Title

Delayed Nrf2-regulated antioxidant gene induction in response to silica nanoparticles

Authors

Zhang, Hongqiao
Zhou, Lulu
Yuen, Jenay
et al.

Publication Date

2017-07-01

DOI

10.1016/j.freeradbiomed.2017.04.002

Peer reviewed

HHS Public Access

Author manuscript

Free Radic Biol Med. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Free Radic Biol Med. 2017 July ; 108: 311–319. doi:10.1016/j.freeradbiomed.2017.04.002.

Delayed Nrf2-regulated antioxidant gene induction in response to silica nanoparticles

Hongqiao Zhang[1,*], **Lulu Zhou**[1], **Jenay Yuen**[1], **Nancy Birkner**[2], **Valerie Leppert**[3], **Peggy A. O'Day**[2], and **Henry Jay Forman**[1]

[1]Leonard Davies School of Gerontology, University of Southern California, Los Angeles, CA 90089

[2]School of Natural Sciences and Sierra Nevada Research Institute, University of California at Merced, Merced, CA 95343

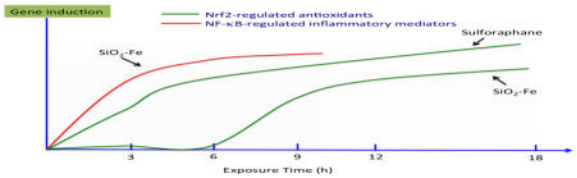
[3]School of Engineering, University of California at Merced, Merced, CA 95343

Abstract

Silica nanoparticles with iron on their surface cause the production of oxidants and stimulate an inflammatory response in macrophages. Nuclear factor erythroid-derived 2 –like factor 2 (Nrf2) signaling and its regulated antioxidant genes play critical roles in maintaining redox homeostasis. In this study we investigated the regulation of four representative Nrf2-regulated antioxidant genes; i.e., glutamate cysteine ligase (GCL) catalytic subunit (GCLC), GCL modifier subunit (GCLM), heme oxygenase 1 (HO-1), and NAD(P)H:quinone oxidoreductase-1 (NQO-1), by iron-coated silica nanoparticles (SiO_2-Fe) in human THP-1 macrophages. We found that the expression of these four antioxidant genes was modified by SiO_2-Fe in a time-dependent manner. At 6 h, their expression was unchanged except for GCLC, which was reduced compared with controls. At 18 h, the expression of these antioxidant genes was significantly increased compared with controls. In contrast, the Nrf2 activator sulforaphane induced all antioxidant genes at as early as 3 h. The nuclear translocation of Nrf2 occurred later than that for NF-κB p65 protein and the induction of proinflammatory cytokines (TNFα and IL-1β). NF-κB inhibitor SN50 prevented the reduction of GCLC at 6 h and abolished the induction of antioxidant genes at 18 h by SiO_2-Fe, but did not affect the basal and sulforaphane-induced expression of antioxidant genes, suggesting that NF-κB signaling plays a key role in the induction of Nrf2-mediated genes in response to SiO_2-Fe. Consistently, SN50 inhibited the nuclear translocation of Nrf2 caused by SiO_2-Fe. In addition, Nrf2 silencing decreased the basal and SiO_2-induced expression of the four reprehensive antioxidant genes. Taken together, these data indicated that SiO_2-Fe induced a delayed response of Nrf2-regulated antioxidant genes, likely through NF-κB-Nrf2 interactions.

Graphical Abstract

[*]Correspondence to: Andrus Gerontology Center of the Leonard Davis School of Gerontology University of Southern California 3715 McClintock Ave, Los Angeles, CA 90089-0191, USA. Tel.: + 626 272 7968. hongqiaz@usc.edu.



Keywords

Silica; iron; nanoparticles; NF-κB; inflammation; Nrf2; antioxidant

Introduction

Ambient nanoparticulate matter (nPM), i.e. particles with a diameter less than 100 nm, exhibit greater toxicity compared to particles of similar composition in the ~1–10 micrometer size range [1]. Nanoparticles are capable of freely entering alveoli, penetrating cells, evading alveolar microphage phagocytosis, and crossing through epithelium to enter the circulatory and lymphatic systems [2, 3]. Although the underlying mechanism remains to be further elucidated, it is postulated that oxidative stress plays a critical role in the pathogenesis and development of ambient particle-caused health disorders [2, 4–9]. Oxidants produced upon particle exposure may arise from several sources [10, 11], including redox cycling of surface-adsorbed quinones [12, 13], Fenton-like reactions of transition metals [12, 14–16], altered mitochondrial function [17], and an inflammatory response [11].

Nuclear factor erythroid 2 -like 2 (NFE2L2; more commonly known as Nrf2) is a redox sensitive transcription factor that regulates the basal and inducible expression of an array of antioxidant and detoxifying enzymes, including glutamate cysteine ligase (GCL) [18, 19], NADPH:quinone oxidoreductase 1 (NQO-1) [20, 21] and heme oxygenase-1 (HO-1) [22, 23]. Under non-stimulated conditions, Nrf2 is rapidly degraded in the cytosol through ubiquitination mediated by Keap1. In response to electrophiles, Keap1 is modified so that it cannot assist in Nrf2 degradation. Non-degraded Nrf2 is phosphorylated and translocated into the nucleus where it binds to the electrophile response element (EpRE) in the promoter of target genes, and increases gene expression [24]. The Nrf2 signaling system plays a critical role in maintaining cellular redox homeostasis and also in protecting against oxidative injuries in response to oxidative stress [25]. Studies using animal models found that upon the exposure of ambient nanoparticles, Nrf2 signaling and its regulated antioxidant genes were increased [26–28], reflecting the effective response of this antioxidant system to ambient nanoparticle exposure. Regardless, it remains unclear how Nrf2 signaling is regulated upon nanoparticle exposure in macrophages, the first cells to encounter particles in both the airway and alveoli. Macrophages remove inhaled particles, but also play a central role in the inflammatory response [29, 30].

In this study using iron-coated silica nanoparticle (SiO_2-Fe) as a model, we investigated how Nrf2 signaling is regulated in response to nanoparticles in macrophages. Silica particles are a component of ambient particulate matter [31, 32], and a major occupational hazard in

construction and farming [33]. Studies of the insoluble fraction of natural particulate matter show that the non-carbonaceous component is composed mostly of silica, aluminosilicate minerals, and Fe^{3+} oxides [34–36], and that particles are complex mixtures with different types of surface coatings [37]. Like ambient particulate matter, silica particles can stimulate oxidant production and, when iron is on the surface, trigger an inflammatory response [17, 38, 39].

Our data demonstrates that the Nrf2 signaling and its regulated antioxidant genes are induced by SiO_2-Fe in human THP-1 macrophages, but in a delayed manner compared to Nrf2 activators that directly modify Keap1, such as sulforaphane. The delayed response in Nrf2 signaling requires the activation of NF-κB signaling. In addition, a dual phase of GCLC regulation is observed in response to SiO_2-Fe nanoparticle exposure. GCLC expression is reduced in the early phase, but increased along with other Nrf2-reguated genes in the later phase. Therefore, a complex interaction between Nrf2 and NF-κB signaling appears to exist in macrophages in response to ambient nanoparticle exposure.

Methods and Materials

Chemicals and reagents

Unless otherwise noted, analytical chemicals were from Sigma (St. Louis, MO). Antibodies to Nrf2, NF-κB p65, and lamin B1, and β-tubulin were from Cell Signaling Technology, Inc. (Danvers, MA). TriZol reagent, NE-PER Nuclear and Cytoplasmic Extraction Reagents, Reverse Transcription kit, SYBR Green PCR master mixture, and RPMI 1640 cell culture medium were from Thermal Fisher Scientific Inc. (Thermal Fisher, Rockford, IL). NF-κB SN50 inhibitor was from EMD Millipore (Billerica, MA). Nrf2 siRNA, negative control siRNA and Nrf2 primers were from Santa Cruz Biotechnology (Dallas, TX). Nucleofactor™ Kits for THP-1 was purchased from (Lonza, Rockland, ME).

Synthesis of SiO_2-Fe nanoparticles

Silica synthesis—Colloidal silica was synthesized using a modified method after Stober *et al.* [40]. Briefly, 29 ml of ethanol (Anhydrous, Fisher Scientific) and 1.5 ml of 29% ammonium hydroxide (Sigma Aldrich) were combined under rapid and continuous stirring at ambient conditions in a glass bottle. To this was slowly dropwise added 0.75 ml of tetraethyl orthosilicate (Sigma Aldrich). The solution was continuously stirred for an additional 12 h and the resulting colloids were collected and washed with centrifugation (14,000 rpm for 60 minutes, three times) using 18 Mega-ohm water. The final suspension was oven dried (85–90 °C overnight) to a fine white powder. Specific surface area was measured on dried samples by the N_2-BET (Brunauer–Emmett–Teller) method using a surface area analyzer (Tri-Star 3000, Micrometrics) and five point calibration. Particle size was characterized using a JEOL JEM-2010 Transmission Electron Microscope equipped with LaB_6 filament and operated at 200 kV.

Fe-coating procedure—All reagents were ACS Reagent grade or trace metal grade. All containers were soaked in 5% nitric acid for 24 hours and thoroughly rinsed with 18 Mega-ohm ultrapure deionized (DI) water to avoid trace metal contamination. Silica nanoparticles

were suspended in DI water (2 mg solid/1 mL water; pH 7) and mixed with an aliquot of 1 mM $FeCl_3$ solution (pH 6) under continuous stirring to produce a final solution concentration of 0.5 μM. The pH was slowly adjusted to 7–7.5 using trace metal grade NaOH. The final mixtures were equilibrated for 2 h with continuous mixing using a horizontal agitator. Following equilibration, suspensions were washed with degassed deionized water (pH 7–7.5) and then centrifuged at 14,000 g for 1 h in 50 mL ultracentrifugation tubes. The Fe-coated silica nanoparticles were dried in open centrifuge tubes in a clean drying oven in air at 30–35 °C. Total iron concentrations were determined by microwave-assisted digestion of 29.7 mg of solid in concentrated aqua regia (HCl + HNO_3), dilution with ultrapure water, and elemental analysis by ICP-OES (Perkin-Elmer Optima 4000 DV; limit of detection for Fe = 1 ppb). Total Fe concentrations were normalized to solid dry weight and to the measured BET surface area of dry SiO_2 nanoparticles.

Cell culture and treatment

THP-1 cells (Human acute monocytic leukemia cell line) purchased from American Type Culture Collection were cultured in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 100 μg/ml penicillin and 100 μg/ml streptomycin, and 0.05 mM β-mercaptoethanol at 37°C in a humidified 5% CO_2 incubator. Prior to experiments, THP1 cells at a density of 3×10^5 cells/cm^2 were differentiated into macrophages in medium containing 7.5 ng/ml phorbol 12-myristate-13-acetate (PMA) for 2 days, and then the medium was replaced with normal medium one day before further treatment. SiO_2-Fe was suspended in deionized water at 1 mg/ml and sonicated for 2 min before adding to cells.

Western Analysis

Briefly, cell lysate was extracted with NE-PER and 20 μg protein was electrophoresed on a 4–20% Tris-glycine acrylamide gel (Thermal Fisher Scientific, Rockford, IL) and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% fat-free milk and then incubated with primary antibody overnight at 4°C in 5% BSA dissolved in Tris-buffered saline (TBS). After being washed with 1XTBS containing 0.05% Tween 20 (TTBS), membranes were incubated with secondary antibody at room temperature for 2 h. After TTBS washing, membranes were treated with an enhanced chemiluminescence reagent mixture (ECL Plus; Thermal Fisher Scientific, Rockford, IL) for 5 min and then imaged using Syngene PXi6 imaging system (Syngene, Cambridge, UK). The blots were analyzed with ImageJ.

mRNA assay

RNA extraction, cDNA reverse transcription, measurement of mRNA and calculation of mRNA levels relative to control were performed according to a procedure as described before [41]. The primer sequences for real time PCR assay were listed in Table 1.

Transfection of THP1 cells with siRNAs by electroporation

Nrf2 expression was silenced by transient transfection of THP1 cells with human Nrf2 siRNA using Nucleofactor™ Kits for THP-1 (Lonza, Rockland, ME), as described by Maess

et al. [42]. The Nrf2 siRNA was a pool of three different siRNA duplexes consisting of A) 5′-GCAUGCUACGUGAUGAAGAtt-3′; B) 5′-CUCCUACUGUGAUGUGAAAtt-3′; and C) 5′-GUGUCAGUAUGUUGAAUCAtt-3′. In brief, differentiated THP1 cells were detached with Accutase I (37°C for 30 min) and re-suspended in electroporation solution (1×10^6 cells/100 μl). Three microgram of Nrf2 or negative control siRNA was mixed with 1 × 10^6 THP-1 cells (in 100 μl of electroporation solution). Cells were then transferred into a 2-mm electroporation cuvette and electroporated using a BTX ECM399 Electroporation System (BTX, Holliston, MA) at the setting of 125 V of voltage pulse and 1050 μF of capacitance. Transfected cells were immediately transferred to 3 ml of cell medium (one transfection per well in a 6-well plate). After 4 h, medium was replaced with complete medium containing 2.5 ng/ml PMA. The medium was replaced again after 24 h before cells were treated with or without nanoparticles.

Statistical analysis

Data were expressed as mean ± standard error. One-way ANOVA with post hoc analysis was used for significance testing. Wilcoxon rank-Sign test was used for statistical analysis of Western densitometry data. Statistical significance was accepted when $p < 0.05$.

Results

SiO_2-Fe induced pro-inflammatory cytokines

Previously we reported that SiO_2-Fe induced pro-inflammatory cytokine expression in human macrophages [39]. The mean size (60±12 nm) and surface area (56.15±0.35 m^2/g) of SiO_2 nanoparticles used here were similar to those used in the prior study (42±11 nm; 54.5 ±0.34 m^2/g). Furthermore, total Fe and surface-area normalized Fe concentrations in a bulk sample were lower than those of SiO_2-Fe nanoparticles used in Premasekharen *et al.* [39]. Total Fe in bulk SiO_2-Fe nanoparticles was 13.16 μM Fe/g solid, which gave an average surface-area normalized Fe concentration of 0.234 μM Fe/m^2 SiO_2 surface (±1.7%), compared with 54.03 μM Fe/g solid and 0.991 μM Fe/m^2 SiO_2 surface for particles. Electron micrographs of the iron-coated silica particles used in the present study are shown in Supplemental Fig. A.

We first confirmed the pro-inflammatory effects of SiO_2-Fe nanoparticles by measuring the mRNA levels of TNFα and IL-1β, to establish the appropriate dose and exposure times for subsequent experiments. SiO_2-Fe nanoparticles induced the expression of both TNFα and IL-1β in 6 h at 10 and 40 μg/ml, with a higher induction at 40 μg/ml than lower doses (Fig. 1A). The mRNA induction of both cytokines occurred as early as 3 h and persisted at least until 9 h after SiO_2-Fe exposure (Fig. 1B), indicating that the pro-inflammatory cytokines were induced in a time- and dose-dependent manner.

Delayed induction of Nrf2-regulated antioxidant genes by SiO_2-Fe

SiO_2-Fe nanoparticles stimulated $O_2^{\cdot-}$ production and a small amount of lipid peroxidation in macrophages [39]. An increase in Nrf2-regulated antioxidant and detoxifying genes is a critical adaptive response to oxidative stress. Therefore, we wondered whether Nrf2 signaling was also activated in response to SiO_2-Fe in macrophages. To investigate this, we

determined the mRNA and protein levels of four representative genes that are regulated by Nrf2 signaling (GCLC, GCLM, HO-1, and NQO-1) after THP1 macrophages were treated with SiO_2-Fe for different time. As shown in Fig. 2A, the expression of these antioxidant genes was differentially and time-dependently altered by SiO_2-Fe exposure. At 6 h, SiO_2-Fe decreased GCLC mRNA, but had no effect on the mRNAs of other genes; however, at 18 h, SiO_2-Fe increased the mRNA levels of all four genes. Changes of the protein level of these 4 genes in response to SiO_2-Fe exposure followed a similar pattern as their mRNA levels: the protein levels started to increase from 9 h and remained higher (than control) at least until 18 h of exposure, while GCLC protein decreased at 3 h and 6 h of exposure before its increase at 9 h. (Fig. 2B). The temporal change of the induction of Nrf2-regulated antioxidant genes by SiO_2-Fe was markedly different from that by sulforaphane, a well-documented Nrf2 activator. Following sulforaphane exposure (5 μM), the mRNA level of these antioxidant genes was increased at as early as 3 h and maintained at least until 18 h (Fig. 2C). These data indicate that SiO_2-Fe exposure induced the expression of Nrf2-regulated antioxidant genes in THP-1 cells, but in a delayed manner compared to the direct Nrf2 activator sulforaphane.

The NF-κB inhibitor SN50 inhibited SiO_2-Fe-induced expression of cytokine and antioxidant genes

Given the finding that NF-κB activation (pro-inflammatory cytokine induction) occurred earlier than the induction of Nrf2-regulated antioxidants genes, we hypothesized that the early induction of NF-κB-regulated pro-inflammatory cytokines or mediators was responsible for the delayed increase in Nrf2-regulated antioxidant genes caused by SiO_2-Fe exposure. To test it, THP1 macrophages were pretreated with SN50, a specific NF-κB peptide inhibitor [43], before exposure to SiO_2-Fe (20 μg/ml). As expected, SN50 (50 μg/ml) effectively inhibited the induction of both TNF-α and IL-1β caused by SiO_2-Fe (Fig. 3A), suggesting that it blocked NF-κB-mediated induction of pro-inflammatory cytokines or mediators. Meanwhile, SN50 pretreatment inhibited SiO_2-Fe-mediated induction of Nrf2-regulated antioxidant genes at 18 h (Fig. 3B). Interestingly, SN-50 also prevented the decrease of GCLC mRNA by SiO_2-Fe at 6 h (Fig. 3B).

To exclude the possibility of direct regulation of these antioxidant genes via NF-κB signaling in THP-1 macrophages, the effect of NF-κB antagonist SN50 on both baseline expression and sulforaphane induction of GCLC, GCLM, HO-1 and NQO-1 was determined. As shown in Fig. 3C, SN50 had no effect on either the basal expression or the induction of these antioxidant genes by sulforaphane at 18h (Fig. 3C), indicating they are not the direct targets of NF-κB signaling in contrast with the pro-inflammatory cytokines.

Taken together, data from above indicate that an NF-κB mediated product, rather than NF-κB itself, is required for the induction of Nrf2-regulated antioxidant genes by SiO_2-Fe nanoparticles.

Nrf2 silencing inhibited SiO_2-Fe-induced expression of antioxidant genes

To further confirm the role of Nrf2 in the induction of these antioxidant genes by SiO_2-Fe nanoparticles, Nrf2 expression in THP1-differentiated macrophages was silenced with Nrf2

siRNA (Fig. 4A). Nrf2 knockout decreased the basal expression of the four antioxidant genes significantly, and it also abrogated the induction of these genes in response to SiO_2-Fe exposure (Fig. 4B). Interestingly, Nrf2 mRNA was also increased by SiO_2-Fe, and its induction was abolished with Nrf2 silencing. Considering Nrf2 gene is auto-regulated through Nrf2 signaling [44], this adds further evidence that Nrf2 was required for the induction of antioxidant genes by SiO_2-Fe nanoparticles.

Nrf2 activation by SiO_2-Fe

To further elucidate the underlying mechanism of delayed induction of Nrf2-regulated antioxidant genes, we determined Nrf2 activation (nuclear translocation) upon exposure to SiO_2-Fe nanoparticles. Following the exposure to SiO_2-Fe, nuclear Nrf2 content (marker of Nrf2 activation) was increased from 3 h and returned to the basal level at 9 h (Fig. 5A). In contrast, the nuclear content of NF-κB p65, a marker of NF-κB activation, was increased at as early as 30 min, reached at maximal level at 3 h, and returned to baseline at 9 h. In comparison, sulforaphane activated Nrf2 at as early as 30 min after exposure (Fig. 5B), suggesting that the delayed antioxidant gene induction by SiO_2-Fe could be due to the late Nrf2 activation (compared to sulforaphane).

NF-κB inhibitor blocked Nrf2 activation by SiO_2-Fe

To further confirm that NF-κB activation was responsible for the delayed induction of Nrf2-regulated antioxidant genes by SiO_2-Fe, we determined the effect of NF-κB inhibitor SN-50 on Nrf2 activation (nuclear translocation). As shown in Fig. 6, SN50 pretreatment inhibited SiO_2-Fe-caused Nrf2 activation (Fig. 6), suggesting that NF-κB activation is essential for the following Nrf2 activation by SiO_2-Fe.

Discussion

Alveolar macrophages play a central role in particulate toxicology. In addition to initiating the pro-inflammatory response upon the exposure to ambient particles and being involved in tissue repair, they are also the primary cells that phagocytize and clear particles [2, 45]. To understand how macrophages attempt to maintain their own redox homeostasis is important for elucidating their response to nanoparticle exposure and the mechanism of nanoparticle toxicology. In this study we found that Nrf2-regulated antioxidant and detoxifying enzymes were induced in macrophages in response to SiO_2-Fe nanoparticles, suggesting that these cells could increase their adaptive antioxidant enzymes for protection against oxidative injury. In comparison to activation by the direct Nrf2 stimulator sulforaphane, the activation of Nrf2 signaling by SiO_2-Fe was delayed. Using an NF-κB inhibitor, we showed that NF-κB activation played a critical role in SiO_2-Fe-induced Nrf2 activation and antioxidant gene induction. It was also found that GCLC expression was reduced, apparently through an NF-κB dependent mechanism, in the early phase of SiO_2-Fe exposure. These findings suggest that there is a complex interaction between the inflammatory (NF-κB signaling) and antioxidant (Nrf2 signaling) responses upon silica nanoparticle exposure.

The activation of Nrf2 signaling involves multiple steps, including oxidative modification of Keap1 to prevent its facilitation of Nrf2 degradation, Nrf2 phosphorylation and nuclear

translocation, and interaction of Keap1 and Nrf2 with several other proteins that modify their turnover [24]. Nrf2 level is constantly fine-tuned to maintain redox homeostasis [25], and its activation in response to electrophiles is rapid. In response to activators such as 4-hydroxy-2-nonenal [46], resveratrol [19], and sulforaphane [47] (Fig.5B), a significant increase in nuclear Nrf2 protein level (marker of Nrf2 activation) is usually observed in 1 h or less, and the induction of Nrf2 target genes becomes evident at as early as 3 h (Fig. 2C). SiO_2-Fe was able to increase $O_2^{\cdot-}$ production and lipid peroxidation at as early as 10 min after exposure of THP-1 macrophages [39], and thus a quick response of Nrf2 signaling was expected. In contrast, the increase of nuclear Nrf2 became significant only at 3 h (Fig. 5A) and the induction of Nrf2-regulated genes was observed at as early as 9–12 h (Fig. 2) in response to SiO_2-Fe exposure. Therefore, although SiO_2-Fe initiated a rapid pro-inflammatory response [39] (Fig. 1B and Fig. 5), the activation of Nrf2 signaling occurred in the later phase, which may explain the delayed increase in the Nrf2-regulated genes.

Nonetheless, the current study showed that NF-κB activation might also be required for Nrf2 signaling in response to SiO_2-Fe. The classical NF-κB (p65/p50) is a dimer transcription factor that regulates the gene expression of pro-inflammatory cytokines and mediators including TNFα and IL-1β. In resting cells, the NF-κB dimer is requested in the cytosol by IκB or exists as precursors that can be proteolytically cleaved [48]. In response to inflammatory triggers, IκB is degraded and NF-κB is released and translocated into nucleus, where it binds to κB sites in promoter region and increases gene expression [48]. The p65/p50 NF-κB dimer plays a critical role in SiO_2-Fe triggered pro-inflammatory response as evidenced in Fig. 3A and Fig. 5, which showed that p65 was activated by SiO_2-Fe and that the p50 inhibitor SN50 blocked the induction of pro-inflammatory cytokines by SiO_2-Fe. SN50 pretreatment also abrogated the SiO_2-Fe mediated Nrf2 activation (Fig. 6) and target gene induction (Fig. 3B) suggesting that Nrf2 activation by SiO_2-Fe nanoparticles required the induction of some NF-κB-regulated gene.

There are some reports that these four antioxidant genes could also be regulated through NF-κB signaling [49–52]. These reports however, are not supported by later studies based on chromatin immunoprecipitation (CHIP)-seq technology [53, 54]. A recent research demonstrated that the purported NF-κB inhibitor Pyrrolidine Dithiocarbamate (PDTC) actually up-regulated the expression of these antioxidant genes, through Nrf2 signaling [55], extending the earlier study by Mulcahy and coworkers that PDTC activated GCLC and GCLM through a copper-dependent pro-oxidant mechanism rather than through NF-κB inhibition [56]. In the current study, Nrf2 silencing decreased the basal mRNA levels of the four reprehensive Nrf2-regulated antioxidant genes and abrogated their induction by SiO_2-Fe (Fig.4), indicating that these genes were regulated by Nrf2 signaling in response to SiO_2-Fe exposure. Furthermore, the induction of these antioxidant genes did not occur until hours after proinflammatory cytokine mRNAs were increased (Fig.1). Indeed, GCLC mRNA level was negatively correlated with cytokine mRNA transcription. In addition, the NF-κB antagonist SN50 showed no effect on the induction of the antioxidant genes by sulforaphane and did not affect their basal expression (Fig. 3C). Taken together, this evidence indicate that the delayed induction of these antioxidant genes is not by direct NF-κB signaling, but through Nrf2 signaling, although some NF-κB–dependent gene products appear to be required for the later Nrf2 activation and antioxidant gene induction.

There are many NF-κB regulated genes, including the pro-inflammatory cytokines, chemokines, and other stress response proteins [57]. Without going through a survey of which NF-κB-regulated gene product entirely was responsible for the induction of Nrf2, we can only speculate which protein(s) was responsible for the delayed increase in Nrf2-regulated gene expression. The induction and release of NF-κB-regulated pro-inflammatory cytokines (TNFα and IL-1β) [58–61] or other inflammatory mediators such as cyclooxygenase-2 (COX-2) [62, 63] usually occurs as rapidly as 1 h following exposure to particles or other inflammatory triggers [64]. These cytokines or other inflammatory mediators could then activate NADPH oxidases [65] to produce H_2O_2, and/or increase production of electrophiles that activate or induce other redox-sensitive proteins and genes [66, 67]. This hypothesis is supported by studies from Barrett el al., who found that TNF-α induction by silica was required for the subsequent up regulation of chemokines in alveolar type II cells [68, 69]. Herseth *et al.* also found that the early induction of IL-1β and COX-2 genes was required for a later up regulation of IL-6 by silica [70]. In addition, 15-deoxy-delta-12,14-prostaglandin J2 (15d-PGJ2), a product of COX-2, is a well-established Nrf2 activator [71]. Nonetheless, as stated above, this is speculation as many other NF-κB-regulated proteins including NADPH oxidase 4 [72] and iNOS [73] that produce oxidants may also activate Nrf2 through direct oxidation of Keap1 cysteine residues.

Our evidence does, however, clearly suggests that NF-κB plays the role of a double-edged sword in SiO_2-Fe induced Nrf2 signaling and regulation of antioxidant genes. One side of the NF-κB signaling sword appears to interfere with baseline Nrf2 signaling while the other side appears to cause a delayed Nrf2 activation. A possible mechanism for the initial suppression of Nrf2 is that NF-κB and Nrf2/EpRE transcription compete for the transcriptional co-activator cAMP response element binding protein (CREB) binding protein (CBP)/p300, which can be rate limiting because of limited abundance [74]. Although competition between the two signaling pathways cannot be excluded as also playing a role in the delayed up regulation of Nrf2-regulated genes, that seems less likely as NF-κB signaling was required for the nuclear translocation of Nrf2 (Fig. 6). Therefore, the induction of an NF-κB-mediated protein that stimulates Nrf2 activation seems more likely be responsible for the participation of NF-κB in Nrf2 activation by SiO_2-Fe.

Among the four representative Nrf2-regulated antioxidant genes examined in this study, the expression of GCLC was different from other three genes (GCLM, HO-1 and NQO-1) in response to SiO_2-Fe nanoparticles. Unlike the other genes whose expression remained unchanged, the expression of GCLC was initially reduced in the early phase (3–6 h), and then increased at 9 h following exposure as did the other genes (Fig. 2). The reduction of GCLC expression by SiO_2-Fe was prevented by inhibiting NF-κB signaling (Fig. 3A), indicating another potential regulatory mechanism of NF-κB on GCLC expression other than Nrf2 signaling. GCLC mRNA stability is regulated through the binding of HuR to the 3′-untranslated region [75]. HuR is also involved in the mRNA binding of nearly half of the cytokines [76], whose mRNAs are significantly increased during the inflammatory process. The competition of a large amount of cytokine mRNAs for HuR could result in less HuR binding to GCLC mRNA and lead to its rapid decay. The underlying mechanism of how GCLC expression was down regulated by SiO_2-Fe was however, beyond the scope of the present investigation.

It should be noted that the interaction between NF-κB and Nrf2 signaling network is more complex than described in the brief discussion above. Several interaction points have been reported. RAC1, a small G-protein of the Rho family, for instance, is involved in the activation of both NF-κB and Nrf2 signaling pathways. By inducing Nrf2/EpRE signaling, RAC1 could block RAC1-dependent NF-κB activation [77]. NF-κB/p65 can also antagonize Nrf2 binding to DNA by competing for co-activators and promoting a co-repressor (histone deacetylase 3) [78]. Considering the overlapping time of the increased presence of both NF-κB and Nrf2 in the nucleus after SiO2-Fe exposure (Fig. 5), antagonistic between these two pathways could result in less capacity of both to enhance gene transcription. The involvement of these interactions in the delayed induction of Nrf2-regulated genes by SiO2-Fe remains to be determined.

In summary, our data suggest that NF-κB signaling is required for the activation of Nrf2 and the induction of Nrf2-regulated genes in response to SiO_2-Fe nanoparticles in macrophages (Fig. 7). It remains for future investigation to determine which NF-κB-regulated genes are involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant ES023864. Work at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Thanks to Alexander Leven and Stefanie Helmrich for assistance with nanoparticle preparation and analysis. We also thank Dr. Andrew Gracey at the Department of Marine Environmental Biology, University of Southern California, to kindly provide the electroporation system.

References

1. Buzea C, Pacheco, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases. 2007; 2(4):MR17–71. [PubMed: 20419892]
2. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005; 113(7):823–39. [PubMed: 16002369]
3. Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect. 2001; 109(Suppl 4):547–51.
4. Fubini B. Surface chemistry and quartz hazard. Ann Occup Hyg. 1998; 42(8):521–30. [PubMed: 9838865]
5. Dye JA, Adler KB, Richards JH, Dreher KL. Role of soluble metals in oil fly ash-induced airway epithelial injury and cytokine gene expression. Am J Physiol. 1999; 277(3 Pt 1):L498–510. [PubMed: 10484457]
6. Aust AE, Ball JC, Hu AA, Lighty JS, Smith KR, Straccia AM, Veranth JM, Young WC. Particle characteristics responsible for effects on human lung epithelial cells. Res Rep Health Eff Inst. 2002; (110):1–65. discussion 67–76.
7. Dick CA, Singh P, Daniels M, Evansky P, Becker S, Gilmour MI. Murine pulmonary inflammatory responses following instillation of size-fractionated ambient particulate matter. J Toxicol Environ Health A. 2003; 66(23):2193–2207. [PubMed: 14669776]

8. Lomnicki S, Truong H, Vejerano E, Dellinger B. Copper oxide-based model of persistent free radical formation on combustion-derived particulate matter. Environ Sci Technol. 2008; 42(13): 4982–8. [PubMed: 18678037]
9. Yi S, Zhang F, Qu F, Ding W. Water-insoluble fraction of airborne particulate matter (PM10) induces oxidative stress in human lung epithelial A549 cells. Environ Toxicol. 2014; 29(2):226–33. [PubMed: 22331617]
10. Tao F, Gonzalez-Flecha B, Kobzik L. Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med. 2003; 35(4):327–40. [PubMed: 12899936]
11. Risom L, Moller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutat Res. 2005; 592(1–2):119–37. [PubMed: 16085126]
12. Valavanidis A, Fiotakis K, Bakeas E, Vlahogianni T. Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter. Redox Rep. 2005; 10(1):37–51. [PubMed: 15829110]
13. Charrier JG, McFall AS, Richards-Henderson NK, Anastasio C. Hydrogen peroxide formation in a surrogate lung fluid by transition metals and quinones present in particulate matter. Environ Sci Technol. 2014; 48(12):7010–7. [PubMed: 24857372]
14. Sorensen M, Schins RP, Hertel O, Loft S. Transition metals in personal samples of PM2.5 and oxidative stress in human volunteers. Cancer Epidemiol Biomarkers Prev. 2005; 14(5):1340–3. [PubMed: 15894700]
15. Charrier JG, Anastasio C. Rates of Hydroxyl Radical Production from Transition Metals and Quinones in a Surrogate Lung Fluid. Environ Sci Technol. 2015; 49(15):9317–25. [PubMed: 26153923]
16. Loxham M, Morgan-Walsh RJ, Cooper MJ, Blume C, Swindle EJ, Dennison PW, Howarth PH, Cassee FR, Teagle DA, Palmer MR, Davies DE. The effects on bronchial epithelial mucociliary cultures of coarse, fine, and ultrafine particulate matter from an underground railway station. Toxicol Sci. 2015; 145(1):98–107. [PubMed: 25673499]
17. Deshpande A, Narayanan PK, Lehnert BE. Silica-induced generation of extracellular factor(s) increases reactive oxygen species in human bronchial epithelial cells. Toxicol Sci. 2002; 67(2): 275–83. [PubMed: 12011487]
18. Zhang H, Court N, Forman HJ. Submicromolar concentrations of 4-hydroxynonenal induce glutamate cysteine ligase expression in HBE1 cells. Redox Rep. 2007; 12(1):101–6. [PubMed: 17263920]
19. Zhang H, Shih A, Rinna A, Forman HJ. Resveratrol and 4-hydroxynonenal act in concert to increase glutamate cysteine ligase expression and glutathione in human bronchial epithelial cells. Arch Biochem Biophys. 2009; 481(1):110–5. [PubMed: 18983812]
20. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci USA. 1996; 93(25):14960–5. [PubMed: 8962164]
21. Jeyapaul J, Jaiswal AK. Nrf2 and c-Jun regulation of antioxidant response element (ARE)-mediated expression and induction of gamma-glutamylcysteine synthetase heavy subunit gene. Biochem Pharmacol. 2000; 59(11):1433–9. [PubMed: 10751553]
22. He CH, Gong P, Hu B, Stewart D, Choi ME, Choi AM, Alam J. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. J Biol Chem. 2001; 276(24):20858–65. [PubMed: 11274184]
23. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. EMBO J. 2002; 21(19):5216–24. [PubMed: 12356737]
24. Zhang H, Davies KJ, Forman HJ. Oxidative stress response and Nrf2 signaling in aging. Free Radic Biol Med. 2015; 88(Pt B):314–36. [PubMed: 26066302]
25. Ursini F, Maiorino M, Forman HJ. Redox homeostasis: The Golden Mean of healthy living. Redox Biol. 2016; 8:205–215. [PubMed: 26820564]
26. Zhang H, Liu H, Davies KJ, Sioutas C, Finch CE, Morgan TE, Forman HJ. Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments. Free Radic Biol Med. 2012; 52(9):2038–46. [PubMed: 22401859]

27. Lovera-Leroux M, Crobeddu B, Kassis N, Petit PX, Janel N, Baeza-Squiban A, Andreau K. The iron component of particulate matter is antiapoptotic: A clue to the development of lung cancer after exposure to atmospheric pollutants? Biochimie. 2015; 118:195–206. [PubMed: 26419736]
28. Brown DM, Kanase N, Gaiser B, Johnston H, Stone V. Inflammation and gene expression in the rat lung after instillation of silica nanoparticles: effect of size, dispersion medium and particle surface charge. Toxicol Lett. 2014; 224(1):147–56. [PubMed: 24466574]
29. Hogg JC, van Eeden S. Pulmonary and systemic response to atmospheric pollution. Respirology. 2009; 14(3):336–46. [PubMed: 19353772]
30. Hiraiwa K, van Eeden SF. Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants. Mediators Inflamm. 2013; 2013:619523. [PubMed: 24058272]
31. Schoning P, Abraham JL, Burnett BR. Silicate and metal dust in lungs of Greyhounds. Am J Vet Res. 1996; 57(7):1006–9. [PubMed: 8807011]
32. Ripanucci G, Grana M, Vicentini L, Magrini A, Bergamaschi A. Dust in the underground railway tunnels of an Italian town. J Occup Environ Hyg. 2006; 3(1):16–25. [PubMed: 16482974]
33. Vallyathan V, Pack D, Leonard S, Lawson R, Schenker M, Castranova V. Comparative in vitro toxicity of grape- and citrus-farm dusts. J Toxicol Environ Health A. 2007; 70(2):95–106. [PubMed: 17365569]
34. Cwiertny DM, Young MA, Grassian VH. Chemistry and photochemistry of mineral dust aerosol. Annu Rev Phys Chem. 2008; 59:27–51. [PubMed: 18393675]
35. Posfai M, Buseck PR. Nature and Climate Effects of Individual Tropospheric Aerosol Particles. Annu Rev Earth Pl Sc. 2010; 38:17–43.
36. Shi ZB, Krom MD, Jickells TD, Bonneville S, Carslaw KS, Mihalopoulos N, Baker AR, Benning LG. Impacts on iron solubility in the mineral dust by processes in the source region and the atmosphere: A review. Aeolian Research. 2012; 5:21–42.
37. Takahama S, Liu S, Russell LM. Coatings and clusters of carboxylic acids in carbon-containing atmospheric particles from spectromicroscopy and their implications for cloud-nucleating and optical properties. J Geophys Res-Atmos. 2010; 115:32–45.
38. Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med. 2003; 34(12):1507–16. [PubMed: 12788471]
39. Premasekharan G, Nguyen K, Contreras J, Ramon V, Leppert VJ, Forman HJ. Iron-mediated lipid peroxidation and lipid raft disruption in low-dose silica-induced macrophage cytokine production. Free Radic Biol Med. 2011; 51(6):1184–94. [PubMed: 21741475]
40. Stober W, Fink A, Bohn E. Controlled Growth of Monodisperse Silica Spheres in Micron Size Range. J Colloid Interf Sci. 1968; 26(1):62–69.
41. Zhang H, Dickinson DA, Liu RM, Forman HJ. 4-Hydroxynonenal increases gamma-glutamyl transpeptidase gene expression through mitogen-activated protein kinase pathways. Free Radic Biol Med. 2005; 38(4):463–71. [PubMed: 15649648]
42. Maess MB, Wittig B, Lorkowski S. Highly efficient transfection of human THP-1 macrophages by nucleofection. J Vis Exp. 2014; (91):e51960. [PubMed: 25226503]
43. Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J. Inhibition of nuclear translocation of transcription factor NF-kappa B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem. 1995; 270(24):14255–8. [PubMed: 7782278]
44. Kwak MK, Itoh K, Yamamoto M, Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cell Biol. 2002; 22(9):2883–92. [PubMed: 11940647]
45. Peters A, Veronesi B, Calderon-Garciduenas L, Gehr P, Chen LC, Geiser M, Reed W, Rothen-Rutishauser B, Schurch S, Schulz H. Translocation and potential neurological effects of fine and ultrafine particles a critical update. Part Fibre Toxicol. 2006; 3:13. [PubMed: 16961926]
46. Zhang H, Liu H, Dickinson DA, Liu RM, Postlethwait EM, Laperche Y, Forman HJ. gamma-Glutamyl transpeptidase is induced by 4-hydroxynonenal via EpRE/Nrf2 signaling in rat epithelial type II cells. Free Radic Biol Med. 2006; 40(8):1281–92. [PubMed: 16631518]

47. Gan NQ, Mi LX, Sun XY, Dai GF, Chung FL, Song LR. Sulforaphane protects Microcystin-LR-induced toxicity through activation of the Nrf2-mediated defensive response. Toxicol Appl Pharm. 2010; 247(2):129–137.
48. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009; 1(4):a000034. [PubMed: 20066092]
49. Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A, Abraham NG. Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. Proc Natl Acad Sci U S A. 1994; 91(13):5987–91. [PubMed: 8016102]
50. Brouard S, Berberat PO, Tobiasch E, Seldon MP, Bach FH, Soares MP. Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. J Biol Chem. 2002; 277(20):17950–61. [PubMed: 11880364]
51. Yao KS, O'Dwyer PJ. Involvement of NF-kappa B in the induction of NAD(P)H:quinone oxidoreductase (DT-diaphorase) by hypoxia, oltipraz and mitomycin C. Biochem Pharmacol. 1995; 49(3):275–82. [PubMed: 7857313]
52. Peng Z, Geh E, Chen L, Meng Q, Fan Y, Sartor M, Shertzer HG, Liu ZG, Puga A, Xia Y. Inhibitor of kappaB kinase beta regulates redox homeostasis by controlling the constitutive levels of glutathione. Mol Pharmacol. 2010; 77(5):784–92. [PubMed: 20159942]
53. Sharif O, Bolshakov VN, Raines S, Newham P, Perkins ND. Transcriptional profiling of the LPS induced NF-kappaB response in macrophages. BMC immunology. 2007; 8:1. [PubMed: 17222336]
54. Satoh J. Molecular network of ChIP-Seq-based NF-kappaB p65 target genes involves diverse immune functions relevant to the immunopathogenesis of multiple sclerosis. Multiple sclerosis and related disorders. 2014; 3(1):94–106. [PubMed: 25877979]
55. Liddell JR, Lehtonen S, Duncan C, Keksa-Goldsteine V, Levonen AL, Goldsteins G, Malm T, White AR, Koistinaho J, Kanninen KM. Pyrrolidine dithiocarbamate activates the Nrf2 pathway in astrocytes. J Neuroinflamm. 2016; 13(1):49–63.
56. Wild AC, Mulcahy RT. Pyrrolidine dithiocarbamate up-regulates the expression of the genes encoding the catalytic and regulatory subunits of gamma-glutamylcysteine synthetase and increases intracellular glutathione levels. Biochem J. 1999; 338(Pt 3):659–65. [PubMed: 10051436]
57. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene. 1999; 18(49):6853–66. [PubMed: 10602461]
58. Driscoll KE, Howard BW, Carter JM, Asquith T, Johnston C, Detilleux P, Kunkel SL, Isfort RJ. Alpha-quartz-induced chemokine expression by rat lung epithelial cells: effects of in vivo and in vitro particle exposure. Am J Pathol. 1996; 149(5):1627–37. [PubMed: 8909252]
59. Rader CP, Sterner T, Jakob F, Schutze N, Eulert J. Cytokine response of human macrophage-like cells after contact with polyethylene and pure titanium particles. J Arthroplasty. 1999; 14(7):840–8. [PubMed: 10537260]
60. Garrigues GE, Cho DR, Rubash HE, Goldring SR, Herndon JH, Shanbhag AS. Gene expression clustering using self-organizing maps: analysis of the macrophage response to particulate biomaterials. Biomaterials. 2005; 26(16):2933–45. [PubMed: 15603788]
61. Schremmer I, Brik A, Weber DG, Rosenkranz N, Rostek A, Loza K, Bruning T, Johnen G, Epple M, Bunger J, Westphal GA. Kinetics of chemotaxis; cytokine; and chemokine release of NR8383 macrophages after exposure to inflammatory and inert granular insoluble particles. Toxicol Lett. 2016; 263:68–75. [PubMed: 27565850]
62. Williams JA, Shacter E. Regulation of macrophage cytokine production by prostaglandin E-2 - Distinct roles of cyclooxygenase-1 and -2. Journal of Biological Chemistry. 1997; 272(41):25693–25699. [PubMed: 9325293]
63. Hofer TPJ, Bitterle E, Beck-Speier I, Maier KL, Frankenberger M, Heyder J, Ziegler-Heitbrock L. Diesel exhaust particles increase LPS-stimulated COX-2 expression and PGE(2) production in human monocytes. J Leukocyte Biol. 2004; 75(5):856–864. [PubMed: 14966191]

64. Chanput W, Mes J, Vreeburg RAM, Sayelkoul HFJ, Wichers HJ. Transcription profiles of LPS-stimulated THP-1 monocytes and macrophages: a tool to study inflammation modulating effects of food-derived compounds. Food Funct. 2010; 1(3):254–261. [PubMed: 21776474]

65. Chen X, Andresen BT, Hill M, Zhang J, Booth F, Zhang C. Role of Reactive Oxygen Species in Tumor Necrosis Factor-alpha Induced Endothelial Dysfunction. Curr Hypertens Rev. 2008; 4(4): 245–255. [PubMed: 20559453]

66. Sato M, Miyazaki T, Nagaya T, Murata Y, Ida N, Maeda K, Seo H. Antioxidants inhibit tumor necrosis factor-alpha mediated stimulation of interleukin-8, monocyte chemoattractant protein-1, and collagenase expression in cultured human synovial cells. J Rheumatol. 1996; 23(3):432–8. [PubMed: 8832978]

67. Rahman A, Kefer J, Bando M, Niles WD, Malik AB. E-selectin expression in human endothelial cells by TNF-alpha-induced oxidant generation and NF-kappaB activation. Am J Physiol. 1998; 275(3 Pt 1):L533–44. [PubMed: 9728048]

68. Barrett EG, Johnston C, Oberdorster G, Finkelstein JN. Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-alpha. Am J Physiol. 1998; 275(6 Pt 1):L1110–9. [PubMed: 9843848]

69. Barrett EG, Johnston C, Oberdorster G, Finkelstein JN. Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-alpha-induced oxidant stress. Am J Physiol. 1999; 276(6 Pt 1):L979–88. [PubMed: 10362723]

70. Herseth JI, Refsnes M, Lag M, Schwarze PE. Role of IL-1 beta and COX2 in silica-induced IL-6 release and loss of pneumocytes in co-cultures. Toxicology in Vitro. 2009; 23(7):1342–1353. [PubMed: 19580863]

71. Itoh K, Mochizuki M, Ishii Y, Ishii T, Sekizawa K, Uchida K, Yamamoto M. Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-D12,14-prostagiandin J2. Faseb Journal. 2004; 18(8):C94–C94.

72. Manea A, Tanase LI, Raicu M, Simionescu M. Transcriptional regulation of NADPH oxidase isoforms, Nox1 and Nox4, by nuclear factor-kappa B in human aortic smooth muscle cells. Biochem Bioph Res Co. 2010; 396(4):901–907.

73. Koga T, Zhang WY, Gotoh T, Oyadomari S, Tanihara H, Mori M. Induction of citrulline-nitric oxide (NO) cycle enzymes and NO production in immunostimulated rat RPE-J cells. Experimental Eye Research. 2003; 76(1):15–21. [PubMed: 12589771]

74. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-kappaB response pathways. Biochem Soc Trans. 2015; 43(4):621–6. [PubMed: 26551702]

75. Song IS, Tatebe S, Dai W, Kuo MT. Delayed mechanism for induction of gamma-glutamylcysteine synthetase heavy subunit mRNA stability by oxidative stress involving p38 mitogen-activated protein kinase signaling. J Biol Chem. 2005; 280(31):28230–40. [PubMed: 15946948]

76. Asirvatham AJ, Gregorie CJ, Hu Z, Magner WJ, Tomasi TB. MicroRNA targets in immune genes and the Dicer/Argonaute and ARE machinery components. Mol Immunol. 2008; 45(7):1995–2006. [PubMed: 18061676]

77. Cuadrado A, Martin-Moldes Z, Ye JP, Lastres-Becker I. Transcription Factors NRF2 and NF-kappa B Are Coordinated Effectors of the Rho Family, GTP-binding Protein RAC1 during Inflammation. Journal of Biological Chemistry. 2014; 289(22):15244–15258. [PubMed: 24759106]

78. Liu GH, Qu J, Shen X. NF-kappa B/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. Bba-Mol Cell Res. 2008; 1783(5):713–727.

Highlights

- Silica nanoparticles with iron induced pro-inflammatory cytokines at early phase of exposure in human macrophages
- Nrf2 signaling and its target antioxidant genes were activated or induced at a later phase of exposure to silica nanoparticles with iron
- NF-κB inhibition blocked the induction of pro-inflammatory cytokines by silica nanoparticles with iron
- NF-κB inhibition blocked the activation of Nrf2 and the induction of Nrf2-regulated antioxidant genes by silica nanoparticles with iron

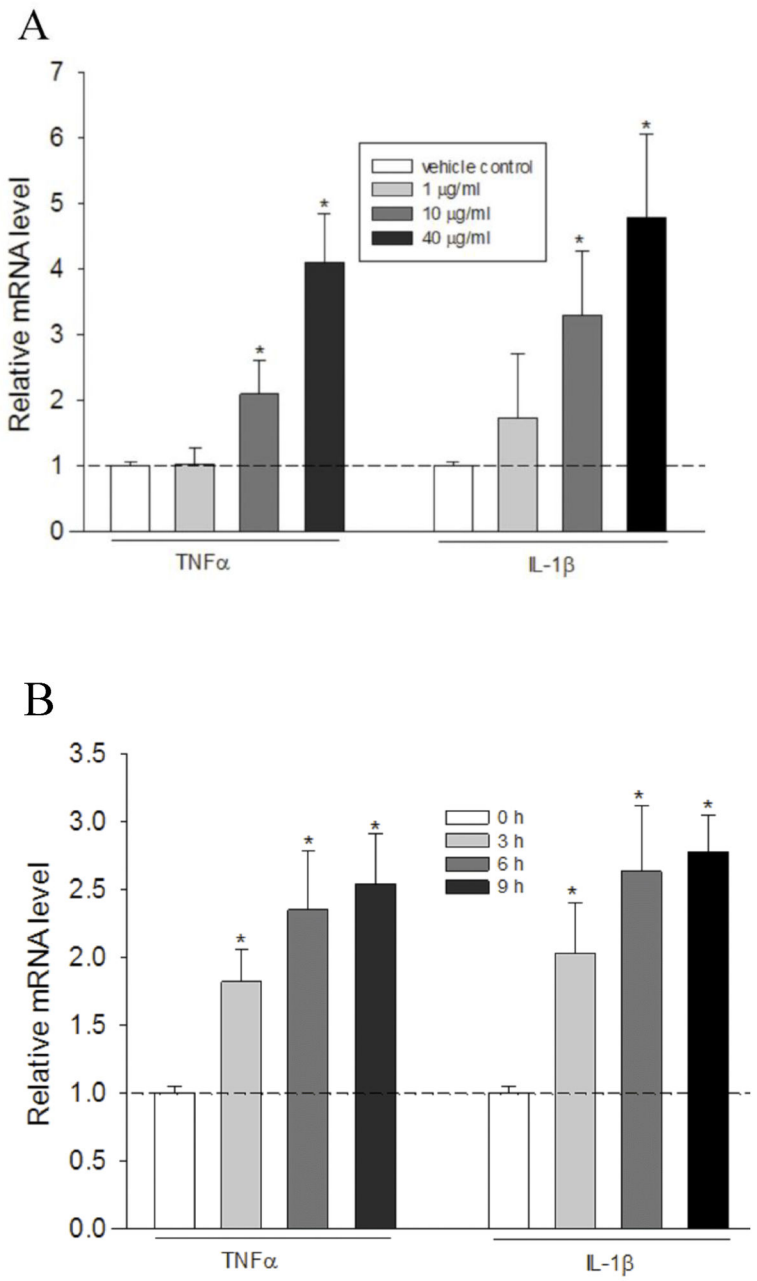
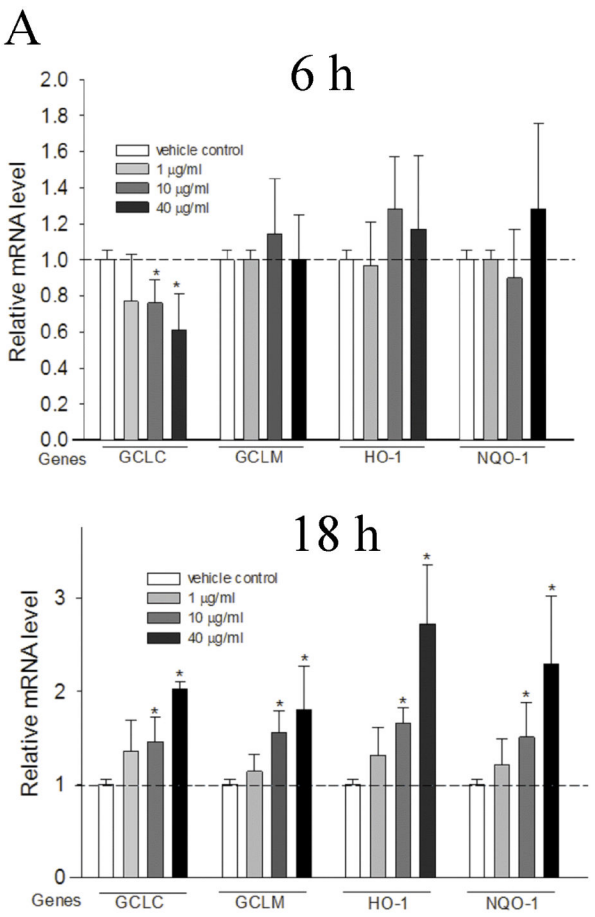


Figure 1.

SiO_2-Fe stimulated induction of pro-inflammatory cytokines. (A) SiO_2-Fe-induced TNF-α and IL-1β in a dose dependent manner. Differentiated THP1 macrophages cells were treated with SiO_2-Fe for 6 h with indicated concentration of Si-Fe and the mRNA levels of TNF-α and IL-1β were measured with real time PCR assay. (B) Change of TNF-α and IL-1β mRNAs with time after SiO_2-Fe exposure. Cells were treated with 20 μg/ml SiO_2-Fe for indicated time and the mRNA level was measured. N=3; *, $P<0.05$ compared to vehicle control.



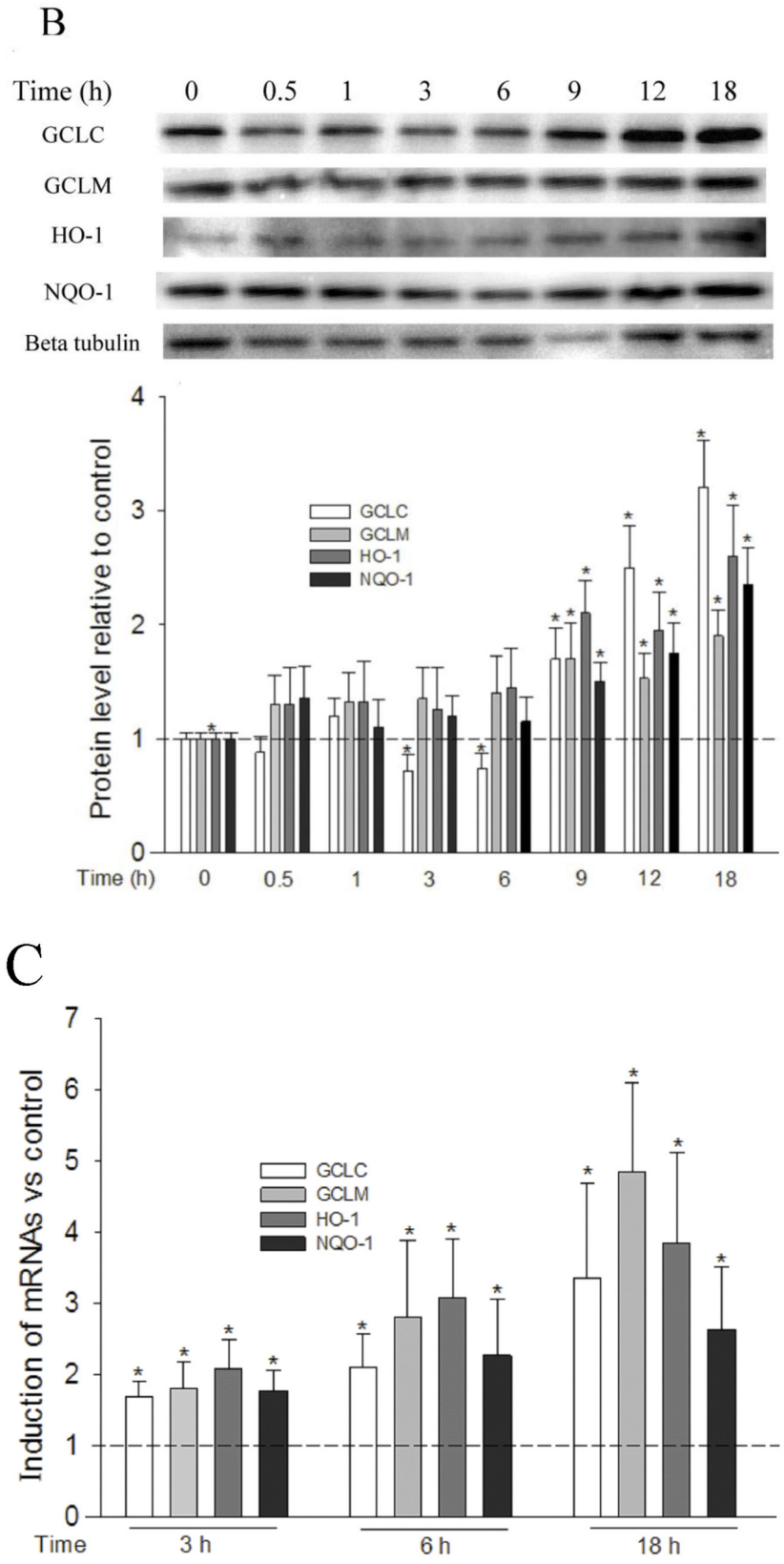
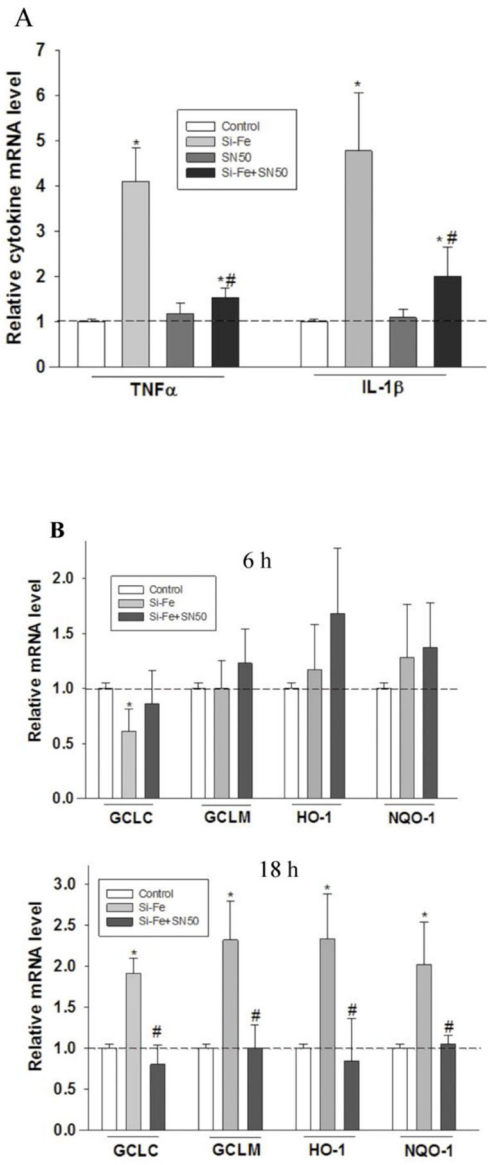


Figure 2.

SiO_2-Fe increased Nrf2-regulated antioxidant and detoxifying genes. (A) mRNA level at 6 h and 18 h; (B) Protein level; (C) mRNA induction by sulforaphane. Differentiated THP1 macrophages cells were treated with Si-Fe with indicated doses for 6 h (A), or 20 μg/ml Si-Fe (B) or 5 μM sulforaphane (C) for indicated time. The mRNA of target genes was measured with real time PCR and the protein was determined with Western Blotting. N=3, *, $P<0.05$ compared to vehicle control.



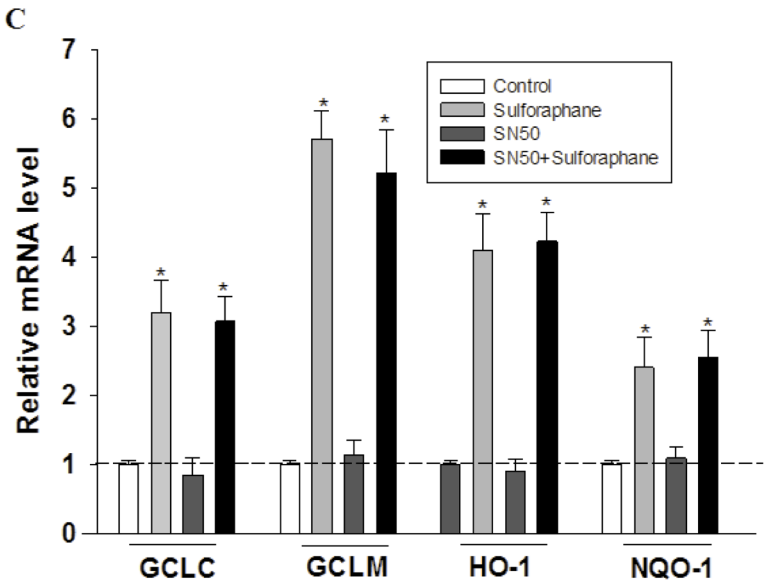


Figure 3.

SN-50 inhibited the effects of SiO_2-Fe on Nrf2-regulated antioxidant genes. (A) SN50 inhibited Si-Fe-induced pro-inflammatory cytokines. (B) SN-50 effects on antioxidant gene regulation by SiO_2-Fe. (C) NF-κB antagonist SN50 had no effect on sulforaphane induction or baseline expression of Nrf2-regulated antioxidant genes. Cells were pretreated with 50 μg/ml SN-50 for 15 min and then exposed to 20 μg/ml of SiO_2-Fe for 6 h (A) and 18 h (B) respectively, or to 5 μM sulforaphane for 18 h (C). The mRNA levels of cytokines and antioxidant genes were measured with real time PCR. Si-Fe, SiO_2-Fe. N=3; *, $P<0.05$ compared to vehicle control; #, $P<0.05$ compared with SiO_2-Fe exposure.

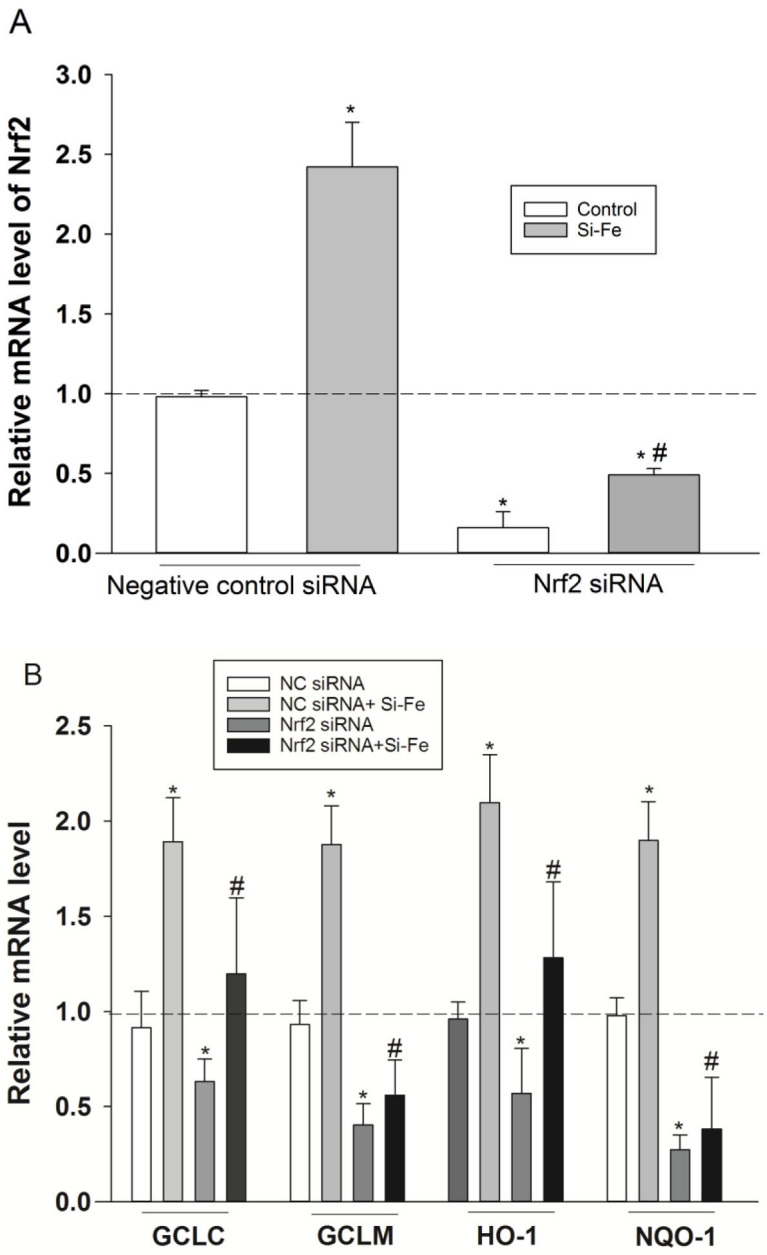


Figure 4.

Nrf2 silencing abrogated the induction of antioxidant genes by SiO_2-Fe in THP-1 macrophages. Differentiated THP1 macrophages were transfected with negative control siRNA (NC siRNA) or Nrf2 siRNA for 24.before being treated with vehicle control or 20μg/ml SiO_2-Fe nanoparticles (Si-Fe) for 18 h. The mRNA levels of Nrf2 (A) and GCLC, GCLM, HO-1, and NQO-1 were measured using real time PCR assay. *, $P<0.05$ compared with vehicle control of NC siRNA, N=4; #, $P<0.05$ compared with SiO_2-Fe of NC siRNA, N=4.

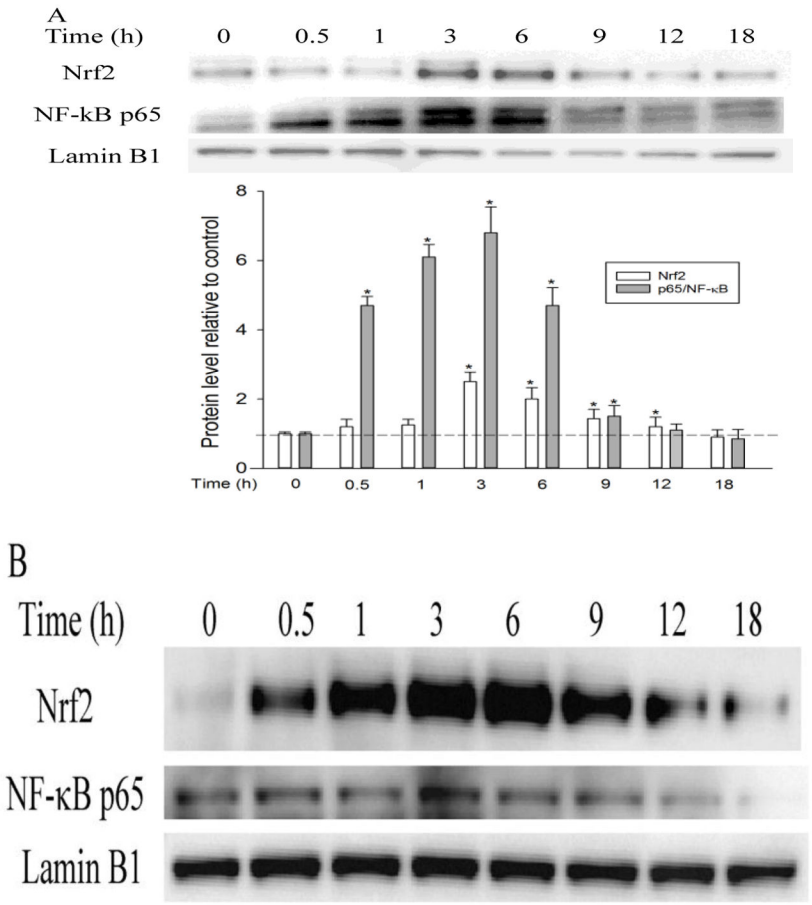


Figure 5.

Nrf2 activation by SiO_2-Fe required NF-κB signaling. (A) Time-dependent increases of nuclear Nrf2 and NF-κB/p65 with Si-Fe treatment. (B) Activation of Nrf2 and NF-κB/p65 by sulforaphane. THP1 macrophages were treated with 20 μg/ml of SiO_2-Fe (A) or 5 μM sulforaphane (B) for indicated time and the nuclear protein was extracted, then Nrf2 and p65 in the nucleus were measured as a function of time after exposure using Western blotting. N=3; *, P<0.05 compared to vehicle control; #, P<0.05 compared to SiO_2-Fe.

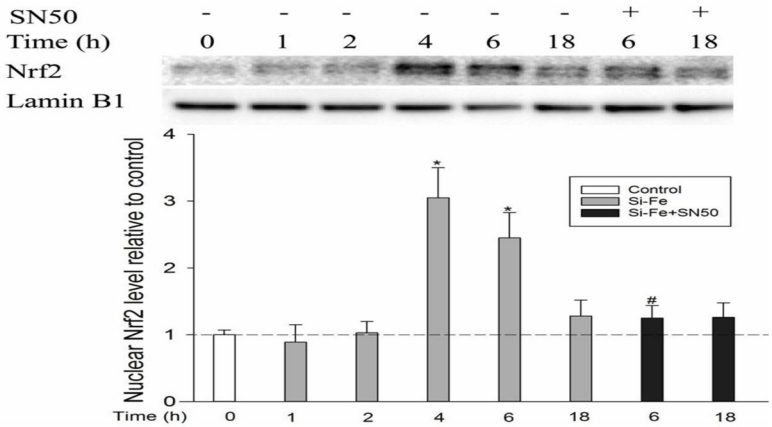


Figure 6.

SN50 inhibited SiO_2-Fe-caused Nrf2 activation. Cells were pretreated with 50 μg/ml SN-50 for 15 min and then exposed to 20 μg/ml of SiO_2-Fe for indicated times, the nuclear protein was extracted and Nrf2 was determined with Western blotting. *, $P<0.05$ compared with vehicle control, N=3; #, $P<0.05$ compared with SiO_2-Fe, N=3.

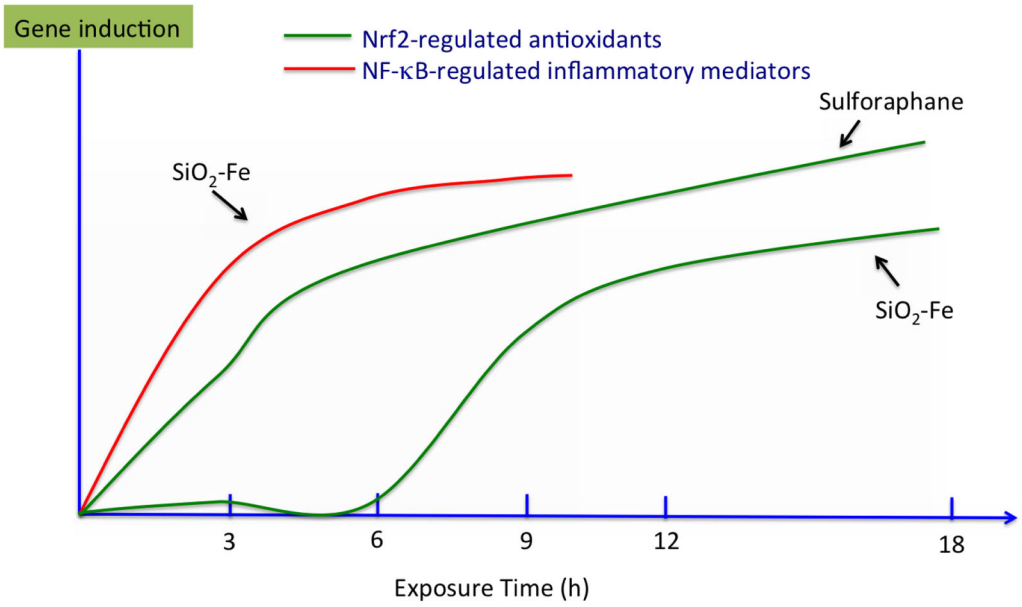


Figure 7.

Summary of the temporal effect of SiO_2-Fe on the activation of NF-κB and Nrf2 signaling. X Axis shows the exposure time and Y Axis shows the induction of genes regulated by NF-kB (red line) or Nrf2 signaling (Green line).

Table 1

Primer sequences for mRNA assay

Genes	Primers
Actin	Forward 5′-CATGGAGTCCTGTGGCATC-3′; Reverse 5′-GGAGCAATGATCTTGATCTTC-3′
GCLC	Forward 5′-ATGGAGGTGCAATTAACAGAC-3′; Reverse 5′-ACTGCATTGCCACCTTTGCA-3′
GCLM	Forward 5′-GCTGTATCAGTGGGCACAG-3′; Reverse 5′-CGCTTGAATGTCAGGAATGC -3′
HO-1	Forward 5′-TCTCTTGGCTGGCTTCCTTAC-3′; Reverse 5′-GGCTTTTGGAGGTTTGAGACA-3′
NQO-1	Forward 5′-TGCAGCGGCTTTGAAGAAGAAAG-3′; Reverse 5′-TCGGCAGGATACTGAAAGTTCGC-3′
TNF-α	Forward 5′-CCCAGGGACCTCTCTCTAATCA-3′; Reverse 5′-AGCTGCCCCTCAGCTTGAG-3′
IL-1β	Forward 5′-CGACACATGGGATAACGAGGCTT-3′; Reverse 5′-TCTTTCAACACGCAGGACAGGTA-3′