Proteotoxic stress and circulating cell stress proteins in the cardiovascular diseases

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Abstract The cardiovascular system is one of the major body systems and probably the one most exposed to stress. There is clear evidence that increasing levels of cell stress proteins within the heart is cardioprotective. In addition, there is rapidly emerging evidence that secreted cell stress proteins play a role in the function of the cardiovascular tissues. Those secreted proteins have three potential functions: (1) as normal homeostatic cardiovascular signals (e.g. protein disulphide isomerase); (2) as anti-inflammatory molecules, which are able to inhibit cardiovascular pathology (e.g. Hsp27); and (iii) as pro-inflammatory signals that can induce and promote cardiovascular pathology (e.g. Hsp60). As all of these various proteins may be released—at different rates—and in different cardiovascular diseases—we need to consider the cohort of potential secreted cell stress proteins as a dynamic system (network) that can aid and/or damage the equally dynamic cardiovascular system.

Keywords Cardiovascular disease · Molecular chaperone · Protein-folding catalyst · Circulating cell stress proteins · Inflammation

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Introduction

There is a growing recognition of the contribution of the cell stress response to human health and the association of the failure of this response with human disease and ageing (Anckar and Sistonen 2011). This is opening up new therapeutic approaches, specifically targeting the cell stress response in different cellular compartments, such as the endoplasmic reticulum (McLaughlin and Vandenberg 2011). This focus on the cell stress response in human disease, generally, is recapitulating the story of the role of this response in cardiovascular disease, which began about 20 years ago. The prevalence of cardiovascular disease is still striking. It is estimated that in the USA, coronary artery disease (CAD) is still responsible for one third of deaths in those over 35 (Rosamond et al. 2008; Lloyd-Jones et al. 2010), and one half of middle-aged men and one third of equivalent women will develop some aspects of CAD (Towfighi et al. 2009). While there is still active interest in the role of intracellular cell stress proteins in cardiovascular pathology, evidence is emerging to suggest that secreted cell stress proteins also play important roles in the cardiovascular system in health and disease. It is the potential role of secreted circulating cell stress proteins that is the focus of this shortened review, which will start with a brief historic overview of our understanding of the role of the cell stress response in the cardiovascular tissues and its potential cardioprotective actions.

The cell stress response in the heart

The potential therapeutic role of upregulating cell stress proteins in cardiovascular tissues has been studied since the early 1990s (e.g. Williams and Benjamin 1991). This arose from the finding that heating animals to above normal body temperature

protected them from subsequent myocardial ischaemia (Donnelly et al. 1992). Similar protection occurred in transgenic mice expressing high levels of cardiac Hsp70 (HSPA1A), revealing that this protein is a key cardio-protective agent (Marber et al. 1995). In vitro studies of the upregulation of HSPA1A expression in rat heart cell lines supported the in vivo studies—in that overexpression of this protein protected cells from subsequent exposure to thermal or ischaemic stress (Heads et al. 1994; Mestril et al. 1994). However, overexpression of Hsp56, Hsp60 (HSPD1), or Hsp90 (HSPC1) in a rat heart cell line, or in primary cardiac cells, showed less efficacy, with only HSPC1 overexpression protecting cells against subsequent thermal, but not ischaemic, stress (Cumming et al. 1996a,b). In contrast, overexpression of the small heat shock protein, Hsp27 (HSPB1), was as effective as overexpression of HSPA1A in cultured cells (Martin et al. 1997).

It was subsequently shown that other molecular chaperones were involved in ischaemic preconditioning in whole animals. These include Hsp20 (HSPB6) (Fan et al. 2005), the Hsp70 co-chaperone, CHIP (Zhang et al. 2005), and the Hsp70 protein family member BiP (or Grp78 [HSPA5]) (Shintani-Ishida et al. 2006). Hsp40 (DNAJB1), the co-chaperone of HSPA1A, has also been shown to be involved in the pathology of dilated cardiomyopathy (Hayashi et al. 2006). Thus, inactivation of the gene encoding mitochondrial DNAJB1 in mice resulted in the development of cardiomyopathy, with animals dying within 10 weeks of birth, probably due to the role played by DNAJB1 in mitochondrial biogenesis (Hayashi et al. 2006).

There is also experimental evidence for the deleterious effects of cell stress proteins in cardiac preconditioning and myocardial infarction. Thus, inactivation of the genes coding for two of the major small heat shock proteins, CRYAB (HSPB5) and HSPB2 resulted in significantly decreased infarct size in mice subject to coronary occlusion/reperfusion (Benjamin et al. 2007). Furthermore, recent evidence that the peptidylprolyl isomerase, cyclophilin D, may have negative effects on cardiac survival after stress has emerged. This centres around the finding that the mitochondrial permeability transition pore (MPTP), if opened, results in mitochondrial breakdown, loss of ATP synthesising activity and, if prolonged, necrotic cell death (Halestrap 2010). Cyclophilin D is a crucial component of the MPTP, and there is experimental evidence that targeting/ablation of this protein confers resistance to acute ischaemia–reperfusion injury and post-myocardial infarction heart failure (Lim et al. 2011).

This very limited introduction to the potential role of the cell stress response in the cardiovascular tissues is leading to the viewpoint that targeting the cell stress response in the heart could be of therapeutic benefit. Currently, most interest has focused on endoplasmic reticulum stress (e.g. Minamino et al. 2010) with there being less of an effort to study cell stress

responses in other cell compartments. One agent that is being considered as a potential modulator of the cardiac cell stress response is the plant stilbene resveratrol (3,5,4'-trihydroxy-trans-stilbene). Its potential use in cardiovascular disease is linked to its presence in red wine and its potential for inducing a pre-conditioned state in the heart by triggering the cell stress response (Putics et al. 2008; Csiszar 2011).

A new paradigm emerging suggests that secreted cell stress proteins represent an important new signalling system (Panayi et al. 2004). Currently, there is debate as to whether these secreted stress proteins are part of a so-called damage-associated signalling system or whether they are part of a novel stress-regulated homeostatic mechanism.

Circulating cell stress proteins and their relationship to DAMPs/RAMPs/SAMPs

The cellular immunologist, Charles Janeway, presciently predicted that the innate immune system would have receptors [which he termed pattern-recognition receptors (PRRs)] for recognising evolutionarily conserved components of microbial pathogens. These pathogen molecules [which he termed **pathogen-associated molecular patterns (PAMPs)**] are so essential for microbial life that their structures cannot evolve, and thus, they are the key molecules that are recognised by innate immune cells (Janeway 1989). Another cellular immunologist, Polly Matzinger, introduced the concept that the immune system is controlled by responding to signals that induce damage, rather than non-self signals, and created the Danger Model (hypothesis) of Immunity (Matzinger 2002). Matzinger suggested that the initiation of immune responses was induced by endogenous components released by stressed, damaged or necrotic tissues. These were called 'danger signals', and Matzinger included heat shock proteins, nucleotides, reactive oxygen species, matrix breakdown products and certain cytokines in this category (Gallucci and Matzinger 2001). Other workers have combined the thoughts of Janeway and Matzinger and have proposed the term 'danger-associated molecular patterns (DAMPs)', which is used to describe situations when *host* PRRs recognise *host* components (in this case, DAMPs) (McDermott and Tschopp 2007). These DAMPs have been proposed to be involved in atherogenesis (Miller et al. 2011). Confusingly, the acronym DAMP is now also being used for 'damage-associated molecular patterns'. For this, the functional DAMP can be virtually any form of molecule ranging from protein to glycan to free radical to urate crystal. The primary feature that unites the DAMPs is that they are the product of tissue damage and are recognised by host PRRs. It is assumed that the release of these factors is not under any major form of biological control (Bianchi 2007; Manfredi et al. 2009; Piccinini and Midwood 2010). Other acronyms that are creeping into the

literature are RAMPs for resolution-associated molecular patterns (Shields et al. 2011) and SAMPs for stress-associated molecular patterns (Rubartelli and Sitia 2009).

Precise nomenclature is essential for scientific discussion. However, this –AMP nomenclature is becoming confusing and is also starting to become detached from the original meaning of the term PAMP. The authors would suggest that as cell stress proteins are often found in the circulation of the healthy individual and may, in fact, decrease in concentration in the circulation in patients with disease (e.g. Martin-Ventura et al. 2004; Shamaei-Tousi et al. 2007), they are not classifiable as DAMPs. Furthermore, as will be described, cell stress proteins are released through normal secretory pathways (Table 1), suggesting that their secretion is not pathological. Indeed, one recent review has suggested that extracellular ubiquitin is actually an ‘endogenous opponent’ of the DAMPs (Majetschak 2010). Finally, DAMPs are now being classified on the basis that they bind to the receptors that Janeway termed PRRs, such as the Toll-like receptors, the NOD receptors and so on (Kumar et al.

2011). However, many secreted cell stress proteins bind to receptors other than the PRRs. Thus, we would suggest that it is premature to classify exogenous cell stress proteins as DAMPs, RAMPs or SAMPs. While still premature, the authors would hypothesize that extracellular cell stress proteins function as a unified homeostatic system, which mirrors the intracellular stress response of the body cells, and that these networks of proteins function to: (1) broadcast the fact that there is stress within the organism (Maguire et al. 2002) and (2) modulate the actions of cells in order to facilitate the return to normal homeostasis (Fig. 1). Of course, this only relates to situations in which ‘normal’ levels of the cell stress proteins are present. The induction of tissue pathology could ensue if ‘normal levels’ of these stress proteins are either exceeded or not reached. It is important to emphasize that the authors are proposing the hypothesis that secreted cell stress proteins are part of an extracellular homeostatic signalling system and that it is the entire system (i.e. all the secreted cell stress proteins), which needs to be understood in order to comprehend the

Table 1 Secreted molecular chaperones and protein folding catalysts and mechanism of secretion

Protein	Secreted in culture	Present in blood	Secretion mechanism
Ubiquitin	Yes	Yes	?
Trx80	Yes	Yes	?
Chaperonin 10	Yes	Yes	?
Thioredoxin	Yes	Yes	Unique mechanism ^a
Glutaredoxin	?	Yes	?
Peroxiredoxin	Yes	Yes	Brefeldin-insensitive non-classical pathway ^b
PPIs	Yes	Yes	Vesicle-mediated process ^c
Hsp27	Yes	Yes	?
Protein disulphide Isomerase (PDI)	Yes	Yes	?
Hsp60	Yes	Yes	Secreted in exosomes ^d
Hsp70 (HSPA1A)	Yes	Yes	Potentially by two pathways—one exosomal, the other similar to thioredoxin ^{e,f}
HspB1	Yes	Yes	Possibly two mechanisms including classical secretion ^g
BiP	Yes	Yes	Release inhibited by brefeldin, an inhibitor of the classic pathway of protein secretion ^h
Gp96	Yes	Yes	Vesicular transport pathway ⁱ
Hsp90	Yes	Yes	Possibly via exosomes ^h
Clusterin	Yes	Yes	Classical secretion pathway ^k

^a Rubartelli et al. (1992)

^b Chang et al. (2006)

^c Suzuki et al. (2006)

^d Gupta and Knowlton (2007)

^e Lancaster and Febbraio (2005)

^f Mambula and Calderwood (2006)

^g Evdonin et al. (2009)

^h Xiao et al. (1999)

ⁱ Robert et al. (1999)

^j Cheng et al. (2008)

^k Bhat and Brunngraber (1985)

actions of the individual proteins in cardiovascular health and disease. The analysis of proteins in isolation is unlikely to be a good-and-true-reflection of the *in vivo*, physiological system.

Circulating cell stress proteins in cardiovascular disease

On the basis of the literature, the authors have proposed a hypothetical role for the various cell stress proteins found within the human circulation (Table 2). These fall into the proposed categories: homeostatic, cardioprotective, cardiopathologic and (currently) unknown. Note that any one cell stress protein can fit into more than one category. The literature on these proteins will be subdivided into these four categories. To conform to space limits of the journal, much of this material will be included in a supplement.

Circulating cell stress proteins with a homeostatic function

The information on the cell stress proteins with a potential homeostatic role in the cardiovascular system is found in the Supplement to this article. These proteins include protein disulphide isomerase, which plays a major role in platelet aggregation and fibrin generation, and the peroxiredoxins, which have anti-inflammatory properties and a protective

role in aortic abdominal aneurysm and play a unique role as circadian rhythm controllers in erythrocytes.

Circulating cell stress proteins with cardioprotective functions

A number of unusual secreted cell stress proteins have been identified as having potential cardioprotective function as a result of their agonist–receptor interactions (Table 3). These include the small ubiquitous protein, ubiquitin, whose major function is to designate intracellular unfolded/damaged proteins for proteosomal degradation. Surprisingly, this protein is also secreted and can be found in relatively high concentrations in the blood, where it is proposed to have anti-DAMP properties (Majetschak 2010). Thioredoxin is both a cell stress protein and a redox protein, and there are multiple studies in animals and humans suggesting the therapeutic potential of this protein. The small heat shock protein, Hsp27 (HSPB1), is an inducer of IL-10 and inhibits monocyte differentiation into dendritic cells (De et al. 2000). Clinical studies suggest that it is atheroprotective, and animal models are revealing the connection between secreted Hsp27 and oestrogen signalling (Rayner et al. 2008). The major heat shock protein, Hsp70 (HSPA1A) is thought to exhibit pro-inflammatory actions, and there is controversy in the literature over its role in cardiovascular pathology. However, in one carefully controlled study an inverse correlation between

Fig. 1 Schematic representation of the role played by secreted molecular chaperones in maintaining a relationship between intracellular cellular stress and extracellular responses. It is proposed that different stressors produce different patterns of molecular chaperones and PFCs and that this is somehow linked to the secretion of these protein-folding proteins. These different patterns of secreted proteins generate a stress-focused system, which can feed back onto the producing cells (autocrine

signalling) and can also interact with nearby cells (paracrine signalling) and, depending on the circulating life of the proteins, can exert agonist effects in other parts of the organism (endocrine signalling). The key to understanding the physiological and pathophysiological role of these secreted cell stress proteins is as a system (network) of cellular signals analogous to the cytokine networks that permeate the body

Table 2 Hypothesised functions of the various secreted cell stress proteins

Homeostatic	Cardioprotective	Cardiopathologic	Unknown
PDI	Ubiquitin	MIF	Trx80
Peroxiredoxins	Thioredoxin	Cyclophilins	Cpn10/Hsp10
	Peroxiredoxin	Cpn60/Hsp60	Glutaredoxin
	Hsp27	Gp96	BiP
	Hsp70	Hsp70	HspB1
	Clusterin	Hsp90	

circulating levels of HSPA1A and coronary artery pathology has been defined, suggesting that this protein is cardioprotective (Zhu et al. 2003). Finally, clusterin, an intriguing molecular chaperone (Wyatt et al. 2011), which is found naturally in the circulation and is likely to be the first of many such proteins (to inhibit formation of particular DAMPs), also has biological actions that make in cardioprotective. More detailed information of each of these proteins is provided in the appropriate [Supplementary section](#).

Circulating cell stress proteins with a cardiopathological function

The circulating cell stress proteins described in this section have been associated with cardiovascular disease (Table 4), and it would have been sensible to include the HSPA1A family proteins as there is evidence both for and against the role of these proteins in heart disease. However, the HSPA1A protein has been included in the cardioprotective grouping and described above. Other secreted and circulating cell stress proteins with potential for inducing cardiopathological changes include the unexpected protein, macrophage migration inhibitory factor (MIF), which has been shown to be a molecular chaperone with a major influence on the vasculature—define largely as a result of animal studies (Schober et al. 2004). Cell culture, animal model and

clinical studies of the peptidylprolyl isomerase, cyclophilin (Cyp)A, also indicate that the agonist receptor pairing of CypA and its receptor, CD147 is involved in cardiovascular pathology and a potential therapeutic target in cardiovascular disease (Yurchenko et al. 2010). The molecular chaperone, chaperonin 60, has had a long history as a putative pathogenic factor in atherosclerosis. More recent clinical studies are revealing strong correlations between levels of this protein and cardiovascular pathology. Finally, there is emerging evidence for the potential role of the large molecular chaperones, Gp96 and Hsp90, in cardiovascular disease. More details of the biomedical roles of these proteins is found in the [Supplementary section](#).

Circulating cell stress proteins with uncertain roles in cardiovascular pathology

A number of cell stress proteins found in the circulation have not, as yet, been shown to have any role in the operation of the cardiovascular system. However, their biological actions suggest that they could have such function. The description of these proteins is found in the Supplement to this article.

Conclusion

Stress is ubiquitous and leads to protein misfolding. All cells are subject to stress at some time in their life spans. However, it is likely that the cardiovascular system is the most stressed of the bodies organ systems, as from birth, it is subject to: (1) mechanical stress as a result of the pulsatile blood flow with concomitant shear stress generated by the heart beat (Shyu 2009), (2) oxidative stress that seems particularly important in cardiovascular pathology (Touyz and Briones 2011) and (3) all the other stresses that the other tissues of the body are subject, including infections. To combat the proteotoxic effects of cellular stress, a large number of molecular chaperones and protein-folding catalysts (collectively cell stress proteins) have evolved. The former are involved in the folding of nascent proteins, the refolding or unfolded

Table 3 Circulating cell stress proteins with proposed cardioprotective function

Protein	Protective ability	Reference
Ubiquitin	Protein with anti-DAMP/anti-inflammatory activity	Majetschak 2010
Thioredoxin	Circulating levels are inversely correlated with cardiovascular pathology. Animals studies show potent anti-inflammatory actions	e.g. Martinez-Pinna et al. 2010
Hsp27	Animal and patient studies suggest anti-inflammatory action of Hsp27 can inhibit cardiovascular pathology	e.g. Martin-Ventura et al. 2004
Hsp70	Conflicting data, but one clinical study reveals inverse correlation Between circulating Hsp70 levels and cardiovascular pathology	e.g. Zhu et al. 2003
Clusterin	Animal and clinical studies suggest a cardioprotective function to this naturally circulating molecular chaperone	e.g. van Dijk et al. 2010

Table 4 Circulating cell stress proteins with proposed cardiopathological function

Protein	Cardiopathological function	References
MIF	Largely based on cardiopathological studies in animals	e.g. Noels et al. 2009
Cyclophilins	CypA and its receptor, CD147, involved in foam cell formation CypA knockout unable to develop AAA	Seizer et al. 2010 Sato et al. 2009
Chaperonin 60	Largely clinical studies revealing positive correlations between circulating Cpn60 levels and signs of cardiovascular pathology	Many references (see text)
Gp96	Administration prolongs cardiac allografts	Slack et al. 2007
Hsp90	Increased secretion in patients with atherosclerosis	Businaro et al. 2009

proteins, the inhibition of protein aggregation and the solubilisation of protein aggregates (Gershenson and Gierasch 2011). The PFCs are also involved in protein folding, but some can also function in an anti-oxidant capacity (Yamawaki and Berk 2005). Cell stress proteins are also well known antigens and immunogens with a wide range of immunomodulatory actions, which can contribute to homeostasis and/or tissue pathology. This is, to some extent, a reflection of the fact that infectious microbial pathogens also contain homologs of the eukaryotic cell stress proteins, which can give

rise to cross-reactive immunity and potential autoimmunity (Yokota and Fujii 2010).

In addition to acting as antigens and immunogens, it is now well established that a growing number of cell stress proteins are secreted from cells, by a variety of known secretion mechanisms (Table 1), and can exist in the circulation and function, mainly as intercellular signalling proteins, by binding to agonist receptors on target cells (Henderson and Pockley 2010). It is this most recently discovered facet of cell stress protein biology that has

Fig. 2 Role of molecular chaperones and PFCs in cardiovascular physiology and pathophysiology. This diagram reveals the history of our developing understanding of cell stress proteins over the past 20 years. Originally, these proteins were believed to be only found within cells (1). However, soon after the discovery of the cell stress proteins, it was discovered that some, at least, could be secreted (2) and function as autocrine (3) and paracrine (4) signals for a variety of cells including leucocytes and vascular endothelial cells. Like the cytokines,

it was then found that cell stress proteins could exist on the outer plasma membrane where they act as receptors for a variety of ligands (5). In more recent years, it has been established that a growing number of cell stress proteins can be secreted into the blood and other body fluids where they function as endocrine signals (6) able to influence end-organ function. At the present time, we know most about the interaction of these proteins with the cells and tissues of the cardiovascular system

begun to attract the attention of the cardiovascular community. It is this ability to act as signalling agonists that marks these molecules as moonlighting proteins (Henderson and Martin 2011). It must be emphasised that it is still too early to make sensible predictions as to the role of secreted cell stress proteins in cardiovascular biology and medicine. However, at this particular juncture, the available evidence from the literature can be interpreted as suggesting that secreted molecular chaperones and PFCs have, as a minimum, three functions in relation to the cardiovascular system (Table 2). The first is as homeostatic signals, which control particular aspects of cardiovascular physiology (Fig. 2). The current best example of this is the role of secreted PDI in thrombus formation and its role in controlling the function of tissue factor (Furie 2009). It is expected that, based on the study of human cardiovascular disease, that other secreted cell stress proteins may be found to have homeostatic functions. The second function for secreted cell stress proteins is as anti-inflammatory proteins. This is emphasised by the current clinical testing of a number of molecular chaperones and PFCs including HSP1A, an HSPD1 peptide, HSPA5 and thioredoxin. In the cardiovascular situation, the available evidence would suggest that secreted ubiquitin, thioredoxin, HSPB1, HSPA1A and possibly clusterin are ‘cardioprotective’ or can inhibit cardiovascular pathology. This leaves the third function for the secreted cell stress protein—as a potential pathological factor in cardiovascular disease. Again, the evidence is still weak, but it appears as if secreted MIF, the cyclophilins, HSPD1 and HSPC1 contribute to cardiovascular pathology (Table 2).

To add to the complication of the secretion of cell stress proteins, it must be emphasised that a number of these proteins can also act as receptors when they are on the cell surface. In this context, these proteins largely act as receptors for the binding of bacteria and viruses or for the binding of bacterial components such as LPS. Cell surface HSPA5 binds to a number of viruses including Borna virus (Honda et al. 2009). HSPD1 on human cells can bind to pathogenic, but not non-pathogenic *L. monocytogenes* (Jagadeesan et al. 2010). The binding of bacteria by HSPC4 has already been described. In addition to this, HSPA1A and HSPC1 are reported to be part of a cell surface complex that is able to bind to and respond to LPS (Triantafilou et al. 2001).

We now have the situation where cellular stress proteins are secreted and may have distinct roles to play in promoting or inhibiting cardiovascular pathology. To fully understand what is happening, we need to treat these secreted proteins, not singly, but as a system (network) and to measure all of these distinct proteins in the same patients and under the same conditions. Other factors that need to be taken into account is the titres of antibodies to these various proteins (these may inhibit or exacerbate biological activity), other binding proteins and ligands (of which there are many) and

the rate of turnover of these secreted proteins in the blood. A key requirement is that the nature of the cell surface signalling receptors for each of these secreted cell stress proteins be identified to generate the other half of the biological signal. With this information, it will be possible to start to model the secreted cell stress system—the stress-ome and to define its role in the initiation and development of cardiovascular disease and the response to cardiovascular therapies.

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