Vitamin D₃–Upregulated Protein-1 (VDUP-1) Regulates Redox-Dependent Vascular Smooth Muscle Cell Proliferation Through Interaction With Thioredoxin

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Abstract—Reactive oxygen species are important cellular signaling molecules, and thioredoxin (TRX) is a key regulator of cellular redox balance. We investigated the interaction of TRX with its endogenous inhibitor, vitamin D₃–upregulated protein (VDUP)-1, in human aortic smooth muscle cells (SMCs). Adenoviral gene transfer of TRX enhanced TRX enzyme activity 2.7±0.4-fold (P<0.05 versus cells infected with adenoviral vector expressing green fluorescent protein [AdGFP]) and resulted in a 3.8±0.5-fold increase of cellular DNA synthesis as detected by methyl-[³H]thymidine incorporation (P<0.001). Platelet-derived growth factor (PDGF) also increased TRX enzyme activity 2.5±3.3-fold (P<0.05 versus no stimulation) and DNA synthesis 6.5±0.3-fold (P<0.001 versus no stimulation) without significant changes in TRX expression. PDGF and H₂O₂ time-dependently suppressed VDUP-1 expression (13-fold and 30-fold reduction after 1 hour, respectively; P<0.001), and this was inhibited by the cell-permeable antioxidants N-acetylcysteine and 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron). Overexpression of VDUP-1 (AdVDUP-1) reduced TRX activity at baseline (−61±23% versus control cells, P<0.05) and abolished PDGF-induced TRX activity (−9±27% in AdVDUP-1–infected cells; P=NS versus control cells). In addition, overexpression of VDUP-1 blocked PDGF-induced DNA synthesis (1.3±0.4-fold increase in AdVDUP-1–infected cells versus 6.5±0.4-fold increase in AdGFP-infected cells, P<0.001). In conclusion, VDUP-1 has marked antiproliferative effects in SMCs through the suppression of TRX activity, suggesting that the regulation of VDUP-1 is a critical molecular switch in the transduction of pro-oxidant mitogenic signals. These data also demonstrate that activation of the reductase TRX plays a pivotal role in the redox-dependent proliferation of SMCs. (Circ Res. 2002;91:689-695.)

Key Words: oxidative stress ■ growth factors ■ smooth muscle cells

Reactive oxygen species (ROS) are important signaling molecules during cellular growth and differentiation, and a number of mitogenic factors, such as angiotensin II (Ang II), thrombin, and platelet-derived growth factor (PDGF), induce ROS generation during their growth-promoting actions. ROS may activate intracellular pathways, including protein kinases such as p38 mitogen-activated protein kinase and Akt. In addition, ROS play a pivotal role in the response of vascular smooth muscle cells (SMCs) after stimulation by PDGF that results in cellular proliferation, including the activation of activator protein (AP)-1, a transcription factor.

The regulation of cellular redox balance is critically determined by the activity of several antioxidant systems. The ubiquitously expressed thiol-reducing systems include the thioredoxin (TRX) system and the glutathione system. The TRX system (TRX, TRX reductase, and NADPH) reduces ROS through an interaction with the redox-active center of TRX and is highly conserved in almost all species from bacteria to higher eukaryotes. TRX functions by the reversible oxidation of two TRX-specific redox-active cysteine residues (-Cys-Gly-Pro-Cys-) to form a disulfide bond that in turn can be reduced by the action of TRX reductase and NADPH. The TRX system protects against H₂O₂ and tumor necrosis factor (TNF)-α–mediated cytotoxicity, in which the generation of ROS is thought to play a crucial role. In vascular SMCs, the involvement of TRX in the lipopolysaccharide-induced and interleukin (IL)-1β–induced increase in DNA-binding activity of the transcription factor AP-1 after nuclear translocation of TRX has been reported, and the importance of oxidative stimuli for this mechanism has been demonstrated.

Recently, the regulation of TRX function by an endogenous inhibitor, vitamin D₃–upregulated protein (VDUP)-1 (also called TRX-binding protein), has been reported. Interestingly, the redox-active site of TRX mediates this interaction, which leads to a reduction in TRX activity, suggesting that the TRX–VDUP-1 interaction may be an important regulatory mechanism of cellular redox processes.
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increased [3H]thymidine incorporation in AdGFP-infected cells (P

0.001). Stimulation with PDGF increased TRX activity in AdGFP-infected SMCs (P

0.01 vs baseline) and AdTRX-infected SMCs (P

0.05, 0.01 vs baseline). Vertical bars represent SEM. *P

0.05, **P

0.01 vs baseline). C, In nonstimulated SMCs, overexpression of TRX increased methyl-[3H]thymidine incorporation compared with that in AdGFP-infected cells (P

0.001). Stimulation with PDGF increased [3H]thymidine incorporation in AdGFP-infected cells (645%, P

<0.001 vs baseline) and in AdTRX-infected cells (51%, P

<0.01 vs baseline). Vertical bars represent SEM. *P

<0.05, **P

<0.01, and ***P

<0.001.

In the present study, we show that TRX overexpression induces robust growth and proliferation of vascular SMCs, a surprising finding because the redox-sensitive cysteine residues of TRX would be anticipated to antagonize mitogenic effects of ROS.10 We explain this paradox by showing that activation of TRX is a critical event during mitogenesis and that this activation occurs at the posttranslational level through redox-controlled regulation of VDUP-1, the endogenous TRX inhibitor.

Materials and Methods

Cell Culture

Human aortic SMCs were isolated from surgical specimens and cultured in DMEM with 10% FCS by following previously described methods.15 For experiments after adenoviral gene transfer of VDUP-1 and TRX as well as green fluorescent protein (GFP, for control cells), the cells were infected with adenoviral vectors at a multiplicity of infection of 200 (200 infectious particles per cell for infection of >99% of the cells) after 24 hours of incubation in DMEM with 1% ITS supplement (Sigma Chemical Co). Efficiency of infection was confirmed by fluoromicroscopic visualization of GFP. In addition, cells were incubated for different time intervals with platelet-derived growth factor (PDGF)-BB (4 ng/mL) and H2O2 (0.1 to 200 μmol/L) in ITS in the presence or absence of a 1-hour pretreatment with N-acytelycysteine (NAC, 10 mmol/mL) or 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron, 10 mmol/mL). Further experiments were performed with the use of thiorubin (3 μmol/L), Ang II (10 mmol/mL), l-α-lysophosphatidic acid (LPA, 5 μmol/L), epidermal growth factor (EGF, 10 ng/mL), TNF-α (10 ng/mL), and IL-1β (50 ng/mL) for the stimulation of SMCs.

Flow Cytometry

Cellular DNA content was assessed as described elsewhere,16 and the percentage of cells with a sub-G1 DNA content was determined as a measure of apoptotic rate of the total cell population.

Northern and Western Analyses

For the detection of mRNA transcripts by Northern analysis, specific cDNA probes were synthesized using the following oligonucleotide: TRX, 5'-AGCAGCATGTTGAAGCAGA-3' and 5'-GCTCCAGAAAAATTTCCACCAC-3'; VDUP-1, 5'-TCTGCCAA-AAAGGAGAAAGAAA-3' and 5'-GGCGTACATAAGATAGG-GCTG-3'; glutathione peroxidase (GPx), 5'-CGAAGTCGACGGGGGCGGCTCTG-3' and 5'-CGCGAGAAGGCAGAGGGG-3'; and glutathione reductase (GR), 5'-CCGACGTTGGTCAGCTGC-3' and 5'-GCTCTCCTGGTGACTTGTTG-3'. Total RNA was isolated, and identical amounts of RNA were loaded to a 1% agarose gel containing formaldehyde. After transfer to membranes, incubation of the membranes with radiolabeled labeled probes was performed, and specific binding after hybridization was visualized with autoradiography.

Protein expression of VDUP-1 was analyzed by Western analysis using a specific polyclonal rabbit anti–VDUP-1 antibody that was affinity-purified from serum after injection of a VDUP-1 peptide. For the detection of adenovirally overexpressed TRX, we used a polyclonal rabbit anti–TRX antibody that was generated by injection of a TRX peptide (GAN KEK LEA SIT EYA). Endogenous levels of TRX in human SMCs were detected using a commercially available monoclonal mouse anti-TRX antibody (MBL). Protein expression of GPx was detected using a monoclonal anti-human GPx antibody (Medical and Biological Laboratories Co). After incubation with horseradish peroxidase–conjugated secondary antibody, specific bands were visualized by enzymatic chemiluminescence reaction (Perkin-Elmer).

Adenoviral Vectors for Gene Transfer

Construction of recombinant replication-deficient adenoviral vectors (AdTRX and AdVDUP-1) was performed using the pAdTrack-CMV system (Stratagene). By polymerase chain reaction, a specific cDNA for full-length TRX (primer, 5'-GAACTTCTACAGAGCTTTAATGGTGAAGCGT-3' and 5'-GCTCTAGATCTTTCAGAAACGAGG-3') and VDUP-1 (primer, 5'-GAACTTCTACAGAGCTTTAATGGTGAAGCGT-3' and 5'-GCTCTAGATCTTTCAGAAACGAGG-3') was generated and cloned into the pAdTrack-CMV shuttle vector that contains a cytomegalovirus (CMV) promoter driving the expression of GFP in tandem with a second CMV promoter for the expression of the gene of interest. After selection by kanamycin and confirmation by sequencing, the clones were linearized and coinfected with the adenoviral plasmid PadEasy-1 in electocompetent BJ5183 cells. After selection of the construct with ampicillin, 293 cells were transfected, and viruses were purified.

TRX Activity Assay

TRX activity was measured using the insulin disulfide reduction assay as described elsewhere.11 Total cellular protein was extracted using lysis buffer, and 50 μg of cellular protein extracts were
incubated at 37°C for 15 minutes with 1 μL of activation buffer in a total volume of 35 μL to reduce TRX. Reaction buffer (20 μL) was then added to the samples. The reaction was started by the addition of 5 μL bovine TRX reductase (American Diagnostica Inc) or 5 μL water to control cells, and the samples were incubated for 20 minutes at 37°C. The reaction was terminated by adding 250 μL of stop mix. Finally, the absorption at 412 nm was measured spectrophotically. TRX activity was compared with standards and expressed as micrograms TRX per milligram total protein.

Methyl-[3H]Thymidine Incorporation

Five hours before termination of the cell culture experiments, methyl-[3H]thymidine (6.7 mCi/mmol, Perkin-Elmer) was added to the cell culture medium (final concentration, 2 μCi/mL). Cells were washed three times with ice-cold PBS (pH 7.4) and harvested in 10% trichloroacetic acid (Sigma) for 45 minutes at 4°C, and precipitates were solubilized in 0.2N NaOH at 37°C. This solution was analyzed in a liquid scintillation chamber for incorporation expressed as counts per minute.

GPx and GR Activity Assay

Total cellular proteins were extracted using a buffer containing 20 mmol/L HEPES (pH 7.9), 300 mmol/L NaCl, 100 mmol/L KCl, 10 mmol/L EDTA, and 0.1% Nonidet P-40, plus protease inhibitors (Sigma). Cellular GPx and GR were determined using assay kits (Sigma) according to the manufacturer’s instructions. Briefly, to assess GPx activity, the cellular lysate was added to a solution containing reduced glutathione, GPx, and NADPH, and the enzyme reaction was initiated by adding tert-butyl hydroperoxide. To assess GR activity, the cellular lysate was added to a solution containing oxidized glutathione and 5,5’-dithiobis(2-nitrobenzoic acid), and the enzyme reaction was initiated by NADPH. The absorbance at 340 nm for GPx or 412 nm for GR was recorded. Activity was expressed as units per milligram protein.

Immunostaining Procedures

For immunocytochemistry, SMCs were plated on coverslips and grown in DMEM with 10% FCS until 70% confluence. Cells were then placed in ITS for 48 hours and stimulated with IL-1β (10 ng/mL), H2O2 (200 μmol/L), or PDGF-BB (4 ng/mL) for 24 hours. Cells were washed three times with PBS and fixed in 4% paraformaldehyde at 4°C overnight. Cells were then permeabilized with 0.1% Triton X in PBS for 10 minutes at room temperature. After two more washes with PBS, a blocking solution containing 10% horse serum in PBS was added. After 1 hour, the cells were washed and incubated with rabbit anti-TRX antibody (1:100 in PBS) overnight at 4°C. The secondary antibody (goat anti-rabbit IgG, conjugated with TRITC) was diluted 1:200 in PBS, and the incubation was carried out at room temperature for 1 hour. Finally, the slides were covered with medium (Vectashield) and coverslips. Positive immunoreactivity was visualized by fluorescence microscopy. Cells with nuclear localization of TRX were microscopically counted and expressed as percentage of the total cell number per field. A minimum of 80 cells per condition was counted.

Statistical Analysis

All experiments were performed at least three times, and data are expressed as mean±SEM. The data were analyzed by Student t test or, for nonparametric distribution, by the Mann-Whitney U test. One-way ANOVA with post hoc analysis was used for the analysis in data sets of >2 groups, with post hoc testing as appropriate for further comparison. A value of P<0.05 was considered statistically significant.

Results

Effects of Adenoviral Gene Transfer of TRX on SMCs

To assess the effects of TRX on cellular DNA synthesis, we overexpressed TRX by adenoviral gene transfer in SMCs. Gene transfer using an adenovirus (AdTRX) that forced expression of both TRX and the expression of reporter GFP enhanced TRX activity 2.7±0.4-fold (P<0.05 versus cells infected with an adenoviral vector that expressed only GFP [AdGFP]), as assessed by an insulin reduction assay.17 Overexpression of TRX in SMCs resulted in a 3.8±0.5-fold increase of cellular DNA synthesis as detected by methyl-[3H]thymidine incorporation (P<0.001) (Figure 1).

Redox-Dependent Regulation of TRX–VDUP-1 Interaction

PDGF, a powerful SMC growth factor that induces growth through ROS generation,6 increased TRX activity 2.5±3.3-fold (P<0.05 versus nonstimulated cells) and methyl-[3H]thymidine incorporation 6.5±3.0-fold (P<0.001) compared with no stimulation (Figure 1). However, no significant
Modulation of TRX Activity and Cellular Growth by VDUP-1

To test this hypothesis, an adenoviral vector for VDUP-1 was constructed that dramatically increased VDUP-1 protein levels and reduced TRX activity by \(-61 \pm 23\%\) compared with AdGFP infection \((P<0.05)\) (Figure 2). In addition, overexpression of VDUP-1 completely blocked the PDGF-induced increase in TRX activity \((-9 \pm 27\%\) in AdVVDUP-1-infected cells \([P=NS\) versus baseline\]) and \(248 \pm 33\%\) in AdGFP-infected cells \([P<0.05\) versus baseline\]; \(P<0.05\) for difference between stimulated AdVVDUP-1-infected and AdGFP-infected cells) (Figure 3).

After establishing that gene transfer of VDUP-1 and TRX had the anticipated effects on gene expression, protein expression, and TRX activity, we assessed specific effects of VDUP-1 on cellular growth. At baseline, cells overexpressing VDUP-1 compared with AdGFP-infected control cells exhibited no significant changes in methyl-[3H]thymidine incorporation (1.1 \(\pm\) 0.1-fold in AdVVDUP-1-infected cells compared with AdGFP-infected cells, \(P=NS\)). However, overexpression of VDUP-1 completely inhibited PDGF-induced growth (1.3 \(\pm\) 0.4-fold increase in AdVVDUP-1-infected cells versus 6.5 \(\pm\) 0.4-fold increase in AdGFP-infected cells after PDGF stimulation, \(P<0.001\)) (Figure 3). In addition, overexpression of VDUP-1 did not induce apoptosis (sub-G1 DNA content, 1.4 \(\pm\) 0.5% in AdVVDUP-1-infected cells versus 2.3 \(\pm\) 2.1% in AdGFP-infected cells; \(P=NS\)). Thus, our findings demonstrate that downregulation of VDUP-1 expression is an essential component of PDGF-induced cellular growth.

To investigate the specificity of VDUP-1 in the regulation of cellular proliferation, we assessed the effects of VDUP-1 on the proliferation induced by other agents that have been shown to induce proliferation of human SMCs. Serum-starved SMCs were stimulated with 10% FCS in DMEM, \(\text{H}_2\text{O}_2\) (1 \(\mu\)mol/L/mL), PDGF (4 ng/mL), thrombin (3 U/mL), Ang II (10 nmol/L/mL), LPA (5 \(\mu\)mol/L/mL), EGF (10 ng/mL), TNF-\(\alpha\) (10 ng/mL), and IL-1\(\beta\) (50 ng/mL). A decrease of VDUP-1 mRNA expression was found in all samples compared with nonstimulated cells. However, two agents (Ang II and EGF) that have previously been shown to involve the upregulation of ROS during their growth-promoting action induced only a weak downregulation of VDUP-1 mRNA after 4 hours of incubation (see Figure 4).

This argues for the involvement of additional mechanisms besides levels of ROS in the regulation of VDUP-1 transcription or a delayed action of these agents on regulation of VDUP-1 mRNA levels.

We used EGF as an agent that only slightly reduces VDUP-1 mRNA levels to induce proliferation of SMCs. Incubation with EGF induced a 2.1-fold increase in [3H]thymidine incorporation after 24 hours in AdGFP-infected cells. Overexpression of VDUP-1 only partially inhibited the EGF-induced increase in
the proliferation of SMCs compared with AdGFP infection (1.6-fold versus 2.1-fold, respectively; Figure 4). This finding supports the assumption of a specific pathway by which VDUP-1 regulates the redox-dependent proliferation of vascular SMCs and the additional signaling cascades that induced proliferation after stimulation with EGF.

We next tested whether overexpression of VDUP-1 affects antioxidant proteins of the glutathione system, another major thiol-reducing system of the cell. Overexpression of VDUP-1 showed no effects on the expression of GPx or GR in SMCs. Using Western analysis, we found no significant differences in protein levels of GPx in cells overexpressing VDUP-1 compared with control cells. In addition, GPx or GR activity was not significantly influenced by the overexpression of VDUP-1 compared with control AdGFP infection. Therefore, we conclude that overexpression of VDUP-1 does not alter GPx or GR expression or protein activity levels (Figure 5).

Inhibition of TRX Nuclear Translocation by VDUP-1
To test the underlying mechanisms for the regulation of cellular growth through VDUP-1, we visualized the cellular distribution of TRX in SMCs by immunofluorescence. In serum-starved (48-hour) quiescent cells, a homogeneous cytoplasmic localization of TRX was found (<10% of the cells showing a nuclear localization of TRX). PDGF and H$_2$O$_2$ as well as IL-1β induced rapid nuclear translocation of TRX as early as 4 hours after exposure (PDGF, 58±4%; H$_2$O$_2$, 85±4%; and IL-1β, 67±8% of the cells with nuclear immunostaining for TRX; P<0.0001 versus nonstimulated cells). Overexpression of VDUP-1 prevented translocation of TRX (PDGF, 16±4%; H$_2$O$_2$, 26±6%; and IL-1β, 19±4% of the cells with nuclear immunostaining for TRX; P<0.001 versus AdGFP-infected cells) (Figure 6). Immunoprecipitation of TRX did not reveal a specific phosphorylation of the molecule upon stimulation, therefore suggesting a different mechanism responsible for regulation of nuclear translocation of TRX (data not shown). Together, these data show that VDUP-1 inhibits the nuclear translocation of TRX. A model is shown in Figure 7.

Discussion
The ubiquitously expressed TRX system participates in the regulation of cellular redox potential and is functionally regulated by the endogenous inhibitor VDUP-1. In the present study, we demonstrate the suppression of VDUP-1 expression in SMCs by PDGF and the regulation of this mechanism by intracellular levels of radical oxygen species. We also demonstrate mitogenic effects of TRX and inhibition of PDGF-induced proliferation of SMCs by overexpression of VDUP-1. These findings reveal a key role for VDUP-1 in redox-mediated control of cell proliferation.

Accumulating evidence indicates the importance of ROS for a wide variety of biological mechanisms. The TRX system participates in the response to oxidative stimuli and cellular proliferation, and TRX is overexpressed in a number of malignancies. TRX is essential for early embryonic development, inasmuch as targeted disruption of the TRX gene leads to early lethality in utero. Recently, independent groups reported a protein-protein interaction between TRX and VDUP-1 and selective inhibition of TRX function by VDUP-1. The TRX–VDUP-1 interaction is mediated by the thiol groups of the TRX protein and is lost when the cysteine residues at positions 32 and 35 are substituted by serines. In addition, these cysteine residues are essential for interaction with redox factor (Ref)-1, leading to the activation of AP-1 and the binding of apoptosis signal-regulating kinase-1 to TRX. Previous studies investigating the functional effects of the TRX–VDUP-1 interaction have shown that overexpression of VDUP-1 rendered the cells more vulnerable to oxidative stress and apoptosis. These findings show that VDUP-1 serves as an endogenous inhibitor of TRX function.

The expression of VDUP-1 in SMCs depended on intracellular oxidative stress. An increase in oxidative stress either mediated through the intracellular pathways of PDGF signaling or directly by incubation with H$_2$O$_2$ resulted in the downregulation of VDUP-1 mRNA and protein expression. To clarify the role of ROS in this
process, cells were pretreated with the cell-permeable antioxidants NAC or Tiron. NAC is a thiol-based antioxidative agent that directly scavenges ROS and also increases the intracellular levels of reduced glutathione, a hydroxy radical scavenger and a substrate of GPx that directly degrades H₂O₂. Tiron is a cell superoxide scavenger that is directly oxidized by superoxide radicals. Pretreatment of the cells with these agents led to an inhibition of VDUP-1 downregulation after PDGF stimulation, further indicating the importance of ROS for the expression of VDUP-1.

In agreement with previous reports on the regulation of TRX expression in response to oxidative stress, we did not find increased expression of TRX in SMCs after pretreatment with H₂O₂ or PDGF, indicating that the expression of TRX in SMCs is not regulated by ROS. In contrast, PDGF and H₂O₂ reduced the expression of VDUP-1 in SMCs, and this was associated with increased TRX activity. This effect was completely blocked by overexpression of VDUP-1. In addition, the reducing activity of TRX at baseline was inhibited in AdVDUP-1–infected SMCs. Therefore, our findings show the redox-dependent regulation of TRX activity in SMCs through VDUP-1, the endogenous inhibitor of TRX function.

Overexpression of TRX resulted in increased proliferation of SMCs, in agreement with reports on growth-promoting effects of TRX as well as increased TRX expression in several malignomas. At first glance, the growth-promoting effects of the reductase TRX seem counterintuitive given the well-established but incompletely understood promitogenic effects of ROS. This paradox can now be explained by the posttranslational modulation of TRX activity through the redox-dependent downregulation of VDUP-1. By suppressing VDUP-1 expression, growth factors and ROS-signaling events activate TRX and trigger cell growth. Therefore, the redox-dependent regulation of VDUP-1 by mitogenic factors through ROS and the specific binding of VDUP-1 to the redox-sensitive cysteine-sulfide center of TRX modulate intracellular levels of ROS and the mitogenic activity of TRX.

Our results show that overexpression of VDUP-1 inhibits the nuclear translocation of TRX and blocks the PDGF-induced increase in cellular proliferation. This may be important for the effects of TRX–VDUP-1 interaction inasmuch as a nuclear interaction of TRX with several transcription factors, such as nuclear factor (NF)-κB, Ref-1, and AP-1, has been described. By reduction of its redox-active half-cysteine residues, TRX binds to the transcription activator Ref-1, which increases the DNA-binding activity of AP-1. The transcriptional activation of AP-1 is critically involved in PDGF-induced intracellular signaling cascades, leading to an increased proliferation of SMCs. On the other hand, the interaction of TRX with VDUP-1 increases the levels of free oxygen species known to be growth-promoting at low concentrations but to be proapoptotic at higher concentrations, which also may mediate the antiproliferative effects of VDUP-1.

In Figure 5, we show the effects of VDUP-1 on GPx and GR. Overexpression of VDUP-1 showed no significant effects on GPx or GR mRNA expression. Protein levels of GPx did not change in AdVDUP-1–infected cells compared with AdGFP-infected cells. GPx and GR activity showed no significant differences between the 2 groups.

Figure 6. Inhibition of nuclear translocation of TRX by VDUP-1. Green fluorescence indicates adenovirus-mediated expression of the reporter gene GFP. Red fluorescence indicates positive immunoreactivity for TRX after incubation with rabbit anti-human TRX antibody visualized by binding of TRITC-conjugated mouse anti-rabbit secondary antibody (×40). Serum-starved nonstimulated SMCs (Con) show cytoplasmic localization of TRX (top). In SMCs infected with AdGFP (controls, left), 4 hours of incubation with H₂O₂ or PDGF induced nuclear translocation of TRX. Adenovirus-mediated overexpression of VDUP-1 inhibited the translocation of TRX in response to these stimuli (right).
Figure 7. Model figure. Increased levels of ROS generated by receptor-mediated processes or internal ROS-generating mechanisms suppress the expression of VDUP-1. In addition, binding of VDUP-1 to TRX is regulated by internal levels of ROS. Upon stimulation, TRX translocates to the nucleus and modulates redox-dependent transcriptional activity through interaction with Ref-1 and NF-κB. Rec. indicates receptor.

In conclusion, we describe a new regulatory mechanism by which vascular SMCs regulate levels of oxidative stress and redox-dependent proliferation. The suppression of VDUP-1 expression results in an increase of TRX activity, a mechanism that plays a key role in the endogenous regulation of the cellular redox state and mitogenic activity. In addition, we demonstrate that the proliferative effects of PDGF involve an increase in TRX function and are mediated by a downregulation of VDUP-1 expression.

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