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Review

The thioredoxin system in retroviral infection and apoptosis

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Abstract

Human thioredoxin (TRX) was first identified in human T-cell leukemia virus type I (HTLV-I)-positive T-cell lines and is associated with the pathophysiology of retroviral infections. TRX is a vital component of the thiol-reducing system and regulates various cellular function (redox regulation). Members of the TRX system regulate apoptosis through a wide variety of mechanisms. A family of thioredoxin-dependent peroxidases (peroxiredoxins) protects against apoptosis by scavenging hydrogen peroxide. Thioredoxin 2 is a critical regulator of cytochrome *c* release and mitochondrial apoptosis; transmembrane thioredoxin-related molecule (TMX) has a protective role in endoplasmic reticulum (ER) stress-induced apoptosis. TRX interacts with apoptosis signal-regulating kinase 1 (ASK1) and is a sensor of oxidative stress. Thioredoxin binding protein-2/vitamin D_3 upregulated protein 1 is a growth suppressor and its expression is suppressed in HTLV-I-transformed cells. Studies of these molecules of the TRX system provide novel insights into the apoptosis associated with retroviral diseases.
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Abbreviations: HTLV-I, human T-cell leukemia virus type I; ATL, adult T-cell leukemia; TBP-2, thioredoxin-binding protein-2; VDUP1, vitamin D_3 upregulated protein-1; ASK1, apoptosis signal-regulating kinase 1; ROS, reactive oxygen species; TMX, transmembrane thioredoxin-related protein; ER, endoplasmic reticulum; GSH, glutathione; TRX, thioredoxin; AIF, apoptosis-inducing factor; IAPs, inhibitor of apoptosis proteins; TRXR, thioredoxin reductase; IL-2, interleukin-2; IL-2Rα, α chain of IL-2 receptor; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; ANT, adenine nucleotide translocator

The Thioredoxin (TRX) System and Regulation of Apoptosis Signal

TRX

The cellular reducing environment is provided by two mutually interconnected systems; the TRX system and the glutathione (GSH) system. Under physiological conditions, the intracellular reducing environment is maintained by the disulfide/dithiol-reducing activity of the GSH and TRX systems. GSH is a cysteine-containing tripeptide (γ-glutamyl-cysteinyl-glycine), which is a major component of cytosolic antioxidant. TRX is a small protein with two redox-active cysteine residues in its active center (–Cys–Gly–Pro–Cys–) and operates together with NADPH and thioredoxin reductase as an efficient reducing system for exposed protein disulfides (Figure 1).[1] While the amount of TRX (micromolar concentration) is much less than that of GSH (millimolar concentration), TRX and GSH play distinct roles in maintaining cellular environment. TRX enhances the binding of transcription factors to the target DNA more efficiently than GSH. TRX can directly associate in the nucleus with redox factor 1 (Ref-1), which is identical to a DNA repair enzyme, AP endonuclease, and both molecules through their redox-active cysteine residues augment the DNA-binding activity of transcription factors, such as activator protein 1 (AP-1) and p53.[2–4] The components of the TRX system not only scavenge reactive oxygen species (ROS) but also play regulatory roles in a variety of cellular function through protein–protein interaction.[5] Mice carrying the homozygously deleted *TRX* gene died shortly after implantation, suggesting that TRX is essential for cell survival and early development.[6] TRX transgenic mice, which ubiquitously overexpress human TRX under the control of β-actin promoter, display various phenotypes, such as an elongated lifespan[7] and protection against ischemic injury,[8] acute lung failure,[9] diabetes mellitus,[10] and the toxicity caused by environmental stressors.[11] Since oxidative stress has been implicated in these conditions, TRX seems to play an important role in protection against oxidative stress-associated diseases. In cooperation with peroxiredoxins (described below), TRX has an antiapoptotic effect by scavenging intracellular ROS through the dithiol at its active site (Figure 2). Intriguingly, S-nitrosylation at cysteine 69 is required for the reducing activity and antiapoptotic function.[12]

Oxidative stress and apoptosis

Our group reported that a thiol-oxidizing reagent, diamide, induces cell death. The cell death is either apoptosis or

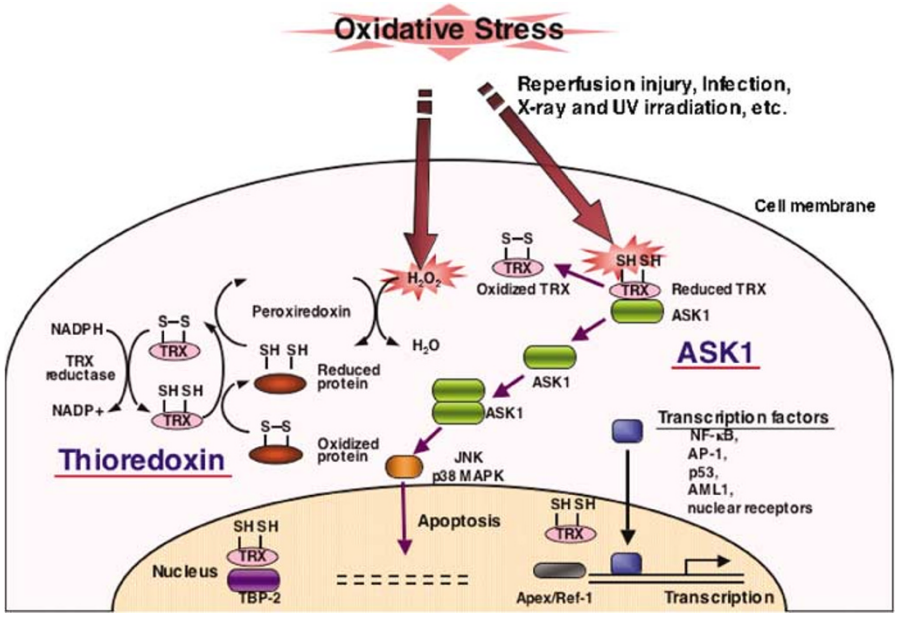

Figure 1 The role of the thioredoxin (TRX) system and related molecules in the regulation of apoptosis. Host cells exert multiple defense responses such as cell cycle control, apoptosis, and antioxidant induction against environmental stressors. The TRX system is composed of several related molecules forming a network of interactions with its active site cysteine residues, maintaining the cellular reducing environment and protecting cells from oxidative stress. TRX operates together with NADPH and TRX reductase as an efficient reducing system for exposed protein disulfides and cooperates with families of TRX-dependent peroxidases (peroxiredoxins) to scavenge intracellular hydrogen peroxide. The binding of transcription factors to DNA is positively modulated by TRX and apurinic/apyrimidinic endonuclease (Apex)/redox factor-1 (Ref-1). TRX also exerts its role through interaction with its binding proteins. TRX-binding protein-2 (TBP-2)/vitamin D_3 upregulated protein 1 (VDUP1) plays an important regulatory role in growth control. TRX also interacts with apoptosis signal-regulating kinase 1 (ASK1), an important regulator of apoptosis. When TRX is oxidized in response to oxidative stress, ASK1 is dissociated from the oxidized TRX and activated to induce an apoptotic signal

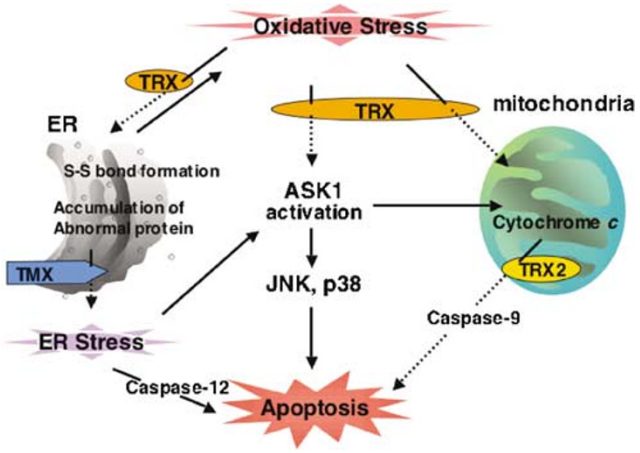

Figure 2 Interconnection among oxidative stress, endoplasmic reticulum (ER) stress, and mitochondrial apoptosis. Mammalian thioredoxin 2 (TRX2) is an essential regulator protecting cytochrome *c* release and apoptosis, whereas transmembrane thioredoxin-related molecule (TMX) plays a role counteracting ER-mediated apoptosis. Apoptosis signal-regulating kinase 1 (ASK1) seems to not only sense oxidative stress but regulate both ER- and mitochondria-derived apoptosis signals

necrosis, depending on the concentration.[13,14] Intriguingly, diamide caused release of mitochondrial cytochrome *c* into the cytosol regardless of whether apoptosis or necrosis is induced. Since each caspase has a cysteine residue in its active site, the activity of caspase is regulated by the redox state (mainly by the TRX system).[14] A disruption of caspase activity induced by oxidative stress resulted in a shift from apoptosis to necrosis. Taken together with the results of the hydrogen peroxide-induced apoptosis–necrosis shift,[15] the intracellular redox state and redox-sensitive caspase activity seem to regulate the morphological changes of cell death. Cell death induced by oxidative stress appears to be apoptosis as long as the intracellular reducing status is maintained by the TRX system.[14,16]

Proapoptotic signal including oxidative stress converges on mitochondria to induce mitochondrial outer membrane permeabilization, which is lethal because it results in the release of proapoptotic caspases-activating molecules and caspases-independent death effectors and metabolic failure in mitochondria.[17] Permeability transition pore complex consists of voltage-dependent anion channel (VDAC) localized in outer membrane, adenine nucleotide translocator (ANT) in inner membrane, and cyclophilin D in the matrix, and opening of the pore leads to loss of the mitochondrial transmembrane potential and swelling of the matrix.[18] Since diamide-induced crosslinking of ANT mediates the membrane permeabilization, the function of ANT is regulated by the redox.[19] Oxidative stress seems to decrease the threshold of the pore opening to induce mitochondria-mediated apoptosis.

Peroxiredoxins

The members of the TRX system form a network and maintain the cellular reducing environment. TRX scavenges intracel-

lular hydrogen peroxide in collaboration with a family of thioredoxin-dependent peroxidases, peroxiredoxins.[20] The mammalian peroxiredoxin family has six members expressed in several subcellular compartments, including peroxisomes and mitochondria, while catalase is present only in peroxisomes. Peroxiredoxins I and II are cytosolic proteins, whereas peroxiredoxin III is specifically expressed in mitochondria. Mammalian peroxiredoxin IV is found in the endoplasmic reticulum and lysosomes and also secreted into the extracellular space. Peroxiredoxin V is located in peroxisomes and mitochondria, while peroxiredoxin VI is in cytoplasm and mitochondria. All peroxiredoxins except type III also exsist in the nucleus. Peroxiredoxins I–V contain two cysteines, whereas peroxiredoxin VI has one cysteine for the catalytic activity. Peroxiredoxins protect cells against apoptotic stimuli.[21–23] Mice deficient in peroxiredoxin I or II have hemolytic anemia, showing that erythrocytes are susceptible to oxidative stress. Peroxiredoxin knockout mice thus display milder phenotypes than TRX knockout mice, suggesting that the function of peroxiredoxins is redundant and can be partly compensated.[24,25]

Thioredoxin 2 (TRX2), a regulator of apoptosis in mitochondria

Two independent pathways of apoptosis converge in mitochondria.[26] Stress as well as death receptor-mediated activation of caspase-8 triggers the release of proapoptotic proteins, such as cytochrome *c* and apoptosis-inducing factor (AIF), from mitochondria, leading to the activation of downstream caspases (ex. caspase-3) and subsequent execution of apoptosis.[27,28] There is also a link between nuclei and mitochondria. In response to DNA double-strand breaks, histone H1.2 is released from the nucleus into the cytosol and induces cytochrome *c* release.[29] Smac/DIABLO and HtrA2/Omi are also released from mitochondria upon receiving apoptotic stimuli and inhibit the functions of inhibitor of apoptosis proteins (IAPs; endogenous inhibitors of caspases) by direct binding, leading to the activation of caspases.[30–32] HtrA2/Omi has the serine protease activity, which seems to be required for the execution of cell death[32] and could have other targets than IAPs.

Since mitochondria are at the center of several stress-induced apoptotic signaling pathways,[17,26] mitochondria have several protective mechanisms. Mitochondria contain large amount of GSH which may serve as a buffer against oxidative stress. Mammalian TRX2 is specifically expressed in mitochondria and essential for cell survival.[33,34] (Figure 2) TRX2 has a mitochondrial translocation signal peptide at the N-terminus and a conserved active disulfide/dithiol-like cytosolic TRX. In mammals, there are three thioredoxin reductases (TRXRs): cytosolic TRXR (TRXR1), mitochondrial TRXR (TRXR2),[35–37] and testis-specific TRX glutathione reductase (TGR).[38] In exquisite contrast to the cytosolic TRX system, which is composed of cytosolic peroxiredoxin (mainly peroxiredoxins I and II)–TRX–TRXR1, the TRX system in mitochondria consists of mitochondrial peroxiredoxin III–TRX2–TRXR2. In a conditional TRX2-deficient chicken B-cell line, DT-40, suppression of TRX2 expression caused the accumulation of intracellular ROS and induced cytochrome *c* release and subsequent apoptosis.[34] TRX2 prevents mitochondria-mediated cell death by scavenging ROS generated in mitochondria, which are a major physiological source of ROS during respiration and pathological conditions (Figure 2). In addition, TRX2 might inhibit apoptotic signaling by anchoring cytochrome *c* in mitochondria, given that TRX2 associates directly with cytochrome *c in vitro* and *in vivo*,[34] or interacting with another molecule in mitochondria (Wang *et al*., unpublished observation). Other reports also indicate the regulatory role of TRX2 in mitochondrial cell death. Overexpression of TRX2 confers an increase in mitochondrial membrane potential and resistance to etoposide-induced cell death.[39] The knockout of *TRX2* gene in mice is embryonic lethal, further indicating that TRX2 is indispensable for cell survival and that TRX and TRX2 cannot compensate for each other.[6,40] Recently, it was reported that mice lacking mitochondrial thioredoxin reductase (TRXR2) also die in the embryonic stage because of reduced myocardial function and perturbed hematopoiesis in the liver.[41] The proapoptotic activity of another redox-active inducer of apoptosis, AIF, might be regulated by TRX or TRX2, although its function is reported to be independent of its NADH oxidase activity.[42]

Apoptosis signal-regulating kinase 1 (ASK1)

ASK1 was identified by Ichijo *et al*,[43] as one of the mitogen-activated protein (MAP) kinase kinase kinases that activates c-Jun N-terminal kinase (JNK) and p38 MAP kinase and induces stress-mediated apoptotic signaling. TRX acts as a sensor of oxidative-stress-induced ASK1 activation. Reduced TRX binds to ASK1 and inhibits the activity of ASK1. Under oxidative stress, TRX is oxidized and dissociated from ASK1, resulting in the activation of ASK1.[44] ASK1 is also activated in cells treated with TNF-α or *cis*-diamminedichloroplatinum (CDDP), indicating that ASK1 is involved in stress-induced apoptosis.[44,45] Constitutively activated ASK1 induces apoptosis through cytochrome *c* release and caspase activation, and caspase-9-deficient murine embryonic fibroblasts are resistant to ASK1 activation-induced cell death, indicating that ASK1 induces apoptosis through mitochondrial pathway.[46]

Endoplasmic reticulum (ER)-mediated stress activates caspase-12 and ASK1, resulting in mitochondria-mediated apoptosis.[46–49] ER stress induced by polyglutamine, which is the underlying cause of neurodegenerative diseases such as Huntington's disease, activates ASK1 through the formation of an IRE1–TRAF2–ASK1 complex, resulting in JNK activation and cell death.[48,50] Amyloid-β, a cause of Alzheimer's disease, induces ER stress through caspase-12 and ROS-mediated ASK1 activation, resulting in neuronal cell death.[47,51] These results show that ER stress is also partly linked to mitochondria through the activation of ASK1 during apoptosis. As discussed in this section, ASK1 interacts with various partners and is regulated through multiple mechanisms upon each stimulus. The precise molecular mechanism of ASK1 activation and how TRX regulates ASK1 in a physiological context should be further elucidated (Figure 2).

Transmembrane thioredoxin-related protein (TMX)

The protective role of TRX-related molecules against ER stress has been emphasized rather recently. TMX, a novel member of the TRX-related protein localized in ER, possesses a signal peptide at the N-terminus, followed by a TRX-like domain with an unique –Cys–Pro–Ala–Cys– sequence at the active site.[52] TMX was abundant in membrane fractions and exhibited a similar subcellular distribution to calnexin localized to the ER. The N-terminal region containing the TRX-like domain was present in the ER lumen. Recombinant TMX showed protein disulfide isomerase (PDI)-like activity to refold scrambled RNase.[53] Cells overexpressing TMX showed resistance to apoptosis induced by an ER–Golgi transport inhibitor, suggesting that TMX relieves ER stress.[52] Since maturation of protein through disulfide bond formation and disulfide isomerization occurs in ER, the redox regulation plays a crucial role in protein quality control in ER and ER stress-mediated apoptosis.

Human T-cell Leukemia Virus Type-I (HTLV-I) Infection and the TRX System

Adult T-cell leukemia (ATL) was identified in 1970s as a distinct clinical entity based on its unique clinical features and is caused by human T-cell leukemia virus type I (HTLV-I).[54–57] ATL cells are $CD3^+CD4^+CD8^-$ in the majority of ATL cells with a characteristic constitutive expression of interleukin-2 (IL-2) and the α chain of the IL-2 receptor (IL-2Rα). The mean age of ATL patients at disease onset is 55 years. ATL is clinically classified into four types: acute, chronic, smoldering, and lymphoma types. Chronic type ATL is characterized by milder symptoms and signs and a longer clinical course, eventually leading to either an abrupt exacerbation of the disease or in fatal complications. ATL develops in 1–3% of infected individuals. The mechanisms of HTLV-I transformation and leukemogenesis are not yet fully elucidated. HTLV-I are also known to cause diseases such as HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP), HTLV-I associated arthropathy (HAAP), and HTLV-I-associated uveitis.[58] The viral oncoprotein Tax, a 40-kDa transcriptional activator of HTLV-I, transactivates not only the genes of its own virus but also a set of cellular genes, including IL-2, IL-2Rα, IL-15R, IL-1, IL-6, IL-15, GM-CSF, Bcl-X_L, and several immediate early response genes (*c-fos, c-jun, fra-1, and c-myc*).[59–62] Tax represses the transcription of certain genes such as DNA polymerase β, *lck*, cyclin-dependent kinase inhibitor (*p18*), and *p53* genes and functionally suppresses cyclin-dependent kinase (CDK)4, $p16^{INKa}$. Tax also interacts with CREB, p300, CBP, and PCAF.[63] Tax is reported to protect cells from stress-induced apoptosis, whereas Tax is also reported to sensitize cells to stress-induced apoptosis. The cellular environment may influence the decision between proliferation and death by Tax.[60] Although Tax seems to play a key role in T-cell transformation, ATL cells frequently lost the expression of Tax to escape from immune surveillance by cytotoxic T lymphocytes (CTL).[62] The low incidence of ATL among HTLV-I-infected carriers, together with a long latent period, suggests that multiple host–viral events in addition to Tax are involved in the progression of HTLV-I-dependent transformation and the subsequent development of ATL (Figure 3).[61,62]

Human TRX was identified in HTLV-I-positive T-cell lines[64] as well as a growth-promoting factor derived from B-cell lines immortalized by Epstein–Barr virus (EBV).[65] Thioredoxin expression was upregulated in HTLV-I-transformed T-cell lines.[66] The enhanced TRX expression may cause augmented growth of HTLV-I- transformed cells and inhibit apoptosis. The mechanism of the upregulation may be partly explained by Tax-mediated transactivation[67] and suppression of thioredoxin binding protein-2 (TBP-2), a negative regulator of TRX (described in detail in the next chapter).

TBP-2/VDUP1

We isolated TBP-2/vitamin D_3 upregulated protein 1 (VDUP1), which was originally reported as the product of a gene whose expression was upregulated in HL-60 cells stimulated with 1α, 25-dihydroxyvitamin D_3.[68,69] The interaction of TBP-2/VDUP1 with TRX was observed *in vitro* and

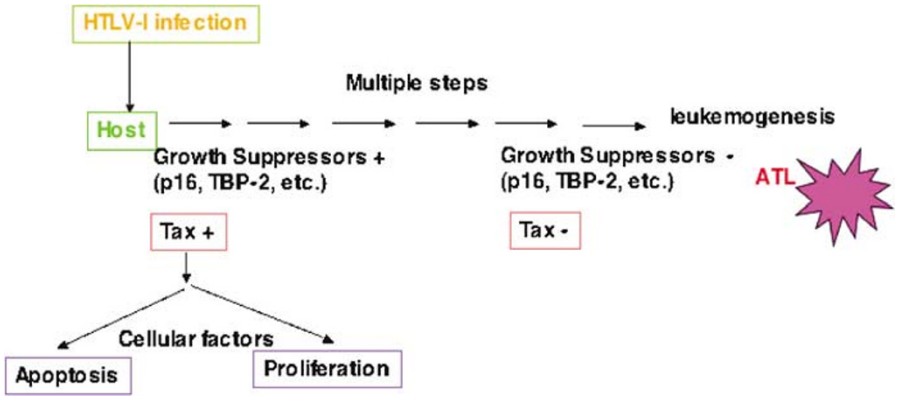

Figure 3 Role of cellular factors in the leukemogenesis of adult T-cell leukemia (ATL). ATL is caused by human T-cell leukemia virus type I (HTLV-I). Multiple host–viral events, including loss of growth suppressors such as thioredoxin-binding protein-2 (TBP-2), are involved in the progression of HTLV-I transformation and the subsequent development of ATL. The cellular environment may influence the decision between proliferation and apoptosis induced by Tax. ATL cells frequently lose the expression of Tax

in vivo. Interestingly, TBP-2/VDUP1 only binds to the reduced form of TRX and acts as an apparent negative regulator of TRX.[68] Later, other groups also reported the interaction between TRX and this protein.[70,71] Although the mechanism is unknown, a reciprocal expression pattern of TRX and TBP-2 was often reported upon various stimulation.[72,73]

TBP-2 has a growth suppressive activity. Overexpression of TBP-2 was shown to result in growth suppression.[74–77] TBP-2 expression is upregulated by vitamin D_3 treatment, serum or IL-2 deprivation, leading to growth arrest. However, the precise molecular function of TBP-2 is currently unclear. The role of TBP-2 expression in apoptosis caused by growth factor deprivation should be investigated. TBP-2 was found predominantly in the nucleus[77] and may be a component of a transcriptional repressor complex.[74] TBP-2 mRNA expression is downregulated in several tumors,[72,78] suggesting a close association between the reduction and tumorigenesis. TBP-2 expression is downregulated in melanoma metastasis.[79]

Loss of TBP-2 seems to be an important step of HTLV-I transformation. In an *in vitro* model, HTLV-I-infected T cells require IL-2 to proliferate in the early phase of transformation, but subsequently lose cell cycle control in the late phase, as indicated by their continuous proliferative state in the absence of IL-2. The change of cell growth phenotype has been suggested to be one of the oncogenic transformation processes.[80] The expression of TBP-2 is lost in HTLV-I-positive IL-2-independent T-cell lines, but maintained in HTLV-I-positive IL-2-dependent T-cell lines as well as HTLV-I-negative T-cell lines.

Human Immunodeficiency Virus (HIV) Infection and the TRX System

HIV infection causes apoptosis in $CD4^+$ T cells.[81] The mechanism of HIV-induced apoptosis is complex and multifactorial.[82] Dysregulation of the TRX system seems to be involved in apoptosis in HIV infection. Cells highly producing TRX, such as dendritic cells and activated macrophages in lymph nodes, decreased in number in HIV-infected lymph nodes. An *in vitro* infection of HIV on T-cell lines (SKT-1 and MT-2 cells) caused downregulation of protein expression of TRX 3 days after infection.[83] HIV infection caused transient downregulation of the Bcl-2 and TRX protein expression in Jurkat and U937 cells, followed by restoration to the initial levels. Cells with decreased levels of these proteins were susceptible to apoptosis. The upregulation of Bcl-2 expression repressed viral replication, therefore, the state of low level viral replication may be favorable for cell survival, resulting in persistent infection.[84] In monocytes from asymptomatic patients, the levels of Bcl-2 and TRX decreased, which is associated with enhanced hydrogen peroxide production, whereas in cells from AIDS patients the levels returned to normal.[85] Peroxiredoxin family proteins NKEF-A and NKEF-B were upregulated in activated $CD8^+$ T cells with HIV infection. T cells transfected with NKEF-A or NKEF-B were resistant to HIV infection.[86] Protein expression of peroxiredoxin IV (AOE372) was reduced in T-cell lines (C81 and MT-2) that are acutely infected with high titers of HIV-1. Overexpression of peroxiredoxin IV (AOE372) in T cells inhibited HIV transcription through inactivation of HIV-1 long terminal repeat (LTR) and suppressed the level of HIVp24.[87] The level of peroxiredoxin IV in T cells (C81) chronically infected with HIV was reduced. Although the regulatory mechanisms of the expression of TRX and peroxiredoxins in HIV infection may be varied and should be further elucidated, the redox balance affected by the intracellular TRX system seems to be important for cell survival and low viral production, probably leading to chronic persistent infection of HIV (Figure 4).

As for extracellular TRX, the plasma TRX level was found to be elevated in HIV-infected patients,[88] although the source of the increase remains unclear. Survival was significantly impaired when the plasma TRX level is chronically elevated in HIV-infected subjects with $CD4^+$ T-cell counts below 200/μl blood.[89] The elevated TRX level may reflect an involvement of oxidative stress or enhanced susceptibility to infection in advanced disease, according to the results that oxidative stress enhances the secretion of TRX. The impairment of survival in AIDS patients with chronically elevated TRX levels may be because normal immune defenses have been severely disrupted.

The redox system has been implicated in the pathophysiology of HIV infection as well as the regulation of apoptosis. Glutaredoxin (thioltransferase) is detected within HIV-1 and can regulate the activity of glutathionylated HIV protease *in vitro*. Although the physiological significance remains to be elucidated, the cysteine residues of the HIV protease coupled with glutaredoxin and GSH may optimize HIV protease activity particularly under conditions of oxidative stress.[90] The conserved cysteines of the HIV protease are involved in the

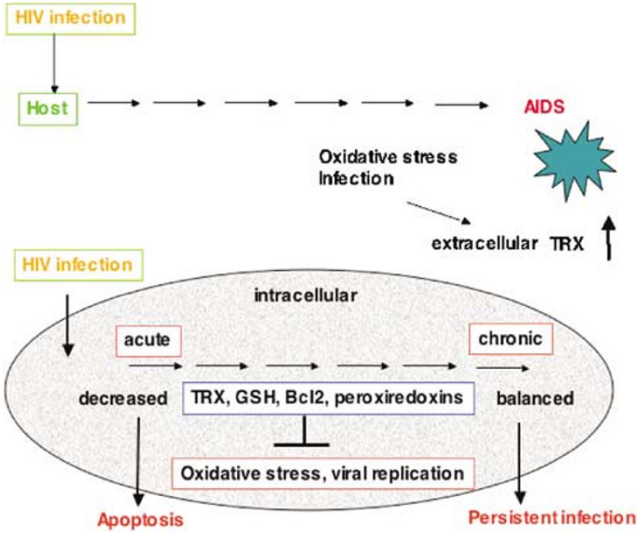

Figure 4 Human immunodeficiency virus (HIV) infection and the thioredoxin (TRX) system. HIV infection causes changes of the expression of cellular defensive factors such as Bcl-2, TRX, and peroxiredoxins. The compensated redox balance seems to be important in cell survival and low viral production, probably leading to chronic persistent infection of HIV. As for the extracellular TRX, the plasma TRX level was elevated in acquired immune deficiency syndrome (AIDS) patients and may reflect an involvement of oxidative stress or enhanced susceptibility to infection in advanced disease

redox regulation of polyprotein processing and viral maturation of immature virions.[91] The activity of HTLV-I protease can be also regulated through reversible glutathionylation of its conserved cysteine residues. The regulation of retroviral proteases may be a mechanism to prevent the premature activation of retroviral proteases with the cytoplasm of infected cells.[92] The immunoglobulin-like domain D2 disulfide bond of CD4 is reported to be redox-active and reduced by TRX, indicating that the redox changes in CD4 are important for HIV entry.[93] The physiological significance of these regulatory mechanisms by the redox system should be further investigated.

Summary and Future Perspectives

Here, we discussed that members and related molecules of the TRX system regulate apoptotic signaling through a wide variety of mechanisms and are closely associated with the pathophysiology of retroviral diseases. In spite of an accumulating knowledge on the molecular biology of HTLV-I, the mechanism of HTLV-I-mediated cellular transformation and leukemogenesis still remains unsolved. The molecular mechanism of persistent infection should be also elucidated to counteract the problem of resistance to intensive combinatory antiretroviral therapy. Since viruses seem to take advantage of the host defense machinery, the investigation of basic cellular regulatory mechanisms such as redox may provide novel insights into apoptosis research and approaches to treat retroviral diseases.

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