The protein-disulfide reductase thioredoxin is critical for redox signaling during apoptosis and growth. In this study, we demonstrate that vitamin D₃-up-regulated protein-1 regulates thioredoxin in conditions of biomechanical or oxidative stress and critically regulates cardiomyocyte viability. Expression of vitamin D₃-up-regulated protein-1 but not of thioredoxin in rat cardiomyocytes was rapidly suppressed by biomechanical strain or hydrogen peroxide at both mRNA and protein levels. Mechanical suppression of vitamin D₃-up-regulated protein-1 gene expression was blocked by N-acetylcysteine. The half-life of vitamin D₃-up-regulated protein-1 transcripts in cardiomyocytes was only 1.1 h and remained unchanged after mechanical stimulation, suggesting that rapid responses in vitamin D₃-up-regulated protein-1 gene expression occur through transcriptional control. Vitamin D₃-up-regulated protein-1 down-regulation by strain or hydrogen peroxide led to increased thioredoxin activity, whereas adenovirus-mediated overexpression of vitamin D₃-up-regulated protein-1 suppressed thioredoxin activity. Overexpression of vitamin D₃-up-regulated protein-1 but not of thioredoxin induced cardiomyocyte apoptosis. Furthermore, overexpression of vitamin D₃-up-regulated protein-1 sensitized cells to hydrogen peroxide-induced apoptosis, whereas overexpression of thioredoxin protected against injury. These data identify vitamin D₃-up-regulated protein-1 as a key stress-responsive inhibitory switch of thioredoxin activity in cardiomyocytes and demonstrate that the vitamin D₃-up-regulated protein-1/thioredoxin axis has an important role in the preservation of cellular viability.

Biomechanical strain, hypoxia, and other types of stress induce hypertrophy, apoptosis, contractile failure, and other myocardial changes that directly or indirectly predispose to cardiac failure. The molecular pathways responsible for these changes are only partly understood, but oxidative reactions, either by injurious levels of reactive oxygen species (ROS)

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Vitamin D₃-up-regulated Protein-1 Is a Stress-responsive Gene That Regulates Cardiomyocyte Viability through Interaction with Thioredoxin*

Received for publication, March 4, 2002, and in revised form, May 6, 2002
Published, JBC Papers in Press, May 14, 2002, DOI 10.1074/jbc.M202133200

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This work was supported by Grants HL64858 (to R. T. L.) and HL67554 (to Y. W.) from the NHLBI, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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* This work was supported by Grants HL64858 (to R. T. L.) and HL67554 (to Y. W.) from the NHLBI, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: ROS, reactive oxygen species; TRX, thioredoxin; VDUP1, vitamin D₃-up-regulated protein; NRVM, neonatal rat ventricular myocytes; GFP, green fluorescent protein; MOI, multiplicity of infection; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling; FACS, fluorescence-activated cell sorter.

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for 15 min with 1
membrane. Western analysis was performed using polyclonal antisera
on SDS-PAGE and electrotransferred to a polyvinylidene difluoride
precipitation assay buffer. An equal amount of protein was separated
strain at 1 Hz for varied periods of times and lysed in radioimmune
(91
against TRX(91
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VDUP1 mRNA by strain (2.5 h, 8%, 1 Hz) was completely inhibited.

To further characterize stress-induced VDUP1 mRNA down-regulation in cardiomyocytes, we measured VDUP1 mRNA half-life. NRVM were exposed to 0 or 8% strain for 3 h and then incubated with actinomycin D (5 μg/ml) to inhibit transcriptional activity. The half-life of VDUP1 mRNA was only 1.1 ± 0.1 h and remained unaltered after strain (0.9 ± 0.2 h). These experiments suggest that biomechanical strain regulates VDUP1 by suppressing synthesis of VDUP1 mRNA and that inhibitory transcriptional events induced by strain participate in the regulation of this redox-regulatory switch.

Effect of Biomechanical Strain and Hydrogen Peroxide on TRX Activity in Cardiomyocytes—We demonstrated that either biomechanical strain or oxidative stress suppressed expression of the endogenous TRX inhibitor, VDUP1. After exposures to biomechanical strain or hydrogen peroxide, TRX activity was consistently increased. Using insulin-reducing assays, mechanical strain (1 Hz, 8%, 24 h) increased TRX activity by 37 ± 4% and hydrogen peroxide (50 μM, 24 h) by 56 ± 6% (Fig. 2, p < 0.05, n = 3 independent experiments).
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Effects of VDUP1 on TRX-reducing Activity—To explore further the functional interaction between VDUP1 and thioredoxin, we prepared replication-defective adenovirus constructs expressing wild type VDUP1 or TRX. Transduction of cardiomyocytes with VDUP-Ad resulted in a 7-fold increase in VDUP1 protein (compared with control GFP-Ad-infected cells) but did not change TRX protein expression (Fig. 3A). On the other hand, transduction of cardiomyocytes with TRX-Ad resulted in a 2.5-fold increase in TRX protein expression (compared with control GFP-Ad-infected cells) but did not change VDUP1 expression (Fig. 3A).

To determine whether VDUP1 affects TRX activity, TRX-specific insulin-reducing assays were performed. As shown in Fig. 3B, overexpression of VDUP1 suppressed TRX activity by 43% (data are representative of three separate experiments, p < 0.01), whereas overexpression of TRX increased TRX activity by 144% (data are representative of three separate experiments, p < 0.01). These data confirmed that gene transfer of VDUP1 or TRX affects TRX activity as anticipated.

Role of the VDUP1/TRX Pathway in Preservation of Cardiomyocyte Viability—Primary cardiomyocytes transduced with VDUP-Ad or TRX-Ad were serum-starved for 2 days, and apoptosis was evaluated by measurements of DNA content with FACS analysis and TUNEL assays. Strikingly, overexpression of VDUP1 induced apoptosis compared with cells transduced with control GFP-Ad (TUNEL assays: from 11.5 ± 1.4 to 18.5 ± 1.8%, n = 3, p < 0.05; sub-G1: from 12.2 ± 0.7 to 19.6 ± 0.6%, n = 3, p < 0.05). Overexpression of TRX, however, had no effect on apoptosis (TUNEL assays: 10.4 ± 1.4%; sub-G1: 11.6 ± 1.0% (Figs. 4 and 5).

When cells were exposed to hydrogen peroxide for 24 h, the pro-apoptotic effects of VDUP1 were even more pronounced, and protective effects of TRX became evident. TUNEL assay showed that hydrogen peroxide dose-dependently increased the proportion of apoptotic cells transduced with the GFP-Ad from 11.5 ± 1.4 to 26.7 ± 1.3 and 47.8 ± 1.6% and from 18.5 ± 1.8 to 45.1 ± 3.3 and 60.7 ± 2.8% in cells transduced with VDUP-Ad (data are representative of three separate experiments that were performed in triplicate, p < 0.05), whereas overexpression of TRX inhibited hydrogen peroxide-induced cardiomyocyte apoptosis (Fig. 4B). Similar results were obtained when apoptosis was quantified with FACS analysis (Fig. 5).

DISCUSSION

In the present study, we focused on VDUP1, an intrinsic TRX inhibitory protein, as a stress-responsive gene in cardiomyocytes that regulates cell viability. We found that biomechanical strain and oxidative stress rapidly down-regulated VDUP1 expression at mRNA and protein levels. Given the known interactions between VDUP1 and TRX (9–11), these observations argued for a role of VDUP1 as a stress-responsive regulatory switch of TRX in cardiomyocytes. Suppression of VDUP1 expression allowed strain and oxidative stress to induce TRX activity at unchanged levels of TRX protein. Importantly, overexpression of VDUP1 inhibited TRX activity and induced spontaneous cardiomyocyte apoptosis. Furthermore, in conditions of oxidative stress, forced expression of VDUP1 induced apoptosis. These findings demonstrate that VDUP1 is a pivotal gene in the control of a TRX-dependent survival pathway in cardiomyocytes.

Expression of VDUP1 was suppressed by hydrogen peroxide and its mechanical down-regulation inhibited by antioxidants. These observations suggest that VDUP1, in addition to regulating cellular redox state through TRX, acts as a sensor of reactive oxygen species and as a critical mediator between environmental stress and adaptive cellular responses. Consistent with previous reports (9–11), overexpression of VDUP1 inhibited TRX activity. TRX is a major ubiquitous disulfide reductase that controls rapid thiol-disulfide exchange reactions of structural or catalytic SH groups of many proteins. TRX has many biological activities, including protection against oxidative stress and inhibition of apoptosis (7, 8). In this study, inhibition of endogenous TRX by VDUP1 induced apoptosis even in the absence of imposed oxidative stress. TRX is thus more than a defense system in cardiomyocytes against exogenous oxidative stress and may be a critical regulator of cell viability in non-oxidative conditions. These conditions likely include biomechanical overload, as suggested by our observation that mechanical deformation induced VDUP1 down-regulation and activation of TRX. Intriguingly, mechanical deformation of cardiomyocytes has been reported previously to induce functionally relevant changes in the cardiac muscle redox state (12, 16).

The mechanisms through which TRX acts as an anti-apoptotic regulator are still under investigation, but recent reports have shown that TRX inhibits ASK-1, a mammalian mitogen-activated protein kinase kinase kinase that delivers apoptotic signals by activating c-Jun NH2-terminal kinase and p38 pathways (11, 17). VDUP1 competes with ASK-1 to bind to TRX (11). TRX may, however, promote survival by other mechanisms, including the modulation of nuclear factor κB activity, a stress-related survival-related transcription factor in cardiomyocytes and other cell types (18, 19).

These data suggest that the regulation of VDUP1 may participate in protection against apoptosis from mechanical overload or oxidative stress. It is important to consider this protective mechanism within the context of potential pro-apoptotic effects of mechanical strain and oxidative stress. We and others have found that there are large deformations (25% or greater) that can promote apoptosis (16, 20). However, we have found this pro-apoptotic effect only in association with obvious partial cell detachment from the culture substrate; we have not observed an increase in cardiac myocyte apoptosis by any measure with the smaller deformations that regulate gene expression that were used in this study. In addition, our data suggest that overexpression of TRX and an increase in TRX activity can partially protect against oxidative stress, but this protection is not complete. Thus, we would anticipate that even with complete absence of VDUP1, extreme conditions of oxidative stress could exceed the beneficial effects of increased TRX activity.

This study argues for a role of the VDUP1/TRX pathway in myocardial remodeling, and previous reports further suggest a role of TRX in cardiac disease (21–23). Serum levels or local expression of TRX are enhanced in heart failure and myocarditis (22, 23), and TRX prevents reperfusion-induced arrhythmias (21). The present study demonstrates that the earliest regulatory event in the recruitment of TRX may be stress-induced down-regulation of its inhibitory partner VDUP1 rather than increased expression of TRX. Delayed or deficient down-regulation of VDUP1 may result in incomplete adaptation in the early phases of cardiac disease and further alter disease progression.

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