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# Intracellular heat shock protein 70: a possible therapeutic target for preventing postoperative atrial fibrillation

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*Received December 14, 2011, accepted January 6, 2012*

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*Pharmazie 67: 747–755 (2012)* *doi: 10.1691/ph.2012.1844*

Postoperative atrial fibrillation is a frequent complication after cardiac surgery, associated with an increased risk of mortality and morbidity; thus, additional treatment and increasing costs of postoperative care are required. Inflammation and oxidative stress caused by ischemia-reperfusion during cardiac surgery may play an important role in the pathogenesis of postoperative atrial fibrillation. Stress-inducible heat shock proteins act as molecular chaperones that maintain cell homeostasis against stress in these events. Heat shock protein 70, the 70-kDa family of heat shock proteins, has been shown to closely associate with the incidence of postoperative atrial fibrillation in patients undergoing cardiac surgery. Extracellular heat shock protein 70 may be pro-inflammatory in the myocardial innate immune response caused by ischemia-reperfusion. In contrast, intracellular heat shock protein 70 exerts primarily anti-inflammatory and anti-apoptotic effects by preventing response to inflammatory cytokines, and inhibiting the nuclear factor Kappa B signaling pathway and different stages of mitochondrial-dependent pathways. Furthermore, the intracellular molecule can inhibit the induction and the maintenance of atrial fibrillation by attenuating $Ca^{2+}$ overload in injuried myocardial cells. It is the intracellular heat shock protein 70, but not the extracellular molecule that holds as a therapeutic strategy for preventing postoperative atrial fibrillation.

## 1. Introduction

Open heart surgery is the most frequent surgical procedure performed with the use of cardiopulmonary bypass (CPB) and cardioplegic arrest. Though technical and technological improvements have made the operation safer, postoperative atrial fibrillation (POAF) remains the most common complication after cardiac surgery. The incidence of POAF after coronary artery bypass graft (CABG) ranges from 15% to 40% (Aranki et al. 1996; El-Chami et al. 2010; Elahi et al. 2003; Nazeri et al. 2010). These events usually occur within 2 to 5 days and often resolved within several weeks. Though generally well tolerated and seen as a temporary problem related to surgery, POAF increases the risk of stroke and is associated with significant morbidity and long-term mortality. In the meantime, POAF is invariably associated with prolonged hospitalization and significant additional costs to patient care. It was reported that patients developing POAF after CABG were hospitalized about 4.9 days longer than patients remaining in sinus rhythm, with $10,000 to $11,500 of additional hospital charges for CABG in the USA (Aranki et al. 1996).

## 2. Pathophysiology and mechanisms of postoperative atrial fibrillation (POAF)

The pathophysiology of POAF after cardiac surgery is not precisely known and the mechanisms are thought to be multifactorial and are influenced by preoperative, intraoperative and postoperative factors (Fig. 1). Different risk factors have been reported, included increasing age, a previous history of atrial fibrillation (AF), male gender, hypertension, obesity, decreased left-ventricular ejection fraction, left atrial enlargement, chronic obstructive pulmonary disease, prolonged ventilation, chronic renal failure, diabetes mellitus, and rheumatic heart disease (Girerd et al. 2009; Magee et al. 2007; Mathew et al. 2004).

Inflammation and oxidative stress caused by ischemia-reperfusion during cardiac surgery may play an important role in the pathogenesis of POAF (Fig. 1). Although the relative strength of the association of inflammation and oxidative stress markers with POAF remains unclear, the role of inflammation and oxidative stress on structural and electrical remodeling is under investigation. Previous studies showed that POAF is a probable consequence of the electrophysiological disturbances associated with inflammation and reperfusion injury in cardiac surgery patients (Ascione et al. 2000; Wu et al. 2003). Inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-alpha, monocyte chemoattractant protein-1 (MCP-1), N-terminal pro-brain B-type natriuretic peptide (NT-proBNP) and the renin-angiotensin system have all been investigated for an association with AF (Boos et al. 2006; Li et al. 2010). Inflammation can alter atrial conduction, facilitating reentry and then predisposing to the development of POAF (Beere et al. 2000; Ishii et al. 2005).

Oxidative stress is a major contributory factor representing the unavoidable consequences of ischemia-reperfusion cycle

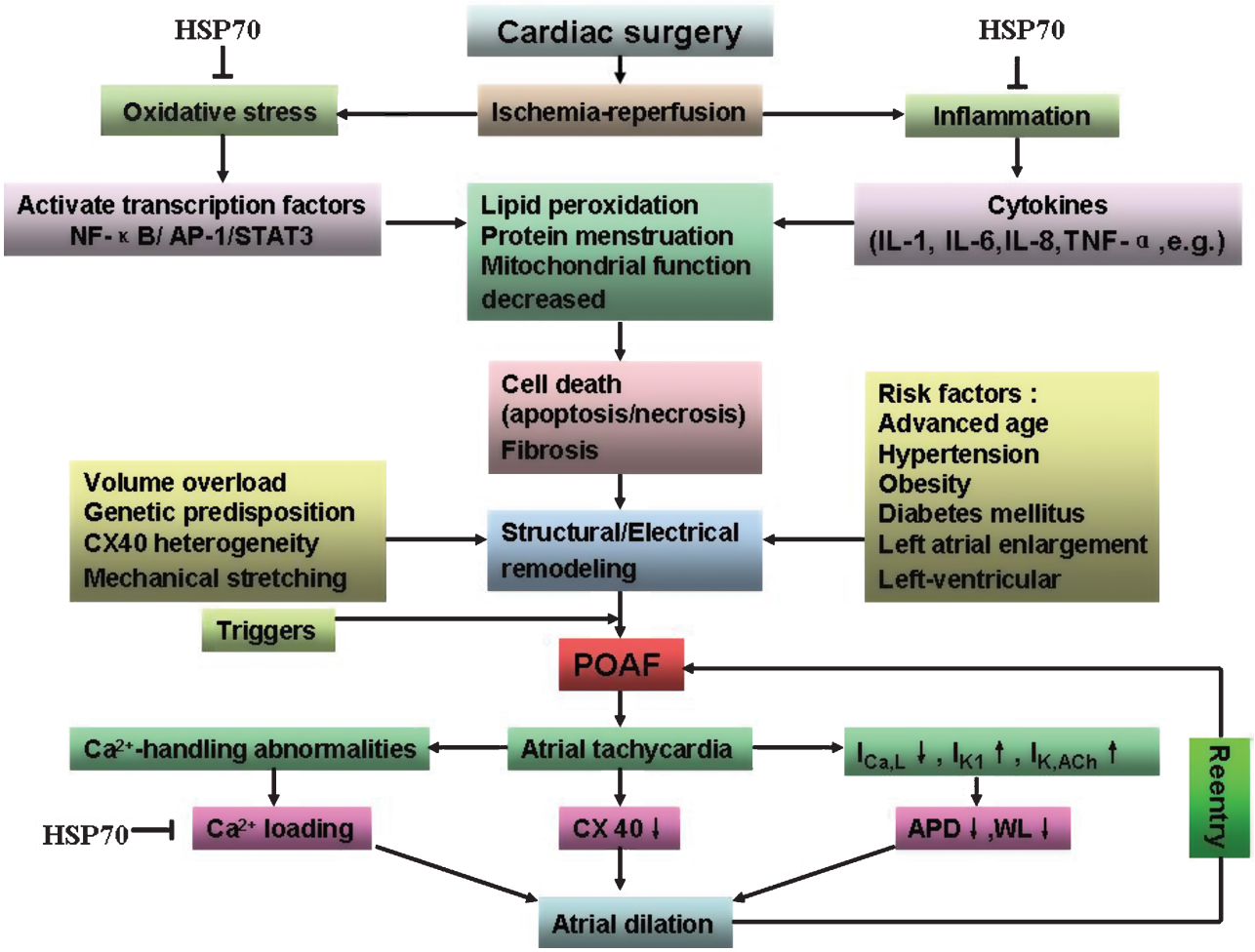


Fig. 1: Proposed hypothesize the pathogenesis of postoperative atrial fibrillation in patients undergoing cardiac surgery. NF-κB = nuclear factor Kappa B; AP-1 = transcriptional factor activating protein-1; STAT3 = signal transducer and activator of transcription 3; IL-1 = interleukin-1; IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α=tumor necrosis factor-α; POAF = postoperative atrial fibrillation; $I_{Ca,L}$ = inward L-type $Ca^{2+}$ channel; $I_{K1}$ = inward rectifier $K^+$current; $I_{K,Ach}$ = acetylcholine-dependent $K^+$ current; CX40 = connexin 40; APD = action potential duration; WL = wavelength

occurring in cardiac surgery. Many studies have strongly suggested a link between oxidative stress and cardiac arrhythmias, especially AF (Li et al. 2010; Neuman et al. 2007). Oxidative stress triggers inflammation by activating nuclear factor Kappa B (NF-κB) signaling pathways and activator protein-1 (AP-1) transcription factors (Bowie and O'Neill 2000). Signal transducer and activator of transcription-3 (STAT-3) has also been reported to participate in the regulation of this signaling pathway (Hilfiker-Kleiner et al. 2005). Ischemia-reperfusion injury during cardiac surgery leads to the formation of reactive oxygen species (ROS), causing oxidative stress and a systemic inflammatory response (Matata et al. 2000). The increased ROS may lead to lipid peroxidation, breakdown of cell membranes, mitochondrial function decrease, calcium overload, cells death (apoptosis/necrosis), or fibrosis in the myocyte. Thus atrial oxidative injury can lead to the development of POAF by impairing atrial contraction, altering myofibrillar energetics and reducing atrial effective refractory period after cardiac surgery (Kim et al. 2008). Although both oxidative stress and inflammation could be separately involved in the mechanism of POAF, there is a close relation between the two processes. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity is potently stimulated by cytokines, suggesting that this oxidase system may be an important link between the systemic inflammatory response to cardiac surgery and myocardial oxidative stress (Kim et al. 2008). Other pathophysiological mechanisms might also intervene as contributed factors in the development of POAF, including volume overload (Kalus et al. 2004), genetic predisposition assessed by the IL-6 promoter gene variant (Gaudino et al. 2003), and increased expression of the gap-junctional protein connexin 40 (CX40) (Dupont et al. 2001). In addition, mechanical stretching of the atrium can alter cellular electrophysiologic properties suggesting that increased intravascular volume due to postoperative mobilization of interstitial fluid could contribute to POAF (Bruins et al. 1997).

Atrial tachycardia promotes the induction and maintenance of AF, a phenomenon called atrial tachycardia remodeling. Rapid electrical activation reduces atrial refractoriness and decreases the wavelength (WL), primarily due to inward L-type $Ca^{2+}$ channel ($I_{Ca,L}$) down-regulation (Yue et al. 1997) but also via increasing inward-rectifier $K^+$ currents such as the background current $I_{K1}$ and a constitutively active form of acetylcholine-dependent $K^+$ current ($I_{K,ACh}$). The reduced WL caused by action potential duration (APD) abbreviation decreases the size of functional reentry circuits and promotes the induction and maintenance of AF by multiple circuit reentry (Nattel 2002; Nattel et al. 2007). Furthermore, atrial tachycardia appears likely to suppress expression of the atrial-selective CX40 (van der Velden et al. 2000), which associated with AF. In addition, atrial tachycardia remodeling also leads to atrial contractile dysfunction, mainly through $Ca^{2+}$-handling abnormalities, including increased cellular $Ca^{2+}$ loading and reduced systolic $Ca^{2+}$ transients (Sun et al. 2001; Sun et al. 1998), which causes atrial dilation that further promotes reentry (Fig. 1).

## 3. Intracellular HSP70, but not extracellular molecule prevents POAF

Heat shock proteins (HSPs) are characterized as molecular chaperones, playing a dominant role in the preservation and protection of cells and organs from oxidative stress and ischemia-reperfusion injury. The heat shock protein 70 (HSP70) family includes a number of proteins, with slightly different molecular masses and cellular localization. Two members of this family, HSP78 (glucoseregulated protein (Grp)78 or immunoglobulin-binding protein and HSP75 (mitochondrial (mt)HSP70), perform chaperone functions in the endoplasmic reticulum and mitochondria. The remaining member proteins, HSC70 (sometimes called HSP73), and its inducible isoform, HSP70 (often referred to as HSP72), are usually distributed in the cytoplasm and nucleus. Intracellular HSP70 and HSC70 function as molecular chaperones, playing important roles in protein folding and transport.

The expression of HSP70 is very low under normal physiological conditions. HSP70 may be released into the blood stream under a number of pathological conditions that lead to widespread cell death. Cardiac surgery is not only associated with an up-regulation of intracellular HSP70 in leukocytes but also may result in increasing liberation of HSP70. Many studies have reported that inducible HSP70 is released into the circulation by the myocardium due to myocardial ischemia-reperfusion after cardiac surgery (Dybdahl et al. 2004, 2002; Szerafin et al. 2008). CPB machine used in cardiac surgery may cause physical damage to the cells in circulation, leading to the induction and release of HSP70. A significant increase of serum levels of HSP70 concentration was observed in the on-pump CABG patients shortly after surgery (Lin et al. 2010). Significantly more HSP70 is released into the circulation after on-pump CABG than after off-pump CABG (Dybdahl et al. 2004, 2002; Lin et al. 2010). This difference may be explained by increased leukocyte and tissue damage or increased complexity of surgical procedures in the on-pump group. Leukocytes, including monocytes, granulocytes, and lymphocytes, are sources of elevated serum HSP70 in the inflammatory response of CABG patients (Fehrenbach et al. 2000; Lin et al. 2010).

Intracellular function of HSPs is molecular chaperone, activity counteracting the unfolding, misfolding and pathological modification of critical proteins in ischemic injury (Williams and Benjamin 2000). Glutamine has been shown to induce HSP70 in the heart, lung and liver, leading to increased nitric oxide (NO) production in an attempt to reduce the CPB-induced inflammatory response (Hayashi et al. 2002). Myocardial biopsies showed up-regulation of HSP70 transcripts and protein at the end of CABG (Schmitt et al. 2002; Szerafin et al. 2008). It appears more likely that most of HSP70 induced in the heart are not released into the circulation, but localized in the heart, which may be due to the surgical trauma, or the local ischemia caused by the clamping of coronary arteries (Lin et al. 2010). Moreover, myocardial stretch or prevention of systolic shortening may lead to increase of myocardial HSP70 in rabbits (Knowlton et al. 1991), indicating that manipulation and stretching of the heart during surgery may cause an increase of HSP70 in the heart. Intracellular HSP70 not only prevents the adverse atrial remodeling leading to AF, but also inhibits its progression from paroxysmal to persistent AF. Both Mandal et al. (2005) and Rammos et al. (2002) studied HSP70 expression levels in atrial tissues of patients in sinus rhythm undergoing cardiac surgery. They consistently showed a lower incidence of POAF in patients with higher atrial intracellular HSP70 expression levels, but not serum HSP70. HSP70 has protective abilities only when it localizes intracellularly and loses its protective role when it is released into the blood (Oc et al. 2008). Higher serum HSP70 levels may be correlated with worsened clinical outcome in various critical conditions (Ganter et al. 2006). Extracellular HSC70 induces myocardial expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, MCP-1 and keratinocyte-derived chemokine (KC, a human IL-8 analog) (Ao et al. 2009; Zou et al. 2008), which may lead to the development of POAF in patients undergoing cardiac surgery. The extracellular molecule can lead to inflammatory cytokine production and has a pro-inflammatory effect in the myocardial innate immune response caused by ischemia-reperfusion, and these effects are interacted with multiple transmembrane immune receptors, such as Toll-like receptor (TLR) 2, TLR4, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), CD91, CD94, CD40 and CC-chemokine receptor 5 (CCR5) (de Jong et al. 2009). The mechanisms of intracellular HSP70 preventing POAF may be due to its preservation of myocardial tissue and cells from stress and ischemia-reperfusion injury.

## 4. Intracellular HSP70 anti-oxidative stress

Previous studies strongly suggested a link between myocardial oxidative stress and AF (Li et al. 2010; Neuman et al. 2007). Products of oxidative stress increased in the plasma and myocardium of patients undergoing cardiac surgery, and before and for 5 days following surgery, while supplementing patients with ascorbate, a known antioxidant, could reduce the incidence of POAF more than 2-fold (Carnes et al. 2001). Right atrial appendages of patients with AF express higher levels of the oxidative markers 3-nitrotyrosine and protein carbonyls compared to the patients with sinus rhythm (Mihm et al. 2001). Left atria of patients with AF exhibit up-regulation of Ras related C3 botulinum toxin substrate-1 (Rac-1) correlating with increased NADPH oxidase activity (Adam et al. 2007). NADPH oxidases are major sources of ROS in myocytes and vascular cells, and involved in hypertension and inflammation states such as atherosclerosis (Griendling et al. 2000), and heart failure (Heymes et al. 2003). Atrial NADPH oxidase activity was shown to be independently associated with an increased risk of AF in patients undergoing conventional CABG (Kim et al. 2008; Oral 2008). ROS produced by NADPH oxidases can promote ROS generation by other sources thereby amplifying total levels of ROS (Murdoch et al. 2006). ROS-mediated activation of p38MAPK which in turn activates Akt, results in over-expression of HSP70 in heat-stressed V79 fibroblasts (Banerjee Mustafi et al. 2009). Previous studies showed that HSP70 inhibits NADPH oxidase activity by regulating superoxide production (Maridonneau-Parini et al. 1988; Polla et al. 1995). HSP70 over-expressing astrocyte cultures down-regulated matrix metalloproteinase-9 after oxygen glucose deprivation, compared to wild-type cell cultures (Lee et al. 2004); in the meanwhile, via activation of the janus tyrosine kinase (JAK)/STAT pathway that may help vascular smooth muscle cells adapt to oxidative stress (Gaudino et al. 2003).

## 5. Intracellular HSP70 has anti-inflammatory effect

### *5.1. Intracellular HSP70 regulates inflammatory response*

In recent years, mechanisms of POAF have been proposed. Increased IL-6 and CRP levels in patients with AF suggest a role of inflammation in the pathogenesis and recurrence of AF (Hatzinikolaou-Kotsakou et al. 2006; Madamanchi et al. 2001; Ucar et al. 2007). Interestingly, there is also increasing evidence to support an association between inflammatory response and POAF after cardiac surgery (Amar et al. 2006; Fontes et al. 2009). Pro-inflammatory cytokines, such as TNF-α,

IL-1, IL-6, and IL-8, play a dominant role in the upstream of the inflammatory cascade. TNF-α, IL-1β, and IL-6 contributed to myocardial injury after ischemia-reperfusion. Systemic inflammatory response after on-pump CABG is involved in the pathogenesis of POAF (Ishida et al. 2006). Long half-life for monocytes in circulation and their ability to transform into long-lived tissue macrophages may also participate in the inflammatory response leading to POAF. In addition, monocyte CD11b up-regulation perioperatively was significantly associated with POAF (Fontes et al. 2005). The molecular is the $\beta_2$-integrin that mediates leukocyte adhesion to vascular endothelial cells and leukocyte migration from the vasculature into tissues (Smith et al. 1989).

Acting as a molecular chaperone in cardiac tissues, intracellular HSP70 is also associated with the orchestration of inflammatory responses after cardiac surgery and plays a role in modulating inflammation caused by ischemia-reperfusion. It was demonstrated that preoperative induction of HSP70 may attenuate CPB-induced inflammatory response by regulating NO synthase (NOS) activity (Hayashi et al. 2002). Intracellular HSP70 can inhibit responses to inflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-10. The experiment of Van Molle et al. showed that heat shock treatment coincides with a strong induction of HSP70, which protects against TNF-induced toxicity in the whole-body mice, yet mice missing the HSP70.1 gene are no longer protected (Van Molle et al. 2002). Liposomally delivered HSP70 protected against IL-1β-induced impaired pancreatic beta-cell function in a rat's diabetes model (Margulis et al. 1991). In addition to regulating the response to inflammatory cytokines, intracellular HSP70 also down-modulates their production. Inflammatory cytokines such as TNF-α and IL-1, are potent, multifunctional cytokine mediators of inflammation and immune responses that are produced primarily by activated monocytes and macrophages. LPS-induced increases in the production of TNF-α, IL-1β, IL-10 and IL-12 are significantly inhibited by the over-expression of HSP70 in human peripheral blood monocyte-derived macrophages (Ding et al. 2001). The study of Su et al. (2010) suggested that HSC70 preserves cardiac function involves the suppression of TLR4 signaling and production of intercellular adhesion molecule-1 (ICAM-1), which has a central role in endotoxemic myocardial dysfunction.

### *5.2. Intracellular HSP70 disrupts NF-κB signaling pathway*

Activation of the NF-κB pathway acts as a functional switch and is critical to the initiation of inflammatory responses by innate cells. Intracellular HSP70 anti-inflammatory activity could account for ischemia-reperfusion injury, for its function of regulating the NF-κB signaling pathway (Voegeli et al. 2008). This may be due to direct interaction of HSP70 with NF-κB proteins, or due to interactions with other proteins in the NF-κB regulatory pathway (Fig. 2). Three NF-κB/Rel family members p65, c-Rel, p50 have been shown to co-precipitate with HSP70 (Guzhova et al. 1997). The p50/p65 heterodimer that is most well studied, is normally sequestered in the cytoplasm by its interaction with inhibitor of κB (I-κB). Phosphorylation of I-κB by the I-κB kinase (IKK) leads to ubiquitination and degradation of I-κB (Gilmore 2006; Matthews and Hay 1995; Tang et al. 2007), which ultimately results in the translocation active NF-κB p50/p65 heterodimers from the cytosol to the nucleus (de Jong et al. 2009). The p65 subunits bind to promotor regions on the chromosome, where it induces expression of a multitude of pro-inflammatory cytokines and enzymes, such as TNF-α, IL-1β, IL-6 and inducible NOS (iNOS) (de Jong et al. 2009). Previous studies suggested that anti-inflammatory

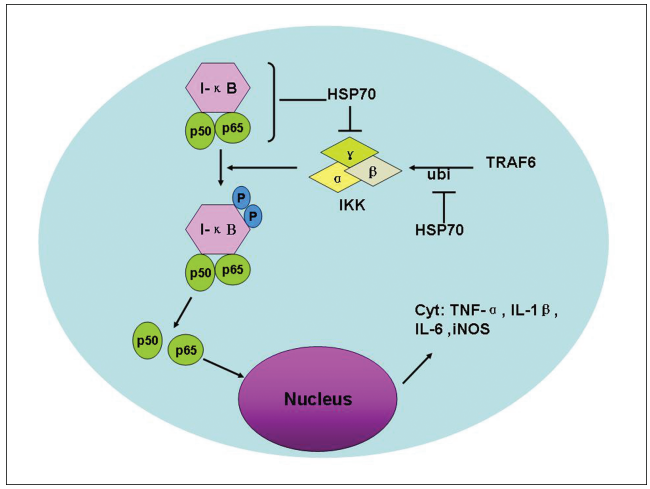


Fig. 2: Intracellular HSP70 disrupts the activation of the transcription factor NF-κB reducing inflammatory cytokine production. It can inhibit the activation of IKK and the ubiquitination of TRAF6, keep the stabilization of the inhibitory complex with I-κB. NF-κB = nuclear factor Kappa B; I-κB = inhibitor of NF-κB; TNF = tumor necrosis factor-α; TRAF6 = TNF associated factor 6; ubi = ubiquitination; IKK = I-κB kinase; IKK complex is composed of three subunits including IKKα, IKKβ and IKKγ. IKKγ is an essential regulatory component of the IKK complex that is necessary for NF-κB activation. p50 and p65 are two of the NF-κB subunits which after release move to the nucleus to act as a transcription factor resulting in activation of inflammatory genes. Cyt = cytokines; IL-1β= interleukin-1β; IL-6 = interleukin-6; iNOS = inducible nitric oxide synthase

effects of intracellular HSP70 may also involve in preserving I-κB complex by interacting with IKK, thereby inhibiting the degradation of I-κB and the subsequent activation of the NF-κB pathway (Chen et al. 2004a; Chen and Currie 2006; Chen et al. 2004b). IKK is composed of two catalytic subunits including IKKα and IKKβ, which could phosphorylate I-κBα and cause its rapid degradation by the ubiquitin-proteasome pathway and thereby mediates NF-κB activation. IKKγ is an essential regulatory component of the IKK complex that is necessary for NF-κB activation. It was reported that the ability of HSP70 to promote TNF-mediated apoptosis was attributed to binding with IKKγ and impairing NF-κB survival signaling (Ran et al. 2004).

In addition, NF-κB activation induced by various stimuli is mediated by members of the TNF receptor-associated factor (TRAF) adapter family. TRAF6 is essential for the activation of NF-κB (Kobayashi et al. 2001). Intracellular HSP70 was demonstrated to inhibit LPS-induced NF-κB activation by binding TRAF6 via the TRAF-C domain and preventing its ubiquitination, thus resulting in inhibition of inflammatory mediator production (Cao et al. 1996). The ubiquitination of TRAF6 is a key step in the activation of the NF-κB pathway via activating downstream kinase IKK (Baud et al. 1999; Cao et al. 1996; Kobayashi et al. 2001). TRAF6 may also play an important role in TLR signaling, which can activate NF-κB by inducing I-κB kinase activity and degradation of I-κBα (Lee and Kim 2007). It appears likely that intracellular HSP70 involves in many levels of the NF-κB pathway to regulate its activation. Apparently independent of its effects on inflammatory responses, the NF-κB pathway has been proved to be involved in cell survival. But the mechanism of intracellular HSP70 modulation on NF-κB activity is still in its infancy and needs further research.

## 6. Intracellular HSP70 inhibits cell death signaling pathways

Ischemia-reperfusion injury during cardiac surgery may lead to cells death (apoptosis/necrosis) or fibrosis in the myocyte, which could associate with the development of POAF. Several

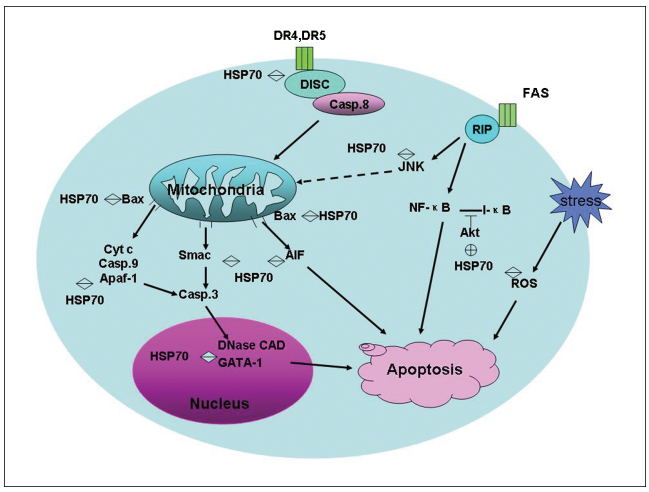


Fig. 3: HSP70 is an important regulator of apoptosis by inhibiting the mitochondrial-dependent pathway at different levels. At the extrinsic cell death receptors level, HSP70 binds to DR4 and DR5, thereby inhibiting TNF-related apoptosis-inducing ligand (TRAIL)-induced assembly and activity of death inducing signaling complex (DISC). At the pre-mitochondrial level, HSP70 inhibits stress-activated kinases. At the mitochondrial stage, HSP70 prevents mitochondrial membrane permeabilization through the blockage of Bax translocation. At the post-mitochondrial level, HSP70 interacts with AIF and Apaf-1 or protects essential nuclear proteins from caspase-3 cleavage. FAS, DR4 and DR5 all belong to the transmembrane receptors of TNF family, which can activate caspase-8 and can proceed independently of the intrinsic pathway, and they also can lead to activation of the intrinsic pathway. JNK is implicated in apoptosis triggered by FAS. Cyt c = cytochrome c; casp = caspase; AIF = apoptosis inducing factor; RIP = receptor interacting protein; JNK = c Jun N terminal Kinase; Akt = PKB = protein kinase B; Apaf-1 = apoptosis protease activating factor 1; DNase CAD = caspase activated DNase; ROS = reactive oxygen species; Smac/DIABLO is a mitochondrial protein that potentiates some forms of apoptosis; Bax is a ember of Bcl-2 family that acts as an anti-apoptotic protein; GATA-1 is a member of the GATA transcription factor family, it plays a role in erythroid development by regulating the switch of fetal hemoglobin to adult hemoglobin

studies have shown that intracellular HSP70 may directly interfere with cell death pathways (Lanneau et al. 2008; Mokhtari et al. 2009; Zhao et al. 2007), which are described for mammals as the extrinsic and the intrinsic pathways. The extrinsic or death receptor-mediated pathway plays a major role in, for example, the immune system, while the intrinsic pathway, also called the mitochondrial-dependent pathway, is activated in response to extracellular or intracellular injury such as DNA damage. Mitochondria are known to have a great impact on the regulation of apoptosis; it relies on the release of mitochondrial pro-apoptotic molecules, opening of the mitochondrial permeability transition pore, and activation of caspases (Gogvadze and Orrenius 2006). Intracellular HSP70 acts as an important regulator of apoptosis, by inhibiting the apoptotic pathways at the extrinsic receptor pathway and at different levels of the mitochondrial-dependent pathway (Fig. 3).

### *6.1. HSP70 targets at the extrinsic cell death receptor pathway*

The extrinsic cell death pathway is initiated upon ligand-receptor interactions at the cell surface including FAS ligand-FAS/APO1 (CD95), TNF-TNF receptors, and TNF-related apoptosis-inducing ligand (TRAIL)-TRAIL receptors (Ozoren and El-Deiry 2003). They can activate caspase-8 and can proceed independently of the intrinsic pathway, and also can lead to activation of the intrinsic pathway (Thorburn 2004). Overall eight extrinsic death receptors have been identified and all belong to the receptors of TNF family, such as TNF-R1, FAS, DR3, TRAIL-R1 (DR4), TRAIL-R2 (KILLER/DR5) and DR6 (Locksley et al. 2001). TRAIL-death receptors can activate both the extrinsic and intrinsic death signaling cascades (Ozoren and El-Deiry 2003). At the extrinsic cell death receptors level, HSP70 binds to DR4 and DR5, thereby inhibiting TRAIL-induced assembly and activity of death inducing signaling complex (DISC) (Guo et al. 2005).

### *6.2. HSP70 targets at the pre-mitochondrial level*

At the pre-mitochondrial level, intracellular HSP70 blocks the activation of c-Jun N-terminal kinase (JNK), which plays a critical role in mitochondrial intrinsic apoptotic pathways by directly modulating the activities of mitochondrial pro- and anti-apoptotic proteins through distinct phosphorylation events (Dhanasekaran and Reddy 2008). HSP70 inhibits JNK dephosphorylation thereby blocking its activation (Lee et al. 2005; Park et al. 2001; Yaglom et al. 1999). The ATPase domain of HSP70 is dispensable for this binding. The deficiency of HSP70 induces JNK and caspase-3 activation in hyperosmolarity-induced apoptosis (Lee et al. 2005). In the meantime, JNK is implicated in apoptosis triggered by FAS (Aggarwal 2003). HSP70 is capable of protecting the cardiomyocyte from stress-induced injury by inhibiting FAS-mediated apoptosis (Zhao et al. 2007). In addition, HSP70 binds to nonphosphorylated protein kinase C (PKC) and Akt, stabilizing both proteins and allowing re-phosphorylation of PKC (Gao and Newton 2002), both of which are well known for protecting from damage-induced cells death. It has been discussed above that HSP70 acts as a regulator for the important anti-apoptotic prosurvival kinase Akt/PKB (Barati et al. 2006; Rafiee et al. 2006), which is involved in inhibiting the activation of NF-κB signaling pathway in the upstream.

### *6.3. HSP70 targets at the mitochondrial level*

At the mitochondrial level, intracellular HSP70 primarily helps to maintain mitochondrial membrane potential. This effect may contribute to the preservation of mitochondrial function and mitochondrial protein import, by preventing mitochondrial membrane permeabilization through the blockage of Bax translocation. Bax belongs to the Bcl-2 family, which comprise both anti-apoptotic members, for example, Bcl-2, Bcl-XL, and Mcl-1, and pro-apoptotic molecules, such as Bax, Bak, and BH3 domain only molecules (Adams and Cory 2007). The balances between pro- and anti-apoptotic members of the Bcl-2 family determine whether cells undergo apoptosis by interacting with intrinsic mitochondrial proteins or regulating the mitochondrial membrane permeability transition. Bcl-2 acts as a key anti-apoptotic protein by its increased expression blocks the release of cytochrome c and apoptosis inducing factor (AIF), and reduces caspase activation. Cytochrome c translocates from the mitochondria to the cytosol, thereby interacts with the CED-4 homologue, apoptosis protease activating factor-1 (Apaf-1), and dATP, to form the apoptosome and activate caspase-9 (Gogvadze and Orrenius 2006; Leist and Jaattela 2001). Caspase-9 activates caspase-3, thereby activates caspase-activated DNase. With cleavage of multiple targets within the cell and DNA fragmentation, apoptotic cell death results. HSP70 can inhibit Bax translocation and insertion into the outer mitochondrial membrane, thereby preventing mitochondrial membrane permeabilization and release of cytochrome c and AIF (Stankiewicz et al. 2005).

### *6.4. HSP70 targets at the post-mitochondrial level*

At the post-mitochondrial level, intracellular HSP70 interacts with AIF and Apaf-1 or protects essential nuclear proteins from caspase-3 cleavage. It interferes with the activity of AIF,

inhibits AIF nuclear translocation and chromatin condensation; the interaction involves a domain of AIF between aminoacids 150 and 228 (Gurbuxani et al. 2003). Furthermore, HSP70 has been shown to interfere with recruitment of procaspase-9 into the apoptosome (Beere et al. 2000; Saleh et al. 2000), and to associate with EndoG and to prevent DNA fragmentation (Kalinowska et al. 2005). Since EndoG can form complexes with AIF, its association with HSP70 could involve AIF as a molecular bridge. It also inhibits release of the pro-apoptotic protein Smac/DIABLO from myocyte mitochondria and apoptosis (Jiang et al. 2005). HSP70 also binds directly to Apaf-1, thereby inhibiting the recruitment of procaspase-9 to the apoptosome and preventing activation of the caspase-dependent apoptosis cascade (Beere et al. 2000; Saleh et al. 2000). In addition, HSP70 rescues cells from a later phase of apoptosis than any known survival protein, downstream caspase-3 activation (Jaattela et al. 1998). During the final phases of apoptosis, chromosomal DNA is digested by the DNase CAD (caspase activated DNase), following activation by caspase-3. The enzymatic activity and proper folding of CAD has been reported to be regulated by HSP70 (Sakahira and Nagata 2002). Another final target of caspase-3 is the transcription factor GATA-1. HSP70 can protect the transcription factor from caspase-3 cleavage in the nucleus (Ribeil et al. 2007).

A growing number of data show now that ROS, mainly produced by the mitochondria (Ali et al. 2010; Criscuolo et al. 2010; Pouvreau 2010), can also be involved in cell death. On the one hand, they can produce oxidative stress leading to cell destruction, as observed during necrosis or the post-mitochondrial phase of apoptosis. On the other hand, ROS derived from mitochondria are also involved in the initiation phase of apoptosis contributing to cell death signaling. Over-expression of HSP72 was shown to enhance the activity of the mitochondrial antioxidant enzyme manganese superoxide dismutase in myocardial cells (Suzuki et al. 2002). HSP70 inhibits NADPH oxidase activity by regulating superoxide production (Maridonneau-Parini et al. 1988; Polla et al. 1995).

## 7. Intracellular HSP70 attenuates $Ca^{2+}$ overload

Under normal conditions, $Ca^{2+}$ enters cardiomyocyte primarily through $I_{Ca,L}$. $Ca^{2+}$ can also enter via the reverse-mode $Na^+/Ca^{2+}$ exchanger (NCX). This $Ca^{2+}$ influx triggers much larger $Ca^{2+}$ release via ryanodine-receptor channels (RyR2) in the sarcoplasmic reticulum through $Ca^{2+}$-induced $Ca^{2+}$ release. Abnormalities in intracellular $Ca^{2+}$ handling may constitute key missing links in AF-initiating focal activity and AF perpetuation by rapidly firing foci and reentry. AF is associated with phosphorylation of phospholamban and RyR2, which might endorse cellular $Ca^{2+}$ overload (El-Armouche et al. 2006; Vest et al. 2005). Atrial tachycardia promotes the induction and maintenance of AF. When AF persists, tissue adaptive responses in the form of structural remodeling will occur. Previous experiments suggested that pre-induction of HSPs by a mild non-toxic heat shock or by the drug geranylgeranylacetone (GGA) prevented atrial remodeling processes induced by tachypacing (Brundel et al. 2006a, b). Activation of intracellular HSP70 attenuated $Ca^{2+}$ overload by increasing the release of $Ca^{2+}$ via RyR2, sped up the uptake of $Ca^{2+}$ via sarcoplasmic reticulum $Ca^{2+}$-ATPase, and removal of $Ca^{2+}$ via NCX in the rat cardiomyocytes (Liu et al. 2006). But Brundel et al.'s study suggested that HSP27, not HSP70 induction by GGA protected HL-1 myocytes against suppression of cellular $Ca^{2+}$ release and contractility resulting from tachypacing (Brundel et al. 2006b). The precise mechanism of intracellular HSP70 attenuates $Ca^{2+}$ overload is still unclear and needs further research.

## 8. Summary

POAF is associated with significant morbidity and increased health care costs. It is a probable consequence of the electrophysiological disturbances that are mainly associated with reperfusion injury and inflammation in patients undergoing cardiac surgery patients. HSP70 is a group of proteins which facilitate the folding of newly, synthesized polypeptides in an ATP dependent manner and play an important role in maintaining the dynamic stability of the intracellular proteome. Besides its function as a molecular chaperone in cardiac tissues, the intracellular molecule is also involved in the orchestration of inflammatory responses after myocardial ischemia reperfusion and can inhibit the pathogenesis of POAF. HSPs could be induced by a mild non-toxic heat shock and the drug GGA. And there is an experiment support that oral GGA is cardioprotective against ischemia reperfusion injury insult through its induction of HSP72 (Ooie et al. 2001). Since intramyocardial HSP70 acts as an anti-arrhythmic and a protective function in cardiac surgery, preoperative induction of HSP70 by myocardial precondition or any drug could be a new and useful strategy for decreasing the incidence of POAF and its associated complications. But more clinical proofs are needed to support that HSP70 holds as a novel target for the prevention and treatment of POAF. In addition, for its extracellular action as pro-inflammatory, the use of plasma HSP70 as a marker in the development of POAF and its possible use in prognosis are being investigated.

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