p53 and Metabolism: Inside the TIGAR

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The p53 tumor suppressor pathway coordinates DNA repair, cell-cycle arrest, apoptosis, and senescence to preserve genomic stability and prevent tumor formation. The discovery of three new target genes for p53 reveals unexpected functions for this tumor suppressor in the regulation of glucose metabolism and autophagy.

Life is fire—at least aerobic life is. Mitochondria consume oxygen and burn metabolites to provide chemical power to cells, and the availability of nutrients and oxygen are important constraints on the growth of cells and tissues. However, many tumors toss these constraints aside in their rush to proliferate and to form metastases, gorging on sugar and using glycolysis as their major energy pathway (Warburg, 1956). But how do cells switch from a life of moderation to one of gluttony en route to oncogenesis? One way may be to mutate the tumor suppressor protein p53. In response to cellular stressors such as oncogene activation or DNA damage, wild-type p53 becomes stabilized and switches on the expression of target genes. These target genes drive a variety of cellular responses to stress including DNA repair, cell-cycle arrest, senescence, and apoptosis. Several new studies—two published in this issue of *Cell* (Bensaad et al., 2006; Crighton et al., 2006) and one published in a recent issue of *Science* (Matoba et al., 2006)—highlight a new metabolic role for p53. This versatile protein not only drives damaged cells to undergo apoptosis but also coordinates how cells use nutrients to preserve their survival.

In this issue, Bensaad and colleagues (2006) report that p53 plays a direct role in cellular metabolism. They identify the product of a p53 target gene, *TIGAR* (*T*P53-*i*nduced *g*lycolysis and *a*poptosis *r*egulator), and show that it alters the pathway in which a cell uses glucose (see Figure 1). TIGAR shares functional sequence similarities with the bisphosphatase domain (FBPase-2) of the bifunctional enzyme PFK-2/FBPase-2 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase), which degrades fructose-2,6-bisphosphate (Fru-2,6-P_2). Fru-2,6-P_2 stimulates 6-phospho-1-kinase to convert fructose-6-phosphate to fructose-1,6-bisphosphate at the third step in glycolysis; when Fru-2,6-P_2 decreases, the formation of fructose-6-phosphate is favored. Similarly, TIGAR causes a decline in Fru-2,6-P_2 levels and thereby blocks glycolysis at this step, directing the pathway into the pentose phosphate shunt to produce NADPH (see Figure 1).

As the authors note, one consequence of the pentose phosphate shunt and increased NADPH generation is an increase in glutathione (GSH) levels, which promote the scavenging of reactive oxygen species (ROS). Indeed, the investigators found that expression of

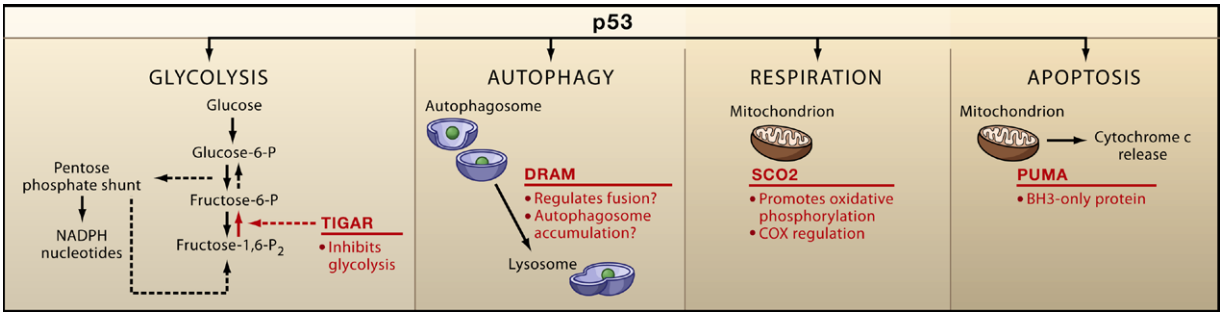

Figure 1. The Myriad Functions of p53
The p53 tumor suppressor pathway coordinates multiple functions to promote genomic stability and to prevent the formation of tumors. The p53 target DRAM may regulate autophagy by participating in the fusion of the autophagosome with the lysosome. The pathways responsible for glucose metabolism and mitochondrial respiration are regulated by the p53 targets, TIGAR and SCO2, respectively. TIGAR blocks glycolysis thus promoting activity of the pentose phosphate shunt. SCO2 may enhance oxygen consumption and mitochondrial respiration by regulating the assembly of cytochrome c oxidase (COX). As is the case for glycolysis, mitochondrial respiration and autophagy are controlled by metabolism. A molecular circuit may exist that links these three processes to ensure cell survival. The p53 protein can also engage apoptosis by inducing expression of PUMA, which leads to release of cytochrome c from mitochondria and apoptosis of the cell. (p53 target genes, red; dotted arrows indicate pathways that promote the pentose phosphate shunt.)

TIGAR protected cells from ROS and moderately protected cells from DNA damage-induced apoptosis (although the extent to which the latter involves ROS is uncertain). Other findings support a model in which p53 target genes preserve genomic stability by decreasing the oxidation of DNA (Sablina et al., 2005). In addition, cells that have a bias toward the pentose phosphate shunt may have improved DNA repair (Zhang et al., 2003). Intriguingly, NADPH blocks activation of caspase-2 (Nutt et al., 2005), which may partly mediate p53-induced apoptosis in some cells through expression of the p53 target, PIDD. However, the extent to which p53-mediated apoptosis proceeds through caspase-2 is unclear. Most p53-induced apoptosis depends on expression of the p53 target protein, PUMA, a proapoptotic BCL-2 family protein (Jeffers et al., 2003). Currently, we do not know whether NADPH or the pentose phosphate shunt affects the function of p53 or its target genes, directly or indirectly (Figure 1).

Is the predominant role of TIGAR to block apoptosis? More than serving to increase GSH levels, TIGAR's most important role may be to direct glucose away from energy production and toward the synthesis of nucleotides and other products that might be important for the repair of DNA lesions. Indeed, at least one other p53 target gene, *p53R2*, appears to be involved in regulating nucleotide pools within damaged cells (Tanaka et al., 2000). Although the pentose phosphate shunt can produce downstream products for glycolysis, it is likely that a decrease in Fru-2,6-P_2 tends to shift the pathway toward fructose-1-phosphate and away from energy production. But if this is so, how does the cell fulfill its metabolic demand for energy? Another recent study (Matoba et al., 2006) suggests how this may be accomplished. The p53 protein induces production of a copper transporter, SCO2 (*s*ynthesis of *c*ytochrome c *o*xidase *2*), which participates in the assembly of cytochrome c oxidase (COX) in mitochondria (Matoba et al., 2006). Cells lacking p53 have diminished oxygen consumption, which could be restored by ectopic expression of SCO2. Remarkably, mice lacking p53 were much more prone to fatigue than wild-type animals, suggesting that p53 performs this function in the absence of appreciable cellular stress.

This finding in primary cells and in mice, for what appears to be a non-tumor-suppressive role for p53, raises the question of what induces p53 function and subsequent metabolic changes during day-to-day cellular activities. Under conditions where mitochondrial oxygen consumption becomes faulty, ROS may be generated resulting in activation of p53 to halt glycolysis (thus restricting the fuel to drive transfer of electrons to molecular oxygen). The action of p53 via TIGAR also provides NADPH to increase GSH levels that are needed to scavenge ROS; this feeds back to stop the damage signals, presumably while DNA repair intermediates are maintained. Meanwhile, during oncogenesis, all of these steps are bypassed because in cells that accumulate defects in the p53 pathway, glycolysis proceeds at full steam and the normal restraints on tumor growth are lost.

In a related paper in this issue, Crighton et al. (2006) provide evidence for another unexpected function of p53: involvement in the autophagy pathway. They show that the p53 target, DRAM (*d*amage-*r*egulated *a*utophagy *m*odulator), engages autophagy, a conserved lysosomal-mediated catabolic pathway involved in the turnover of long-lived proteins and organelles (Lum et al., 2005). DRAM is a lysosomal protein with six membrane-spanning regions. Its exogenous expression leads to the accumulation of autophagosomes, whereas RNAi-mediated knockdown of DRAM prevents the p53-mediated accretion of autophagosomes (Figure 1). Remarkably, knockdown of DRAM also preserves clonogenic survival of cells treated with a DNA-damaging agent. The authors provide evidence that DRAM is required for p53-induced apoptosis and suggest that DRAM-dependent autophagy acts upstream of cytochrome c release from mitochondria and is required for apoptosis to proceed.

Autophagy has received much attention recently, but there is still confusion about whether autophagy is exclusively a mechanism for cell survival when nutrients are limiting, or whether, under some conditions, it causes nonapoptotic cell death referred to as "autophagic" or "type 2" cell death. Intriguingly, type 2 cell death induced by chloroquine in some cell types is p53 dependent (Zaidi et al., 2001), and we speculate that DRAM may be involved in this process. Crighton et al. (2006) suggest that autophagy operates upstream of apoptosis and provide evidence that knockdown of an element in the autophagic pathway, ATG5, prevents apoptosis induced by p53. It will be important to elucidate whether this knockdown can preserve clonogenic survival of DNA-damaged cells, as shown for knockdown of DRAM. Curiously, another study suggests that knockdown of ATG5 can itself induce apoptosis (Boya et al., 2005). The resolution may lie in the actual effect of DRAM. The authors propose that DRAM induces autophagy, but it is also possible that DRAM interferes with autophagy at a later stage, as is consistent with its localization. Such late-stage inhibition of autophagy would lead to the accumulation of autophagosomes (as the authors observed), thereby preventing the cell from gaining a catabolic benefit from autophagy, a condition that could be lethal.

Collectively, these observations suggest that the effects of p53 on metabolism are responses to environmental conditions. Are these effects such that, in some circumstances, p53 promotes cell viability by regulating metabolism and repair pathways that are distinct from its control of apoptosis? This possibility is certainly something to ruminate about.

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