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The Cardiomyokine Story Unfolds: Investigating Stress-induced Protein Secretion in the Heart

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Abstract

Cell-cell communication is largely dependent upon secreted proteins. The repertoire of cardiac-derived secreted proteins, or cardiomyokines, is dynamic. For example, stresses, such as ischemia, shift the cardiomyokine repertoire from those in the healthy heart, to those required for the cardiac stress response. How can this shift occur when conditions in the ischemic heart are unfavorable for the expression, folding and secretion of most cardiomyokines? Perhaps cardiomyokines whose secretion is maintained or increased during ischemia are unusual, in that they can withstand the unfavorable conditions. Since the stress-induced cardiomyokines could impact how the heart responds to potentially lethal stresses, such as ischemia, identifying them and characterizing their functions will broaden our understanding of how to heal the damaged heart.

Keywords

cardiomyokines; protein secretion; ER stress response; myocardial infarction; ischemia; protein folding

1-Introduction – Potential Effects of Stress on Cardiomyokine Secretion

A fundamental mechanism that governs the successful interaction of all cells with the environment is secretion. Cell and tissue communication, as well as optimal organismal function are dependent upon the secretion of biologically active proteins. Numerous proteins are secreted from every organ and tissue, such as adipose and skeletal muscle. Proteins secreted from adipose and skeletal muscle tissue, which include the anti-diabetic, anti-atherogenic cytokine, adiponectin¹, and the cardioprotective peptide, Fstl1^{2, 3}, are called adipokines and myokines, respectively^{4, 5}. In this review, we use the term **cardiomyokine** to describe proteins secreted from any cell type in the healthy, stressed or diseased heart, that have autocrine/paracrine, and potentially endocrine functions (Fig. 1A).

Cardiomyokines play many roles, including maintaining healthy heart and cardiovascular function (e.g. growth factors and endocrine hormones), sending signals of distress, exacerbating pathology (e.g. cytokines), and remodeling and healing the damaged heart (e.g.

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extracellular matrix proteins, stem cell homing factors). Thus, a change in the repertoire of cardiomyokines released from the stressed or diseased heart not only serves as a marker of the pathology, but may also play a critical role in determining how the heart responds, acutely and chronically, to stress.

Certain diseases, including ischemic heart disease, affect protein release from the heart⁶. Ischemia, which is defined as deprivation of oxygen and nutrient delivery, usually due to vascular impairment, is not lethal, by definition. However, it can be severe enough to lead to tissue damage, which in the heart is known as myocardial infarction. Myocardial infarction causes the release of proteins from the damaged portions of the heart into the circulation. These proteins are commonly used as markers that aid diagnosticians in assessing the extent of tissue damage. Several proteomic studies using isolated perfused rat or mouse hearts have identified numerous proteins released from the infarcted heart. The release of most proteins from the infarct is not due to active secretion, but instead, most likely represents a passive release of cellular contents following necrotic tissue damage^{7, 8}. In contrast, tissues exposed to less severe, non-damaging ischemia, which include the surviving myocardium bordering infarct, are stressed, but the cells comprising them remain mostly intact and are, therefore, able to actively secrete cardiomyokines. In fact, stress-induced cardiomyokines (Fig. 1A) play critical roles in preserving myocardial tissue health during ischemia, and so their active secretion is imperative. However, the effects of ischemia on biomolecular processes, such as decreasing intracellular calcium levels⁹, altering redox state¹⁰, and reducing ATP¹¹, conspire to potentially impair the secretion of many cardiomyokines⁶. Fortunately, cells in the heart have cellular machinery that ensures the secretion of functional, stress-induced cardiomyokines that are required for myocardial preservation during ischemia^{12, 13} (Fig. 1B), and may play roles in stem cell-mediated myocardial regeneration.

In this review, we examine the mechanisms of cardiomyokine secretion, explore recent concepts in the relationship between protein folding and function, and summarize some cellular mechanisms that could help preserve the secretion of functional cardiomyokines during stresses, such as ischemia.

2- Mechanisms of Protein Secretion

As with proteins released from any tissue, heart-derived proteins are secreted via either the classical/conventional ER-dependent secretory pathway¹⁴, or the nonclassical/unconventional ER-independent secretory pathway¹⁵.

Classical Secretory Pathway

A defining characteristic of most proteins secreted via the classical secretory pathway is that they are synthesized on ER-associated ribosomes (**Fig. 3A, Step 1**). Many nascent proteins secreted via the classical secretory pathway contain ER-targeting signal sequences that are recognized by the macromolecular signal recognition particle complex, which facilitates ribosome docking to the ER, followed by co-translational translocation into the ER lumen (**Fig. 3A, Step 2**), where synthesis is completed, and folding and disulfide bond formation begin. From the ER lumen, classically secreted proteins are usually routed to the Golgi lumen (**Fig. 3A, Step 3**), where glycosylation initiation and maturation take place, and disulfide bond formation and folding are completed. Classically-secreted proteins are then packaged into Golgi membrane-derived secretory vesicles that fuse with the plasma membrane (**Fig. 3A, Step 4**), thus releasing the mature properly folded, and in some cases glycosylated, cardiomyokines.

Nonclassical Secretory Pathway

In contrast to proteins released via the classical pathway, those secreted via the nonclassical secretory pathway are synthesized on cytosolic ribosomes (**Fig. 3B, Step 1**). This secretory pathway was initially discovered for interleukin-1 β ¹⁶ and galectin-1¹⁷, thus, establishing the first proteins to be secreted via a nonclassical secretory pathway. Since then, the list of proteins secreted through the nonclassical secretory pathway has grown to include hormones, growth factors, cytokines, chemokines, viral proteins, and parasitic proteins. At least four main types of nonclassical secretion have been characterized¹⁸. Three types of nonclassical secretion involve import of the protein of interest into various types of vesicles, including endocytic secretory lysosomes (**Fig. 3B, 2**), exosome-derived multivesicular bodies (**Fig. 3B, 3**), and microvesicles which are shed from the cell surface (**Fig. 3B, 4**). One type of nonclassical secretion is non-vesicular and involves the direct translocation of cytosolic proteins across the plasma membrane (**Fig. 3B, 5**).

3- Roles of Cardiomyokine Folding and Unfolding During Stress

Tight regulatory control of all cellular functions is especially important for survival under limiting conditions, such as ischemic stress. Preservation of biologically active macromolecules, in particular, proteins, is key for maintaining this regulatory control. Although dogma holds that protein function requires a stable folded structure, studies fifty years in the making show that disordered/unfolded proteins may be functional. Hence, the *functional* conformation of all proteins is not the 'native' state; the functional conformation of some proteins may be the 'native' state, while the functional conformation of other proteins can be an intermediate equilibrium between the low-energy 'native' and the high-energy 'denatured' states—an intermediate state known as the 'molten globule'. (See below for more on molten globule.)

While this dynamic concept has explained functions for certain unfolded proteins, it is known that many cellular functions are affected by the accumulation of unfolded and misfolded proteins that result from stresses, such as ischemia. As part of their survival-oriented strategy, eukaryotic cells have developed ways of resolving such protein trafficking stress. The discussion below on the preservation of functional cardiomyokines during stress highlights the possibility that the secretion of certain proteins is upregulated during protein trafficking crises, when protein unfolding/misfolding is generally thought to hinder protein secretion.

Classical Secretory Pathway

In the absence of stress, or during mild stress, nascent proteins can misfold, and existing proteins can unfold. Under these conditions, protein quality control machinery is responsible for proteasome-mediated degradation of the misfolded and unfolded proteins, thus avoiding the toxicity associated with the accumulation of dysfunctional proteins¹⁹. However, during stresses of greater strength, and/or duration, these protein degradation pathways may become overwhelmed, leading to accumulation of misfolded proteins in most cellular locations, each of which mounts a response. In the ER, the accumulation of misfolded proteins activates the ER stress response (ERSR)^{20, 21}. The ERSR, which is activated in the ischemic myocardium²²⁻²⁴, is a complex intracellular signaling process that plays an essential role in ensuring the proper functioning of the classical secretory pathway. To achieve this, the ERSR collaborates with other cellular machinery to meet the biosynthetic and secretory needs of some cardiomyokines that must be secreted during stress. One way this is accomplished is via ER stress-mediated decreases in the translation of mRNAs that code for proteins that do not play essential roles in the stress response. ER stress also leads to the activation of several transcription factors, such as ATF4, ATF6 and XBP-1, which

increase transcription of genes that encode numerous proteins, many of which fortify the protein folding and secretory machinery. Moreover, these transcription factors are responsible for upregulating the expression of some stress-induced cardiomyokines. Importantly, ER stress-inducible gene transcripts have structural features that allow them to efficiently escape translational inhibition, which effectively focuses cellular attention on the needs of stress-inducible cardiomyokines.

Nonclassical Secretory Pathway

Perhaps one of the reasons that a nonclassical secretory pathway exists is that during stress, some proteins must be secreted at a time when not only the cytostructure of the classical secretory pathway is affected, but also, the higher order structures of many cellular proteins are disrupted. Nevertheless, just as the classical secretory pathway has developed complex protein quality control characteristics, protein synthesis in the cytosol is also bound by restrictions on protein quality. In the cytosol, misfolded proteins are targeted and degraded via proteasome-mediated degradation. Since the discovery that some proteins, such as polypeptide hormones and transcription factors, can be functional in the unfolded state, it has been postulated that higher order folding actually can limit functionality of certain proteins, and therefore some proteins must remain unfolded to retain function²⁵. What then delineates a *functional* unfolded protein from a *nonfunctional* unfolded protein, the latter of which should be degraded to avoid proteotoxicity? It is thought that any unfolded protein targeted for degradation must contain solvated hydrophobic amino acids, which are detected by protein-degradation systems such as the ubiquitin-proteasome pathway. This signifies a relative instability for functional unfolded proteins, such as transcription factors p53 and HIF1 α , as well, which is thought to play a role in the dynamic regulation of the levels of these proteins by proteasome-mediated degradation. Functional unfolded proteins that must retain stability, or unstructured regions, such as linkers of proteins, which must necessarily be unfolded, lack hydrophobic residues that could target them for degradation, however they could intrinsically be unstructured, i.e. their unfolded state is not dependent on the presence of hydrophobic residues.

In order for some nonclassically secreted cardiomyokines to translocate in a non-vesicular manner across the plasma membrane, they must adopt a 'molten globule' conformation. This is a partially unfolded intermediate conformation that increases hydrophobicity, which is required 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 appears that unfolded, or a partially folded protein conformation is important for secretion of at least a subset of nonclassically secreted cardiomyokines that pass directly through the plasma membrane. Moreover, if a protein must be folded to be functional, recent evidence has shown the secretion of cytosolic chaperones such as members of the Hsp70 family, might facilitate refolding after secretion²⁶. Other proteins with chaperone activity are also seen to complex with nonclassical proteins and to be involved in the secretion process. An example is S100A13, which complexes with the nonclassically secreted FGF1 and in so doing, assists in its secretion.

It is also possible that the secretion of some nonclassically secreted proteins that use one of the vesicular routes of release may be enhanced by unfolding²⁶. One example is α -synuclein, which resides in a folded state in the cytosol in the absence of stress²⁷. Stresses that cause widespread protein unfolding, such as proteasome inhibition or oxidative stress, also unfold α -synuclein; this unfolding causes its translocation from the cytosol into vesicles, from which it is released via the nonclassical secretory pathway. This is, perhaps, the first example of enhanced release of a nonclassically secreted protein by proteotoxic or

oxidative stresses that increase protein misfolding of classically and nonclassically secreted proteins.

4- Examples of Ischemia-induced Cardiomyokine Secretion

The heart is comprised of four major cell types (i.e. cardiac myocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells), and intercellular communication amongst 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 ischemia²⁸. To the best of our knowledge, there have been no proteomic analyses of the effects of ischemia on cardiomyokine secretion. However, some studies that focused on specific proteins have shown that ischemia augments the release of cardiomyokines through the classical and nonclassical pathways (see Table 1).

Classical Pathway

Table 1 shows a list of cardiomyokines whose release was experimentally shown to be increased upon ischemia. Two cardiomyokines of particular interest are the relatively recently discovered, protective protein, follistatin-like 1 (Fstl1) and TNF α (Table 1; **entries 11 and 16**). In addition to its relatively recent discovery, Fstl1 is of interest because it is a myokine, as well as a cardiomyokine⁴. Moreover, Fstl1 is induced by ER stress in the heart²⁹. Fstl1, which is a member of the activin family of proteins, is released from skeletal muscles of transgenic mice that overexpress Akt in a skeletal muscle-specific manner, implying that Akt induced the expression and secretion of Fstl1. In that same study it was also shown that skeletal muscle-derived Fstl1 exerted apparent endocrine functions on the heart, where it binds to a novel receptor that differs from all other activin family member receptors³⁰. Moreover, it was shown that activation of this novel Fstl1 receptor fosters cardioprotection in response to ischemia/reperfusion injury.

TNF α is of interest for a variety of reasons, including its unusual mechanism of secretion and its multiple roles, and in some cases, apparently paradoxical functions in the cardiac context. Like most proteins released via the classical secretory pathway, TNF α is originally synthesized in the ER and then enters the Golgi network; but, in contrast to most classically secreted proteins, a TNF α precursor, pro- TNF α , is routed to the cell-surface, where it resides until activation^{31, 32}. TNF α converting enzyme, TACE, cleaves the extracellular domain of pro-TNF α , releasing it to interact with receptors (TNF-R1 or TNF-R2)^{31, 33}. Thus, TNF α is routed to the cell surface via the classical secretory pathway, but it exhibits an unusual release mechanism. Moreover, TNF α was originally thought to be a pro-inflammatory cytokine with negative inotropic and pro-apoptotic effects, which would exacerbate heart failure, while other studies suggested that TNF α could also be cardioprotective³⁴. However, it is now known that TNF α can exert either damaging or protective effects on the heart, depending on which receptor is activated³⁵. Moreover, recent studies have demonstrated a potentially novel mechanism of action for TNF α by showing that some of its protective effects are exerted in TNF receptor-independent manner, via direct interaction with cardiac myocyte mitochondria³⁶.

Nonclassical Pathway

There are also several cardiomyokines whose release via the nonclassical secretory pathway have been shown to be increased by ischemia. 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; amphoterin), and S100 proteins (Table 1; **entries 24 and 28**). Despite its reputation as a proinflammatory cytokine, of the nonclassical cardiomyokines induced by ischemia, the non-histone DNA-

binding protein, HMGB1, stands out as one with diverse and potentially important functions associated with inflammation, myocyte protection, and regeneration in the ischemic heart. HMGB1 has been identified as a ligand for TLR2, TLR4, and TLR9 receptors, as well as for the multi-ligand receptor for advanced glycation endproducts (RAGE), the latter of which has been implicated in cancer and inflammation. Following the discovery of elevated levels of HMGB1 in patients with myocardial infarction, several groups have focused on studying the effects of HMGB1 in the heart. Although active, nonclassical secretion of HMGB1 by several cell types including neurons, macrophages, and activated monocytes has been reported, given that cell death is a feature of the pathophysiology of permanent myocardial infarction and ischemia/reperfusion injury, the groups who study HMGB1 in the cardiac context do not explicitly describe its secretion as active. Although there is currently a disagreement regarding the role of HMGB1 in ischemic heart disease, considering the differences in the experimental conditions, the conclusion can be reached that the effects of HMGB1 are context dependent. A group of studies relevant to this issue were compared and recently reviewed³⁷; a brief summary of that review follows. One study showed that when HMGB1 was administered intramyocardially at a relatively low dose (200ng/mouse), infarcted heart tissue was restored, in part, through the proliferation and differentiation of c-kit⁺ progenitor cells. In contrast, a different study showed that systemic administration of HMGB1 at a relatively high dose (10µg/mouse) exacerbated cardiac dysfunction and remodeling after ischemia reperfusion. Regarding the role of HMGB1 in modulating inflammation and c-kit⁺ progenitor cell function, another study concluded that HMGB1-stimulated cardiac fibroblasts, which have been shown to physically interact with cardiac-derived c-kit⁺ cells, produce increased levels of cytokines and growth factors, the combination of which affected the proliferation and differentiation of c-kit⁺ progenitor cells. Moreover, with regard to effects of HMGB1 on inflammation in the heart, another study has shown that intramyocardial injection of HMGB1 (2.5µg/mouse) three weeks after left coronary artery ligation decreased local inflammation by reducing the accumulation of CD45⁺ dendritic cells, attenuating cardiac remodeling and hypertrophy. Effects of extracellular HMGB1 on isolated cardiac myocytes have also been studied. For example, it was shown that extracellular HMGB1 decreased sarcomeric shortening and L-type Ca²⁺ current in cardiac myocytes suggesting that HMGB1 can mediate cardio-depression, which can be potentially protective during stress³⁸. Given the growing number of HMGB1 binding partners, it has been speculated that there are different types of HMGB1 complexes that form in the context of different pathologies³⁹. Furthermore, there is evidence that different receptors are preferentially engaged by HMGB1, depending on the context of its release. For example, while nucleosome-associated HMGB1 released during secondary necrosis (late apoptosis) preferentially binds to TLR2 receptors, highly purified HMGB1, and HMGB1 released during apoptosis, the latter of which harbors an oxidized cysteine at position 106, lack inflammatory activity.³⁹

Also known to bind to RAGE are members of the EF-hand Ca²⁺-binding S100 protein family. In the cardiac context, the myocyte-specific S100A1, which has been extensively studied by Most, and colleagues⁴⁰, is a regulator of cardiovascular function. During myocardial infarction, S100A1 is secreted via the nonclassical secretory pathway. Although it may affect cell function via binding to RAGE, RAGE-independent, Ca²⁺-dependent, clathrin-mediated endocytosis of extracellular S100A1 was shown to protect cardiac myocytes from apoptosis, establishing a putative protective role for this novel cardiomyokine. This protective effect was mediated by signaling through PLC and PKC associated with endosomes. While intracellular S100A1 plays an important role in Ca²⁺-handling in cardiac myocytes, extracellular S100A1 affects Ca²⁺-handling, as well. Extracellular S100A1 was shown to enhance cardiomyocyte Ca²⁺-transient amplitude by decreasing intracellular diastolic Ca²⁺, while intracellular S100A1 enhanced Ca²⁺-transient amplitude by increasing systolic Ca²⁺. Interestingly, overexpressed S100A1 colocalizes with

SERCA2a and other Ca^{2+} -regulatory proteins at the sarcoplasmic reticulum, whereas endocytosed S100A1 protein does not colocalize with sarcoplasmic reticulum proteins, but it instead localizes to endosomes.

5- Concluding Thoughts

Cardiomyokines released from the stressed heart are likely to play a central role in establishing an environment that provides maximum protection from damage, and/or optimal conditions for repair. Due to the limited self-renewal capacity of cardiac myocytes, repairing the damaged myocardium and improving cardiac function following an infarction pose major challenges in preventative and regenerative medicine. Capitalizing on engrafting self-renewing cells, such as bone marrow-derived cells and recently, cardiac progenitor cells, into the damaged heart has proven successful in animal studies, and has led to clinical experimentation. To enhance the benefits demonstrated in pioneer trials, and address the challenge of repairing the damaged myocardium, identification of additional novel, effective strategies to induce cardiac repair is necessary. This challenge could be met, in part, through a better understanding of the identities and functions of cardiomyokines released by the stressed myocardium. Stimulating endogenous repair-promoting cardiac proteins may be one effective strategy to enhance cardiac repair. Stress-induced proteins, including ischemia-inducible cardiomyokines, are likely endogenous, pathologically upregulated candidate proteins. Such stress proteins, induced by the very same stress that damages the heart, i.e. ischemia, serve as the heart's natural defense and may be viable therapeutic candidates. These proteins, secreted by cardiac myocytes and non-myocytes, can have paracrine effects on all cell types in the heart, thereby enhancing cardiac repair. Thus, the ischemia-inducible cardiomyokines expressed in, and released by the heart may exert autocrine, paracrine and/or endocrine effects that might not only moderate damage, but could enhance stem cell-mediated repair. Characterizing ischemia-inducible cardiomyokines will shed light on the modulation of endogenous repair machinery of the heart.

TEXT BOX 1

Delineating the Secretory Route of a Protein of Interest

Classical Secretory Pathway

If a protein has an N-terminal structure that conforms to the predictive rules for ER-targeting signal sequences, it is straightforward to predict whether a protein is secreted via the classical pathway. A commonly used initial paradigm is to examine the predicted pro-protein sequence for an N-terminal signal sequence using SignalP 3.0⁶², or a similar algorithm. Given a positive result, a transmembrane domain search can be carried out using TMHMM 2.0⁶³. Given a negative result from the transmembrane domain search, a search for the presence of a C-terminal ER-retention signal, which is the four amino acid motif, KDEL, or a KDEL-like motif⁶⁴, can be performed. Generally, 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. For most proteins structural and biological predictions are available in databases, such as UniProt (www.uniprot.org/). It should be noted that some ER-targeted proteins do not have N-terminal signal sequences, thus, predicted absence of such a sequence may still be consistent with ER-targeting⁶⁵. Also, 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/Golgi resident proteins that have transmembrane domains or KDEL sequences.

Nonclassical Secretory Pathway

Several algorithms, such as SecretomeP⁶⁶, have been developed to identify nonclassically secreted proteins. SecretomeP uses a combination of features to obtain information about posttranslational modifications and subcellular localization of a protein of interest. The determining score is the 'NN-score', for which a value above 0.5 indicates possible secretion; secretomeP assigns scores based on features shared by secreted proteins, not the process by which proteins are secreted. Accordingly, although SecretomeP is trained to predict nonclassical secretion, it usually gives high scores to proteins entering the classical secretory pathway, as well. Therefore, in order to filter out proteins that receive high scores that are secreted via the classical pathway, the user must check for the presence of a signal sequence, the absence of which, in combination with an NN-score above 0.5, is consistent with possible nonclassical secretion. SecretomeP was used to survey the human proteome; 33 novel proteins, and 30 unannotated/hypothetical proteins fit the criteria for nonclassical secretion⁶². In a more recent study a different algorithm identified 566 candidates for nonclassical secretion⁶⁷.

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Figure 1. Cardiomyokines and Effects of Stress on Cardiomyokine Secretion

Panel A: By analogy to the release of adipokines from adipose tissue, and myokines from skeletal muscle tissue, the term, cardiomyokine, is used to describe proteins released by the heart that exert autocrine, paracrine and/or endocrine functions. Stress-induced cardiomyokines are a subset of cardiomyokines whose expression and secretion increase upon stresses that impair the release of other cardiomyokines.

Panel B: No Stress- Stress-free conditions favors the synthesis of functional cardiomyokines involved in the maintenance of normal heart structure and function.

Panel C: Ischemic Stress- During stress, such as sublethal ischemia, synthesis of non-functional cardiomyokines is moderated via cellular stress response machinery.

Figure 2. Classical and Nonclassical Secretory Pathways

Panel A: Classical Secretory Pathway- Stresses, such as sublethal ischemia, can induce cardiomyokine (CMK) genes, and in the case of the classical secretory pathway, those CMK mRNAs are translated on ER-associated ribosomes (**Step 1**). The resulting proteins, depicted here in red (CMK-1), are initially localized to the ER lumen (**Step 2**), then transported in vesicles to the Golgi lumen (**Step 3**), after which they are released via secretory vesicle fusion with the plasma membrane (**Step 4**).

Panel B: Nonclassical Secretory Pathway- Some transcripts encode CMKs that will be synthesized on cytosolic ribosomes (**Step 1**) and released via the nonclassical secretory pathway. The resulting proteins, depicted here in blue or green (CMK2, 3, 4 and 5) are destined for secretion via one of four nonclassical secretory pathways. Nonclassical CMK secretion takes place via vesicle-mediated (2-4), or non-vesicle-mediated pathways, as follows:

- (2) endocytic secretory lysosomes (CMK-2),
- (3) exosome-derived multivesicular bodies (CMK-3),
- (4) microvesicles which are shed from the cell surface (CMK-4), and
- (5) direct translocation of cytosolic proteins across the plasma membrane through complex protein conducting channels (CMK-5).

A portion of Panel B and the nomenclature used to describe the nonclassical secretory routes were inspired by a review written by Walter Nickel.⁶⁸

TABLE 1**Cardiomyokines Secreted during Ischemia**

Classical Secretory Pathway		
cardiomyokine	gene symbol	reference
1- adrenomedullin	ADM	41
2- atrial natriuretic peptide	Nppa	41
3- apelin	APLN	42
4- brain natriuretic peptide	Nppb	41
5- cardiotrophin-1	CT1	43
6- C-C motif chemokine	CC12 (MCP1)	44
7- clusterin	CLU	45
8- collagen	COL1A1	46
9- c-type natriuretic peptide	Nppc	41
10-endothelin-1	ET1	41
11-follistatin-like 1	FSTL1	2
12-growth differentiation factor-15	GDF-15	47
13-interleukin-6	IL6	48
14-osteopontin	OPN	49
15-petraxin-3	PTX3	50
16-tumor necrosis factor- α	TNF α	48
17-urocortin	UCN	41
18-vascular endothelial growth factor	VEGF	48

Nonclassical Secretory Pathway		
cardiomyokine	gene symbol	reference
19-annexin V	ANXA5	51
20-cyclophilin A	PPIA	52
21-fibroblast growth factor 1	FGF1 (acidic)	53
22-fibroblast growth factor 2	FGF2 (basic)	54
23-heat shock protein 60	HSP60	55
24-high mobility group box 1	HMGB1	56
25-interleukin-1 α	IL1A	57
26-interleukin-1 β	IL1B	58
27-migration inhibitory factor	MIF	59
28-protein S100-A1	S100A1	60

Nonclassical Secretory Pathway		
cardiomyokine	gene symbol	reference
29-thioredoxin	TXN	61

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