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NITRATIVE INACTIVATION RESISTANT HUMAN THIOREDOXIN-1 Y49F MUTANT STRENGTHENS THE THIOREDOXIN-1'S INHIBITION ON ASK-1 MEDIATED APOPTOSIS

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Objectives Thioredoxin-1 (Trx) is an important antioxidant and antiapoptotic molecule and its activity can be altered by the modification of different amino acid sites. The oxidative stress stimulates apoptosis signal-regulating kinase 1 (ASK-1) signaling and thus induces apoptosis, which was inhibited by Trx treatment. This inhibition could be attenuated by nitrative inactivation of Trx induced by the oxidative stress. However, the underlying mechanism remains largely unknown. Therefore, the present study aims to examine whether Y49 in Trx is the nitrative modification site and further investigate

whether the single mutation of Trx at site Y49 (Trx Y49F) resists nitrative inactivation and strengthens the Trx's inhibition on ASK-1 mediated apoptosis.

Methods and Results In contrast to Trx, in vitro exposure of Trx Y49F to SIN-1 (a protein nitration reagent) did not result in protein nitration. The interactions of Trx/Trx Y49F and ASK-1 were studied by cotransfection of Trx/Trx Y49F and ASK-1 plasmids into HEK293A cells. By treating cells with H_2O_2, Trx-ASK-1 complex formation was reduced and activity of ASK-1 and p38 MAPK increased. In contrast, mutation of Trx at site Y49 prevented Trx nitration, increased Trx activity, restored Trx-ASK-1 interaction, reduced ASK-1 and P38 MAPK activity, and attenuated caspase-3 activation ($p<0.01$) under the same treatment of H_2O_2. Further animal experiments confirmed that compared with Trx, Trx Y49F treatment is more effective in protecting MI/R injury by enhancing Trx-ASK-1 interaction, inhibiting p38 MAPK signaling pathway, and subsequently decreasing post-ischemic cardiomyocyte apoptosis ($p<0.05$).

Conclusions Our results demonstrated for the first time that the oxidative stress results in nitration of Trx at site Y49. Nitrative inactivation of Trx can be prevented by Y49F mutation, which strengthens the Trx's inhibition of ASK-1-mediated pathological apoptosis. This finding may lead to a novel therapeutic strategy to attenuate MI/R injury in patients.