



Elucidation of Thioredoxin Targeted Protein Networks in Mouse Heart

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Abstract

Thioredoxin 1 (Trx1) is a key redox modulator that is functionally conserved across a wide range of species. Using a conserved CXXC motif, it catalyzes the reduction of cysteine disulfides and S-nitrosothiols. In contrast to small molecular reductants such as glutathione and cysteine that can reduce a wide range of oxidized proteins, Trx1 reduces only selected proteins via specific protein-protein interaction. Trx1 has been shown to regulate a wide range of signal transduction pathways, and its dysfunctions have been implicated in several diseases, including cancer and cardiovascular diseases. Identification of Trx1 target proteins may help to identify novel signaling mechanisms that are important for Trx1 anti-stress responses. In this study, we performed an isotope-coded affinity tag (ICAT) proteomic study for the identification of Trx1 target proteins from the hearts of a cardiac-specific Trx1-overexpressing transgenic mouse model (Tg-Trx1). Trx1-reduced proteins were distinguished from Trx1-induced proteins by comparison of the ICAT results with those obtained using a parallel isobaric tags for relative and absolute quantitation (iTRAQ) protein expression analysis. We were able to identify 78 putative Trx1 reductive sites in 55 proteins. Our data suggests that Trx1 may be involved in the coordination of a wide array of cellular functions for maintaining proper cardiac energy dynamics and facilitating muscle contraction.

Introduction

> Implication of oxidative imbalance in diseases including cancer, HIV infection, neurodegenerative diseases and cardiovascular diseases.

> Cardioprotective functions of Trx1

- 1) Regulation of gene expression, translation
- 2) Regulation of redox post-translational modifications (PTMs)

> Redox proteomics

- 1) Gauge the redox state of protein cysteines by ICAT method
 - 2) Evaluate potential Trx1 induced protein expression variation by iTRAQ method
- > Validation of putative reduction target of Trx1 by alternative method
- 1) Potential conserved motif in Trx1 reduction targets

> Trx1 reductive protein network and cardiac energetic pathway

Methods

Mice with cardiac-specific overexpression of Trx1 (Tg-Trx1) were generated on a FVB background, using the α -myosin heavy chain promoter. Induction of cardiac hypertrophy was accomplished by surgical constriction of the transverse thoracic aorta on both the control and Tg-Trx1 mice.

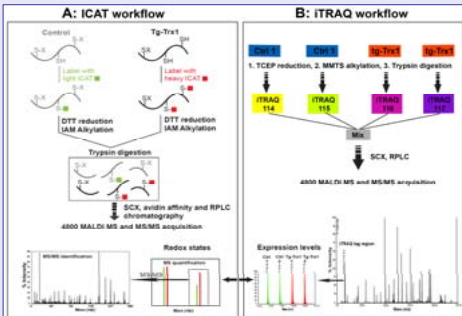


Fig. 1 Illustration of quantitative proteomic workflow. Protein lysates (control and Tg-Trx1) from mice heart left ventricle were labeled with light and heavy ICAT reagents, respectively and were proceeded to tryptic digestion. After a series of chromatography fractionation and enrichment, the RPLC-purified peptides were analyzed on a 4800 MALDI-TOF/TOF mass spectrometer (ABI). Peptide identification was performed with MASCOT (v 1.9) integrated in the GPS explorer software, against a non-redundant Swiss-prot database

Results

Fig. 2 Cys53 of DJ-1 is a putative redox regulated target of Trx1. Trx1 restored ~60% of the free thiols in the DJ-1 peptide 49 DVVICPDTSLSEDAK 62 compared to the control mouse heart (Fig. 2A, insert). A mass difference of 339.1 Da between the y9 and y10 ions corresponded to the addition of a cleaved heavy ICAT label to Cys53. To confirm that the reduction of this specific cysteine was Trx1-dependent, we simulated oxidative stress by treating recombinant human DJ-1 protein with mild H_2O_2 (200 μ M) for 30 min, followed by removal of excess H_2O_2 with catalase (at a final concentration of 0.1 μ g/ml). The oxidized DJ-1 samples were then incubated with either the activated Trx1/TrxR/NADPH system or blank reaction buffer and subjected to ICAT labeling. We observed only trace levels of available protein thiols labeled with the light ICAT reagent, however the Trx1/TrxR/NADPH system reduced a significant amount of the oxidized Cys53 in peptide 49 DVVICPDTSLSEDAK 62 (Fig. 2B, insert). Although the tryptic peptide sequences are slightly different between mouse and human DJ-1, Cys53 is highly conserved in both species. Both *in vivo* and *in vitro* experiments confirmed that Cys53 is a redox-sensitive cysteine, and its redox state can be regulated by the Trx1 reduction system.

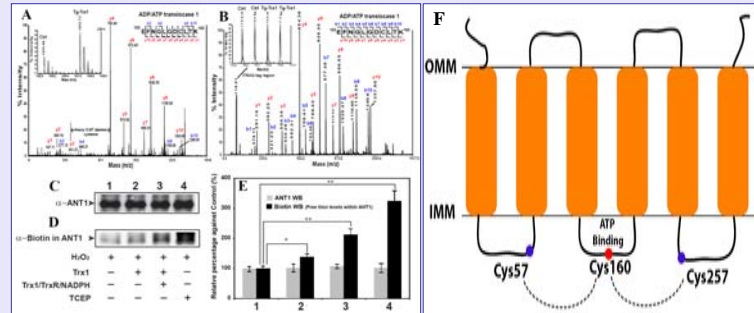
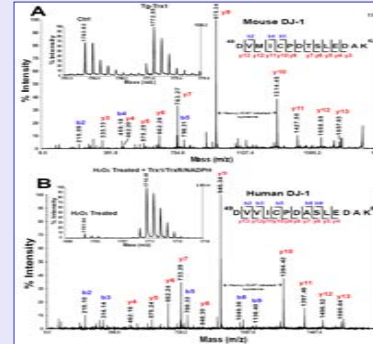


Fig. 3 Cys160 of ANT1 protein is redox regulated target of Trx1 and critical for ANT1 function. Oxidation of Cys160 in mouse ANT1, within the binding site of ADP, has been shown to be detrimental to MPTP function and ATP transport. Although ANT1 expression levels were comparable for all animals, Cys160 was found to be more reduced in Tg-Trx1 than wild type hearts (Tg-Trx1/control ratio of 2.5, Fig. 3A and B). We also found that ANT1 was oxidized by H_2O_2 (Fig. 3D, lane 1), and plain Trx1 was able to partially reverse the oxidation of free thiols (Fig. 3C and D, lane 2). The reduction effect was more pronounced when TrxR and NADPH were included for Trx1 regeneration (Fig. 3C and D, lane 3), suggesting that ANT1 could be a direct target of Trx1 reduction. More importantly, Cys160 was likely to be a reactive cysteine within ANT1 that was reduced by Trx1, given that TCEP was more thorough at potentially reducing all oxidized cysteine thiols non-specifically in ANT1 than Trx1 (Fig. 3C and D, lane 4). The formation of intramolecular disulfide bonds between Cys160 and Cys57/Cys257 has been the cause of ANT conformation alteration (Fig. 3F), leading to leakage of apoptosis factors such Cytochrome C from mitochondria.

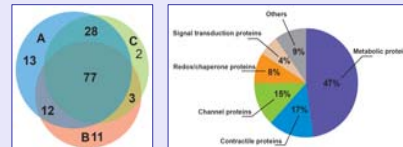


Fig. 4 Venn Diagram of protein ID in three independent ICAT experiments (A, B and C) and functional analysis of putative Trx1 reduction targets. The ICAT results are consistent with high level of overlapping of protein ID and quantitation (left). GO functional analysis reveals that a wide array of functional proteins are under redox-regulation of Trx1 (Right).

Fig. 5 Consensus motifs in Trx1 reductive targets. We have examined all the putative Trx1 target sequences found in this study for possible presence of consensus sequence motifs. Interestingly, we discovered 2 potential CXXC motifs (where either one or both cysteines could be reduced by Trx1) resembling the catalytic site of Trx1. We postulate that the similarity in the peptides sequence may facilitate the recognition and reduction reaction upon Trx1-mediated regulation. (motif-x <http://motif-x.med.harvard.edu/>)

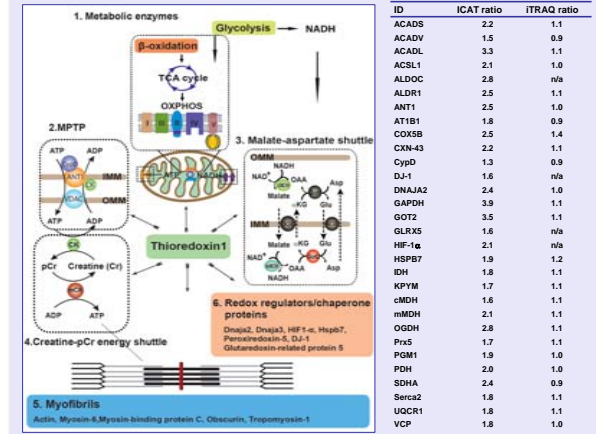
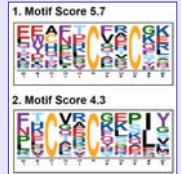


Fig. 6 Trx1 redox-regulated cardiac energetic network. Trx1 reduces metabolic proteins involved in glycolysis, β -oxidation, TCA cycle and oxidative phosphorylation, ensuring sustainable production of ATP for muscle contraction. Trx1 also regulates MPTP, creatine-phosphocreatine energy shuttle and malate-aspartate shuttle for both metabolite and ATP conduction among coordinated protein networks, ensuring the delivery of high energy phosphates to heart muscle. A large number of myofibril components are reduced by Trx1 supporting the contraction ability of the cardiac muscle. Trx1 also modulates a wide variety of redox regulator/chaperone proteins such as Dnaj2, Dnaj3, Hspb7, Pdx5, DJ-1 and glutaredoxin-related protein 5, coordinating cellular anti-stress response. Proteins colored in grey were not found in our ICAT results, they were depicted for enhancing the clarity of the diagram.

Conclusions

In the present study, we performed a proteomic identification of Trx1 reduction target proteins from the hearts of cardiac-specific Tg-Trx1 mice. Using the comparative analysis of both iTRAQ and ICAT results, we were able to reveal many putative Trx1 reduction substrates, several of which were previously unknown. We identified several protein networks whose functions may be regulated by Trx1; including the creatine-phosphocreatine shuttle, the MPTP complex and the sarcomeric contractile apparatus. The results presented here suggest that in addition to its antioxidant function, Trx1 may be involved in the coordination of a wide array of cellular signaling pathways to maintain cardiac function.

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