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Basic Science

Role of SUMOylation and deSUMOylation of mitochondrial fission proteins in myocardial ischaemia-reperfusion injury

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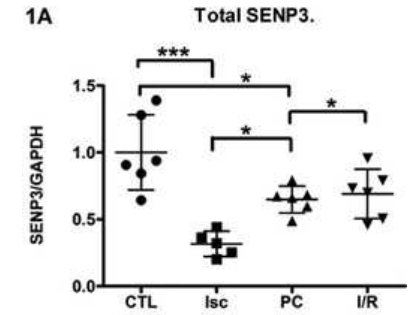
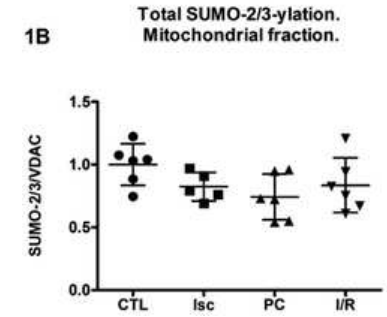
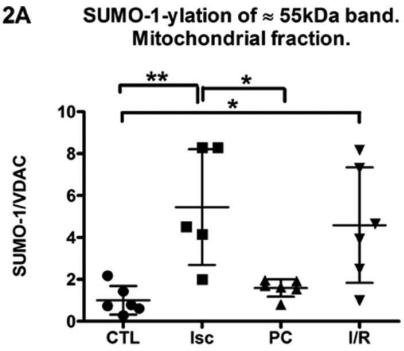
Abstract

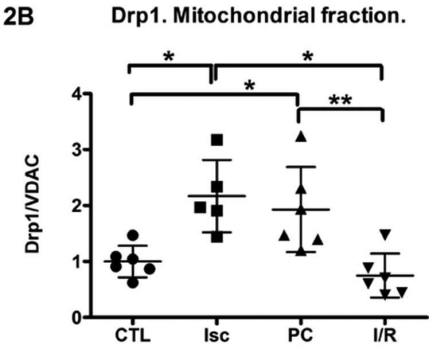
Background Restoration of blood supply to the heart after a prolonged ischaemic episode causes myocardial ischaemia-reperfusion (I/R) injury. Paradoxically, short periods of ischaemia and reperfusion, known as preconditioning, has a potent cardioprotective effect. One key aspect of I/R injury is cytotoxic mitochondrial fission that allows cytochrome c release and promotes apoptotic cell death. Dynamin-related protein 1 (Drp1) is a large GTPase that is recruited from the cytosol to the mitochondrial outer membrane to drive fission. Drp1 function is regulated by posttranslational modifications, including conjugation to small ubiquitin-like modifier (SUMO)−2/3. SUMO-2/3-ylation decreases Drp1 partitioning to the mitochondrial membrane, which reduces stress-induced fission and apoptosis. We have shown previously that, in neurons, Drp1 SUMO-2/3-ylation is controlled by the deSUMOylating enzyme SENP3, levels of which are reduced during ischaemia but restored upon reperfusion.

Objective Here we investigated alterations in SUMOylation and Drp1 partitioning in heart during ischaemia, I/R and preconditioning. Methods: Isolated hearts from male Wistar rats were perfused using Langendorff apparatus with Krebs Henseleit solution. Hearts were randomly divided into 4 groups (with n=5–6 per group): *control* (50 min perfusion); *ischaemia* (20 min perfusion+30 min ischaemia); *preconditioning* (3 short cycles of I/R, 2 and 3 mins, respectively, followed by 30 min of ischaemia); *I/R* (30 min of ischaemia followed by 2 hour of reperfusion). All samples were immediately subjected to subcellular fractionation, then frozen on dry ice and used for Western blot analysis.









Results Levels of SENP3 were reduced in all three groups compared to control, with the greatest reduction seen during ischaemia (Fig 1A). Despite the observed reduction in SENP3 levels no obvious change in overall levels of protein SUMO-2/3-ylation was detected in mitochondria fractions (Fig 1B). An increase of SUMO-1 conjugation to an unidentified protein at about 55 kDa was observed during ischaemia and I/R compared to both the control and preconditioning groups (Fig 2A). Intriguingly, in contrast to our finding in neurons, where mitochondrial partitioning of Drp1 decreases during ischaemia, in cardiac tissue we observed recruitment of Drp1 to mitochondria, with no change in total protein levels. Furthermore, Drp1 recruitment to

mitochondria was increased by preconditioning. In the I/R group, in which cells are undergoing apoptosis, levels of Drp1 at the mitochondria are similar to controls (Fig 2B).

Conclusion Taken together our data suggest a delicate balance between SUMOylation and deSUMOylation that regulates the recruitment of Drp1 to mitochondria. This pathway plays an important role in the vulnerability of cardiomyocytes to ischaemic damage and myocardial reperfusion injury. Interestingly, the interplay between the relevant proteins appears to differ between heart and brain cells.

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