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The Molecular Mechanism of Glucose Metabolism Hampered by p53 Tumor Suppressor Protein

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p53 is the most frequently mutated gene in human cancer cells. It functions as a transcriptional activator and induces apoptosis or cell-cycle arrest by inducing expression of its target gene upon DNA damage, such as that caused by antitumor drugs. Recently, it was reported that activation of oncogenes, such as ras, triggers DNA damage responses and, consequently, induces apoptosis or senescence via a p53-dependent pathway in normal cells. Thus, oncogene-induced transformation is suppressed by p53-mediated surveillance, resulting in the elimination of cells with activated oncogenes.

Numerous studies have demonstrated that constitutive activation of nuclear factor (NF)-κB is frequently observed in many types of cancer cells and that NF-κB plays an important role in oncogenesis. These findings suggest that p53 suppresses NF-κB activation and regulates oncogene-induced transformation.

To investigate the role of p53 in the regulation of NF-κB activity, we first examined the activity of NF-κB in $p53^{-/-}$ mouse embryonic fibroblasts (MEFs) or wild-type MEFs expressing a cancer-associated p53 mutant. It was shown that the activity of NF-κB and that of IkappaB kinases (IKKs), upstream kinases of NF-κB, were increased in these cells. Accordingly, the enhanced DNA binding activity of NF-κB was abolished by knockdown of IKKα or IKKβ, indicating that the loss of p53 function enhances the IKK-NF-κB pathway.

We next investigated whether NF-κB activation affects oncogene-induced cell transformation (i.e., acquisition of a growth advantage in soft agar). The activating form of Ha-Ras (Ha-RasV12) can induce cell transformation in these cells, whereas the ability of transformation was attenuated by a deficiency of p65, a component of NF-κB. Thus, we found that activated NF-κB has an essential role in oncogene-induced cell transformation in cells that lack of p53 function.

Increased dependency on glycolysis for ATP synthesis instead of oxidative phosphorylation in the presence of oxygen is known as the Warburg effect. Cancer cells preferentially utilize aerobic glycolysis for energy provision, and this metabolic change is important for tumor growth. It has been reported that the rate of glycolysis is increased and that of mitochondrial respiration is decreased following the loss of p53 activity and that transformed cells lacking p53 activity acquire a growth advantage.

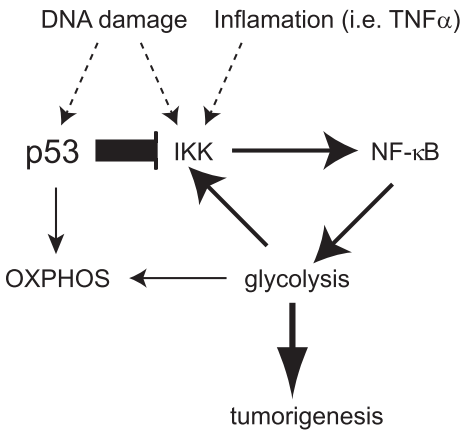

Fig. 1 Model of link between p53, NF-κB and glycolysis

Next, we investigated whether the enhanced NF-κB activation induced by the loss of p53 function contributes to an increased rate of aerobic glycolysis. We found that glucose consumption was increased in *p53*$^{-/-}$MEFs and was reduced by a deficiency of p65, IKKα, or IKKβ. The rate of glucose consumption is strongly correlated with the activity of glucose transporter (GLUT). We found that the expression of *GLUT3*, a high-affinity glucose transporter, is up-regulated in *p53*$^{-/-}$MEFs and that this up-regulation is significantly impaired by a deficiency of p65, IKKα, or IKKβ. Because oxygen consumption in *p53*$^{-/-}$MEFs was not affected by a deficiency of p65, these results indicate that in *p53*$^{-/-}$MEFs, p65 enhances glycolysis through *GLUT3* up-regulation without affecting mitochondrial respiration. Together, these findings show that enhanced activation of IKK-NF-κB, induced by the loss of p53 activity, has a pivotal role in aerobic glycolysis.

Moreover, in Ras-expressing p53-deficient cells, IKK activity was suppressed by p65 deficiency and restored by *GLUT3* expression, suggesting that IKK activity is intensified by glycolysis. In addition, we demonstrated that IKKβ was constitutively modified with O-linked β-N-acetyl glucosamine (O-GlcNAc) in both *p53*$^{-/-}$MEFs and transformed human fibroblasts. In *p53*-deficient cells, the O-GlcNAcylated IKKβ and the activating phosphorylation of IKK were decreased by glucose depletion. We also found that exposure to high glucose induced the O-GlcNAcylation of IKKβ and sustained the TNFα-dependent IKKβ activity. These data indicate the glycolysis-induced IKK activation in *p53*-deficient cells. These findings indicate that p53 restricts activation of the IKK-NF-κB pathway through suppression of glycolysis and suggest the existence of a novel positive feedback loop, whereby glycolysis drives IKK-NF-κB activation; hyperactivation of this loop by the loss of p53 plays an important role in glucose metabolic change in oncogene-induced transformation **(Fig. 1)**.