Thioredoxin System and Redox Signaling; Defence against Stress and Toxicity

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Human Thioredoxin (TRX) with with redox-active dithiol (C-C-Y-C-) in the active site has been cloned as adult T cell leukemia derived factor produced by HTLV-I transformed cells. Thioredoxin (TRX) is one of the major components of the thiol-reducing system and plays multiple roles in cellular processes such as proliferation, apoptosis and gene expression. TRX is induced by a variety of stresses including viral infection(Annu. Rev. Immunol. 15, 351-369, 1997, Current Trends in Immunology 1, 133-140, 1998) and is involeved in the resistance against toxicity by chemicals and anticancer agents such as CDDP and Bleomycin. The promoter sequences of the TRX gene contain a series of stress-responsive elements such as CRE, XRE, ARE, and AP1 but not for HSE. TRX promotes DNA binding of transcription factors such as NF-kB, AP-1 and p53 (Proc. Natl. Acad. Sci. U. S. A. 94, 3633-3638 , 1997; J. Biol. Chem. 274, 35809-35815, 1999). TRX is also secreted from the activated cells as a redox-sensitive cytokine with cytokine-like and chemokine-like activities (H.Nakamura et al. Proc. Natl. Acad. Sci. USA in press).

Redox regulation by TRX plays a crucial role in biological responses against oxidative stress. Transgenic mice overexpressing TRX show resistance against ischemic neuronal injury (Proc. Natl. Acad. Sci. USA 96:4131-4136,1999). Bone marrow cells from TRX transgenic mice also demonstrated resistance against UV- or X-ray irradiation (in collaboration with Drs. Inoue and Hirabayashi, Biological Safety Research Center, Japanese National Institute of Health Sciences). In addition, TRX transgenic mice exhibit up to 30% extension of median life span and one-third of maximum life span. Protective action of TRX against experimental bacterial and viral infection as well as acute inflammatory lung disease (ARDS) involving systemic oxidative stress has been shown in TRX transgenic mice and also by exogenous recombinant TRX.

We have identified thioredoxin binding protein-2 (TBP-2), which was identical to vitamin D3 up-regulated protein 1 (VDUP1)(J. Biol.Chemistry 274:21645-21650, 1999). TBP-2/VDUP1 suppressed the reducing activity of TRX. Treatment of HL-60 cells with vitamin D3 caused an increase of TBP-2/VDUP1 expression, suggesting that the TRX-TBP-2/VDUP1 interaction may be an important redox regulatory mechanism in cellular processes, including differentiation of myeloid /macrophage lineage. We will also discuss the recent data on the increasing number of TRX Superfamily proteins including the anti-apoptotic activity of mitochondoria-specific thioredoxin-2 (TRX-2) based on in vitro knock-out system (coop with G.Spyrou) as well as a new ER-specific TRX family protein (Y. Matsuo et al. J. Biol.Chemistry 2001).

INTRODUCTION

Environmental stresses including oxidative stress, metals, chemicals, heat shock, osmotic stress, pollutants, and ultraviolet irradiation cause various toxic effects on individuals such as defect in the immune, the reproductive, the endocrine system and carcinogenesis. On the other hand, cells have multiple mechanisms to protect themselves from such stresses through an apoptosis, DNA repair, cell cycle arrest, and induction of anti-oxidant enzyme such as thioredoxin. Responses to variety of environmental stressors seem to be mediated by a series of signal transduction pathway resulting in activation of transcription factors, which are regulated by the redox regulating mechanism.

THE THIOREDOXIN SYSTEM

1. Thioredoxin and related molecules

Thioredoxin was originally identified in Escherichia coli and is known to be a dithiol hydrogen donor for a variety of target proteins. The two cystein residues of thioredoxin undergo reversible oxidation-reduction reactions catalyzed NADPH-dependent enzyme, thioredoxin reductase. Human
thioredoxin (TRX) is a 12 kDa protein with redox-active dithiol (Cys-Gly-Pro-Cys-) in the active site has been cloned as adult T cell leukemia derived factor produced by HTLV-I transformed cells. Several cytokine-like factors such as 3B6-IL-1, eosinophil cytotoxicity enhancing factor (ECEF), T-cell hybridoma MP6-derived B-cell growth factor, and early pregnancy factor, are identical to thioredoxin. Thioredoxin (TRX) is one of the major components of the thiol-reducing system and plays multiple roles in cellular processes such as proliferation, apoptosis and gene
expression.

2. TRX induction and TRX-dependent signal transduction

TRX is induced by a variety of stresses including viral infection(Annu.

Rev. Immunol. 15, 351-369, 1997, Current Trends in Immunology 1, 133-140,
1998) and is involeved in the resistance against toxicity by chemicals and anticancer agents such as CDDP, bleomycin and adriamycin. TRX has radical scavenging activity and can protect cell from TNF, H2O2, and ischemic reperfusion injury. The direct association between TRX and redox factror-1 (Ref-1)/ AP-endonuclease has been suggested to play a key role in the AP-1 transcriptional activity. TRX also binds to a mitogen-activated protein kinase kinase kinase, apoptosis signal-regulating kinase 1 (ASK-1) and inhibits the apoptotic process. When TRX is

oxidized by oxidative stress, ASK-1 is dissociated from oxidized TRX and is activated to induce an
apoptosis signal suggesting that redox regulation is involved in the mechanism of the cell death (EMBO J. 17, 2596-2606, 1998). The promoter sequences of the TRX gene contain a series of stress-responsive elements such as CREB, XRE, ARE, and AP-1 and SP-1 but not for HSE. Recent studies in the regulation of the TRX gene have shown that the redox and detoxifying enzymes have common regulatory mechanism and may have co-ordinative role against environmental stressors. (Kim et al. J. Biol. Chem. in press). TRX translocation from cytosol to nucleus was induced by a wide variety of oxidative stresses including UV irradiation, hydrogen peroxide, or hypoxia, treatment with CDDP and may promotes DNA binding of transcription factors such as NF-kB, AP-1, hypoxia inducing factor 1 (HIF-1), PEBP2/AML1, glucocorticoid receptor, estrogen receptor and p53 (Proc. Natl. Acad. Sci. U. S. A. 94, 3633-3638 , 1997; J. Biol. Chem. 274, 35809-35815, 1999) by redox regulation.

3. Extacellular functions of TRX

TRX is also secreted from the activated cells as a redox-sensitive cytokine with cytokine-like and chemokine-like activities (H.Nakamura et al. Proc. Natl. Acad. Sci. USA. in press).

4. TRX knockout and transgenic mice

In mice, heterozygotes carrying a targeted disruption of the mouse TRX gene are viable, fertile and appear normal. In contrast, homozygous mutants die shortly after implication. These results suggest that TRX is essential for early differentiation and morphogenesis of the mouse embrio (Matsui et al. Developmetal Biology 178, 179-185, 1996). Transgenic mice overexpressing TRX show resistance against ischemic neuronal injury (Proc. Natl. Acad. Sci. USA 96:4131-4136,1999). Moreover,
specific-overexpression of human TRX by insulin promoter in pancreatic islet beta-cells in mice prevents autoimmune and streptozocin-induced diabets (Hotta et al J. Exp. Med. 188, 1445-1451, 1998). Bone marrow cells from TRX transgenic mice also demonstrated resistance against UV- or X-ray irradiation (in collaboration with Drs. Inoue and Hirabayashi, Biological Safety Research Center, Japanese National Institute of Health Sciences). In addition, TRX transgenic mice exhibit up to 30% extension of median life span and one-third of maximum life span. Protective action of TRX against experimental bacterial and viral infection as well as acute inflammatory
lung disease (ARDS) involving systemic oxidative stress has been shown in TRX transgenic mice and also by exogenous recombinant TRX.

5. TRX-binding proteins and TRX superfamily

We have identified thioredoxin binding protein-2 (TBP-2), which was identical to vitamin D3

up-regulated protein 1 (VDUP1)(J. Biol.Chem. 274:21645-21650, 1999). Treatment of HL-60 cells with vitamin D3 caused an increase of TBP-2/VDUP1 expression and down-regulation of the expression
and the reducing activity of TRX, suggesting that the TRX-TBP-2/VDUP1 interaction may be an important redox regulatory mechanism in cellular processes, including differentiation of myeloid/macrophage lineages. We will also discuss the recent data on the increasing number of TRX Superfamily proteins including the anti-apoptotic activity of mitochondoria-specific thioredoxin-2 (TRX-2) based on in vitro knock-out system (coop with G.Spyrou) as well as a new ER-specific TRX family protein (Y. Matsuo et al. J. Biol. Chem. 276:10032-10038, 2001).

CONCLUSION AND FUTURE PERSPECTIVE

Environmental stressors cause damages to the cells with production of ROS. Oxidative stress is largely mediated by ROS. Antioxidant enzyme as TRX system can protect cells by scavenging of ROS and is to be involved in a cellular sensing signaling molecule against oxidative stresses. Further analysis of role of TRX system can also help to us against oxidative stress-induced pathogenic effects and will contribute to us new therapeutic approch and developments of novel biotechnological tools by redox regulation in biological response.