Adenine nucleotide translocator, a mitochondrial carrier protein, and fate of cardiomyocytes after ischaemia/reperfusion

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This editorial refers to 'Phosphoproteome analysis of isoflurane-protected heart mitochondria: phosphorylation of adenine nucleotide translocator-1 on Tyr194 regulates mitochondrial function' by Feng et al.,11 pp. 20–29, this issue.

Mitochondria have multiple roles in the development of ischaemia/reperfusion injury. Consumption of ATP by mitochondrial ATPase during ischaemia and generation of reactive oxygen species (ROS) from complex III during ischaemia/reperfusion are detrimental to cardiomyocytes, whereas uptake of cytosolic Ca²⁺ into mitochondria attenuates cytosolic Ca²⁺ overload and thus activation of Ca²⁺-activated proteases such as calpain. However, the most important event in the mitochondria of cardiomyocytes subjected to ischaemia/reperfusion is opening of the mitochondrial permeability transition pore (mPTP).1 Although its molecular structure is still controversial, mPTP apparently consists of an adenine nucleotide translocator (ANT) in the inner membrane, a carrier exchanging matrix ATP with ADP in the intermembrane space; and a voltage-dependent anion channel (VDAC) in the outer membrane of the mitochondria.3 The mPTP is closed under physiological conditions but opens in response to cellular stresses, leading to ATP depletion, increase in inorganic phosphate levels, ROS generation, and mitochondrial Ca²⁺ overload. Opening of mPTP upon reperfusion following ischaemia abolishes mitochondrial membrane potential and compromises ATP generation, which is crucial for restoration of ionic homeostasis and maintenance of cell viability.4

The threshold for opening of mPTP has been shown to be elevated by ischaemic pre- and post-conditioning, though these interventions also modify trigger factors such as Ca²⁺ overload per se.5,6 However, mechanisms of the modification of mPTP opening threshold have not yet been clarified. One possibility is inhibition of glycogen synthase kinase-3β (GSK-3β) activity in the mitochondria by its phosphorylation. Either inhibitory phosphorylation of GSK-3β or reduction of GSK-3β protein by siRNA significantly elevated the threshold for mPTP opening in response to ROS in isolated cardiomyocytes.7 Furthermore, we recently found that phospho-Ser9-GSK-3β in mitochondria physically interact with ANT upon reperfusion, which inhibits interaction of ANT with cyclophilin D, a trigger of mPTP opening, in rat hearts.8 However, there is also evidence suggesting that GSK-3β phosphorylation is not the only mechanism of inhibition of mPTP opening by ischaemic pre- and post-conditioning. A recent study by Nishino et al. showed that ischaemic pre- and post-conditioning successfully limited infarct size after ischaemia/reperfusion in mice lacking the critical N-terminal serine within GSK-3β (Ser9).9 Furthermore, Clarke et al.10 reported that carbonylation of mitochondrial proteins, which would sensitize mPTP to Ca²⁺, was reduced by ischaemic pre-conditioning without any significant change in phosphorylation of mitochondrial proteins. Nevertheless, mechanisms of inhibition of mPTP opening independent of GSK-3β phosphorylation in myocardial protection remain largely unclear.

In this issue of the Journal, Feng et al.11 report intriguing findings suggesting tyrosine-phosphorylated ANT may play a role in cardiomyocyte protection. They performed phosphoproteome analyses of mitochondria from rat hearts subjected to ischaemia with or without pre- and post-conditioning by isoflurane. Using MALDITOF-MS analysis, they identified 45 phosphorylated mitochondrial proteins. Of these, 24 serine/threonine residues and 2 tyrosine residues were identified in 19 proteins by a phosphopeptide-enrichment protocol and LC-MS/MS. Further analyses revealed that the phosphorylation status of five proteins was in parallel with isoflurane-induced protection, among which they focused on ANT1. In Langendorff-perfused rat hearts, phosphorylation of ANT1 at Tyr194 was diminished by ischaemia/reperfusion, and it was maintained by both pre- and post-conditioning, indicating association of tyrosine phosphorylation with cardioprotection. The authors also provide evidence of the physiological importance of Tyr194 phosphorylation using a yeast model; transfection of ADP/ATP carrier-deficient yeast with wild-type ANT1, but not that with a non-phosphorylatable mutant ANT1-Y194F, restored its growth in the presence of non-fermentable carbon sources.11
In contrast with other mitochondrial proteins, little is known about the regulation of ANT by reversible phosphorylation. Lewandrowski et al. recently identified phosphorylation of ANT on Tyr194 and Tyr190 in rat brain mitochondria. Tyr190 and Tyr194 are located inside the cavity of the transmembrane domain. Its surface is positively charged in the basal unstimulated state, but the phosphorylation of Tyr190 and Tyr194 changes it into a negative charge. The results of structural analysis of ANT suggest that ADP translocation along the tyrosine ladder consisting of Y194/Y190/Y186 could be guided by a stacking interaction with tyrosine rings. These findings support the notion that phosphorylation of Tyr194 alters ADP/ATP transport activity and specificity of the transport.

The findings in the study by Feng et al. do not directly indicate a relationship between the level of ANT1 phosphorylation at Tyr194 and mPTP opening threshold or triggers of mPTP opening. However, there are several lines of circumstantial evidence suggesting that modification of ANT could lead to a change in myocardial tolerance against injury. First, H⁺ leak via ANT after ischemia/reperfusion, which reduces mitochondrial membrane potential and uncouples substrate oxidation from ATP production, was suppressed by ischemic pre-conditioning. Secondly, functional coupling between ANT and mitochondrial creatine kinase was improved by ischemic pre-conditioning. Thirdly, functional capacity of ANT was correlated with myocardial protection afforded by protein kinase C ε overexpression, which mimics ischemic pre-conditioning. Fourthly, cardiac-specific ANT1 overexpression improved mitochondrial function and structure, ventricular function and survival in rats with severe hypertension. Whether the beneficial effects associated with modified ANT functions are attributable to modulation of mPTP remains unclear.

The intriguing observation by Feng et al. leaves several important questions unanswered. Is phosphorylation of ANT at Tyr194 sufficient for protection of cardiomyocytes from ischemia/reperfusion injury? Does tyrosine phosphorylation in ANT elevate the threshold for mPTP opening? Is an Src ischaemia/reperfusion injury? Does tyrosine phosphorylation at Tyr194 sufficient for protection of cardiomyocytes from important questions unanswered. Is phosphorylation of ANT afforded by protein kinase C?

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