Review

The cardiokine story unfolds: ischemic stress-induced protein secretion in the heart

Shirin Doroudgar and Christopher C. Glembotski

SDSU Heart Institute and the Department of Biology, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA

Intercellular communication depends on many factors, including proteins released via the classical or non-classical secretory pathways, many of which must be properly folded to be functional. Owing to their adverse effects on the secretion machinery, stresses such as ischemia can impair the folding of secreted proteins. Paradoxically, cells rely on secreted proteins to mount a response designed to resist stress-induced damage. This review examines this paradox using proteins secreted from the heart, cardiokines, as examples, and focuses on how the ischemic heart maintains or even increases the release of select cardiokines that regulate important cellular processes in the heart, including excitation–contraction coupling, hypertrophic growth, myocardial remodeling and stem cell function, in ways that moderate ischemic damage and enhance cardiac repair.

Introduction

A key process in the development, growth, differentiation, function and potentially the repair and regeneration of tissues is cell communication via paracrine, autocrine and endocrine signaling [1]. Such intercellular signaling is of particular importance in the heart, where it is required for normal cardiac development and function [2], and where it plays a central role in remodeling and potential repair of damaged and diseased myocardium [3]. The importance of intercellular signaling is highlighted by the fact that a majority of pharmaceutical therapies developed to date target either the signaling substances directly or the receptors to which they bind [4]. Accordingly, it is likely that most molecular-based therapeutic approaches developed in the near future, including those that will advance the utility and feasibility of stem cells in tissue regeneration, will be based on cell signaling.

Protein secretion and cardiokines

The synthesis and active secretion of molecules, including proteins, are critical for autocrine, paracrine and endocrine signaling. Active secretion is the cellular process by which most proteins destined for release are transferred across the plasma membrane. Active secretion occurs via two major protein secretion pathways, depending on whether the proteins that are released are synthesized on cytosolic or endoplasmic reticulum (ER)-associated ribosomes. By contrast, passive secretion describes the release of cellular contents following necrotic tissue damage.

Proteins secreted from adipose tissue are collectively called adipokines, whereas those secreted from skeletal muscle are called myokines [5,6]. By analogy, the term cardiokine, which has also been called cardiomyokine [7], can be used to describe proteins secreted from the heart. Cardiokines, which are secreted from cardiac myocytes and non-myocytes, as well as from other cells that infiltrate the injured and remodeling heart, have autocrine, paracrine and endocrine functions (Figure 1).

Although the total number of cardiokines is not yet known, it can be estimated. The total number of secreted proteins encoded by the genomes of several species, including the human genome, has been conservatively estimated* to be approximately 1000–2000 [8,9]. Several experimental approaches have estimated the number of putative cardiokines to be between 30 and 60 [10–12]. The cardiokines identified in these and other studies are known or predicted to play many roles, including maintaining normal heart and cardiovascular function (e.g. growth factors and endocrine hormones), sending signals of distress and exacerbating pathology (e.g. cytokines), and remodeling and healing the damaged heart (e.g. extracellular matrix proteins and stem cell-homing factors). Thus, a change in the repertoire of cardiokines not only serves as an indicator of pathology, but might also play a critical role in determining how the heart responds, acutely and chronically, to potentially damaging stress such as ischemia.

Potential effects of ischemia on cardiokine secretion

Ischemia is the deprivation of oxygen and nutrient delivery due to inadequate blood flow, often resulting from atherosclerosis. In the heart, prolonged ischemia can cause tissue damage or myocardial infarction, which can be lethal. The damaged tissue passively releases heart-derived proteins into the circulation [13]. By contrast, surviving myocardium bordering the infarct is exposed to mild ischemia and is stressed, but remains capable of actively secreting cardiokines [14]. In fact, as part of the adaptive response of the

Corresponding author: Glembotski, C.C. (cglembotski@sciences.sdsu.edu)

* This estimate includes only those proteins with N-terminal signal sequences that are targeted or predicted to be targeted to the classical secretory pathway via those signal sequences. However, only approximately half of the proteins that are secreted via this pathway have canonical signal sequences. Moreover, significant numbers of proteins are secreted via the nonclassical pathway and they were not included in these estimates. For more on classical and nonclassical secretion, see Box 1.

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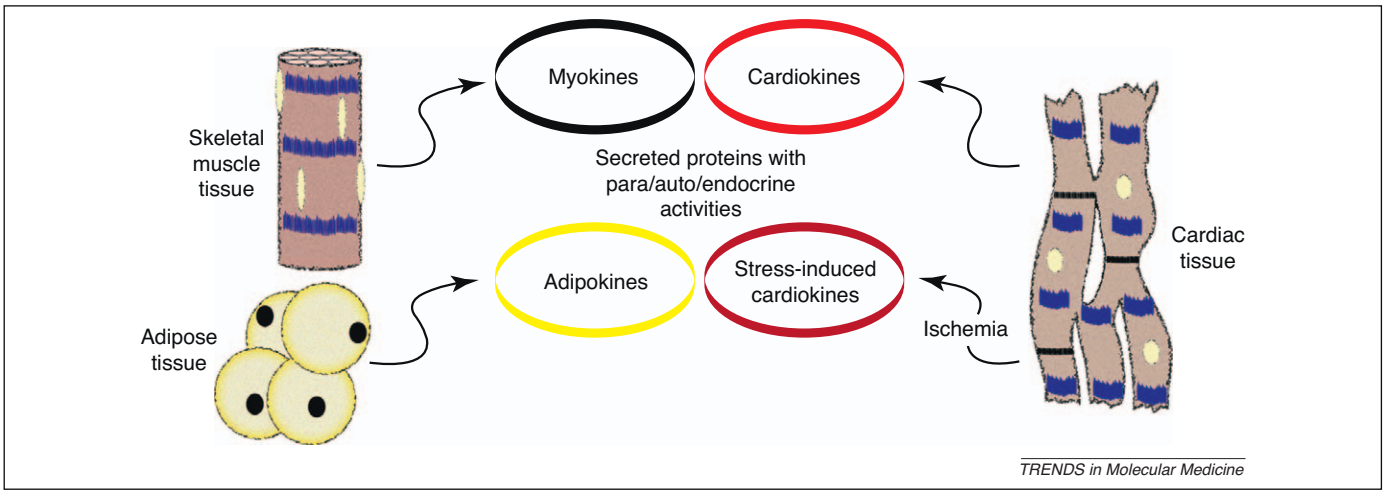

Figure 1. Cardiokines. By analogy to the release of myokines from skeletal muscle tissue and adipokines from adipose tissue, the term cardiokine can be used to describe proteins released by the heart that exert autocrine, paracrine and/or endocrine functions. Stress-induced cardiokines are a subset of cardiokines whose expression and secretion increase upon stresses that impair the release of many other cardiokines.

myocardium, ischemia-inducible cardiokines can help to preserve myocardial tissue structure and function [15], which underscores the importance of their secretion. For example, mild ischemia increases active secretion of the cardiokines enkephalin and calcitonin gene-related peptide, which contribute to preserving cardiac function [16,17]. Accordingly, cardiokines that provide beneficial protective effects can be actively secreted from the ischemic heart. To better understand the impact of ischemia-inducible cardiokines, it is critical to examine the mechanisms of their synthesis and secretion, as well as the potential impact of ischemic stress on cardiokine production and release.

Mechanisms of protein secretion

As for proteins released from any tissue, cardiokines are secreted via either the classical (conventional) ER-dependent secretory pathway [18] or the nonclassical (unconventional) ER-independent secretory pathway [19]; these seem to be distinct and separate pathways. Although the subcellular pathway taken by many secreted proteins has been determined experimentally, there are others for which such evidence does not exist. For proteins in the latter group, informatics approaches can provide an initial determination of whether they are likely to be released through the classical or nonclassical pathway (Box 1).

Secretion: Classical secretory pathway

A defining characteristic of most proteins secreted via the classical secretory pathway is that they are synthesized on ER-associated ribosomes (Figure 2a, Step 1). Approximately half of the proteins secreted via the classical secretory pathway are synthesized initially with ER-targeting signal sequences that are recognized by the macromolecular signal recognition particle complex, which facilitates ribosome docking to the ER. These proteins are co-translationally translocated into the ER lumen (Figure 2a, Step 2), where translation is completed, disulfide bonds are formed and glycosylation begins. Classically secreted proteins are routed through the ER–Golgi intermediate compartment (ERGIC), then through the Golgi (Figure 2a, Step 3) before being packaged into secretory vesicles that eventually fuse with the plasma membrane (Figure 2a, Step 4), which leads to release of the mature, properly folded, and in some cases glycosylated, biologically active cardiokines. In some cases, such as in ventricular myocytes, soon after they are packaged into secretion

Box 1. Predicting the secretory route for a protein

There are numerous secreted protein prediction programs that can be used to determine whether a protein is secreted via the classical pathway; a recent study compared eight such programs [88]. In general, these programs begin with determining whether a protein has an N-terminal structure that conforms to the predictive rules for ER-targeting signal sequences. A commonly used initial paradigm is to examine the predicted proprotein sequence for an N-terminal signal sequence using SignalP 3.0 or a similar algorithm. Given a positive result, a transmembrane domain search can be carried out using TMHMM 2.0. Given a negative result from the transmembrane domain search, a search for the presence of a C-terminal ER-retention signal, the four-amino-acid motif KDEL or a KDEL-like motif [89], can be performed. In general, proteins with a predicted ER-targeting signal sequence, no transmembrane domains, and no C-terminal KDEL or KDEL-like sequence are good candidates for secretion via the classical pathway. Structural and biological predictions are available for most proteins; see, for example, UniProt (www.uniprot.org/). It should be noted that some ER-targeted proteins do not have N-terminal signal sequences; thus, a protein without such a sequence could still be ER-targeted [90]. Moreover, proteins that do not have a KDEL or KDEL-like C-terminus can be retained in the ER or Golgi by associating with other ER or Golgi resident proteins that have transmembrane domains or KDEL sequences. Using a variety of secreted protein prediction programs, it has been estimated that between 1000 and 2000 proteins in the mammalian proteome have features consistent with their secretion via the classical secretory pathway [8,9].

The lack of structural commonality among proteins secreted via the nonclassical pathway makes the development of secretion prediction algorithms much more difficult than for classically secreted proteins. Nevertheless, several algorithms have been developed for this purpose, including SecretomeP [91], SecretP [92] and SPRED [93]. They all use combinations of features to obtain information about structural features, post-translational modifications and subcellular localization of a protein. Although estimates of the total number of proteins secreted via the nonclassical pathway vary, one recent report lists 92 human proteins that have been confirmed as being secreted via this pathway [92].

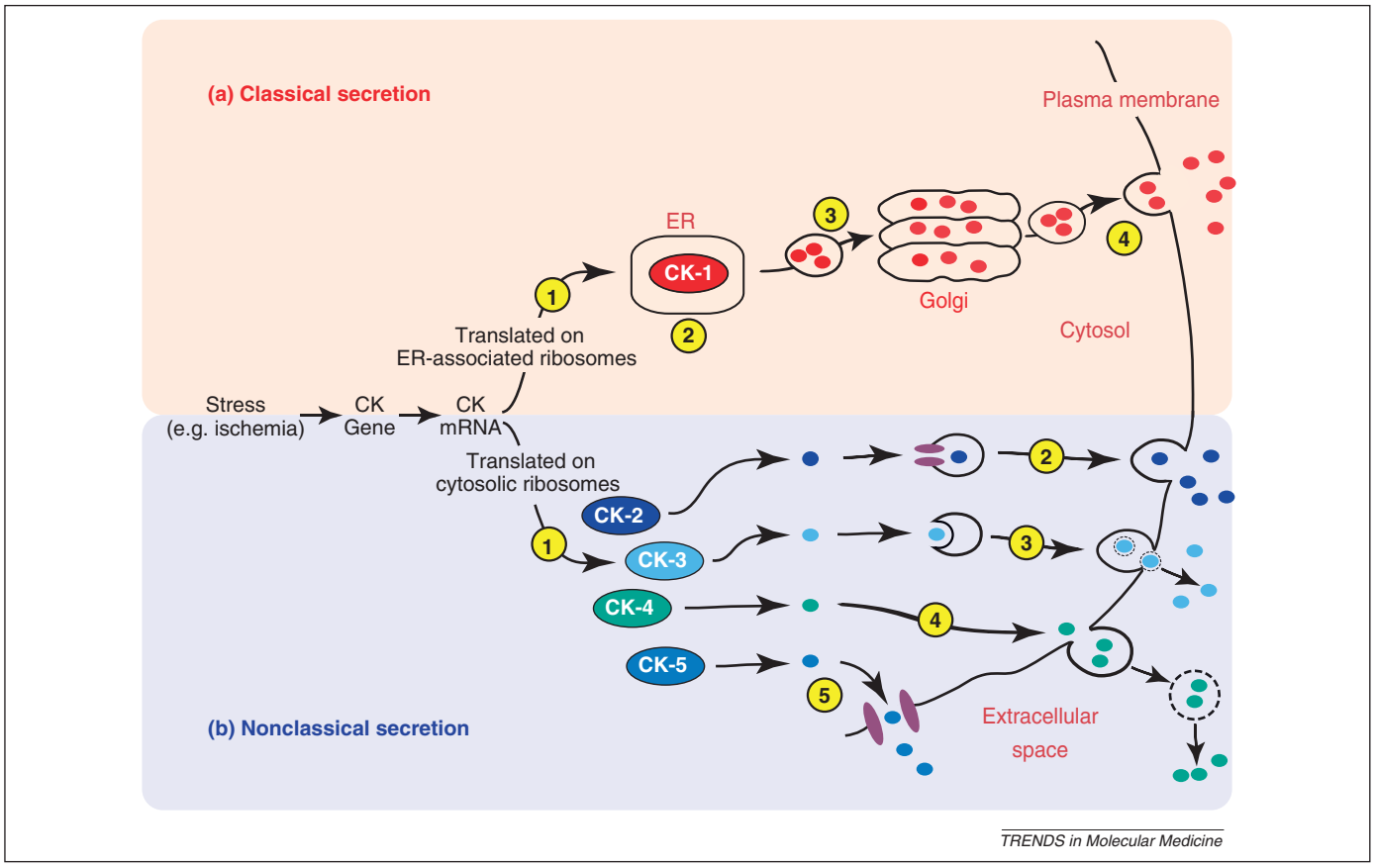

Figure 2. Classical and nonclassical secretory pathways. Proteins are actively secreted via either the classical or nonclassical secretory pathways. **(a)** In the case of the classical secretory pathway, cardiokine (CK) mRNAs are translated on ER-associated ribosomes **(1)**. The resulting proteins, depicted here as CK1, are initially localized to the ER lumen (Step 2), then transported in vesicles of the ER–Golgi intermediate compartment (ERGIC) to the Golgi lumen (Step 3), from which they are eventually released via secretory vesicle fusion with the plasma membrane (Step 4). **(b)** In the case of the nonclassical secretory pathway, CK mRNAs are translated on cytosolic ribosomes (Step 1). The resulting proteins, depicted here as CK2, 3, 4 and 5, are destined for secretion via one of four nonclassical secretory pathways. Nonclassical CK secretion takes place via vesicle-mediated (CK2–4), or nonvesicle-mediated pathways, as follows: **(2)** endocytic secretory lysosomes (CK2); **(3)** exosome-derived multivesicular bodies (CK3); **(4)** microvesicles that are shed from the cell surface (CK4); and **(5)** direct translocation of cytosolic proteins across the plasma membrane through complex protein-conducting channels (CK5). A portion of panel b and the nomenclature used to describe the nonclassical secretory routes were inspired by a review written by Walter Nickel [94].

vesicles, cardiokines are secreted in a constitutive manner. However, in other cases, such as in atrial myocytes, most cardiokines are packaged into secretory granules and stored until the appropriate stimulus activates their secretion [20].

Secretion: Nonclassical secretory pathway

In contrast to proteins released via the classical pathway, those secreted via the nonclassical secretory pathway are synthesized on cytosolic ribosomes and must be transported across the plasma membrane independently of the ER–Golgi pathway (Figure 2b, Step 1). This secretory pathway was initially discovered for interleukin (IL)-1β and galectin-1. Since then, the list of proteins secreted via the nonclassical secretory pathway has increased to include approximately 100 hormones, growth factors, cytokines, chemokines, viral proteins and parasitic proteins. At least four main types of nonclassical secretion have been characterized [21]; in each it is clear that the main barrier to be overcome in the active release of proteins is transfer across the plasma membrane. Accordingly, to assist in this transfer, three types of nonclassical secretion involve import of the protein into various types of vesicles, including endocytic secretory lysosomes (Figure 2b, Step 2), exosome-derived multivesicular bodies (Figure 2b, Step 3) and microvesicles that are shed from the cell surface (Figure 2b, Step 4). The fourth type of nonclassical secretion is nonvesicular and involves the direct translocation of cytosolic protein complexes across the plasma membrane, sometimes through channels or pores (Figure 2b, Step 5).

Cardiokine folding during ischemic stress

Many secreted proteins must fold into the appropriate three-dimensional configuration to be functional [22]. For most of these proteins, folding takes place co-translationally [23]; accordingly, disruptions to this process can cause the nascent proteins to fold improperly or misfold. Numerous studies have focused on determining how ischemic stress affects the function of the folding machinery and client protein folding; these studies have shown that ischemia decreases calcium levels in the ER and sarcoplasmic reticulum, alters redox status and reduces ATP levels, each of which can conspire to cripple the folding and secretion of cardiokines [24]. However, cells have evolved elaborate mechanisms to fortify the secretion machinery to maintain and perhaps even upregulate the secretion of certain proteins that can help to minimize the potentially damaging effects of ischemia [25]. Moreover, studies over the last 50 years have shown that a small number of proteins exhibit intrinsically disordered or unfolded structures, such as the

molten globule (see below), so they might require less folding for optimal function [26]. In fact, it has been postulated that higher-order folding limits the functionality of certain proteins, and therefore some proteins must maintain a partially unfolded state to retain function [27]. The question arises as to what differentiates a functional from a nonfunctional unfolded protein, with the latter needing to be degraded to avoid proteotoxicity. It is believed that any unfolded protein targeted for degradation contains solvated hydrophobic amino acids, which are detected by protein degradation systems such as the ubiquitin–proteasome pathway [27]. Thus, functional unfolded proteins lack the hydrophobic residues that target them for degradation, but they remain intrinsically unstructured.

An example of a secreted protein that must be unfolded is the anti-inflammatory procoagulant protein secreted from the rat seminal vesicle epithelium (SV-IV), which exhibits regions of intrinsically disordered structure on which its biological activity depends [28]. In addition, sclerostin, a secreted glycoprotein that is a negative regulator of Wnt signaling, exhibits unstructured features that are believed to be important for its binding to target proteins [29]. Thus, it seems that secreted proteins exhibit varied requirements for folding; accordingly, a range of strategies are used by eukaryotic cells to respond to conditions that threaten the folding of proteins released via the classical or nonclassical secretory pathways.

Folding: Classical secretory pathway

Although some proteins secreted via the classical pathway require folding for activity, most small peptides do not require folding [30]. However, the proteins responsible for the production of such peptides are large and structurally complex, and therefore they require folding for optimal function. This implies that conditions that affect folding can still impact on the production of small secreted peptides that do not themselves require extensive folding for activity. A case in point is the cardiokine atrial natriuretic peptide (ANP). Although the biologically active form of ANP is 28 amino acids in length and does not adopt a complex three-dimensional structure [31], it is generated by proteolytic cleavage from a 126-amino-acid precursor, pro-ANP [32]. Conditions that affect protein folding in the heart also impact the folding and function of the protease responsible for processing pro-ANP in ways that decrease the efficiency of ANP generation in cardiac myocytes [33]. Therefore, based on this example, it is apparent that ischemic stress can have direct and/or indirect effects on the folding of cardiokines.

To control the potentially damaging effects of protein misfolding in the ER, complex protein quality control machinery is associated with the classical secretion pathway. This machinery facilitates proteasome-mediated degradation of misfolded and unfolded proteins under basal conditions, and thus avoids the cellular toxicity associated with accumulation of dysfunctional proteins [34]. However, during ischemic stresses of greater strength and/or duration, these protein degradation pathways can be overwhelmed, which leads to accumulation of misfolded proteins in the ER and activates a conserved intracellular signaling pathway called the unfolded protein response, also known as the ER stress response [35]. In addition to secreted proteins, the ER stress response can be activated by the accumulation of misfolded transmembrane proteins, which include many receptors that are made in the ER and proceed through the classical secretory pathway to their final membrane destinations. Thus, misfolding of secreted proteins, as well as transmembrane receptors, in the classical secretory pathway can influence cell-to-cell communication.

It was first shown that the ER stress response is activated in cardiac myocytes when sarcoplasmic reticulum calcium stores are depleted [36], a condition that resembles an effect of ischemia. Later studies demonstrated activation of the ER stress response in cultured cardiac myocytes by conditions that simulate ischemia [37] and in ischemic myocardium [38]. In the heart, the ER stress response comprises a complex intracellular signaling pathway that is essential for maintaining a robust classical secretory pathway under adverse conditions. ER stress results in the induction of numerous genes, many of which are anti-growth-oriented; in the heart, this aspect of ER stress exerts anti-hypertrophic growth effects [11], which suggests that the ER stress response might protect against the pathological hypertrophy that often occurs in the diseased heart.

The ER stress response ensures efficient synthesis and folding of cardiokines during ischemic stress. One way this is accomplished is via ER stress-mediated decreases in the translation of mRNAs encoding proteins that are not essential for the stress response. ER stress also leads to the activation of several transcription factors, such as activating transcription factors 4 and 6 (ATF4 and ATF6), as well as X-box binding protein 1 (XBP1); these factors increase the transcription of genes that encode numerous proteins, many of which fortify the protein folding and secretory machinery [35]. Moreover, ATF6 and XBP1 are responsible for upregulating the expression of some ischemia-induced cardiokines [11,38]. Importantly, ER stress-inducible transcripts have structural features that enable them to efficiently escape translational inhibition [35]; this property enables the cell to devote more resources to the synthesis and folding of proteins that play critical roles in the stress response.

Folding: Nonclassical secretory pathway

Perhaps one of the reasons why a nonclassical secretory pathway exists is that some proteins must be secreted during stress, when the cytostructure of the classical secretory pathway is affected, and the higher-order structures of many proteins are disrupted. Nevertheless, just as the classical secretory pathway has complex protein quality control characteristics [39], protein folding in the cytosol is also bound by restrictions on quality [40]. In the cytosol, misfolded proteins are targeted and degraded via a well-studied proteasome-mediated degradation pathway [41]. However, compared to the classical secretory pathway, less is known about the mechanisms by which the accumulation of misfolded proteins destined for secretion via the nonclassical pathway are sensed and targeted for degradation. In addition, analogous to some proteins secreted via the classical pathway, folding might not be required for the function and release of some proteins secreted via the nonclassical pathway.

Some nonclassically secreted proteins might actually have to exhibit a degree of unfolded structure to be secreted. For example, a few nonclassically secreted cardiokines translocate across the plasma membrane in a nonvesicular manner; to do this, they must adopt a molten globule conformation [42]. This is a partially unfolded intermediate conformation that increases hydrophobicity, a property thought to facilitate transport of proteins across lipid bilayers. The molten globule intermediate, which is characterized by the presence of secondary structure but the absence of tertiary structure, is compact and has a loosely packed hydrophobic core. On the basis of these characteristics, it seems that an unfolded or partially folded protein conformation is important for the secretion of at least a subset of nonclassically secreted cardiokines that pass directly through the plasma membrane. Moreover, if a protein that is secreted in the unfolded state must be folded to be functional, the release of cytosolic chaperones such as members of the heat shock protein (HSP)70 family might facilitate refolding after secretion [43]. Other proteins with chaperone activity that assist in the secretion process also form complexes with nonclassically secreted proteins. An example is S100A13, which interacts with the nonclassically secreted fibroblast growth factor (FGF)-1 and, in so doing, assists its secretion [43].

The release of some nonclassically secreted proteins that use one of the vesicular routes for release might also be enhanced by unfolding [43]. One example is α-synuclein, which plays a role in the pathogenesis of Parkinson's disease; in the absence of stress, α-synuclein adopts a folded configuration in the cytosol [44]. Stresses that cause widespread protein unfolding, such as proteasome inhibition or oxidative stress, cause α-synuclein unfolding, which promotes its translocation from the cytosol into vesicles, from which it is released via the nonclassical secretory pathway [44]. Thus, α-synuclein serves as an example of how the release of a nonclassically secreted protein is actually enhanced by proteotoxic and oxidative stresses that increase protein misfolding.

Ischemia-inducible cardiokine secretion

The heart consists of four major cells types (cardiac myocytes, fibroblasts, endothelial cells and vascular smooth muscle cells) and intercellular communication among these cell types plays a crucial role in maintaining cardiac structure and function, as well as determining how the heart responds to potentially pathological stresses, such as ischemia [45]. To the best of our knowledge, there have been no proteomic analyses of the effects of mild ischemia on cardiokine secretion. However, some studies have shown that ischemia augments cardiokine release through both the classical and nonclassical pathways (Table 1).

Ischemia: Classical pathway

Because of their roles in fostering cardioprotection in the ischemic heart, two classically secreted cardiokines of particular interest are follistatin-like 1 (FSTL1) and tumor necrosis factor (TNF)-α (Table 1, entries 13 and 18). FSTL1 is a recently discovered novel member of the activin family of secreted proteins [46]. In the mouse heart, Fstl1 is upregulated by ischemic stress [47] and by the ER stress-induced transcription factor ATF6, which is activated by ischemia [37]. Moreover, FSTL1 exerts a potent cardioprotective effect against ischemic injury in the heart, where it binds a novel receptor that differs from all other activin family receptors [48].

In addition to it being paradoxically pro-inflammatory and cardioprotective, TNFα is of interest because of its unusual mechanism of secretion, which involves cleavage from the extracellular face of the plasma membrane. TNFα is synthesized in the ER and then enters the Golgi network, but in contrast to most classically secreted proteins, the TNFα precursor, pro-TNFα, is routed to the cell surface, where it resides until it is activated [49]. TNFα converting enzyme, TACE, cleaves the extracellular domain of pro-TNFα, releasing it to interact with receptors (TNFR1 or TNFR2) through which it can exert either damaging or protective effects on the heart, depending on which receptor is activated [50]. Moreover, recent studies revealed a potentially novel mechanism of action for TNFα by showing that some of its protective effects are exerted in a TNF-receptor-independent manner via direct interaction with cardiac myocyte mitochondria [51]. Thus, TNFα and FSTL1 represent two examples of cardiokines that are secreted via the classical pathway, even during severe stresses such as ischemia, which usually impairs

Table 1. Cardiokines secreted during ischemia[a]

Cardiokine	Gene symbol	Reference
Classical secretory pathway		
1. Adrenomedullin	*ADM*	[67]
2. Atrial natriuretic peptide	*NPPA*	[67]
3. Apelin	*APLN*	[68]
4. Brain natriuretic peptide	*NPPB*	[67]
5. Calcitonin gene-related peptide	*CALCA/CALCB*	[17]
6. Cardiotrophin-1	*CT1*	[69]
7. C-C motif chemokine	*CCL2* (*MCP1*)	[70]
8. Clusterin	*CLU*	[71]
9. Collagen	*COL1A1*	[72]
10. C-type natriuretic peptide	*NPPC*	[67]
11. Endothelin-1	*ET1*	[67]
12. Enkephalin	*PENK*	[16]
13. FSTL1	*FSTL1*	[73]
14. Growth differentiation factor-15	*GDF15*	[74]
15. IL-6	*IL6*	[75]
16. Osteopontin	*OPN*	[76]
17. Petraxin-3	*PTX3*	[77]
18. TNFα	*TNFA*	[75]
19. Urocortin	*UCN*	[67]
20. Vascular endothelial growth factor	*VEGF*	[75]
Nonclassical secretory pathway		
21. Annexin V	*ANXA5*	[78]
22. Cyclophilin A	*PPIA*	[79]
23. FGF 1	*FGF1* (acidic)	[80]
24. FGF 2	*FGF2* (basic)	[81]
25. HSP60	*HSP60*	[82]
26. HMG 1	*HMGB1*	[59]
27. IL-1α	*IL1A*	[83]
28. IL-1β	*IL1B*	[84]
29. Migration inhibitory factor	*MIF*	[85]
30. Protein S100-A1	*S100A1*	[86]
31. Thioredoxin	*TXN*	[87]

[a]The entries in the table represent proteins whose secretion from the heart or heart cells during ischemia or simulated ischemia has been experimentally confirmed. The table does not include reports of the induction by ischemia of genes that encode secreted proteins.

ER protein synthesis and folding in ways that reduce flux through this secretory pathway.

Ischemia: Nonclassical pathway

There are also cardiokines whose release via the nonclassical secretory pathway is increased by ischemia. Importantly, some of these cardiokines protect the heart and support stem cell-mediated cardiac repair. For example, some proteins originally characterized as pro-inflammatory cytokines are secreted via the nonclassical pathway in response to ischemic stress, including high mobility group box1 (HMGB1, also known as amphoterin) and members of the S100 family of proteins (Table 1, entries 26 and 30). HMGB1, a member of the HMG family of nonhistone DNA-binding proteins [52], exhibits diverse and potentially important functions as a cardiokine [53]. This diversity could be because HMGB1 binds several different receptor types [54], including members of the Toll-like receptor (TLR) and the receptor for advanced glycation endproducts (RAGE) families [55]. Active nonclassical secretion of HMGB1 from several cell types has been reported [56,57]; however, in the cardiac context, following the discovery of elevated levels of HMGB1 in patients who had suffered a myocardial infarction [58], some have postulated that HMGB1 might be passively released from necrotic cells [59]. HMGB1 released passively by necrotic cells preferentially binds TLR2 receptors, which fosters the pro-inflammatory effects of HMGB1; however, HMGB1 released actively lacks inflammatory activity, perhaps because it lacks the structural features required to bind TLR2 receptors [54]. It has also been shown that HMGB1 is protective or damaging in the heart. For example, recombinant HMGB1 administered intramyocardially to mice at a low dose restored infarcted heart tissue, in part through the proliferation and differentiation of c-Kit$^+$ progenitor cells and by decreasing local inflammation [60]. By contrast, systemic administration of HMBG1 to mice at a high dose exacerbated cardiac dysfunction after ischemia reperfusion [60]. Although much remains to be learned about HMGB1, these discrepancies could be due to differences in these studies, including the dose of HMGB1 and the route and timing of administration after myocardial infarction.

Like HMGB1, members of the EF-hand Ca^{2+}-binding S100 protein family also bind RAGE [61]. The cardiomyocyte-specific S100A1, which is cardioprotective, is secreted from the heart via the nonclassical secretory pathway during myocardial infarction. Like many secreted proteins, the S100 proteins exhibit multiple intra- and extracellular functions. Although it might affect certain cell functions via RAGE and possibly other as yet uncharacterized receptor types, RAGE-independent endocytosis of S100A1 protects cardiac myocytes from apoptosis [62]. There are multiple mechanisms by which S100A1 mediates cardioprotection, but in general they converge on optimization of excitation–contraction coupling in ways that facilitate myocardial contraction and pump function during stress [63]. Whereas intracellular S100A1 localizes to numerous cellular sites involved with excitation–contraction coupling, endocytosed S100A1 adopts a different distribution in cardiomyocytes, localizing primarily to endosomes [63]. Thus, S100A1 is an example of a cardiokine that exhibits functions before and after secretion via the nonclassical secretory pathway that enhance myocardial protection from ischemic damage, which makes it an excellent candidate for a heart failure therapeutic [64].

Concluding remarks

Cardiokines released from the stressed heart are likely to play central roles in establishing whether the environment in the injured myocardium is conducive to repair. Given the limited self-renewal capacity of the heart, repair of the damaged myocardium and improving cardiac function following myocardial infarction pose major challenges in preventative and regenerative medicine. Engrafting of self-renewing cells, such as bone-marrow-derived cells and cardiac progenitor cells, into the damaged heart has been successful in animal studies that paved the way for numerous recent clinical trials [65,66]. To enhance the benefits demonstrated in these pioneering trials and to further address the ongoing challenge of repairing the damaged myocardium, additional strategies to augment cardiac repair are needed. This challenge could be met, in part, through a better understanding of the identities and functions of cardiokines released by the stressed myocardium. Stimulation of the expression of endogenous repair-promoting cardiac proteins, such as ischemia-inducible cardiokines, could be one effective strategy for enhancing cardiac repair. Cardiokines induced by the same stress that damages the heart are part of the heart's natural defense and thus could be viable therapeutic candidates. Accordingly, further studies of ischemia-inducible cardiokines could contribute to our understanding of the cellular and molecular mechanisms by which the endogenous repair machinery of the heart can be enhanced.

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