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Mini review

Extracellular thioredoxin: A therapeutic tool to combat inflammation

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ABSTRACT

The manipulation of cellular redox status has emerged as a promising therapeutic strategy to prevent uncontrolled inflammatory response. Thioredoxin is an important regulator of cellular redox homeostasis, which catalyzes the reduction of disulfide bonds. Human thioredoxin, originally identified as a secretory protein ADF, has been implicated in a wide variety of redox regulations in both intracellular and extracellular compartments. This review includes a summary of the evidence available supporting the employment of the beneficial properties of thioredoxin to combat inflammation, an evaluation of the potential of redox-based therapy for the treatment of inflammatory diseases, and a discussion on the conceptual model of a redox-sensitive signaling complex, Redoxisome, consisting of thioredoxin and its redox partners.

1. Introduction

Thioredoxin is a ubiquitously expressed protein with a molecular mass of 12 kDa, which was originally described as the hydrogen donor for ribonucleotide reductase in *Escherichia coli* [1]. Thioredoxin is evolutionarily conserved from prokaryotes to higher organisms, and characterized by the common active site motif, CGPC, in the thioredoxin fold [2]. The enzymatic activity of thioredoxin depends on a pair of cysteine residues in the active site, which exist in the oxidized (disulfide) or reduced (dithiol) state. The reduced form of thioredoxin transfers reducing equivalents to disulfides within the target molecules and catalyzes their reduction (Fig. 1). Oxidized thioredoxin formed in this process is restored to the reduced state by nicotinamide adenine dinucleotide phosphate (NADPH) and thioredoxin reductase [3]. Thus, the thioredoxin system contributes to the maintenance of a cellular reducing environment through the reversible thiol-disulfide exchange reaction (Fig. 2).

Initially, it was thought that thioredoxin is primarily involved in the protection against oxidative stress, scavenging reactive oxygen species (ROS) through the interaction with peroxiredoxin [4], and functioning to control the cellular redox balance. Numerous subsequent studies demonstrated that thioredoxin participates in a wide variety of redox dependent cellular processes, such as gene expression, signal transduction, cell growth and apoptosis. Various kinds of thioredoxin targets and interacting molecules have been identified. A large number of transcription factors, such as nuclear factor κB (NF-κB), p53, hypoxia inducible factor 1 (HIF-1), glucocorticoid receptor and estrogen receptor, contain redox-sensitive cysteines that can be modulated by thioredoxin [5]. Extensively reviewed in recent studies [6,7], the interaction between thioredoxin and signaling molecules, such as apoptosis signal-regulating kinase 1 (ASK-1), phosphatase and tensin homolog (PTEN), as well as thioredoxin interacting protein (Txnip), demonstrates the significance of thioredoxin-dependent intracellular signaling. Especially, Txnip or thioredoxin binding protein 2 (TBP-2), originally described as a vitamin D3 up-regulated protein 1 (VDUP1) [8], has been the focus of many researchers over recent decades. Since its discovery as a negative regulator of thioredoxin in a yeast two-hybrid screening study [9], Txnip/TBP-2/VDUP1 has been thoroughly investigated and a growing number of studies have indicated its involvement in a wide range of diverse biological processes from the regulation of metabolic and immunological pathways to inflammasome activation and tumorigenesis [10–19].

2. Thioredoxin in extracellular space: ADF, a secretory protein from HTLV-I-transformed T cells

Human thioredoxin was originally purified as a secretory protein, adult T cell leukemia-derived factor (ADF), with cytokine-like activities from culture supernatants of human T cell leukemia virus type-I (HTLV-I)-transformed lymphocytes [20–23]. Thioredoxin is released from cells in response to oxidative stress, and extracellular thioredoxin shows cytoprotective effects under oxidative and inflammatory conditions [24]. It has been reported that circulatory thioredoxin shows chemotactic activity for

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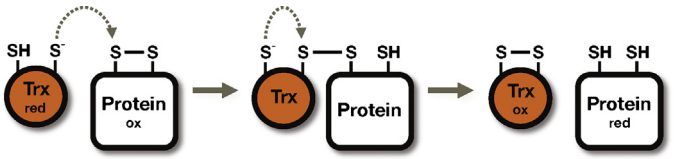

Fig. 1. Mechanism of disulfide reduction by thioredoxin. During the thiol-disulfide exchange reaction, a transient mixed disulfide bond is formed between thioredoxin and its substrate. After completion of the catalytic cycle, a reduced substrate protein is released and thioredoxin is converted to the oxidized form. Trx: thioredoxin; red: reduced; ox: oxidized.

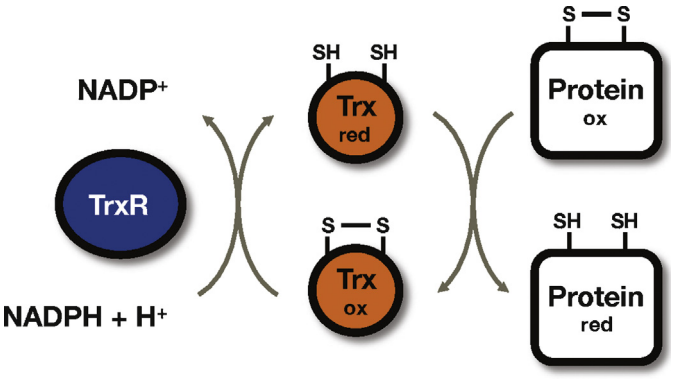

Fig. 2. The reduced form of thioredoxin catalyzes reduction of disulfide bonds in target proteins. Oxidized thioredoxin is regenerated to the reduced state by the NADPH-dependent flavoenzyme thioredoxin reductase. TrxR: thioredoxin reductase.

monocytes, neutrophils, and T lymphocytes [25]. Higher levels of thioredoxin in circulation, however, inhibit the extravasation of neutrophils into inflammatory sites [26,27]. In addition, there is a C-terminally truncated form of thioredoxin, thioredoxin 80 (Trx80), that is released from monocytes. Consisting of 80–84 amino acids, Trx80 lacks oxidoreductase properties, and it is not a substrate for thioredoxin reductase [28]. Although the regulatory mechanisms of the Trx80 generation have not been fully elucidated, recent studies have suggested the involvement of α-secreatases, belonging to a disintegrin and metalloproteinase (ADAM) family, for the cleavage of thioredoxin [29]. Trx80 shows immunostimulatory activities, and especially acts on monocytes to induce the secretion of inflammatory cytokines.

Although thioredoxin lacks an N-terminal signal sequence for its entry into the secretory pathway, it is released from various types of normal and neoplastic cells [30–33]. It may be that thioredoxin is exported to the extracellular space by a non-classical or endoplasmic reticulum/Golgi-independent pathway [34], but the precise mechanism that mediates the secretion of thioredoxin remains unknown. It was demonstrated the secretion process for thioredoxin is distinct from that of another leaderless protein, interleukin-1β (IL-1β). The export of IL-1β appeared to be mediated by intracellular vesicles, whereas no interaction was found between thioredoxin and any membranous elements [30]. Since the mutant forms of thioredoxin lacking the second cysteine (Cys35) in the CGPC motif were exported from cells with comparable efficiency to the wild-type proteins, the redox status of thioredoxin did not seem to affect its secretion [35]. Replacement of both of the two cysteines in the active site, however, blocked the protein secretion induced by hydrogen peroxide in Jurkat cells, suggesting that Cys32 is essential for the release of thioredoxin [32]. A recent study has reported the emerging role of caspase-1 as a regulator of unconventional protein secretion [36]. This isobaric tags for relative and absolute quantitation (iTRAQ)-based secretome analysis identified proteins that are released from human keratinocytes in a caspase-1 dependent manner. Thioredoxin did not meet the criteria for quantitation in this approach, but using an alternative method it was verified that thioredoxin secretion was dependent on the expression and activity of caspase-1. Although the underlying mechanisms remain elusive, the results of this study suggested a general role for caspase-1 in the non-classical secretion of leaderless proteins, including thioredoxin.

3. Thioredoxin as a biomarker for oxidative stress

Multicellular organisms utilize molecular oxygen as an energy source, but at the same time ROS, unavoidable toxic byproducts of aerobic respiration, can be generated through the normal metabolic process of energy extraction. ROS cause oxidative damage to macromolecules such as DNA, lipids, and proteins, and can compromise cell integrity. In general, cells are equipped with intrinsic antioxidant systems, including thioredoxin, which eliminate harmful ROS and control the cellular redox homeostasis under normal conditions. However, an imbalance between ROS production and its detoxification can result in a state of oxidative stress. Accumulating evidence shows that oxidative stress is involved in the pathogenesis of a wide variety of human disorders, including cancer, diabetes mellitus, and inflammatory diseases. It would be beneficial if we could monitor the levels of oxidative stress for the prediction of the disease state and the evaluation of risk indices. Therefore the identification of specific biomarkers for oxidative stress should have significant implications in clinical practice, as they could potentially be used to monitor the progression of the disease and predict the therapeutic response to drugs.

Upon exposure to oxidative stress, it has been shown that thioredoxin is transcriptionally up-regulated and some parts of the protein are released into the extracellular compartment. Increased levels of thioredoxin in biological fluids, such as plasma, have been reported in many pathological conditions associated with oxidative stress (Table 1) [26,37–62]. Considering that basal levels of

Table 1
Potential utility of thioredoxin as a clinical biomarker. The extracellular concentrations of thioredoxin are increased in many pathological conditions associated with inflammation.

Disease	Sample	Reference
Acquired immunodeficiency syndrome (AIDS)	Plasma	[26,37]
Hepatocellular carcinoma	Serum	[38]
Hepatitis C	Serum	[39]
Nonalcoholic steatohepatitis (NASH)	Serum	[40]
Pulmonary sarcoidosis	BALF	[41]
Asthma	Serum	[42]
Acute respiratory distress syndrome (ARDS)	BALF/plasma	[43]
Interstitial lung disease	Serum	[44]
Non-small cell lung cancer	Serum	[45]
Obstructive sleep apnea (OSA)	Plasma	[46]
Rheumatoid arthritis	Plasma/serum/SF	[47–49]
Sjögren's syndrome	Saliva	[50]
Cardiac surgery with cardiopulmonary bypass	Plasma	[51]
Dilated cardiomyopathy	Serum	[52]
Acute coronary syndrome	Serum	[52]
Acute myocardial infarction	Plasma	[53,54]
Chronic heart failure	Plasma	[55]
Unstable angina	Plasma	[56]
Diabetes mellitus	Plasma/serum	[57,58]
Pancreatic ductal carcinoma	Plasma	[59]
Acute pancreatitis	Serum	[60]
Burns	Serum	[61]
Inflammatory bowel disease	Serum	[62]

BALF: bronchoalveolar lavage fluid; SF: synovial fluid.

thioredoxin in plasma or serum are very low compared with its intracellular levels [63], thioredoxin could be used as a noninvasive marker to reflect the oxidative damage in clinical settings.

3.1. AIDS/HIV infection

In acquired immunodeficiency syndrome (AIDS), the progression of the disease is accompanied by alterations in the redox balance, resulting in systemic oxidative stress. It has been shown that intracellular glutathione (GSH) levels are decreased in human immunodeficiency virus (HIV)-infected individuals [64]. The concentration of thioredoxin in plasma is elevated in patients with HIV and negatively correlated with intracellular GSH levels [37]. At the advanced stage of the disease, higher plasma thioredoxin levels are associated with lower CD4 T cell counts, and a connection between elevated thioredoxin and decreased survival of HIV-infected individuals has also been observed [26].

3.2. Liver diseases

Circulating thioredoxin levels were measured in patients suffering from chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC). There was no alteration of thioredoxin levels in CH/LC patients, whereas patients with HCC had elevated serum levels of thioredoxin, compared with healthy subjects [38]. Furthermore, thioredoxin levels significantly decreased after the surgical removal of the tumor, suggesting that the serum thioredoxin value could be a useful parameter to predict the progression of the disease to HCC. It has been shown that hepatitis C virus (HCV)-related chronic liver diseases were associated with the elevation of serum levels of thioredoxin [39]. A tendency was shown for a correlation between the thioredoxin levels and the severity of hepatitis, and patients with higher serum levels of thioredoxin exhibited resistance to interferon. These results suggested that thioredoxin, which is regarded to reflect increased oxidative damage in the liver, could be employed as a marker for the prediction of the efficacy of interferon-based therapy in chronic HCV-infected patients. An elevation of serum thioredoxin levels was also reported in patients with nonalcoholic steatohepatitis (NASH) [40]. The results of that study suggested the potential application of thioredoxin for monitoring hepatic dysfunction associated with oxidative stress, and for the differential diagnosis between simple steatosis and NASH.

3.3. Respiratory diseases

The respiratory system, including the lungs, is constantly exposed to external stimuli, such as toxic materials in the air and virus infection, resulting in the generation of ROS and oxidative tissue damage. In addition to environmental stressors, such as exposure to tobacco smoke and air pollutants, lungs are damaged by ROS generated from inflammatory cells under pathological conditions. Direct links between oxidative stress and the pathophysiology of many lung diseases have been established, and the induction of thioredoxin is regarded as an adaptive response against lung inflammation associated with oxidative stress [65]. The concentration of serum thioredoxin was significantly increased in current smokers [66]. The elevation of thioredoxin levels in plasma/serum or bronchoalveolar lavage fluid (BALF) has been observed in several types of pulmonary diseases, including pulmonary sarcoidosis [41], asthma [42], acute respiratory distress syndrome (ARDS) [43], interstitial lung disease [44], and non-small cell lung cancer [45]. Obstructive sleep apnea (OSA) is a sleep disorder characterized by interrupted breathing caused by obstruction of the airway. Repetitive pauses in breathing can cause systemic oxidative stress, and, in fact, it has been shown that plasma thioredoxin levels were significantly higher in patients with OSA [46]. Thioredoxin levels significantly decreased after clinical treatment in concert with the improvement in OSA symptoms. The elevation of thioredoxin in these pathologies suggests its potential utility as a clinical parameter for respiratory disorders associated with oxidative stress.

3.4. Rheumatoid arthritis and associated diseases

Oxidative stress has been implicated in the pathogenesis of rheumatoid arthritis (RA), and the increased production of ROS in inflamed joints contributes to the tissue damage and chronic symptoms of the disease. Increased levels of thioredoxin have been found in synovial fluid and plasma of patients with RA, compared with other forms of arthritis [47–49]. It has also been reported that thioredoxin levels increased in saliva obtained from patients suffering from Sjögren's syndrome, a systemic inflammatory disorder sharing features with RA [50]. The up-regulation of thioredoxin appeared to be correlated with the severity of these diseases, making it an attractive candidate as a clinical biomarker for the diagnosis of RA and related diseases.

4. Protective effects of thioredoxin against inflammation

The induction of thioredoxin appears to be a physiological response designed to protect cells from oxidative stress. Accumulating evidence indicates that oxidative stress is associated with inflammation, and the cellular redox status can determine the sensitivity and the final outcome in response to inflammatory stimuli. In fact, a number of preclinical studies using animal models have produced evidence revealing the beneficial protective function of thioredoxin against inflammatory tissue injury associated with oxidative stress. Transgenic overexpression of thioredoxin protects mice from a wide variety of inflammatory disorders [67]. Thioredoxin transgenic mice show an increased resistance to oxidative stress, compared with wild-type animals, which would be beneficial for a significant extension of their life span [68,69]. Analyses of thioredoxin transgenic mice have strongly indicated the benefits of the supplementation of thioredoxin proteins for the treatment of inflammatory conditions. The effectiveness of thioredoxin administration against inflammation-induced damage has been extensively reviewed elsewhere [65,67,70]. In the following section, we describe some of the recent findings related to the protective effects of exogenously applied thioredoxin in animal models of inflammatory diseases.

4.1. Lung inflammation

The protective effects of thioredoxin against cigarette smoke-induced inflammation in the lungs have been investigated [71]. Cigarette smoke causes oxidative stress and chronic inflammation in the lungs, which have been shown to be associated with the pathogenesis of chronic obstructive pulmonary disease (COPD) [72]. Overexpression of thioredoxin in transgenic mice ameliorated acute inflammatory lung damage after 3 days exposure to cigarette smoke. The number of neutrophils in BALF was significantly lower in thioredoxin transgenic mice, compared with wild-type mice, after exposure to cigarette smoke. Thioredoxin overproduction also suppressed the expression of inflammatory mediators, such as matrix metalloprotease-12 and tumor necrosis factor α (TNF-α). Consistent with these results, the administration of recombinant thioredoxin prevented acute lung injury induced by cigarette smoke through the suppression of neutrophil influx. Moreover, in chronic inflammatory conditions induced by long-term exposure to cigarette smoke, pulmonary emphysema was attenuated in thioredoxin transgenic animals with decreased

infiltration of neutrophils and macrophages to the lungs. These studies may provide the foundation required for the possibility of the clinical application of thioredoxin for the treatment of COPD.

Influenza virus infection induces a massive pulmonary inflammatory response, and excessive inflammation results in severe tissue damage during acute infection. It has been reported that thioredoxin transgenic mice showed an increased resistance to lethal infection with influenza virus [73]. Transgenic overexpression of thioredoxin prevented viral pneumonia in mice, and thioredoxin appeared to control the magnitude of the inflammation without affecting the systemic immune response against the influenza virus infection. Recent studies have revealed that exogenously administered thioredoxin protects the lungs from acute lung injury induced by influenza virus infection [74]. Thioredoxin injections were started 1 day before the inoculation with influenza A virus (H1N1), and mice receiving repeated administrations of thioredoxin had a higher survival rate, compared with a control group. There was no significant difference in the viral load in the lung between the control and the thioredoxin-treated animals, suggesting that the protection provided by the thioredoxin was not mediated by preventing the infection or the propagation of the virus. Influenza virus-induced pathological changes, such as edema, hemorrhage, and neutrophil infiltration, were attenuated in the lungs of thioredoxin-treated mice. In regard to the pathology of influenza virus-induced lung injury, it has been proposed that pro-inflammatory cytokines are the major effectors of tissue damage [75]. The levels of TNF-α and chemokine (C-X-C motif) ligand 1 (CXCL1) were increased in lavage fluid, as well as in lung tissue, after H1N1 infection. The induction of these inflammatory mediators was suppressed in the lungs of thioredoxin-treated mice, suggesting that thioredoxin may regulate the production and release of pro-inflammatory cytokines, and thereby contribute to the protection against virus-induced inflammation. In fact, *in vitro* studies using murine lung epithelial cell lines provided direct evidence showing that extracellular thioredoxin attenuated the transcriptional up-regulation of TNF-α and CXCL1 in response to H1N1 infection. These studies may raise the possibility of thioredoxin treatment as a novel therapeutic approach to control influenza infection. It should be noted however that the protective effects of thioredoxin were diminished when the mice were treated after H1N1 infection, and therefore, such treatment may not be effective after the onset of symptoms. Further studies will be required to improve the therapeutic efficacy of thioredoxin for the treatment of inflammatory conditions, and it will be important to clarify the precise mechanism by which thioredoxin overcomes excessive inflammation and the associated tissue injury caused by acute virus infection.

4.2. Sepsis/SIRS

It has been shown that the levels of thioredoxin in plasma were significantly increased in patients with sepsis/systemic inflammatory response syndrome (SIRS) [76,77], and thioredoxin levels within 24 h after the onset of septic shock were even higher in non-survivors, compared with survivors. In the mouse model of cecal ligation puncture (CLP), a widely used model for experimental sepsis in rodents, the inhibition of endogenous thioredoxin with neutralizing antibodies significantly impaired the survival of mice, suggesting a protective role played by thioredoxin in sepsis. Furthermore, mice receiving intraperitoneal injections of recombinant thioredoxin immediately after CLP showed an improved survival rate, compared with a control group. Thioredoxin administered after the induction of sepsis still exerted a protective effect and reduced mortality from septic shock. These results suggested that thioredoxin plays a critical role in the protection against inflammation, and it could be a potential therapeutic target for controlling sepsis/SIRS.

4.3. Skin inflammation

Thioredoxin has beneficial effects in the treatment of inflammatory skin diseases. In a mouse model of allergic contact dermatitis, ear swelling induced by dinitrofluorobenzene (DNFB) was suppressed in thioredoxin transgenic mice, compared with wild-type mice [78]. The activation of cutaneous dendritic cells and the subsequent antigen-specific proliferation of lymph node cells were equivalent in both wild-type and thioredoxin transgenic mice after DNFB sensitization. These results suggested that the overproduction of thioredoxin does not affect the primary immune response in the induction phase of allergic contact dermatitis. In contrast, after elicitation challenge with DNFB, neutrophil infiltration to the site of inflammation was attenuated in thioredoxin transgenic mice with the lower expression of IL-17 in the skin. The anti-inflammatory effect of thioredoxin in the elicitation phase was also confirmed by the observation that the administration of recombinant thioredoxin in DNFB-sensitized mice suppressed the contact hypersensitivity response.

The effectiveness of thioredoxin treatment has also been demonstrated in a model of ultraviolet (UV)-induced skin inflammation. Exposure to solar UV radiation induces acute and chronic inflammatory response and may cause adverse clinical effects on the skin. Sunburn is one of the major acute effects of excessive UV exposure, and the involvement of oxidative stress in this process has been postulated. Cutaneous inflammatory response, such as skin erythema and edema induced by UV-B irradiation, was significantly suppressed in mice administered with recombinant thioredoxin [79]. Thioredoxin treatment inhibited the migration of inflammatory cells to the UV-irradiated site, where the apoptotic cell death of keratinocytes was also suppressed by thioredoxin administration. UV-B irradiation has been shown to activate cellular stress responses, such as p38 and the c-Jun N-terminal kinase (JNK) pathway [80,81]. Sustained activation of p38 and the phosphorylation of JNK were both suppressed in the skin epidermis of mice injected with thioredoxin after UV-B exposure. Although the precise mechanism by which thioredoxin exerts its therapeutic effects on skin inflammation awaits further clarification, these studies suggested a broad role of thioredoxin in the host defense against inflammatory conditions.

4.4. Gastric injury

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for the treatment of acute or chronic inflammatory conditions. NSAIDs, however, has been associated with severe adverse effects, and gastrointestinal toxicity is a primary risk factor limiting the usage of NSAIDs. One report showed that thioredoxin overexpression could protect mice from indomethacin-induced gastric injury [82], suggesting the potential of thioredoxin for limiting the side effects of NSAIDs. More recently, yeast-derived thioredoxin has been developed and used for the treatment of gastric inflammation induced by indomethacin. Mice were fed a control or thioredoxin-supplemented diet containing thioredoxin-enriched yeast extracts for 3 days prior to indomethacin administration. Dietary supplementation of thioredoxin ameliorated the pathology of gastric mucosal injury induced by indomethacin [83]. Thioredoxin treatment inhibited the neutrophil infiltration into the mucosa, where the production of pro-inflammatory cytokines, such as IL-1β, IL-6 and CXCL1, was significantly suppressed after the administration of indomethacin, compared with a control group. Furthermore, in *in vitro* studies using murine gastric mucosal cell lines, yeast-derived thioredoxin protected cells against indomethacin-induced cytotoxicity. The results were in agreement with a previous report showing the cytoprotective effects of recombinant human thioredoxin [82].

These studies suggested that oral administration might be an alternative option for thioredoxin delivery for treating gastrointestinal injury induced by NSAIDs.

5. The production of recombinant human thioredoxin and the development of thioredoxin-enriched materials

E. coli is a predominant host for the expression of recombinant proteins, and human thioredoxin produced in *E. coli* has been utilized in most of the previous studies. Protein expression systems in hosts other than *E. coli* has also been developed, including lactobacillus, algae, yeasts, insects, plants and animal cells. Since edible species have a lower risk of contamination from bacterial pathogens, they would be promising candidates for the production of therapeutic materials. Yeast (*Saccharomyces cerevisiae*) is an alternative source for thioredoxin production [84,85]. Yeast thioredoxin was efficiently extracted from various strains used for baking bread or brewing wine, beer and Japanese sake. As described above, the oral administration of thioredoxin-enriched extracts from sake yeast provides protection against gastric inflammation [83], suggesting that yeast-derived thioredoxin has anti-inflammatory activities that are comparable to those found for purified recombinant human thioredoxin.

The expression of target molecules in plants is another option for producing pharmaceutical proteins. Recent advances in genetic engineering have allowed the plant-based expression system producing large amount of foreign proteins. There has been an attempt to develop transgenic plants expressing thioredoxin *via* the chloroplast genome. Lettuce (*Lactuca sativa*) was selected as a host for producing thioredoxin. Edible crops like lettuce are ideal for producing biomaterials that can be used for oral delivery [86]. Human thioredoxin transgene was integrated into the chloroplast genome, and the resulting transgenic lettuce accumulated significant quantities of thioredoxin in chloroplasts [87]. The recombinant proteins purified from lettuce leaves exhibited disulfide reducing activity, and they could attenuate the cytotoxic effects of hydrogen peroxide to a similar extent as bacterially expressed thioredoxin. Considering that even the crude extracts of lettuce leaves expressing thioredoxin showed catalytic activity to reduce disulfide bonds, they could be directly utilized without further purification. In such a case, powdery products like finely ground freeze-dried leaves, rather than fresh vegetables, would be preferable to control the dose. Thus, transplastomic lettuce could provide a feasible source for the production and oral delivery of thioredoxin protein.

6. Mechanistic insight into the redox-based control of cellular processes

Low-molecular-weight antioxidants such as glutathione have been used clinically for preventing oxidative stress. Unfortunately, however, the therapeutic use of antioxidants did not provide successful results in clinical trials. While these small antioxidants serve as nonspecific reducing agents with broad substrate spectra, thioredoxin can interact with the specific targets, which will enable the fine control of cellular processes with the lower risk of unwanted side effects. In this section, we describe the proposed mechanisms of anti-inflammatory action of thioredoxin, and also provide a conceptual model illustrating the role of thiol-disulfide exchange in the control of cell signaling.

6.1. Suppression of leukocyte chemotaxis

The beneficial effects of thioredoxin against inflammation were initially ascribed to its anti-oxidative activity to scavenge ROS generated in inflamed tissues. However, subsequent studies have reported that thioredoxin suppressed the migration of inflammatory cells into the tissues, which would more likely explain the protective effect of thioredoxin under the type of inflammatory conditions. Leukocyte extravasation is a part of a host defense for eliminating pathogens, but it can also cause adverse effects leading to excessive tissue damage. It has been proposed that thioredoxin blocks the activation of neutrophils through the inhibition of p38 pathway [27]. Furthermore, thioredoxin suppressed the interaction between leukocytes and endothelial cells [88], suggesting that the recruitment of leukocytes during inflammation could be regulated by the cellular redox status.

6.2. Regulatory effects on cytokine production

Inflammatory cytokines have been implicated as critical mediators of the inflammatory response. Excessive production of these inflammatory mediators can be detrimental, and it would be a major cause of tissue damage during inflammation. Intriguingly, thioredoxin treatment could inhibit the synthesis of multiple proinflammatory cytokines and chemokines in *in vivo* animal models of inflammatory disorders (please see Section 4). In addition, there is a report showing that thioredoxin blocked the induction of several potent proinflammatory factors, such as IL-1β, IL-6, IL-8, and TNF-α, in human monocyte-derived macrophages treated with lipopolysaccharide (LPS) [89]. Contrasting to the role of thioredoxin in the cytoplasm, extracellular thioredoxin inhibited LPS-induced activation of NF-κB pathway in the cultured macrophages, which could be involved in the down-regulation of the selected cytokines. These results suggest that attenuation of cytokine production is one of the regulatory mechanisms by which thioredoxin exerts its protective function against inflammatory tissue injury. Since the source of inflammatory cytokines may vary depending on the type of inflammation, it should be required to specify the target cells of thioredoxin in a given disease condition.

6.3. Molecular mechanism of thioredoxin action against inflammation

At present, the precise mode of thioredoxin action in the extracellular compartment awaits further clarification. A proteomic approach identified the proteins interacting with thioredoxin in human plasma, including complement factor H, and the results suggested the inhibitory effect of thioredoxin on the activation of the complement pathway [90]. It was also suggested that extracellular thioredoxin could be incorporated into cells, and exert its effect intracellularly [32]. The internalization of thioredoxin was mediated through membrane lipid rafts, and the active site cysteine was supposed to be important for its entry into cells [91]. It is unclear, however, whether exogenously administered thioredoxin can be transported into cytosol and exhibit the protective function in *in vivo* models of inflammatory tissue injury. Considering that the cellular uptake of extracellular thioredoxin seemed to be quite slow [91], it seems implausible that a limited amount of exogenous thioredoxin incorporated into cells exerts the effect superior to that of preexisting abundant endogenous pool. Notably, it has been reported that extracellular thioredoxin could control the expression levels of endogenous thioredoxin. Upon treatment with thioredoxin, the expression of endogenous thioredoxin was significantly reduced in LPS-activated macrophages, leading to the down-regulation of the LPS-induced inflammatory response. Thus, extracellular thioredoxin may control the intracellular redox balance, thereby influencing the cellular response. More recently, the concept of redox-sensitive signaling complex named Redoxisome has been proposed [92]. The change of cellular redox states can induce the assembly or dissociation of protein components of Redoxisome, consisting of

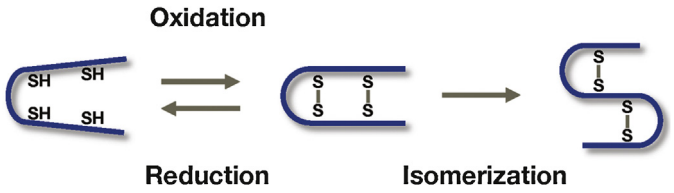

Fig. 3. Thiol-disulfide oxidoreductases catalyze the formation, reduction, and isomerization of protein disulfide bonds. Modification of cysteine residues induces a conformational change, and it can alter the molecular structure and affect the function of proteins.

thioredoxin and its redox partners including Txnip/TBP-2/VDUP-1. It would be intriguing to investigate the possible involvement of this redox-dependent signalosome in the regulation of inflammatory pathway.

It did not appear that the extracellular function of thioredoxin was mediated through its binding to the specific receptor on the cell surface. Alternatively, thioredoxin might act on cell surface molecules to regulate their function through its reducing activity. It has been shown that thiol-disulfide exchange could be a regulatory mechanism that controls the protein function and cellular processes [93–96]. The formation of disulfide bonds is a critical step for stabilizing proteins and also for their assembly. Posttranslational modification of cysteine residues induces a large conformational change, and it can affect the molecular structure and even modify the function of proteins (Fig. 3), similar to that seen in other regulatory systems, such as in phosphorylation and dephosphorylation. In this context, disulfide bonds may be regarded as molecular switches that can be turned on and off by oxidoreductases, *e.g.*, thioredoxin. In fact, thioredoxin has been shown to control the redox state of cell surface receptors, such as CD4 and CD30, thereby influencing cellular behavior [97,98]. In addition, it has been reported that a type of transient receptor potential (TRP) channel, TRPC5, is activated by reduced thioredoxin, which can cleave a disulfide bond in the extracellular loop of the channel [99]. Thus, the reversible thiol-disulfide exchange has emerged as a novel regulatory mechanism to control cellular functions in response to external stimuli (Fig. 4). Although the mechanistic details of the anti-inflammatory action of thioredoxin are still unclear, the identification and classification of thioredoxin target molecules on the cell surface should provide a clue as to how extracellular thioredoxin controls the excessive inflammatory response. It has been shown that exogenously applied thioredoxin was associated with the specialized microdomains of the plasma membrane, lipid rafts [91]. These observations raise the hypothesis that extracellular thioredoxin may specifically interact with the components of lipid rafts, and modulate the redox properties on the cell surface. Given that lipid rafts serve as a membrane platform for the assembly of signaling complexes, redox remodeling by thioredoxin can potentially affect the lipid raft-dependent signal transduction, leading to dynamic changes in the cellular response to inflammatory stimuli.

Another important issue is whether thioredoxin can be reduced and regenerated in the more oxidizing environment of extracellular space. Indeed, thioredoxin has been shown to be readily oxidized in circulation [51]. If the biological properties of thioredoxin rely on its reducing activity, it would be necessary to maintain the protein in a reduced state during the treatment period. Intriguingly, there was an indication that normal and transformed cells secreted thioredoxin reductase, which might be involved in the regeneration of oxidized thioredoxin outside the cells [100].

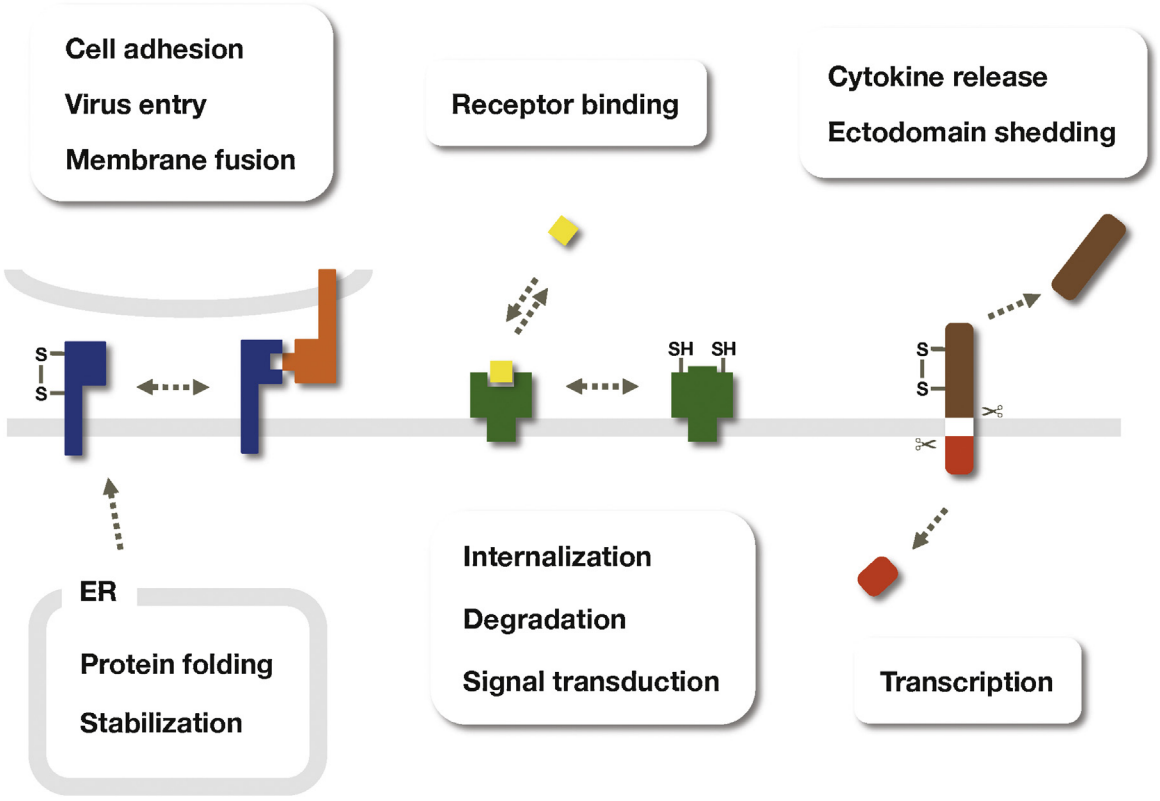

Fig. 4. Reversible thiol-disulfide exchange as a regulatory mechanism to control cellular functions. Potentially, alteration of the redox state of proteins can either positively or negatively regulate their function and thereby influence cellular behavior. ER: endoplasmic reticulum.

7. Conclusion

Increasing lines of evidence have provided support for the concept that the beneficial properties of thioredoxin are involved in the protection against a wide range of inflammatory disorders. Thioredoxin exerts its protective effects with a mode of action apparently distinct from other existing anti-inflammatory agents. In particular, the lack of serious side effects demonstrated in *in vivo* disease models is a unique and valuable trait of thioredoxin, which will make it an attractive alternative to conventional therapies. The longevity of thioredoxin transgenic animals also supports this concept. Furthermore, increased levels of thioredoxin have been associated with disease conditions, suggesting the potential use of thioredoxin as a diagnostic marker. Therefore, it has now become necessary to develop advanced methods for the simple and rapid measurement of thioredoxin in biological fluids.

The modulation of cellular redox status has emerged as a potential clinical approach to block uncontrolled inflammatory processes. Further studies are required to elucidate the mechanisms underlying protein redox modifications and their physiological relevance, and the results of such studies should be crucial for the development of rational strategies for redox therapy against inflammatory diseases.

Conflict of interest

The authors declare that they have no competing financial interests.

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