



# MS Identification of A Redox-Dependent Pathway for Regulating Histone Deacetylase in Cardiac Myocytes

Hong Li \*, Tong Liu, Tetsuro Ago, Wei Chen, Junichi Sadoshima

Center for Advanced Proteomics Research, UMDNJ, Newark, NJ 07103 USA

(http://njms.umdj.edu/proweb)

## Abstract

Thioredoxin 1 (Trx1) facilitates the reduction of signaling molecules and transcription factors via a cysteine thiol-disulfide exchange mechanism, thereby regulating cell signaling and apoptosis. We used tandem MS to delineate the molecular mechanism by which Trx1 regulates gene expression in cardiac hypertrophy. Using MALDI-TOF/TOF MS, we found that both Cys-274/Cys-276 in DnaJb5 (a chaperon) and Cys-667/Cys-669 in HDAC4 are oxidized and form intramolecular disulfide bonds in response to reactive oxygen species (ROS)-generating hypertrophic stimuli; whereas they are reduced by Trx1. The reduction of Cys-274/Cys-276 in DnaJb5 is essential for maintaining the interaction between DnaJb5 and HDAC4; and the reduction of Cys-667/Cys-669 in HDAC4 inhibits its nuclear export. This redox proteomics study reveals a novel regulatory mechanism of class II HDACs via their redox modifications in a Trx1-sensitive manner.

## Introduction

Reduction and oxidation (redox) are important post-translational modifications for modulating protein functions. The redox of specific cysteine residues often induce conformational changes in protein molecules, thereby regulating enzymatic activities, protein-protein interactions, and subcellular localizations. ROS and antioxidant proteins critically affects the function of the heart. Both oxidative and reductive stress have been implicated in the pathogenesis of cardiac hypertrophy (enlargement) and heart failure.

Trx1 is a 12 kD protein that regulates signaling molecules and transcription factors and realted gene expression. During reduction of target proteins, Trx1 is oxidized to form a disulfide bond between cysteine residues 32 and 35 in its catalytic core. The oxidized Trx1 can be reduced and regenerated by a thioredoxin reductase and NADPH. Trx1, Trx reductase, and NADPH are collectively called the Trx system, operating as a powerful protein disulfide reduction system. Class II HDACs are expressed prominently in non-proliferative cells, including myocytes. In the heart, the nuclear export of class II HDACs directly elicits the activation of nuclear factor of activated T cell (NFAT) and myocyte enhancer factor 2 (MEF2), master positive regulators of cardiac hypertrophy. Using MALDI-TOF/TOF MS analyses, we have demonstrated that Trx1 regulates class II HDACs through a redox-dependent mechanism. These results may provide a new insight into the mechanism by which redox pathways regulate the development of cardiac hypertrophy.

## Methods

For the *in vitro* studies, purified GST-DnaJb5 and GST-HDAC domain of HDAC4 were treated with TCEP, H<sub>2</sub>O<sub>2</sub> or Trx1. For *in vivo* analysis, lysates of myocytes overexpressing either DnaJb5 or HDAC4 were subjected to immunoprecipitation and separation by SDS-PAGE. The protein bands were digested with either trypsin or Glu-C. The resulting peptides were analyzed on an ABI 4700 Proteomics Analyzer MALDI-TOF/TOF MS. Positive ion mass spectra were acquired in the reflectron mode. Tandem mass spectra of selected ions were acquired with 1 KV collision energy. Data analysis to determine peptide cysteine-thiol redox status was performed with Data Explorer software.

## Results

### Identification of redox-sensitive cysteines in DnaJb5 (Cys-274/276) and HDAC4 (Cys-667/669)

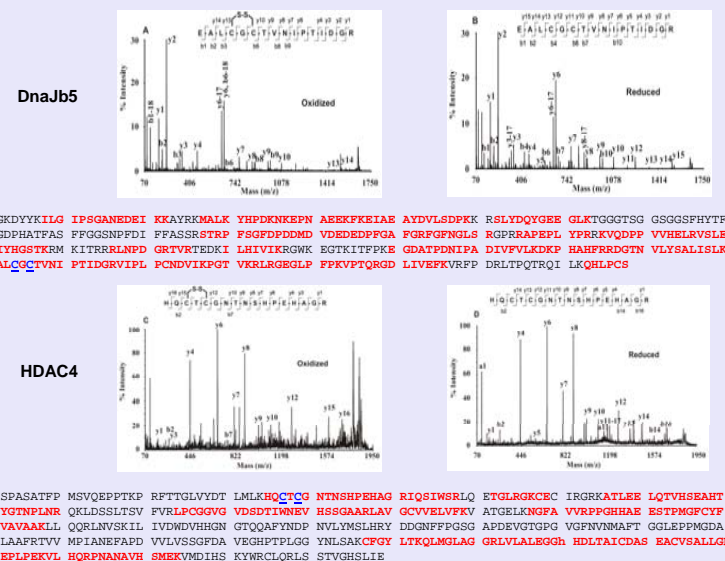


Figure 1. The MS and MS/MS spectra of peptide [271–286] derived from DnaJb5 and peptide [665–681] derived from HDAC4. The peptides were generated from the digestions of the proteins under nonreducing conditions. They each contained a disulfide bond (A and C). The peptides could be reduced by TCEP (B and D).

### DnaJb5 and HDAC4 are reduced by Trx1 but not inactive Trx1, affecting their interactions

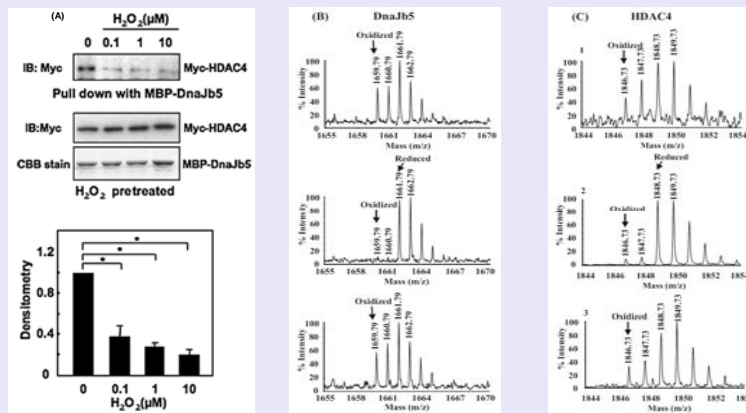


Figure 2. Redox-regulate interaction between DnaJb5 and HDAC4. (A) COS7 lysates with HDAC4 and MBP-DnaJb5 overexpression were treated with the indicated concentration of H<sub>2</sub>O<sub>2</sub> for 30 min and subjected to pull-down assays. MS spectra of DnaJb5 peptide [271–286] (B) and HDAC4 peptide [665–681] (C) incubated with either the reaction buffer alone (1), Trx1 (2), or C32/35S-Trx1 (3). Both peptides were reduced by Trx1, compared with either buffer alone or C32/35S-Trx.

### ROS and Trx1 regulations of DnaJb5 redox-sensitive cysteines *in vivo*

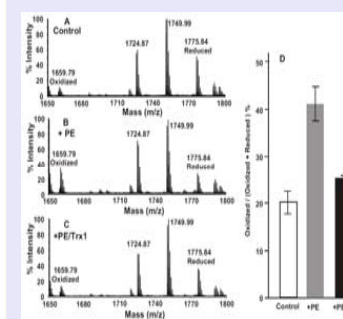


Figure 3. Myocytes transduced with HA-tagged DnaJb5 were treated with PE in the presence or absence of Trx1 overexpression (A) MS spectrum of both oxidized and reduced [271–286] at baseline; (B) after PE treatment and (C) after PE treatment in the presence of excess Trx1. (D) Relative % of the oxidized peptide (m/z 1659.79) to the sum of both the oxidized and IAM-alkylated & reduced peptide (m/z 1775.84).

### DnaJb5 Cys-274/276 are crucial for binding to HDAC4 & nuclear localization

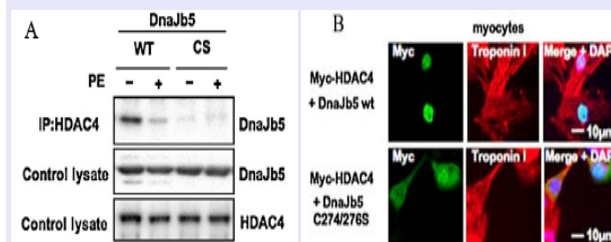
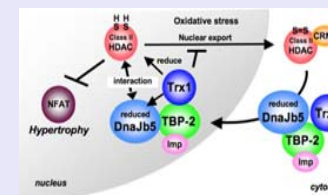


Figure 4. Cys-274/276 of DnaJb5 affects its interaction with HDAC4 and HDAC4 localization. (A) Effects of PE treatment on the interaction between wild-type DnaJb5 and HDAC4 or between DnaJb5 C274/276S mutant and HDAC4 were examined by immunoprecipitation assays. (B) Cys-274/276 of DnaJb5 are important for nuclear localization of HDAC4. Myocytes were stained with a myc antibody (green), a troponin I antibody (red), and DAPI (blue).

## Conclusions



HDAC4 suppresses positive mediators of cardiac hypertrophy, such as NFAT, in the nucleus when in a reduced state. During oxidative stress, HDAC4 is promptly oxidized and exported to the cytosol, where it can no longer suppress positive mediators of hypertrophy. Reduced DnaJb5, upregulated by Trx1, interacts with HDAC4. Trx1 reduces critical cysteines in HDAC4 and DnaJb5 by forming a multiprotein complex, thereby returning HDAC4 to the nucleus.

## Acknowledgement

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