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MINI REVIEW

A pivotal role for p53: balancing aerobic respiration and glycolysis

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Abstract The genetic basis of increased glycolytic activity observed in cancer cells is likely to be the result of complex interactions of multiple regulatory pathways. Here we review the recent evidence of a simple genetic mechanism by which tumor suppressor p53 regulates mitochondrial respiration with secondary changes in glycolysis that are reminiscent of the Warburg effect. The biological significance of this regulation of the two major pathways of energy generation by p53 remains to be seen.

Keywords Synthesis of cytochrome c oxidase 2 · Cytochrome c oxidase · Tumor protein p53

Tumor suppressor p53 serves a protective function against cellular stresses such as DNA damage (Vogelstein and Kinzler 2004), and this important role is underscored by its high inactivation frequency in human cancers (Olivier et al. 2002; Hofseth et al. 2004). Although the mechanisms p53 utilizes to regulate the cell cycle and apoptosis have been extensively studied, its role in other cellular processes such as metabolism is less clear. Nonetheless, such a relationship between p53 and non-cell cycle related genetic pathways may be equally important for tumorigenesis as initially hypothesized by Warburg (1956). We briefly review our recent insights into how p53 affects the mode of energy production (Matoba et al. 2006) and place this in the context of previous observations. Further dissection of this pathway may give us new insight into the genetic mechanisms of energy generation with implications for other global functions.

We have recently found that tumor suppressor p53 directly regulates mitochondrial oxygen consumption through an important assembly protein encoded by the *Synthesis of Cytochrome c Oxidase 2* (*SCO2*) gene in mice and in human cancer cells (Matoba et al. 2006). The basis of alterations in the cytochrome c oxidase (COX) complex of cancers has been studied by a number of investigators (Heerdt et al. 1990; Luciakova and Kuzela 1992; Polyak et al. 1998; Herrmann et al. 2003; Modica-Napolitano and Singh 2004), and associations between p53 and COX enzymatic activity, COX subunits I and II, as well as mitochondrial 16S ribosomal RNA, have been reported (Ibrahim et al. 1998; Okamura et al. 1999; Zhou et al. 2003). In the HCT116 human colon cancer cell line with targeted disruption of p53, reduced COX enzymatic activity and COX subunit II protein levels have previously been described (Zhou et al. 2003). In these same isogenic cell lines, we have now provided direct genetic evidence that p53 regulates mitochondrial respiration through SCO2 with associated changes in glycolytic activity (Matoba et al. 2006). Fittingly, 50 years after the publication of Warburg's (1956) seminal review on tumor metabolism, the direct genetic pathway between a frequently mutated human tumor suppressor and mitochondrial respiration provides a molecular mechanism in support of his observations (Matoba et al. 2006).

We observed that the total amount of ATP generated by the HCT116 cells with wild-type (+/+), heterozygous (+/−) or homozygous (−/−) targeted disruption of p53 was similar when calculated from oxygen consumption and lactate production representing aerobic respiration and glycolysis,

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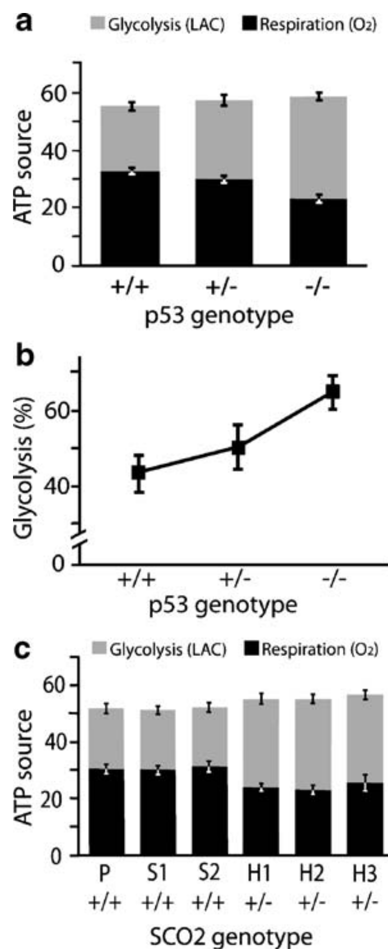

Fig. 1 Dependence of aerobic respiration and glycolysis on p53 and SCO2 (Matoba et al. 2006). **a** The amount of ATP (mean+SD, nmol/min/mg protein) produced by aerobic respiration (*dark bars*) and glycolysis (*light bars*) was calculated by measuring oxygen (O_2) consumption and lactate (LAC) production in the three different p53 genotypes of HCT116 cells: wild-type (+/+), heterozygous (+/−), and homozygous (−/−). **b** The fraction of total ATP generated by glycolysis was inversely proportional to p53 gene dosage (values calculated from **a**). **c** The amount of ATP (mean±SD, nmol/min/mg protein) produced by aerobic respiration (*dark bars*) and glycolysis (*light bars*) was calculated by measuring oxygen (O_2) consumption and lactate (LAC) production in the three wild-type (+/+, P, S1, S2) and three SCO2 heterozygous knockout (+/−, H1, H2, H3) clones

respectively (Fig. 1a; Sariban-Sohraby et al. 1983; Matoba et al. 2006). This estimation was consistent with the nearly equivalent growth rates observed in these isogenic cell lines. However, we noted a reciprocal relationship between the two sources of ATP that varied with p53 gene dosage (Fig. 1a). In fact, the relative fraction of ATP derived from glycolysis was inversely proportional to p53 gene dosage (Fig. 1b). The decreased aerobic respiration in p53-deficient cells was rescued by reintroducing SCO2 at physiologic levels. Finally, as genetic proof of function, we created a heterozygous (+/−) *SCO2* gene knockout cell line by somatic cell homologous recombination, and its ATP-generating metabolic profile phenocopied the p53−/− cell line (Fig. 1c). Remarkably, Warburg hypothesized that cancer cells have a primary defect in respiration with compensatory increases in glycolysis, a phenomenon that we have now reproduced in vitro when cells were made deficient in the p53-inducible gene *SCO2* (Warburg 1956; Matoba et al. 2006).

The regulatory mechanisms underlying aerobic and glycolytic pathways of energy production are complex making the prediction of system-specific cellular responses rather difficult. Glycolysis can dramatically be increased by the constitutive expression of activated Akt, but mitochondrial oxygen consumption is unchanged indicating tight homeostatic control of the respiratory complexes (Fig. 2; Elstrom et al. 2004). In other cellular contexts, oxygen consumption can be increased by overexpressing Myc or mitochondrial protein Frataxin with both increased or decreased cell proliferation, respectively (Schuhmacher et al. 1999; Li et al. 2005; Schulz et al. 2006). These difficulties in predicting the outcome of altered metabolism underscore its complex interaction with the cell cycle. Another lesson from these metabolic studies is that specific observations in one system cannot be extrapolated without taking into account the wide genetic variables that are likely to exist between different species and cell types. In our study, we sought to minimize these confounding factors by utilizing isogenic human colon cancer cell lines and mice with the targeted disruption of one specific gene in the genome (Bunz et al. 1998; Matoba et al. 2006). The aerobic energy deficit introduced by the 20 to 30% reduction in oxygen consumption in the p53−/− and SCO2+/− cell lines would have been predicted to result in reduced cell proliferation, however, empirically this was not observed. The mechanisms governing similar growth rates and total ATP requirements of the isogenic HCT116 cells may have enabled us to observe a reciprocal relationship between the two major energy generating pathways caused by a specific perturbation.

The maintenance of similar proliferation rates in both the p53−/− and SCO2+/− cells with a compensatory increase in glycolysis suggested coordinate regulation of the aerobic and glycolytic pathways. What mechanisms may underlie this balance between the aerobic and glycolytic pathways of energy generation? The connections between loss of cell cycle control and increased glycolysis in cancer cells were first made by the demonstration of mutant p53 transactivating the hexokinase 2 (*HK2*) gene in hepatoma cells (Bustamante and Pedersen 1977; Mathupala et al. 1997). Subsequently, another glycolytic enzyme phosphoglycerate mutase (PGM) was shown to be modulated by p53 and important for immortalizing mouse embryo fibroblasts (Ruiz-Lozano et al. 1999; Kondoh et al. 2005). More recently, a novel p53-transactived gene TP53-induced glycolysis and apoptosis regulator (TIGAR) with homology

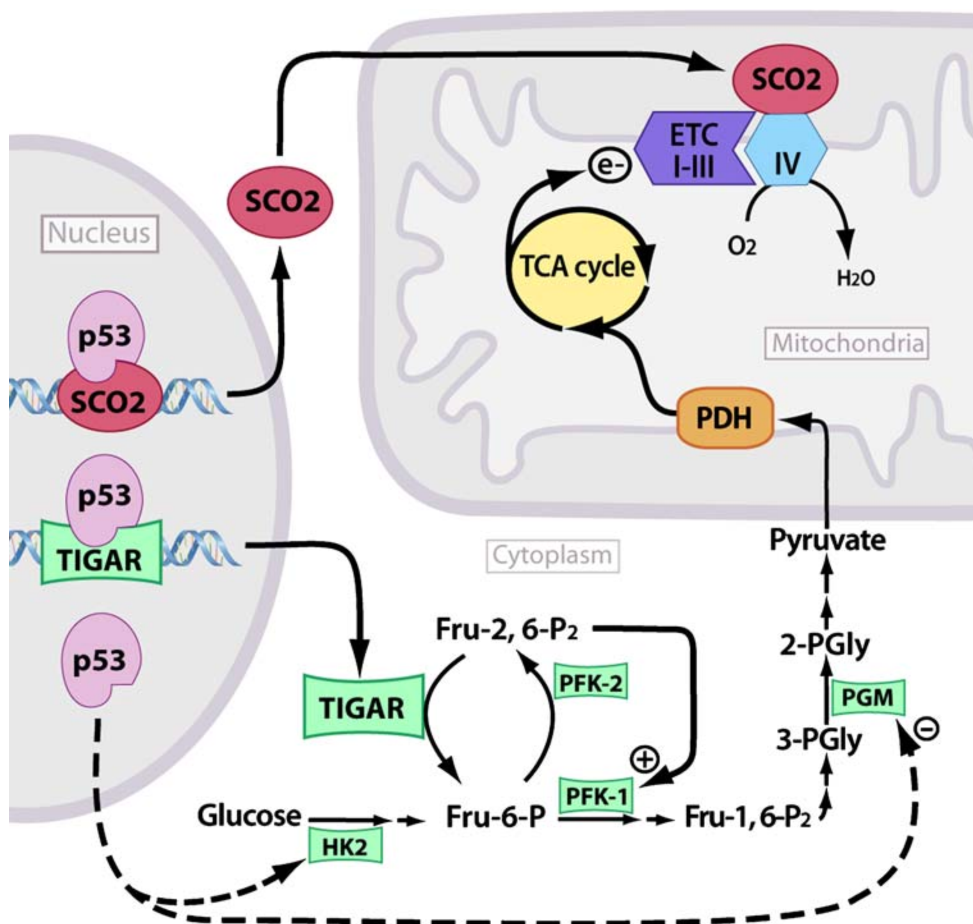

Fig. 2 p53 regulation of mitochondrial respiration and glycolysis. Shown are the nuclear transactivation of *SCO2* (synthesis of cytochrome c oxidase 2) and *TIGAR* (TP53-induced Glycolysis and Apoptosis Regulator) genes by p53. SCO2 is targeted to the inner membrane of the mitochondria where it facilitates the assembly of cytochrome c oxidase (COX) complex in the electron transport chain (ETC). The pyruvate generated from glycolysis enters the tricarboxylic acid (TCA) cycle and donates electrons (e−) to the ETC. TIGAR decreases glycolysis by dephosphorylating fructose-2,6-bisphosphate (Fru-2,6-P_2), an important allosteric effector (+) of the key glycolytic enzyme 6-phosphofructose-1-kinase (PFK-1). *Dashed lines* indicate mutant p53 that has been shown to increase two additional glycolytic enzymes, hexokinase 2 (HK2) and phosphoglycerate mutase (PGM). In contrast, wild-type p53 has been shown to decrease (−) PGM activity and levels by ubiquitination (Kondoh et al. 2005). Other abbreviations: fructose-6-phosphate (*Fru-6-P*), phosphoglycerate (*PGly*), pyruvate dehydrogenase (*PDH*)

to bisphosphatases determined the cellular levels of fructose-2,6-bisphosphate (Fru-2,6-P_2), a potent allosteric effector of glycolytic enzyme 6-phosphofructo-1-kinase (PFK-1; Fig. 2; Bensaad et al. 2006). In this study the expression of TIGAR not only decreased glycolytic activity by dephosphorylating Fru-2,6-P_2 to Fru-6-P, but interestingly it also decreased reactive oxygen species (ROS) generation and apoptosis by promoting glutathione production and redirecting metabolites into the pentose phosphate shunt (Bensaad et al. 2006). Collectively, p53 appears to regulate a number of key enzymes along the glycolytic pathway, but it may also regulate glycolysis through other factors such as plasma membrane glucose transporters (Schwartzenberg-Bar-Yoseph et al. 2004).

Our genetic data indicate that the decrease in aerobic respiration in the p53-deficient HCT116 human colon cancer cell line is primarily mediated by the SCO2 gene and that there is a compensatory increase in glycolysis. A putative mechanism whereby p53 inactivation promotes glycolysis may include reductions in TIGAR level and increases in HK2 and PGM. Whether our specific observation involves enzymes like PGM or TIGAR in cancer or normal cells remains to be determined. Because a primary defect in respiration in the SCO2+/− cells was sufficient to reproduce the p53−/− metabolic phenotype (Fig. 1c), the reciprocal increase in glycolysis may also involve p53 independent pathways. Based on the above findings, how p53 balances the two major energy generating metabolic pathways is multi-factorial and likely extends to a network of metabolic regulators including mammalian target of rapamycin (mTOR) and AMP-activated kinase (AMPK; Feng et al. 2005; Jones et al. 2005). However, we are able

to conclude that a primary defect in aerobic respiration is sufficient to alter glycolysis through both p53 dependent and independent pathways, providing a genetic model for the Warburg effect in cancer cells. Though the alterations in respiration and glycolysis that we observed were modest, the dramatic decrease in exercise capacity of p53−/− mice indicates that our findings have important implications for normal metabolism.

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