Cell-to-cell communications via connexins: function and alterations in endothelial and smooth muscle cells of vessels

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Introduction

Virtually every animal cell type expresses connexins (Cx), the integral membrane proteins that concentrate at gap junction domains of the cell membrane. At these sites, Cx form specialized channels for direct cell-to-cell exchanges of cytoplasmic ions and small molecules between adjacent cells. The functions of these exchanges are still poorly understood[1]. The research effort to elucidate these functions are increasing, particularly since molecular genetic and clinical studies have shown that perturbed expression of specific Cx, due to gene mutation, loss or overexpression, is associated with multiple human diseases, including some affecting vascular functions[1].

Connexins

There are at least 16 genes dispersed throughout the human genome, each coding for a distinct Cx. These genes always contain two exons separated by one intron of variable length. Most Cx are encoded by an uninterrupted region within exon 2. Cx are integral membrane proteins with both ends in the cytoplasm and four transmembrane domains connected by one cytoplasmic and two extracellular loops. They all share a high degree of amino acid identity in the membrane-spanning regions and two extracellular loops, but differ in the size of cytoplasmic loop and C terminus. Hence, their molecular weight ranges from 26 to 50 kDa. Phosphorylation is the only post-translational modification so far detected in some Cx, which appear to share a half-life < 3-6 hours. One or more Cx species hexamerize around a central hydrophilic space, to form a tube-like structure called connexon that spans the entire width of the phospholipid bilayer of a cell membrane. Thus, one end of each connexon extends in the cytoplasm, while the other end projects into the intercellular space. There, two connexons, each contributed by two adjacent cells, assemble end-to-end to form an intercellular channel that bridges the intercellular space in regions (gap junctions) where two cell membranes are closely apposed. Cx channels allow for the diffusion-driven passage from the cytoplasm of one cell into the cytoplasm of an adjacent cell of most current-carrying ions as well as molecules < 900 Da, including glycolytic intermediates, vitamins, amino acids and nucleotides. This passage, which is also referred to as ionic and metabolic (or junctional) coupling, is modulable, at the transcriptional, translational, post-translational and regulatory level, by a variety of factors, including H^+, Ca^{2+}, arachidonic acid metabolites, nucleotides, hormones, kinases, growth factors and drugs, whose relative effect depends on cell type and physiological condition[1].

Coupling is thought to ensure the electrical synchronization of excitable cells and to contribute to the control of growth and differentiation of developing and tumoral tissues. A variety of other functions have been contemplated, most of which, however, are still hypothetical[1]. The mechanism whereby any cell function may be affected by coupling is also largely undetermined. Coupling rapidly equilibrates ionic and molecular electrochemical gradients between nearby cells, thus ensuring their synchronization. It also

provides for the spreading across large cell populations of signals elicited in a few selected cells, thus mediating the functional recruitment of cells distant from the site of signaling[1].

Vascular connexins

At least four Cx species are expressed in the vascular system, in patterns that vary depending on cell type and compartment. We have performed experiments with both endothelial and vascular smooth muscle cells, to elucidate the functional reasons for such a complex distribution.

Arterial endothelial cells are usually coupled by Cx40 and Cx37, but also express some Cx43 in discrete locations[1,2-5]. Due to the necessity of ensuring a continuous, non-thrombogenic covering during the formation and renewal of blood vessels, endothelial cells often move as sheets, in which groups of cells have to migrate coordinatedly. To test whether Cx contribute to this coordination, we have mechanically removed endothelial cells within confluent monolayers, to induce the cells bordering the experimental wound to proliferate and migrate into the denuded area. Using cells derived from microvessels, we have found that this migration is locally associated with an increased expression of Cx43 and coupling, which is not observed in the quiescent cells distant from the wound[2-4]. Experiments indicate that conditions blocking migration, but not cell division, prevented the coupling increase and, conversely, that pharmacological and genetically induced inhibition of Cx channels perturbed the movement of endothelial sheets, without affecting the migration of individual cells, thus delaying the wound closure[2-4]. The occurrence of extensive endothelial cell coupling *in situ*[1] together with the observation that basic fibroblast growth factor increases coupling in monolayers of quiescent endothelial cells[4], indicates that Cx may similarly coordinate the migration of endothelial cells under *in vivo* conditions.

In both resistance and conduit arteries, smooth muscle cells are coupled predominantly by Cx43, even though some Cx45 may also contribute[1,6,7]. Since these cells do not readily generate propagated action potentials[1], coordination of both their contraction and relaxation requires the intercellular exchange of second messengers to ensure the propagation of the impulse and its synchronization over long distances. To test whether this is achieved via Cx channels, we have compared rats rendered hypertensive by different experimental procedures, but featuring a similar increase in blood pressure and in thickness of both aortic and left cardiac ventricle wall. We found that these structural changes did not affect the levels of Cx43 in cardiomyocytes, but were associated with different alterations in the levels of this Cx among the smooth muscle cells of the aorta. Thus, whereas the levels of Cx43 increased in hypertensive rats featuring increased arterial distensibility[1,6], they decreased in rats which featured a close to normal arterial distensibility[7]. The data show that Cx43 is differentially modulated with vessel function, probably depending on local mechanical forces, and suggest that this Cx acts as a mechanical sensor, transcriptionally regulatable by the stretching of the vascular wall[1,6,7].

Conclusions

Cx and coupling participate in the physiological functions of endothelial and smooth muscle cells of vessels. However, how this participation is achieved and what the advantages are for vessel physiology of using Cx rather than other forms of intercellular communication are questions still largely open to future research.

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