A Tumor Suppressor SIRTainty

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Sirtuin deacetylases are linked to longevity, aging, and stress responses. In this issue of *Cancer Cell*, Kim et al. show that SIRT3 functions as a tumor suppressor by enhancing the expression of mitochondrial MnSOD. Loss of SIRT3 leads to increased mitochondrial ROS, which then enhances cellular transformation and tumor growth.

The sirtuins family of histone deacetylases affect diverse biological processes, including longevity, aging, and the response to stress. Humans express seven sirtuins (SIRT1-7), whose functions have become the focus of intense investigation. Three mammalian sirtuins are targeted to mitochondria (SIRT3, 4, and 5), whereas the remainder exhibit cytosolic and/or nuclear localization. Initial excitement regarding sirtuins arose with the realization that they mediate the increase in lifespan arising from caloric restriction (Guarente, 2008), although the underlying mechanism is unknown. SIRT3 is the only member linked genetically to lifespan in humans (Rose et al., 2003), which, together with its localization in mitochondria, has made SIRT3 an especially interesting target for study.

Surprisingly, deletion of *Sirt3* in the mouse produces no overt signs of disorder (Lombard et al., 2007). However, a different picture emerges when these animals are stressed. For example, cardiomyocytes from *Sirt3*-deficient mice exhibit increased levels of reactive oxygen species (ROS), decreased ATP levels, and intolerance to oxidant stress (Sundaresan et al., 2008). Wild-type mice challenged with angiotensin II (to increase cardiac workload) develop myocardial hypertrophy, whereas $Sirt3^{-/-}$ mice exhibit a massively amplified response (Sundaresan et al., 2009). In wild-type animals, treatment with NAD, an activator of SIRT3, blocked the hypertrophic response by suppressing Akt activation via an LKB1-AMPK-mediated pathway (Pillai et al., 2009). Thus, SIRT3 is important in regulating the response to stress.

Given that SIRT3 localizes to mitochondria, that mitochondrial ROS are important in cancer, and that SIRT3 expression decreases with aging (Lanza et al., 2008), one might ask whether SIRT3 plays a role in oncogenic transformation. The answer, detailed in an impressive set of studies in this issue (Kim et al., 2010), is a resounding yes. The authors began by comparing ROS in wild-type and $Sirt3^{-/-}$ murine embryonic fibroblasts (MEFs). Under basal conditions, there were no differences in cytosolic ROS. However, when stressed by treatments that augment ROS generation, the increases in ROS they observed were greater in the $Sirt3^{-/-}$ cells. Even greater differences were seen when they used a probe to detect mitochondrial oxidant stress, during which they detected higher ROS levels even under unstressed conditions. These higher ROS levels correlated with a progressive deterioration in liver mitochondrial DNA integrity, over 58 weeks of age. Although oxidant stress and genomic instability might promote transformation, the SIRT3-deficient MEFs were not immortalized. Thus, loss of *Sirt3* pushes the cell toward a transformed phenotype, but not enough to induce transformation. However, when the stress of expressing Myc and/or Ras was added to $Sirt3^{-/-}$ MEFs, the cells became immortalized, they lost contact inhibition and they grew more quickly. By contrast, wild-type cells required both Myc and Ras to achieve a similar phenotype. Moreover, $Sirt3^{-/-}$ MEFs expressing Myc or Ras grew in soft agar and developed subcutaneous tumors in nude mice. In wild-type MEFs, expression of Myc or Ras alone is insufficient to permit soft-agar or in vivo tumor growth. Thus, SIRT3 functions as a tumor suppressor, and loss of SIRT3 amplifies the phenotypic effects of oncogene expression.

How does loss of SIRT3 alter mitochondrial ROS? On the basis of its mitochondrial localization, and the finding that ROS levels were increased in $Sirt3^{-/-}$ cells, Kim et al. (2010) examined the mitochondria. They found that glycolytic activity was increased, whereas mitochondrial ATP levels and maximal capacity of complexes I and III were reduced in the $Sirt3^{-/-}$ Myc/Ras cells. ROS-mediated damage to mitochondrial DNA might explain these changes, although loss of mitochondrial protein deacetylation in the $Sirt3^{-/-}$ cells might also contribute (Figure 1). In either case, the increase in mitochondrial ROS was an important clue. In mitochondria, a principal antioxidant component is MnSOD, which degrades superoxide into H2O2. Sure enough, they found that MnSOD protein levels were decreased in the $Sirt3^{-/-}$ Myc/Ras fibroblasts. Moreover, enhanced expression of MnSOD in $Sirt3^{-/-}$ Myc/Ras cells slowed their growth, and coexpression of MnSOD prevented the immortalization of $Sirt3^{-/-}$ cells expressing either Myc or Ras. Thus, increased mitochondrial superoxide appears to mediate the transformation of *Sirt3* knockout cells expressing an oncogene.

Why does loss of SIRT3 decrease the expression of MnSOD? SIRT3 activates the expression of MnSOD and catalase by promoting FOXO3a translocation to the nucleus (Sundaresan et al., 2009). Loss of SIRT3 leads to increased FOXO3a phosphorylation, triggering its nuclear export. Thus, the nuclear/cytosolic functions of SIRT3, rather than the mitochondrial, mediate its role in the regulation of this key antioxidant (Figure 1). Indeed, expression of wild-type SIRT3 in $Sirt3^{-/-}$ cells normalized mitochondrial ROS and MnSOD expression, whereas mutant SIRT3 lacking deacetylase activity failed to do so (Kim et al., 2010). Hence, SIRT3

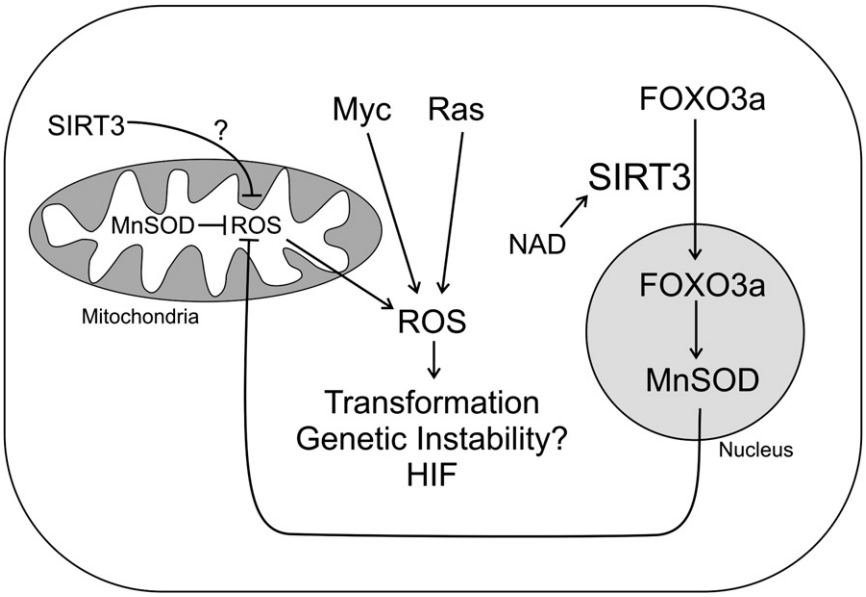

Figure 1. SIRT3 Regulates the Expression of MnSOD
SIRT3, a mitochondria-targeted deacetylase, plays an important role in regulating cell transformation, intriguingly, by its indirect effects in the cytosol, where it controls FOXO3a nuclear trafficking and thereby affects MnSOD expression. The MnSOD is a critical regulator of mitochondrial superoxide levels, which appear to regulate cellular transformation. Thus, loss of SIRT3 pushes the cell toward a transformed phenotype by enhancing mitochondrial ROS, which can also induce genetic instability and the stabilization of hypoxia-inducible factor (HIF).

functions as a tumor suppressor by regulating mitochondrial ROS via the regulation of FOXO3a.

How does the loss of MnSOD promote tumor formation? Kim et al. (2010) found that MnSOD expression declined in the *Sirt3*$^{-/-}$ mice as they aged. Although the underlying mechanism is unclear, they observed a higher incidence of spontaneous mammary gland tumors in the knockouts compared with wild-type mice, along with evidence of higher oxidant stress. Does loss of MnSOD promote tumor formation by itself? Interestingly, mice heterozygous for MnSOD initially appeared to be normal, but later also show an enhanced incidence of mammary tumors (Van Remmen et al., 1999). Thus, partial loss of MnSOD pushes cells toward oncogenic transformation by decreasing the number of other mutations needed to grow tumors. This is precisely the definition of a tumor suppressor.

Kim et al. (2010) reveal novel aspects of SIRT3 in regulating transformation while confirming other studies linking SIRT3 to the regulation of mitochondrial ROS (Sundaresan et al., 2009). However, other issues remain. First, although SIRT3 may localize to mitochondria, its effects on mitochondria are mediated by its regulation of FOXO3a in the cytosol/nucleus. If the principal effects of SIRT3 are unrelated to its localization in mitochondria, what is it doing there? This question could be addressed by targeting the expression of SIRT3 to various mitochondrial compartments in *Sirt3*$^{-/-}$ Myc/Ras cells and examining the consequences for the transformation phenotype.

Another issue concerns the relationship between mitochondrial superoxide and transformation. Kim et al. (2010) show decreased MnSOD levels and increased mitochondrial superoxide in the *Sirt3*$^{-/-}$ cells, which drive proliferation and transformation behavior. Yet ROS levels in the cytosol, intermembrane space, and matrix compartment are regulated independently (Waypa et al., 2009), and it is difficult for superoxide to travel from the matrix to the cytosol because anion channels are needed to traverse membranes. Under basal conditions, the *Sirt3*$^{-/-}$ cells showed no increase in cytosolic oxidant stress. So how does superoxide trapped in the mitochondrial matrix push the cells in the direction of transformation? Could the SIRT3 molecules localized to the mitochondria be playing a complementary role by regulating the release of ROS to the cytosol? Studies using the targeted rescue constructs described above could help to address this question.

Kim et al. (2010) reinforce an important concept in cancer biology—that mitochondrial ROS play an important role in promoting transformation and tumor progression. It seems likely that ROS-mediated inactivation of protein and lipid phosphatases, whose active sites contain redox-sensitive cysteine thiols, may be responsible. Therapeutically, is there a way to exploit SIRT3 to minimize undesirable ROS signaling? One idea is to supply NAD to cells to enhance SIRT3 activity. Recently, Pillai et al. show that NAD administered to wild-type, but not *Sirt3*$^{-/-}$ mice, abolished the cardiac hypertrophic response to angiotensin II (Pillai et al., 2009). In tumors in which loss of SIRT3 may contribute to the transformed phenotype, it is worth considering whether NAD administration could drive the cells in a reverse direction along the transformation pathway.

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