

REVIEW ARTICLE

A critical role of the thioredoxin domain containing protein 5 (TXNDC5) in redox homeostasis and cancer development

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Abstract Correct folding of nascent peptides occurs in the endoplasmic reticulum (ER). It is a complicate process primarily accomplished by the coordination of multiple redox proteins including members of the protein disulfide isomerase (PDI) family. As a critical member of the PDI family, thioredoxin domain containing protein 5 (TXNDC5) assists the folding of newly synthesized peptides to their mature form through series of disulfide bond exchange reactions. Interestingly, TXNDC5 is frequently found overexpressed in specimens of many human diseases including various types of cancer. In this review, we summarized the biochemical function of TXNDC5 in mammalian cells and the recent progress on the understanding of its role and molecular mechanisms in cancer development. Findings of TXNDC5 in the activation of intracellular signaling pathways, stimulation of cell growth & proliferation, facilitation of cell survival and modulation of extracellular matrix to affect cancer cell invasion and metastasis are reviewed. These published studies suggest that strategies of targeting TXNDC5 can be developed as potentially valuable methods for the treatment of certain types of cancer in patients. Copyright © 2018, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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Introduction

Redox imbalance is one of the major contributing factors of many types of human diseases, including cancer. Mechanisms of redox communication and cellular response to the waves of reactive oxygen species under various conditions are yet to be completely understood. Oxygen and hydrogen peroxide are two examples of diametric molecules that can function as pro- as well as anti- survival elements, through either enhancing growth signaling or causing destructive effect on nucleotides, lipids, and proteins. In this process, there are numerous redox enzymes that not only shelter cellular components from oxidative damages, but also recycle damaged molecules through disulfide exchange reactions to maintain redox homeostasis. Among these is the superfamily of thioredoxin, which includes thioredoxin, peroxiredoxin (Prx), ferredoxin, glutaredoxin, quiescin sulfhydryl oxidase, vitamin K, epoxide reductase, PDI, etc. Most of these enzymes share a common structural characteristic of containing thioredoxin or thioredoxin-like domains, through which thiol-disulfide exchange reactions are fulfilled. TXNDC5 is a particular member of the family of PDI. In this review, we summarized the findings of most recent studies on the understanding of the role and molecular mechanisms of TXNDC5 in human diseases including cancer development.

Orthologs of TXNDC5 are present in many eukaryotic organisms including yeast, lancelet, drosophila, zebra fish, lizards, mouse, elephant, and chimpanzee.^{1,2} In human, TXNDC5 gene, alias ERp46, PDIA15, HCC-2, or EndoPDI, is located in chromosome 6p24.3. It encodes five splicing variants, two of which are able to be translated into proteins. The transcript of TXNDC5-001 contains 2964 nucleotides and encodes a full-length protein containing a total of 10 exons. A second protein-coding transcript, named TXNDC5-003, contains only 1301 nucleotides and encodes a protein in which the first exon is truncated due to the absence of 324 nucleotides in the N-terminus.² In addition, in the immediate upstream of the TXNDC5 gene there is MUTED gene. A naturally occurring read-through transcript that contains a MUTED gene splicing variant and the exon 1–4 of TXNDC5 is observed in mammalian cells. However, this fusion transcript is normally removed by nonsense-mediated mRNA decay due to the lack of open reading frame.^{3,4}

Besides TXNDC5, a total of more than 20 PDI family members have been identified in human cells.⁵ As a common structural feature, all members of the PDI family contain one or more of the thioredoxin-like domain that consists of the signature sequence cysteine-X-X-cysteine (CXXC). The existence of multiple PDI members in mammalian cells probably suggests that these members have non-redundant functions or are needed for the reactions with different substrates for specificity. TXNDC5 protein was initially identified in 2003 from the extract of endoplasmic reticulum of hepatic tissue using two-dimensional gel electrophoresis separation and mass spectrometry determination.⁶ The full length version of TXNDC5 contains a total of 432 amino acids (aa), with a signal peptide of 32 aa at the N-terminus and a Lys-Asp-Glu-Leu (KDEL) ER retention signal at the C-terminus. In addition,

there are three thioredoxin-like domains in the full-length TXNDC5. Compared with other members of the PDI family, however, it does not contain recognizable peptide binding domain, denoted as (b) domain, where small protein substrates are expected to bind (Fig. 1A).⁷ Compared with the full length, the truncated version of TXNDC5 does not contain the N-terminal signal peptide, transmembrane domain and the first thioredoxin-like domain due to the absence of the N-terminal 108 aa^{2,8} (Fig. 1B). We performed molecular modeling based on the published partial crystallography data using the I-TASSER software,⁹ the predicted secondary structure of the full length and truncated TXNDC5 are quite similar (Fig. 1C,D). Since there are not sufficient studies on the truncated TXNDC5, the following review is summarized from the studies of the full-length TXNDC5 in mammalian cells.

Primary biochemical function of TXNDC5

Generation of disulfide bond in TXNDC5

Proper folding of newly synthesized plasma membrane and secretory proteins is one of the major functions of the ER in healthy cells. Protein folding occurs when the electron transport takes place between an electron acceptor protein (oxidant) and electron donor protein (reductant). Appropriate formation/breakdown of disulfide bond is one of the critical pathways to ensure the flow of electrons between oxidant and reductant proteins in the cell. These reactions eventually guarantee the synthesis of physiologically functional proteins.¹⁰ In addition to their chaperone activity, members of the PDI family actively participate in the oxidation, reduction, and isomerization of nascent peptides in the ER. To function on a newly synthesized peptide, the PDI needs to be in an oxidized state, which is established through a redox sensitive thiol group in its primary sequence by a number of oxidoreductase and oxygen derived molecules. Each of the sulfur in the thioredoxin-like domain of the PDI is in an oxidative state of -2 . Therefore, to form a disulfide bond with the oxidative state of -1 , two electrons and two protons must be transferred to the oxidant.¹¹ Once the sulfur containing cysteine residues are in close vicinity, thermodynamically favored reaction will be established through molecular oxygen. However, the reaction is slow and requires other enzymes to catalyze the disulfide bond formation within the PDI. There are several well-established pathways involved in generating disulfide bonds in mammalian PDIs through thiol-disulfide exchange reactions. Enzymes required for these pathways including flavoenzyme, ER oxidoreductin 1 (Ero1), Prx, vitamin K epoxide reductase, glutathione peroxidase 7 and 8, as well as reductase for the reduction of dehydroascorbate to ascorbate.^{12–14} Among these, TXNDC5 is found to interact with Ero1 and Prxs, particularly Prx2 and Prx4.^{15,16} It is worth to note that the nascent protein can also be folded into the native form through the involvement of other enzymes such as the quiescin sulfhydryl oxidase pathway, which is independent of the PDI members.¹⁷

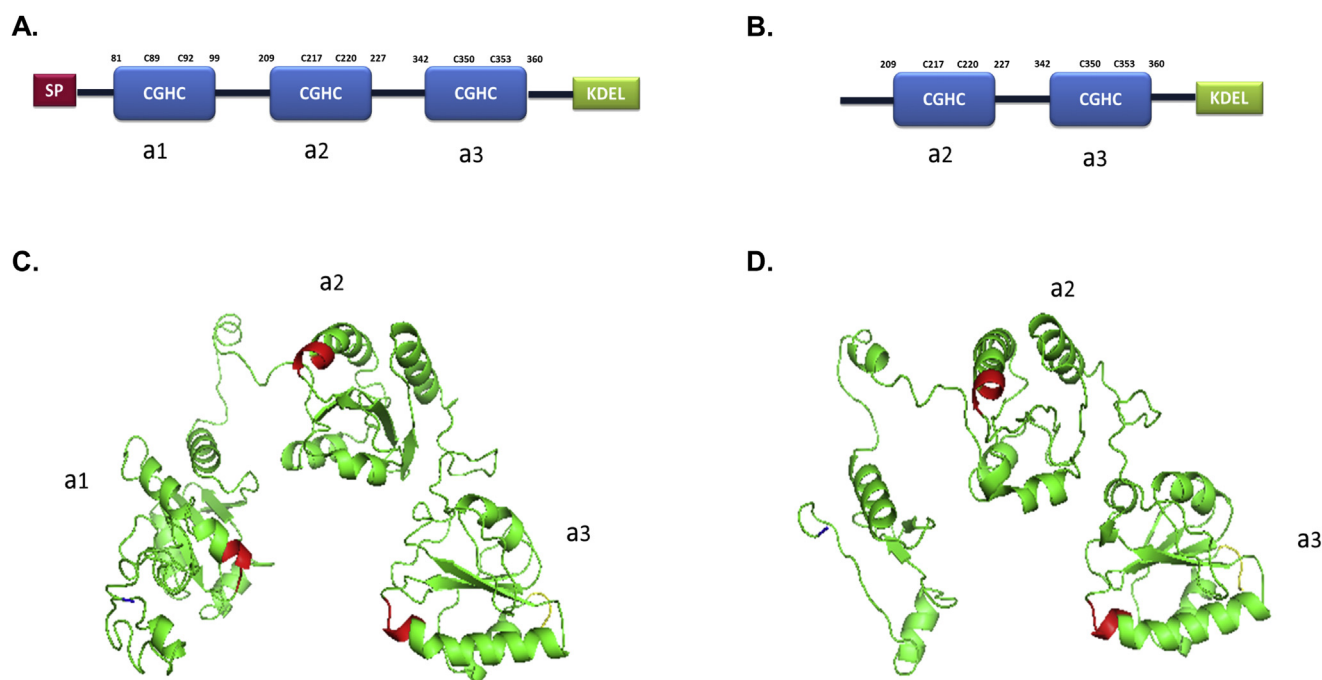


Figure 1 The architecture and structure of TXNDC5 protein in human. (A) The domain architecture of full length TXNDC5 (variant 1) featuring signal peptide (SP), three thioredoxin like domains (CGHC), and ER retention signal (KDEL). (B) The domain architecture of the truncated TXNDC5 (variant 3) featuring two thioredoxin like domains (CGHC), and the KDEL sequence. The secondary structure of the full length (C) and truncated (D) TXNDC5 as predicted from the I-TASSER program. Blue: N-terminus; red: thioredoxin like domain; yellow: C-terminus.

When the ER is shifting toward a reducing environment, it stimulates flavin adenine dinucleotide bound to Ero1. The latter acts on molecular oxygen to generate hydrogen peroxide, leading to the oxidation of the PDI family members including TXNDC5. The thioredoxin-like domains of TXNDC5 enable its enzymatic function including the oxidase and reductase activity, which is essential for the disulfide exchange reaction occurred in the physiologic environment of mammalian cells. Interestingly, three thioredoxin-like domains of TXNDC5 work independently from each other to accelerate the process of protein folding.¹⁸ In contrast, similar domains of other PDIs work cooperatively and resulting in much slower rate of substrate folding. With an extended long loop, the C-terminal thioredoxin-like domain of TXNDC5 acquires a unique V-shape configuration, which distinguishes it from the U-shaped feature of other PDI members, such as ER protein 57 and protein 72.⁷ This unique feature enables TXNDC5 an essential role in disulfide bond exchange and nascent peptide folding.

2-cys containing Prxs, particularly the homodecamer that is composed of five pairs of 2-cys Prx4, are also involved in the oxidation of the PDIs. For this reaction, the affinity of 2-cys Prxs to TXNDC5 is much higher than to the other PDI members.^{15,18} Prx4 contains two cysteine residues, in which the peroxidatic cysteine is responsible for the reaction with hydrogen peroxide and is oxidized to cysteine sulfenic acid (SOH). The oxidized cysteine will then react with the resolving cysteine of the other 2-cys Prx molecule to form the disulfide bond. The disulfide bond is passed to other protein substrates via the exchange reactions through TXNDC5 and PDIA6. In this process, other

PDI family members may also participate in the editing of disulfide bond through their isomerase activity. Therefore, oxidative folding of various protein substrates through disulfide bond formation is largely accomplished by the cooperation of TXNDC5 with other members of the PDI family (Fig. 2).

The functional independence of thioredoxin-like domains in TXNDC5 is demonstrated by utilizing the oxidative ability of Prx4 on each of the isolated domain. Compared with the full length TXNDC5, there are no observable differences in the rate of oxidation of individual thioredoxin-like domain by Prx4.¹⁵ Nevertheless, there is one study reports that TXNDC5-Prx4 interaction is more likely to be through the second thioredoxin like domain.¹⁸ In Jurkat and human umbilical vein endothelial cells, the C-terminal thioredoxin-like domain of TXNDC5 is found to selectively interact with the resolving cysteine of Prx2.¹⁶ This interaction is present in plasma membrane and cytosol. Therefore, TXNDC5 transmits the redox signal from hydrogen peroxide sensors to the substrate through series of disulfide bond formation involving the reduction of Ero1 and dimeric forms of Prx2 or Prx4.

When TXNDC5 passes the disulfide bond to the substrate, its disulfide bond is reduced. In addition to the nascent peptides, glutathione is also known to be capable of reducing the thioredoxin domain of PDI at pH 7.4 in vitro. Furthermore, some members of the PDI family also have the reductase activity. For example, the reductase activity of ERdj5 is involved in the reduction of misfolded proteins in the ER.¹⁹ This function of ERdj5 is important for the degradation of misfolded proteins occurred outside of the

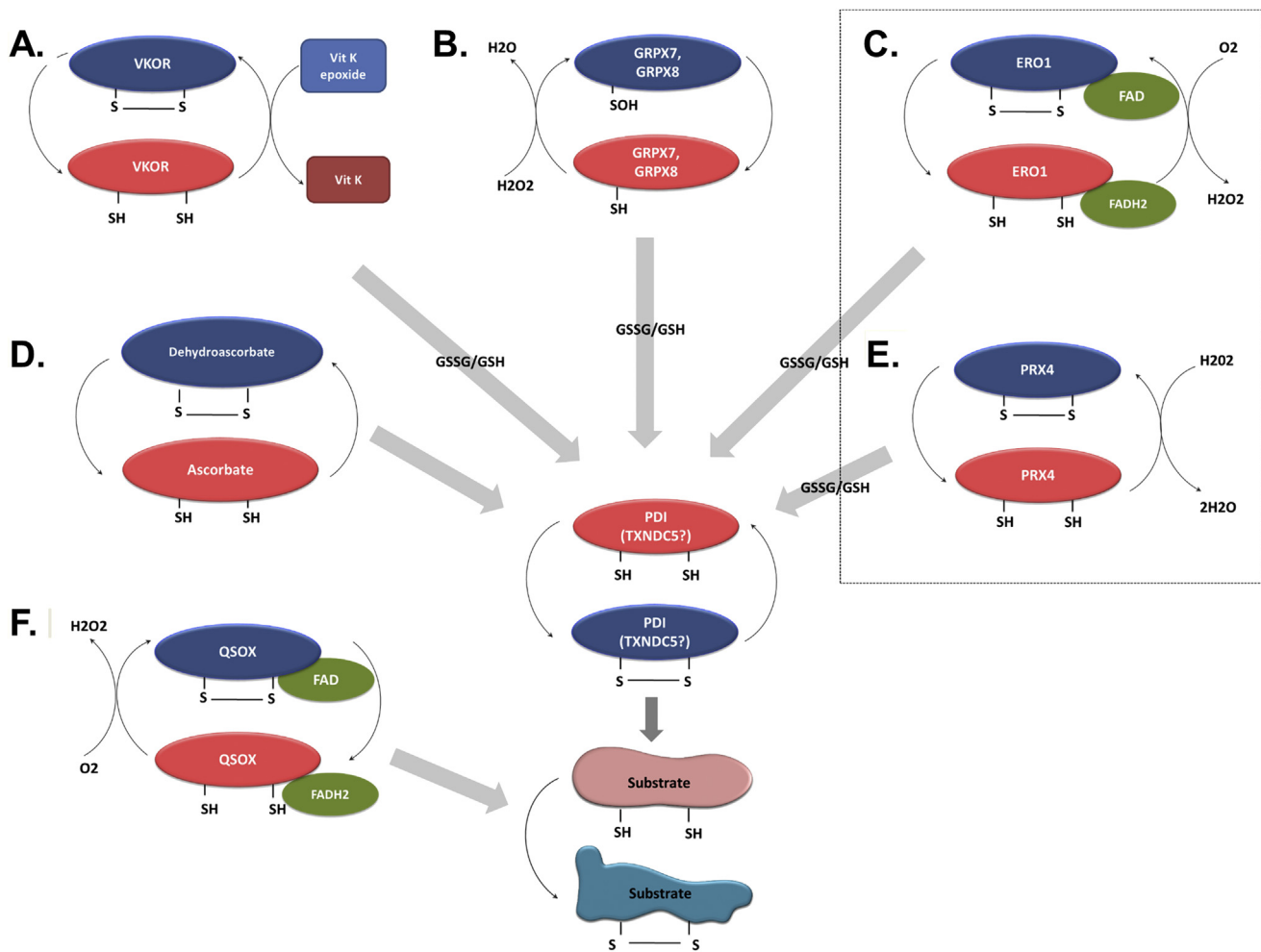


Figure 2 Possible substrates of oxidative folding in endothelial reticulum carried out by TXNDC5. (A) The thioredoxin domain of Vitamin K epoxide reductase (VKOR) is oxidized during the reduction process of vitamin K epoxide to vitamin K. The disulfide bond of VKOR is reduced by TXNDC5. (B) Hydrogen peroxide (H_2O_2) oxidizes glutathione peroxidase 7 and 8 (GPx7, 8) and the disulfide bond is exchanged to TXNDC5. (C) Ero1 used molecular oxygen to generate disulfide bond and releasing H_2O_2 then passes the disulfide bond to TXNDC5. (D) Dehydroascorbate is reduced to ascorbate by TXNDC5. (E) The disulfide bond in Prx4 is reduced by TXNDC5. (F) Quiescin-sulfhydryl oxidase (QSOX) couples the generation of disulfide bonds to the reduction of molecular oxygen, and the disulfide is passed directly to other substrates. Disulfide bond generated through the coupling of TXNDC5 with Ero1 or Prx4 has been experimentally validated (depicted in the dashed box).

ER, as well as the correction of misfolded proteins to their native forms within. In this context, the reductase activity of these PDIs is comparable to the thioredoxin reductase, which employs NAD(P)H to reduce the oxidized cysteine in thioredoxin domain.¹¹ Therefore, it is possible that the disulfide bond of TXNDC5 is reduced through its interaction with glutathione or ERdj5 in the ER. However, there is a lack of experimental evidence to elaborate the exact mechanism by which oxidized TXNDC5 is reduced in cells.

Mechanism of TXNDC5-substrate/thiol-disulfide exchange in cells

The redox status of different cellular compartments is not even, with the general conception of being reducing and hypoxic in the cytosol, oxidizing and normoxic in the

extracellular cell milieu, and oxidizing in the ER.^{20,21} Potential disulfide bond exchange between TXNDC5 and its substrate is determined by the redox status of local environment. In general, members of the PDI family take part in the processes of reduction, oxidation, and isomerization of disulfide bonds to facilitate the maturation of nascent peptides to native proteins in the ER (Fig. 3).

The PDI is activated when the sulfhydryl group (SH) of the cysteine in the thioredoxin-like domain is deprotonated to thiolate anion (S^-). The deprotonation of the SH is dependent on the pKa of the thiol group and the charge of the neighboring amino acids. Under normal physiologic conditions, the pKa of cysteine residue is about 8.5 ± 0.5 and the thiol is in its inactive state. However, the presence of a neighboring positively charged amino acid can increase the propensity of SH to lose the hydrogen ion and form a more stable S^- anion. The S^- is nucleophilic and will attack

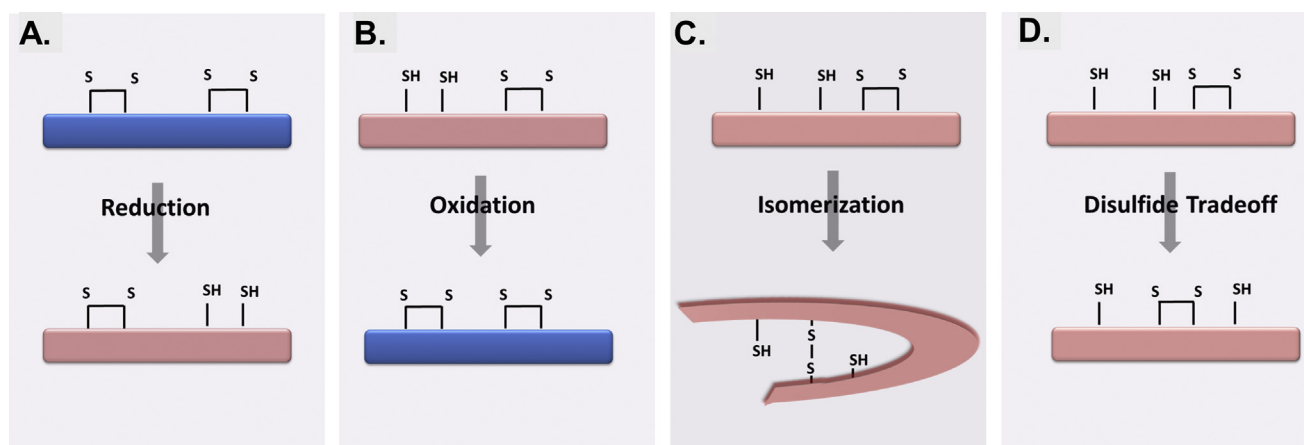


Figure 3 Disulfide exchange reactions on the substrates carried out by TXNDC5 and other PDI family members. Blue, oxidized substrate; red, reduced substrate. **(A)** Reduction of disulfide bond to two sulfhydryl groups. **(B)** Oxidation of the substrate from the reduced form to more oxidized disulfide bond. **(C)** Isomerization involves changing the overall folding of the substrate. **(D)** Disulfide bond alteration with limited changes on the protein conformation. In both cases, exchange of disulfide bond does not change the overall oxidative nature of the substrate.

the sulfur atom of the disulfide bond, through which the thiol-disulfide exchange occurs.^{11,20} Glutathione and glutathione reductase are among the proteins that contribute to the thiol activation of PDI. The pH of normal ER is about 7.2, while the extracellular pH is about 7.4. Therefore, under physiological conditions, the pKa of the N-terminal thiol is about 4.5–6.7. PDI with active S[−] can also break the bond in the form of other oxidative modifications, such as the bond between nitric oxide and sulfur in S-nitrosothiol.²⁰ As to TXNDC5, the oxidation of the nascent peptide takes place when the S[−] ion exerts a nucleophile attack on the sulfhydryl group of the substrate, resulting in a mixed disulfide bond. The latter is resolved when the second cysteine of the thioredoxin-like domain attacks, leading to the release of the substrate from TXNDC5. Thus the substrate is left with an active S[−] ion, which will then attack and deprotonate a nearby sulfhydryl group to form a disulfide bond.¹¹

The formation of appropriate disulfide bond will stabilize the structure of protein and thus increase its half-life.^{11,22} For instance, the formation of disulfide bond between Cys68-Cys98 of subtilisin E increases its half-life for nearly three folds.²³ Such effect of stabilization is resulted from the increase of the melting temperature. Moreover, the formation of disulfide bond can also affect the protein activity, either positively or negatively depending on the location of the disulfide bond. On the other hand, formation of non-native disulfide bonding may create intermediates leading to protein aggregation and precipitation. Such effect will potentially produce undesirable consequences in cells, and should be avoided in the design of proteins aimed for therapeutic purpose.²²

Other possible modifications of TXNDC5

Besides disulfide bond formation, protein thiols are also subjected to other redox related modifications. For example, protein thiols undergo S-nitrosylation in response to nitrosative stress. S-thiolation of TXNDC5 leads to the

formation of disulfide bond with cysteine, homocysteine, cysteinylglycine and glutathione, and such modifications share the characteristic of having low molecular weight. S-nitrosylation can be potentially harmful since increased levels of S-nitrosylation in PDIs have been reported in human disorders such as Parkinson's and Alzheimer's diseases.^{24,25} Moreover, thiols can also be oxidized by reactive oxygen species to produce sulfenic form (SOH) with an oxidation state of zero, or overoxidized to form sulfinic form (SO₂H) with an oxidation state of +2, and even sulfonic acid (SO₃H) with an oxidation state of +4. The oxidized thiol plays a role in cell signaling and is an important intermediate in disulfide bond formation. The overoxidized thiols are intermediates of cysteine metabolism and can function as agonists for certain receptors. In general, the overoxidation of protein thiols are considered irreversible. However, the overoxidation of thiols in Prxs can be reduced back to their oxidized form through the reducing activity of sulfiredoxin.^{26,27} Theoretically, thiols in the thioredoxin folds of TXNDC5 are also likely undergo above mentioned modifications. Currently, there are no sufficient studies involving the thiol modification of TXNDC5. Therefore, the significance of such modifications in TXNDC-5 mediated cell signaling is still not clear.

TXNDC5 and cancer

TXNDC5 is highly expressed in human cancer

As evidenced from results of immunohistochemical staining and western blotting of patient specimens, abnormally high expression of TXNDC5 protein has been found in many types of human tumors, including cancers of lung, prostate, colon, breast, esophagus, liver, gastro and ovary.²⁸ In lung cancer, more than sixty percent of non-small cell lung cancer specimens has elevated TXNDC5 expression compared with adjacent normal tissue. Such increase of TXNDC5 expression is found at both transcriptional and

translational levels, in particular, in the early stage of tumor formation.²⁹ Similar findings were reported in colon cancer, in which nearly eighty five percent of specimens has increased level of TXNDC5; and significant higher level is found in the early stage of cancer development.³⁰ Another study using specimens from colorectal cancer patients also reveals the upregulation of TXNDC5 in the early stage of colon cancer.³¹ Nissom and his group reported that TXNDC5 is upregulated in poorly differentiated hepatocellular carcinoma.⁸ In human gastric adenocarcinoma, TXNDC5 is elevated in most of the examined samples and significantly high level of TXNDC5 is observed especially in poorly differentiated cancer.³² In prostate tumors, TXNDC5 is upregulated in androgen naïve prostate cancer and castration-resistant prostate cancer.^{33,34} Taken together, these studies suggest that TXNDC5 is highly expressed in different types of human tumors and may play a potential role in cancer development.

Mechanisms of TXNDC5 to promote cancer development

TXNDC5 affects tumor cells' response to extracellular oncogenic signaling through interacting with receptors on the plasma membrane. The majority of TXNDC5 protein is localized in the ER and Golgi, where it plays a critical role in protein folding and anterograde transport. However, nearly one fifth of TXNDC5 is present in plasma membrane, where it is presumably involved in the transduction of extracellular signaling events.^{35,36} Other membrane localized PDIs, such as PDIA6, were reported to be involved in signal activation and remodeling through binding to receptors in the plasma membrane. For example, PDI3 binds and participates in the activation of α IIb β 3 fibrinogen and α 2 β 1 collagen receptors, whereas PDIA6 binds to integrin β 3 subunit and participates in platelet aggregation.^{20,21,37} It is thus reasonable to believe that TXNDC5 also plays such a role in the transduction of certain membrane receptor signaling. Indeed, TXNDC5 is found to interact with few membrane receptors such as adiponectin receptor. Specifically, TXNDC5 interacts with the N-terminal domain of AdipoR1 and this interaction is critical for receptor remodeling. In HeLa cells, increased distribution of TXNDC5 on plasma membrane inhibits adiponectin signaling through reducing the distribution of AdipoR1 on the cell surface.³⁵ In renal cell carcinoma, there is a high ratio of TXNDC5/AdipoR1 in metastatic RCC compared to non-metastatic control; and the presence of TXNDC5 in metastatic RCC promotes cell invasion, enhances tumor xenograft growth in nude mice.³⁸ Another example of TXNDC5 in membrane receptor signaling is the activation of androgen receptor (AR). In prostate cancer, TXNDC5 is found to directly interact with AR. The TXNDC5-AR interaction stabilizes AR and protects it from degradation, thus more AR can be translocated into the nucleus upon ligand binding, leading to an enhanced activation of downstream signaling.³³ In particular, this mechanism functions as an alternative pathway to enhance AR activation in castration-resistant prostate cancer when the amount of androgen is limited. By stabilizing AR and increasing the sensitivity of cells to androgen, TXNDC5 promotes abnormal activation of

epidermal growth factor receptor 2, leading to the sustained activation of protein kinase B and extracellular-signal-regulated kinase cascades even when there's limited ligand presence.³³ Moreover, TXNDC5 is also reported to affect mitogen activated protein kinase (MAPK) signaling through the TNF α -TNF receptor activation, and this effect exists upstream of c-Raf in the MAPK cascade.³⁹ Therefore, it is conceivable that TXNDC5 may interact directly with tumor necrosis factor (TNF) receptor. However, further studies are needed to test this interaction and determine how this interaction affects the TNF α -mediated cell signaling.

Expression of TXNDC5 increases tumor cell proliferation. A close examination of TXNDC5 on cell cycle progression reveals that its expression increases the number and percentage of cells in G2/M phase, whereas knockdown of TXNDC5 leads to the accumulation of cells in G0/G1 phase. In gastric cancer cells, elevated level of TXNDC5 contributes to increased rate of cell proliferation, migration, colony formation and reduced rate of apoptosis.⁴⁰ The anti-apoptotic function of TXNDC5 is also supported by findings from pancreatic cancer, where the orphan nuclear receptor 4A1 (NR4A1) and TXNDC5 are overexpressed. In these cells, activation of NR4A1 protects cells from damages caused by ER stress. Such effect of NR4A1 is closed associated with the transcriptional activation of TXNDC5. Down-regulation of TXNDC5 results in the defragmentation of ER and altered expression of many ER proteins, such as ATF-4, GRP78, IDH1, TXNDC5 and CCAAT/enhancer-binding protein homologous protein. Therefore, the protective effect of NR4A1 is mediated at least partially through the expression of TXNDC5, which reduces ER stress and facilitates cell survival.⁴¹

Moreover, TXNDC5 contributes to tumor cell survival and growth under hypoxia environment. In addition to cancer cells, TXNDC5 is also found to be highly expressed in endothelial tissue of tumors and atherosclerotic plaques, particularly under hypoxic condition. Knockdown of TXNDC5 leads to the reduced expression of endothelin-1, adrenomedullin and CD105. These molecules are known to facilitate the survival of endothelial cells. Through enhancing the expression of these molecules, TXNDC5 may protect cancer cells from hypoxia induced cell death.⁶ Treatment of prostate cancer by androgen deprivation often leads to an increased expression of TXNDC5, which is mediated through hypoxia-induced activation of HIF-1 α and miR-200b pathways. The hypoxic condition fortifies the interaction between TXNDC5 and AR and conceivably further enhances downstream signaling including the activation of ERK1/2, AKT and MEK pathways. Mechanistically, TXNDC5 is negatively regulated by miR-200b since it binds and targets the 3' UTR of TXNDC5 mRNA for degradation. However, miR-200b is also negatively regulated by HIF-1 α . Under hypoxia condition, the degradation of HIF1 α is reduced, leading to its stabilization, accumulation and translocation to nuclear. Therefore, the inhibition of miR-200b by HIF1 α leads to the increase of TXNDC5 under hypoxia, which is positively associated with increased cell migration and invasion.^{42,43}

Furthermore, the presence of TXNDC5 leads to the change of extracellular microenvironment that facilitates cancer cell invasion and metastasis. In gastric

adenocarcinoma, expression of TXNDC5 promotes cancer cell invasion and metastasis.³² TXNDC5 is highly expressed in gastric cancer compared with normal surrounding tissue. Knockdown of TXNDC5 in gastric adenocarcinoma MKN45 cells significantly reduces their malignancy. In prostate cancer, TXNDC5 is upregulated in androgen naïve prostate cancer and castration-resistant prostate cancer. Silencing of TXNDC5 in androgen-independent prostate cancer cells inhibits cell invasion, and overexpression of TXNDC5 stimulates tumor xenograft growth in nude mice.³³ Similar observation is also found in HeLa cells, since knockdown of TXNDC5 leads to the reduced activation of p38 mitogen-activated protein kinases.^{35,38}

In human umbilical vein endothelial cells, knockdown of TXNDC5 leads to reduced activation of Ras/Raf and downstream ERK1/2 in response to inflammatory cytokines such as TNF α . The insufficient activation of ERK1/2 results in a decreased activity of activator protein-1 (AP-1). Among the downstream target genes of AP-1 inhibition, there are various protein proteases including matrix metalloproteinase (MMP) 9 and cathepsin B. Down-regulation of these protease has a negative effect on the angiogenesis of endothelial cells.³⁹ Moreover, TXNDC5 also promotes the expression of MMP1 and MMP13, which play significant role in cancer cell invasion and metastasis.⁴⁴ In particular, MMP13 has been associated with tumor angiogenesis through promoting the secretion of VEGF in endothelial cells and fibroblasts.⁴⁵ Through the upregulation of matrix metalloproteinase, TXNDC5 enhances cancer cell invasion and metastasis. Based on these findings, we summarized the possible mechanistic contributions of TXNDC5 in human cancer development (Fig. 4).

TXNDC5 in other diseases

In addition to cancer, TXNDC5 also plays important role in other types of human disease. In patients with rheumatoid arthritis, the levels of TXNDC5 in plasma and synovial fluid are significantly higher compared with normal control.⁴⁶ Epidemiological studies reveals that a single nucleotide polymorphism rs443861 is associated with an increased risk of rheumatoid arthritis.⁴⁷ The increased level of TXNDC5 in rheumatoid arthritis is not resulted from the autoimmune response, but rather is the response to hypoxia. Moreover, TXNDC5 is believed to have a pro-inflammatory function that contributes to synovial membrane angiogenesis, aberrant cell differentiation and bone destruction in rheumatoid arthritis. Mechanistically, adiponectin is found to be significantly increased in the synovial site. TXNDC5 can modulate adiponectin signaling since it directly interacts with adiponectin receptor.⁴⁷ Furthermore, the expression of multiple factors including MMP1, MMP13, VEGF, IL-6, IL-8 and other cytokines is enhanced by TXNDC5-mediated intracellular signaling. Among these, MMP1 and MMP13 facilitate the degradation of collagenous extracellular matrix; and cytokines stimulate further inflammatory cell infiltration and synovial cell death.^{41,47} At the later stage of rheumatoid arthritis, TXNDC5 also contributes to the deterioration of osteoblast and bone destruction.

Genetic polymorphism of TXNDC5 gene is also associated with other types of diseases in humans. For example, certain SNPs of TXNDC5 are positively linked to non-segmental vitiligo, a condition featured by the loss of melanocyte in the skin. In Korean males, a case-control study of 230 subjects reveal that rs1043784, rs7764128 and rs8643, located on TXNDC5 exons, are significantly associated with NSV. As a result of autoimmune response or oxidative stress, these SNPs can negatively affect the anti-apoptotic activity of TXNDC5, leading to the progressive loss of melanocytes.⁴⁸ Moreover, TXNDC5 SNPs are found to be associated with increased vulnerability to schizophrenia. In families with at least two siblings suffered from schizophrenia, genetic analyses found that the locus 6p24.3 is linked to cognitive impairment. In particular, SNP rs13873 of TXNDC5 gene and the haplotype rs1225934-rs13873 of the *BMP6-TXNDC5* genes on chromosome six are associated with a high risk of schizophrenia.⁴⁹

Increased expression of TXNDC5 may also contribute to the progression of diabetes. In patients with autoimmune antibodies that attack insulin producing β -cells, the level of TXNDC5 is increased and positively associated with a higher risk of type I diabetes.⁵⁰ In type II diabetes, TXNDC5 is identified as one of the important response components of glucose toxicity.⁵¹ Interestingly, pancreatic cells' response to high glucose exposure may include a reduced expression of TXNDC5 but increased expression of other PDI family members. For instance, in a study of hepatic insulin resistance in hamster, the level of TXNDC5 is found to be reduced by nearly two folds, whereas expression of other PDIs increases in fructose-fed insulin-resistant animals. Therefore, these studies reveal that there is a possible association between TXNDC5 and hepatic insulin signaling. However, the mechanism by which TXNDC5 affects insulin signaling has yet to be determined.

Other PDIs in cancer

The human PDI family can be divided into three subgroups and some unusual subfamilies.^{5,52,53} The first group includes five typical PDIs (PDIA 1–5) and PDILT, each containing two catalytically active a-type domains (a and a' domains) and two inactive b-type domains (b and b' domains). Proteins in the second group contain only enzymatically active thioredoxin-like domains including ERP44, TXNDC5, PDIA6 and DNAJC10. The third group comprises TMX 1–4, which carrying only one a-type domain are the only PDI proteins with a transmembrane domain. Erp27, Erp29, TXNDC12, AGR2 and AGR3 can be considered as unusual members of the PDI family. These proteins contain single a-type domain thus have lower molecular weight (<30 kDa), except for Erp27, which has two b-type domains.⁵³ CASQ1 and CASQ2 belong to another unique subfamily, which has only b-type domains and no ER-retention sequence.⁵⁴ Although functions of PDI proteins in cancer progression are complicate, many published researches suggested that these proteins are frequently up-regulated in various cancers. Analyses of proteomes and microarray data indicated that PDIA1, PDIA3, PDIA4 and PDIA6 are highly expressed in brain, liver, breast, prostate and

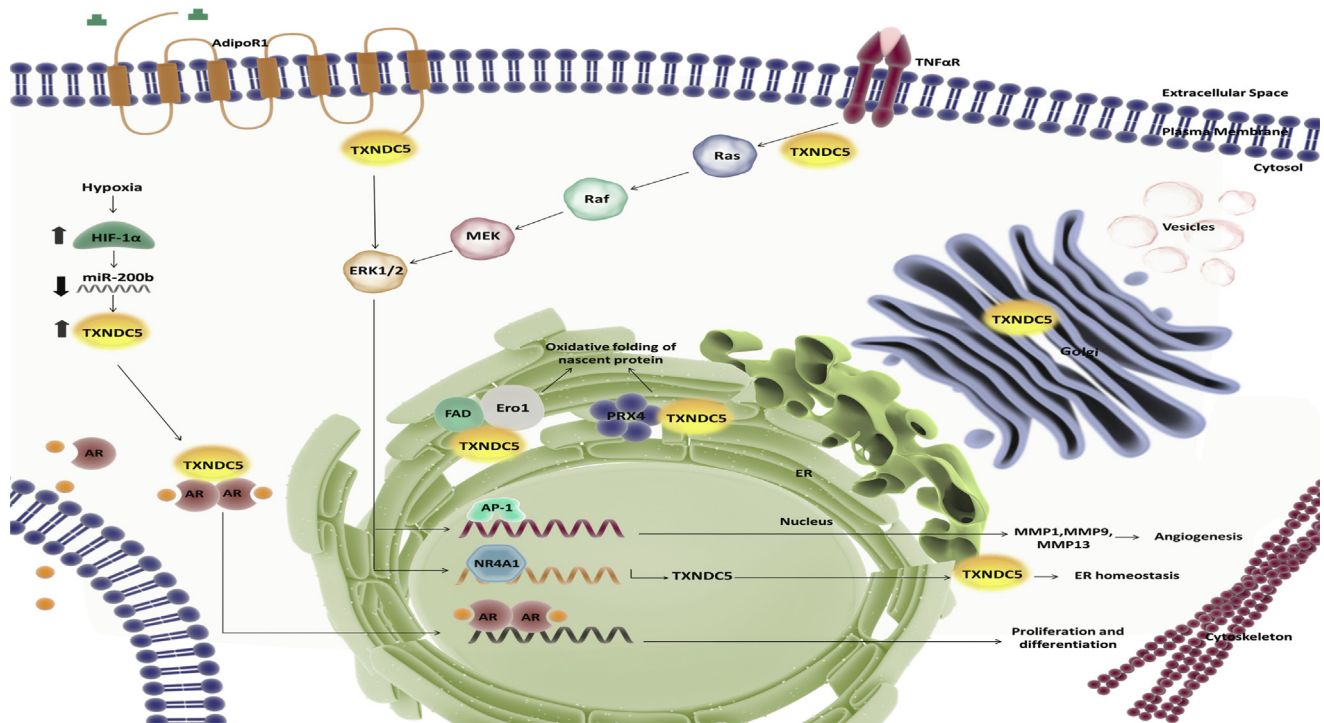


Figure 4 Mechanisms of TXNDC5 in cell signaling and cancer development. Binding of ligands to membrane receptors, such as AdipoR1 and tyrosine kinase receptors, stimulates signaling cascades that activate transcription factors including AP-1 and NR4A1. TXNDC5 is a downstream target of such activation. In addition, binding of miR-200R to the 3'-UTR of TXNDC5 transcripts leads to the degradation of mRNA and low expression under normoxia conditions. Hypoxia leads to the downregulation of miR-200b, which results in the upregulation of TXNDC5. Increased level of TXNDC5 can further enhance membrane receptor signaling through direct interaction or receptor stabilization. The presence of TXNDC5 facilitates the folding of nascent peptides and reduces ER stress through its chaperone activity, and this process may also involve the contribution from other enzymes, such as Prx4 and Ero1. Collectively, TXNDC5 enhances cell growth and proliferation, ER homeostasis and angiogenesis to promote cancer development.

colorectal cancers, and correlated with metastasis and invasiveness.^{1,55–57} A recent study showed that PDIA1 interacts with actin, regulates cytoskeletal organization and cell adhesion.⁵⁸ Schorr-Lenz et al found PDIA6 reduces disulfide bonds of MHC I-related chain protein (MIC, a ligand for NK cell receptor) on tumor cell surface, which will cause absence of immune surveillance.⁵⁹ PDIA5 can activate ATF6, a transcription factor that modulates cellular response to ER stress, by rearrangement of disulfide bonds, and as a consequence promotes cancer development and resistance to chemotherapies.⁶⁰ Although most PDI proteins confined to ER, a recent study showed that PDI on the cell surface can activate metalloprotease ADAM17 to regulate growth factor signaling in cancer cells.⁶¹ Other than TXNDC5, Erp29 can increase E-cadherin in epithelial-mesenchymal transition (EMT) pathway and upregulate Hsp27 in breast cancer cells leading to reduced apoptosis.^{2,62,63} Other studies also showed that PDIA3 and DNAJC10 have a protective role to tumor cells in response to oxidative stress, due to their ability to degrade misfolded proteins in ER.^{64,65} Although study on TMX1 revealed that absence of this protein caused increasing apoptosis in liver cells through p53 signaling pathway, physiological functions of other TMX proteins are still unclear.⁶⁶ Among all human PDI proteins, ARG subfamily, especially ARG2, is the most closely related to oncogenesis and metastasis.

Several publications considered ARG2 as a diagnostically biomarker of various cancers and functionally act as a potential molecular chaperone through its interaction with different proteins (e.g. MUC2, EGFR, GRp78).^{67–72}

Concluding remarks

In nascent peptide folding, there are different PDI members that are specific for certain substrates, and they may also share common functionalities. In cancer cells, multiple disulfide bond formation and isomerization pathways may be present to ensure efficient folding of various substrates. TXNDC5 is found to be increased in the early stage of gastric, colon and liver tumors, and it contributes significantly to cancer progression in the later stage. In addition to participating disulfide bond formation for correct folding of the substrates, increased level of TXNDC5 promotes tumor cell growth, proliferation and survival. The hypoxic environment in the late stage of cancer further stimulates the expression of TXNDC5, which not only contributes to cancer cell survival through the maintenance of redox balance and ER homeostasis, but also enhances oncogenic signaling pathways that promotes metastasis and angiogenesis. Most previous studies indicate that abnormally high level of TXNDC5 has an essential signaling

function that can promote the development of cancer and other types of diseases in humans. Therefore, it can be considered as an important target for the development of cancer therapeutics. In addition to TXNDC5, increased levels of other PDIs are also found in different types of cancer. Expression of these PDIs may contribute to cancer cell proliferation, invasion and metastasis. Currently there are no therapeutic drugs that have been approved to specifically target TXNDC5 or other PDIs for the treatment of cancer in patients.

Conflict of interests

There is no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2018.09.003>.

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