Induction of Cell Membrane Protrusions by the N-terminal Glutaredoxin Domain of a Rare Splice Variant of Human Thioredoxin Reductase 1*

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The human thioredoxin system has a wide range of functions in cells including regulation of cell proliferation and differentiation, immune system modulation, antioxidant defense, redox control of transcription factor activity, and promotion of cancer development. A key component of this enzymatic system is the selenoprotein thioredoxin reductase 1 (TrxR1), encoded by the TXNRD1 gene. Transcription of TXNRD1 involves alternative splicing, leading to a number of transcripts also encoding isoforms of TrxR1 that differ from each other at their N-terminal domains. Here we have studied the TXNRD1_v3 isoform containing an atypical N-terminal glutaredoxin (Grx) domain. Expression of the transcript of this isoform was found predominantly in testis but was also detected in ovary, spleen, heart, liver, kidney, and pancreas. By immunohistochemical analysis in human testis with antibodies specific for the Grx domain of TXNRD1_v3, the protein was found to be predominantly expressed in the Leydig cells. Expression of the TXNRD1_v3 transcript was also found in several cancer cell lines (HCC1937, H23, A549, U1810, or H157), and in HeLa cells, it was induced by estradiol or testosterone treatments. Surprisingly, green fluorescent protein fusions with the complete TXNRD1_v3 protein or with only its Grx domain localized to distinct cellular sites in proximity to actin, and furthermore, had a potent capacity to rapidly induce cell membrane protrusions. Analyses of these structures suggested that the Grx domain of TXNRD1_v3 localizes first in the emerging protrusion and is then followed into the protrusions by actin and subsequently by tubulin. The results presented thus reveal that TXNRD1_v3 has a unique and distinct expression pattern in human cells and suggest that the protein can guide actin polymerization in relation to cell membrane restructuring.

The thioredoxin system involves several thioredoxins (Trx) and thioredoxin reductases (TrxR) that are found in different cellular and subcellular locations where they participate in a wide range of functions in cells, including protection from oxidative stress, synthesis of DNA precursors, regulation of cell proliferation and differentiation, control of apoptosis, modulation of the immune system, redox control of transcription factor activity, and promotion of cancer development (1–6). The many functions of the thioredoxin system are mainly carried out by the interaction of thioredoxins with target proteins, most often involving redox reactions whereby thioredoxin reduces a disulfide in the target protein. For this purpose, the active site disulfide thioredoxin first has to be reduced to a dithiol, which is catalyzed by TrxR using NADPH, and hence, making TrxR a key player for essentially all downstream cellular functions regulated by the thioredoxin system.

In human cells, three genes encode thioredoxin reductases, namely TXNRD1, TXNRD2, and TXNRD3. The classical TrxR1 enzyme, encoded by the TXNRD1 gene, is predominantly cytosolic and is expressed in most, if not all, human cells, where Trx1 is believed to be its major substrate (4, 5, 7, 8). The TXNRD2 gene encodes mitochondrial TrxR2 that reduces mitochondrial Trx2 (9–11), although TrxR2 is also found in the cytosol (12) but seems to have non-ubiquitous lower expression level when compared with TrxR1 (see Ref. 13 and www.proteinatlas.org). Both cytoplasmic and mitochondrial thioredoxin are essential proteins for mammals, as shown by the embryonic lethal phenotypes.
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Scheme 1. Overview of the alternatively spliced transcripts of the TXNRD1 gene studied here. Transcription of the α-transcripts is initiated at the core promoter located upstream of exon I, whereas an alternative promoter upstream of exon β_v3 regulates transcription of the β splice variant. The β1 transcript is translated into the third isoform of TrxR1 (TXNRD1_v3, or v3) containing an atypical N-terminal Grx domain fused to the common TrxR1 module. Arrowheads connected by a dotted line indicate primer pairs used to identify the respective splice variant, and the length of the amplified fragment is given in parentheses. The dashed part of exon I shown in this scheme in transcripts α7,α8 indicates an alternative splice donor site within that exon, resulting in either α7,α8 encoding TXNRD1_v1 or α1,α2 encoding TXNRD1_v2. Filled exons of larger width indicate the open reading frame (ORF) of each transcript. The common TrxR1 module is shown in green, the unique part of the Grx domain of the β1 transcript is indicated in red, and the sequences shared between the Grx domain and the N-terminal part of TXNRD1_v2 are hatched. The scheme is drawn approximately to scale, as indicated in the figure. Note that the complete TrxR domain is encoded by additional exons downstream of exon V. The scheme has been modified from an earlier report, to which the reader is referred for further information including sequences and accession numbers (7, UTR, untranslated region).

All three human thioredoxin reductase genes are subject to extensive splicing, mainly in the 5′-end, leading to a number of transcripts that in several cases encode different protein isoforms (7, 8, 12, 25, 26). In the present study, we have characterized further a rare alternative transcript derived from the TXNRD3 gene, which is similar to TrxR1 and TrxR2 but contains an additional monothiol glutaredoxin (Grx) domain as an N-terminal addition to the TrxR module. The TGR enzyme is mainly expressed in early spermatids in testis and is suggested to participate in the redox reactions required during maturation of sperm (22–24).

Three human thioredoxin reductase genes are subject to extensive splicing, mainly in the 5′-end, leading to a number of transcripts that in several cases encode different protein isoforms (7, 8, 12, 25, 26). In the present study, we have characterized further a rare alternative transcript derived from the TXNRD1 gene that expresses an atypical N-terminal Grx domain fused to the TrxR1 module and encoded by alternative exons located upstream of the core promoter. Adhering to the generally proposed nomenclature for human genes, we originally named the transcript β1 and its encoded protein isoform TXNRD1_v3 (7) and will keep to this nomenclature in the present study.

For ease of readability, the TXNRD1_v3 protein is hereafter referred to as version three ("v3") of TrxR1. In Scheme 1, the exon organization of the 5′-region of the v3 transcript (β1) is depicted and compared with the other main isoforms of human TrxR1, with the localization of the different primers and probes used in this study also shown in the scheme.

The Grx domain of v3 has an atypical CTRC active site sequence (7, 25), instead of the more common CPYC motif found in Gra (27). This Grx domain of v3 was earlier found to lack activity in any of the classical Grx assays, except if the CTRC active site was mutated to the classical CPYC motif whereupon the protein gained typical Grx activity (25). The rare cDNA or expressed sequence tag clones found to be encoding v3 have thus far only been derived from testis (7, 25). Interestingly, the transcripts encoding this isoform can be found only in human, chimpanzee, and dog but not in mouse or rat (25). Furthermore, expression of the v3 protein was recently detected in a human mesothelioma cell line using immunoblotting and mass spectrometry (28). To date, no information exists about the possible function(s) of v3 in human cells, which has been the focus of the present study.

Experimental Procedures

Northern Blot Analysis—Human multiple tissue Northern blots with poly(A+)-RNA from different tissues were purchased from Clontech. The β1 variant cDNA cloned into a pET vector (Novagen) served as a positive control when used as template in the PCR reactions. The cDNA of the different cancer cell lines used here was generously provided by Christopher Horst Lillig, Christoph Hudemann, and Maria Lönn (Karolinska Institutet). The following primers were used: R5, 5′-GCA ACC CAC ATT CAC ACA TGT TCC TCC-3′; R2, 5′-GGA CCA TGG CCG CTT TTT TCT TCT TCT ACA AAC AC-3′; Fl, 5′-GAG TCC TGA AGG AGG GCC TGA TGT CTT CAT CAT CTT C-3′; and FB_vIII, 5′-CAA CAA AAT AAG AAA CAC ACA GAG ATT TAA ATA ACA G-3′. For PCR reactions, 2.5 units/reaction Taq polymerase, 10 μM of each primer, and 2–3 μl of cDNA template were used. Reactions were heated to 96 °C for 1 min before the first of 35 cycles (94 °C for 30 s, 58 °C for 45 s, and 72 °C for 2 min) followed by 72 °C for 3 min after the final cycle and then paused at 4 °C. PCR products were analyzed on 1.2% agarose gels with 5 μl of sample loaded. Sequencing reactions of some DNA bands were performed to verify the sequence of PCR products.

Immunohistochemistry—Paraffin-embedded testis tissue slides (ProSci) were dewaxed and rehydrated by placing the slides in three changes of xylene for 3 min each time followed by three changes of ethanol (99.5% for 2 × 5 min, 95% for 2 × 4 min, 70% for 1 × 5 min) and then hydrated at 37 °C for 3 min. For antigen retrieval, slides were immersed into a solution of
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pH 7.8 containing 0.1 g of CaCl₂ and 0.1 g of chymotrypsin (from bovine pancreas) in water at 37 °C. After removing the slides, they were placed in cold water and rinsed twice with TBS containing 0.025% Triton X-100. Slides were blocked in 10% normal goat serum (DAKO) with 1% bovine serum albumin in TBS for 2 h at room temperature and subsequently drained and wiped around the sections. Polyclonal rabbit antibodies, α-TXNRD1_v3 (α-v3), were made by AgriSera against the synthetic v3-derived peptide (NH₂-CCKGKNGDGRWSAKDH-PGK-COOH), with the underlined cysteine residue added for maleimide-linked conjugation of keyhole limpet hemocyanin protein as an immunization carrier. The peptide sequence is encoded by exons β_vIII and β_vI (Scheme 1). The antibodies were eluted at pH 7.0 from affinity-purified rabbit IgG and kept in PBS with 20% glycerol (0.497 mg/ml) and used as primary antibodies. The slides were incubated overnight at 4 °C with a 1:200 dilution of α-v3 primary antibodies in 200 μl of TBS with 1% bovine serum albumin. For the preabsorption control, the same synthetic peptide antigen (500 μg) used for raising the antibodies was added to the primary antibody solution. Subsequently, the slides were rinsed for 2 × 5 min in TBS with 0.025% Triton X-100. Slides were incubated for 2 h at room temperature with biotinylated goat α-rabbit secondary antibody (DAKO) in a 1:200 dilution in TBS containing 1% bovine serum albumin. Slides were rinsed for 2 × 5 min in TBS. Streptavidin antibody complexed with horseradish peroxidase (DAKO) were applied to the slides in TBS for 30 min at room temperature. Slides were rinsed 2 × 5 min with TBS and developed with chromogen (diaminobenzidine, DAKO) for 10 min at room temperature. Following another rinse under running tap water for 5 min, slides were counterstained with Mayer’s hematoxylin (DAKO) for 2 min. Then, slides were rinsed with water and counterstained with eosin for 2 s, rinsed again with water, and finally mounted using fluorescent mounting medium (DAKO).

Transfection Experiments—v3-GFP, v3(Grx)-GFP or TrxR1 module-GFP fusion constructs were made using a human expressed sequence tag clone for TXNRD1_v3 (GenBank™ accession number BG772375) as template for PCR. The following forward primers were used: v3 GFP forward, 5’-CTG AAT TCG CCA CCA TGG ACG GCC CTG-3’ (introducing a BamHI site). Cloning of the final desired target constructs for transfection experiments was made using the pEGFP-N3 vector (Clontech), fusing the GFP at the C-terminal end of the protein domains and for the TrxR1 module-encoding transcripts excluding both the selenocysteine residue and the 3’-untranslated region of the cDNA, essentially as described previously (29). HEK293 and HeLa cells were grown on glass coverslips until they reached ~80% confluency. Subsequently, cells were transfected with the constructs using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. We used various concentrations of DNA ranging from 0.25 to 1 μg/well on a six-well plate, without any differences observed. Cells were fixed 16 h after transfection in 4% paraformaldehyde for 30 min at room temperature and then permeabilized with PBS-T (PBS with 0.1% Tween 20) for 3 × 5-min washes followed by a blocking step using 5% normal goat serum (Jackson Immunoresearch Laboratories) in PBS-T. Subsequently, after a short wash in PBS-T, cells were incubated with a monoclonal anti-β-tubulin antibody (T4026, Sigma) diluted 1:200 in PBS-T for 1 h followed by 3 × 5-min washes. The tubulin antibody was detected using a goat anti-mouse antibody conjugated to Alexa Fluor 568 (Molecular Probes) diluted 1:500, whereas the actin network was visualized using phalloidin Alexa Fluor 647 (Molecular Probes) diluted 1:40 in PBS-T. After a 1-h incubation, cells were washed briefly and stained with DAPI (Molecular Probes) for 5 min and then washed 3 × 5 min in PBS-T before mounting using fluorescence mounting medium (Calbiochem). For co-staining of estrogen receptors ERα and ERβ, cells were treated with 10 nM estradiol and stained essentially as described before (29).

Fluorescence Microscopy—For the imaging, we employed a Leica AS MDW fluorescence microscope (Leica Microsystems). DAPI, GFP, and Alexa Fluor 568 were excited, and fluorescence was detected using a triple band-pass filter cube (Leica Microsystems) with the excitation wavelengths/band-pass of 420/30, 495/12, and 570/20 and the emission wavelengths/band-pass of 465/20, 530/30, and 640/40. Separation of the fluorophores was achieved by adjusting the excitation wavelength of the monochromator. For the phalloidin Alexa Fluor 647, a separate filter was used, a Cy5 filter (Chroma) with excitation and emission wavelengths of 620/60 and 700/75. Image stacks were obtained with a step size of 0.25 μm in the z-direction and then deconvolved using the AutoQuant blind deconvolution algorithm. Images were then pseudocolored and prepared in PhotoShop software (Adobe). The live cell experiments were performed using the AS MDW as well, with the difference that the z-stacks obtained had a 0.5-μm step size. For production of the movie, the transfected cells were followed for 1 h with a z-stack obtained every minute and were deconvolved as described previously and composed into a single file.

RESULTS

Expression of v3 in Human Tissues and Cells—As the human v3 transcript has only been identified from rare expressed sequence tag clones and partial PCR amplifications (7, 25), we first aimed to analyze the complete size of the v3 transcript and its expression pattern in different human tissues using Northern blot. For this purpose, we used a v3-specific probe containing the first three exons of the Grx domain, which are unique to this variant (Scheme 1, red). This analysis showed a single transcript of ~4.5 kb in testis, whereas all other analyzed tissues were negative (Fig. 1A). This size fits very well with the expected size of the B1 transcript, encoding the TrxR1 module plus the N-terminal Grx domain. Next, using specific PCR analyses with first-strand cDNA, we also found v3 transcript in samples from ovary, spleen, heart, liver, kidney, and pancreas in addition to that of testis but could not detect the transcript in thymus, prostate, small intestine, colon, leukocyte, brain, placenta, lung, or skeletal muscle (Fig. 1B). The PCR product obtained corresponded to the expected size of 468 bp (Scheme 1), and its specificity was verified by sequencing of the PCR products from
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FIGURE 1. Expression of v3 in testis, in particular the Leydig cells. A, a Northern blot using a v3-specific probe, containing the first three exons of the Grx domain unique to v3, identified a single transcript in testis, whereas all other analyzed tissues were negative. P.B.L., peripheral blood leukocytes. B, specific analysis of first-strand cDNA using the FP9/VIII/R2 primer pair (Scheme 1) showed a PCR product corresponding to 468 bp with the strongest expression in testis and weak expression in heart, liver, kidney, pancreas, spleen, and ovary. Skel. Muscle, skeletal muscle; Sm. Intestine, small intestine; Pos. Ctrl, positive control; Neg. Ctrl, negative control. C and D, immunohistochemical staining of tissue sections from testis, derived from a donor in his mid-40s, are shown. C, a polyclonal antibody, α-v3, generated against a synthetic peptide contained in the unique part in the Grx domain of v3, showed a strong signal in the interstitial Leydig cells. D shows a preabsorption control done with the synthetic peptide used to generate the antibody, diminishing the signal in Leydig cells and illustrating the specificity in the staining pattern. E and F, tests tissue sections taken from the Human Protein Atlas, according to its academic usage permission (see Ref. 13 and www.proteinatlas.org), are shown for comparison. Those tests sections were derived from a 26-year-old male and a 68-year-old male, respectively. α-v3, Western blotting controlled, showing the specificity of the v3 signal with the antibody generated against v3.

FIGURE 2. Expression of TrxR1 splice variant in different cancer cell lines. A, a first-strand reverse transcription-PCR of various cancer cell lines was performed to identify the potential concomitant transcription of different splice variants of TrxR1 (Scheme 1). The β1 variant showed a distinct transcription pattern in some of the cell lines, whereas the α7,α8 transcripts were expressed at a high level in all of the investigated cancer cell lines, and α1,α2 was weakly but consistently detected except in HCC1937 and Jurkat. B, PCR results using cDNA from treated HeLa cells showed an up-regulation of β1 by estradiol or testosterone. Estradiol, estradiol (1 μM); Testost., testosterone (0.1 μM); No FCS, medium without fetal calf serum; HU (10), hydroxyurea (10 μM); HU (100), hydroxyurea (100 μM); Pos. Ctrl., positive control; Neg. Ctrl., negative control. The CDNA of v3 in a plasmid was used as a positive template control.

encoded by exons β, VIII and β, V1 (Scheme 1). Immunohistochemical staining of human testis using these antibodies identified interstitial Leydig cells as the most prominent cell type expressing v3 (Fig. 1C). This should be compared with the staining of Leydig cells, Sertoli cells, and spermatids using a commercial antibody against the TrxR1 module as published by others (see Ref. 13 and www.proteinatlas.org) (Fig. 1, E and F).

We subsequently analyzed the expression of the v3 transcript in human cancer cell lines using the same PCR strategy as described for normal tissue (Scheme 1). Several cancer cell lines were found to express v3, i.e. HCC1937, H23, A549, U1810, and H157 (Fig. 2A). Other cell lines, such as U1285, HeLa, or Jurkat, were negative in the analysis. Interestingly, HeLa cells that normally did not express v3 induced the transcript upon treatment with estradiol or testosterone, whereas other treatment such as serum starvation or hydroxyurea had no obvious effect on the expression (Fig. 2B).

Localization of TXNRD1v3 in Transfected Cells—Human TXNRD1v3 has been shown to co-localize with the estrogen receptors ERα and ERβ in the nucleus of transfected cells in the presence of ligand (29). Because of the effect of estradiol and testosterone on v3 expression (Fig. 2B) and since the last exon of the Grx domain of v3 is shared with a part of TXNRD1v2...
Scheme 1), we analyzed whether v3 would also co-localize with estrogen receptors in transfected cells. As shown in Fig. 3, A and B, we found no evidence of such co-localization in transfected HEK293 cells of v3-GFP fusion proteins with either the ERα (Fig. 3A) or the ERβ (Fig. 3B) nuclear receptors in the presence of ligand. However, performing these experiments, we noted a strikingly dotted appearance of the v3-GFP fusion protein fluorescence (Fig. 3, A and B). We thus analyzed that pattern in further detail, using GFP fusion constructs with the complete v3 protein, the TrxR1 module alone, or the Grx domain of v3 alone, also comparing their respective pattern of localization with that of actin and tubulin. This approach showed that all constructs containing the Grx domain of v3 display a dotted cytosolic appearance as well as membrane-associated fluorescence in the two cell lines used (HEK293 and HeLa) (Fig. 4, A, C, and F). This could not be seen neither with the TrxR1 module alone in fusion with GFP, which showed a more diffuse cytosolic localization (Fig. 4D), nor with only GFP alone, which was used as control and found in both cytosol and nucleus in these cells (Fig. 4, B and E). In a mitotic cell, fluorescence of the GFP-coupled Grx domain of v3 appeared strongly localized to the cell membrane (Fig. 4G). Comparing the localization of the Grx domain of v3 fused to GFP with the staining patterns of actin and tubulin in the same cells, it was clear that the Grx domain of v3 (either when expressed as the complete protein carrying the TrxR1 module or in the form of the isolated Grx domain) closely resembled the staining of actin but without a complete overlap. Comparing the green fluorescence of the v3-derived GFP fusion proteins with the red staining of actin and analyzing the merged figures, it seemed like the green fluorescence was more pronounced at the cell membrane, whereas in the case of the dotted cytosolic staining, the actin-derived staining was more pronounced than the fluorescence of v3, although a clear overlap (yellow in the merged pictures) could be seen (Fig. 4, A, C, F, and G).

**Induction of Cell Membrane Protrusions**—While performing the localization experiments shown in Fig. 4, we noted that the cells transfected with GFP in fusion with the Grx domain of v3 (either alone or followed by the TrxR1 module) not only showed a dotted or membrane-associated pattern of fluorescence but also gained a novel phenotype consisting of a strong tendency to form cell membrane protrusions. Interestingly, these protrusions displayed green fluorescence, suggest-
ing that the Grx domain of v3 is directly involved in driving the formation of these novel structures. Analyzing one of the protrusions in more detail (Fig. 4C, dashed white box, merged picture), we observed that the v3-derived fluorescence appeared to be present in the whole membrane protrusion (Fig. 5A), similarly to what is seen with actin staining (Fig. 5B), whereas tubulin staining was mainly detected at the base of the protrusion (Fig. 5C). The merged figure clearly showed that a smaller protrusion at the side of the major protrusion was only fluorescent for v3 (Fig. 5D). Using computer analyses of a three-dimensional isosurface model from an AS MDW fluorescence microscope, it appeared clear that the v3-derived green fluorescence in fact seemed to “lead” the formation of membrane protrusions, with the red-stained actin following the protrusion and tubulin growing last into the newly formed structure (Fig. 5E). Indeed, we noted that cells transfected with a construct expressing the Grx domain of v3, either alone or in fusion with the TrxR1 module, displayed rapid formation of dynamic cell membrane protrusions. This is further illustrated in a time-lapse movie (see supplemental Movie 1).

**DISCUSSION**

Here we found that the third splice variant of human TrxR1, v3, exhibits a prominent expression in Leydig cells of the testis and is expressed in a few additional tissues including ovary and several cancer cell lines. Its expression in HeLa cells may be induced by treatment with steroid sex hormones. We also found that the Grx domain of v3 seems to induce actin and tubulin polymerization, thus promoting a prominent formation of cell membrane protrusions. These findings suggest that v3 is a uniquely specialized splice variant of TrxR1. This adds further complexity to the human thioredoxin system and gives rise to a number of questions regarding both the molecular mechanisms for regulation of expression of v3 and the potential role of this splice variant for human cell function.

The transcripts for mammalian thioredoxin reductase isoenzymes are known to display significant alternative splicing in the 5' -region, resulting in some cases in alternative N-terminal domains of the encoded protein (7, 8, 12, 25, 26, 29–31). The capacity to induce cell membrane protrusions as found here is, however, the hitherto first demonstrated function of the Grx domain encoded by the v3 transcript. Prior knowledge of the v3 transcript included its expression in testis (7, 25), which was shown here to be predominantly derived from the Leydig cells. It was also shown earlier that the v3 transcript is present in human, chimpanzee, and dog (where the encoded protein was also demonstrated using testis extract), but it is not found in mouse or rat (25). The human v3 protein was also recently demonstrated to be expressed in human mesothelioma cells (28). The encoded Grx domain of v3 lacks classical glutaredoxin activity and, interestingly, it furthermore inhibits the inherent thioredoxin reductase activity of the TrxR1 module to which it is fused (25).

The presently available results regarding v3 suggest that its functions in cells may be highly specialized. Based on the transfection experiments shown herein, the protein was found to catalyze cell membrane restructuring with promotion of actin polymerization. Such properties may reflect a role in certain...
cell type-related growth conditions and could, possibly, be related to cellular transport functions. The role of v3 may also be related to steroid sex hormone signaling considering the strong expression in Leydig cells and its induction in HeLa cells upon steroid treatment. Many questions are yet unanswered, but certain considerations can be made regarding the potential relations between v3 and the membrane protrusions here found to be provoked by this protein. It is well known that the polymerization and functions of actin or tubulin are redox-regulated, and earlier experiments have also demonstrated links to the glutathione and thioredoxin systems, shortly summarized as follows. For more than 15 years, tubulin has been recognized as a protein with reactive sulfhydryl groups, the assembly of which may be regulated by the thioredoxin (32, 33) or glutaredoxin system (34). It was also shown that de-glutathionylation of Cys-374 in actin increases its polymerization potency in relation to epidermal growth factor signaling and that this de-glutathionylation is catalyzed by glutaredoxin (35). This effect was further demonstrated to have a physiological function using small interfering RNA for knockdown of Grx1, which hampered actin polymerization and translocation close to the cell membrane in NIH-3T3 cells (36). It should be noted that those results may be seen as resembling or strengthening the findings presented in our study but that knockdown of Grx1 (36) should presumably not affect v3 expression. These results indicate that different glutaredoxin isoforms may have either redundant function in relation to actin polymerization, or rather highly specified functions in relation to different cell types or growth stimuli. The structural basis for redox regulation of actin was also recently delineated in further detail, showing the importance of both Cys-374 and Cys-272 in actin and paving the way for more detailed studies of actin regulation in relation to redox active proteins (37). It should be emphasized that although we found in this study a prominent capacity of v3 to induce actin polymerization and cell membrane protrusions, the protein is, as already mentioned, inactive in regular glutaredoxin assays (25). It may be possible that it has a restricted substrate specificity interacting only with actin or some actin-associated protein(s). We have tried to measure its activity in vitro with actin polymerization assays, but for now, without findings of any such activity and the molecular basis for the cellular effects reported here, we must therefore await further studies.

It was surprising to us that v3 was expressed in the Leydig cells of the testis. Several unique members of the thioredoxin system are known to be expressed in testis, including TGR (22), Txl-2 (38), and Sptrx-1, -2, and -3 expressed specifically in sperm (39–41). None of these other proteins, however, are being prominently expressed in the Leydig cells (42). What could the function of v3 be in the Leydig cells? It could be noted that testicular macrophages and the Leydig cells seem to be functionally coupled, with the macrophages stimulating testosterone secretion by the Leydig cells and physically interacting by membrane projections from the Leydig cells being located within membrane invaginations of the macrophages, at least in rat testis (43). Specific structures of actin are also involved in the clustering of the Leydig cells around blood vessels (44). Perhaps v3 could be involved in keeping the integrity or forming such structural components required for human Leydig cell function. It is also noteworthy that we found v3 expression to be induced in HeLa cells by testosterone or estradiol, suggesting that v3 could also be involved in pathways related to the testosterone synthesis or secretion by the Leydig cells of the testis or similar sex hormone-related functions in the ovary. It should be noted, however, that v3 did not co-localize with estrogen receptors in the nucleus, in contrast to the second splice isoform of TrxR1, TXNRD1_v2 (29). It should also be noted that, similarly to the earlier study of TXNRD1_v2 (29), we performed those analyses using constructs lacking the selenocysteine residue of the TrxR1 module. These localization studies lead us to the finding that the Grx domain of v3 by itself can promote cell membrane protrusions, thereby evidently being a function not dependent upon the integrity of the selenocysteine residue in the TrxR1 module of v3. It is not possible to exclude, however, that the Grx domain of v3 can interact with endogenous selenocysteine-containing TrxR1 expressed in the transfected cells, which needs to be analyzed in future studies. It is also possible that some naturally expressed transcripts indeed encode the Grx domain of v3 without fusion to the complete TrxR1 module, as may be suggested by many incomplete expressed sequence tag clones found in GenBank. This is another factor that should be analyzed in future studies.

The expression of v3 in a number of cancer cells as found here is of potential interest in relation to actin polymerization, with possible importance for cell mobility or invasiveness, which should also be the focus of future studies. Moreover, since the expression of v3 is regulated by an alternative promoter located upstream of the core promoter, which is otherwise guiding expression of the more common TrxR1 splice variants (7, 31), this alternative promoter should be studied in terms of its transcriptional mechanisms.

To conclude, this study is the most detailed study thus far regarding the tissue-specific expression of the third splice variant of human TrxR1, discovering the highest expression in Leydig cells of the testis and a steroid sex hormone-responsive induction in a cancer cell line otherwise not expressing this splice variant. Ovary and several other cancer cell lines also expressed the transcript, without hormone treatment. This study is also the first to show a function of the Grx domain of this v3 splice variant using transfection experiments, which induced a polymerization of actin and tubulin with formation of cell membrane protrusions. Based upon the findings presented here, we suggest that the v3 splice variant of human TrxR1 is likely to have a highly specialized expression regulation and functional role in specific human cells and tissues.

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