Endothelial connexins are down-regulated by atherogenic factors

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1. ABSTRACT

Connexins are single polypeptides that assemble to form paired connexon hexamers participating in gap-junctional intercellular communication. In addition, unpaired connexons at cell membrane also act as channels connecting cytosols and extracellular space. These channels' properties plus other unique functions of connexins give the molecules significant roles in endothelial cells, which mainly express connexin43 (Cx43), Cx40, and Cx37. In vitro studies have shown that expression of endothelial connexins are regulated by both physiological and pathological factors, a majority of which are involved in atherogenesis. In vascular disorders, endothelial connexins are differentially regulated. However, down-regulation of gap junctions is a common phenomenon. These findings suggest that reduced expression of endothelial gap junctions is a potential indicator of endothelial dysfunction and warrant investigators to explore the molecular mechanisms as well as therapeutic implications.

2. INTRODUCTION

Vascular endothelial cells interconnect with one another to form a continuous single layer surrounding a central lumen throughout the whole vascular network. This monolayer of cells forms a barrier and mediates the interaction between the blood and the vascular wall, participating in the regulation of a variety of critical functions, including vascular tone, hemostasis, and inflammation (1). To facilitate the regulation for maintenance of homeostasis, integrity of the function of this monolayer is essential. Such teamwork of endothelium requires coordination of the activity between individual cells, in which numerous signaling mechanisms are involved, including gap junctional intercellular communication. This review article focuses on the endothelial gap junctions, the component proteins, and the physiological and pathophysiological role of these proteins in vascular disorders related to atherosclerosis.

3. ENDOTHELIAL GAP JUNCTION, CONNEXON AND CONNEXIN

3.1. Structure and nomenclature

Gap junctions are cell membrane protein channels, which span a tiny 2 nm gap of the extracellular space to bridge the neighboring cells (2). Construction of the channels is equally contributed by the connected cells, each of which donates hemichannels named connexons. One connexon is a hexameric array of connexins delineating a central pore. When connexons from apposing cells meet end to end at the contacted cell borders, the central pores merge to form a tunnel linking the cytoplasmic compartments of connected cells (3). Apart from formation of channels insulated from extracellular space by paired connexons, unpaired connexons located at the cell membrane can also serve as pathways between the cytoplasm and extracellular space (4).

Connexin, the unit protein of gap junctions, belongs to a multigene family. In mammals, at least 20 members of connexins (21 in humans) have so far been identified (5). Each connexin molecule is a single polypeptide with 4 segments embedded in the cell membrane, leaving the other 5 domains at both sides of the membrane: a cytoplasmic C-terminus, first extracellular loop, cytoplasmic loop, second extracellular loop, and an cytoplasmic N-terminus. Two systems of nomenclature are widely used in the literature to distinguish the members of the family. One of the systems is based on the predicted molecular mass of connexin protein, in kDa, deduced from the cDNA. For example, connexin43 (Cx43) has a molecular weight about 43 kDa. The other system emphasizes the sequence similarity and length of the cytoplasmic loop. With the latter system, all connexins are divided into 4 groups: alpha, beta, gamma, and delta followed by a number according to the order of discovery. In this system, Cx43 is named alpha 1 connexin, the first one found in the alpha group.

Connexin molecules of the same or different isoforms can be assembled into connexons before being transported to the cell membrane. For each gap junction channel, the counterpart connexons can be of the same or different component connexins. Such an arrangement enables construction of numerous different channel structures far beyond the total number of connexin isoforms. However, compatibility is required between connexins to form connexons and between connexons to form gap junctions, i.e., not all randomly selected connexins can form connexons or gap junctions (6)

3.2. Functional properties of gap junction, connexon and connexin

The traffic of gap-junctional channels allow passage of ions and small molecules (~1,000 Daltons), including metabolic and electrical signals such as amino acids, ions, and second messengers, the flux of which through gap junctions is driven by the chemical and electrical gradient between the connected cytoplasmic compartments. Recent studies also showed that large linear molecules, such as short interfering RNA (7) and polypeptide (8), may be able to permeate the channels. Gating of the channels is regulated by a variety of factors, including pH, voltage, calcium level, and phosphorylation (9-10), the last of which occurs at the cytoplasmin terminus of all connexin members, except Cx26 (11). Gap-junctional intercellular communication is involved in the regulation of a variety of biological functions, such as growth, proliferation, differentiation and development (12-13). Connexon hemichannels located at cell membrane also allow passage of ions and small molecules. Knowledge of connexon hemichannels mainly came from cultured cells, which showed that under normal conditions, hemichannels generally remain in a closed state. Hemichannels are opened by a variety of factors, such as low calcium medium, high intracellular calcium (14), hypoxic or ischemic stress, mechanical stimulation (15-16), and dephosphorylation (17). The functions of hemichannels include regulation of cell volume, release of ATP (18-19), NAD+ (20), glutamate (21), and prostaglandins (22) as paracrine or autocrine signals, and activation of cell survival pathways (23). Gating of connexin hemichannels and gap junction channels of the same cell may vary by the same regulators. For example, lipopolysaccharide and basic fibroblast growth factor inhibited gap junction channels, but they stimulated ATP release via hemichannels in C6 glioma cells, which express Cx43 (14).

Apart from the channel properties of gap junctions and connexons, the cytoplasmic tail of connexins can interact with other molecules, including Src, ZO-1, and components of microtubule (24), which give connexins' channel-independent effects. Such a role of connexins has been implicated in intracellular signalling involved in transcriptional and cytoskeletal regulation. Recently, Cx43 was found in cardiomyocyte mitochondria. The function of Cx43 there is involved in a mechanism of cardioprotection characterized by diazoxide-induced preconditioning (25-26).

3.3. Endothelial gap junction, connexon, and connexin

Of the 20 more connexins present in mammalian cells, the endothelial cells mainly express 3 connexins of the alpha group, Cx43 (alpha 1), Cx40 (alpha 5), and Cx37 (alpha 4) (27-28). Cx45 transcripts are also found in the endothelial cells, however, the protein is undetectable. Once located at cell membrane, connexons of endothelial cells can be paired with connexons of adjacent cells, including endothelial cells, subjacent smooth muscle cells, or attached blood cells, to form gap junctions. The existence of gap junctions between endothelial cells and between endothelial cells and smooth muscle cells (also named myoendothelial gap junctions) are well documented, while the existence of those between endothelial cells and blood cells are still questionable, though gap junctions appear to form between leukocytes and endothelial cells in a model of ischemia-reperfusion (29). Distribution and size of gap junctions between endothelial cells vary along the vascular network. In general, the number and size decrease as the diameter of vessels become smaller, and the arterial side has more and larger gap junctions compared to the venous side. Expression patterns of connexins is more complicated, even on the arterial side. In large arteries of

mammals, the expression of connexins is species-specific. For example, in aortic endothelium, all 3 connexins, Cx37, Cx40, and Cx43, exist in the Sprague-Dawley and Wistar-Kyoto rats. In contrast, only Cx37 and Cx40, but not Cx43, exist in C57BL/6 mice. In addition, the expression of connexins is site-specific, and the transition can be dramatic. For example, in the endothelium of the proximal portion of rat aorta, Cx43 is abundantly expressed, while the expression decreases steeply to total absence in the intramural coronary arterial endothelium (30). Other factors related to the expression include physical forces, such as shear stress or mechanical load, which were also reported to regulate the expression of Cx43 (31), but not Cx40 or Cx37. On the other hand, in the cultured endothelial cells, Cx43 is by far predominantly expressed (32-34). In our laboratory, we found that this rule also applies to endothelial cells cultured from the mononuclear cells of peripheral blood named late-type endothelial progenitor cells.

Although connexons of myoendothelial gap junctions are half contributed by the endothelial cells, the distribution and size of myoendothelial gap junctions do not follow the rules of those between endothelial cells. In general, myoendothelial gap junctions are small (often less than 100 nm) and more frequently seen in resistance arteries with fewer layers of smooth muscle cells, while rare in large arteries (35). However, since gap junction channels must cluster in a certain amount to be detected, the absence of myoendothelial gap junctions by electron microscopy or immunofluorescence microscopy does not exclude the existence of small gap junctions.

Unpaired connexons manifesting channel properties are also present in endothelial cells (36). Theoretically, connexon hemichannels can exist at any part of the cell membrane, however, whether they have preferential locations or are restricted to only the luminal side of endothelial cells, and whether hemichannels located at the luminal side and aluninal side have distinct properties, remain unclear.

3.4. Endothelial function contributed by connexins

Regulation of blood flow is ranked as perhaps the most important role of connexins in the endothelial cells, and the mechanisms have been extensively studied (35,37). This role is mainly achieved by construction of gap junctions for passage of vasodilatory or vasoconstricting signals though those between endothelial cells and via myoendothelial gap junctions to affect the subjacent smooth muscle cells. Release of vasoactive signals through connexon hemichannels may as well be involved. Because control of blood flow mainly takes place at the level of resistance arteries, the preferential distribution of myoendothelial gap junctions in the resistance arteries is in agreement with the physiological need. Although both vasodilatory and vasoconstricting signals are conducted through gap junctions, vasodilatation is considered the dominant work. The signals going through the junctions that have been examined include Ca^{2+}, ATP, IP3, NO, and endothelium-derived hyperpolarization factor (38). Other

roles of gap junctions that have been suggested in endothelial cells include maintenance of monolayer topology, transport of nutrients, and regulation of angiogenesis, endothelial growth, and senescence (39-43).

4. ENDOTHELIAL CONNEXINS ARE ALTERED IN ATHEROSCLEROSIS

4.1. Endothelial connexins and vascular disorders related to atherosclerosis

Atherosclerosis is a complex and multifactorial disease, involving excessive oxidative stress, endothelial dysfunction, inflammation, platelet activation, and thrombosis. A common pathway for various cardiovascular risk factors, such as hypertension, hyperlipidemia, and smoking, to trigger the initiation and contribute to the progression of the disease has been established (44-47). At the beginning of the disease, the presence of risk factors is associated with excessive oxidative stress in the vascular wall, which attenuates the availability of nitric oxide generated by the endothelial cells, leading to insufficient vasodilation, a status called endothelial dysfunction. Existing data showed that endothelial gap junctions are also changed in vascular disorders known to manifest endothelial dysfunction (48-49). As the process goes on, the elevated oxidative stress activates the endothelial cells to express adhesion molecules, which attract the circulating inflammatory cells to infiltrate the vascular wall. The existence of gap junctions between endothelial cells and attached white blood cells has been proposed (29). Thereafter, the infiltrated inflammatory cells release growth factors and cytokines, which induce migration and proliferation of smooth muscle cells and synthesis of extracellular matrix. In addition, the released growth factors and cytokines recruit more inflammatory cells. The result is remodeling of vascular wall with the change of flow, which activates the circulatory platelets. The milieu becomes prothrombotic, and once the subendothelial tissue is exposed to the blood, thrombosis occurs. Numerous factors which actively participate in atherosclerosis, including growth factors and cytokines, were found to change the endothelial connexins (Table 1). The following sections will discuss the effects of each factor on endothelial connexins and the change of connexins in different vascular disorders.

4.2. Alteration of endothelial connexins by oxidative stress

Ischemia/reperfusion, or hypoxia/reoxygenation, is known to increase the production of superoxide, which possesses multiple biological effects, including alteration of connexins. In human umbilical endothelial cells hypoxia/reoxygenation was reported to inhibit gap junctional intercellular communication. The effect was partly prevented by protein kinase C inhibitor calphostin C and completely abrogated by the protein tyrosine kinase inhibitor genistein, but not altered by free radical scavenger DMSO or antioxidant superoxide dismutase (50). Another study investigating the role of Cx40 showed that hypoxia followed by abrupt reoxygenation reduced protein kinase A activity as well as cell-cell coupling in cultured microvascular endothelial cells of wild type mice.

Table 1. Effects of oxidative stress, inflammation, growth factors, and cytokines on endothelial gap junctions and connexins

Condition/factor	Cell	Effect	Reference
hypoxia/reoxygenation	HUVEC	↓GJIC	50
hypoxia/reoxygenation	mouse microvascular EC	↓GJIC in wild-type cells, but not Cx40$^{-/-}$ cells	51
sepsis by cecal ligation and perforation	mouse aortic EC	↑Cx40	52
Lipopolysaccharide (i.p.)	mouse aortic EC	↓GJIC and ↓Cx37, Cx40	53
diet-induced hyperhomocysteinemia	rat EC	↓Cx40 mRNA	54
TGF-beta	bovine aortic endothelial cells	↑Cx43 protein, ↓Cx37 mRNA	55
VEGF	HUVEC, coronary capillary endothelial cells,	↓GJIC, ↑internalization of Cx43	56, 57
Epidermal growth factor	young HUVEC	↓GJIC	42
bFGF	bovine microvascular endothelial cells	↑Cx43 protein and GJIC	40
TNF-alpha	HUVEC	↓Cx37 and Cx40 mRNA	28

HUVEC, human umbilical vein endothelial cell. GJIC, gap-junctional intercellular communication. EC, endothelial cell. i.p., intraperitoneal. TGF, Transforming growth factor. VEGF, vascular endothelial growth factor. bFGF, basic fibroblast growth factor. TNF, Tumour necrosis factor.

However, such an uncoupling effect was absent in Cx40 knockout mice, indicating that Cx40 is required to translate the effect of oxidative stress (51). In the same report, protein kinase A activator 8-bromo-cAMP was shown to prevent the reduction in the coupling, while antioxidant ascorbate abolished all the effects of hypoxia/reoxygenation. (51). These findings indicate that different protein kinases are involved in oxidative stress-induced gap junction inhibition and not all antioxidants prevent the inhibition.

4.3. Alteration of endothelial connexins by inflammation and prothrombotic molecules

Endothelial cells play a key role in the regulation of inflammation and hemostasis. Literature regarding endothelial gap junctions and inflammation is in a dearth, and is even rarer regarding the junctions and hemostasis. Nevertheless, the effects of inflammation and prothrombotic factors on endothelial connexins has been studied in animals. In rats woth sepsis by cecal ligation and perforation, the aortic endothelial Cx40 was found elevated in expression (52). In contrast, in mice treated with lipopolysaccharide to induce inflammation, intercellular communication and connexin expression in the aortic endothelium were found decreased (53). These contradictory results indicate that models of inflammation rather than inflammation itself have distinct effects on the expression of endothelial connexins. Hyperhomocysteinemia is associated with increased risk of thrombosis. In rats made hyperhomocysteinemic, the endothelial Cx40 mRNA and EDHF-mediated vasodilatation were found reduced (54).

4.4. Alteration of endothelial connexins by growth factors, cytokines, and vasoactive peptides

The effects of growth factors, cytokines, and vasoactive peptides on the expression of endothelial connexins has been widely examined. Transforming growth factor (TGF)-beta is a growth-inhibitory cytokine. Activin receptor-like kinase 1 (ALK-1) is a TGF-beta type I receptor expressed in endothelial cells. In cultured bovine aortic endothelial cells, TGF-beta increases the content of Cx43 protein. The mechanisms involve enhanced synthesis and decreased degradation, the latter of which is due to impaired lysosomal activity (41). In contrast, TGF-beta suppressed the expression of Cx37 transcripts (39). However, in human umbilical vein endothelial cells (HUVEC) infected with recombinant adenoviruses carrying a constitutively active form of ALK-1, Cx37 transcripts were increased (55). Vascular endothelial growth factor (VEGF) is an important angiogenic factor, which stimulates angiogenesis and induces blood vessel formation by directly acting on endothelial cells. In HUVEC, VEGF transiently disrupts gap junctional communication (56). The signalling mechanisms involved in this phenomenon depend on activation of VEGFR-2. In coronary capillary endothelial cells, VEGF also disrupts the Cx43 gap junctions, and the action involves internalization of Cx43 and its tyrosine phosphorylation (57). A similar effect was seen with bradykinin (57). In young but not senescent HUVEC, epidermal growth factor decreases the gap-junctional coupling (42). In bovine microvascular endothelial cells, basic fibroblast growth factor (bFGF) increases the expression of Cx43 protein and gap-junctional communication. Administration of antibodies to bFGF abolishes the increase in coupling and Cx43 expression in mechanically wounded cells (40). Tumour necrosis factor alpha (TNF-alpha) is a potent pro-inflammatory cytokine which activates endothelial cells to pathological status in many aspects, including the expression of adhesion molecules and promotion of migration. In HUVEC treated with TNF-alpha, Cx40 and Cx37 transcripts were reduced while Cx43 remained unchanged (28).

4.5. Alteration of endothelial connexins by cardiovascular risk factors

We and other investigators have, using cultured human endothelial cells and animal models, extensively examined the effects of traditional risk factors for atherosclerotic cardiovascular diseases, such as aging (58), hypertension (48), hyperlipidemia (49,59), smoking (33), and diabetes (59-60) on endothelial connexins. In addition, the influence of arsenic (34), which causes blackfoot disease and accelerates atherosclerosis (61-62), was also studied. In animal studies, the expression of aortic or carotid endothelial Cx43, Cx37, and Cx40 in rat or Cx37 and Cx40 in mice were examined using *en face* immunoconfocal microscopy, which enables examination of much larger endothelial area, compared to the section view (Figure 1). Interestingly, aging (by observation of animals of different ages (58), hypertension (induced by adding eNOS inhibitor L-NAME in the drinking water (48), hyperlipidemia (induced by cholesterol-enriched diet or ApoE gene knockout (49,59)), and exposure to arsenic (by continuous intravenous infusion of arsenic trioxide using osmotic pump (34)) are associated with reduced
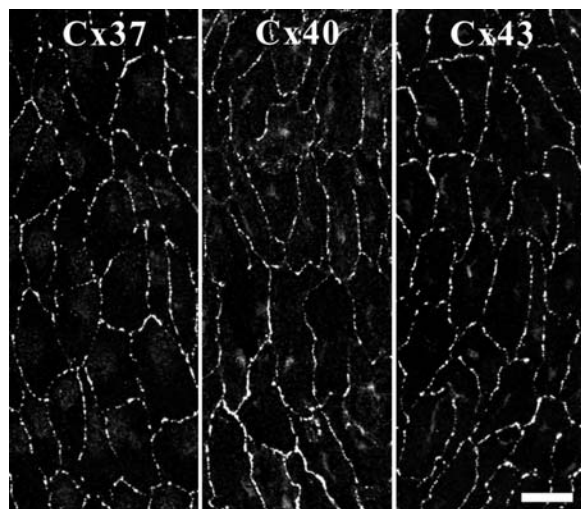
Figure 1. Gap junctions made of Cx37, Cx40, and Cx43 are abundantly expressed and delineate the endothelial cell borders. Images are from *en face* immunoconfocal microscopy of rat aortic endothelium. Bar, 0.02 mm.

expression of gap junctions. In rats exposed to arsenic trioxide for 8 weeks, all 3 connexins are consistently reduced (34). However, in other animal models, not all endothelial connexins respond in the same manner or are affected at the same pace. In aged rats, though all 3 connexins are reduced in expression after 20 months of age, Cx40 is the last one to decrease (58). In L-NAME-induced hypertension, while the expression of Cx43 and Cx37 are decreased within 2 months after the addition of L-NAME in the water, the expression of Cx40 remains stationary (48). In addition, the effects of antihypertensive drugs and lipid lowering therapy vary on the depressed connexins. In hyperlipidemic mice induced by cholesterol-enriched diet, a 7-day treatment with simvastatin lowers the serum cholesterol level and nearly recovers Cx37 expression, but Cx40 remains depressed (49). In L-NAME-induced hypertension, carvedilol and atenolol lower the blood pressure to a compatible level, while the recovery of depressed Cx37 and Cx43 by carvedilol is better compared to that by atenolol (48).

In the *in vitro* experiments, nicotine (one of the major contributors to the harmful effect of smoking) (33), high concentration of glucose (mimicking the milieu of diabetes) (60), and arsenic trioxide (34) were added to the medium. The results showed that the expression of endothelial Cx43 and gap junction communication are similarly reduced. Regarding the mechanisms underlying the reduced expression of Cx43 in the cultured cells, the reduction in response to nicotine and arsenic is mainly contributed by an accelerated degradation of the Cx43 protein, as evident from the effects being reversible by addition of protease inhibitors N-acetyl-Leu-Leu-Norleu-al and leupeptin. Unlike the same enhanced degradation of protein, the Cx43 transcripts were either increased (with nicotine) or decreased (with arsenic). The cell culture experiments also allow examination of drugs on the expression of Cx43. In nicotine-treated cells, various

HMG-CoA reductase inhibitors, including pravastatin, simvastatin, fluvastatin, and lovastatin, are able to reverse the effect of nicotine. Interestingly, the effect of statins are not blocked by supplement of mevalonate, the main product of HMG-CoA reductase (33). This indicates that the mechanism underlying the action of statins on Cx43 protein does not involve the mevalonate pathway. Apart from statins, carvedilol also increases the expression of Cx43 in the cultured endothelial cells (48). Such an action of carvedilol on Cx43 protein is independent of the blood pressure lowering property, since in the cultured cells the effect of blood pressure does not exist.

4.6. Alteration of connexins in endothelial cells exposed to mechanical injury and grown on vascular stents

In human atherosclerotic disease, organ ischemia due to stenosis of supplying arteries can be relieved by angioplasty, the standard procedures of which include balloon dilatation with or without stent placement. However, restenosis following the procedures in the treated artery is a major drawback. Pathological examination has shown that after the procedures, endothelial cells are denuded, with the formation of a thrombotic layer on the luminal surface followed by the growth of neointima mainly composed of smooth muscle cells (63-65). Animal studies have shown that complete coverage of endothelial cell in injured vessels is associated with attenuation or even stopping of the growth of neointima, attributable to the anti-thrombotic and anti-proliferative properties of endothelial cells (66-67). To understand the effects of angioplasty and stent on endothelial connexins, we injured the rat carotid artery by denudation, grew endothelial cells on stent materials, and examined the expression of connexins in the endothelial cells (68-69). In the animal study, after injury, the regenerating endothelium initially expresses small, sparse gap junctions, within 28 days the expression levels then progressively increase to values equivalent to (Cx40) or exceed (for Cx37 and Cx43) those of controls (68). In addition, co-localization of connexins to the same gap junction plaque is a common feature. In the *in vitro* experiment, Cx43 was reduced in endothelial cells grown on various stent materials, associated with retarded growth and reduced expression of eNOS and von Willebrand factor, which indicates the existence of endothelial dysfunction (69). Taken together, these two studies demonstrate that reduction of endothelial gap junctions may reflect impaired endothelial function. On the other hand, an escalating expression of connexins in the regenerating arterial endothelium implies the requirement of enhanced intercellular communication during the vascular healing process.

5. DOWN-REGULATION OF ENDOTHELIAL CONNEXINS IS A MARKER FOR IDENTIFYING ENDOTHELIAL CELLS SITUATED AT PATHOLOGICAL STATUS

From the above review, it is clear that, in response to factors contributing atherogenesis and therapeutic interventions, the expression of endothelial connexins is consistently inhibited (Figure 2). This can be
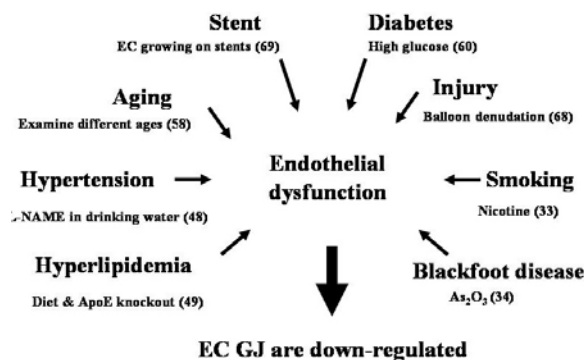
Figure 2. Summary of animal studies and cell culture experiments examining the effects of risk factors for atherosclerotic cardiovascular diseases and therapeutic interventions on endothelial cells (EC) gap junctions (GJ). All the factors and interventions are known to impair endothelial function and are consistently shown to reduce the endothelial gap junctions. References are in parentheses.

explained in part by the fact that some of the atherogenic molecules, including exogenous toxicants, for example, nicotine and As2O3, as well as stent materials, directly inhibit the expression of endothelial connexins, as revealed by *in vitro* examination (33-34). For other risk factors, such as hypertension and hyperlipidemia, and balloon denudation, the mechanisms may be not as simple. Many endogenous molecules, for examples, growth factors and cytokines, known to alter the expression of endothelial connexins are potentially involved, and the down-regulation may be a net result. Nevertheless, since the properties of endothelial cells are changed to be proatherogenic in the presence of the above mentioned factors and after the therapeutic interventions, reduced expression of endothelial connexins may reflect such a change. Implication from this is that reduced expression of endothelial connexins may be used as a marker to identify endothelial cells situated at proatherogenic status.

Another feature regarding the expression of endothelial connexins at pathological status is that individual members of connexin do not behave in the same manner. Examples are that during the aging process, the expression of Cx40 is reduced temporally later compared to Cx43 (58), while in L-NAME-induced hypertension, the expression of endothelial Cx40 remains stationary, in contrast to the decline of Cx43 and Cx37 (48). The different responses of individual connexin members can serve as the basis for compensation between the members. Interestingly, Cx43 is always reduced in the presence of risk factors. As mentioned before, the function of Cx43 is not only limited to the cell membrane as conduit protein; it also participates in molecular interaction and the regulation of mitochondrial function (24-26). This raises a question whether down-regulation of Cx43 itself may impair the function of endothelial cells. To answer this question, examination of the properties of endothelial cells in Cx43 knockout mine is one approach. However, in the genetically manipulated animals, the compensation of loss

of Cx43 by other connexins starts from the embryonic stage, and this may mask the significance of deficient Cx43 expression. Another approach is to examine endothelial cells in an *in vitro* system using the technique of small interference RNA, which is currently conducted in our laboratory. Preliminary results showed the viability and proliferation of endothelial cells were impaired by the controlled knockdown of Cx43.

6. CONCLUDING REMARKS AND PERSPECTIVE

Accumulated information has indicated that connexins in endothelial cells participate in numerous vascular activities under physiological conditions. In the past decade, data regarding the changes of endothelial connexins in various vascular disorders and pathological status have come out, and down-regulation of connexins is found to be a common phenomenon in these pathological conditions. Understanding the molecular signaling underlying the down-regulation and clarification of whether rectification of the down-regulation is beneficial to the endothelial cells are important future directions for research.

7. ACKNOWLEDGMENTS

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Abbreviations: ALK-1: activin receptor-like kinase 1, bFGF: basic fibroblast growth factor, Cx: connexin, GJIC:

gap-junctional intercellular communication, HUVEC: human umbilical vein endothelial cells, TGF: transforming growth factor, TNF: tumour necrosis factor, VEGF: vascular endothelial growth factor

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