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The Regulation of Energy Metabolism: An Important Facet of P53 Function

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Objectives: p53 is a key tumor suppressor protein that has a diverse range of functions which help to prevent cancer development. Given that metabolic alterations are common features of cancer cells it has been recently suggested that p53 has an important role in controlling metabolic pathways. The aim of this review is to provide an update of our current understanding of the role and mechanisms of p53 in maintaining the homeostasis of cellular energy metabolism. **Methods:** the studies which are reported, focus on the regulation of p53-targeted genes which are mainly involved in the glycolytic pathway, oxidative phosphorylation and the signaling pathway regulating cell proliferation. **Results:** To meet the high demand of energy and precursors for macromolecule biosynthesis, cancer cells markedly increase their glucose uptake to fuel:1) the glycolytic pathway in order to rapidly generate energy (ATP) and 2) the metabolic pathways which give rise to macromolecules to support uncontrolled cell growth. The net effect of p53 is to repress the glycolytic flux at different steps through multiple mechanisms, to maintain the oxidative phosphorylation, to decrease the fatty acid synthesis and to partly inhibit the growth signaling pathway of IGF1. Taken together these effects are detrimental for the cell survival and participate to the tumor suppressive effect of p53. **Conclusion:** This review clearly indicates that p53 has the capacity to control, in physiological conditions and in cancer cells, the expression of metabolism-related genes that are important regulators of metabolic pathways, including glycolysis, oxidative phosphorylation, fatty acid metabolism and mTOR signaling. Consequently, a better understanding of the complex network connecting p53 and the metabolic pathways may allow the discovery of novel anticancer tools.

Keywords: P53, Tumor Suppressor, Metabolism, Cell Growth, Mutant P53

Introduction

p53 was discovered in 1979 and it was suggested that the protein might act as a cellular oncogene but was later identified as a tumor suppressor protein [1-6] . Several studies confirmed this role: 1) many individuals affected by a germline mutation of p53 display an abnormally high incidence of tumor development (Li-Fraumeni syndrome) and 2) the transformation of cells from normal to cancerous initiates its activation due to stress signals which accompanied malignant progression. Its activation

prevents tumor progression by its ability to induce apoptosis or senescence [7-9]. Broadly defined, stress is the state when cellular homeostasis is disrupted due to environmental changes or fluctuations in environmental factors. A multitude of different types of stress often encountered during tumor development initiate its activation, such as DNA damage, oncogene activation, hypoxia, telomere erosion and others [10]. Once activated, p53 stimulates (or in some cases represses) the expression of a large network of genes involved in many cellular processes aiming at restoring cellular homeostasis to the former state or to cope with the new environment. Stress responses are mediated via multiple mechanisms, depending on the type, severity and duration of stress encountered. These specific responses to any stress exert a protective effect on the organism or the cell. For instance, in response to a severe DNA damage, p53 induces apoptosis which eliminates cells with mutated genes. In response to mild DNA damage p53 can temporarily arrest the cell cycle, which allows for the repair of any damaged DNA, preventing mutations from being passed on to daughter cells [11]. Oncogene activation also leads to p53 activation, resulting in senescence thus limiting the oncogenic potential of preneoplastic cells [9].

During the past decade it has been reported that p53 is a master regulator of many biological processes, including aging, innate and adaptive immunity, development, reproduction and neuronal degeneration [12-16]. There is now growing evidence that p53 plays a key role in metabolism. This review article provides an update of our current understanding of the role and mechanisms of p53 in maintaining the homeostasis of cellular energy metabolism.

It should be pointed out that there are several excellent reviews on this subject [17-22].

p53 characteristics and activation

In the normal homeostatic tissues p53 is maintained at low intracellular level, due essentially to two mechanisms: 1) an interaction with an E3 ubiquitin ligase (MDM2: murine double minute 2) whose major role is to target p53 for a proteosomal degradation [23]. Other ubiquitin ligases have been identified but their relative contribution to regulating p53 level *in vivo* needs further investigation and 2) MDM2 is a transcriptional target of p53, establishing a negative feedback loop which can keep the intracellular level of p53 is quite low [24]. p53 is a short-lived protein, as its half life is~5-10 min. However, when cells are exposed to stress signals (γ irradiation, UV, Reactive Oxygen Species, oncogene activation, hypoxia, nutrients deprivation) there is an increase in p53 cellular levels and stability. It is achieved through uncoupling of the p53-MDM2 interaction, which therefore prevents its degradation. Then, p53 undergoes post-translational modifications particularly, phosphorylation, methylation, acetylation, glycosylation and ribosylation, which results in increased activity. p53 is then translocated to the nucleus and plays a role of transcription factor. It binds to its response element (RE) of the target genes in association with other proteins (co-activators) such as CBP/p300 (histone acetyltransferases) which fine-tune its activity. Depending on the cells and tissue types, the nature and intensity of stress signals it selectively regulates the expression of genes involved in cell-cycle arrest, apoptosis, senescence, autophagy, DNA repair, and antioxidant activities (Figure1). These cellular responses prevent tumor formation and maintain genomic integrity. The molecular mechanisms leading to a cell-cycle arrest or apoptosis have been well described after DNA lesions caused by exposure to chemotherapeutic agents, genotoxic agents, ultraviolet, irradiation and which generate DNA double strand breaks (DSBs) and single-strand breaks (SSBs). These lesions trigger a cascade of events generated in the following order: 1) interaction of the kinase ATM (Ataxia Telangiectasia Mutated) with the damaged DNA, 2) phosphorylation of p53 and MDM2 by ATM allowing their dissociation, thereby promoting p53 stabilization and activation [25-29]. 3) p53 stimulates the expression of p21 (cyclin dependent kinase inhibitor = CKI) which binds and inactivates the cyclin-dependent kinase/cyclin complexes, indispensable to the progression of the cell cycle and 4) DNA repair genes (p48 and p53 R2) [30]. Taken together these responses allow cells to survive until the damage has been repairedor stress of cells has been removed [31-33]. These processes permit the cell to re-enter the cell cycle without incurring errors that result in a high mutation rate. When cells are exposed to severe or chronic stress signals, p53 initiates the transcription of genes involved in apoptosis (Puma, Bax Fas) so eliminating cells that may have acquired DNA markedly damaged and which could subsequently be extremely prone to tumor development [31-33].

It should be emphasized that severe DNA damage or oncogene activation are likely to be amongst the most predominant mechanisms of p53-mediated tumor suppression. However, a novel function of p53 in regulation of cellular energy

metabolism has recently been identified, suggesting that this is another mechanism that contributes to the role of p53 in tumor suppression. (Figure 1)

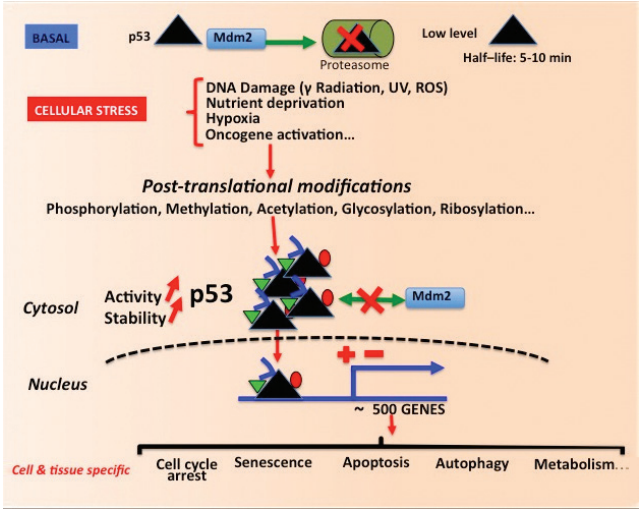

Figure 1. p53 characteristics and activation.

In normal homeostatic tissues p53 is maintained at low intracellular level due essentially to an interaction with an ubiquitin ligase (MDM2 : murine double minute 2) whose major role is to target p53 for a proteosomal degradation. When cells are exposed to extrinsic and intrinsic stress signals,there is an increase in p53 cellular levels and stability. It is achieved through uncoupling of the p53-MDM2 interaction, followed by post-translational modifications. p53 is then translocated to the nucleus and plays a role of transcription factor. Depending on the cells and tissue types, the nature and intensity of stress signals p53 selectively regulates the expression of genes involved in cell cycle arrest, apoptosis, senescence, autophagy, DNA repair, metabolism.

p53 and the regulation of cellular metabolism

Unlike normal cells, cancer cells have lost the ability to control their proliferation. They grow and divide in an uncontrolled manner. Consequently, the cell must synthetize biomolecules at a higher rate so as to meet the increased metabolic demands of proliferation. To cope with this challenge tumor cells display marked changes in pathways of energy metabolism and nutrient uptake [34]. Metabolic adaptation was one of the first aspects of cancer studied. In the 1920s Otto Warburg noted that the glycolytic flux, which normally increases under anaerobic conditions was enhanced in cancer cells in the presence of oxygen. This phenomenon is known as "aerobic glycolysis" or the "Warburg effect". The switch from oxidative phosphorylation to glycolysis is associated with a marked release of lactate by the cancer cell [35-37]. The view which then prevailed has been that the Warburg effect is a mechanism to produce cellular energy in the form of ATP. In the presence of robust glucose transport the glycolytic flux drives ATP production at a faster rate and in greater quantities than that generated via mitochondrial oxidative phosphorylation [38]. Furthermore, the major function of aerobic glycolysis is to maintain high levels of glycolytic intermediates to feed anabolic processes so as to meet the high demand of macromolecules required for a rapid growth and proliferation of cancer cells.

The molecular mechanism underlying the Warburg effect is not well-understood [34, 39, 40]. The activation of several oncogenes in cancer cells has been shown to contribute to the Warburg effect, including Myc, Akt and hypoxia inducible factor 1 (HIF-1). Myc transcriptionally activates many of the glycolytic enzymes, and activation of Akt increases both glucose uptake and metabolism [41, 42]. Activation of HIF-1 is also involved in mediating the switch to aerobic glycolysis through its ability to increase the expression of genes encoding glucose transporters and glycolytic enzymes [43, 44]. Furthermore, HIF-1 induces pyruvate dehydrogenase kinase 1 (PDK1), which phosphorylates and inactivates pyruvatede hydrogenase and thus suppresses the TCA cycle and aerobic respiration [45].

The net effect of p53 is a repression of the glycolytic flux at different steps through multiple mechanisms, here briefly reported (Figure 2). Glucose uptake is the first rate-limiting step that fuels the glycolysis and it is mediated by glucose transporters GLUT 1-4 localized in the plasma membrane. The role of p53 is to transcriptionally repress the expression of these transporters, which consequently decreases the glucose uptake in cells and it also decreases GLUT-3 expression by antagonizing the NF-kB pathway [46-47].

Once internalized the glucose is phosphorylated by the hexokinase II into glucose-6-phosphate (G6P) which can fuel two alternative pathways: glycolysis and the pentose phosphate pathway (PPP). In the glycolytic pathway, p53 stimulates the expression of TIGAR (TP53-induced glycolysis and apoptosis regulator), whose role is to promote the conversion of F2,6P to F6P (it stimulates the phosphatase activity of the bifunctional enzyme phosphofructokinase-2-kinase/fructose-6-kinase), thereby attenuating the allosteric effect of F2, 6P on the glycolytic regulatory enzyme PFK1[48-49]. Consequently, it slows

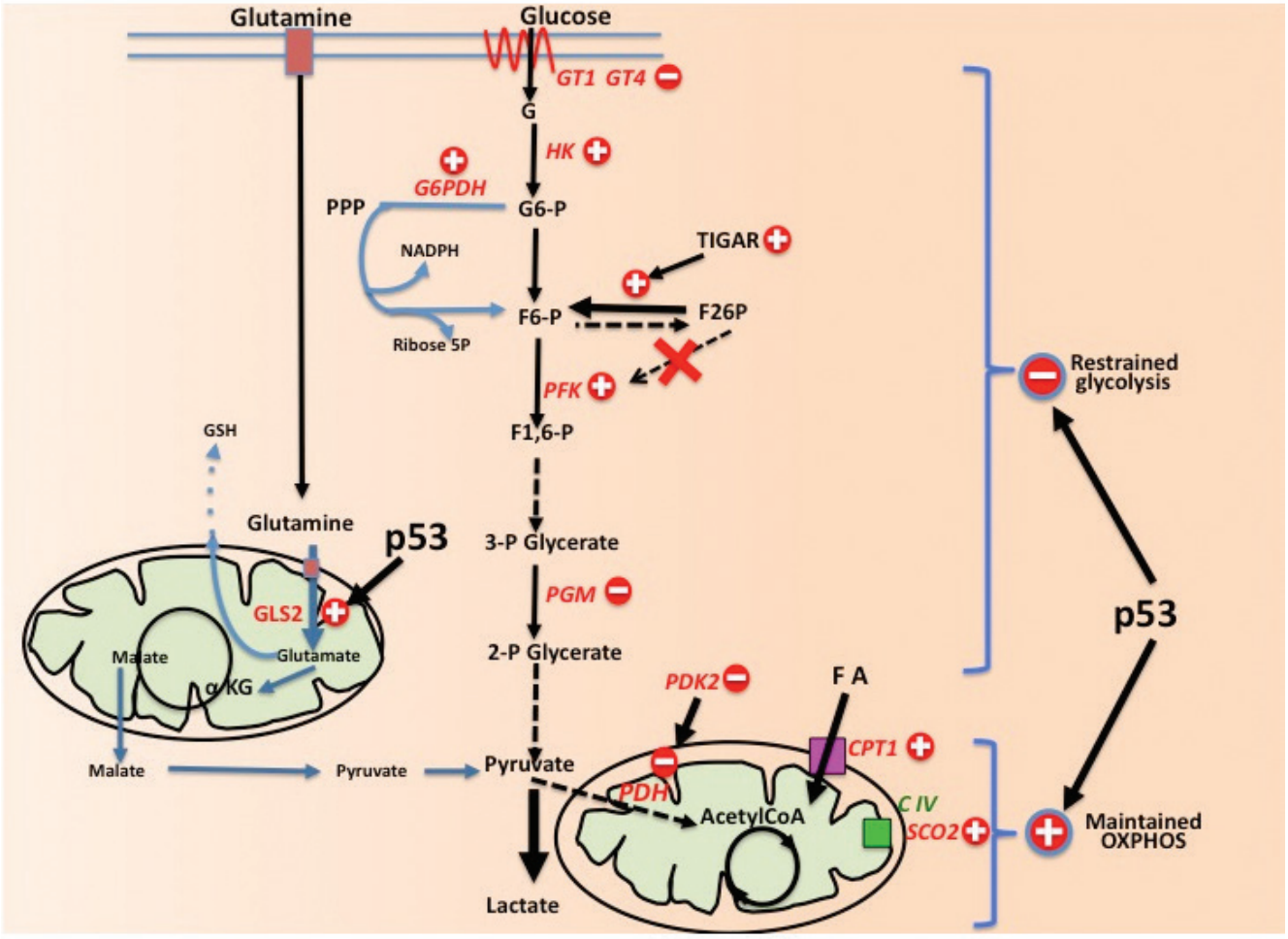

Figure 2. p53 and energy metabolism.

The protein has a role in the regulation of both glycolysis and oxidative phosphorylation. p53 impedes the glycolytic flux through various steps of the pathway. It decreases the expression of the GLUT1 and GLUT4 transporters and the level of the phosphoglycerate mutase (PGM) while stimulating the expression of TIGAR. The latter promotes the conversion of F2,6-P to F6-P, thereby attenuating the allosteric effect of F2,6-P on PFK1. It leads to a partial accumulation of G6P which can fuel the pentose phosphate pathway (PPP). p53 represses this flux by inhibiting the G6PDH activity, first enzyme of this pathway. These mechanisms counteract the increase of glycolysis that is characteristic of cancer cells. p53 also maintains the oxidative phosphorylation by stimulating the expression of SCO2 (Synthesis of Cytochrome c Oxidase 2) which is involved in the assembly of mitochondrial complex IV in the electron transport chain. Glutamine is also a crucial nutrient during cell proliferation as it provides carbon to the TCA cycle that plays a key role in the energy metabolism. Glutamine metabolism requires a conversion to glutamate and it is regulated by the mitochondrial glutaminase 2 (GLS2) and its expression is induced in response to DNA damage or oxidative stress in a p53-dependent manner.

down the conversion of F6P to F1,6-P and it leads to a partial accumulation of G6P. It ensues that a fraction of the G6P pool feeds the pentose phosphate pathway (PPP) to generate ribose-5-phosphate, a key intermediate in nucleotide biosynthesis and reducing equivalents in the form of NADPH. The latter is a required electron donor, for reductive steps in lipid synthesis in nucleotide metabolism and in maintaining glutathione (GSH) in its reduced state. GSH allows cells to resist to the oxidative stress. The role of p53 is to bind the G6PDH (converts G6P in 6-phosphogluconate = 6PG) the first and rate-limiting enzyme of PPP and inhibitits activity [50]. This mechanism might represent a way to block PPP and thus inhibiting NADPH production, in case of accumulation of TIGAR in cancer cells.

p53 also reduces the protein level of the glycolytic enzyme phosphoglycerate mutase (PGM) which converts 3-phosphoglycerate into 2-phosphoglycerate [51]. The effects of

p53 on glycolysis are well documented but it should be pointed out that they are tissue-specific. Thus in muscle it promotes glycolysis by inducing the expression of PGM, M isoform and of hexokinase II [52-53].

Increased glycolytic flux generates a large amount of pyruvate which is mainly converted in lactate. It is attributed to a partial block of the PDH activity which cannot convert pyruvate in Acetyl-CoA, whose fate is to fuel the TCA cycle. The inhibition of the PDH activity is a consequence of its phosphorylation by PDK2. p53 represses the expression of PDK2 and thus reduces its inhibitory effect on the PDH, thereby promoting the conversion of pyruvate to Acetyl-CoA [54].

Taken together, these findings link the p53 protein with glucose metabolism. Given that most mutations observed in human tumors abrogate or attenuate p53 functions (loss of function), it strongly suggests that this genetic change might contribute to the Warburg effect (enhanced glycolytic flux).

p53 and oxidative phosphorylation

While repressing glycolysis, p53 promotes oxidative phosphorylation atmultiple levels. It up-regulates the expression of SCO2 (Synthesis of Cytochrome c Oxidase 2) which is involved in the assembly of mitochondrial complex IV in the electron transport chain [55]. It also increases the expression of AIF (Apoptosis-Inducing Factor) which is required for the assembly and function of complex I.

p53 also reduces TCA cycle intermediates for biosynthesis and NADPH production by repressing the expression of the two malic enzymes ME1 and ME2 which recycle malate and pyruvate [56]. Consequently, lowered intermediates inhibit cancer cell proliferation.

Glutamine is a crucial nutrient during cell proliferation (normal and cancer cells) as it is a source of both nitrogen and carbon and it protects against oxidative stress. It provides carbon to the TCA cycle in order to supply biosynthetic pathways such as lipid synthesis. This process, termed Anaplerosis represents an additional source of carbon in order to replenish the pool of mitochondrial citrate which otherwise would be depleted due to a high rate of lipid synthesis. To provide carbon, the glutamine is converted to glutamate, which then gives rise to α-ketoglutarate. The latter enters the TCA cycle to provide oxaloacetate (OAA), which combines with acetyl-CoA to generate citrate. This metabolic pathway plays also an important role in the control of energy supply. It is regulated by the mitochondrial glutaminase 2 (GLS2) and its expression is induced in response to DNA damage or oxidative stress in a p53-dependent manner. It is the first enzyme which catalyzes the hydrolysis of glutamine to glutamate [57, 58]. The fate of glutamate is to fuel the TCA cycle via the α-ketoglutarate and also to be the glutathione precursor whose function is to protect the cell against oxidative stress.

p53 and lipid metabolism

There is an increased fatty acid synthesis in proliferative cells, in order to meet the high demand of lipids, such as phospholipids required for the membrane formation. The precursor of fatty acids is the citrate that originates from the mitochondria. Once in the cytosol, it is converted by the citrate lyase (CL) and yields OAA and Acetyl CoA. The latter is converted to malonyl-CoA via the reaction catalyzed by the Acetyl-CoA carboxylase (ACC) and it is further metabolized by the fatty acid synthase (FAS) to give rise to a fatty acid. This is called, *in vivo* lipogenesis. In many tumors there is an over expression of these two lipogenic enzymes (CL and FAS) and their inhibition results in the inhibition of the tumor development.

p53 has been reported to repress fatty acid synthesis and it would block cancer cell proliferation. The mechanism is as follows: p53 represses the transcription of SREBP1c (sterol regulatory element binding protein 1c), which is the transcription factor responsible for the stimulation of the expression of CL and FAS.

Additionally, p53 stimulates fatty acid oxidation in the liver and it occurs in response to starvation. It is attributed to an activation of p53 in response to low glucose which in turn enhances the expression of the guanidinoacetate methyltransferase (GAMT), a critical enzyme involved in the creatine synthesis, which plays an essential role in the regulation of ATP synthesis [59]. Though the creatine action mechanism is unclear, its elevation leads to an activation of AMPK, which in turn phosphorylates and inactivates the ACC enzyme, which converts the Acetyl-CoA to Malonyl-CoA. It results in a lowered intracellular level of Malonyl-CoA, thus blunting its allosteric inhibitory effect on CPT1. It ensues an increased FA influx in the mitochondria through the carnitinepalmitoyltransferase 1 (CPT1) to fuel the FA oxidation pathway. p53 also stimulates CPT1 when cells are exposed to glucose deprivation, thereby increasing the FA influx in the mitochondria. It should be pointed

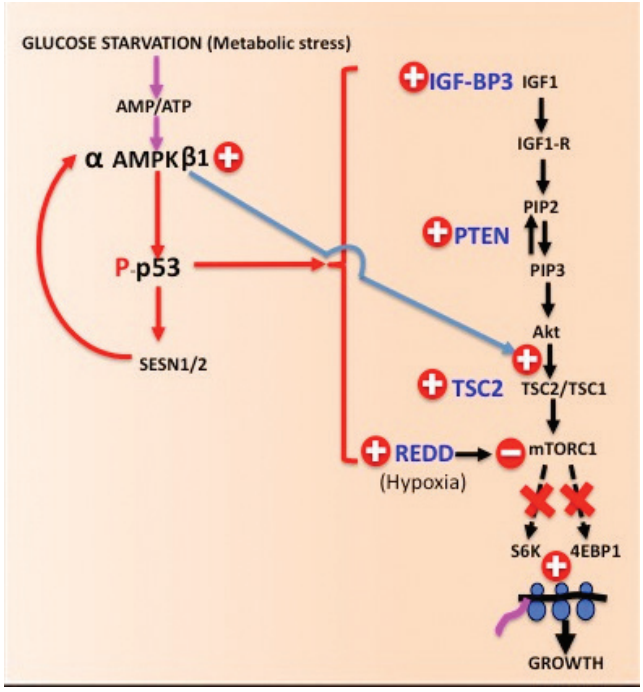

Figure 3. p53 and IGF1/Akt/mTOR pathways.

mTOR plays a major role in the control of cell growth and can sense and respond to various signals, including nutrients, energy, mitogens. In presence of an adequate level of nutrients and a positive mitogen signal this pathway is stimulated and mTOR activates S6K and 4EBP1, thus promoting cell growth. In response to a metabolic stress (glucose deprivation), p53 stimulates the transcription of negative regulators of these pathways including IGF-BP3, PTEN, TSC2. p53 induces the expression of , Sestrins 1/2, that interact with the α-subunit of AMPK and also stimulates the expression of the β1 subunit of AMPK. This leads to the activation of AMPK and TSC2 resulting in the inhibition of mTOR activity.

out that increased fatty oxidation, results in a partial inhibition of the glycolysis (glucose utilization) which might contribute to tumor suppression [60].

p53 and IGF1/Akt /mTOR pathways

Availability of adequate nutrients and mitogens are the major signals that can turn on or off the molecular mechanisms of cell growth and proliferation. Recent studies have revealed that various signals including nutrients (glucose and amino acids), intracellular energy (AMP/ATP ratio) and growth factors (IGF1, insulin) are sense by the kinase mTOR (the mammalian target of rapamycin), which controls cell growth and division. It is now well established that p53 is also implicated in metabolic control through two key regulatory factors AMPK and mTOR.

Thus, under the condition of low levels of glucose and ATP (energy stress), two kinases, LKB1 and AMPK (AMP-activated protein kinase), sense the levels of these stress signals. Thus, when AMP/ATP ratio is elevated, it elicits an activation of AMPK and it can be explained by the binding of AMP to the Y subunit of AMPK and its phosphorylation by LKB1. In response to the AMPK activation, mTOR activity is turned off and it is due to a phosphorylation and activation of theTSC2 (Tuberous Sclerosis heterodimer) protein, a GTPase negatively regulating Rheb (conversion of Rheb-GTP to Rheb-GDP), which can no longer bind and stimulate mTOR.

Energy stress (glucose deprivation) in the presence of activated oncogene results in AMPK phosphorylation. Activated AMPK results in a stimulation of p53 transcription and activation, which then stimulates the expression of the proteins encoded by the Sestrin 1 & 2 genes (anti-oxidants genes) [61]. They bind to the α subunit of AMPK, which leads to its kinase activation and then it phosphorylates TSC2 which acts as a negative regulator of mTOR.

In response to mitogen signals (IGF-1 or insulin) and adequate availability of nutrients the IGF-1/AKT/mTOR signaling pathway is activated to stimulate cell growth and division [62]. In the absence of stress signal, to stimulate the pathway the ligand IGF-1 binds to its receptor which then recruits and activates the PI3 kinase (PI3K) to the plasma membrane. It phosphorylates the phosphatidylinositol 4,5 bisphosphate (PIP2) and gives rise to the phosphatidylinositol 3,4,5 triphosphate (PIP3) that activates PDK1 which in turn phosphorylates and activates the kinase Akt [63, 64]. A full activation requires also a phosphorylation by PDK2 (mTORC2). Akt phosphorylates two proteins that play a major role in cellular outcomes: 1) BAD, a proapoptotic molecule which will be inactivated, thereby promoting a cell survival signal, due to an increase of antiapoptotic/proaptotic molecules ratio and 2) TSC2 (Tuberous Sclerosis heterodimer) a GTPase molecule (Rheb-GAP) which will be inactivated leading to an enhanced Rheb-GTP form that activates mTOR [65-68]. The outcome is a stimulation of cell growth, division and energy metabolism.

This pathway is monitored by p53 because any cell stress occuring when it is activated can introduce errors during cell growth and division. To prevent the accumulation of errors

during these processes, p53 induces the expression of genes that encode proteins which negatively regulate the IGF-1/AKT/mTOR signaling pathway. For instance, under the condition of energy stress (glucose starvation and low intracellular ATP) the p53 target genes include IGF-BP3, PTEN, TSC2, REDD, AMPK β1 subunit and Sestrins 1&2 [69-73]. Thus, IGF-BP3 binds to IGF1, which prevents its binding to the IGF-1 receptors. PTEN a phosphatase that converts PIP3 to PIP2, results in a decrease in intracellular PIP3, which no longer activates PDK1 and thus lead to a decreased AKT activity. TSC2 which functions as RhebGTPase activator, prevents the formation of Rheb-GTP whose binding to mTOR is required to enable its activation. REDD is also involved during cell hypoxia and negatively regulates mTOR activity.

Mutant p53 and metabolism

Given the high mutation rate of p53 (> 50%) in human tumors, these findings suggest that the mutation of the p53 gene and the resultant loss of function of the p53 protein in tumors could be an important genetic change contributing to the marked metabolic changes. It is now widely acknowledged that the most common mutant forms arise from missense mutations (single base-pair substitutions) [74]. Many of these stable mutant forms of p53 (~75% of all mutant forms in humans) can exert a dominant negative effect on the remaining wild-type allele, serving to abrogate the ability of wild-type p53 to inhibit cellular transformation, particularly when the mutant protein is expressed in excess of its wild-type counterpart [75, 76]. Beside the dominant-negative effect of p53 mutants there is also clear evidence that mutant p53 can exert oncogenic or gain-of-function activity independent of its effects on wild-type p53 [76-79]. There are multiple proposed mechanisms that account for different mutant p53 gain-of-function activities. These include both transcriptional and non-transcriptional mechanisms [79].

The novel gain of function of mutant p53 promotes tumor metabolic changes resulting in tumor development. For instance, the mutant activates SREBP 1 and 2, which then induces the transcription of genes encoding enzymes regulating the mevalonate pathway. This pathway represents one aspect of lipid metabolism which gives rise to cholesterol and isoprenoid [80]. Mutant p53 also induces the hexokinase II gene that could stimulate the glycolytic flux.

As mentioned above, the normal functionality of p53 is abrogated, in the vast majority of human tumors. In past years there have been attempts to restore its activity as a novel cancer therapy strategy. This approach is potentially highly promising in the long term.

Conclusion

Initially, it was demonstrated that the p53 protein is essential to counteract tumor formation and progression and it was attributed to its capacity to induce cell cycle arrest and apoptosis. However, over the past decade, numerous studies have now indisputably demonstrated that the tumor suppressive function of p53 also relies on its ability to control and regulate cellular metabolism. It modulates the glycolytic flux , the lipid synthesis and the mitochondrial functions . Given that metabolic changes are common to a broad range of cancer cell types they are attractive potential targets for anticancer therapy. Therefore, a characterization of cancer cells metabolism shoud yield useful information that can be translated into therapeutic tools to partly or totally inhibit the tumor growth.

Conflict of Interest

The authors state no conflict of interest.

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