transcriptional activation of E2F and cell cycle suppression. The E2F family proteins have recently been demonstrated to be unnecessary for proliferation in vivo. Since E2Fs are less commonly mutated in cancer, RB may have other functions besides controlling E2F-dependent transcription. All in all, RB has been demonstrated to be integral in segregating chromosomes, controlling checkpoint, apoptosis, senescence, and terminal differentiation. These RB functions could be facilitated through post-translational changes on the C-terminal domain of RB, such as acetylation and methylation. RB suppresses tumor formation by receiving various signals, and mediates between CDK regulatory pathways and E2F activators [112].

The Rb tumor suppressor family of proteins negatively regulate glutamine uptake. Loss of Rb family proteins can increase the entrance and use of glutamine through the E2F-dependent upregulation of ASCT2 and GLS1 [63]. C-myc and E2F, both which are major coordinators of cell division, allow cells to gain access to glutamine in order to satisfy biosynthetic demands of DNA replication [18].

The phosphor retinoblastoma protein (pRb) is a key mediator of oxidative metabolism as it blocks cell cycle progression by repressing the E2F1 transcription factor [27,113]. Subsequently, pRb is phosphorylated by cyclin D-CDK4/6, which deactivates Rb and induces E2F1-mediated transcription. Among the many signals that control pRb expression, AMPK directly phosphorylates pRb, controlling the G1/S phase transition based on the energetic state of the cell. Rb also blocks SLC1A5 expression [63].

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Previously, pRb was shown to direct stress response due to starvation in Caenorhabditis elegans and a Drosophila model, suggesting that pRb was involved in cancer metabolism [114,115]. This study indicated that flies with mutant RBF1 (Drosophila Rb homolog) were hypersensitive when starving and displayed an increased flow of glutamine and nucleotide metabolism. Furthermore, inactive pRb in humans also showed elevated glutamine flow due to increased control of glutamine expression [115].

3.4 P53 Inhibits Anabolism And Promotes Mitochondrial Metabolism

The tumor suppressor p53 is a transcription factor that acts as the primary defender against tumor formation. *TP53* is mutated or deleted in 50% of human cancers [26,116]. However, recently it was suggested that p53 tumor-suppressive activities may be independent of the well-established p53 actions and dependent on control of metabolism and oxidative stress [117]. p53 regulates various functions including impaired DNA, apoptosis, and aging. p53 repairs damaged DNA by activating genes that facilitate nucleotide excision repair and base excision repair [112]. If DNA is too severely damaged, wild-type p53 can relay the cell into cell cycle arrest, senescence, or even apoptosis, by activating genes associated with apoptosis such as PUMA. Thus, p53 plays a critical role in responding to various cellular stresses signals [112]. Loss of p53 increases flow of glucose to support anabolism and redox balance, thus promoting tumor formation [118].

p53 also plays a key role in responding to metabolic stress, since p53 controls a metabolic checkpoint. While RB receives growth-inhibitory signals usually from outside of the cell, TP53 receives stress and abnormal sensory signals from inside the cell—including impaired DNA, loss of nutrients, glucose, oxygen, or oxygenation, or growth-promoting signals—in which TP53 can halt cell-cycle progression until these conditions have stabilized [119]. Cells without p53 and glucose cannot undergo this cell cycle arrest, making p53-impaired cells more sensitive to metabolic stress than normal cells [120].

P53 regulates the transcription of four genes: PTEN, IGF- binding protein-3 (IGF-1BP-3), tuberous sclerosis protein 2 (TSC-2), and the beta subunit of AMPK, which all negatively regulate AKT kinase and mTOR. p53 activates PTEN to indirectly inhibit the glycolytic pathway, thereby blocking the PI3K-AKT pathway, which activates protein synthesis through mTOR [121]. All these activities block cell growth, lower the Warburg effect and HIF levels, and thus reverse the cancer phenotype [110].

The metabolic shift to OXPHOS by p53 is partly due to the p53-dependent transcriptional control of TP53-induced glycolysis and apoptosis regulator (TIGAR) and formation of cytochrome c oxidase 2 (SCO2) [122]. The TIGAR gene is an enzyme that lowers flow of glucose by regulating ROS levels, glycolysis, and apoptosis in the cell through fructose-2,6-bisphosphate (Fru-2,6-P2). Fru-2,6-P2 is a key allosteric activator of PFK1, an essential glycolytic enzyme, and is produced by PFK2 from fructose 1-phosphate. Enhanced levels of TIGAR converts Fru-2,6-P2 back to fructose 1-phosphate, thereby lowering Fru-2,6-P2 levels and slowing tumor glycolysis by diverting glucose through the PPP, possibly resulting in lower ROS levels and lower cellular sensitivity to ROS-associated apoptosis [12].

Another function of p53 is to regulate glutamine metabolism, which is an important pathway since the enzyme which converts glutamine to glutamate, glutaminase 1 (GLS1), has been shown to promote tumor formation [4]. p53 transcribes the expression of another isoform of glutaminase (GLS2), which promotes increased mitochondrial OXPHOS and energy production from glutaminolysis. The two glutaminases (GLS1 and GLS2) have opposite effects on the cell: downregulated Gls1 inhibits oncogenic transformation and cancer cell

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proliferation, while overexpressed Gls2 suppresses tumor formation [123]. Myc induces the expression of Gls1, while p53 induces the expression of Gls2 (Fig. 5). Furthermore, p53 is known to block glucose uptake by directly inhibiting Glut1 and Glut4 transcription, and suppressing Glut3 expression [12]. Glut3 is an NF-κB target gene and p53 is found to block NF-κB stimulation, thus reducing transcription and expression of Glut3 [12]. In addition, p53 has been shown to suppress expression of malic enzymes ME1 and ME2 in order to control glutamine-dependent NADPH production [124].

P53 also control several miRNAs that regulate cancer metabolism, and restrains the expression of miR-34, the miR-194/miR-215 cluster, let-7 and miR-107, all of which further block expression of p53's target genes including LDHA, MYC, sirtuin-1 (SIRT1), and HIF [38]. p53 blocks transcription of some tumorigenic miRNAs which directly target p53 3'-UTR and thus blocks p53 response, and thus takes part in controlling cell proliferation through cell cycle arrest by targeting KRAS and CDK6. Furthermore, p53 regulates the expression of p21 gene, which indirectly controls responses to high ROS and modified redox potentials through the Nrf2 transcription factor [27]. When DNA get damaged, p53 induces expression of p21Cip1 genes to halt cell cycle progression at G1 phase [112].

Mutant p53 is able to block the function of p53 family proteins p63 and p73 through protein-protein interaction [125]. Mutant p53 is found to only inhibit p73 and p63 when mutant p53 is in greater quantities compared to p63 and p73, which usually occurs in cancers [125]. P63 and p73 have high sequence homology with p53 and controls the expression of similar genes by linking to p53 responsive elements and having similar functions to p53. Thus, p63 and p73 are able to functionally replace p53. The same approach of gene therapy using adenovirus delivered wild-type p53 has been expanded to p73 and p63 [125].

Previous studies suggest that the adenovirus-mediated delivery of p63 and p73 (Ad-p63/p73) into tumor cells is an efficient method of gene therapy [125]. Ad-p73 activates p21 and stimulates cell cycle arrest and apoptosis in several cancer cell lines. Ad-p73 alerts p53 mutant cancer cells to adriamycin with a higher efficiency than Ad-p53. Ad-p73 infection does not stimulate apoptosis in normal human cells. Ad-p63 leads to apoptosis in osteosarcoma cells that are resistant to Ad-p53-mediated apoptosis. Ad-p63 is found to have greater apoptosis-inducing effects than Ad-p53 in osteosarcoma cells. Intra-tumoral injection of Ad-p63 greatly reduced tumor growth in human osteosarcoma xenografts. p63 stimulates osteosarcoma cells to the chemotherapeutic agents doxorubicin and cisplatin [125].

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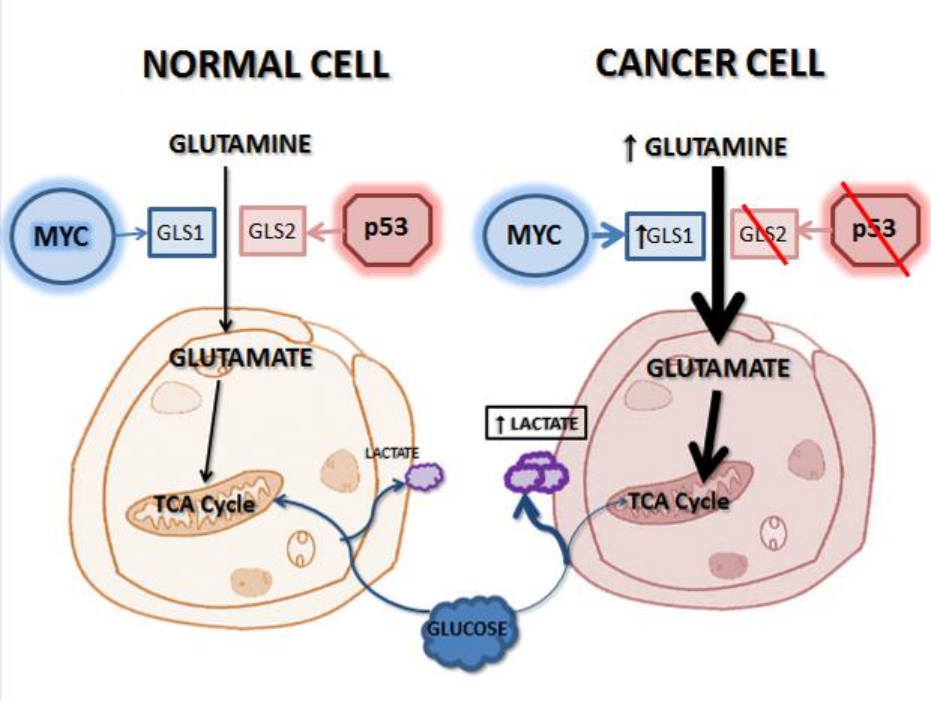

FIGURE 5: p53 regulates glutamine metabolism and opposes MYC

One of the roles of p53 is to regulate glutamine metabolism, which is an important pathway since the enzyme which converts glutamine to glutamate, glutaminase 1 (GLS1), has been shown to promote tumor formation. p53 transcribes the expression of another isoform of glutaminase (GLS2), which promotes increased mitochondrial OXPHOS and energy production from glutaminolysis. The two glutaminases—GLS1 and GLS2—have opposite effects on the cell: downregulated Gls1 inhibits oncogenic transformation and cancer cell proliferation, while overexpressed Gls2 suppresses tumors. Myc induces the expression of Gls1, while p53 induces the expression of Gls2.

4. THERAPEUTICS AND FUTURE PROSPECTS

4.1 Targeting Kras For Cancer Therapy

KRASG12D-transformed MEFs is able to proliferate without leucine, an essential amino acid, when the culture medium is supplemented with physiological levels (20–30 mg/mL) of serumalbumin [79]. Proliferation of *KRASG12D*-driven mouse pancreatic cancer line can be restored by albumin supplementation in a medium that is missing all free amino acids [126]. Contrary to *KRASG12D*, PI3K/Akt signaling does not support the cellular use of extracellular protein. In treating a *KRASG12D*-driven mouse model of pancreatic cancer , rapamycin is able to suppress cancer cell proliferation where there is sufficient vascular delivery of nutrients, and also enhance cell proliferation where there is poor vascularization by enhancing lysosomal breakdown of extracellular proteins [79].

Recent studies demonstrated that progressive lung tumors from *KrasG12D* mice usually exhibit *KrasG12D* allelic enhancement (*KrasG12D*/Kras wild-type), suggesting that mutant Kras copy gains are chosen positively during progression. Mutant Kras homozygous and heterozygous mouse embryonic fibroblasts and lung cancer cells have phenotypically different genotypes. Specifically, *KrasG12D/G12D* cells switch to glycolysis and and increase channeling of glucose-derived metabolites into the TCA cycle and glutathione production, causing increased glutathione-facilitated detoxification. This metabolic change is reiterated in mutant *KRAS* homozygous nonsmall-cell lung cancer cells and in vivo, in

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uncontrolled advanced murine lung tumors with higher incidence of *KrasG12D* copy gain, but not in the early *KrasG12D* heterozygous tumours. Mutant Kras copy gain creates distinct metabolic necessities that can be utilized to target these aggressive mutant Kras tumors [127].

Cancer cells can withstand long periods of nutrient deprivation via macroautophagy, or the degradation of intracellular macromolecules and organelles when fused with lysosomes in order to liberate free amino and fatty acids [128]. Deletion of Atg7, a core component of autophagy, dramatically changes the nature of lung tumors driven by *KrasG12D* and *BrafV600E* oncogenes from malignant adeno-carcinomas to benign onco-cytomas [129].

Melanoma is a heterogenetic disease with several subdividsion due to specific genetic variations. About half of cutaneous melanomas have mutations in *BRAF*, a protein kinase that is part of the RAS/RAF/MEK/ERK pathway and which controls cell proliferation and survival [16]. The most common *BRAF* mutation is *BRAF(V600E),* a glutamine for valine substitution at position 600, which produces an active kinase that drives signaling and cell proliferation of its component MEK/ERK [131]. Drugs that block *V600EBRAF* (such as vemurafenib and dabrafenib) or drugs that inhibit MEK (such as trametinib and cobimetinib) can extend survival in melanoma patients a *V600EBRAF* mutation in the tumor [131]. Mutant *BRAF(V600)* tends to be relatively dependent on mitochondrial metabolism when administered for malignant melanoma cells to survive and proliferate [41]. Since *BRAF* blocks OXPHOS, MRD cells stimulate proliferator-activated receptor-gamma coactivator-1 (PG C1-alpha). The *BRAF(V600E)*-MITF-PGC1-alpha axis supports formation of mitochondria and causes BRAF-mutant melanoma cells to become dependent to mitochondrial metabolism [41].

In a previous study, PLX4720 lowered lactate levels in all *BRAF* mutant melanomas. Lactate levels did not change despite treating melanoma cell line that did not have *BRAF* mutation, validating that PLX4720 is unable to suppress ERK signaling in these cells. Thus, *BRAF* suppresses OXPHOS gene expression and mitochondrial density in melanoma [130].

A study observed that *BRAF(V600E)* expression suppressed PGC1a, a major regulator of mitochondrial biogenesis and metabolism. When treating a series of *BRAF* mutant melanomas and non-melanoma cell lines with PLX4720, it was found that PLX4720 induced 3- to 14-fold increases in PGC1a mRNA of all melanomas with *BRAF* mutations. MITF overexpression or treatment with PLX4720 led to the induction of the wild-type promoter, whereas mutation of either of the two E boxes significantly inhibited this response. Thus, MITF binds and directly regulates the PGC1a gene in the melanocyte lineage. In addition, treatment with PLX4720 strongly induced PGC1a mRNA in M14 cells and 3-fold in UACC62 cells. This induction was absent in cells with MITF knocked down by siRNA, indicating that BRAF regulates PGC1a via MITF [130].

Recently it has been found that activating *BRAF* leads to lower oxidative enzymes, lower mitochondria and function, and higher lactate formation. Metabolic reprogramming by *BRAF(V600E)* is followed by MITF and PGC1a suppression. Overall, the study suggests that MITF is a major regulator of mitochondrial respiration in the melanocyte lineage by directly facilitating *BRAF*-regulated PGC1a transcription. Unregulated PGC1 may significantly affect melanoma cells metabolism, and may contribute to oncogenesis in some cases. *BRAF* mutant melanomas treated with PLX4720 were found to be dependent on ATP generation by mitochondria, suggesting that blocking mitochondrial metabolism may be most effective as initial therapy, since patients whose health deteriorated with *BRAF* inhibitors have reactivation of the MAPK pathway. In addition, mitochondrial uncouplers were found to increase the effectiveness of PLX4720 in *BRAF* mutant melanomas. Since the drugs are

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highly toxic, alternative OXPHOS inhibitors should be further developed. Although *BRAF* inhibitors recently demonstrated clinical successes, the recurrence rates are still high and survival is only increased by several months [130].

BRAFV600E inhibition in melanoma cells have been reported to overtake expression suppresses glycolytic enzyme expression, causing lower glucose uptake and growth prevention [132]. Aerobic metabolism regulates opposition to *BRAF* inhibitors, implying that these drugs pressure cancer cells to restore aerobic metabolism and proliferation. Removing *Q61KNRAS* expression due to *BRAF* inhibitors reestablishes glycolytic enzyme expression in *BRAFV600E* melanoma cells [132,133].

Several studies show that loss of AMPK activity can help oncogenes promote tumor progression. One example is AMPK suppression in cancer is through mutated *B-RAF (V600E)* blocking the LKB1 function in melanoma. Mutant *B-RAF V600E* supports ERK and RSK-dependent phosphorylation of LKB1 in melanoma cells, leading to AMPK suppression [135]. Reversal of LKB1 inhibition causes suppression of *B-RAF V600E*-mediated conversion. Recently, AMPK has been shown to return to *B-RAF* to lower MEK–ERK signaling [135].

4.2 Targeting the PI3K/Akt/mTOR Pathway

Clinically, PI3K therapy is powerful in adapting to tumors, reprogramming mitochondrial functions in metabolism, and apoptosis for cell survival and resistance to treatment. Gamitrinib, a combination of a small-molecule inhibitor of mitochondrial-localized Hsp90s which is currently in preclinical development, transformed the cytostatic effects of PI3K antagonists into strong, symbiotic anticancer activity in vivo [136]. Focusing on targeting the mitochondria for cancer therapy, regulators of Bcl-2 proteins, OXPHOS, and redox pathways have undergone preclinical development [137]. Gamitrinib has great potential since it is able to concurrently disable several pathways of mitochondrial metabolism, homeostasis, gene expression, and redox balance specifically for tumors [136]. In addition, combining with Gamitrinib reverses tumor reprogramming through PI3K therapy, with respect to Akt reactivation, growth factor receptor signaling, cell growth, and tumor inhibition. Small molecule inhibitors of PI3K, Akt, or MTOR are shown to stimulate several types of gene expression in tumor cells [136]. However, Gamitrinib—or other agents with similar activity—is not yet available for clinical testing, since it currently in the final stages for preclinical and safety evaluation [136].

Several therapeutic strategies for the PI3K-AKT-mTOR pathway in RCC have been studied. Stimulating mutations in p110 and p85 subunits of PI3K and disabling mutations in the PTEN phosphatase was done to allow disposal of tumors to targeted inhibitors. Positive results with PI3K-inhibitors include NVP-BEZ235, GDC-0980, and LY294002 in RCC model [31,76,138,139,140]. Perifosine (KRX-0401) is an AKT inhibitor that can decrease production of RCC cells [31]. Rapalogs, temsirolimus and everolimus, administered clinically in patients with RCC stimulated formation of next generation mTOR inhibitors. Specifically, increased activity against mTORC2 shows improved utilization and therefore will undergo clinical trials. WYE-125132, WYE-354, P7170, and AZD8055 are initial examples of mTOR inhibitors that prompted tumor reduction in preclinical RCC models [31,141].

PI3K is a striking therapeutic target being a downstream facilitator of receptor tyrosine kinase (RTK) signaling. Several inhibitors, including NVP-BEZ235, GDC-0980, and SF1126 drugs, have entered clinical trials. Multiple pan-PI3K targeting drug inhibitors passed phase 1 and 2 clinical testing, displaying low toxicity and moderate clinical activity.31 Limiting dosage caused hyperglycemia, maculopapular skin rash, nausea, anorexia, and diarrhea

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[142,143]. AKT phosphorylation in blood, skin, or tumor tissue was used as a pharmaco-dynamic biomarker, showed low metabolic responses in a small subset of patients [142]. It is questionably whether these effects are enough to achieve long-lasting treatment responses in patients with RCC.

With the recent success of δ-isoform-specific PI3K-inhibitor idelalisib in hematological malignancies, specific inhibitors in solid tumors were investigated in order to avoid potential limitation of pan-PI3K inhibition [144]. RCC tumors are known to frequently contain *PTEN* and *PIK3CA* mutations. Previous studies found that loss of *PTEN* should be targeted by p110β-inhibitors, and *PIK3CA* mutations should be targeted by p110α selective inhibitors [145]. Initial clinical outcomes of p110α selective (BYL719, MLN1117) and p110β-selective (AZD8186, GSK2636771, SAR260301) inhibitors are now developing, so it is too early to further explain the role of these inhibitors in patients with RCC.

AKT acts as critical downstream mediator of PI3K. Examples of AKT inhibitors include Perifosine and MK-2206, which are currently under phase 1 clinical trials [31]. AKT inhibitors, GSK690693 and GDC-0068, are ATP-competing targets of all three isoforms and currently under investigation. Toxicities with limited dosage included skin rash, nausea,diarrhea, pruritus, and hyperglycemia. AKT phosphorylation lessened in tumor surgeries when treated with MK-2206. Perifosine underwent two phase 2 trials in patients with RCC, displaying low clinical activity of the drug. Preclinical studies suggested that there is limited clinical activity of perifosine, and proposed to improve anti-tumor activity of PI3K/mTOR or mTORC1/ mTORC2 [31].

Mutation of *PIK3CA* allows for positive response to rapalogs.146 A previous study showed that increased systemic LDH level prior to treatment was associated with overall survival of patients with RCC treated with temsirolimus.31 The findings of this study were used to create dual PI3K/mTOR inhibitor drugs, including BEZ235, XL765, GDC-0890, and GSK1059615. The results of Phase 1 clinical trials with BEZ235 and XL765 show that toxicity profiles are comparable with pan-PI3K inhibitors [147-148]. Examples of dual mTORC1/2 inhibitors are AZD8055 and AZD2014, both of which underwent phase 1 testing as well [149]. AZD2014 was shown to block p-S6 in tumor biopsies. A randomized phase 2 trial has been conducted with AZD2014, but there were no results describing pharmacodynamics analysis of the tumor tissue [150].

MTORC1 inhibitors significantly increase ability for cells to recover amino acids from outer protein and improve their growth without essential amino acids [79]. Thus, mTORC1 suppresses use of extracellular proteins for nutrients when amino acids are full, and only use it in emergency when there are not enough free amino acids. The rapalogs everolimus and temsirolimus block mTOR signaling in tumor cells. Resistance mechanisms include activation of MAPK pathway via PI3K mediation and increased expression of survival [31]. TSC1/2 mutations were shown to be inclined to a positive treatment response [151]. Moreover, inhibition of mTOR causes stimulation of recovery pathways to generate energy, including autophagy or using extracellular amino acids [31].

4.3 Targeting MYC

MAX, which is required for MYC DNA-binding activity, has been used to create inhibitor drug compounds. Inhibitors that directly target the MYC/MAX interaction include compounds like 10058-F4, a molecule that blocks hetero-dimerization and can and is probe cells with low non-specific toxicity, and KJ-Pyr-9, a compound discovered in a pyridine library screen. To date, 10058-F4 and KJ-Pyr-9 have proven unsuccessful *in vivo*. However, Omomyc, a mutant basic helix-loop-helix domain that acts like a powerful negative molecule by seizing

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MYC and preventing MAX/MYC DNA binding, has proven informative. Unfortunately, these compounds do not have positive pharmacokinetics and pharmacodynamics *in vivo*. However, this suggests that directly blocking MYC by controlling MYC/MAX interaction is promising but needs to by further studied in order to establish specificity and efficiency in humans [152].

Recent studies have also reported indirectly suppressing MYC by developing inhibitory compounds JQ1 and THZ1, which target factors involved in distinct stages of transcription. JQ1, a potent suppressor of BRD4 (bromodomain protein), attaches to the Ac-K-binding site of BET bromodomains and dislocates BRD4 from chromatin, blocking elongation of transcription. THZ1 was the first developed inhibitor of CDK7, and has high selectivity for CDK7 due to chemical linkage to a cysteine residue outside of the canonical kinase domain [153]. Both JQ1 and THZ1 seem to be highly therapeutic for cancers with high MYC levels, although some effects are independent of MYC [152].

4.4 Targeting LKB1/AMPK

Significant efforts have been made to discover drugs that activate LKB1/AMPK, specifically in metabolic therapy. The most widely studied molecule is metformin, a well-known oral anti-diabetic drug that stimulates AMPK by at least two LKB1-dependent mechanisms. By inhibiting complex I of the mitochondrial electron-transport chain, metformin causing higher AMP/ADP ratio in the cell, and thus stimulating LKB1-AMPK pathways [87]. Blocking OXPHOS causes lower ATP levels and metabolic reprogramming of cells to preserve energy and restore ATP levels, eventually leading to negative control of cell growth and division [154]. This causes a decrease in blood glucose levels, higher sensitivity to insulin, and blocks AMPK-mediated mTOR activation even in CSCs [12,155]. This unregulation of metformin is facilitated by lowering protein synthesis by inhibiting mTOR and lowering fatty-acid production through unrestrained expression of fatty-acid synthase [154].

Currently it is not clear whether metformin improves clinical outcomes for cancer patients by reducing blood glucose levels and insulin/insulin-like growth factor production, or by directly targeting cancer cells [156,157]. Nonetheless, metformin has been well-documented to improve survival of cancer patients, be harmful for cancer stem cells, and prevent tumor growth and development [12,41,87]. Phase 2 trials were done, estimating full anti-cancer effects at regularly used antidiabetic doses. No prospective clinical trials were conducted in RCC. Disease reduction had the best response in patients with prostate cancer, but no clinical progress was shown in pancreatic cancer patients [31].

Like metformin, the biguanide phenformin displays anti-cancer effects by inhibiting mitochondrial complex I and has been shown to inhibit mTORC1 in both AMPK-dependent and independent mechanisms [158,159,160]. However, unlike metformin, phenformin is readily transferred into tumor cells and was withdrawn from clinical use due to increased incidence of lactic acidosis. In a recent study, phenformin seemed to be more effective in treating non-small cell lung cancer (NSCLC), since phenformin has greater effects on ATP level and apoptosis in tumors without a functional LKB-AMPK pathway [96,161]. With its favorable pharmacokinetic characteristics of higher potency and wider tissue distribution, several studies have suggested phenoformin as an anti-neoplastic agent. Further clinical investigations are required to determine tolerable dosage and duration needed to treat cancer [154].

Recent studies have shown that cancer stem cells are dependent on mitochondrial metabolism, and various cancer stem cells are preferentially killed by metformin and phenformin, suggesting that AMPK stimulations could have more pro-survival effects in a

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therapeutic setting [162,163,164]. Furthermore, recent studies are showing that LBK1 is vital for hematopoietic stem cell survival (HSC), suggesting that LKB1 stimulation could also improve leukemic stem cell (LSC) survival. Although this possibility has not been tested yet, LKB1's effects on HSC are most likely not linked to AMPK and mTORC1, suggesting that the therapeutic targeting of AMPK may not improve LSC survival [161,165,166].

A recent study demonstrated that sunitinib—a multiple tyrosine kinase inhibitor used clinically to treat advanced renal cellcarcinoma (RCC) and gastrointestinal stromal tumor (GIST)—directly attaches to the AMPKa subunit to inhibit AMPK activity [167]. AMPKa1 was shown to be pulled-down with sunitinib and midostaurin when treated in melanoma cell lines, demonstrating that these two inhibitors can block AMPK causing MITF break-down, and prompting cell death in melanoma cell lines [168]. Therefore, the cytotoxic effects of sunitinib and midostaurin could possible to linked to their inhibition of AMPK, with one drawback being hyperactivation of mTORC1 [167]. Compound C, the only one molecule inhibitor, is also known to selectively inhibit AMPK by binding the the AMPKa subunit. However, several studies show that Compound C can also block many other kinases and bone morphogeneticprotein (BMP) receptor, suggesting that it has opposing roles [169]. However, sunitinib was found to be a more powerful than compound C, both in vitro andin vivo [167].

The topoisomerase II inhibitor etoposide, which facilitates in breaking DNA to prevent re-forming of DNA, was shown to promote ATM-dependent stimulation of AMPK, which induces apoptosis prostate cancer cells compared to cells without functional LKB1-AMPK [170]. Additionally, cisplatin, which damages DNA by creating intra-strand crosslinks, was reported to stimulate ATM-AMPK pathway in several tumors, especially in conditions of metabolic stress (i.e., nutrient deprivation). Contrarily, unregulated ATM-mediated DNA damage in oral cancers was associated with cisplatin resistance [16]. Doxorubicin, an anthracycline antibiotic that inserts between base pairs of DNA, also recently displayed ability to activate AMPK through increased ROS production. Other AMPK agonists, such as AMP mimetic 5-aminoimidazole-4-carboxamide-1-b-4-ribofuranoside (AICAR), salicylate, and 2DG have also displayed inhibition of tumorigenesis in vitro [87]. AICAR has been known to signal through ATM to control AMPK activity [16].

4.5 Targeting p53

Compounds NSC279287 and NSC66811 have been found to disrupt the interactions with p53 proteins and MDM2, an E3 ubiquitin ligase which regulates p53 and promotes polyubiquitination and subsequent proteasome- dependent breakdown of p53 [125]. MI219, a second class of Mdm2 inhibitors, inhibits p53 interaction with MDM2 by imitating key residues of the p53-Mdm2 complex interface. MI-219 stimulates the p53 pathway and promotes apoptosis in p53 wild-type cancer cells. MI-219 is known to prompt tumor suppression with low toxicity in normal tissues of a mouse model with wild-type p53 human cancer xenografts [125]. RG7112 tightly binds MDM2, blocking its contact with p53. RG7112 stimulates the p53 pathway, causing halt in cell cycle and apoptosis in wild-type p53 expressing cancer cells. Currently, phase I clinical trials were done in patients with progressive solid tumors, hematologic neoplasms, or liposarcomas before debulking surgery. RG7112 seemed tolerable for patients in the initial clinical data, suggesting that clinical activity is consistent with targeting the MDM2-p53 interaction [171]. The limitation with the p53-MDM2 interaction inhibitors is that it is only effective in wild-type p53 expressing cancer cells instead of mutant p53-expressing cancer cells. In addition, p53 over-expression in normal cells may be toxic. The risk of p53 expression in MDM2-null mice shows the risk of inducing p53 in normal tissues in development [125].

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PhiKan083, a carbazole derivative, can selectively attach to a distinct pocket in p53 Y220C mutant protein, and neutralize the p53 Y220C mutant. PhiKan083 increases the melting temperature of Y220C mutant protein, and lowers its rate of denaturation. The complete biological functions of this compound have not been studied yet [125]. NSC319726 is another compound that can restore activity of wild-type p53 in R175H-mutant cancer cell lines. NSC31397 has anti-tumor activity in particular p53 R172H mutant genetically engineered mice, and specifically blocks xenograft tumor growth of R175H-mutant p53 cancer cells [125].

Other compounds for mutant p53 include CP31398, SCH529074, Ellipticine, WR1065, p53R3. CP31398 neutralizes the central domain of mutant p53 protein, increases binding and transcription of DNA, and shows anti-tumor ability in colon cancer and melanoma mice models. SCH529074 attaches to the DNA binding region of mutant p53 and stabilizes it, causing p53-dependent apoptosis. Ellipticine builds up the transcriptional activity of mutant p53. WR1065, the active metabolite of amifostine repairs the wild-type conformation of the thermo-sensitive V272M p53 mutant, increasing transcription of p21, GADD45 and MDM2, and causing G1 cell cycle arrest. Finally, p53R3 repairs DNA binding of R175H and R273H p53 mutants, stimulates DR5 expression, and excites cancer cells to TRAIL-induced apoptosis [125].

5. CONCLUSION

Mutations in oncogenes and tumor suppressor genes result in various changes to intracellular signaling pathways that affect cancer cell metabolism and restructure it for increased survival and growth [27,172]. Previous studies have identified a good number of oncogenes and tumor suppressors that function as regulators of metabolism. While this paper reviews only a few of those genes, research and literature in this area is quickly growing, and many other proteins involved in cancer metabolism are emerging [4].

Previous studies continue to emphasize the significance of metabolic changes in cancer cells, and how this knowledge could be utilized to stop tumor cells in their track. Some targets are already well-established or going through clinical trials; for example, metformin, which is a well-known diabetic drug and activator of AMPK, is being tested for cancer therapy. Other possible targets are still under way.

Only through understanding the metabolic processes will we be able to discover the Achilles heels of tumor metabolism and utilize this information to identify and develop new targets for treatment. The ultimate goal is to design treatment strategies that inhibit tumor progression, improve therapeutic response, and produce positive clinical outcomes.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

The authors has declared that no competing interests exist.

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