CHAPTER THREE

Regulation of Cancer Metabolism by Oncogenes and Tumor Suppressors

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Abstract

Cell proliferation requires the coordination of multiple signaling pathways as well as the provision of metabolic substrates. Nutrients are required to generate such building blocks and their form of utilization differs to significant extents between malignant tissues and their nontransformed counterparts. Thus, oncogenes and tumor suppressor genes regulate the proliferation of cancer cells also by controlling their metabolism. Here, we discuss the central anabolic functions of the signaling pathways emanating from mammalian target of rapamycin, MYC, and hypoxia-inducible factor-1. Moreover, we analyze how oncogenic proteins like phosphoinositide-3-kinase, AKT, and RAS, tumor suppressors such as phosphatase and tensin homolog, retinoblastoma, and p53, as well as other factors associated with the proliferation or survival of cancer cells, such as NF-κB, regulate cellular metabolism.

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ABBREVIATIONS

AMPK AMP-activated protein kinase
COX cytochrome c oxidase
GLS1 glutaminase 1
HIF-1 hypoxia-inducible factor 1
IκB inhibitor of κB proteins
LDH lactate dehydrogenase
LKB1 liver kinase B1
mTOR mammalian (or mechanistic) target of rapamycin
PDH pyruvate dehydrogenase
PDK1 pyruvate dehydrogenase kinase 1
PHD prolyl-4-hydroxylase domain protein
pRb retinoblastoma protein
PtdIns(3,4,5) P3 phosphatidylinositol-3,4,5-trisphosphate
PTEN phosphatase and tensin homologue
SCO2 synthesis of cytochrome c oxidase 2
SREBP sterol regulatory element-binding protein
TIGAR TP53 (tumor protein 53)-induced glycolysis and apoptosis regulator
TSC1/2 tuberous sclerosis 1/2
VHL von Hippel–Lindau

1. INTRODUCTION

Most oncogenes and tumor suppressor genes encode proteins that promote cellular proliferation or cell cycle arrest. In recent years, we are learning that proliferation is tightly coupled with metabolic changes. For this reason, cancer metabolism is an area of intense research, since the metabolism of cancer cells can be exploited for therapeutic purposes (Munoz-Pinedo, El Mjiyad, & Ricci, 2012). In accordance to the normal function of their encoded proteins, oncogenes or tumor suppressors regulate cellular metabolism (Vander Heiden, Cantley, & Thompson, 2009). This is an intrinsic part of their program to reduce or promote cell proliferation. Oncogenes promote glucose and amino acid uptake and metabolism in order to make new lipids, nucleotides, and proteins. Conversely, tumor suppressors upregulate mitochondrial respiration and Krebs (TCA) cycle (see review by Frezza and colleagues, Chapter 1 of this volume). We will discuss how several oncogenes and tumor suppressors regulate cellular metabolism.

2. HIF-1: REGULATOR OF HYPOXIC RESPONSES AND CANCER METABOLISM

Highly proliferating tumor cells are characterized by a hypoxic microenvironment due to the increased oxygen consumption, which stimulates metabolic reprogramming (Vaupel, Thews, & Hoeckel, 2001). The master regulator of cellular responses to low oxygen is hypoxia-inducible factor 1 (HIF-1), a transcription factor induced by hypoxic conditions and whose levels are increased in many human cancers even under normoxia (Semenza, 2010). Under normal oxygen conditions, HIF-1 is degraded by the proteasome after prolyl hydroxylation by prolyl-4-hydroxylase domain proteins (PHDs) and ubiquitination by the tumor suppressor von Hippel–Lindau (VHL) (Kaelin & Ratcliffe, 2008; Fig. 3.1). HIF-1 can also be constitutively activated by genetic alterations, such as the loss of function of VHL in renal cancer cells, or due to the accumulation of metabolites such as fumarate or succinate (Boulahbel, Duran, & Gottlieb, 2009). Cancer cells frequently undergo oxygen shortage which inhibits the prolyl hydroxylases and stabilizes HIF-1, which induces the expression of hundreds of genes involved in angiogenesis, metabolism, apoptosis, and proliferation.

The major metabolic effect of HIF-1 is to trigger the switch from mitochondrial oxidative phosphorylation (OXPHOS) to anaerobic glycolysis. HIF-1 induces the expression of glucose transporters (GLUT-1, GLUT-3) to enhance glucose uptake and it upregulates glycolytic enzymes and the lactate dehydrogenase A (LDHA) subunit to stimulate the conversion of pyruvate into lactate (Brahimi-Horn, Chiche, & Pouyssegur, 2007; Semenza, 2011; Fig. 3.1). Importantly, HIF-1 activates the pyruvate dehydrogenase kinase 1 (PDK1; Kim, Tchernyshyov, Semenza, & Dang, 2006; McFate et al., 2008), a negative regulator of pyruvate dehydrogenase (PDH). PDH converts pyruvate into acetyl-CoA to enter the Krebs cycle in the mitochondria (Fig. 3.1). The effect of inhibiting PDH is the inhibition of mitochondrial oxygen consumption and reduction of ROS production, and this promotes anaerobic glycolysis and thus the Warburg effect (Papandreou, Cairns, Fontana, Lim, & Denko, 2006).

HIF-1 also controls respiration by regulating expression and stability of the cytochrome oxidase subunits cytochrome c oxidase (COX)4-1 and COX4-2 (Fukuda et al., 2007). Additionally, HIF-1 upregulates the expression of the proteins BNIP3 and BNIP3L, which trigger mitochondrial

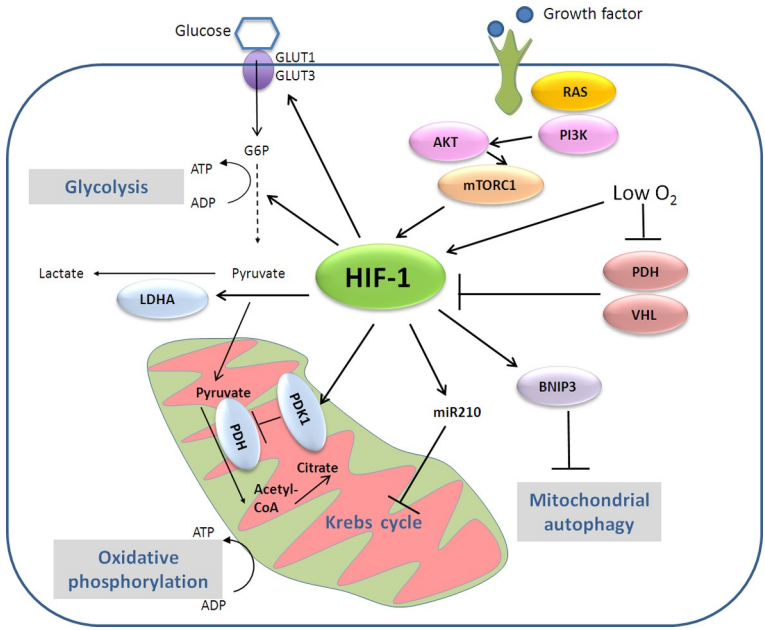

Figure 3.1 *Regulation of cancer metabolism by HIF-1*. HIF-1 switches metabolism from oxidative respiration to anaerobic glycolysis. Hypoxia induces HIF-1 by blocking its inhibitors prolyl-4-hydroxylase domain proteins (PHDs) and von Hippel–Lindau (VHL) protein that need O_2 to exert their functions. Once activated, HIF-1 upregulates the glucose transporters GLUT1 and GLUT3, thus enhancing glucose uptake. HIF-1 induces the expression of almost every enzyme of the glycolytic pathway and lactate dehydrogenase A (LDHA), thus resulting in lactate production. Importantly, HIF-1 induces the pyruvate dehydrogenase kinase 1 (PDK1) that phosphorylates pyruvate dehydrogenase (PDH) blocking the entry of pyruvate into the mitochondria. HIF-1 also induces the expression of miR210, inhibiting important enzymes of Krebs cycle, and upregulates the protein BNIP3 that promotes mitochondrial autophagy.

autophagy, another possible mechanism by which HIF-1 reduces oxidative metabolism (Zhang et al., 2008). HIF-1 can also activate the transcription of miR-210, a microRNA which blocks the expression or activity of some enzymes of the Krebs cycle and the Complex I of the electron transport chain (Chen, Li, Zhang, Huang, & Luthra, 2010; Favaro et al., 2010; Fig. 3.1).

3. THE PI3K–AKT–PTEN PATHWAY REGULATES METABOLISM

The PI3K–AKT pathway is one of the main prosurvival pathways activated in human cancers. The phosphatidylinositol 3-kinases (PI3Ks) are a

family of proteins that phosphorylate phoshoinositides at the D-3 position of the inositol ring, and their functions are linked to different biological roles, like regulation of cell growth, organismal metabolism, cell proliferation, and vesicle trafficking (Cantley, 2002; Engelman, Luo, & Cantley, 2006).

The best known effector downstream of PI3K is AKT (also known as Protein Kinase B, PKB). Oncogenic mutations in PI3K increase the PI3K and AKT signaling, promoting factor-independent growth and increasing cell invasion and metastasis (Manning & Cantley, 2007). Activated AKT is also an important driver of oncogenic metabolism. It was recognized early that AKT activation drives the glycolytic metabolism of tumor cells (Fig. 3.2; Elstrom et al., 2004). Activation of AKT increases cellular glucose uptake by inducing the expression and membrane translocation of glucose transporters (Barthel et al., 1999; Kohn, Summers, Birnbaum, & Roth, 1996). AKT also

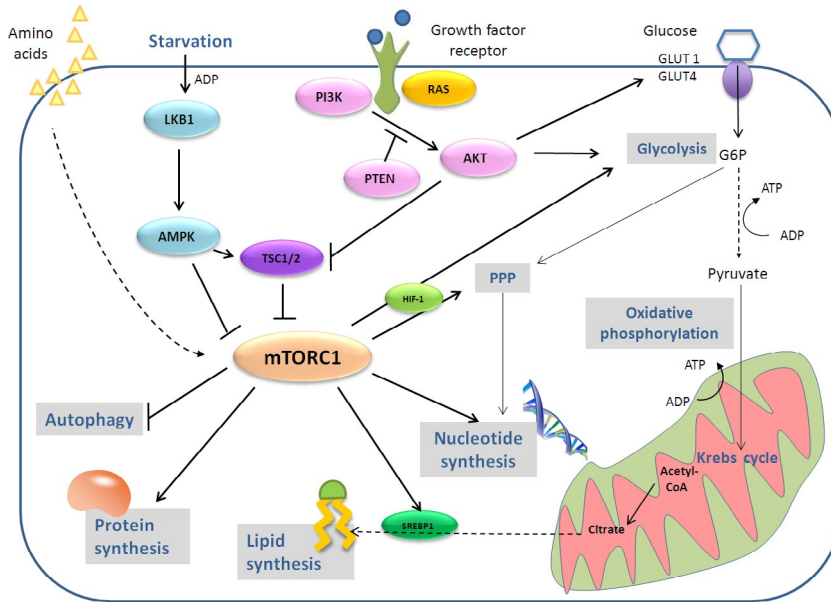

Figure 3.2 *Regulation of cancer metabolism by the PI3K–AKT–PTEN and LKB1–AMPK–mTORC1 pathways*. Growth factor receptors activate Ras and phosphatidylinositol 3-kinase (PI3K) leading to the activation of AKT. Once activated, AKT induces glycolysis by regulating glycolytic enzymes and glucose transporters. These effects are counteracted by the phosphatase and tensin homologue (PTEN). AKT can indirectly activate the mTORC1 pathway that promotes lipid, protein, and nucleotide synthesis, contributing to the building of bioblocks necessary for tumor proliferation. Under stress conditions, the AMP-activated protein kinase (AMPK) activation through the liver kinase B1 (LKB1), opposes glycolytic metabolism in part by inhibiting mTORC1. PPP, pentose phosphate pathway.

increases glycolysis by activating the enzyme phosphofructokinase-1 (PFK1) through phosphorylation of phosphofructokinase-2 (PFK2) (Deprez, Vertommen, Alessi, Hue, & Rider, 1997), which leads to allosteric activation of PFK1. In addition, AKT stimulates the mammalian (or mechanistic) target of rapamycin (mTOR) pathway, thus promoting many other metabolic branches as we will discuss below.

PI3K/AKT signaling pathway can be inhibited by the tumor suppressor gene phosphatase and tensin homologue (PTEN). PTEN dephosphorylates phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5) P3), the second messenger generated by the activation of PI3K, and the main activator of AKT, thereby inhibiting the PI3K–AKT–mTOR pathway. The main functions of PTEN are the regulation of cell growth, metabolism, and survival, and thus it has an important tumor-suppressive ability (Carracedo & Pandolfi, 2008). Even a slight decrease of PTEN levels, or a fine change in *PTEN* gene expression, is sufficient to induce cancer susceptibility (Alimonti et al., 2010). Consistently, loss of PTEN promotes glycolysis (Tandon et al., 2011) and elevation of PTEN levels can reverse the cancer metabolic reprogramming from glycolysis to OXPHOS (Garcia-Cao et al., 2012). For example, transgenic mice carrying additional copies of *PTEN* (referred to as Super-PTEN mice), are less prone to cancer development. In this model, PTEN elevation resulted in a healthier metabolism, with systemic metabolic reprogramming; mice display increased oxygen consumption and energy expenditure, higher mitochondrial biogenesis increasing the mitochondrial ATP production, and an important reduction of body fat accumulation. Cells derived from these mice show reduced glucose and glutamine uptake, increased mitochondrial OXPHOS, and resistance to oncogenic transformation (Garcia-Cao et al., 2012). Conversely, in nontransformed thyrocytes of a PTEN-deficient mouse model, the constitutive PTEN deficiency caused a downregulation of Krebs cycle and OXPHOS, defective mitochondria and reduction of respiration with compensatory glycolysis. In this case, the metabolic switch to glycolysis is driven by PI3K-dependent AMP-activated protein kinase (AMPK) inactivation (Antico Arciuch, Russo, Kang, & Di Cristofano, 2013).

4. mTOR CONTROLS ANABOLISM AND IT IS INHIBITED BY AMPK UPON METABOLIC STRESS

mTOR is a serine/threonine kinase that is part of two distinct complexes, TORC1 and TORC2, which have different sensitivity to rapamycin. We will discuss the role of the rapamycin sensitive complex,

mTORC1, which controls cell growth and metabolism in response to environmental signals (Wullschleger, Loewith, & Hall, 2006). The mTOR pathway is one of the most deregulated signaling pathways in human cancer, and growth-factor-independent activation of mTORC1 is observed in up to 80% of tumors, across nearly all lineages (Guertin & Sabatini, 2007; Menon & Manning, 2009). mTOR is also deregulated in metabolic disorders, such as obesity and type 2 diabetes. Mice with hyperactive mTORC1 signaling in the liver display metabolic abnormalities, including defects in glucose and lipid homeostasis, and subsequently develop hepatocellular carcinoma (Menon et al., 2012).

mTOR integrates diverse signals to regulate cell growth: growth factors, nutrients, oxygen, energy, and several forms of stress. mTOR, downstream of PI3K, responds to growth factors via the inactivation of tuberous sclerosis (TSC)1 and TSC2 by AKT; these proteins are negative regulators of mTORC1 (Manning & Cantley, 2007; Fig. 3.2). Nutrients, particularly amino acids, also regulate mTORC1 signaling, which controls protein translation. The molecular mechanism by which mTORC1 senses intracellular amino acids is not fully understood, but it requires the Rag GTPases (Kim, Goraksha-Hicks, Li, Neufeld, & Guan, 2008; Sancak et al., 2010).

mTOR regulates many anabolic pathways. Through regulation of HIF1 it activates glycolysis and the pentose phosphate pathway (PPP) (Figs. 3.1 and 3.2), and by activating the transcription factor sterol regulatory element-binding protein (SREBP)1, it also stimulates lipid synthesis (Düvel et al., 2010; Fig. 3.2). Nucleotide synthesis is also regulated by mTOR in two different manners: through regulation of the PPP and by activation of an enzyme of pyrimidine synthesis (Ben-Sahra, Howell, Asara, & Manning, 2013; Robitaille et al., 2013). Thus, cells with active mTOR are stimulated to proliferate by making all necessary building blocks.

mTOR is inhibited in conditions of nutritional stress by the AMPK. Tumors under metabolic stress adapt to these conditions by altering the liver kinase B1 (LKB1)–AMPK pathway (Sebbagh, Olschwang, Santoni, & Borg, 2011). As a result, the LKB1–AMPK pathway works as a metabolic checkpoint and inhibits cancer metabolic reprogramming (Jones et al., 2005; Kuhajda, 2008). AMPK is an ATP sensor that checks and regulates cellular energy homeostasis. AMPK is activated in response to nutrient deprivation or hypoxia, when ATP levels decline and the AMP and ADP levels increase (Fig. 3.2) (Hardie, 2011; Xiao et al., 2011). Under conditions of energy stress, LKB1 (serine–threonine kinase LKB1) acts as the main upstream kinase that activates AMPK (Shaw, Bardeesy, et al., 2004; Woods et al.,

2003). Once activated, AMPK can target a wide range of downstream metabolic pathways, especially the mTOR pathway. During energetic stress, AMPK can inhibit mTORC1 through two different mechanism; phosphorylating TSC2 (Corradetti, Inoki, Bardeesy, DePinho, & Guan, 2004; Inoki, Zhu, & Guan, 2003; Shaw, Kosmatka, et al., 2004) or by direct phosphorylation of Raptor, a component of mTORC1 (Scott, Norman, Hawley, Kontogiannis, & Hardie, 2002). LKB1-deficient cells and mutant mice for LKB1, or MEFs deficient for TSC2, show hyperactive mTORC1 signaling in response to energy stress (Shaw, Bardeesy, et al., 2004). Thus, AMPK alters important cellular responses, like cell growth, proliferation and autophagy (Shackelford et al., 2009). The lack of AMPK signaling increase tumorigenesis and enhances the glycolytic metabolism in cancer cells (Faubert et al., 2012). However, AMPK can also promote survival of tumor cells: LKB1 deficiency reduces the AMPK signaling in tumor cells (Godlewski et al., 2010; Shackelford & Shaw, 2009; Zheng et al., 2009), and deletion of LKB1 makes the cells more sensitive to nutrient deprivation (Shaw, Bardeesy, et al., 2004). Additionally, by inhibiting lipid synthesis and promoting lipid oxidation, AMPK contributes to maintenance of NADPH levels thus mitigating redox stress (Jeon, Chandel, & Hay, 2012).

5. c-MYC PROMOTES AEROBIC ANABOLISM

c-Myc has been reported to be the master regulator of metabolic processes involved in cell proliferation. Myc is deregulated in many human cancers in which it triggers tumorogenesis through the transcriptional modulation of many genes. In fact, it has been recently proposed that Myc is a "general" transcription factor, in the sense that high levels of c-Myc in tumor cells produce elevated levels of transcripts from the existing gene expression program of tumor cells (Lin et al., 2012). This includes genes involved in glucose metabolism, nucleotide, lipid, amino acid, and protein synthesis (Dang, 2013; Li & Simon, 2013). Once activated, c-Myc binds, with its cofactor Max, to the consensus sequences called "E-boxes" present in genes driven by all three RNA polymerases, resulting in ribosomal RNA synthesis and ribosome biogenesis, necessary to build the increasing cell mass (Grandori et al., 2005; van Riggelen, Yetil, & Felsher, 2010).

c-Myc also regulates mitochondrial biogenesis by inducing the expression of genes involved in mitochondrial structure and function, such as *TFAM* which encodes a protein involved in mitochondrial transcription and mitochondrial DNA replication (Li, 2005; Fig. 3.3). To trigger biomass

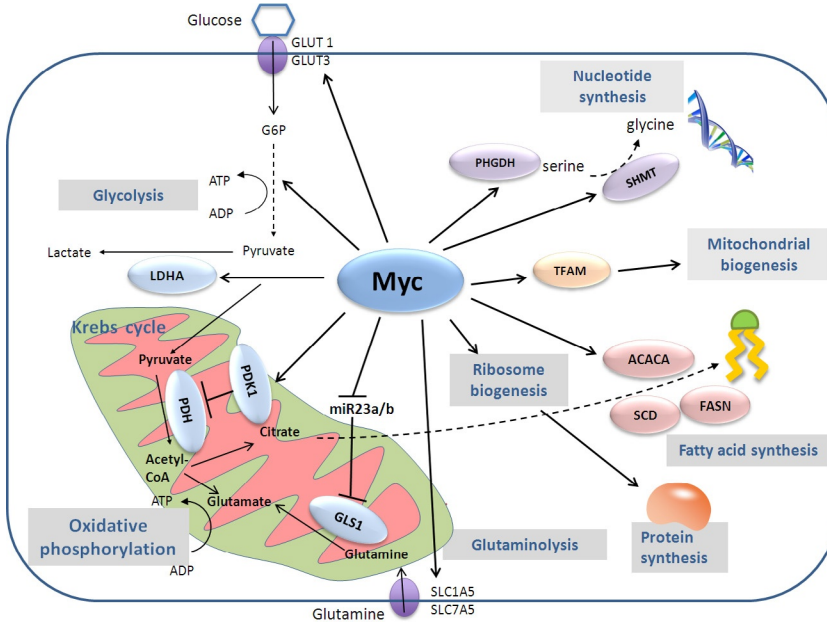

Figure 3.3 *Myc regulates cancer metabolism*. Myc promotes cancer cell metabolism at several levels. Myc upregulates the glucose transporters GLUT1 and GLUT3 increasing glucose uptake. It induces several glycolytic enzymes such as the lactate dehydrogenase A (LDHA) resulting in lactate production. Like HIF-1, Myc induces pyruvate dehydrogenase kinase 1 (PDK1) expression, which prevents pyruvate entry into the mitochondria. Myc also regulates glutaminolysis: it upregulates glutamine transporters SLC1A5 and SLC7A5 and induces glutaminase 1 (GLS1) expression. Myc also promotes biomass accumulation essential for proliferating tumor cells. It regulates ribosome biogenesis, mitochondrial biogenesis, and several enzymes involved in fatty acids synthesis such as acetyl-CoA carboxylase (ACACA), fatty acid synthetase (FASN), and stearoyl-CoA desaturase (SCD). Additionally, Myc regulates enzymes involved in nucleotide synthesis such as phosphoglycerate dehydrogenase (PHGDH) and serine hydroxymethyltransferase (SHMT).

accumulation necessary for cell proliferation, c-Myc induces the expression of almost every glycolytic gene, redirecting cells to glucose consumption for ATP but also for biomolecule production. c-Myc also stimulates the transcription of LDHA that is necessary for c-Myc mediated tumorigenesis in some models (Shim et al., 1997; Fig. 3.3).

Like HIF-1, c-Myc regulates other important glycolytic enzymes such as hexokinase 2 -that phosphorylates glucose to make glucose-6-phosphate- and PDK1 -which phosphorylates and inhibits PDH, blocking the entry of pyruvate into the mitochondria (Kim, Gao, Liu, Semenza, & Dang,

2007; Fig. 3.3). It has been shown by *in vivo* imaging techniques that in c-Myc-driven liver tumors pyruvate is converted preferentially to lactate (Hu et al., 2011). Interestingly, metabolic changes were detected prior to the appearance of tumors: in pretumor tissues, an accumulation of alanine due to increased expression of transaminases was observed.

c-Myc also controls glutamine metabolism, achieved through regulation of mitochondrial glutaminase 1 (GLS1) expression (Gao et al., 2009). 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. There are evidences that GLS1 is regulated by c-Myc also at posttranscriptional level. c-Myc suppresses the expression of two miRNAs, miR-23a and miR-23b, which target GLS1 in its 3′UTR, resulting in increased glutaminase expression and glutamine metabolism. c-Myc also stimulates the transport of glutamine inside the cell by increasing the expression of the glutamine transporters SLC1A5 and SLC7A5 (Fig. 3.3).

It has been shown that c-Myc can regulate nucleotide biosynthesis by transcriptional regulation of several key enzymes, redirecting glycolysis to the synthesis of serine and glycine that are essential for nucleotide building (Mannava et al., 2008). Recently, Myc has also been associated to lipid synthesis as many enzymes of fatty acid biosynthesis are its direct targets and they contribute to the building of bioblocks needed in the c-Myc-driven proliferation program (Loven et al., 2012; Fig. 3.3). Thus, Myc has been shown to activate all pathways necessary to build new cells.

6. RAS STIMULATES GLYCOLYSIS AND THE PPP

The Ras family encompasses a number of small GTPases that transduce signals to induce proliferation, including the metabolic switch. Transfection of a constitutively activated form of Ras is sufficient to stimulate glycolysis and the PPP (Vizan et al., 2005). Ras proteins are activated downstream of growth factors or they are constitutively active in tumors, and they signal through MAP kinases and/or through PI3K. Some of the metabolic effects of Ras, thus, may be mediated through the PI3K/AKT/mTOR pathway, while other effects can be due to stimulation of Myc. H-Ras, for instance, upregulates Glut-1 mRNA through the PI3-kinase pathway. This effect is indirect, through the PI3K-mediated upregulation of HIF-1 (Chen, Pore, Behrooz, Ismail-Beigi, & Maity, 2001). Since Ras can indirectly regulate HIF-1, it can regulate metabolism in the same manner,

and this is for instance the case in colon cancer cells with hyperactivated KRas, in which KRas inhibits mitochondrial metabolism through activation of HIF-1 (Chun et al., 2010).

Pancreatic tumors often carry activating KRAS mutations. In these cells, KRas regulates multiple metabolic pathways at the transcriptional level. It stimulates glucose uptake and it channels glucose intermediates into the hexosamine biosynthesis and PPPs. These effects are mediated by MAP kinases and Myc (Ying et al., 2012). Additionally, pancreatic ductal adenocarcinomas have recently been shown to depend on a nonclassical glutamine utilization pathway stimulated transcriptionally by Kras. Kras directs the metabolism of these cells in toward the use of glutamine as a source of pyruvate and NADPH to maintain the cellular redox balance (Son et al., 2013).

Ras is also a regulator of autophagy, a cellular process that can provide nutrients by self-digestion of intracellular components. This process is also responsible for clearance of damaged mitochondria. Ras-mediated transformation induces autophagy, which is required to maintain mitochondrial metabolic functions in Ras-driven tumors (Guo et al., 2011). In these tumors, knockdown of essential autophagy genes can promote the accumulation of abnormal mitochondria unable to metabolize lipids through fatty acid oxidation (White, 2013). Similarly, tumors driven by a Ras downstream effector, the oncogene BRAF, rely on autophagy to maintain healthy mitochondria and glutamine metabolism (Strohecker et al., 2013).

7. NF-kappaB REGULATES INFLAMMATION AND PROLIFERATION BUT ALSO METABOLISM

NF-κB is a transcription factor of the Rel-homology-domain family. Its subunit p65/RelA is the most important in transactivation of several target genes involved in immunity, inflammation, and proliferation. Its activity is tightly regulated by the inhibitors of κB proteins (IκBs) and the IκB kinase proteins (IKKs), and it results in the expression of growth factors, cytokines, and promotion of cell proliferation (Hayden & Ghosh, 2004). Although NF-κB is not considered a classical oncogene, its expression can be regulated by several oncogenes, suggesting a role of NF-κB in promotion of tumorogenesis (Basseres & Baldwin, 2006). It has been reported that oncogenic H-Ras activates NF-κB (Finco et al., 1997) inducing lung tumor progression *in vivo* in a p53-dependent (Meylan et al., 2009) or independent manner (Bassères, Ebbs, Levantini, & Baldwin, 2010). In cells with mutated

p53, the activation of Ras induces a metabolic switch from oxidative mitochondrial phosphorylation to aerobic glycolysis that has been related to NF-κB activation (Kawauchi, Araki, Tobiume, & Tanaka, 2008). In this model, the loss of p53 activity resulted in transcriptional activation of NF-κB that was essential for the enhanced glucose consumption and lactate production. GLUT3 expression was directly regulated by NF-κB, accordingly with the observed increase of glucose uptake in those cells. Recently, it has been shown that NF-κB activation by the epidermal growth factor receptor (EGFR) in cancer cells induces the expression of pyruvate kinase M2 (PKM2), triggering lactate production and glucose uptake (Yang et al., 2012). However, NF-κB has also been shown to contribute to tumorogenesis by sustaining mitochondrial function. This effect was mediated through p53 and its target synthesis of cytochrome c oxidase 2 (SCO2), which increases OXPHOS (Mauro et al., 2011). Although NF-κB is not a typical oncogene, all these findings suggest an involvement of NF-κB in metabolic reprogramming and tumorigenesis. However, the manner by which NF-κB regulates cancer metabolism is still unclear and may be context dependent.

8. RETINOBLASTOMA: SUPPRESSING TUMOROGENESIS AND ANABOLISM

The retinoblastoma protein (pRb) is one of the tumor suppressors whose role in cancer metabolism has been most extensively studied (Nicolay & Dyson, 2013). The major function of pRb is the inhibition of cell cycle progression exerted through repression of the E2F1 transcription factor. This function is reverted by pRb phosphorylation by cyclin D-CDK4/6, which inactivates Rb and promotes E2F1-mediated transcription. Many signals can regulate pRb expression; among those, AMPK has been shown to phosphorylate directly pRb controlling the G1/S phase transition based on the energy status of the cell (Dasgupta & Milbrandt, 2009). Recently, pRb was shown to regulate starvation-induced stress response in *Caenorhabditis elegans* (Cui, Cohen, Teng, & Han, 2013) and similar results have been recently provided in a Drosophila model, suggesting an involvement of pRb in cancer metabolism (Nicolay et al., 2013). This study shows that flies with mutant RBF1 (Drosophila Rb homolog) are hypersensitive to fasting conditions and present deregulated glutamine and nucleotide metabolism. Also human cancers with inactivated pRb show an increase in glutamine uptake due to upregulation of expression of the glutamine

transporter ASCT2, and an increase in glutamine utilization in the Krebs cycle resulting in glutathione accumulation (Reynolds et al., 2014). pRb and E2F1 can regulate in an opposite way the oxidative metabolism, modulating the expression of different genes at their promoters. pRb deletion in murine erythrocytes causes a block in differentiation and impairs mitochondrial biogenesis uncovering a positive role of pRb on mitochondrial activity (Sankaran, Orkin, & Walkley, 2008), while other studies show that E2F1 induces a switch from oxidative to glycolytic metabolism by repressing multiple genes involved in mitochondrial function (Blanchet et al., 2011). Some studies have described a role of pRb in lipid metabolism, showing that pRb deletion induces E2F-dependent expression of fatty acid biosynthesis enzymes and SREBP (Shamma et al., 2009). Additionally, pRb has been shown to play a role in nucleotide metabolism by inhibiting enzymes such as dihydrofolate reductase and thymidylate synthase (Angus et al., 2002). All these data indicate a connection of pRb in cell cycle progression and regulation of tumor metabolism.

9. p53 REGULATES MULTIPLE METABOLIC PATHWAYS

p53 function is lost in most human cancers (Soussi & Beroud, 2001). p53 exerts an important defense mechanism against tumor development (Vousden & Ryan, 2009). It is a transcription factor that regulates a large range of functions like DNA damage response, apoptosis, and senescence. Mutations in p53 found in tumors can produce a variety of biological effects, for example: lack of control in cell cycle, defective apoptosis, and inefficient DNA repair (Resnick & Inga, 2003). In p53 knockout mice, tumor development is rapid and spontaneous (Donehower et al., 1992). p53 also plays an important role in metabolic stress response (Vousden & Ryan, 2009). Cells lacking p53 and deprived of glucose cannot undergo cell cycle arrest, since p53 controls a metabolic checkpoint. This makes p53-defective cells more sensitive than nontransformed cells to metabolic stress, what has led to propose the use of antiglycolytic drugs for therapy of p53-deficient tumors (Jones et al., 2005). p53 also responds to lack of serine and allows *de novo* synthesized serine to be channeled to production of reduced glutathione to counter oxidative stress (Maddocks et al., 2013). For this reason, p53-deficient cells are more sensitive to serine depletion.

As part of the antitumor activity of p53, it promotes glucose OXPHOS and it inhibits glycolysis (Fig. 3.4). Disruption of TP53 in mice promotes a significant decrease in oxygen consumption that closely correlates with p53

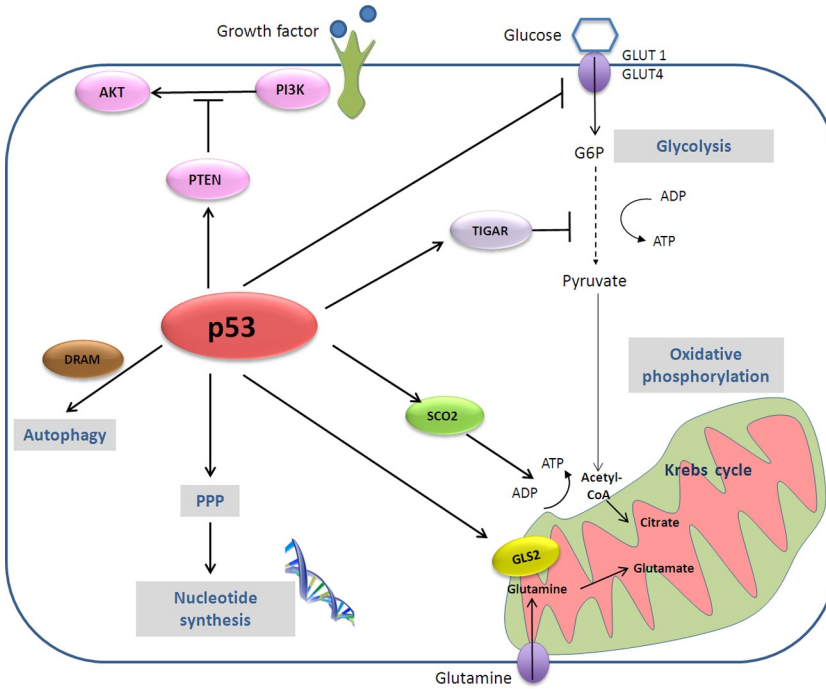

Figure 3.4 *p53 regulates multiple metabolic pathways*. p53 responds to metabolic stress and it can inhibit the tumorigenic metabolic switch by suppressing glycolysis and activating the phosphatase and tensin homologue (PTEN). p53 inhibits the transcription of GLUT1 and GLUT4 reducing glucose uptake and it upregulates the TP53 (tumor protein 53)-induced glycolysis and apoptosis regulator (TIGAR), which results in glycolysis inhibition. p53 increases the mitochondrial metabolism by activation of the synthesis of cytochrome c oxidase 2 (SCO2), thus promoting oxidative phosphorylation. p53 can also induce, contradictorily, prosurvival responses in cancer cells, for instance when it increases the flux through the pentose phosphate pathway (PPP) or glutamine utilization. P53 can regulate positively autophagy by increasing the expression of DRAM.

deficiency, as p53 increases OXPHOS through upregulation of the gene SCO2, whose product participates in the assembly of COX in the mitochondria (Matoba et al., 2006). p53 upregulates TP53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme that decreases the levels of the glycolytic activator fructose-2,6-bisphosphate (Bensaad et al., 2006). It also inhibits glucose uptake by inhibiting the transcription of GLUT1 and GLUT4 (Schwartzenberg-Bar-Yoseph, Armoni, & Karnieli, 2004). p53 can also inhibit the glycolytic pathway indirectly by activating PTEN, thus inhibiting the PI3K pathway (Stambolic et al., 2001).

p53 is also involved in somewhat contradictory responses, since it has been associated with pathways that may support tumor growth and survival. For example, in some tumor cells it can increase the flux through the PPP, reducing oxidative stress and promoting anabolism, thus helping the growth of cancer cells (Vousden & Ryan, 2009). p53 is also able to contribute to glutaminolysis, an alternative fuel bioenergetic pathway, where glutamine is metabolized to produce α-ketoglutarate from glutamate in the Krebs cycle. This pathway is important in the process of oncogenic transformation: the enzyme which converts glutamine to glutamate, glutaminase 1 (GLS1/KGA) has been shown to help tumor development (Wang et al., 2010). p53 can play a role in the regulation of glutaminolysis by the activation of another isoform of glutaminase (GLS2/LGA), helping the cells produce ATP in periods of glucose deprivation (Hu et al., 2010; Suzuki et al., 2010). Both the activation of the PPP and glutaminolysis could have a function in reduction of oxidative stress.

Another function of p53 is related to autophagy. The control of p53 in autophagy is context specific, and it could work like a prodeath or cell survival mechanism. One of the ways by which p53 regulates autophagy is by upregulating damage regulated autophagy modulator (DRAM), a lysosomal protein that positively regulates autophagy (Crighton et al., 2006).

The family of transcription factors of p53 includes p63 and p73, both functional homologs with high sequential and structural similarity (Kaghad et al., 1997; Yang et al., 1998). These two members of the p53 family have functions that are markedly different from those of p53 (Allocati et al., 2012), but they also have many similarities and overlapping activity with p53, including the regulation of cellular metabolism (Berkers, Maddocks, Cheung, Mor, & Vousden, 2013). Tp63 and Tp73 genes are transcribed from two different promoters, and the final product can be either full length proteins that retain a full transactivation (TA) domain (TAp63 and TAp73) or N-terminally truncated isoforms (ΔNp63 and ΔNp73) (De Laurenzi & Melino, 2000). TAp63 can control fat and glucose metabolism, because is a positive regulator of the transcription of Sirt1, AMPKa2, and LKB1. TAp73 can promote cancer cell proliferation, controlling biosynthetic pathways and cellular antioxidant capacity through the regulation of glucose metabolism. TAp73 regulates the expression of glucose-6-phosphate dehydrogenase (G6PD), an enzyme involved in glucose metabolism through the PPP (Du et al., 2013). p73 can be negatively regulated by AMPKα by direct interaction without affecting p53, which represses the TAp73 transcription program (Lee, Lee, Sin, Kim, & Um, 2008).

And recently it was discovered that like p53, TAp73 is implicated in the maintenance of mitochondrial Complex IV (Rufini et al., 2012).

In summary, p53 opposes the PI3K pathway to inhibit anabolism, it promotes mitochondrial metabolism and it regulates oxidative stress. The metabolic roles of p53 may well be more important for its tumor suppressor abilities than its roles as a proapoptotic or prosenescent proteins, as recently revealed by a study employing a mutant that had lost these functions and still suppressed tumorogenesis (Li et al., 2012).

10. CONCLUSIONS

To date, a good number of oncogenes and tumor suppressors have been shown to play a role as regulators of metabolism. The vast literature is growing quickly, and we have only summarized here the roles of a few of these genes. However, many other proteins involved in cancer have been shown to play roles in metabolism, from the breast cancer associated receptor tyrosine kinase ErbB2 (Her2/neu) (Zhao et al., 2009) to the promyelocytic leukemia tumor suppressor (Carracedo et al., 2012) or many of the Bcl-2 family of antiapoptotic proteins (reviewed by Fulda and colleagues, Chapter 4 of this volume). Metabolic rewiring is such an important part of the cellular growth process that we will likely see this field expanding in the future.

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