p53 aerobics: The major tumor suppressor fuels your workout

In addition to its role as the central regulator of the cellular stress response, p53 can regulate aerobic respiration via the novel transcriptional target SCO2, a critical regulator of the cytochrome c oxidase complex (Matoba et al., 2006). Loss of p53 results in decreased oxygen consumption and aerobic respiration and promotes a switch to glycolysis, thereby reducing endurance during physical exercise.

p53, often referred to as the ''guardian of the genome,'' is the most commonly mutated gene in human cancer (Vogelstein et al., 2000). In response to DNA damage or other types of stress, p53 acts as a sequence-specific transcription factor and orchestrates the appropriate cellular response by inducing cell cycle arrest, apoptosis, senescence, or differentiation. While most p53 studies have focused on its role as a tumor suppressor, recent work suggests that p53 might play a role in other aspects of mammalian life, including metabolism as reported by Matoba et al. (2006) in *Science*. Most cancer cells display altered energy metabolism, primarily utilizing glycolysis rather than the more efficient aerobic respiration for their energy needs, a switch known as the Warburg Effect (Warburg, 1930). Although Warburg's classic hypothesis that cancer results from a change in cellular metabolism has been questionable, his observation of increased glycolysis in cancer is commonly used in clinical practice where positron emission technology (PET) scanning is used to detect tumors through their increased incorporation of the glucose analog ^{18}fluorodeoxyglucose tracer (Gambhir, 2002). Based on the hypothesis that an alteration in the most commonly disturbed pathway in cancer might cause metabolic changes in cancer cells, Matoba et al. (2006) found that p53 plays a direct role in the assembly of the cytochrome c oxidase complex, the major site of oxygen utilization in the eukaryotic cell.

To test their hypothesis, the authors initially looked at oxygen-consumption and ATP production in both mice and cell lines with hetero- and homozygous loss of *p53* and show that p53 loss results in a dose-dependent defect in oxygen consumption, suggesting decreased mitochondrial respiration. Overall ATP production is not altered and lactic acid levels increase with loss of p53, indicating that glycolysis compensates for the reduction in aerobic energy production. Strikingly, $p53^{-/-}$ mice, while having nearly identical body composition and overall ATP production as wild-type mice, have a marked decrease in exercise stamina as shown by a swim stress test (Matoba et al., 2006). These results suggest that p53 plays a crucial role in ensuring efficient ATP production by aerobic respiration for prolonged exercise (see Figure 1). How does a tumor suppressor involved in cell cycle control and apoptosis regulate energy metabolism? The canonical p53 targets could not explain this glycolytic switch, but careful analysis of p53 expression databases led the authors to identify *SCO2* (Synthesis of Cytochrome c Oxidase 2) as a gene upregulated in a p53-dependent manner. SCO2 is required for the assembly of a critical component of the COXII complex, and mutations in the gene result in aerobic respiratory failure. The authors establish *SCO2* as a transcriptional target for p53 and demonstrate that p53 loss leads to reduced levels of SCO2 expression in both mice and cell lines. Rescue of endogenous SCO2 levels by expression of exogenous SCO2 in p53-deficient cells restores aerobic respiration, establishing the biological relevance of this regulatory circuit. Hetero- or homozygous deletion of *SCO2* phenocopies the impaired respiration in p53 deficient cells, indicating that p53 directly regulates mitochondrial respiration through SCO2.

The observation that cells with mutated p53 have impaired respiration could have profound effects on cancer cells, as they require large amounts of energy to allow for continuous uncontrolled proliferation. Loss of p53 and the resulting SCO2-dependent reduction in oxidative respiration might drive cells into glycolysis in order to support increased ATP demand. During tumor growth, cells distal to vasculature have limited oxygen supply, and switching to glycolysis may give these cells a growth advantage, allowing them to continue to proliferate before the onset of angiogenesis. A role in controlling cancer cell metabolism complements recent advances in the p53 field that started to establish the protein as a central control point for stress and nutrient response networks in addition to its role in cell cycle control and apoptosis (reviewed by Levine et al., 2006). p53, IGF-1/AKT and TOR are all involved in sensing and integrating nutrient and stress signals and their cross-talk allows an intricate regulation of cell-growth, proliferation and programmed cell death. The study by Matoba et al. (2006) identified SCO2 as the first direct transcriptional target of p53 that modulates energy metabolism, adding a layer of complexity by connecting the stress response to energy metabolism. To determine the molecular mechanisms of complicated biological processes such as aging and tumor development, the cross-talk between these pathways will need to be further dissected.

The p53-SCO2-respiration axis that connects the tumor suppressor to metabolism is an intriguing addition to the long-established connection between the metabolic state of an organism and cancer development. Calorie restriction (CR) has long been known to negatively regulate both tumor formation, growth, and development (reviewed by Hursting et al., 2003). This observation has been of great interest, as CR has also been established as the only exogenous way to extend the lifespan of a number of organisms, including yeast, worms, flies, and rodents (reviewed by Bordon and Guarente, 2005). Members of the SIR2 protein family (Sirtuins), originally identified in yeast, have emerged as key players in regulating longevity, their enzymatic activity being regulated by the NAD/NADH ratio within the cell. Mammalian Sirtuins regulate a number of proteins

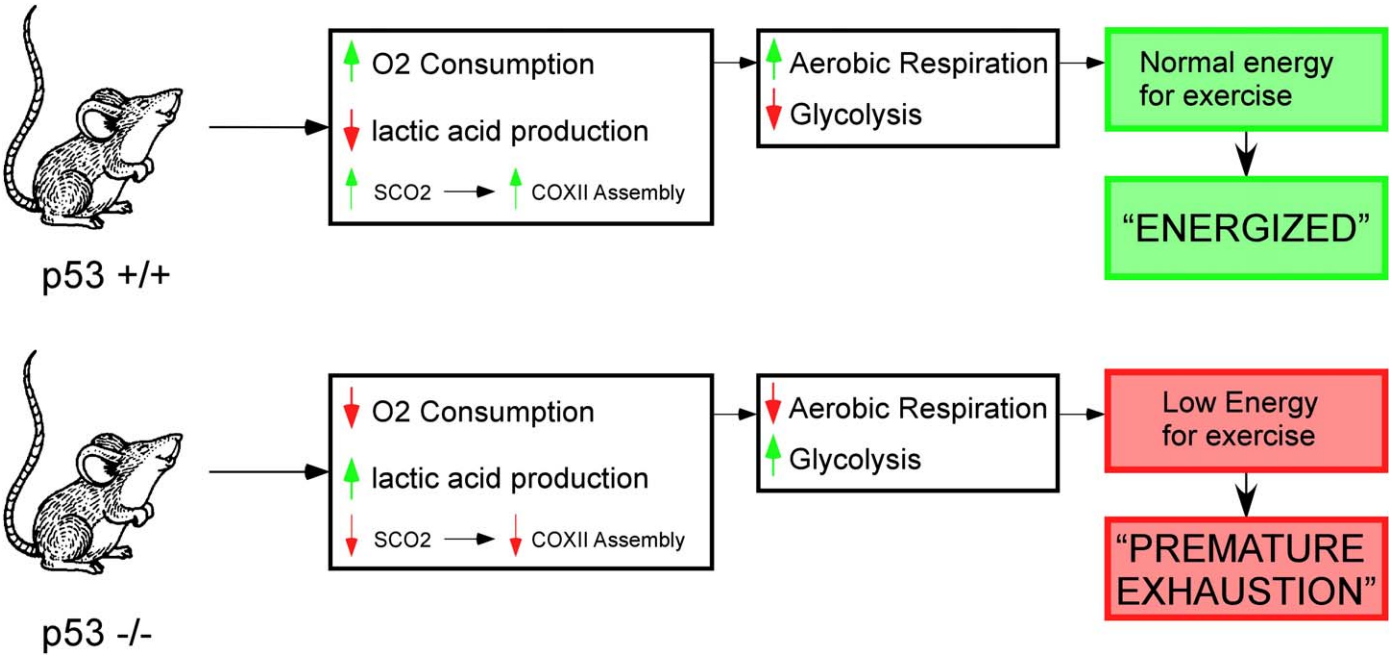

Figure 1. Mice that are wild-type for p53 primarily use aerobic respiration for the production of ATP, a more efficient way to generate energy for physical activity than glycolysis

p53 regulates the energy metabolism by transcription of SCO2, promoting the assembly of the cytochrome c oxidase complex (COXII). In mice with homozygous deletion of p53, the level of SCO2 is low, thereby preventing COXII assembly and concomitant decreased aerobic respiration, promoting the switch to glycolysis. Glycolytic energy production is not sufficient to generate ATP for prolonged exercise, resulting in premature exhaustion.

involved in metabolism and the stress response, including p53 (Luo et al., 2001; Vaziri et al., 2001) and the Forkhead transcription factors (Brunet et al., 2004; Motta et al., 2004). It will be interesting to see whether SIRT1, the mammalian SIR2 analog, can modulate the p53-dependent expression of SCO2 and thereby regulate the switch from aerobic respiration to glycolysis.

It has been argued that the metabolic switch to glycolysis and an acquired acid resistance of cancer cells could yield a powerful growth advantage, promoting unconstrained proliferation and invasion (Gatenby and Gilles, 2004) and the data presented by Matoba et al. (2006) suggest that loss of p53 might contribute to this acquired growth advantage. Hanahan and Weinberg have described evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, issue invasion/metastasis, limitless replicative potential, and sustained angiogenesis as the six hallmarks of cancer development (Hanahan and Weinberg, 2000). While a loss of p53 has long-established roles in the evasion of apoptosis and the regulation of replicative potential via cell cycle control, the connection of p53 to the regulation of metabolism constitutes a new aspect of how p53 loss and changes in energy metabolism in general might affect tumor cell growth. This raises the possibility that the energetic switch from aerobic respiration to glycolysis adds an additional hallmark of cancer development or could provide further explanations for existing ones, such as the limitless replicative potential or insensitivity to anti-growth signals. Demonstrating a direct metabolic target for p53 could be of great interest for cancer treatment, as the switch to glycolysis is predominantly observed in cancer cells. Specific activation of p53, for example by drugs like Nutlin-3 (Vassilev et al., 2004), could result not only in targeting its cell cycle and apoptotic responses, but it could also prevent or inhibit the glycolytic metabolism of cancer. It will be interesting to see whether metabolic changes can be observed in cancer patients with mutations for *p53* and whether tumors from these patients have reduced SCO2 and aerobic respiration levels. It would also be intriguing to know whether the loss of functional p53 in humans also reduces their energy and exercise potential as seen in mice. The study by Matoba et al. (2006) adds aerobic respiration and endurance control to the already extensive array of p53 functions, establishing that p53 may be more than "the guardian of the genome."

Jan-Philipp Kruse[1,2] and Wei Gu[1,2]
[1] Institute for Cancer Genetics, and Department of Pathology
College of Physicians & Surgeons
Columbia University
1150 St. Nicholas Ave
New York, New York 10032
[2] Integrated program in Molecular, Cellular and Biophysical Studies
Columbia University
New York, New York 10032

Selected reading

Bordon, L., and Guarente, L. (2005). Nat. Rev. Mol. Cell Biol. *6*, 298–305.

Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., et al. (2004). Science *303*, 2011–2015.

Gambhir, S.S. (2002). Nat. Rev. Cancer *2*, 683–693.

Gatenby, R.A., and Gilles, R.J. (2004). Nature Rev. Cancer *4*, 891–899.

Hanahan, D., and Weinberg, R.A. (2000). Cell *100*, 57–70.

Hursting, S.D., Lavigne, J.A., Berrigan, D., Perkins, S.N., and Barrett, J.C. (2003). Annu. Rev. Med. *54*, 131–152.

Levine, A., Feng, Z., Mak, T.W., You, H., and Jin, S. (2006). Genes Dev. *20*, 267–275.

Luo, J., Nikolaev, A.Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L., and Gu, W. (2001). Cell *107*, 137–148.

Matoba, S., Kang, J.G., Patino, W.D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P.J., Bunz, F., and Hwang, P.M. (2006). Science *312*, 1650–1653. Published online May 25, 2006. 10.1126/science.1126863.

Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M., and Guarente, L. (2004). Cell *116*, 551–563.

Warburg, O. (1930). The Metabolism of Tumors (London: Constable).

Vassilev, L.T., Vu, B.T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., et al. (2004). Science *303*, 844–848.

Vaziri, H., Dessain, S.K., Ng Eaton, E., Imai, S.I., Frye, R.A., Pandita, T.K., Guarente, L., and Weinberg, R.A. (2001). Cell *107*, 149–159.

Vogelstein, B., Lane, D., and Levine, A.J. (2000). Nature *408*, 307–310.

DOI 10.1016/j.cmet.2006.06.004

Xenobiotic metabolism in the fourth dimension: PARtners in time

A significant portion of the transcriptome in mammals, including the PAR bZIP transcription factors DBP, HLF, and TEF, is under circadian clock control. In this issue of *Cell Metabolism*, Gachon and colleagues (Gachon et al., 2006) show that disruption of these three genes in mice alters gene expression patterns of many proteins involved in drug metabolism and in liver and kidney responses to xenobiotic agents. Triple mutant mice have severe physiological deficits, including increased hypersensitivity to xenobiotic agents and premature aging, highlighting the profound effect the circadian clock has on this important response system.

Humans normally only notice their circadian clocks when they are disrupted—when struggling to stay awake upon arrival in a new time zone or when trying to perform optimally at a difficult job while working the night shift. At these times, this internal clock seems to be an inconvenience, making it difficult to function optimally when out of phase with one's environment. However, it has become increasingly clear that circadian clocks control a vast array of physiological functions and behaviors that are critically important to an organism's well being. This has come into sharper focus in recent years as genetic disruption of circadian systems has revealed a number of serious health consequences (Hastings et al., 2003). In this issue of *Cell Metabolism*, Gachon et al. demonstrate that loss of three circadian-controlled PAR bZIP transcription factors in mice causes disruption of a rhythmic transcriptional program that regulates circadian detoxification. The mice exhibit hypersensitivity to xenobiotic compounds and display signs of premature aging, providing a compelling example of the importance of the circadian system.

Circadian clocks are found in a wide spectrum of organisms ranging from cyanobacteria to humans, with many well-conserved properties (reviewed in Bell-Pedersen et al., 2005). In mammals, the circadian oscillator consists of a core negative feedback loop in which the transcription factors CLOCK and BMAL1 activate the *Period* (*Per1, Per2*) and *Cryptochrome* (*Cry1, Cry2*) genes via E box enhancers in their promoters (Figure 1; reviewed in Lowrey and Takahashi, 2004). The products of these genes form complexes with each other and with other proteins and eventually translocate into the nucleus and repress the CLOCK/BMAL1 complex, shutting off their own transcription. This primary negative feedback loop is augmented by an interlocking loop in which CLOCK/BMAL1 also drive transcription of other transcription factors (REV-ERBα and RORA) that act to drive rhythmic transcription of the *Bmal1* gene. The circadian mechanism is cell autonomous, and the majority of cells and tissues in the body contain circadian oscillators. At the organismal level, temporal organization is achieved by a hierarchical order in which a circadian pacemaker in the suprachiasmatic nucleus (SCN) synchronizes and coordinates peripheral tissue oscillators throughout the body (Yoo et al., 2004).

So, how do circadian clocks composed of interlocking feedback loops control such various output pathways? Microarray analyses have shown that ~3%–10% of expressed transcripts are under circadian regulation (reviewed in Lowrey and Takahashi, 2004). In the liver, basic cellular pathways such as glycolysis, fatty-acid metabolism, cholesterol biosynthesis, and xenobiotic and intermediate metabolism are under circadian regulation. Importantly, rate-limiting steps in these various pathways are key sites of circadian control, highlighting the fundamental role that circadian clocks play in cellular and organismal physiology (Panda et al., 2002). Gachon et al. provide new insight into the complexities of circadian gene regulatory networks using genetic and biochemical approaches (Gachon et al., 2006). In this paper, they examine the role of three PAR-domain basic leucine zipper (PAR bZip) transcription factors in regulation of rhythmic gene expression in liver. One of these proteins, DBP, has definitively been shown by this group to be a direct transcriptional target of CLOCK/BMAL1 (Ripperger and Schibler, 2006; Ripperger et al., 2000). The other two, TEF and HLF, are reported here to also be under similar control. These three proteins can form homo- or heterodimers and activate transcription of genes containing the appropriate PAR response element (PARRE).

In order to evaluate how these rhythmic transcription factors contribute to circadian function, all three PAR bZIP genes were inactivated by gene targeting