SIRT3 Is a Mitochondrial Tumor Suppressor: A Scientific Tale That Connects Aberrant Cellular ROS, the Warburg Effect, and Carcinogenesis

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Abstract

Tumors exhibit metabolic reprogramming characterized by increased cellular reactive oxygen species (ROS) and the preferential use of glucose, which is known as the Warburg effect. However, the mechanisms by which these processes are linked remain largely elusive. Murine tumors lacking Sirt3 exhibit abnormally high levels of ROS that directly induce genomic instability and increase hypoxia-inducible factor 1α (HIF-1α) protein levels. The subsequent transcription of HIF-1α-dependent target genes results in cellular metabolic reprogramming and increased cellular glucose consumption. In addition, agents that scavenge ROS or reverse the Warburg effect prevent the transformation and malignant phenotype observed in cells lacking Sirt3. Thus, mice lacking Sirt3 provide a model that mechanistically connects aberrant ROS, the Warburg effect, and carcinogenesis. Cancer Res; 72(10); 2468–72. ©2012 AACR.

Introduction

Although the first identified tumor suppressors were confined to the nucleus and/or cytoplasm, it seemed logical to hypothesize that the mitochondria also contain fidelity-sensing proteins that serve as tumor suppressors. A central role of the mitochondria is to generate ATP from ADP and organophosphate via oxidative phosphorylation, which generates reactive oxygen species (ROS) as a toxic byproduct. Failure to match the rate of oxidative phosphorylation with the nutrient supply or energy demand would cause decreased energy availability or excess ROS production, resulting in cellular stress. Therefore, fidelity proteins within the mitochondria should be critical for sensing and responding to changes in ATP demand, as well as elevations in ROS production. Furthermore, the processes of ATP production, ROS production, and clearance and/or removal of damaged molecules should be regulated by specific sensing or watchdog proteins to ensure that energy production closely matches the cellular energy requirements.

Mitochondrial aerobic respiration is an efficient method for generating energy in biological systems. However, as a side product of electron transfer reactions, aerobic cells continuously produce ROS from the incomplete reduction of dioxygen molecules (1). The steady-state levels of ROS are a function of ATP production and incomplete removal by detoxification enzymes. Oxidative stress occurs when the physiological balance between the production and scavenging of ROS is disturbed (2). Although low levels of cellular ROS are well tolerated by the cell and are a key part of homeostatic signaling pathways, abnormally high levels can induce oxidative stress, which has been implicated as a causative agent in several degenerative diseases, such as amyotrophic lateral sclerosis and rheumatoid arthritis, as well as in genomic instability, aging, and most importantly, carcinogenesis (1).

Cellular signaling networks often contain critical or central sensor proteins that activate downstream targets in response to environmental conditions (1, 3, 4). In this regard, lysine acetylation has emerged as an important posttranslational modification that is employed to activate mitochondrial signaling proteins (5, 6). The idea that the acetylome may be critical for regulating mitochondrial metabolism is based on several proteomic surveys that identified a high number of acetylated proteins that direct mitochondrial metabolism (6, 7). Given the tight link among metabolism, energy production, and ROS, it is likely that the mitochondrial acetyltransferases and deacetylases may coordinate, at least in part, the balance between energy production and ROS detoxification.

Sirt3 is a Mitochondrial Tumor Suppressor and Metabolic Regulator

Sirtuin genes are the homologs of the Saccharomyces cerevisiae Sir2 gene that directs downstream processes involved in longevity, and although it has not been shown that these genes determine longevity in mammals, they do appear to regulate critical pathways and physiologies that are implicated in age-related diseases (8, 9). Sirtuins share a 275-amino-acid catalytic deacetylase domain and are localized to the nucleus (SIRT1, SIRT6, and SIRT7), cytoplasm (SIRT2), and mitochondria (SIRT3, SIRT4, and SIRT5), respectively (9). Sirt3 is a

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mitochondrial deacetylase that acts on numerous substrates to activate fat oxidation, amino-acid metabolism, and electron transport (10). Several studies published in the last year provided convincing evidence that Sirt3, the primary mitochondrial deacetylase, is a bona fide tumor suppressor (11–13).

In support of Sirt3’s role as a fidelity or tumor-suppressor gene, in one study (11), mouse embryonic fibroblasts (MEF) lacking Sirt3 exhibited stress-induced genomic instability and were immortalized by infection with a single oncogene. By contrast, wild-type cells required both Myc and Ras to achieve a similar phenotype. Moreover, Sirt3 functions as an in vitro tumor suppressor, and loss of Sirt3 amplifies the phenotypic effects of oncogene expression. In vivo overexpression of Sirt3 was shown to decrease tumorigenesis in xenografts, even when induction of the Sirt3 occurred after tumor initiation (13). In addition, mice lacking Sirt3 developed estrogen- and progesterone-positive mammary tumors (11). Finally, human breast cancer data sets consisting of genomic, RNA, and tissue data from 992 human breast cancer samples also showed that SIRT3 is decreased in human breast cancers (11). Together, the knock-out mice, tissue culture, and human tumor data provide genetic evidence that a mitochondrial protein can function as a tumor suppressor.

With respect to its role in mitochondrial metabolism, MEFs lacking Sirt3 exhibited increased ROS (11), and in vivo overexpression of Sirt3 suppressed cellular ROS levels (13). These findings raise the question: What is the mechanistic link between loss of Sirt3 and aberrant mitochondrial ROS production? Several studies have shown that cells lacking Sirt3 exhibit aberrant or decreased activity of oxidative phosphorylation proteins, including complex I (14) and complex III of the electron transport chain (11, 12). Altered flux through the electron transport chain directly influences ROS production: electrons can leak out of complexes I and III, resulting in one-electron reductions of oxygen to produce the superoxide radical (15). Thus, when electrons are flowing quickly and efficiently through electron transport chains, opportunities for ROS production are diminished. In contrast, when electrons are flowing slowly or inefficiently, as has been proposed for cells lacking Sirt3, there is a greater opportunity for one-electron reductions of oxygen and, hence, formation of ROS (11–14).

Although these results account for increased ROS production, cells also contain detoxification enzymes that should scavenge the increased ROS in cells lacking Sirt3. Thus, in accord with recent studies, cells lacking Sirt3 may have dysfunctional coordination of both electron transport and detoxification enzymes, which can result in aberrant and potentially damaging levels of ROS.

Two recent studies (16, 17) showed that MnSOD, the primary mitochondrial superoxide detoxification enzyme, contains a lysine that is deacetylated by caloric restriction, fasting, and SIRT3 overexpression. Further analysis by Tao and colleagues (17) showed that lysine 122, which is conserved in multiple species, is directly deacetylated by SIRT3. When lysine 122 was changed to arginine (to mimic the deacetylated state; MnSOD^K122R), enzymatic activity was increased, intracellular ROS were decreased, and stress-induced genomic instability was prevented. In contrast, when lysine 122 was changed to a glutamine (to mimic the acetylated state; MnSOD^K122Q), MnSOD activity was decreased, suggesting that the acetylation status directs MnSOD enzymatic activity and cellular ROS levels.

The role of acetylation in cells lacking Sirt3 was confirmed by experiments that showed that Sirt3^−/− MEFs expressing MnSOD^K122R, but not MnSOD^K122Q, are resistant to in vitro transformation by infection with a single oncogene or exposure to irradiation (17). Thus, decreased Sirt3 deacetylation activity appears to increase ROS levels by 2 mechanisms: decreased electron transport and decreased MnSOD enzymatic detoxification activity. These results establish a connection between mitochondrial metabolism (i.e., increased production and decreased detoxification of ROS by MnSOD in cells lacking Sirt3) and tumor suppression (i.e., the genomic instability observed in Sirt3 knockouts [Fig. 1A]).

Sirt3 Directs Cellular Metabolic Reprogramming That Mirrors the Warburg Effect

A fundamental observation in oncology is that tumor cells exhibit a reprogramming of cellular metabolism that involves an increase in glucose consumption. This phenomenon, first described by Otto Warburg (18) in 1956, is known as the Warburg effect. Finley and colleagues (12) recently linked aberrant ROS regulation by SIRT3 to the Warburg effect, and they showed that the increased glucose consumption in cells lacking Sirt3 promoted a tumor-permissive phenotype both in vitro and in vivo. They also showed that elevated glucose metabolism was the direct result of increased hypoxia-inducible factor 1α (HIF-1α) stability in response to aberrant increased ROS levels.

HIF-1α is stabilized in response to low oxygen levels and subsequently increases the expression of more than 200 genes (19). The activation of HIF-1α protein is linked to multiple cellular pathways, such as metabolic reprogramming, cell survival, proliferation, progression, and metastasis, all of which are essential to the process of carcinogenesis. Finally, increased HIF-1α levels are associated with a poor prognosis in breast cancer (20).

Two independent studies by Finley and colleagues (12) and Bell and colleagues (13) revealed a new link between Sirt3 and HIF-1α. The authors found that loss of Sirt3 increased cellular HIF-1α levels by inhibiting prolyl hydroxylase via a mechanism that depended on an increase in cellular ROS levels (Fig. 1B). They also showed that this regulatory node regulated glucose metabolism. SIRT3 deletion increased HIF-1α target gene expression, glycolytic metabolism, and glucose-dependent cellular proliferation. In vivo, brown adipose tissue of the Sirt3^−/− mice had elevated glucose uptake, and the gene expression profile was very similar to that observed in cells exposed to low oxygen, suggesting a direct regulation of gene expression. In this regard, one could hypothesize that the induction of HIF-1α in response to increased ROS may be an adaptive response to increase glucose metabolism and thus detoxify hydroperoxides. This response could activate a series of necessary adaptive prosurvival signaling pathways to protect against cell
death while also increasing metabolic oxidative stress due to increased flux of ROS. Thus, over time, this compensative increase in cellular ROS could lead to genomic instability, a mutator phenotype, and progression to the malignant transformed phenotype. Finally, at least one copy of the SIRT3 gene is deleted in 20% of all human cancers and 40% of breast and...
ovarian cancers, and the majority of genomic \textit{SIRT3} deletions are heterozygous. Thus, these 2 studies (12, 13) suggest a mechanism of transformation where an increase in ROS stabilizes HIF-1$\alpha$, reprogramming the cell toward Warburg, and drives cells that already contain genomic instability toward cell proliferation that amplifies or propagates DNA damage (Fig. 1A).

\textbf{Model Connecting ROS, HIF-1$\alpha$, and the Warburg Effect in Carcinogenesis}

The results presented above suggest that an increase in the levels of cellular ROS, stemming from both increased production and decreased detoxification, may be an early initiating event that allows genomic instability and establishes a DNA-damage–permissive phenotype. This is based on the Hanahan and Weinberg (21) multi-hit model for carcinogenesis, which proposes that transformation requires an initiating event (Fig. 1C). On the basis of the above studies, we propose that Sirt3 is well poised to mediate the balance between energy generation and ROS scavenging. A loss of or decrease in Sirt3 activity results in increased mitochondrial ROS, due to decreased ROS detoxification or hyperacetylation of proteins such as MnSOD (16, 17), and creates, via the inefficient electron transport chain function (11–13), a cellular environment that favors the development of genomic instability. This model is well supported by 3 studies (11–13) in which ROS-scavenging agents were shown to prevent \textit{in vitro} transformation, as measured by contact inhibition, proliferation, and tumor cell growth in soft agar and nude mice. These observations are consistent with studies (1, 2) showing that abnormal levels of cellular ROS exert a strong genotoxic stress that, over time, results in genomic instability. Thus, increased ROS in cells lacking Sirt3 plays a role in the establishment of a DNA-damage–permissive cellular phenotype.

Although it has been proposed that increased ROS and genomic instability are potential early events in tumorigenesis, it is clear that additional genetic promoting events are also required to induce proliferation. It has been suggested that the hallmarks of carcinogenesis (Fig. 1C) comprise a series of biological capabilities that are acquired via a multistep dedifferentiation pathway (21). In this regard, loss of Sirt3 stabilizes HIF-1$\alpha$ via a ROS-dependent mechanism, resulting in the reprogramming of cellular metabolism that may be one such promoting event. The subsequent increase in HIF-1$\alpha$ increases the expression of a series of HIF-1$\alpha$–dependent proproliferative and pro-survival genes/proteins (Fig. 1B and C). Over time, cell proliferation expands the pool of cells and allows for the eventual selection of one or more cells with additional genetic mutations, leading to the activation of oncogenes and/or inactivation of tumor suppressors. Thus, the combination of genomic instability and proliferation results in a promutation and prodivison phenotype that may allow an individual cell with the necessary carcinogenic combination of mutations to progress through several additional events that ultimately lead to a malignant cell phenotype (Fig. 1C).

Although the results reviewed here suggest a mechanism by which absent or decreased Sirt3 activity results in a HIF-1$\alpha$–dependent proproliferative phenotype, they do not \textit{a priori} explain the genomic instability observed in murine cells lacking Sirt3. One possible mechanism may be ROS leakage into the cytoplasm and/or nucleus. These oxidative ROS could physically interact with any of a number of cellular targets and damage cells by peroxidizing lipids, disrupting proteins, and/or inducing DNA damage, as well as by activating transformation driver gene mutations (1). Alternatively, increased cellular ROS may induce prometabolism and/or proliferative signaling networks that activate downstream oncogenes that are transformation driver proteins. Although these and other mechanisms are possible, it is clear that conditions that scavenge ROS, such as exposure to N-acetylcysteine or enforced MnSOD expression, reverse both the increased cellular ROS levels and the tumor-permissive phenotype.

\textbf{Conclusions}

Investigations of the mechanism of carcinogenesis in mice lacking Sirt3 have resulted in several important observations regarding the mechanistic connection between aberrant mitochondrial metabolism and carcinogenesis: (i) Sirt3 regulates HIF-1$\alpha$ activity, resulting in an altered cellular metabolism that supports cell proliferation; (ii) Sirt3 directly regulates MnSOD enzymatic activity via acetylation, and the aberrant acetylation of MnSOD in cells lacking Sirt3 increases cellular ROS; and (iii) Sirt3 knockout mice develop estrogen-positive mammary tumors. Furthermore, \textit{Sirt3} expression is decreased in many different types of human cancers, and heterozygous loss of \textit{SIRT3} occurs in 40% of human breast malignancies, suggesting that knockout mice are the first murine model for the most common subtype of breast cancer observed in older, postmenopausal women. However, if this is the case, why is spontaneous tumorigenesis found only in mammary tissue? Although this could be due, at least in part, to functional redundancy of different members of the sirtuin family, especially those localized to the mitochondrial, recent studies have shown that the deacetylase activity of sirtuins can be changed under the influence of different forms of cellular stress (11–13, 16, 17). Thus, it is plausible that the tumor-suppressor functions of Sirt3 in other cell types may only become obvious under various stress conditions and/or after the accumulation of different oncogenic driver mutations. In conclusion, these new findings reveal several therapeutic opportunities, including the identification of potential molecular targets and biomarkers to determine tumor risk, and the development of agents that can selectively inhibit ROS-dependent aberrant cellular signaling networks in cancer.

\textbf{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

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