

## Review

# Study progress of homocellular and heterocellular gap junctions in vessel wall

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There were four different degrees which were related to organs, tissues, cells and molecules in the study of physiopathologic process and treatment of vessel remodeling diseases. More people concentrated on the research of one kind of cell, for example, the vascular smooth muscle cell (VSMC) or adventitial fibroblast (AF) and their effect on the vessel rebuilding. However, the human body is an organic whole, so the structural and functional contacts among vascular endothelial cell, smooth muscle cell (SMC) and AF should be paid more attention to. Books and articles on the gap junction (GJ) were collected and studied. Gap junction between and among cells of the vessel wall played a critical role in many aspects of vascular function and pathology. There was homocellular communication in intima, tunicae media and adventitia singly, and also, heterocellular communication among different cells of the vessel wall. As a result, a link network was formed among cells of the vessel wall and the vessel had integrated function in the physiological and pathological process. Hence, a summary was made about the homocellular and heterocellular gap junctions, hoping that there would be more people concentrating on the whole function of vessels.

**Key words:** Homocellular, heterocellular, gap junctions, vessel wall.

## INTRODUCTION

Gap junctions, extensively existing in organization and cell of animal, are direct pathway of exchange intercellular matter and information (Yuling and Liping, 2008). Cells of most invertebrate and vertebrate tissues can communicate with their neighbors via a low-resistance gap junction. In vertebrates, a few cell types do not form gap junctions in their fully differentiated state, including red blood cells, spermatozoa, and skeletal muscle (Saez et al., 2003). Nevertheless, the progenitors of these cells do express gap junctions (Saez et al., 2003).

The basic structural unit of gap junction is connexon or termed hemi-channel. The wall of the connexon is formed of six protein subunits, or named connexins (Cxs). Dock-

ing of two connexons separately inlay in two cells can form a cylindrical pore diameter which is hydrophilic. When the connexon is formed of the same connexins, it is called homomeric. Reversely, different connexins form heteromeric. Homotypic channel is formed by docking of the same connexons, while heterotypic channel is composed of different connexons. The channel pore narrows from ~40 Å diameter at the cytoplasmic side to ~15 Å at the extracellular side of the bilayer and then widens to ~25 Å in the extracellular region (Perkins et al., 1998). Small molecules and second messengers (molecular mass is lesser than 1 kDa and diameter is lesser than 10 nm) are free to pass the gap junction. Six connexin subunits of the hemi-channel may coordinately change configuration to open and close the hemi-channel, then adjust the gap junction. For example, integrin  $\alpha 5 \beta 1$  induces the opening of Cx43 hemi-channel through transforming the conformation of Cx43 (Batra et al., 2012; Chen et al., 2012). Gap junction are the intercellular

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pathway of electrical, chemical and metabolic coupling, characterizing fast-transmission, low-resistance and short delaying time. They are responsible for direct intercellular transfer of ions and small molecules and involved in coordinating the electrical and metabolic responses of heterogeneous cells (Saez et al., 2003).

## CONNEXINS AND PHOSPHORYLATION

Connexins are part of the membrane protein family which is polygenic and highly conserved. In recent days, there are at least 20 members in mammalian cells (Sohl and Willecke, 2004).

Each of the connexins appears to fit the general topological model for gap junction protein. In this model, the polypeptide traverses the lipid bilayer four times, with both the N- and C-termini facing the cytoplasm (Kumar and Gilula, 1996). Finally, there are four transmembrane domains (M1 ~ M4), two extracellular loops and one cytoplasmic loop (Kumar and Gilula, 1996). Cxs are named by molecular mass. Cx37, Cx43, Cx40 and Cx45 definitely exist on the wall of blood vessels.

Pannexin (Px) is a protein family which generally exists in vertebrates and invertebrates. There is a hypothesis that pannexins, rather than providing a redundant system to gap junctions formed by connexins, exert a physiological role as non-junctional membrane channels (Dahl and Locovei, 2006).

There lies Cx37, Cx43 and Cx40 in the endothelial cells and Cx37 have a high-expression (Kwak et al., 2003). Cx43 and Cx40 are usually observed in smooth muscle cells (SMC) (Yuling and Liping, 2008). Specifically speaking, Cx43 is expressed on SMC in most great and medium vessels (Yeh et al., 1997), while Cx40 is observed in small resistance vessels (Little et al., 1995; Kempen and Jongasma, 1999). But Cx45 and Cx37 are reported in recent literature (Li and Simard, 2002; Horan et al., 2006). All studied connexins with the exception of Cx26 are phosphoproteins. Connexin phosphorylation and dephosphorylation regulate the expression and distribution of gap junction proteins through a number of mechanisms, including connexin biosynthesis. Protein kinases and signal transduction pathway affect the phosphorylation of connexin. Take Cx43 for an example, Cx43 contains several consensus sites for phosphorylation in the carboxy terminus, several of which have been identified as target sites for specific protein kinases (Saez et al., 2003). In these clear sites, serine 368 site (Ser-368) and Ser-372 are phosphorylated by protein kinase C (PKC) (Saez et al., 1997), while mitogen-activated protein kinase mitogen-activated protein kinases (MAPK) can phosphorylate Cx43 at Ser255, Ser279, and Ser282 (Warn-Cramer et al., 1998, 1996). In addition, Akt (protein kinase B) activity controlled gap junction stability and was necessary to form larger stable gap junctions. Akt activation was increased upon proteasomal inhibition and resulted in phosphorylation of Cx43 at Akt

phosphorylation consensus sites (Dunn et al., 2012). The carboxy terminus of Cx43 are adjusted by a Ca<sup>2+</sup>/calmodulin-dependent mechanism (Xu et al., 2012; Guerineau et al., 2012). Recent studies pay attention to Cx43, sodium channel and the linking between ankyrin-G (AnkG, one of the composition of sodium channel) and gap junction. Cx43 down-regulation may disturb trafficking of sodium channel components, and conversely, loss of AnkG expression may prevent the arrival of Cx43 to its final destination (Delmar, 2012).

## HOMECELLULAR GAP JUNCTION

### Gap junction between endothelial cells

Among the functions in which endothelial gap-junctional intercellular communication has been implicated, is the migratory behavior of endothelial cells after injury, angiogenesis, endothelial growth and senescence, and the coordination of vasomotor responses (Yeh et al., 1998). Connexins may be expressed in vascular endothelium according to different species, parts of the body and types of vessels. For example, rat aortic and pulmonary arterial endothelia were found to express all 3 connexin types, whereas coronary artery endothelium expressed Cx40 and Cx37, but lacked Cx43 (Yeh et al., 1998). Porcine aortic endothelia expressed Cx37 and Cx43, but not Cx40 (Yeh et al., 1998). Not all the connexins can form functional gap junction. It has been proved that *in vitro* expression systems, connexons composed of Cx43, but do not form functional channels with those composed of Cx40 (Bruzzone et al., 1993). However, Cx37 channels are compatible with both Cx43 channels and Cx40 channels (Brink et al., 1997).

Atherogenesis is a kind of vascular remodeling diseases. The expression of Cx40, an endothelial gap junction protein is decreased during atherogenesis. Meanwhile, Cx40-mediated gap junctional communication contributes to a quiescent non-activated endothelium by propagating adenosine-evoked anti-inflammatory signals between endothelial cells (Chadjichristos et al., 2010). Alteration in the aforementioned mechanisms by targeting Cx40 promotes leukocyte adhesion to the endothelium, thus accelerating atherosclerosis (Chadjichristos et al., 2010).

Asymmetric dimethylarginine (ADMA), a major endogenous inhibitor of nitric oxide synthase, is recently defined as a novel atherogenic factor. ADMA down regulates Cx43 expression through the phosphorylation of p38. Low-expressed Cx43 affects the development of atherosclerosis via inhibiting endothelial gap junction intercellular communication (GJIC) function (Jia et al., 2009).

There is a high morbidity rate among the diabetic patients. Advanced glycation end products generated in the circulation of diabetic patients were reported to affect the function of vascular wall and to speed the process of vascular remodeling, mainly through reduced Cx43

transcription and down-regulated Cx43 expression (Wang et al., 2011).

Connexins and gap junction that affect the function of vascular walls play an important role in the process of vascular rebuilding. So gap junction coupling in endothelial cells is a suitable therapeutic target for the treatment of cardiovascular diseases. For example, dipyridamole-induced increase in gap junction coupling in endothelial cells is related to a cAMP-protein kinase A (PKA) dependent phosphorylation pathway (Begandt et al., 2010).

### Gap junction between SMC

The gap junctions in SMC maintain the stasis of circulation, adjust the vessels and affect the modulation of homeophenotype.

The phenotype of SMC in tunica media will change from contracting to secreting type in pathological state. Then, the changed cells take part in the vessels rebuilding through proliferation, migration to intima and secretion. Increased expression of Cx43 gap junctions in SMC is implicated in the response to primary arterial injury and in the early stages of human coronary atherosclerosis (Plenz et al., 2004). Also, the enhanced gap junction formation may contribute to the coordination of the response of SMC in the early phase of restenosis (Plenz et al., 2004).

SMC populations exhibit distinct phenotypes: spindle-shaped (S) and rhomboid (R). S-SMCs are predominant in the normal media, whereas R-SMCs are recovered in higher proportion from stent-induced intimal thickening, suggesting that they participate in the restenotic process (Chadjichristos et al., 2008). Limiting Cx43 expression in S-SMCs prevents platelet-derived growth factor-BB-induced S-to-R modulation (Chadjichristos et al., 2008).

After the acute lesion of the vessels, decreased expression of Cx43 will inhibit the inflammatory reaction and control the proliferation, migration to intima and secretion of SMC, in order to repress the thick intima (Chadjichristos et al., 2008).

When gap junction between human saphenous vein SMCs was observed using the technique of dye transfer, someone found that angiotensin II (AngII) acted on AngII type 1 receptor, increased the expression of Cx43 via MAPK-AP-1 signaling pathway and enhanced communication through gap junction (Jia et al., 2008). As a result, the proliferation of saphenous vein SMCs was markedly increased (Jia et al., 2008).

### Gap junction between fibroblasts

In this experiment, neonatal rat heart myofibroblasts and simulated ischemia with  $\alpha$ 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer were used.

Short period with simulated ischemia reduced the ability to transfer a dye between neighbouring cells, which indicated reduced GJIC (Johansen et al., 2011). Prolonged exposure to simulated ischemia caused opening of hemichannels, while gap junction channels remained closed (Johansen et al., 2011). Opening of Cx43 hemichannels seems to be an important mechanism for ischemia-reperfusion injury in the heart (Johansen et al., 2011).

## HETEROCELLULAR GAP JUNCTION

### Gap junction between endothelial cell and SMC

The myoendothelial junction (MEJ) was first described in 1957 in a transmission electron microscopy (TEM) study of small arteries in the dog heart (Moore and Ruska, 1957). It was the place that endothelial protrusions extended through windows of the elastica interna to make direct contact with smooth muscle plasma membranes (Moore and Ruska, 1957). In fact, there are three distinct "types" of MEJs: (1) endothelial cell (EC) extensions through the internal elastic lamina (IEL) that make contact with the VSMC, (2) VSMC extensions through the IEL that make contact with the EC, or (3) both EC and VSMC extensions into the IEL (Michel et al., 1995). Now, the MEJ is commonly described as the structural location at which an EC or VSMC extension protrudes through the IEL, resulting in plasma membrane juxtaposition with the opposite cell type. The MEJ is approximately 0.5  $\mu$ m in width by 0.5  $\mu$ m in depth (although this is highly variable depending on the IEL thickness), as shown by TEM (Heberlein et al., 2009).

Johnson et al., (1973) demonstrated for the first time that cellular extensions >10  $\mu$ m from two different cells could form heterocellular gap junctions, as revealed by freeze-fracture TEM images. There may be heterocellular gap junctions in MEJ. Using the technique of dye transfer demonstrates that there is a transfer of dye between EC and SMC, while the application of gap junction blocker proves the existence of gap junction between EC and SMC. Most people consider that there are two ways in the signal transduction between EC and SMC. First, is the communication through body fluid. EC releases a series of cytokines and acts on the homologous receptor of SMC (Straub et al., 2010). Second, is the communication through gap junctions existing in MEJ which is called the myoendothelial gap junction (MEGJ). MEGJ plays an important part in the vessels adjustment (Xuejun and Zuoyun, 2010). The MEGJ is mainly composed of Cx43. Various compounds up to a molecular mass of 1000 Da that can be exchanged by passive diffusion through gap junction, that is, metabolites, ions, second messengers, water and electrical impulses.

In MEGJ, the phosphorylation of Cxs changes the number, type and distribution of gap junction protein. As a

result, the exchange of signal and transfer of material can be adjusted. For example, lipoprotein-derived phospholipid oxidation products (OxPAPC) play a critical role in atherosclerosis. In the *vitro* coculture model of EC and SMC, biocytin dye transfer between EC and VSMC coupling was dramatically reduced by OxPAPC. The decrease in dye transfer after OxPAPC treatment was correlated with an increase in tyrosine 265 phosphorylation of Cx43, especially at the *in vitro* myoendothelial junction (Isakson et al., 2006). Application of adenosine 3',5'-cyclic monophosphate sodium (pCPT) to mouse cremasteric arterioles reduces the detection of Cx43 phosphorylated at its carboxyl terminal Ser-368 at the MEJ *in vivo* (Straub et al., 2010). Using a vascular cell co-culture (VCCC) and applying the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (PMA) or fibroblast growth factor-2 (FGF-2) induce phosphorylation of Cx43 Ser-368 (Straub et al., 2010). The result shows that PMA and FGF-2 both inhibited movement of inositol 1,4,5-triphosphate (IP(3)), but to a lesser extent Ca(2+) (Straub et al., 2010).

MEGJs play a central role in endothelium-derived hyperpolarizing factor (EDHF) dilation and highlight a primary role for Cx40 in the radial transfer of this signal. A powerful EDHF is transferred partly through MEGJs (Boettcher and de Wit, 2011). Herein, a less potent EDHF depends on Cx40 and may represent signaling through myoendothelial gap junctions (Boettcher and de Wit, 2011).

### Building of EC-SMC coculture model

In order to better understand the pathologies and potential cellular physiology that occurs at the MEJ, a *vitro* EC-SMC coculture model is required.

Initially bovine endothelial cells were grown on a preformed layer of cultured rat SMC (Jones, 1979). The endothelial cells anchored more firmly to the SMC than to plastic, and electron microscopy showed the existence of an irregular, dense, basal lamina-like structure between the two cell types (Jones, 1979). Then, there is a microcarrier technique to build a model of EC-SMC coculture. In the 80's of the 20th century, Navab laboratory builds human aortic EC-SMC coculture model successfully to study the mechanism of atherosclerosis. In this model, polycarbonate filters with 1- $\mu$ m-pore-diameter were used (Navab et al., 1988). Human aortic SMC were seeded on filters and grew for 2 day, at which time they covered the entire surface of the filter (Navab et al., 1988). Collagen type 1 was layered over the SMC and the collagen layer was added in human fibronectin (Navab et al., 1988). Then, the excess fibronectin was washed away and human aortic EC were seeded on collagen layer (Navab et al., 1988).

There is physiological IEL between the intima and media of vascular walls. Using microporous PET membranes which simulates IEL is better to develop the *vitro* EC-SMC model. In this *vitro* model, cells grow separately

and contact with each other through micropores to form gap junctions (Kinard et al., 2001). Now, building the *vitro* EC-SMC coculture model takes the methods developed by Isakson and Duling (2005).

### PROSPECT OF INTERACTIONS BETWEEN VASCULAR SMOOTH MUSCLE CELLS (VSMC) AND ADVENTITIAL FIBROBLAST (AF)

VSMC traditionally play a central role in vascular remodeling and the adventitia has an effect of nutritional support. But more and more evidences demonstrate that AF is the important pathological foundation in vascular remodeling diseases such as hypertension, atherosclerosis and restenosis. After injury of vascular intima, the AF will express  $\alpha$ -SM-action and change into myofibroblast, the latter can proliferate, migrate to intima, secrete massive extracellular matrix to result in the vascular rebuilding (Torsney et al., 2005; Xu et al., 2007). In general, AF should be paid more attention to in the process of hypertension, atherosclerosis and restenosis.

The building of developed EC-SMC coculture model offers a good method to study the communication between AF and SMC. Someone use the coculture model of AF and SMC to study subarachnoid hemorrhage (Tao et al., 2006). The results demonstrate that heterocellular gap junction probably exists between AF and SMC (Tao et al., 2006). This may offers a new approach to research the vascular remodeling diseases. We can forecast that there are significant physiological and pathological relations between AF and SMC. If we can intensively study these relations, there will be high value for the mechanism of vascular remodeling diseases.

### Conclusions

Important implications on the interactions of the gap junctions between homolytic and heterotypic cells can be seen. The three-layer structure of vascular wall forms an organic whole by these gap junctions, which plays an important role in the pathological and physiological processes of the vascular remodeling diseases. Through animal laboratory experiment and the use of PET membrane as a vector in this co-culture model *in vitro*, we wish to investigate the important role of interaction between the gap of AF and SMC. Together with MEGJ, we will further explain the physiopathological mechanism of the blood vessel reconstruction and the method on prevention and cure. This will be the new direction for research.

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