Maintenance of intracellular redox homeostasis by an antioxidant enzyme glutaredoxin 1 (Grx1) in human cells

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Abstract

Backgrounds

Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism as well as in cellular response to ionizing radiation, heat exposure or oxidizing agents. ROS act as the critical signaling molecules in cellular metabolism. However, excessive accumulation of ROS within cells results in oxidative stress that leads to oxidative damages to proteins, lipids and DNA, which is implicated in many disease states.

In particular, oxidative modification in protein thiols can affect the catalytic or structural function of a protein. Cells have specific enzyme systems that can reduce most of these forms of oxidized thiols. Glutaredoxin 1 (Grx1) is a reductase that belongs to the thiol-disulfide oxidoreductase superfamily (Glutaredoxin family), which catalyze the reduction of oxidized protein thiols (disulfides in protein or mixed disulfides between protein and glutathione). A number of studies had reported the biochemical function of Grx1 in regulating sulfhydryl homeostasis *in vitro*. However, the physiological regulation of human cells by Grx1 is still poorly understood. In the present study, I clarified the role of Grx1 in maintaining intracellular redox homeostasis.

Methods

Grx1-deficient HeLaS3 cell lines were generated by CRISPR / Cas9 system and Grx1-overexpressing cell lines were generated by transfecting prepared pcDNA4/TO/Grx1-Myc-HisA plasmids into T-REx HeLa cells and using drug

screening (zeocin). I investigated the survival of cells exposed to γ-rays irradiation, heat shock and H_2O_2 by colony formation assay. I analyzed the cell division capacity under oxidative stress exposure by performing a cell proliferation assay. The accumulation of total oxidants in cells was investigated by a fluorescent probe (2'-7'dichlorofluorescin diacetate, DCFH-DA) staining. ROS production level in mitochondria was determined by MitoSox Red staining assay. Apoptosis and necrosis were observed under a fluorescence microscope after Annexin-V/EthD-III staining, and then apoptosis and necrosis rates were calculated. Multinucleated cells were observed and analyzed after MitoTracker staining. Genomic stability was examined by micronucleus formation assay. The level of NADPH was measured using a spectrophotometer. The protein levels in this study were determined by western blotting. The protein bands were detected using Las 3000 (Fuji film). Detected bands were captured and quantified using ImageJ software.

Results

First, Grx1-deficient isogenic cell lines were established and screened. Then, the results in survival assay showed that Grx1-deficient HeLaS3 cells were more sensitive to γ-rays irradiation, heat shock or H_2O_2 exposure than HeLaS3 wild-type cells. Next, I found that oxidative stress exposure induced more oxidant accumulation and higher levels of oxidized ROS scavenging enzyme peroxiredoxin 2 (Prx2) or higher levels of total oxidized protein in Grx1-deficient HeLaS3 cells compared to HeLaS3 wild-type cells. This indicated that Grx1 deficiency was associated with the disruption of intracellular redox balance and the dysregulation of protein redox state. Furthermore, I found that Grx1 deficiency caused higher levels of ROS production in mitochondria, higher levels of cytochrome c, and higher rates of apoptosis after cells were exposed to oxidative stress. This suggested that deficiency of Grx1 contributed to the oxidative stress-induced mitochondrial dysfunction and mitochondria-mediated apoptotic cell death. Genomic stability was investigated by micronucleus formation assay, and there was no significant difference in micronucleus formation rate between Grx1-deficient HeLaS3 cells and HeLaS3 wild-type cells after oxidative stress exposure. Besides,

Grx1 deficiency had no effect on the p38/p53/p21 DNA damage response signalling axis as there was no significant difference in p38, P-p38, p53 and p21 levels between HeLaS3 Grx1-deficient cells and HeLaS3 wild-type cells under oxidative stress exposure. On the other hand, I found that deficiency of Grx1 resulted in a significant decrease in cell proliferation after oxidative stress exposure. Additionally, a higher rate of multinucleated cell formation was also found in Grx1-deficient HeLaS3 cells than in HeLaS3 wild-type cells, suggesting that deficiency of Grx1 was associated with inhibited proliferation induced by cytoplasmic division disorder during oxidative stress exposure.

Grx1-overexpressing T-REx HeLa cell lines were established and screened. The proliferation ability of Grx1-overexpressing T-REx HeLa cells was lower than that of wild-type cells under γ-rays irradiation, heat shock and H_2O_2 exposure. Next, I found that total ROS levels in Grx1-overexpressing cells were higher than those in wild-type cells after experimental treatment. Furthermore, the levels of γH2AX in Grx1-overexpressing cells were higher than those in wild-type cells under oxidative stress exposure, suggesting a higher level of DNA damage in Grx1-overexpressing cells. In addition, I measured intracellular levels of NADPH, an important substance in the reductase system involved in regulating intracellular redox homeostasis. I found that levels of NADPH were reduced in Grx1-overexpressing cells after oxidative stress exposure.

Discussion

Under oxidative stress exposure, excessive accumulation of ROS and increased levels of oxidized Prx2 in Grx1-deficient cells suggested that Grx1 could maintain intracellular redox homeostasis by regulating the redox state of ROS scavenging enzyme. On the other hand, increased ROS levels and decreased NADPH levels in Grx1-overexpressing cells suggested that Grx1 overexpression could lead to disruption of intracellular redox homeostasis by affecting the stability of the reductase system, and may further affect redox signaling, indirectly causing DNA damage.

Conclusion

Grx1 plays an important role in regulating redox homeostasis in human cells and affects the cellular physiological response to oxidative stress. Either deficiency or overexpression of Grx1 adversely affects the physiological response of cells to oxidative stress by causing disruption of intracellular redox homeostasis and oxidative damage to intracellular macromolecules.