Cancer Cell
Article

SIRT3 Is a Mitochondria-Localized Tumor Suppressor Required for Maintenance of Mitochondrial Integrity and Metabolism during Stress

Hyun-Seok Kim,[1,7] Krish Patel,[2,7] Kristi Muldoon-Jacobs,[2] Kheem S. Bisht,[2] Nukhet Aykin-Burns,[3] J. Daniel Pennington,[2] Riet van der Meer,[4] Phuongmai Nguyen,[2] Jason Savage,[2] Kjerstin M. Owens,[6] Athanassios Vassilopoulos,[1] Ozkan Ozden,[2] Seong-Hoon Park,[2] Keshav K. Singh,[6] Sarki A. Abdulkadir,[4] Douglas R. Spitz,[3] Chu-Xia Deng,[1,*] and David Gius[2,5,*]

[1]Genetics of Development and Disease Branch, NIDDK, NIH, Bethesda, MD 20892, USA
[2]Molecular Radiation Oncology, Radiation Oncology Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892, USA
[3]Free Radical and Radiation Biology Program, Department of Radiation Oncology, University of Iowa, Iowa City, IA 52242, USA
[4]Department of Pathology
[5]Department of Radiation Oncology
Vanderbilt University Medical Center, Nashville, TN 37232, USA
[6]Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA
[7]These authors contributed equally to this work
*Correspondence: chuxiad@mail.nih.gov (C.-X.D.), david.gius@vanderbilt.edu (D.G.)
DOI 10.1016/j.ccr.2009.11.023

SUMMARY

The sirtuin gene family (*SIRT*) is hypothesized to regulate the aging process and play a role in cellular repair. This work demonstrates that SIRT3$^{-/-}$ mouse embryonic fibroblasts (MEFs) exhibit abnormal mitochondrial physiology as well as increases in stress-induced superoxide levels and genomic instability. Expression of a single oncogene (*Myc* or *Ras*) in SIRT3$^{-/-}$ MEFs results in in vitro transformation and altered intracellular metabolism. Superoxide dismutase prevents transformation by a single oncogene in SIRT3$^{-/-}$ MEFs and reverses the tumor-permissive phenotype as well as stress-induced genomic instability. In addition, SIRT3$^{-/-}$ mice develop ER/PR-positive mammary tumors. Finally, human breast and other human cancer specimens exhibit reduced *SIRT3* levels. These results identify *SIRT3* as a genomically expressed, mitochondria-localized tumor suppressor.

INTRODUCTION

An emerging theme in aging research is that sirtuin genes appear to regulate longevity in a wide variety of living systems from yeast to mammals (Sinclair, 2005; Tissenbaum and Guarente, 2001). Sirtuin genes are the human and murine homologs of the *Saccharomyces cerevisiae Sir2* gene, which has been shown to regulate both replicative and overall life span (Guarente and Kenyon, 2000). The sirtuin genes are also central to the regulation of longevity in *C. elegans* and *D. melanogaster* (Rogina and Helfand, 2004). The mammalian sirtuin family consists of seven NAD+-dependent protein deacetylases that are localized to the nucleus (*SIRT1*, *SIRT6*, and *SIRT7*), mitochondria (*SIRT3*, *SIRT4*, and *SIRT5*), and cytoplasm (*SIRT2*), respectively, and that regulate a wide range of intracellular process (Haigis and Guarente, 2006).

The incidence of human malignancies increases exponentially as a function of aging, suggesting a mechanistic connection between aging (longevity) and carcinogenesis (Finkel et al., 2009). Mammalian cells contain fidelity proteins or tumor

SIGNIFICANCE

The incidence of human malignancies increases significantly with age, suggesting a mechanistic connection between aging (longevity) and carcinogenesis. One aspect of that connection is impaired mitochondrial function, which is observed in both aging cells and cancer cells as aberrant oxidative metabolism. Sirtuin family genes regulate longevity in yeast, *C. elegans*, and *D. melanogaster*, and in mammals, three of the seven sirtuin genes are localized to the mitochondria, including *SIRT3*. These observations led us to hypothesize that *SIRT3* might be a tumor suppressor that protects against carcinogenesis by maintaining mitochondrial integrity and efficient oxidative metabolism. The current work demonstrates that the loss of function of SIRT3 results in a cellular environment permissive for carcinogenesis and characterized by aberrant oxidative metabolism.

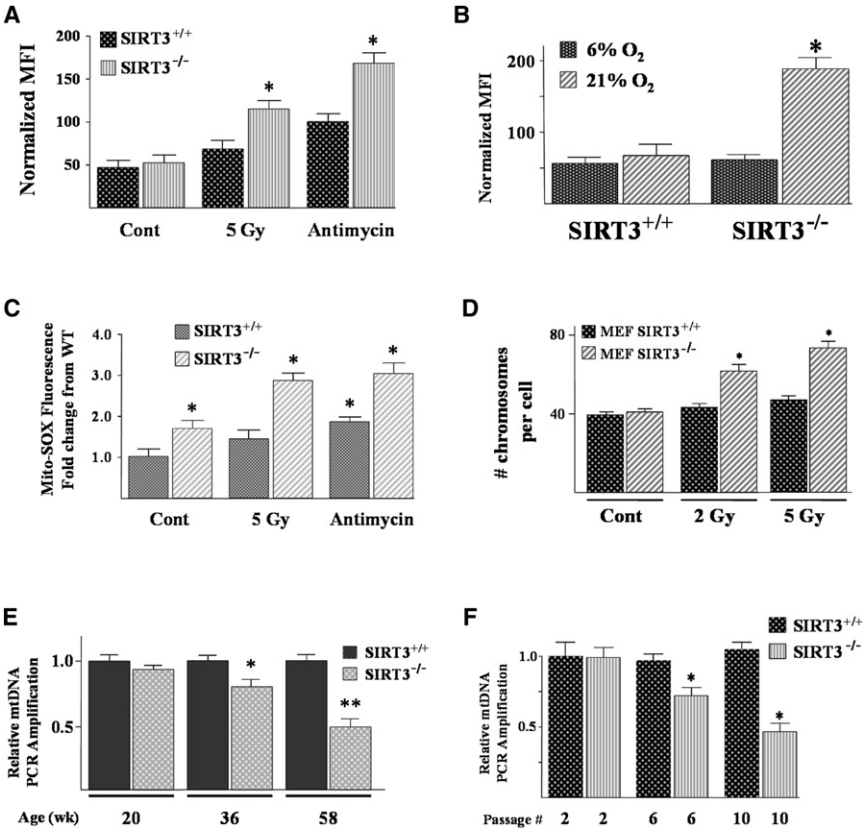

Figure 1. *SIRT3* Knockout MEFs Exhibit Increased Superoxide Levels, Aneuploidy in Response to Exogenous Stress, and Decreased Mitochondrial Integrity with Increasing Age

(A) Superoxide levels were elevated in *SIRT3* knockout cells exposed to agents that induce mitochondrial damage. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs were cultured in 6% oxygen and exposed to either 5 Gy of IR or 5 μM antimycin A for 3 hr, and superoxide levels were monitored by DHE oxidation as compared to control, untreated cells (Cont). For all DHE oxidation experiments, the results were the normalized mean fluorescence intensity (MFI) for three independent replicates.

(B) $SIRT3^{-/-}$ superoxide levels were elevated when cultured in 21% oxygen. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEF cells were cultured at 21% O_2 for 6 hr, and superoxide levels were monitored by DHE oxidation, as compared with control cells grown at 6% O_2.

(C) Mitochondrial superoxide levels are elevated in *SIRT3* knockout MEFs and increase following exogenous stress. Mitochondrial superoxide levels were determined by the addition of Mito-SOX (3 μM) to the culture medium and cells were incubated for an additional 10 min before being trypsinized and resuspended. Fluorescence was measured via flow cytometry, and 20,000 and 40,000 cells were counted for each sample.

(D) *SIRT3* knockout MEFs exhibited aneuploidy following exposure to IR. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs were exposed to either 2 or 5 Gy. Whole-mount chromosomes were counted in a blinded fashion. Bars show the mean chromosome number per cell from 100 separate counts.

(E) Livers from *SIRT3* knockout mice have increased mtDNA damage with age. DNA was isolated from the livers of *SIRT3* wild-type and knockout mice at 20, 36, and 58 weeks, and mtDNA primers that amplify either the 10 kb amplicon or a 117 bp region (Figure S4A) were used for PCR. Primers to the genomic β-globin gene were used as a control.

(F) *SIRT3* knockout MEFs have decreased mtDNA integrity. DNA was isolated from $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs at passage 2, 6, and 10, and mtDNA primers that amplify either the 10 kb amplicon used for PCR. All the results in this figure are from at least three separate experiments. Data are presented as the average ± SD; *$p < 0.05$ by t test. See also Figure S1.

suppressor (TS) genes, such as p53, and loss of function of these proteins results in a damage-permissive cell phenotype (Sherr, 2004). As such, the loss of function of these fidelity proteins is considered an early event in carcinogenesis. Because cancer is a disease of aging, and sirtuin genes appear to play a role in repair of cellular damage during aging, it is reasonable to propose that sirtuin genes may also have an anticarcinogenic role and function as TSs (Saunders and Verdin, 2007; Wang et al., 2008). If so, it follows that loss of function of sirtuin genes may contribute to a tumor-permissive phenotype (Deng, 2009).

It has also been suggested that the mitochondria play a central role in aging and carcinogenesis by generating reactive oxygen species as a byproduct of respiration (Singh, 2006). Mitochondrial abnormalities associated with altered oxidative metabolism are observed in tumor cells in vitro and in vivo and appear to contribute to a chronic condition of oxidative stress (Hsu and Sabatini, 2008). SIRT3 is one of the three genomically expressed sirtuins that localize to mitochondria (Onyango et al., 2002; Schwer et al., 2002) and is the primary mitochondrial protein deacetylase (Lombard et al., 2007). In this regard, it is proposed that SIRT3 is ideally situated to function as a mitochondrial fidelity protein, and by extension, loss of function could result in a damage-permissive and tumorigenic cellular environment.

RESULTS

SIRT3 Knockout MEFs Exhibit Increased Superoxide Levels and Chromosomal Instability in Response to Exogenous Stress

We have previously shown that HCT116 cells genetically altered to express a deacetylation-null mutant *SIRT3* gene ($SIRT3^{dn}$) have difficulty responding to increased reactive oxygen species (Jacobs et al., 2008). In addition, it has previously been shown that $SIRT3^{-/-}$ livers and MEFs have decreased total ATP levels and mitochondrial respiration (Ahn et al., 2008). As such, steady-state levels of superoxide were determined in $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs by following the oxidation of dihydroethidium (DHE) as mean fluorescence intensity. No differences in total cellular DHE oxidation levels were seen between the wild-type and *SIRT3* knockout MEFs that are cultured in 6% oxygen for these studies unless otherwise stated (Figure 1A). However,