AMPK, MAPK and Bax in the heart: some questions answered

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The question of how Bax is activated during apoptosis to perform its role in permeabilization of mitochondrial membranes is intriguing for investigators in the wide field of cell death research. In their paper published in the Biochemical Journal in 2006, Capano and Crompton presented their discovery that simulated ischaemia causes rapid activation of AMPK (AMP-activated protein kinase) which phosphorylates and activates p38 MAPK (mitogen-activated protein kinase) leading to Bax activation and translocation to mitochondria in isolated cardiac myocytes. This was the first report on the molecular mechanism of Bax activation and migration during ischaemia-induced apoptosis in cardiomyocytes.

Key words: AMP-activated protein kinase (AMPK), apoptosis, Bax, heart ischaemia, mitochondrion, mitogen-activated protein kinase (MAPK), protein kinase.

Programmed cell death, or apoptosis, is an active, energy-requiring and precisely tuned process that plays an essential role in development of organisms and maintenance of tissue homeostasis; however, this form of cell death also contributes to the pathogenesis of various diseases. Among other related issues, understanding of mechanisms of apoptosis in the heart is a rapidly expanding area; however, a number of key questions are still controversial and remain to be answered.

Is apoptosis important in the heart? This question was raised a decade ago and it still remains intriguing in 2008. Most of the controversy comes from the findings that apoptosis, although observed in diseased hearts, is usually not extensive, being just a few per cent of the total number of myocardial cells [1]. Adult finally differentiated cardiac myocytes seem to be quite well protected against apoptosis: they contain a variety of endogenous IAPs (inhibitors of apoptosis) [IAP, FLIP (FADD (Fas-associated death domain)-like inhibitory protein)], some of which are expressed mainly in myogenic cells [such as ARC (apoptosis repressor with caspase recruitment domain)]. Moreover, the levels of expression of Apaf-1 (apoptotic protease-activating factor 1), the main component of caspase-activating machinery, the apoptosome, are down-regulated in adult cardiomyocytes [2]. Nevertheless, apoptosis does occur in the heart. It is considered to be a possible source of a cumulative loss of myocytes and it is thought to play an important role in certain heart pathologies such as ischaemia/reperfusion, infarction, heart failure and aging [3].

The second important question is how apoptosis can be triggered by ischaemia/reperfusion in the heart? Although components of the death receptor pathway of apoptosis [Fas and TNF receptors (tumour necrosis factor α) receptors and ligands, caspase 8, etc.] are present in cardiac myocytes, most of the recent research points to mitochondria as a central player at the stage during ischaemia/reperfusion (reviewed in [1,3]). In response to apoptotic signals, mitochondria undergo alterations resulting in the release of cytochrome c and other apoptogenic intermembrane space proteins involved in activation of caspases [cytochrome c, SMAC (second mitochondrial-derived activator of caspase)/DIABLO (direct IAP-binding protein with low pi), etc.] or nuclear fragmentation [AIF (apoptosis-inducing factor)]. Release of cytochrome c from mitochondria may lead not only to the formation of apoptosome and caspase activation, but also to the suppression of oxidative phosphorylation, resulting in energy depletion which may cause necrosis rather than apoptosis.

Here, the third burning question comes: what makes mitochondria leaky enough to cause apoptosis, but not too much to prevent killing the cells by explosive necrosis owing to lack of energy? At least two mechanisms have been proposed to explain the outer mitochondrial membrane permeabilization during ischaemia/reperfusion. One puts the opening of the MPTP (mitochondrial permeability transition pore) at the centre of events [4]. Another hypothesis claims that the cytosolic protein Bax when translocated to mitochondria co-operates with another mitochondrial protein, Bak, and forms pores in the outer mitochondrial membrane, permitting escape of cytochrome c and other pro-apoptotic proteins into cytosol (see [5] for a review). In support of this mechanism, are reports that Bax-knockout mice are resistant to myocardial ischaemia/reperfusion-induced apoptosis and cardiac dysfunction [6,7]. In addition, recent experimental evidence suggests that both MPTP and Bax may be related and may co-operate in providing means for cytochrome c release from mitochondria at least in certain models of apoptosis [8,9].

Assuming that Bax is an essential player in ischaemia-induced apoptosis, another intriguing question was raised by Capano and Crompton [10] in the Biochemical Journal: what forces Bax to migrate during ischaemia towards mitochondria and to permeabilize the mitochondrial outer membrane causing leakage of cytochrome c? This is a major issue not only with respect to heart ischaemic injury, but also in the wider field of apoptosis research in general. Despite the widely accepted notion that Bax is a key component of the intrinsic apoptotic pathway, the precise molecular mechanisms controlling Bax activation during apoptosis are poorly understood. The study by Capano and Crompton [10] shed light on this mechanism. They investigated how Bax moves to mitochondria during simulated ischaemia in the cardiomyocytes and whether other influential players in the ischaemic injury, e.g. p38 MAPK (mitogen-activated protein kinase) and AMPK (AMP-activated protein kinase) have any impact on that.

Bax is a pro-apoptotic member of so-called Bcl-2 family proteins. In healthy cells, it resides in the cytosol as a monomeric...
soluble protein; however, after induction of apoptosis, Bax changes conformation and translocates to mitochondria, where it inserts into the mitochondrial outer membrane causing the release of cytochrome c (reviewed in [5]). This ‘change of location’ step is usually irreversible and reflects the commitment of the cell to undergo apoptosis. All is well, but what actually forces Bax to change shape and move towards mitochondria? Some protein kinases [p38 MAPK, AMPK, JNK (c-Jun N-terminal kinase)] were suspected to have a role in doing that job as there was previous evidence of their involvement in ischaemic heart injury.

Capano and Crompton [10] have reported finding the missing connection between AMPK, p38 MAPK and apoptotic cell death in the ischaemic heart. In their study, they applied a model of simulated ischaemia (glucose deprivation plus inhibition of the mitochondrial respiratory chain by cyanide) in cultured rat neonatal cardiomyocytes and investigated the dynamics of movement of GFP (green fluorescent protein)-tagged Bax from the cytosol to mitochondria [10]. Translocation of Bax was found to be a gradual process beginning within 20 min of simulated ischaemia and progressing for at least 3 h. Furthermore, the researchers investigated how ischaemia can trigger apoptotic events in cardiomyocytes. They observed that Bax accumulation in mitochondria was preceded by rapid (5 min) phosphorylation and activation of p38 MAPK. Interestingly, AMPK was activated even more rapidly: the increased levels of phospho-AMPK were detectable after 1–3 min of simulated ischaemia and even more rapidly: the increased levels of phospho-AMPK in mitochondria was preceded by rapid (5 min) phosphorylation events in cardiomyocytes. They observed that Bax accumulation in mitochondria was preceded by rapid (5 min) phosphorylation and activation of p38 MAPK. Interestingly, AMPK was activated even more rapidly: the increased levels of phospho-AMPK were detectable after 1–3 min of simulated ischaemia and remained high for at least 3 h. The mimetic of AMP [a product of AICAR (5-amino-4-imidazolecarboxamide 1-remained high for at least 3 h. The mimetic of AMP [a product [10] suggested that the chain of events during ischaemia should include: ischaemia-induced mild depletion of ATP/accumulation of AMP → AMPK activation → p38 MAPK activation → migration of Bax to mitochondria → apoptosis.

As discussed in the paper by Capano and Crompton [10], p38-induced Bax activation seems to be a universal mechanism operating not only during ischaemia in the heart, but also in other models of apoptosis such as NO-induced neuronal cell death or UV-induced apoptosis in keratinocytes (see [10] for references).

The question of when apoptosis is initiated, during ischaemia or reperfusion, is one often discussed. Apoptosis is an energy-requiring process, therefore it is usually assumed to be activated during reperfusion when oxygen supply to the heart and mitochondrial oxidative phosphorylation are restored. However, there were reports that the release of cytochrome c from mitochondria is the earliest event during ischaemia and can be observed already after 20 min of total heart ischaemia (without reperfusion) [1,11]. The difficulty at that time was to explain what was the real cause of cytochrome c loss from mitochondria. The work by Capano and Crompton [10] has shown that the earliest step in apoptosis, Bax activation and translocation to mitochondria (preceding the release of cytochrome c), may be initiated during the ischaemic period itself, which may cause depletion of cellular energy resources and subsequent activation of AMPK.

We usually judge the significance and quality of papers according to their impact on the research advance in the field. The paper by Capano and Crompton [10] has stimulated the research and in 1 year, it has been cited more than 20 times. But good papers also raise new questions. It would be interesting to learn whether p38 phosphorylates Bax directly or whether other intermediate protein kinases are involved? Is Bax actually phosphorylated and at which sites? Are ROS (reactive oxygen species) involved in activation of the protein kinase–Bax chain of events, as inhibition of the mitochondrial respiration by cyanide to simulate ischaemia could cause a rapid ROS production rather than ATP depletion (a relatively slower process)?

In the meantime, we can conclude that the paper by Capano and Crompton [10] was a timely, well-designed and conclusive study which increased our understanding about the cellular journey of Bax during heart ischaemia.

REFERENCES


