HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight January 2012

Institute / Independent Department / Clinical Co-operation Group / Junior Research Group:
Institute of Toxicology, AG Zischka

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Title of the Highlight:
Mitochondrial thioredoxin reductase is essential for early postischemic myocardial protection

Keywords:
Reactive oxygen species, redox regulation, ischemia reperfusion, infarct size

Central statement of the Highlight:
Myocardial infarction is the leading cause of death in most industrialized nations throughout the world. High levels of oxygen radicals (ROS) in response to infarction (ischemia/reperfusion) are a major trigger of cardiomyocyte cell death, although their mode of action is only beginning to be understood. Here, we show that targeted disruption of a key mitochondrial ROS-detoxifying enzyme sensitizes to myocardial ischemia/reperfusion injury through opening of the mitochondrial transition pore. Importantly, pharmacological intervention, either through inhibition of the mitochondrial pore or by regeneration of the ROS-sensitive cellular thiol-pool, allowed restoring myocardial function and preventing tissue damage.

Text of the Highlight:
Background: Excessive formation of ROS contributes to tissue injury and functional deterioration after myocardial ischemia/reperfusion (I/R). Especially, mitochondrial ROS are capable of opening the mitochondrial permeability transition pore, a harmful event in cardiac I/R. Thioredoxins are key players in

the cardiac defense against oxidative stress. Recently, we identified the first mutations in the mitochondrial thioredoxin reductase (Thioredoxin reductase-2, Txnrd2) to cause dilated cardiomyopathy in patients. Here, we investigated whether Txnrd2 protects against myocardial I/R injury.

Results: In mice, α-MHC-restricted Cre-mediated Txnrd2 deficiency, induced by tamoxifen (Txnrd2-/-ic), aggravated systolic dysfunction and cardiomyocyte cell death after I/R (Figure 1). Txnrd2-/-ic was accompanied by a loss of mitochondrial integrity and function, which was resolved on pre-treatment with the ROS scavenger N-acetylcysteine and the mitochondrial permeability transition pore blocker cyclosporin A (Figure 2). Likewise, Txnrd2 deletion in embryonic endothelial precursor cells and embryonic stem cell-derived cardiomyocytes, as well as introduction of Txnrd2-shRNA into adult HL-1 cardiomyocytes, increased cell death on hypoxia and reoxygenation, unless N-acetylcysteine was coadministered (Figure 3).

Conclusions: We report that Txnrd2 exerts a crucial function during postischemic reperfusion via thiol regeneration. The efficacy of cyclosporin A in cardiac Txnrd2 deficiency indicates a role for Txnrd2 in reducing mitochondrial reactive oxygen species, thereby preventing opening of the mitochondrial permeability transition pore (Figure 4).

Publication:
"Mitochondrial thioredoxin reductase is essential for early postischemic myocardial protection".

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Taking account of the HMGU mission:
This study strengthens the mission of the HMGU to characterize and understand the detrimental mechanisms of response that culminate in cell death and tissue failure upon genetic predispositions, metabolic disorders or environmental impacts.
CLINICAL PERSPECTIVE of this study:
Although clinical data on successful treatment of myocardial ischemia and reperfusion injury are scarce, the role of unbalanced ROS caused by an imbalance of oxygen supply and proper mitochondrial function is well established in this potentially fatal disease. Cellular defence mechanisms against the reperfusion-triggered wave of ROS depend on regenerating enzymes to maintain the most important redox molecules, glutathione and thioredoxin, in their reduced and thus active state throughout the whole period of enhanced ROS formation.
In the current experimental study, we provide conclusive evidence that heart-specific deletion of the selenium dependent mitochondrial enzyme thioredoxin reductase (Txnrd2) results in increased cardiomyocyte vulnerability in adult mice during early postischemic reperfusion. Cardiomyocyte mitochondria isolated from these animals challenged by different respiratory substrates demonstrate a

significantly higher emergence of ROS compared to mitochondria from control counterparts. In vivo, Txnrd2 deletion caused increased infarct size and cardiomyocyte apoptosis, whereas functional recovery of postischemic hearts was significantly impaired by the absence of Txnrd2. Moreover, a significantly stronger decrease in the activity of the redox-sensitive mitochondrial enzyme aconitase occurs upon I/R damage in Txnrd2-depleted myocardial mitochondria as compared to mitochondria derived from control animals subjected to I/R. Of note, none of these hallmarks of reperfusion was obtained in mice lacking the cytosolic counterpart, thioredoxin reductase 1 (Txnrd1). The observation that pharmacological intervention with the mitochondrial permeability transition pore inhibitor cyclosporin A and the cysteine-rich antioxidant N-acetyl-cysteine were able to rescue the Txnrd2 phenotype indicates that indeed ROS scavenging and direct or indirect prevention of mitochondrial permeability transition pore opening are highly relevant mechanisms to alleviate I/R induced stress and ensure tissue function. Currently, it is unknown if functional impairment of Txnrd2, e.g., by mutation, is clinically associated with increased myocardial I/R injury, although a phenotype of dilated cardiomyopathy has been recently associated with mutations in *TXNRD2*. Yet it is noteworthy that chronic selenium deficiency is a key factor in Keshan-Beck disease, a potentially fatal cardiomyopathy disease endemic in certain rural areas of China where the selenium content was found to be low in foods. Hence, selenium malnutrition may impair Txnrd2 activity and consequently aggravate I/R induced tissue detriment.

The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:

- Research Unit Comparative Medicine, AVM, Brielmeier
- Research Unit Gene Vectors, Junior Research Group Apoptosis, AGV, Jeremias
- Institute of Developmental Genetics; DZNE Modelling Neurodegeneration, Conrad

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Horstkotte et al., Circulation 2011

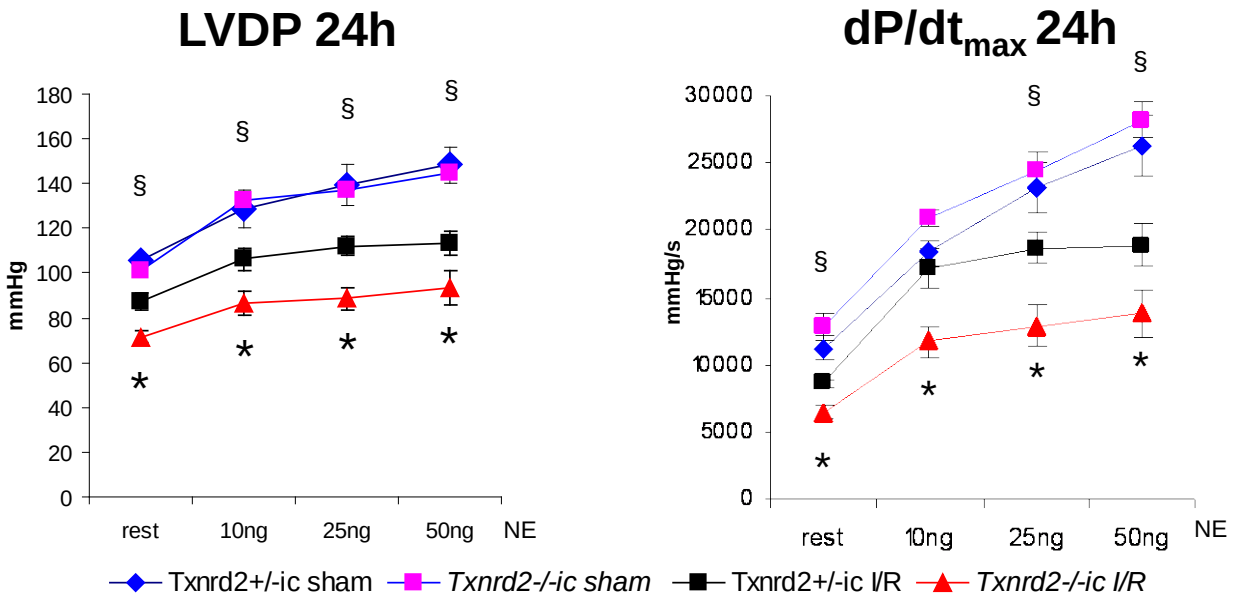

Figure 1: Inducible cardiospecific thioredoxin reductase-2 deficiency impairs cardiac function during ischemia / reperfusion (I/R) as determined by Millar tip catheter analysis. Although no impairment of LV-function in Txnrd2-/-ic (knockout) mice was observed without I/R stress (left panel), an aggravated loss of left ventricular developed pressure (LVDP) was evident compared to Txnrd2+/-ic (control) at rest and after stimulation with norepinephrine (NE). The deterioration of systolic function in Txnrd2-/-ic mice at 24h was mirrored in decay of contraction velocity dP/dtmax (right panel).

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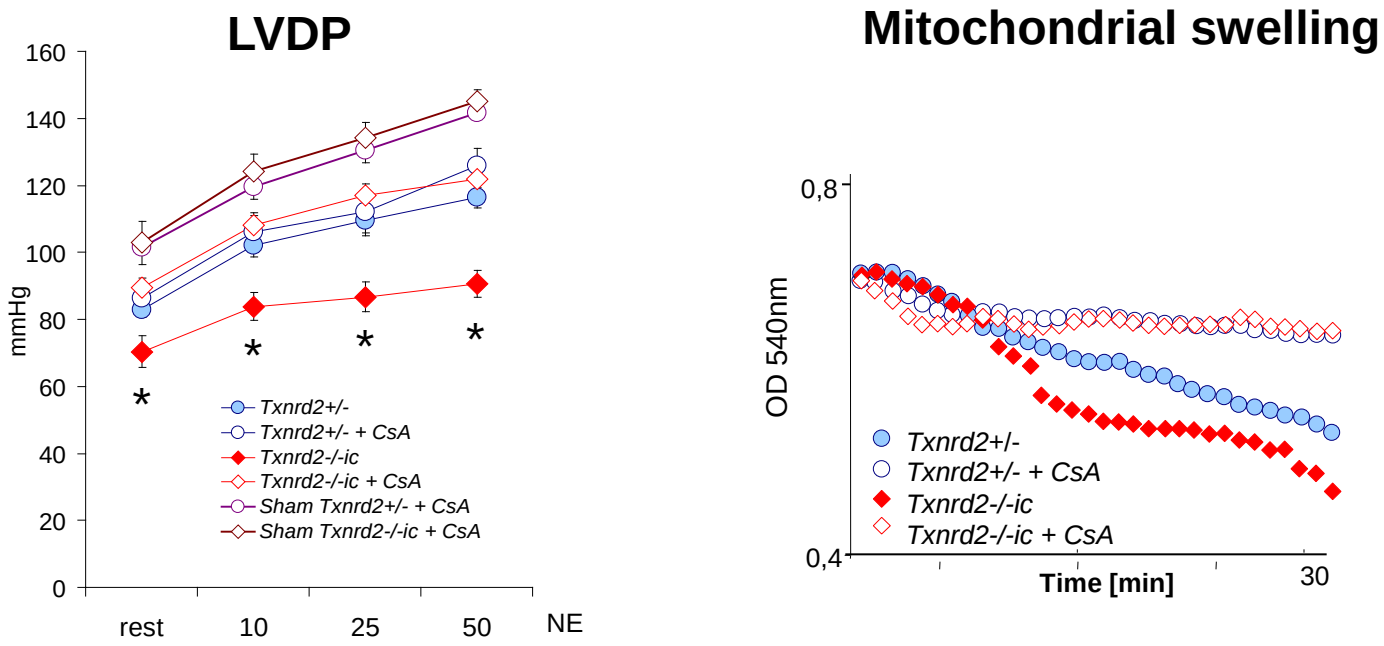

Figure 2: Pharmacological inhibition of mitochondrial pore opening by cyclosporin A mitigates increased sensitivity of thioredoxin reductase-2 deficient hearts towards I/R induced tissue dysfunction. Millar tip catheter analysis displayed a significant improvement of postischemic left ventricular developed pressure (LVDP) of Txnrd2-/-ic (knockout) hearts after cyclosporin A (CsA) treatment (left panel). CsA prevented Ca^{2+}-induced mitochondrial swelling in both, mitochondria isolated from Txnrd2-/-ic and Txnrd2+/- hearts (right panel).

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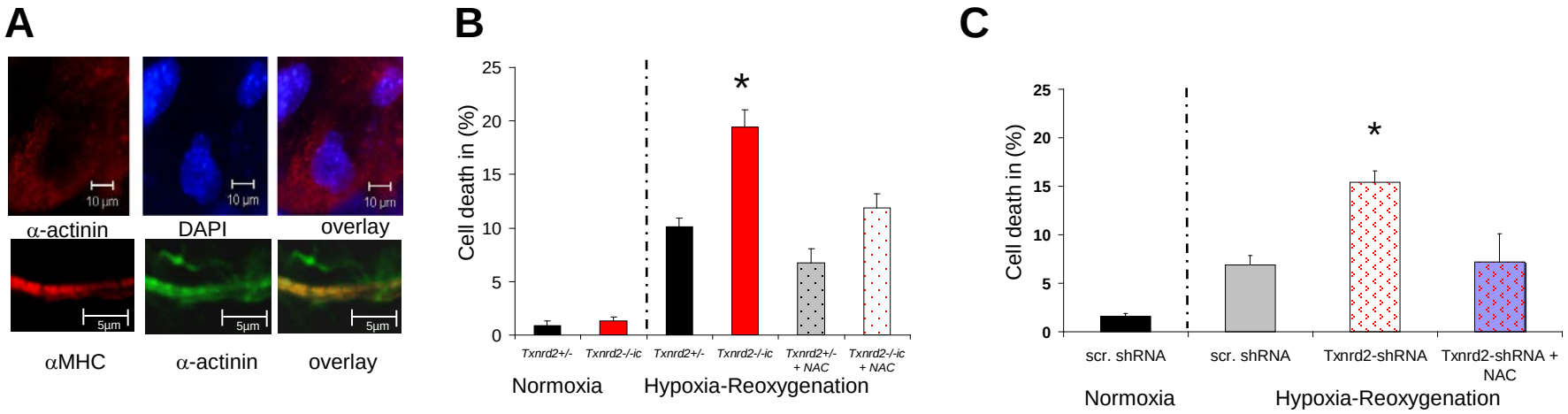

Figure 3: In vitro rescue of Txnrd2 deletion via thiol supplementation. (A) Fluorescence microscopy indicates α-actinin staining (upper panel) and α-MHC-expression (lower panel) of embryonic stem (ES) cell-derived cardiomyocytes, the latter being in a sarcomer-like structure. (B) Inducible Txnrd2 knockout cardiomyocytes (Txnrd2-/-ic) were protected against hypoxia/reoxygenation induced cell death by the thiol provider N-acetylcysteine (NAC). (C) Accordingly, HL-1 cells (a human cardiomyocyte cell line) were more susceptible to hypoxia-reoxygenation induced cell death after knockdown of Txnrd2 comopared to scrambled (scr.) shRNA, an effect prevented by co-treatment with NAC.

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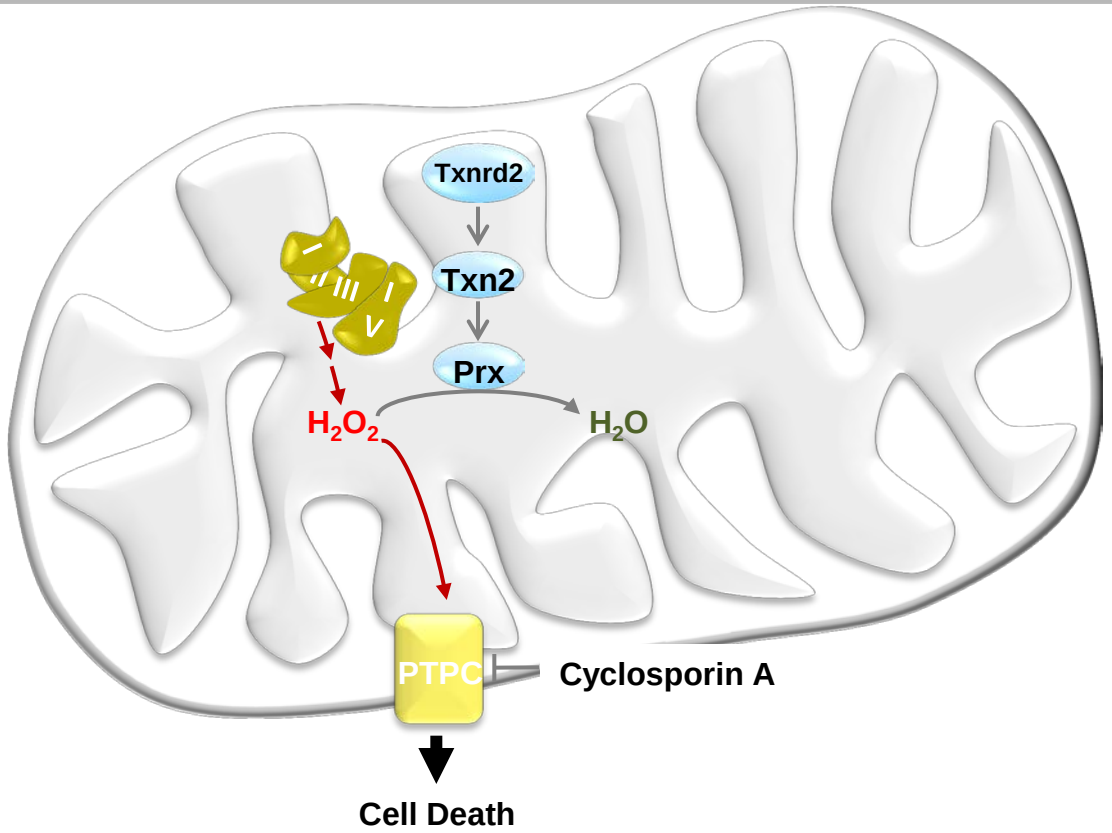

Figure 4: Thioredoxin reductase-2 is an essential redox node in cardiomyocytes by maintaining mitochondrial antioxidant capacity. Reactive oxygen species (ROS, i.e superoxide anion and hydrogen peroxide, H_2O_2) produced particularly in response to cardiac infarction are counteracted by the thioredoxin reductase-2/thioredoxin-2/peroxiredoxin (Prx) system. Impaired activity of this system may lead to high ROS levels, sensitation to opening of the permeability transition pore complex (PTPC) and consequently cell death and tissue malfunction.