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DEFENSE OF THE DOCTORAL

DISSERTATION

"The role of Trx1 in S-nitrosylation of cardiac proteins"

by

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Abstract

Thioredoxin1 (Trx1) is a 12kDa cytosolic antioxidant that participates in redox reactions to reduce or oxidize other protein molecules through formation of disulfide bridges at Cys32 and Cys35. Other studies have shown that Trx1 can be nitrosylated at Cys69 and/or Cys73 and have also elaborated on Trx1's transnitrosylase and denitrosylase activities. These findings suggest that Trx1, in addition to its oxidoreductase activity, can function as a transfer molecule for NO. We identified that Trx1 is S-nitrosylated at Cys73 in the heart. Furthermore, Cys73 of Trx1 mediated cardioprotection during myocardial ischemia by regulating autophagosome formation. Using the Trx1 trapping mutant (Trx1Cys35Ser) mice, we identified that Trx1 interacts with Atg7, a key autophagosome protein. Atg7 is an E1 ubiquitin-like enzyme that binds LC3 and Atg12 to activate them and thus stimulate autophagy. Mechanistically, Trx1 formed disulfide linkages with Cys545 and Cys548 of Atg7. This interaction was significantly enhanced during oxidative stress conditions, such as treatment with H_2O_2 or myocardial ischemia. Also, we discovered that Atg7 was S-nitrosylated at Cys294, Cys354, Cys364, and Cys402 in response to 30 minutes of ischemia or glucose deprivation conditions and that Trx1 was essential for ischemia-induced S-nitrosylation of Atg7. To the contrary, Trx1 Cys73Ser was unable to transnitrosylate Atg7. Using mass spectrometry analysis, we found that Trx1 transnitrosylates Atg7 at sites Cys294 and Cys402. Both these cysteine residues are located in the adenylation domain of Atg7 which is important for Atg7 to bind to Atg8 (LC3) and MgATP. In Atg7-KO MEF cells, Atg7 WT was able to rescue autophagy while Atg7 C294S/C402S was unable to stimulate autophagy. Also, the ability of Atg7 to bind to its targets -- Atg12 or Atg8 -- was reduced with Atg7 C294S/C402S, indicating that S-nitrosylation of Atg7 at Cys294 and Cys402 is required for Atg7 activity and, thus, autophagosome formation. Taken altogether, we conclude that Trx1 via Cys73 promotes transnitrosylation of cellular proteins, including Atg7, in order to regulate the nitroso-redox balance, thereby promoting cell survival during energy stress in the heart.