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# The Control of the Metabolic Switch in Cancers by Oncogenes and Tumor Suppressor Genes

Arnold J. Levine<sup>1,2\*</sup> and Anna M. Puzio-Kuter<sup>2</sup>

Cells from some tumors use an altered metabolic pattern compared with that of normal differentiated adult cells in the body. Tumor cells take up much more glucose and mainly process it through aerobic glycolysis, producing large quantities of secreted lactate with a lower use of oxidative phosphorylation that would generate more adenosine triphosphate (ATP), water, and carbon dioxide. This is the Warburg effect, which provides substrates for cell growth and division and free energy (ATP) from enhanced glucose use. This metabolic switch places the emphasis on producing intermediates for cell growth and division, and it is regulated by both oncogenes and tumor suppressor genes in a number of key cancer-producing pathways. Blocking these metabolic pathways or restoring these altered pathways could lead to a new approach in cancer treatments.

In 1926, Otto Warburg demonstrated that cancer cells did not metabolize glucose in the same way that glucose was catabolized in normal, adult differentiated cells (1, 2). The cancer cells relied on glycolysis, even in the presence of abundant oxygen (aerobic glycolysis) with a reduced use of the tricarboxylic acid (TCA) cycle, so that the pyruvate made in glycolysis was commonly converted to lactate, which was secreted from the cell (Fig. 1). Warburg suggested that this observation explained the cancer phenotype and was possibly a causal event in cancer formation (1, 2). There were several reasons why these observations were not understood and did not promote additional research. First, metabolizing glucose by glycolysis to produce pyruvate and secreted lactate is energetically inefficient. Most of the adenosine triphosphate (ATP) generated by glucose catabolism (34 out of 36 ATP molecules per molecule of glucose) occurs during the TCA cycle, which is used less often in cancer cells (Fig. 1). Second, it was unclear how this observation could contribute to the cancer phenotype. Third, possible mechanisms to mediate a switch to glucose utilization in glycolysis from the more efficient movement of pyruvate into the mitochondria to produce acetyl-coenzyme A (CoA) and enter the TCA cycle were only poorly understood. Fourth, with the discovery of the mutational activation of oncogenes and inactivation of tumor suppressor genes as causal steps in cancer, the relationship between these mutant genes and metabolic regulation was unclear. Remarkably, after a long absence of interest, research done in the past 10 years has

begun to answer these questions. Although our understanding of each question is still imperfect, it is becoming clear that both oncogenes and tumor suppressor gene products can influence the switch between aerobic glycolysis and a more extensive use of the TCA cycle to generate ATP. Furthermore, the altered metabolic processing of glucose observed by Warburg may well contribute to some of the causal changes in the cancer phenotype. There have been a number of reviews that emphasize different aspects of this question and provide a diverse set of answers (3–9).

Normal cells and cancer cells use both glucose and glutamine as substrates to generate energy for the cell (ATP); to produce substrates to synthesize amino acids, nucleosides, and fatty acids; and to regulate the redox potential (number of oxidized molecules in a compartment divided by number of reduced molecules) so as to minimize the effects of reactive oxygen species (ROS) that damage membranes and proteins and cause mutations in a cell. Glucose contributes carbon, oxygen, and hydrogen for both anabolic processes and energy, whereas glutamine contributes nitrogen for synthesis of purines, pyrimidines, and nonessential amino acids. Metabolism of glutamine also produces the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) for the synthesis of fatty acids and the modulation of the redox potential in a cell. Glucose passing through the pentose phosphate pathway (PPP) also generates NADPH and ribose-5-phosphate for the synthesis of nucleotides (Fig. 1). Normal adult differentiated cells have a low cell division rate (low turnover) and predominantly metabolize glucose to CO<sub>2</sub> and H<sub>2</sub>O through glycolysis and the TCA cycle. This satisfies the needs of these cells for free energy supplied by efficient ATP generation during oxidative phosphorylation (complexes 1 to 4 in the oxidative phosphorylation chain) linked to the TCA cycle in mitochondria.

There are several times, however, when regulated rapid cell division is required, such as during embryonic development, in wound healing (liver regeneration), or in the immune responses to specific antigens, where clonal selection provides increased cell numbers with increased immune specificity. Cancer cells share many of these same requirements for energy, substrates to grow and divide, and control of the redox potential and ROS in the cell.

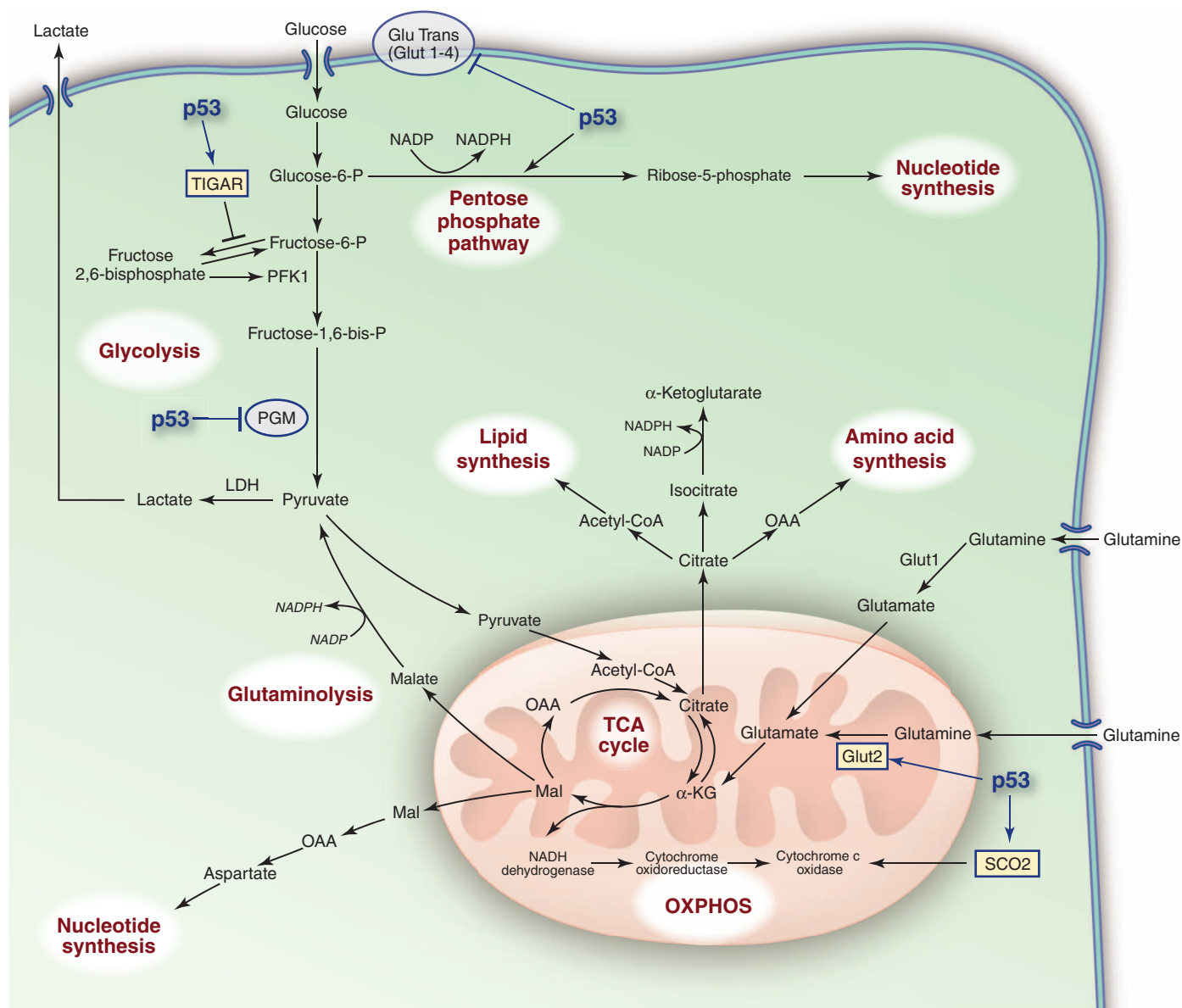
What these processes have in common is a need to synthesize substrates for membranes, nucleic acids, and proteins (increase mass), which means not metabolizing all of the glucose to CO<sub>2</sub> and H<sub>2</sub>O but instead providing the proper intermediates for cell growth. This is accomplished, in part, by slowing the entry of pyruvate into mitochondria, decreasing the conversion to acetyl-CoA, and slowing the rate of the TCA cycle. The pyruvate that builds up in aerobic glycolysis is, in part, converted into lactate that is secreted, eliminating it from the pool and keeping glycolysis active. The secreted lactate lowers the pH of the cellular environment and the extracellular matrix. This may influence remodeling of the matrix, permitting blood vessel invasion in response to angiogenic factors produced by the tumor (10). Furthermore, as a consequence of glycolysis, tumor lesions can become acidotic, which allows for the selection of motile cells that can break through the basement membrane and metastasize. The last step in glycolysis is catalyzed by pyruvate kinase, which receives input about both anabolic precursors and the energy status of the cell. Cancer cells make the fetal isoform of pyruvate kinase (the M2 isoform), which is a spliced variant of the gene that adds several amino acids, one of which is a tyrosine. This tyrosine is phosphorylated in cells with activated tyrosine kinase signaling, a hallmark of actively growing cells. Pyruvate kinase M2 is stimulated in a feedforward loop by fructose 1,6-bisphosphate, but the phosphotyrosine inhibits this positive regulation. Thus, in cancer cells the last step of glycolysis is slowed, resulting in a buildup of phosphorylated intermediates that can be used in anabolic synthesis and cell growth (11).

Rapidly dividing cells require favorable energetics [that is, higher ATP/adenosine diphosphate (ADP) and ATP/adenosine monophosphate (AMP) ratios]. Many cancer cells satisfy this problem by taking up much larger amounts of glucose than do normal cells. This results from facilitated glucose transport by one or more of several isozymes of membrane glucose transporters (GLUT 1 to 9). Once inside the cell, glucose is phosphorylated by one of several hexokinase enzymes (the first step in glycolysis) to keep it in the cell because of the charge added to glucose (Fig. 1). The high concentrations of glucose in the cells of a cancer may be observed by positron emission tomography (PET) scans of radioactive F-19-2-deoxyglucose (FDG is not metabolized but is located in the

<sup>1</sup>Institute for Advanced Study, Princeton, NJ 08540, USA.

<sup>2</sup>Cancer Institute of New Jersey, New Brunswick, NJ 08903, USA.

\*To whom correspondence should be addressed. E-mail: alevine@ias.edu



**Fig. 1.** Signaling networks and their regulation of metabolism in proliferating cells. The figure shows aspects of metabolism in proliferating cells including glycolysis; lactate production; TCA cycle; oxidative phosphorylation; PPP; glutaminolysis; and the biosynthesis of nucleotides, lipids, and amino acids. Glucose can be processed through glycolysis for production of ATP and pyruvate, pass through the PPP to generate ribose 5-phosphate and NADPH, and also enter into the mitochondrion-localized TCA cycle. Glucose-derived citrate is exported to the cytosol and processed to acetyl-CoA, oxaloacetate (OAA), or  $\alpha$ -ketoglutarate ( $\alpha$ -KG). Glutamine

is deaminated to form glutamate, which is processed to generate  $\alpha$ -ketoglutarate and maintain the TCA cycle. p53 induction of key players is boxed, and p53 inhibition is circled. p53 induces TIGAR, inhibits phosphoglycerate mutase (PGM), and represses GLUT1 and GLUT 4, resulting in inhibition of glycolysis and opposing the Warburg effect that is seen in many cancers, whereas p53 induction of SCO2 and GLS2 enhances mitochondrial respiration. Glut Trans indicates glucose transporters; Glut 1, glutaminase 1; Glut 2, glutaminase 2; LDH, lactate dehydrogenase; Mal, malate; and OXPHOS, oxidative phosphorylation.

cell), which is indicative of enhanced glucose uptake by cells. Many, but not all, cancers have this property (3–9) of increasing glucose uptake, and this is a confirmation of the Warburg effect.

With large amounts of glucose available in a cell, glucose is metabolized through the PPP, producing nucleosides and generating NADPH. The NADPH is essential for fatty acid synthesis, along with acetyl-CoA (which is made from some

of the pyruvate in mitochondria that is not converted to lactate). NADPH also contributes to a proper redox control and protects the cell from ROS. There are several ways the cell responds to lower ROS levels, but by far the major molecule involved is glutathione (GSH), which eliminates ROS by accepting an electron and is converted to its oxidized form, GSSG (glutathione disulfide). The enzyme glutathione reductase uses NADPH

to reduce GSSG to GSH. Thus, NADPH is a major source of cellular “coolant” when oxidative reactions run too “hot” (high ROS levels) by using large amounts of glucose to produce both substrates and energy. However, high levels of ROS can be advantageous for cancer cells when they allow for the stimulation of cell proliferation, induction of genetic instability, and evasion from senescence. Although if levels are too high, then

cancer cells undergo oxidative damage-induced cell death. Thus, ROS levels can be exploited to selectively kill cancer cells and therefore be used as a potential therapeutic.

Glutamine contributes both to substrate needs of a dividing cell and to control of redox potentials through the synthesis of NADPH. As with glucose, excessive amounts of glutamine are taken up (by a glutamine transporter) and used by cancer cells. After glutamine is taken into the cell, a mitochondrial-associated enzyme, glutaminase-1, converts it to glutamate (a transaminase can use the amino group and capture the nitrogen for synthesis of nucleosides, and amino acids or ammonium is produced). Glutamate is converted to  $\alpha$ -ketoglutarate and enters the TCA cycle in the mitochondria. The malate and citrate produced in the TCA cycle leave the mitochondria, where malate is converted to pyruvate plus NADPH and citrate is converted to isocitrate and then to  $\alpha$ -ketoglutarate, generating another molecule of NADPH. Citrate also is converted to acetyl-CoA for fatty acid synthesis and oxaloacetate for the synthesis of nonessential amino acids (Fig. 1). The pyruvate generated in these reactions can be used to produce glucose (reverse glycolysis), which enters the PPP, maximizing the production of NADPH. The glutamate can be converted to aspartate, which contributes to nucleoside synthesis. The excessive quantities of glutamine taken into and used by the cell results in the secretion of alanine and ammonium, which together with lactate bathe the extracellular matrix.

Glutamine is also a major cancer cell energy and anabolic substrate that requires functional mitochondria. However, Warburg's hypothesis had its basis in the theory that glycolysis is predominately used in cancer cells because of a dysregulation of mitochondrial oxidative phosphorylation. Research has shown that most cancer cells do not have defects in mitochondrial metabolism except for rare mutations in succinate dehydrogenase (SDH) or fumarate hydratase (FH), both enzymes of the TCA cycle and both initiating events of familial paraganglioma or leiomyoma and of papillary renal cell cancer.

Thus, cancer cells maximize their ability to synthesize substrates for membranes, nucleic acids, and proteins. This results in increased cell mass and allows cell division when needed. This cannot be accomplished without large amounts of energy (ATP), which are obtained by increasing the use of glucose and glutamine many fold. Penalties for this increased flux are an increase in oxidative intermediates, an altered redox potential, and excessive ROS. The metabolic response is to focus reactions on producing NADPH, a coolant that feeds into many chemical systems that reduce the ROS activity. This high rate of glucose and glutamine flux must be handled by increased metabolic enzyme levels or increased enzyme activities. Remarkably, this is accomplished by oncogenes and tumor suppressor genes

as well as regulators of the response to hypoxia. All of these metabolic pathways (TCA, PPP, and glycolysis) contain complex regulatory circuits at the levels of transcription, mRNA splicing, translation, and small molecule feedforward and feedback loops. A deeper understanding of these regulatory pathways that connect the genetics of cancer to the biochemical metabolic pathways may reveal selected metabolic processes that might be good drug targets for slowing or reversing cancers.

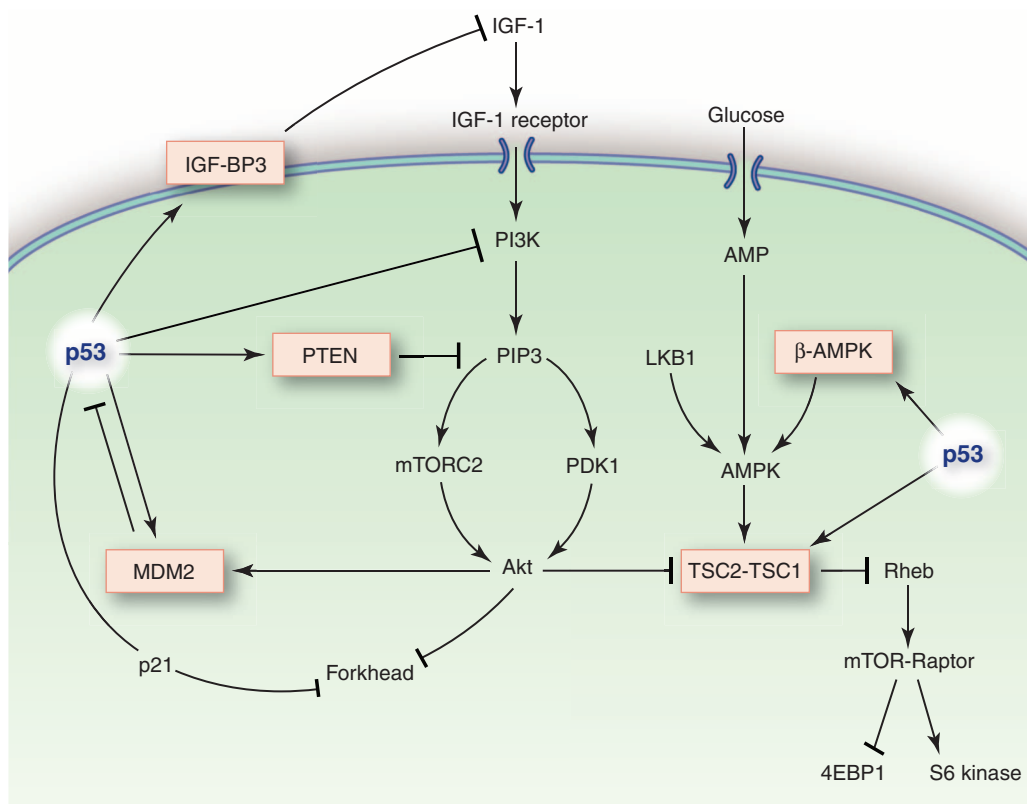
Over the past 10 years, evidence has accumulated that the oncogenes *myc*, nuclear factor  $\kappa$ B (NF- $\kappa$ B), AKT, and the tyrosine kinase receptors (epidermal growth factor, EGF; insulin-like growth factor 1, IGF-1; Her-2; etc.), which turn on Ras, RAF-mitogen-activated protein kinase (MAP kinase), and the phosphatidylinositol 3-kinases (PI3Ks) and mammalian target of rapamycin (mTOR) pathways (Fig. 2) along with hypoxia-induced factor (HIF), can stimulate the transcription of a number of genes that encode the proteins that mediate the glycolysis and glutaminolysis pathways (Fig. 1). The rate of glycolysis can vary over 100-fold. High AKT and mTOR activities result in high HIF activity. Both the *myc* and HIF-1 transcription factors increase the rate of transcription of some of the GLUT transporters and hexokinase 2, enhancing both glucose uptake and its retention in the cell (11). HIF increases the rate of transcription of over 100 genes, resulting in angiogenesis, cell migration, cell survival, and energy metabolism.

Among the HIF-regulated genes are 9 of the 10 enzymes that function in glycolysis (12, 13). HIF is regulated by the cellular hypoxia response. Acute hypoxia stabilizes the HIF-1 $\alpha$  and HIF-2 $\alpha$  proteins, which form dimers with HIF-1 $\beta$  and HIF-2 $\beta$ . The stability of HIF $\alpha$  subunits is controlled by HIF prolyl-hydroxylases (PHDs), which use O<sub>2</sub> and  $\alpha$ -ketoglutarate to convert a prolyl residue to hydroxyproline plus succinate and CO<sub>2</sub> (14, 15). The hydroxyl-proline residues are bound by the von Hippel-Lindau protein complex (VHL tumor suppressor lost in some types of cancers) containing an E3 ubiquitin ligase, which results in the HIF $\alpha$  subunit being polyubiquitinated and degraded (16). The absence of oxygen stabilizes the HIF transcription factor. In addition, lactate dehydrogenase A and pyruvate dehydrogenase kinase are transcriptionally regulated by HIF, both of which keep pyruvate away from the mitochondria. The loss of PTEN (phosphatase and tensin homolog, a tumor suppressor gene) and concurrent increase of AKT-1 and mTOR lead to HIF activation and the Warburg effect (Fig. 2). The *myc* transcription factor activates the transcription of more than 1000 genes involved in all phases of cell growth and metabolism. *Myc* enhances the transcription of glutaminase-1, the first enzyme in glutaminolysis producing glutamate (17), and it transcribes the ribosomal RNA genes and the ribosomal protein genes, increasing the rate of

protein synthesis and mass of a cell (18). *Myc* also regulates glutaminolysis at the microRNA (miRNA) level by transcriptionally repressing miR-23a and miR-23b, which results in greater expression of their target protein, mitochondrial glutaminase-1, and thus up-regulation of glutamine catabolism.

Similarly, the loss of p53 functions lead to the Warburg effect (Fig. 2). The p53 protein represses the transcription of the GLUT 1 and 4 transporters (18). The p53 protein induces the transcription of the TIGAR gene, which lowers the intracellular concentrations of fructose 2,6 biphosphatase (FBPase) and thus decreases glycolysis by diverting glucose through the PPP (19) (Fig. 1). TIGAR also has functional similarities to the biphosphate domain of PFK-2/FBPase-2 in regulating glycolysis, ROS levels, and apoptosis and is structurally similar to FBPase2. The activation of p53 also increases the ubiquitination of phosphoglycerol mutase, which decreases the activity of this glycolytic enzyme. p53 increases the use of the TCA cycle and oxidative phosphorylation. The p53 protein enhances the transcription of the gene for synthesis of cytochrome c oxidase 2 (SCO2), which, along with synthesis of cytochrome c oxidase 1 (SCO1), assembles into oxidative phosphorylation complexes (20). Cells with mutant p53 have compromised oxidative phosphorylation chains. p53 also promotes synthesis of a number of proteins that reduce the high ROS load in cells. Sestrins 1 to 4 are p53-regulated genes and produce proteins that react with and neutralize ROS (21). p53 also regulates the p21 gene, and the p21 protein binds to and stabilizes the Nrf2 transcription factor, which regulates a set of complex responses to altered redox potentials and high ROS. P53 transcribes the glutaminase 2 gene, a nuclear gene that produces a glutaminase localized in the internal compartment of mitochondria (22, 23). Unlike glutaminase 1, glutaminase 2 converts glutamine to glutamate, which can be used to enhance the rate of the TCA cycle and oxidative phosphorylation (22, 23). Thus, these two glutaminases, regulated by *myc* (glutaminase 1) and p53 (glutaminase 2), have opposite effects on the cell. Just why this is the case remains to be elucidated. An activated p53 protein also inhibits the activities of the phosphatidylinositol-3 kinase (PI3K)-AKT and mTOR pathways (Fig. 2). 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 AMP-activated protein kinase (AMPK), which all negatively regulate AKT kinase and mTOR (24, 25). In addition, sestrins 1 and 2, which are p53-regulated genes, stimulate AMPK activity (26). All of these activities shut down cell growth, decrease the Warburg effect, lower HIF levels, and thus reverse the cancer phenotype. In some cases, this results in a p53-directed apoptosis and the activation of autophagy (27).





**Fig. 2.** p53 regulation of PI3K, Akt, and mTOR pathways. p53 functions in a complex network to mediate a cell's adaptation to stress. To do this, p53 regulates the transcription of four genes, PTEN, IGF-BP3, TSC2, and AMPK $\beta$ , which then all negatively regulate Akt kinase and mTOR, leading to a decrease in cell growth and a reversal of the cancer phenotype. Coordinately, there is an inhibition of proliferation that is promoted through an activation of p53 and LKB1. In addition to slowing cell growth, p53-dependent inhibition of the mTOR pathway promotes autophagy, a way of helping cells survive. 4EBP1, 4E-binding protein 1; IGF-1 receptor, insulin-like growth factor-1 receptor; LKB1, liver kinase B1; MDM2, murine double minute 2; PDK1, phosphoinositide-dependent kinase-1; PIP3, phosphatidylinositol 3,4,5-trisphosphate; Raptor, regulatory associated protein of mTOR; Rheb, Ras homolog enriched in brain; S6 kinase, ribosomal protein S6 kinase; TSC, tuberous sclerosis complex.

A number of mutations in genes that encode enzymes in the TCA cycle have been shown to lead to some types of cancers. Mutations in succinate dehydrogenase and fumarate hydratase alter the complex 2 oxidative phosphorylation chain, which generates reduced flavine adenine dinucleotide (FADH<sub>2</sub>). These mutations force a switch to the Warburg effect and contribute to selected inherited and sporadic cancers (27). There is some evidence that these mutations result in the inactivation of the PHDs, leading to increases in HIF-1 and an enhanced glycolytic pathway. In glioblastoma multiforme, up to 12% of these tumors have spontaneous point mutations in the gene for cytosolic isocitrate dehydrogenase 1 (IDH1) (28). This enzyme converts isocitrate to  $\alpha$ -ketoglutarate, generating NADPH. Likewise, mutations have been observed in IDH2 at residue Arg<sup>172</sup>, in the active site, in patients of low-grade gliomas (29) and acute myeloid leukemia (AML) (30), as well as other diseases (31). The somatic mutations in IDH1 and IDH2 identified in gliomas and AML result in a new ability of the enzyme to catalyze the

NADH-dependent reduction of  $\alpha$ -ketoglutarate to 2-hydroxyglutarate, an oncometabolite that can be a correlative marker for mutations occurring in isocitrate dehydrogenase enzymes (32). Just why this is so strongly selected for in these tumors may well be more complex than simply generating more NADPH.

The large number of genetic alterations observed in human cancers in the oncogenes and tumor suppressor genes involved in the IGF-1/mTOR pathways (Fig. 2) suggest that drugs may be developed that alter the Warburg effect and some of its consequences. Inhibitors of TOR complex1 (TORC1), which controls protein synthesis and cell cycle progression, are already approved for use in selected cancers. TORC1 is regulated by AMPK, which measures ATP/AMP ratios and nutrient availability. Metformin, which is used as a treatment for type 2 diabetes, stimulates AMPK. Diabetic patients treated with metformin have lower incidences of cancer than diabetics not treated with this drug (33, 34). Indeed, metformin acts as a synthetic lethal drug on cells in culture

that contain p53 mutations, demonstrating the close interactions between these two pathways (35). The inhibition of lactate dehydrogenase in cancer cells slows their growth, suggesting the importance of making and secreting lactate from cancer cells. Most hepatocellular carcinomas have lost the expression of glutaminase-2 in mitochondria, even though most of these tumors do not contain p53 mutations (p53 regulates this gene). Returning a cDNA for glutaminase-2 to these cells in culture and expressing this protein, which enhances the use of the TCA cycle, inhibits cell division (22, 23). Thus, glutaminase-2 is acting like a tumor suppressor gene in these situations. Indeed, a wild-type p53 gene and protein are required for efficient mitochondrial DNA replication and mitochondrial maintenance in cells (36). These types of observations suggest that the extensive alterations of metabolic processes in cancer can contribute to the phenotypes of the tumor cells and as such are themselves causal (necessary but not sufficient) for these cancers.

## Conclusions

The observations and ideas reviewed here suggest a unity in the genes and pathways involved in several diseases. The interrelationships of the p53, AKT, and mTOR pathways (Fig. 2) bring together stress responses and diabetes. Indeed, p53 in adipose tissue can regulate insulin resistance (37). There is a similar overlap among several genes whose mutations predispose an individual to Parkinson's disease and the functions of those genes in the p53, AKT, and mTOR pathways (38). The connections among chronic inflammatory responses of the immune system, with the activation of NF- $\kappa$ B and its associated metabolic changes (Warburg effect) and PET scan-positive cells, and the formation of cancers of those cells are well established (39). It should not be surprising to observe such a central role of metabolic processes in many disorders and the integration of metabolic pathways with many diverse signal transduction pathways. Metabolic pathways comprise an evolutionarily conserved underlying feature for most functions of a cell and an organism.

## References

1. O. Warburg, K. Posener, E. Negelein, *Biochem. Z.* **152**, 309 (1924).
2. O. Warburg, *Science* **123**, 309 (1956).
3. P. P. Hsu, D. M. Sabatini, *Cell* **134**, 703 (2008).
4. G. Kroemer, J. Pouyssegur, *Cancer Cell* **13**, 472 (2008).

5. M. G. Vander Heiden, L. C. Cantley, C. B. Thompson, *Science* **324**, 1029 (2009).
6. R. J. DeBerardinis, N. Sayed, D. Ditsworth, C. B. Thompson, *Curr. Opin. Genet. Dev.* **18**, 54 (2008).
7. J. W. Locasale, L. C. Cantley, M. G. Vander Heiden, *Nat. Biotechnol.* **27**, 916 (2009).
8. D. A. Tennant, R. V. Durán, H. Boulahbel, E. Gottlieb, *Carcinogenesis* **30**, 1269 (2009).
9. E. Gottlieb, K. H. Vousden, in *The p53 Family*, A. J. Levine, D. Lane, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2010), pp. 187–198.
10. T. K. Hunt *et al.*, *Antioxid. Redox Signal.* **9**, 1115 (2007).
11. H. R. Christofk, M. G. Vander Heiden, N. Wu, J. M. Asara, L. C. Cantley, *Nature* **452**, 181 (2008).
12. G. L. Semenza, *Nat. Rev. Cancer* **3**, 721 (2003).
13. M. L. Macheda, S. Rogers, J. D. Best, *J. Cell. Physiol.* **202**, 654 (2005).
14. R. K. Bruick, S. L. McKnight, *Science* **294**, 1337 (2001); 10.1126/science.1066373.
15. K. S. Hewitson, L. A. McNeill, J. M. Elkins, C. J. Schofield, *Biochem. Soc. Trans.* **31**, 510 (2003).
16. P. H. Maxwell *et al.*, *Nature* **399**, 271 (1999).
17. R. J. DeBerardinis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19345 (2007).
18. C. V. Dang, *Mol. Cell. Biol.* **19**, 1 (1999).
19. K. Bensaad *et al.*, *Cell* **126**, 107 (2006).
20. S. Matoba *et al.*, *Science* **312**, 1650 (2006); 10.1126/science.1126863.
21. A. V. Budanov, A. A. Sablina, E. Feinstein, E. V. Koonin, P. M. Chumakov, *Science* **304**, 596 (2004).
22. W. Hu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7455 (2010).
23. S. Suzuki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7461 (2010).
24. Z. Feng *et al.*, *Cancer Res.* **67**, 3043 (2007).
25. Z. Feng, in *The p53 Family*, A. J. Levine, D. Lane, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2010), pp. 199–298.
26. A. V. Budanov, M. Karin, *Cell* **134**, 451 (2008).
27. Z. Feng, H. Zhang, A. J. Levine, S. Jin, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8204 (2005).
28. D. W. Parsons *et al.*, *Science* **321**, 1807 (2008); 10.1126/science.1164382.
29. H. Yan *et al.*, *N. Engl. J. Med.* **19**, 765 (2009).
30. P. S. Ward *et al.*, *Cancer Cell* **16**, 225 (2010).
31. M. Kranendijk *et al.*, *Science* **330**, 336 (2010); 10.1126/science.1192632.
32. L. Dang *et al.*, *Nature* **17**, 966 (2010).
33. J. M. Evans, L. A. Donnelly, A. M. Emslie-Smith, D. R. Alessi, A. D. Morris, *BMJ* **330**, 1304 (2005).
34. S. Jiralerspong *et al.*, *J. Clin. Oncol.* **27**, 3297 (2009).
35. M. Buzzai *et al.*, *Cancer Res.* **67**, 6745 (2007).
36. A. Bourdoin *et al.*, *Nat. Genet.* **39**, 776 (2007).
37. T. Minamino *et al.*, *Nat. Med.* **15**, 1082 (2009).
38. R. H. Kim *et al.*, *Cancer Cell* **7**, 263 (2005).
39. P. Ak, A. J. Levine, *FASEB J.* **24**, 3643 (2010).

10.1126/science.1193494

## REVIEW

## Autophagy and Metabolism

Joshua D. Rabinowitz<sup>1,2</sup> and Eileen White<sup>2,3,4</sup>

Autophagy is a process of self-cannibalization. Cells capture their own cytoplasm and organelles and consume them in lysosomes. The resulting breakdown products are inputs to cellular metabolism, through which they are used to generate energy and to build new proteins and membranes. Autophagy preserves the health of cells and tissues by replacing outdated and damaged cellular components with fresh ones. In starvation, it provides an internal source of nutrients for energy generation and, thus, survival. A powerful promoter of metabolic homeostasis at both the cellular and whole-animal level, autophagy prevents degenerative diseases. It does have a downside, however—cancer cells exploit it to survive in nutrient-poor tumors.

Living organisms from yeast to humans are capable of eating parts of themselves in order to survive. This involves the degradation of cellular components, either because they are deleterious (e.g., damaged organelles and microbial invaders) or because the resulting breakdown products are needed to support metabolism. This process was aptly termed autophagy from the Greek “auto” or oneself and “phagy” or to eat. It has gained attention recently as an essential contributor to human health and disease.

There are several forms of autophagy, each of which involves delivering intracellular cargo to lysosomes for degradation. The predominant form, macroautophagy (autophagy hereafter), produces vesicles called autophagosomes that capture and deliver cytoplasmic material to lysosomes (*1*). The autophagy-related genes (the *atg* genes) are

conserved from yeast to mammals and regulate the cannibalism of intracellular cytoplasm, proteins, and organelles.

Autophagy is the only mechanism to degrade large structures such as organelles and protein aggregates. In the absence of stress, basal autophagy serves a housekeeping function. It provides a routine “garbage disposal” service to cells, eliminating damaged components that could otherwise become toxic. Such cellular refreshing is particularly important in quiescent and terminally differentiated cells, where damaged components are not diluted by cell replication. In starvation, autophagy provides a nutrient source, promoting survival. Autophagy is induced by a broad range of other stressors and can degrade protein aggregates, oxidized lipids, damaged organelles, and even intracellular pathogens. Although it is not always possible to resolve the metabolic and garbage disposal roles for autophagy, it is clear that autophagy prevents disease. Defects in autophagy are linked to liver disease, neurodegeneration, Crohn’s disease, aging, cancer, and metabolic syndrome.

## Process of Autophagy

A series of protein complexes composed of *atg* gene products coordinate the formation of auto-

phagosomes. The Atg1/ULK1 complex (Atg1 in yeast and ULK1 in mammals) is an essential positive regulator of autophagosome formation (*1*). When nutrients are abundant, binding of the ULK1 complex by the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) inhibits autophagy. mTORC1 is an important regulator of cell growth and metabolism. It is composed of five subunits that include Raptor, which binds ULK1, and mTOR, a serine-threonine kinase. By phosphorylating ULK1 and another complex member (the mammalian homolog of yeast Atg13), mTOR inhibits autophagy initiation. In starvation, mTORC1 dissociates from the ULK1 complex, freeing it to trigger autophagosome nucleation and elongation.

Autophagosome nucleation requires a complex containing Atg6 or its mammalian homolog, Beclin 1, that recruits the class III phosphatidylinositol 3-kinase VPS34 to generate phosphatidylinositol 3-phosphate (*2*). Expansion of autophagosome membranes involves two ubiquitin-like molecules, Atg12 and Atg8 (called LC3 in mammals), and two associated conjugation systems. The E1-like Atg7 and E2-like Atg10 covalently link Atg12 with Atg5, which together bind Atg16L1 to form pre-autophagosomal structures. In the second ubiquitin-like reaction, LC3 is cleaved by the protease Atg4. Phosphatidylethanolamine is conjugated to cleaved LC3 by Atg7 and a second E2-like enzyme, Atg3, and this lipidated LC3-II associates with newly forming autophagosome membranes. LC3-II remains on mature autophagosomes until after fusion with lysosomes and is commonly used to monitor autophagy.

The process beginning with the Beclin 1 complex gives rise to nascent autophagosome membranes. These membranes assemble around cargo, encapsulating the cargo in a vesicle that subsequently fuses with a lysosome, generating an autolysosome. The contents are then degraded by proteases, lipases, nucleases, and glycosidases. Lysosomal permeases release the breakdown products—amino acids, lipids, nucleosides, and carbohydrates—into the cytosol, where they are

<sup>1</sup>Department of Chemistry and Lewis-Sigler Institute for Integrative Genomics, 241 Carl Icahn Laboratory, Washington Road, Princeton University, Princeton, NJ 08544, USA. E-mail: joshjr@genomics.princeton.edu <sup>2</sup>Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903, USA. <sup>3</sup>Department of Molecular Biology and Biochemistry, Rutgers University, 604 Allison Road, Piscataway, NJ 08854, USA. <sup>4</sup>Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854, USA. E-mail: whiteei@umdnj.edu