Thioredoxin/Adult T-Cell Leukemia-Derived Factor (ADF) and Redox Regulation

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Introduction

Adult T-cell leukemia-derived factor (ADF) was first defined as an IL-2R α chain inducing factor produced by human T cell lymphotropic virus-I (HTLV-I)-transformed T cells. Subsequent purification and gene cloning have revealed that ADF is human thioredoxin (TRX). TRX was originally isolated from *Escherichia coli* as a hydrogen donor for ribonucleotide reductase, an essential enzyme for DNA synthesis, and has been purified and characterized from a wide variety of prokaryotic and eukaryotic species. TRX is a small ubiquitous protein (M. W. 12 kDa) having a redox-active disulfide/dithiol within the conserved active site sequence : -Cys-Gly-Pro-Cys-. TRX/ADF has been reported to function as a reducing factor through its dithiol group and have multiple biological activities via the regulation of intracellular redox status.

TRX/ADF as a stress-inducible protein

Originally, the expression of TRX/ADF was reported to be up-regulated in HTLV-I- and Epstein-Barr virus (EBV)-transformed lymphoid cells. However, TRX/ADF has been shown to be a stress-inducible protein, whose expression is enhanced by not only viral infection but also a variety of cellular stresses. Especially, TRX/ADF can be strongly induced by various oxidative stresses, including oxidative agents (hydrogen peroxide, diamide), X-ray and ultraviolet (UV) irradiation, and ischemic reperfusion. We have recently demonstrated that the promoter of TRX/ADF gene contains a novel cis-regulatory element for oxidative stresses, and that this element is involved in the transactivation of the TRX/ADF gene. In addition, other various types of cellular stimuli, such as a viral gene product (HTLV-I Tax), sex hormone (estrogen), cytokines (IL-2), prostaglandins, and anti-cancer agents (cisplatin and bleomycin), have been shown to induce the enhanced expression of TRX/ADF. These observations suggest that TRX/ADF has important roles in the cellular responses against various cellular stresses including oxidative stress.

The biological activities of TRX/ADF as a reducing factor

TRX/ADF is reported to be one of the important factors to maintain the redox environment of the cell. Regulation of intracellular redox environment is critical for various aspects of cellular functions. For example, exogenous administration of TRX/ADF can augment the growth promoting effects of other cytokines on lymphocytes as well as on non-lymphoid cells. TRX/ADF can also enhance the expression of several cytokines, such as IL-1 α , IL-2, IL-6, IL-8 and TNF. Site-

directed mutagenesis of the active site cysteines of TRX/ADF has revealed that the reducing activity is necessary for these biological effects of TRX/ADF. In addition, exogenous TRX/ADF promotes the uptake of cystine into cells and up-regulates the intracellular levels of glutathione. Since cystine is a source of glutathione synthesis, the effects of TRX/ADF may be partly explained by the increased levels of glutathione.

TRX/ADF also plays crucial roles in the cytoprotection against cellular stresses. Exogenous TRX/ADF has been shown to protect cells from the cytotoxicity induced by oxidative stresses, including TNF, hydrogen peroxide, and ischemic reperfusion injury. Moreover, it is suggested that TRX/ADF is essential for the early differentiation and morphogenesis of embryos. Recent our report describing the phenotype of mice carrying a targetted disruption of the TRX/ADF gene has revealed that homozygous mutants die shortly after implantation whereas heterozygotes are viable, fertile, and appear normal. In the tissues of pregnant uterus, TRX/ADF is overexpressed in the decidua and trophoblast cells, suggesting that TRX/ADF may be beneficial in protecting the fertilized egg and placental trophoblasts from the cytotoxic effects of oxygen radicals.

Redox regulation of intracellular signaling by TRX/ADF

It is suggested that TRX/ADF is involved in various cellular processes as an endogenous thiol-related reducing agent, by modulating protein-protein or protein-nucleic acid interactions through the reduction/oxidation of protein cysteine residues. For example, TRX/ADF has been known to be required for the DNA binding of glucocorticoid receptor and for the optimal interaction between iron responsive element-binding protein and the transferrin receptor mRNA. In addition, the binding of Jun/Fos complex to the AP-1 site is enhanced by TRX/ADF through the activation of the redox-sensitive nuclear factor, Ref-1/APEX/HAP-1. The activity of NF- κ B is also redox-regulated and the cysteine residue 62 of NF- κ B p50, which can be reduced by TRX/ADF, is critical site for the binding of NF- κ B to DNA. Recently, we have demonstrated that TRX/ADF can regulate the biological activities of its target molecules, such as NF- κ B p50 and Ref-1/APEX/HAP-1, through the direct interaction.

TRX/ADF has been found to translocate from the cytosol into the nucleus by various stresses. For example, we demonstrated that UV irradiation and PMA induced nuclear translocation of TRX/ADF in some human cell lines. Although untreated cells express TRX/ADF mainly in the cytosol, diffuse nuclear localization of TRX/ADF is observed after treatment with UV or PMA. Therefore, it is suggested that TRX/ADF, which translocates from the cytosol into the nucleus by cellular stresses, may be involved in the transactivation of various genes through the redox regulation of its target molecules, such as some transcription factors.

The role of TRX/ADF on the biological activity of cytotoxic agents

It is suggested that the TRX system can modulate the biological activity of several cytotoxic agents. For example, we and others have recently demonstrated that TRX/ADF is involved in the cellular resistance against anti-cancer agents. TRX/ADF-overexpressed cells are more resistant to anti-cancer agents such as cisplatin compared to the control cells, and TRX/ADF-antisense transfected cells are more sensitive to them. The expression of TRX/ADF can be up-regulated by these anti-cancer agents, suggesting that TRX/ADF plays important roles in the cytoprotection against them. In addition, TRX reductase, which reduces and activates TRX/ADF using NADPH as a cofactor, is found to be one of the crucial target molecules for several cytotoxic agents. We have demonstrated that TRXR inhibitors, including 13-cis-retinoic acid, azelaic acid, and menadione, prevent the DNA synthesis and cell cycle progression in HTLV-I-transformed T cell lines. Although further studies are needed to clarify the precise role of the TRX system on the biological activity of cytotoxic agents, the development of pharmacological or genetical methods to regulate the activity of the TRX system may be a therapeutic strategy to modulate the cellular sensitivity to them.

Conclusion

TRX/ADF is a stress-inducible protein with multiple biological functions. It is suggested that TRX/ADF may be involved in the cellular responses against various stresses through the regulation of intracellular redox status. TRX/ADF translocates from the cytosol into the nucleus by a variety of cellular stresses and regulates the biological activities of its target molecules. Further studies to elucidate the regulatory roles of TRX/ADF and its target molecules may clarify the intracellular signaling pathways in the responses against various stresses including oxidative stress.