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Research Paper

Absence of manganese superoxide dismutase delays p53-induced tumor formation ☆

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ABSTRACT

Background: Manganese superoxide dismutase (MnSOD) is a mitochondrial antioxidant enzyme that is down-regulated in a majority of cancers. Due to this observation, as well as MnSOD's potent antioxidant enzymatic activity, MnSOD has been suggested as a tumor suppressor for over 30 years. However, testing this postulate has proven difficult due to the early post-natal lethality of the MnSOD constitutive knock-out mouse. We have previously used a conditional tissue-specific MnSOD knock-out mouse to study the effects of MnSOD loss on the development of various cell types, but long-term cancer development studies have not been performed. We hypothesized the complete loss of MnSOD would significantly increase the rate of tumor formation in a tissue-specific manner.

Results: Utilizing a hematopoietic stem cell specific Cre-recombinase mouse model, we created pan-hematopoietic cell MnSOD knock-out mice. Additionally, we combined this MnSOD knock-out with two well established models of lymphoma development: B-lymphocyte specific Myc over-expression and conditional pan-hematopoietic cell p53 knock-out. Mice were allowed to age unchallenged until illness or death had occurred. Contrary to our initial hypothesis, the loss of MnSOD alone was insufficient in causing an increase in tumor formation, but did cause significant life-shortening skin pathology in a strain-dependent manner. Moreover, the loss of MnSOD in conjunction with either Myc overexpression or p53 knock-out did not accelerate tumor formation, and in fact delayed lymphomagenesis in the p53 knock-out model.

Conclusions: Our findings strongly suggest that MnSOD does not act as a classical tumor suppressor in hematological tissues. Additionally, the complete loss of MnSOD may actually protect from tumor development by the creation of an unfavorable redox environment for tumor progression. In summary, these results in combination with our previous work suggest that MnSOD needs to be tightly regulated for proper cellular homeostasis, and altering the activity in either direction may lead to cellular dysfunction, oncogenesis, or death.

Introduction

In 1979, Larry Oberley and Garry Buettner outlined the free radical theory of oncogenesis [1]. The theory stemmed from numerous observations that the superoxide dismutase class of enzymes, particularly the mitochondrial isoform manganese superoxide dismutase (MnSOD), was shown to be expressed at low levels in cancers of various cell types compared to their normal tissues of origin. The theory frameworks three predictions in regards to the role of MnSOD and cancer: (1) all cancers should possess decreased amounts of MnSOD activity compared to their normal tissue counterparts, (2) replacement of MnSOD in tumor cells should abrogate the malignant phenotype, and (3) normal cells that lose MnSOD will in turn become malignant. With very few exceptions, the first two postulates have overwhelming evidence demonstrating their validity in regards to MnSOD and cancer. In contrast, the third postulate defines MnSOD as a genuine tumor suppressor protein, and is the only postulate that remains unanswered to date.

The major limitation in addressing the third prediction has been a lack of a viable MnSOD knock-out model. Two separate constitutive MnSOD knock-out mice strains have been developed, and both demonstrate early post-natal lethality due to overt

Abbreviations: MnSOD, manganese superoxide dismutase; FoxO, Forkhead family of transcription factors

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oxygen toxicity and system-wide organ failure [2,3]. These observations have elucidated the critical function of MnSOD in mammalian development, but do not address the role of MnSOD in oncogenesis. To date, the most convincing evidence that MnSOD acts as a tumor suppressor protein is from MnSOD heterozygous mice. While these mice possess the same lifespan of wild-type mice, they have significantly more cancer (primarily lymphoma) at the end of their life [4]. While this evidence proposes a compelling argument for MnSOD as a tumor suppressor protein, the heterozygous mice still retain at least 50% MnSOD activity, and thus have not addressed the hypothesis that complete loss of MnSOD initiates tumor formation in a dose-dependent fashion and therefore acts as a classical tumor suppressor protein.

In 2002, Ikegami et al. created a conditional MnSOD knock-out mouse utilizing Cre/loxP technology [5]. Since this time, our group has been at the forefront of characterizing the effects of tissue-specific loss of MnSOD in an array of cell types [6–9]. We have observed significant phenotypes due to the loss of MnSOD including severe immunodeficiency (T-lymphocytes), aberrant iron homeostasis (pan-hematopoietic cells), and epigenetic dysregulation (liver) in several models we have developed [7–9]. To our surprise, the complete loss of MnSOD in a tissue specific manner alone did not cause a significant increase in tumor formation in any of the animal models when aged [5–8]. These data argue that MnSOD does not act as a tumor suppressing protein in regards to the initiation of oncogenesis, and is in direct contradiction with the third prediction of the free radical theory of cancer. Therefore, we hypothesized that MnSOD alone does not act as a classical tumor suppressor, but the loss of MnSOD may exacerbate the formation of cancer in an established model of oncogenesis. To address this hypothesis, we crossbred our mouse model of conditional pan-hematopoietic cell MnSOD knock-out with two respective mouse models of lymphoma development to understand if the loss of MnSOD accelerated the formation of cancer. We show for the first time that the loss of MnSOD either had no effect or prolonged lymphoma development in the respective models. Taken together, these data strongly suggest that the loss of MnSOD is likely not a casual event in the initiation of cancer, but instead may be down-regulated in fully initiated cancer cells to aid in tumor progression.

Methods

Mice homozygous for the floxed MnSOD allele (*i.e.* B6.Cg-*Sod2*^{tm1}, shorthand MnSOD$^{L/L}$) were initially bred to mice expressing Cre-recombinase under the control of the vav promoter (*i.e.* B6.Cg-Tg-*Vav1-iCre*^{A2Kio}/J, shorthand vav-Cre mice, Jackson Laboratories) to create hematopoietic stem cell specific knock-outs of MnSOD [7,10]. These mice were sequentially bred to either an oncogenic model of splenic lymphoma (*i.e.* B6.Cg-Tg-*iMyc*$^{E\mu}$, shorthand iMyc$^{+/-}$) or a conditional tumor suppressor knock-out model of thymic and splenic lymphoma (*i.e.* FVB.129-*Trp53*^{tm1Brn}, shorthand p53$^{L/L}$, NCI-Frederick Mouse Repository) [11,12]. In all experiments, littermate controls were used to limit the effects of genetic variation amongst strains. Upon weaning, mice were analyzed by tail DNA to confirm appropriate genotype. Mice were observed until death with no additional challenges. Upon death or illness, all mice were examined by full necropsy and cause of death recorded. Kaplan-Meier with Log-Rank analysis was performed and non-cancer deaths were appropriately censored. Causes of death were compared between groups and analyzed by unpaired two-tail Student's *t*-test. A p value of less than 0.01 was considered significant. All work was performed under the approval of the Institutional Animal Care and Use Committee at the University of Iowa.

Results and discussion

We first examined the effects of MnSOD loss in an oncogenic model of splenic lymphoma. We have previously demonstrated that our hematological stem cell MnSOD knock-out is devoid of MnSOD and possesses increased mitochondrial oxidative stress in all hematological tissues such as bone marrow, spleen, and thymus [7]. By combining our hematological stem cell knock-out of MnSOD (MnSOD$^{L/L}$ vav-Cre$^{+/-}$) with a model of conditional B-lymphocyte Myc over-expression (iMyc$^{+/-}$), we assessed the rate in which these animals developed splenic lymphoma. One-hundred percent of mice possessing MnSOD and the iMyc allele (MnSOD$^{L/L}$ vav-Cre$^{-/-}$ iMyc$^{+/-}$) developed splenic lymphoma with a mean tumor free-survival time of approximately 45 weeks (Fig. 1A, Table 1). Surprisingly, the loss of MnSOD in conjunction with Myc over-expression (MnSOD$^{L/L}$ vav-Cre$^{+/-}$ iMyc$^{+/-}$) did not significantly affect the rate of tumor formation (Fig. 1A, Table 1). These data suggest the loss of MnSOD does not act to promote tumor initiation in a Myc-driven model of B-cell lymphoma.

It was observed that the loss of MnSOD alone (MnSOD$^{L/L}$ vav-Cre$^{+/-}$ iMyc$^{-/-}$) did not significantly increase the incidence of any type of hematological malignancy (Fig. 1A, Table 1). In contrast, while malignancy was not increased in these animals, significant life-shortening severe dermatitis was observed (Table 1). We have previously reported that the MnSOD$^{L/L}$ vav-Cre$^{+/-}$ suffer from stark anemia, porphyria, and potential immunodeficiency [7,8]. At this time we cannot rule out any of these pathologies in the contribution to this skin pathology. Porphyria is a significant contributor to skin pathology, as porphyrin rings (macrocycles) are not properly metabolized and building up in dermal tissues [13]. In our MnSOD knock-out model, macrocycle build-up may be a major contributor to the skin pathology, but further studies are needed to confirm the

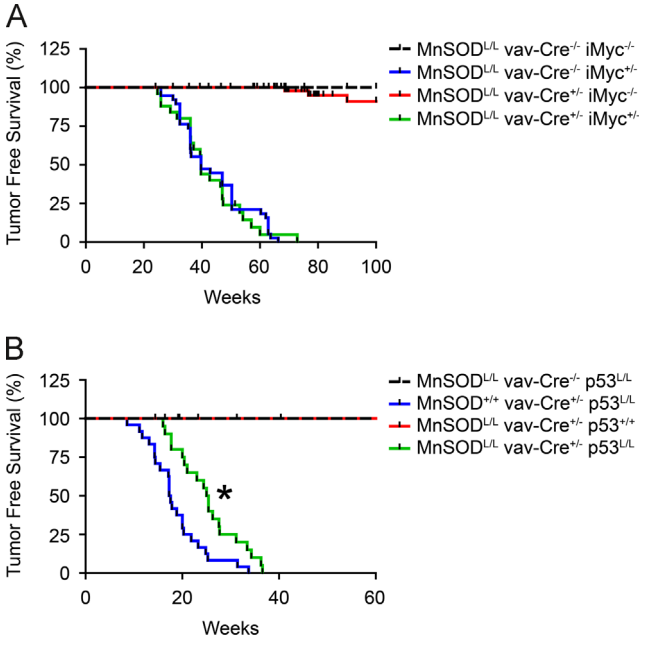

Fig. 1. MnSOD loss prolongs tumor development in a p53 knock-out cancer model. (A) Conditional hematopoietic MnSOD knock-out mice (MnSOD$^{L/L}$ vav-Cre$^{+/-}$) were crossed with mice over-expressing Myc targeted specifically to B-lymphocytes (iMyc$^{+/-}$). Survival studies demonstrate no significant changes in tumor formation with the loss of MnSOD in combination with Myc over-expression. (B) Conditional MnSOD knock-out mice (MnSOD$^{L/L}$) were crossed with conditional p53 knock-out mice (p53$^{L/L}$). Further breeding to the hematopoietic stem cell driven Cre-recombinase mouse (vav-Cre$^{+/-}$) creates double conditional knock-outs for MnSOD and p53 targeted to hematopoietic tissues. Survival studies demonstrate the loss of MnSOD prolongs tumor approximately 10 weeks. Hash marks above lines indicate censored non-tumor deaths. *$p < 0.01$ by Log-Rank analysis.