Expanding the Paracrine Hypothesis of Stem Cell–Mediated Repair in the Heart

When the Unconventional Becomes Conventional

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Recent interest in mechanisms of stem cell–mediated repair in the heart have spawned the paracrine hypothesis, which posits that stem cells release beneficial substances that improve regeneration and function of the injured and diseased myocardium. In support of this hypothesis are studies showing that stem cells release small membranous vesicles called exosomes that deliver beneficial cargo to other cells in the heart. However, in addition to exosomes, which are released by the unconventional secretory pathway, are many other potentially beneficial factors released by the unconventional and the conventional secretory pathways. Therefore, a broader perspective of mechanisms of secretion, as well as an appreciation for the ways in which the secretion of a wide range of different types of molecules can be regulated, will spawn new avenues of thought necessary to move us beyond the exosome-centric view that drives much of the current thinking of those who study of stem cell–mediated repair in the heart.

What Is Secretion and Why Do Cells Do It?

Secretion is what cells do; excretion is what the kidney does. Secretion is an active process by which cells release materials into the surrounding environment. Among the first secretion mechanisms studied were those involved in the regulated release of peptide hormones from endocrine cells. After release, substances can signal to cells afar via the blood stream (endocrine) and to neighboring cells (paracrine), as well as the cell of origin (autocrine). Additionally, some substances signal within the cell of origin (intracrine). Endocrine, paracrine, and autocrine signaling all involve the release of communicator substances directly into the interstitial spaces surrounding the cells of origin. Secretion into a duct, such as salivary or digestive enzyme secretion, is exocrine.

Cells secrete many different substances, including proteins, lipids, steroids, nucleic acids, nucleotides, metabolites, and ions. Generally, these substances are secreted to facilitate communication with other cells and to affect the structure and content of the extracellular matrix. However, secretion is also useful for ejecting cellular waste. Most substances secreted from cells are hydrophilic; thus, for them to be released, they must overcome the hydrophobic barrier of the plasma membrane. To do this, cells have developed many mechanisms, most of which can be considered part of the conventional or unconventional secretory pathways.

How Do Cells Secrete? Conventional and Unconventional Secretion

Perhaps the best understood secretion mechanism is the conventional secretory pathway, which is also known as the endoplasmic reticulum (ER)–dependent pathway and is responsible for the secretion of most peptides and proteins.[1] Proteins destined for the conventional secretory pathway usually have N-terminal ER signal sequences and are cotranslationally translocated into the lumen of the rough ER, after which they are transported to the lumen of the Golgi and then to vesicles that are either retained in the cell until an appropriate stimulus for secretion, that is, regulated secretion (Figure [A]), or are released in a stimulus-independent manner, that is, constitutive secretion (Figure [B]). Typically, regulated secretion is a specialized form of conventional secretion performed by endocrine cells, neuroendocrine cells, and neurons. In the heart, the most studied case of regulated conventional secretion is atrial natriuretic peptide released from atrial myocytes in response to stretch and adrenergic stimulation.[2] In contrast to regulated conventional secretion, constitutive conventional secretion is a characteristic of essentially all cells and does not require specialized secretory granules/vesicles and, in general, does not require a stimulus. For example, atrial natriuretic peptide is expressed in ventricular myocytes of the diseased adult heart and is secreted by the constitutive conventional pathway because ventricular myocytes do not have secretory granules. Collagen is secreted by fibroblasts via the conventional secretory pathway, which is particularly relevant in the diseased heart because inappropriately high levels of collagen secretion can cause maladaptive fibrosis. In neurons, the nonprotein neurotransmitters or their precursors are transported into secretory vesicles using specialized transporter proteins (Figure [A]).

Compared to conventional secretion, relatively fewer proteins are secreted by the unconventional secretory pathway. Unconventional secretion was originally defined as secretion that does not depend on the Golgi apparatus.[3] Certain cytokines and growth factors, such as interleukin and fibroblast growth factor were the first substances found to be secreted by this pathway. Since then it has been found that other substances, including proteins, microRNAs and other noncoding

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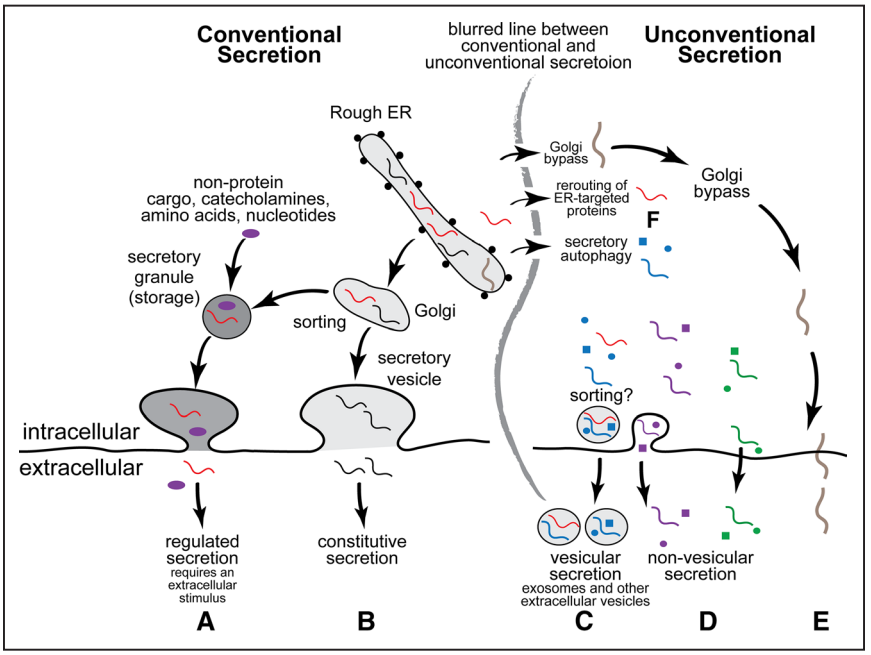

Figure. Shown are the conventional (**left**) and unconventional (**right**) secretory pathways. **A** and **B**, Conventional secretion. **A**, Regulated conventional secretion and (**B**) constitutive conventional secretion. **C–F**, Unconventional secretory pathways. **C**, Vesicular secretion; (**D**) nonvesicular secretion; (**E**) Golgi bypass; and (**F**) secretory autophagy. ER indicates endoplasmic reticulum.

RNAs, nucleotides, and metabolites use the unconventional pathway. Unlike the conventional secretory pathway, which requires rough ER, Golgi, and secretory granules or vesicles, unconventional secretion can facilitate the translocation of hydrophilic substances across the plasma membrane using a variety of mechanisms including the packaging of substances into vesicles that can fuse with the plasma membrane and release their contents, or in vesicles that can traverse across the plasma membrane intact, some of which are exosomes, and transport their cargo in a protected membrane-sealed vault for delivery to other cells (Figure [C]).[4] Some substances are released via nonvesicular secretion mechanisms involving the movement of released substances through specialized channels in the plasma membrane (Figure [D]). These forms of unconventional secretion occur independently of exosomes and, in the heart, examples of substances secreted via nonvesicular pathways are cytokines, such as interleukin-6, interleukin -1β, as well as other signaling substances, such as transforming growth factor-β, fibroblast growth factor, and transforming growth factor-α.

Although discovered some time ago, exosomes gained popularity recently when it was found that they contain many different types of molecules, they are relatively stable in the plasma, and they can deliver their cargo to a variety of cell types, both near and far from their tissue of origin. Moreover, exosome cargo varies, depending on the health status of the tissue of origin, thus serving as biomarkers of specific tissue health status. This was first discovered in cancer studies, where it was shown that plasma levels of substances peculiar to cancer cells could serve as biomarkers of disease progression.[5] Since then, identifying the contents of exosomes from plasma has revealed molecular signatures of numerous pathologies, including heart failure and myocardial infarction. Moreover, some of the contents of exosomes, including microRNAs, have been shown to moderate or exacerbate the pathology phenotype in the heart.[6,7] Although the mechanism by which exosomes are made in cells is well studied, only relatively recently have discoveries been made about how cargo is targeted to exosomes and whether the release of exosomes, and thus, their cargo is regulated.[8]

Conventional Secretion Meets Unconventional Secretion

Recent advances in our understanding of both the conventional and unconventional secretory pathways have revealed interactions and overlap that blurs the line between them. For example, some proteins made at the rough ER can bypass the Golgi apparatus on their way to the cell surface[9] (Figure [E]). According to the original definition, this format of secretion should be classified as unconventional, even though the released protein began life in the conventional secretory pathway; it is clear that it represents a nuance of the conventional secretory pathway. Another nuance that blurs these lines is the finding that when the ER membrane buds off to form an autophagosome, it can fuse with the plasma membrane and lead to release of the autophagosome contents, some of which are in exosomes,[10] a process called secretory autophagy[11] (Figure [F]). Secretory autophagy provides yet another example of an intersection between the conventional and unconventional secretory pathways.[12] Thus, it has become apparent that cargo originally localized to the rough ER, and theoretically bound for the conventional secretory pathway, can be diverted to the unconventional secretory pathway and possibly to exosomes and other vesicles.

Another example of intersections between these 2 pathways can be found in the alternate routing to the cytosol of secretory proteins that normally target to the rough ER,[13–15] which presumably makes it possible for them to become part of the unconventional secretory pathway. Evidently, this rerouting from the conventional to unconventional secretory pathway involves the N-terminal ER-targeting sequence. Many proteins that are targeted to the conventional secretory pathway have N-terminal signal sequences that target them to the ER by facilitating the docking of the nascent protein at the rough ER in preparation for engagement with the ER translocon machinery, which moves proteins cotranslationally from the cytosolic

face of the ER to the ER lumen. However, there are circumstances when the ER-targeting sequence on such proteins is not made, usually as a result of alternative transcript splicing that removes the region of the RNA coding for the signal sequence. By default, such a variant form of the transcript encodes a protein that will localize to the cytosol, where it may be possible for it to be secreted by the unconventional secretory pathway.

When Does the Unconventional Become Conventional?

The blurring of the line that separates conventional from unconventional secretion suggests that there are likely to be multiple mechanisms by which any one substance can be released from cells. And while exosomes are intriguing because of their small, nanoparticle-like size, as well as their diverse payload and stability in the plasma, it is likely that many other substances secreted by stem cells, as well as other cells in the heart, via exosome-independent, vesicular, and nonvesicular mechanisms might influence myocardial repair and regeneration, as well as other functions under physiological and pathological conditions. Although the conventional secretory pathway was probably named this because it was studied first, and while anything other than the conventional is, by definition, unconventional, perhaps a more functional nomenclature for the conventional and unconventional secretory pathways might be the ER/Golgi-dependent and ER/Golgi-independent secretory pathways, respectively.

Broader Perspective of the Secretome in Cardiac Physiology and Pathology

The breadth of secretion mechanisms depicted in the Figure underscore the need to consider ER/Golgi-dependent and ER/Golgi-independent secretory pathways as potential contributors to stem cell–mediated myocardial repair and regeneration, as well as other important functions in the heart. Broadening our perspective on cell communication to include the multifold mechanisms by which substances are released from, and received by cells, will reveal potentially new therapeutic avenues for cardiac repair and regeneration. Moreover, the possibility that secreted substances function in a combinatorial manner to exert either adaptive or maladaptive changes in the heart further emphasizes the need to better understand subclasses of exosomes, as well as the entire spectrum of substances secreted by stem cells to fully grasp the regenerative potential in the heart.

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Disclosures

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