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**ZnT-1 Protects Cardiac Myocytes from Ischemia\Reperfusion Injury trough the activation of Raf-1/MEK/MAPK signaling pathway**

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BACKGROUD: ZnT-1 is a protein that confers cellular resistance from heavy metal toxicity, but its function in the myocardium is not clear. Our group recently demonstrated that ZnT-1 regulates calcium influx into cardiomyocytes by interacting with the regulatory $\beta$ -subunit of the L-type calcium channel. In addition, in non-cardiac cells ZnT-1 was found to interact with Raf-1 kinase leading to downstream activation of MEK/MAPK signaling. In the present study we investigated the role of ZnT-1 in myocardial ischemia\reperfusion injury (I\R). METHODS AND RESULTS: Cultured cells of cardiomyocyte origin (HL-1 cells) were exposed to ischemic conditions for 60 min using sodium cyanide and 2-Deoxi Glucose, followed by 60 min of washout mimicking reperfusion. I\R injury was detected by measuring LDH release and staining for pro-apoptotic proteins activation (caspase 3 and 7). Overexpression of ZnT-1 reduced the LDH release following I\R injury to 50.1±2.5 % of control (n=6, $p<0.01$) and markedly reduced caspases staining. Consistently, ERK phosphorylation was increased in the ZnT-1 transfected cells to 266±27.8 % of control ($p<0.01$). Knockdown of endogenous ZnT-1 by shRNA blocked the phosphorylation of ERK and markedly augmented LDH release following I/R injury to 287.4±36.9 % of control (n=5, $p<0.01$). The protective effect of ZnT-1 following I\R injury was apparent following pretreatment of HL-1 cells with the L-type calcium channel blocker nifedipine (1 μM)In contrast, pretreatment with the MEK inhibitor PD98059 (10 μM)completely abolished the protective effect of ZnT-1 following I\R injury (n=3). Moreover, a mutated form of ZnT-1 lacking the ability to bind Raf-1, failed to protect HL-1 cells from I\R injury (n=3). CONCLUSIONS: ZnT-1 confers cellular resistance from I\R injury trough its ability to interact with Raf-1 and its ability to stimulate the Raf/MEK/MAPK signaling pathway. Our findings suggest an important new role for ZnT-1 in the myocardium.