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Original article

Mitochondrial catalase induces cells transformation through nucleolin-dependent *Cox-2* mRNA stabilization

Xin Liao[a,b,1], Chao Huang[a,1], Dongyun Zhang[a,1], Jingjing Wang[a,c], Jingxia Li[a], Honglei Jin[c], Chuanshu Huang[a,*]

[a] Nelson Institute of Environmental Medicine, New York University, School of Medicine, Tuxedo Park, NY 10987, USA
[b] Department of Geriatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
[c] Zhejiang Provincial Key Laboratory for Technology & Application of Model Organisms, School of Life Sciences, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China

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ABSTRACT

It's well documented that over-production of reactive oxygen species (ROS) causes detrimental damages to cells. While a low level of ROS, such as H_2O_2, functions as signaling transducer and motivates cell proliferation in both cancer and non-transformed stem cells. As a double-edged sword, the direct evidence for demonstrating the function of H_2O_2 in the cause of tumor is barely characterized in intact cells. In our current study, we found that targeted expression of mitochondrial catalase (mCAT), but not catalase, could significantly reduce the accumulation of H_2O_2 in mouse epithelial JB6 Cl41 cells, consequently led to the cell malignant transformation and anchorage-independent cell growth. Further study revealed that this reduction of H_2O_2 resulted in the translocation of nucleolin from the cytoplasm to nuclear, and maintaining the nucleolin nuclear location status, and in turn stabilizing the *cox-2* mRNA and consequently leading to a COX-2 protein upregulation, as well as malignant transforming mCAT-overexpressed Cl41 cells. Collectively, our studies here provide direct experimental evidence demonstrating a novel function and molecular mechanisms of mCAT in transforming mouse Cl41 cells, and high significance insight into understanding the beneficial aspect of H_2O_2 in circumventing tumor promotion and the theoretical basis for the management of H_2O_2 in the clinic implementation as a chemotherapeutic strategy.

1. Introduction

Reactive oxygen species (ROS) is constantly produced in aerobic organisms through normal oxygen metabolism [1]. Although ROS are generated in many intracellular compartments by multiple enzymes, the mitochondrial respiratory chain is the major steady source of ROS production [2]. Superoxide anion radical ($O_2^{\bullet -}$) is formed when oxygen molecule accepts one electron during auto-oxidation of reduced flavin components of NADH dehydrogenase (in complex I) and energy transfer from reduced ubisemiquinone to molecular oxygen (in complex III) [2]. The prematurely formed $O_2^{\bullet -}$ in the inside of mitochondria has a very short half-life due to the rapid conversion into hydrogen peroxide (H_2O_2) by matrix space localized mitochondrial superoxide dismutase (MnSOD) [3]. Catalase (CAT) and glutathione peroxidase (GPx) together with small antioxidant molecules, such as glutathione (GSH), vitamin C and E, are the main salvage enzymes that reduce H_2O_2. Although CAT can efficiently catalyze H_2O_2 to H_2O and oxygen, it is mainly located at peroxisome in the cytoplasm in most types of cells, leaving GPx as the major scavenger in mitochondria to deal with H_2O_2 reduction. However, the activity of GPx relies on the viability of GSH, which needs regeneration from GSSG by flavoenzyme GSH reductase and NADPH [4]. This limitation makes GPx-GSH system inefficient to remove H_2O_2. The accumulated H_2O_2 in the inside of mitochondria can readily interact with $O_2^{\bullet -}$ to produce extremely reactive hydroxyl radical or singlet oxygen through Haber-Weiss reaction [1]. When the production of ROS outstrips the scavenge capability of the antioxidant systems, the detrimental damages will be cast on bio-molecules, such as mitochondrial DNA and membrane, resulting in dysfunction of respiratory chain system, insufficient energy production, and even cell death [5,6].

In order to ameliorate H_2O_2 accumulation in mitochondria, targeted expression of CAT to mitochondria (mCAT) has been experimentally introduced into both animal models and cultured cells [7–9]. Ectopic expression of mCAT has been proven to prevent lung fibrosis [9],

* Correspondence to: Nelson Institute of Environmental Medicine, New York University, School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA.
E-mail address: chuanshu.huang@nyumc.org (C. Huang).
[1] First three authors contributed equally to this work.

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extend animal lifespan [10], enhance exercise performance of mice [11], prevent age-associated whole-body energy imbalance and muscle insulin resistance [12], protect insulin-producing cells against oxidative attack caused by proinflammatory cytokines [13], and prevent radiation-induced cognitive dysfunction [14]. Therefore, it is anticipated that expressing catalase in mitochondria improves cellular defense against oxidative injury, and it might promise an effective strategy to ameliorate the oxidative damage.

Although it's well documented that over-production of ROS causes detrimental damages to cells [15], low levels of ROS, such as H_2O_2, also affect cellular signaling and proliferation in both cancer and non-transformed stem cells [16]. Inactivation of phosphatases and activation of tyrosine kinases or redox-sensitive transcription factors by reversible oxidation of cysteine residues are thought to be three principal mechanisms in this modulation [16]. In addition, H_2O_2 functions as important second messengers in regulating the physiological activity of macrophages during wound healing and phagocytic respiratory burst in response to pathogen invasion at even relatively high levels [1]. Therefore, the double-edged sword nature of H_2O_2 is dependent on its concentration, cell types as well as stimulation essence.

Neoplastic transformation is the conversion of normal cells into tumor cells, which involves a series of genetic and epigenetic alterations. Using fluorescent light exposure as an inducer, Tarone et al. show that addition of catalase into culture medium can prevent photo-product initiated cell transformation by alleviating H_2O_2-originated chromatid damage [17], suggesting that catalase has a protective role in the initiation stage of transformation by attenuating H_2O_2-induced genetic damages. However, up to now, there is no report to define the role of mitochondrial H_2O_2 itself in cell transformation. Mouse Balb/C JB6 Cl41 cell line is a well-characterized model for neoplastic transformation in response to tumor promoters [18,19]. Distinct from several other rodent cells in culture which undergo spontaneous neoplastic transformation due to chromosomal aberrations, the Cl41 cell model is stably free of spontaneous transformation and responsive to tumor promoters [20]. Therefore this model was chosen to exploit the effect of H_2O_2 in the mediation of tumor promotion by ectopic expression of mCAT in the current study.

Cyclooxygenase-2 (COX-2, also known as the Prostaglandin endoperoxide synthase 2, PTGS-2) is an inflammatory response enzyme and has been found to be up-regulated in many cancers, whereas overexpressed COX-2 in cancer cells plays essential roles in promoting stem cell renewal, proliferation and differentiation [21–24]. COX-2 is also reported to promote the tumorigenecity in pancreatic cancer [25], and inhibition of COX-2 results in suppression of the cell motility and metastasis of prostate cancer [26]. In the current study, by using anchorage-independent growth as the indicator to assess transformation capability, we were unexpected to observe that ectopic expression of mCAT in Cl41 cells significantly initiated cell transformation through up-regulation of COX-2 expression. The mechanistic investigation further revealed that expression of mCAT leading to a translocation of nucleolin from the cytoplasm to nuclear, which stabilized the COX-2 mRNA.

2. Results

2.1. Targeted expression of mitochondrial catalase (mCAT) remarkably promoted cell transformation in Cl41 cells

Although the dichotomous role of H_2O_2 has been widely investigated in both normal and tumor cells, its direct association in neoplastic transformation is barely explored. Here we investigated effect of H_2O_2 by stably introduction of CAT or mCAT into Cl41 cells. The stable transfectants of CAT and mCAT were identified by determination of their protein expressions (Fig. 1A). Anchorage-independent growth is one of the hallmarks of transformation, which is considered as an accurate and stringent in vitro assay for characterization of cell malignant transformation. Therefore, CAT, mCAT, and their scramble control vector transfectants were subjected to soft agar assay to assess their capabilities in anchorage-independent growth. As shown in Figs. 1B-1E, in a continuous observation, there was no colonies observed in the scramble control vector transfectants throughout of experiments, confirming that Cl41 cells were steadily free of spontaneous transformation, whereas remarkable colonies were observed in mCAT transfectants as early as 3 days after the cells were seeded into soft agar and kept persistent growth until 7 days during our observation period (Figs. 1B-1C). In contrast to mCAT, overexpression of CAT contributes nothing to colonies formation under same experimental conditions (Figs. 1D and 1E). Our results demonstrate that in contrast to the inhibitory effect in the genetic instability associated initiation of cell transformation [17], mCAT alone was strong enough to initiate the promotion stage of cell transformation, corroborating the beneficial aspect of H_2O_2 in circumventing tumor promotion.

Distinct from normal cells whose proliferation is dependent on both external and internal growth signals, transformed cells show reduced requirements for extracellular growth promoting factors, and are not restricted by cell-cell contact or anchorage support. Given the fact that anchorage-independent growth mirrors the in vivo endothelial cell detachment and loss of cell-matrix contact, under which conditions the intracellular ROS levels are increased due to integrin signalization [27], we hypothesize that when the normal cells are grown in suspension in soft agar, they suffer from enhanced H_2O_2 level so as to halt their cell proliferation cycle; whereas mCAT expression alleviates H_2O_2 level, reliefs the cell growth hamper and finally leads to neoplastic transformation. To test this hypothesis, we examined the reduction of H_2O_2 in CAT, mCAT, and their control transfectant. As shown in Fig. 1F, overexpression of mCAT had a dramatical reduction of H_2O_2 (10.96% vs 50.35%), while CAT only showed a slightly reduction of H_2O_2 (43.73% vs 50.35%), which is more clear-cut shown in the composite graph of Fig. 1G. These results indicate that the mitochondrial H_2O_2 plays a crucial role in inhibiting tumor initiation, and the reduced accumulation of mitochondrial H_2O_2 is a strong driving force for transforming of normal epithelial cells.

2.2. Cell transformation by mCAT attributed to its upregulation of COX-2 expression in Cl41 cells

As mCAT, not CAT, can significantly transform Cl41 cells, mCAT and its control transfectants, were utilized for further mechanistic studies. Several key molecules that have been proven essential for cell transformation, including cell cycle regulator Cyclin D1 [28] as well as proinflammatory factors, Cyclooxygenase-2 (COX-2) [29], and tumor necrosis factor α (TNFα) [30] were detected. As shown in Fig. 2A, elevated expression of COX-2, but not Cyclin D1 or TNFα was observed in mCAT transfectants as compared to scramble control cells, revealing that COX-2 rather than Cyclin D1 or TNFα might be involved in mCAT-mediated cell transformation. To this end, we employed COX-2 chemical inhibitors, NS398 and indomethacin. As shown in Figs. 2B and 2C, inhibition of COX-2 by NS398 profoundly reduced colony formation in mCAT transfectants in a dose-dependent manner. Similar results were also obtained by using another COX-2 inhibitor, indomethacin (Figs. 2D and 2E). We further employed molecular approach, knocking down COX-2 by using two sets of shRNAs in mCAT transfectants, to reinforce the findings obtained using chemical inhibitor. The identification of stable transfectants of shRNA COX-2 in mCAT cells was shown in Fig. 2F. Consistent with results obtained from using COX-2 chemical inhibitor, knocking down of COX-2 by both sets of shRNA almost completely reversed cell transformation potential due to mCAT overexpression (Figs. 2G and 2H), strongly indicating that mCAT-mediated promotion of cell transformation was reversible and dependent on COX-2 induction in Cl41 cells. Taken together, these results clearly prove that COX-2 is one of major factor responsible for mCAT-induced cell transformation.