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Silica nanoparticle releases SIRT6-induced epigenetic silencing of follistatin

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ABSTRACT

Follistatin (FST) plays a protective role during silica nanoparticle (SiO_2 NP) exposure. SiO_2 NP treatment induces FST transcription with an unknown mechanism. We herein reported that SIRT6, one of the sirtuin family members, induced epigenetic silencing of FST. The expression of FST was elevated after SIRT6 knockdown while reduced after SIRT6 overexpression. Chromatin immunoprecipitation revealed a direct interaction between SIRT6 with FST promoter. Knockdown of SIRT6 increased both Ac-H3K9 level and Ac-H3K56 level at FST promoter region. SiO_2 NP treatment de-stabilized SIRT6 mRNA and reduced SIRT6 expression, leading to the activation of FST transcription. Finally, over-expression of SIRT6 increased SiO_2 NP-induced apoptosis. Collectively, this study provided evidence that SIRT6 is a negative regulator of FST transcription and participates in the regulation of cell survival during silica nanoparticle exposure.

1. Introduction

Nanoparticles (NPs) are engineered structures with less than 100 nanometers in at least one dimension, among which silica nanoparticle (SiO_2 NP) is one of the most widely applied (Li et al., 2012; Nel et al., 2006). The wide use of SiO_2 NP has raised serious concerns about their safety for human health. The respiratory system is one of the main routes by which SiO_2 NPs access human body (Nel et al., 2006; Nel et al., 2009; Oberdorster, 2010). SiO_2 NP exposure induces the generation of reactive oxygen species (ROS) in airway epithelial cells and macrophages, which damages cellular proteins, lipids, and DNA (Brown et al., 2014; Nel et al., 2006). The elevated ROS triggers cellular oxidative stress responses which finally induce the expression of protective proteins including antioxidant enzymes, detoxification enzymes, and stress-response proteins (Ma, 2013). These SiO_2 NP-responsive proteins exert antioxidant and cytoprotective effects in respiratory system (Ma, 2013; Nel et al., 2006). However, besides a few Nrf2 target genes, the regulation of other SiO_2 NP-responsive genes and their functions in SiO_2 NP response remain to be identified.

Follistatin (FST) is widely expressed in higher animals and participates in a variety of processes such as cell growth, development, differentiation, and secretion (Hemmati-Brivanlou et al., 1994). FST was firstly identified as a secretory protein that binds and inactivates transforming growth factor (TGF)-β family members including activin, bone morphogenetic proteins (BMPs), and myostatin. The TGF-β-like molecule-neutralizing effect of FST contributes to most of its functions (Amthor et al., 2004; Glister et al., 2004; Nakamura et al., 1990). Recent studies showed that FST also plays a protective role under oxidative stress (Lim et al., 2015). It was reported that activin induces endothelial cell oxidative stress and endothelial cell dysfunction, and these effects are mitigated by follistatin (Lim et al., 2015). Follistatin has also been reported to suppress BMP4-induced ROS production (Jiang et al., 2015). We have demonstrated that SiO_2 NP induced the expression of FST in mouse lung tissue as well as in human lung epithelial cells. The elevated FST inhibited SiO_2 NP-induced ROS production and cell apoptosis. Interestingly, the levels of Ac-H3(K9/18), an active gene marker, at FST promoter region was significantly increased during SiO_2 NP treatment, indicating an activation of FST transcription (Lin et al., 2016). However, the detailed mechanism underlying SiO_2 NP-induced FST transcription remains elusive.

Sirtuins are nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases that regulate lifespan in lower organisms. To date, seven mammalian homologues (SIRT1 ~ SIRT7) have been identified with varied subcellular localizations and enzymatic activities (Michishita et al., 2005). SIRT6, one of the sirtuins, is mainly localized in the nucleus of the cells. It deacetylates histones H3 at either lysine 9 (H3K9) or lysine 56 (H3K56) and represses its target gene expression (Michishita et al., 2008; Michishita et al., 2009; Yang et al., 2009). Through its deacetylation activity, SIRT6 regulates a variety of cellular processes including chromosome stability, inflammation, cell metabolism, apoptosis, and senescence.

Recent studies have shown that SIRT6 participates in cellular response to stresses. In mammalian cells subjected to oxidative stress SIRT6 is recruited to the sites of DNA double-strand breaks (DSBs) and

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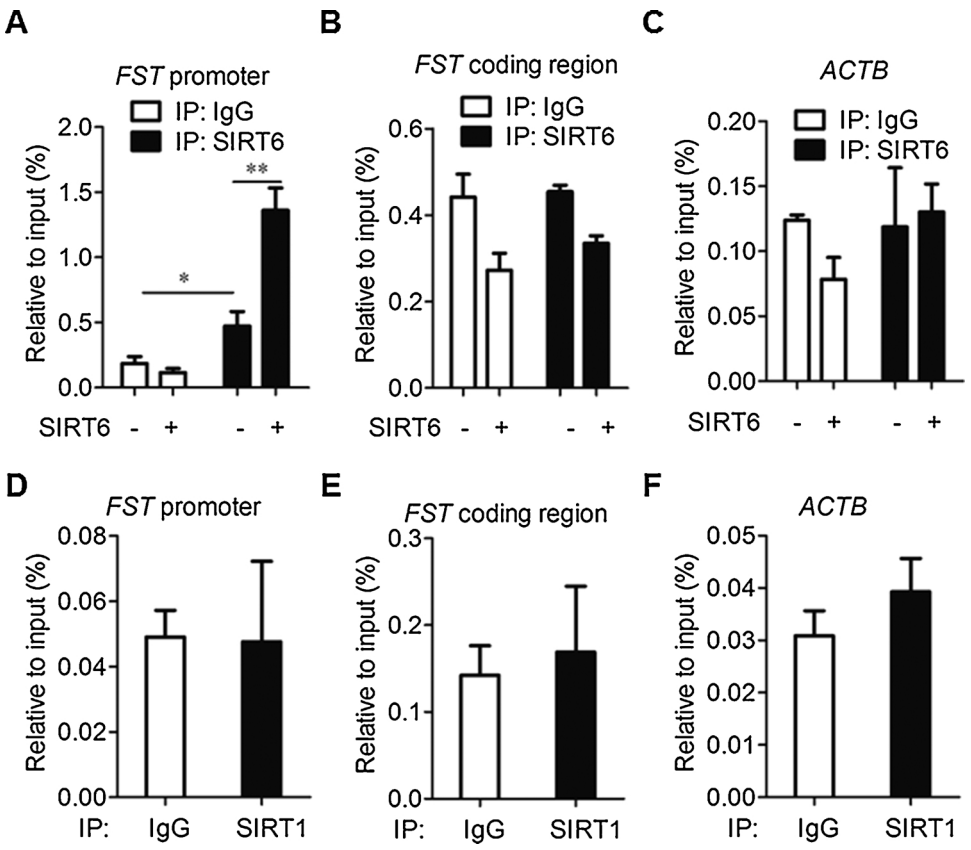

Fig. 1. SIRT6 binds to the promoter region of FST. (A,B,C) A549 cells infected with virus expressing SIRT6 or control vector. ChIP analysis was performed with antibodies against SIRT6 or control IgG and analyzed by qPCR. The occupancies of SIRT6 at FST promoter region (A), FST coding region (B), or ACTB gene (C) were normalized to the input DNA. The values are the mean ± SD of three independent experiments. $^{**}P < 0.01$. (D,E,F) ChIP analysis was performed with antibodies against SIRT1 or control IgG and analyzed by qPCR. The occupancies of SIRT1 at FST promoter region (D), FST coding region (E), or ACTB gene (F) were normalized to the input DNA.

stimulates DSB repair, through both nonhomologous end joining and homologous recombination (Mao et al., 2011). Knockout of SIRT6 in human mesenchymal stem cells (hMSCs) showed dysregulated redox metabolism and increased sensitivity to the oxidative stress. The function of SIRT6 in maintaining hMSC homeostasis relies on its interaction with Nrf2 and transactivation of NRF2-regulated antioxidant genes (Pan et al., 2016). SIRT6 is also involved in camptothecin-induced DNA damage response. Knockdown of SIRT6 up-regulates TRF2 protein levels and counteracts its down-regulation during DNA damage response, leading to cell survival (Rizzo et al., 2017). However, the function of SIRT6 under SiO_2 NP stress remains unidentified.

In this study, we evaluated the regulation of SIRT6 on FST expression and its role under SiO_2 NP stress. We found that SIRT6 negatively regulates FST through deacetylation of H3K9 and H3K56 at FST promoter. SiO_2 NP decreased SIRT6 expression, which accounts for the up-regulated FST level.

2. Materials and methods

2.1. Silica nanoparticles

SiO_2 nanoparticle was from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, United States). Nanoparticles were dispersed in double distilled water at the concentration of 10 mg/mL. Nanoparticles were sonicated at 200 W for 30 s prior to cell or mouse treatment.

2.2. Cells culture and nanoparticle treatment

Lung epithelial cells (A549) were obtained from ATCC and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, United States). Cells were maintained at 37 °C in an atmosphere containing 5% CO_2 and 100% humidity.

For SiO_2 NP treatment, cells were seeded in 6-well plates. After 24 h, cells were treated with silica nanoparticles suspended in RPMI-1640 supplemented with 1% fetal bovine serum at different concentration for indicated time and then subjected to further experiments.

2.3. Plasmids

For the construction of SIRT6 expressing lentiviral plasmid, SIRT6 coding region was amplified by PCR and cloned to vector pCDH between *Eco*RI and *Bam*HI sites.

For the construction of SIRT6 promoter plasmid, SIRT6 promoter region was amplified by PCR and cloned to vector pGL3-basic between *Xho*I and *Hind*III sites.

For the construction of shRNA plasmid targeting SIRT6, lentviral vector pLKO was used. shRNA oligonucleotides were designed using sigma online protocol. Synthesized DNA oligos were annealed and ligated into the *Age*I/*Eco*RI-digested pLKO vector.

All the oligo sequences were listed in supplemental Table 1.

2.4. shRNA lentivirus production

To produce lentivirus expressing SIRT6/SIRT6-shRNA, the plasmid pCDH-SIRT6/pLKO-shSIRT6, the envelope plasmid pMD2G and the packaging plasmid psPAX2 were co-transfected into 293T cells. Lentiviral supernatants were harvested through a 0.45 μm filter 48 h after transfection.

2.5. RNA purification and reverse transcription reaction

Total RNA was isolated with Trizol reagent (Thermo Fisher Scientific) following the manufacturer's protocol. 0.5 μg of total RNA was reverse transcribed using random hexamers and the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific).