Review

The functional implication for endothelial gap junction and cellular mechanics in vascular angiogenesis

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Abstract: Angiogenesis, the sprout and growth of new blood vessels from existing vasculature, is an important process of tumor development for the supply of oxygen and nutrition to cancer cells. Endothelial cell is a critical player in angiogenic process by modulating cell proliferation, cell motility, and cell morphology in the response to pro-angiogenic factors and environments provided by tumor and cancer cells. Recent *in vivo* and *in vitro* studies have revealed that gap junction of endothelial cells also participates in the promotion of angiogenesis. Pro-angiogenic factors modulate gap junction function and connexins expression in endothelial cells, whereas endothelial connexins involve in angiogenic tube formation and cell migration of endothelial cells via both gap junction channel function dependent or independent mechanisms. In particular, connexin might have the potential to regulate cell mechanics such as cell morphology, cell migration, and cellular stiffness that are dynamically changed during angiogenic processes. Here, we review the implication for endothelial gap junction and cellular mechanics in vascular angiogenesis.

Keywords: gap junction; connexin; angiogenesis; cell mechanics; cell migration; cellular stiffness

1. Introduction

The vascular network which supplies oxygen and nutrition is necessary for the tumor growth and cancer cell proliferation. In order to promote angiogenesis from existing blood vessels, tumor and cancer cells secret high levels of pro-angiogenic factors and provide pro-angiogenic hypoxic environments [1, 2]. In the response to these pro-angiogenic factors and environments, vascular endothelial cells (ECs) initiate angiogenic process including vascular sprouting, cell proliferation, cell migration, tube formation, and vascular stabilization [3, 4]. Notably, during these angiogenic process, ECs dynamically changes of cell mechanics that are mechanical and physiological characters determined by cytoskeletal rearrangement [5], focal adhesion formation [6], and contractile force [7], have been also observed.

Gap junctions (GJs) are consisted of connexin (Cx) family protein which has four transmembrane domains and two extracellular loop domains [8, 9]. The hexameric Cx forms a hemichannel (connexon) that docks to another connexon on the adjacent cell via extracellular domains resulting in the formation of GJ channel [8, 9]. GJ channel directly connects each cytoplasm of adjacent cells and allow the intercellular movement of small molecules and electron coupling [10]. Thus, GJ intercellular communication (GJIC) is essential for the transfer and synchronization of the intracellular environment between adjacent cells. It has been considered that GJ-mediated transfer

and synchronization of intracellular mediators such as ions, amino acids, small metabolites, and secondary messengers are essential for orchestration of multicellular responses [10]. In addition, the C-terminal domain of Cx protein interacts with several intracellular protein such as signaling molecules [11], cytoskeletal proteins [12], and cell junctional proteins [13], indicating the possibility of GJ- and Cx-mediated regulation of cell mechanics and mechanotransduction.

EC plays a critical role in the regulation of vascular inflammation [14], blood coagulation [14] [16, 17], leukocyte adhesion and extravasation [15] [18], and angiogenesis [16] [19], thereby, the EC dysfunction is a conceivable cause of the development of cardiovascular diseases [17]. ECs predominantly express three Cxs: Cx37, Cx40, and Cx43 [18, 19] and essentially regulate GJ function and Cx expression in the response to pro-inflammatory stimuli [20, 21]. Conversely, alteration of GJ function and Cx expression in ECs is able to influence on a multiple EC functions under physiological and pathological condition [20, 22, 23]. Recent studies have indicated that abnormality of GJ and Cx expression in vascular component cells including ECs, smooth muscle cells and monocytes/macrophages contributes to atherosclerosis associated with excessive inflammation and vascular remodeling [22, 23]. In addition, more than a decade of research on GJ in ECs and angiogenesis has provided evidences of the interplay between endothelial Cxs and angiogenesis. Here, we mainly focus on GJs and Cxs in ECs and will discuss the implications of cellular mechanics for vascular angiogenesis.

2. Endothelial Cx expression and its role in vascular diseases

Cx expression pattern in ECs is dependent upon vessel type, be it arteries, veins, or lymphatic vessels. Cx37 and Cx40 are co-expressed in arterial ECs of the healthy vessels [24], whereas Cx43 has been observed characteristically in ECs of the microvasculature and at branch points of arteries that experience turbulent blood flow [24]. Cx32, Cx37 and Cx40 are present in venous ECs [25, 26]. *In vitro* studies have demonstrated Cx32, Cx37, Cx40, and Cx43 expression in both cultured human vein and artery ECs [27-29]. It has been known that alteration of each Cx expression and GJ function in ECs upon pro-inflammatory stimuli is closely correlated with EC activation. Indeed, pro-inflammatory tumor necrosis factor-α (TNF-α) reduces GJ function in EC at early phase (4hours) and then decreases the expression of Cx32, Cx37 and Cx40, but not Cx43 at late phase (24 hours) [21, 30]. LPS, is an important activator of inflammation in ECs via toll-like receptor 4, also induces serine-dephosphorylated Cx40 [31] and reduced GJ function between microvascular ECs [31, 32]. Pro-coagulant factor thrombin, which is a major trigger of thrombus formation and increased vascular endothelial permeabilization, induces rapid and acute internalization of Cx43-mediated GJ in primary pulmonary artery ECs [33]. On the other, opposite effect by which thrombin induces Cx43 expression and GJ function associating with the disruption of the endothelial barrier has been reported [34]. In this way, although some different phenotypes have been observed, these results have indicated the dynamic regulation of GJ function and Cxs expression in ECs upon pro-inflammatory stimuli at both post-translational modifications and transcriptional level.

Several studies have revealed the contribution of aberrant GJs function and Cxs expression in ECs to the promotion of endothelial dysfunction and vascular inflammatory diseases such as atherosclerosis. For example, Cx37 and Cx40 are decreased in early stage of atherosclerosis [20], while deletion of Cx40 from ECs in mice, as well as the dysfunction of Cx37, can promote the development of atherosclerosis by enhancing both monocyte adhesion and transmigration [22, 35]. Moreover, Cx37-deficient mice enhance the expression of a number of pro-inflammatory genes involved in advanced atherosclerosis [36]. Cx43 is increased in early stage of atherosclerosis [20], whereas reduced expression of Cx43 by smooth muscle cells inhibits the formation of atherosclerotic lesions [37]. Furthermore, endothelium specific deletion of Cx43 modulates renin secretion, thereby inducing hypertension [38]. A Cx43 mutation in patients with cardiac infarction has been identified as a risk factor [39]. We have previously shown not only that reduced Cx32 expression in HUVECs facilitates pro-inflammatory cytokines expression upon inflammation [30], but also that Cx32-deficient mice enhances activation of vascular inflammation and blood coagulation in septic model [30, 40]. Taken together, these studies have suggested that abnormality of GJs function and Cxs

96 expression may be a trigger of various endothelial dysfunction leading to the development of
97 atherosclerosis and vascular inflammatory diseases.

98 **3. Alteration of GJ function and Cxs expression in ECs under pro-angiogenic stimuli**

99   Pro-angiogenic factors have also been likely to modulate GJ function and Cx expression of ECs
100 [41] (Fig.1).   Vascular endothelial growth factor (VEGF), which plays a central role in vasculogenesis
101 and angiogenesis [42], implicates in diverse physiologic processes including tumor angiogenesis [43,
102 44], diabetic retinopathy [45], wound healing [46], and tissue repair following ischemic injury [47].
103 VEGF-induced VEGF-receptor 2 (VEGF-R2) activation of ECs in existing vasculature is primarily an
104 initiation step of angiogenesis and then induces sprouting, cell proliferation, and cell migration of EC
105 [48].   *In vitro* model experiments, VEGF-induced c-Src tyrosine kinase and MAP kinases activation
106 results in the rapid disruption of GJ function of ECs [41], and increases paracellular endothelial
107 permeability associating with reduction of cell-cell junction [49].   Furthermore, it has been reported
108 that the VEGF-induced disruption of GJ function correlates with the rapid internalization of Cx43
109 and Cx43 tyrosine phosphorylation in rat coronary capillary endothelium [50, 51].   Therefore, pro-
110 angiogenic VEGF stimulation negatively modulate GJ function and Cxs expression in ECs in a
111 consequence of angiogenesis-related signaling.
112   In addition to VEGF, basic fibroblast growth factor (bFGF) and hypoxia are well-known as the
113 pro-angiogenic factor and environment.   It has been reported that microvascular ECs facilitate GJ
114 function and Cx43 expression in the response to bFGF stimulation [52].   The stimulation with bFGF
115 not only increases Cx43 mRNA expression but also facilitates Cx43 localization at cell-cell interface
116 [52].   Hypoxia condition observed in tumor tissue activates HIF pathways and induces the
117 expression of a number of pro-angiogenic genes in cancer cells [1].   In the case of ECs, hypoxia
118 upregulates the Notch ligand Dll4 expression and promotes activation of Notch signaling which is
119 an essential pathway for vascular development and stabilization [53, 54].   The upregulation of Cx40
120 expression has been reported under hypoxia-mediated Notch signaling in ECs [54].   Recent study
121 has shown that a Notch-Cx37-p27 axis promotes EC cycle arrest leading to vascular regeneration
122 under shear stress [13].   These suggest that endothelial Cx and Notch might coordinate the
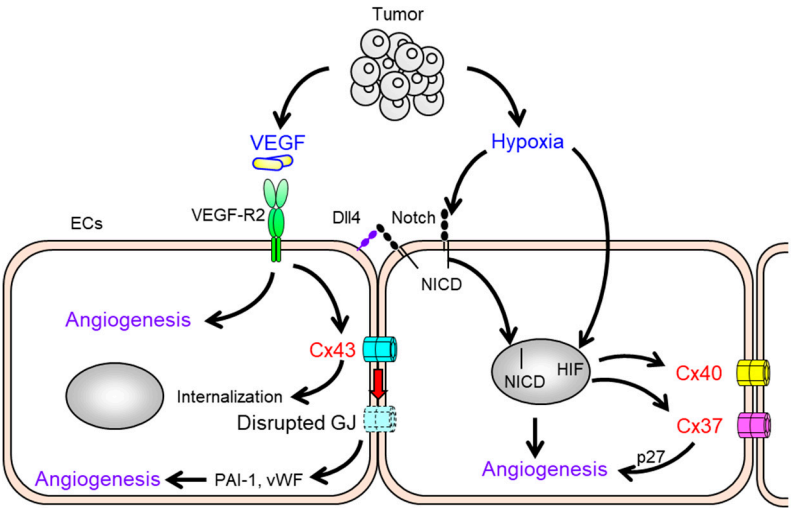123 appropriate EC proliferation and angiogenesis.
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Figure 1. Alteration of GJ function and Cxs expression in ECs under pro-angiogenic stimuli.   VEGF
is an essential initiator of angiogenesis.   ECs induce internalization and disruption of GJ formed by
Cx43 under VEGF-VEGF-R2 signaling.   Hypoxic condition in tumor tissue activates Notch and HIFs
in EC.   Notch signal including the nuclear translocation of the intracellular domain of the notch
protein (NICD) induces EC function and cell mechanics that involved in angiogenesis.   HIF
pathways are angiogenic-related genes expression in ECs.   Both signaling pathways results in
angiogenesis associating with upregulation of Cx37 and Cx40 in ECs.

132   Although endothelial GJ function and Cx expression are assuredly regulated by pro-angiogenic
133 factors and environments, and further, it has been reported that Cxs expression and GJ function in
134 tumor cell [55], myocadiac cell [56], and mesenchymal stem cell [57], tightly link to VEGF expression
135 from these cells.   For example, Cx43 knock-down in tumor cell lines increases VEGF expression and
136 enhanced the proliferation of ECs [55].   Thus, in order to understanding the role of GJ and Cx in
137 angiogenesis, it is necessary to elucidate the basic biology of GJ and Cx in these type cells at the
138 interplay of angiogenesis and tumor development.

139 4. The impact of endothelial Cxs in vascular endothelial angiogenesis

140   Several groups have investigated the impact of Cxs for development of cardiovascular system
141 which is closely related to angiogenesis.   Mutations in the gene for Cx43 (GJA1) were found to cause
142 a hypoplastic left heart syndrome [58].   Cx43-deficient mice, which die at birth from connatural
143 heart malformations, have shown a reduction in the distal branching complexity and length of
144 coronary arteries [59].   In Cx40-deficient mice, cardiac malformations have been observed [60].
145 Additionally, both endothelial Cx40- and Cx37-knockout mice develop severe abnormalities of the
146 vascular function and structure [61].   Recently, loss of endothelial Cx40 leads to a reduction in
147 vascular growth and capillary density in the neovascularization of the mouse neonatal retina [62].
148 We have also demonstrated that aortic vascular tissue from Cx32-deficient mice exhibit suppressed
149 vascular sprouting of ECs [28].   Cx37 knock-out mice enhance vasculogenesis and remodeling
150 resulting in improvement from an ischemic hindlimb injury [63].   These studies have indicated the
151 contribution of endothelial Cxs to angiogenesis in the physiological or pathological condition.
152   Some reports have shown the relevance of endothelial Cxs expression and vascular angiogenic
153 potential in ECs *in vitro* angiogenesis assay.   Knockdown of Cx43 using specific siRNAs reduces
154 tube formation and cell proliferation of human aortic ECs [64].   The downregulation of Cx43
155 increases angiogenesis-related factors [64], such as plasminogen activator inhibitor-1 [65] and von
156 Willebrand factor [66], suggesting that Cx43 might directly and/or indirectly contributes to
157 angiogenesis.   Knockdown of Cx37, Cx40, or Cx43 using siRNAs has shown suppressed endothelial
158 angiogenesis including the branching of HUVECs, elongation of cell length, and tube formation by
159 an *in vitro* matrigel assay [67].   In gain-of-function experiments employed stable Cx-transfectants,
160 we have demonstrated that increased expression of Cx32 markedly enhances tube length and the
161 number of branching of EA. hy926 cells which is ECs line derived from HUVECs in matrigel tube
162 formation [28].   On the other hand, Cx37- or Cx43-transfected EA. hy926 cells impairs tube length
163 and the number of branching [28].
164   These studies have provided many evidences that endothelial Cxs expression modulate
165 angiogenesis, however the specific role of each Cxs on angiogenesis remains unclear.   Notably, it
166 has been considered that any endothelial Cx expression may modifies other Cxs expression [28, 67,
167 68].   Indeed, Cx43 siRNA induces increased both Cx37 and Cx40 expression in aortic ECs.   In
168 HUVECs, Cx43 siRNA does not alter the expression of other Cxs, whereas Cx40 siRNA and Cx37
169 siRNA reduce Cx43 and Cx40 expression, respectively [67].   In addition, Cx32-transfected EA. hy926
170 cells reduce Cx43 expression and has exhibited highly angiogenic potential such as tube formation
171 and branching [28].   Gain-of function and loss-of function assay remain to be experimentally tested,
172 however these have indicated that alteration Cx expression patterns and their relative network of Cx
173 expression may elicit different ECs phenotypes during angiogenic processes.   This interrelated Cx
174 regulatory network have make difficult to understand specific role of each endothelial Cxs in
175 angiogenesis.

176 5. Endothelial Cxs-mediated regulation of cell migration in angiogenesis

177   ECs dynamically change cell mechanics such as cell morphology, cell proliferation, and cell
178 migration during angiogenesis process [69, 70].   EC activation by pro-angiogenic factors allows tip
179 cells to extend filamentous actin (F-actin)-rich filopodial protrusions migrating toward the required
180 site [3, 71].   Tip cells are the leading cells of the sprouts and guide following stalk cell which
181 proliferates to elongate the sprout [4].   A proper tuning of continued migration of tip cells and

182   proliferation of stalk cells is crucial for angiogenesis [4].   Notably, the implication of endothelial Cxs
183   in the control of EC migration has been progressively known.   We have shown impaired cell
184   migration of ECs both *in vitro* wound healing assay by using Cx32 blocking ECs and *in vivo* matrigel
185   plaque implant assay in Cx32-deficient mice [28].   Other groups have reported that GJIC and Cx43
186   expression are increased in the region of cell migration and at localized to cells at the wound edge by
187   using wounded monolayer repair assay [72].   Cx43 specific siRNA markedly suppresses cell
188   migration of endothelial progenitor cells by transwell chamber migration assay that allowed cells to
189   migrate through the filter membrane upon pro-angiogenic factors [73].   In addition to ECs, several
190   type of cell such as leukocyte, epithelial cell, and tumor cell also regulate their migration via GJ
191   channel dependent and independent function (reviewed by Matsuuchi [74] and Kameritsch [75]).
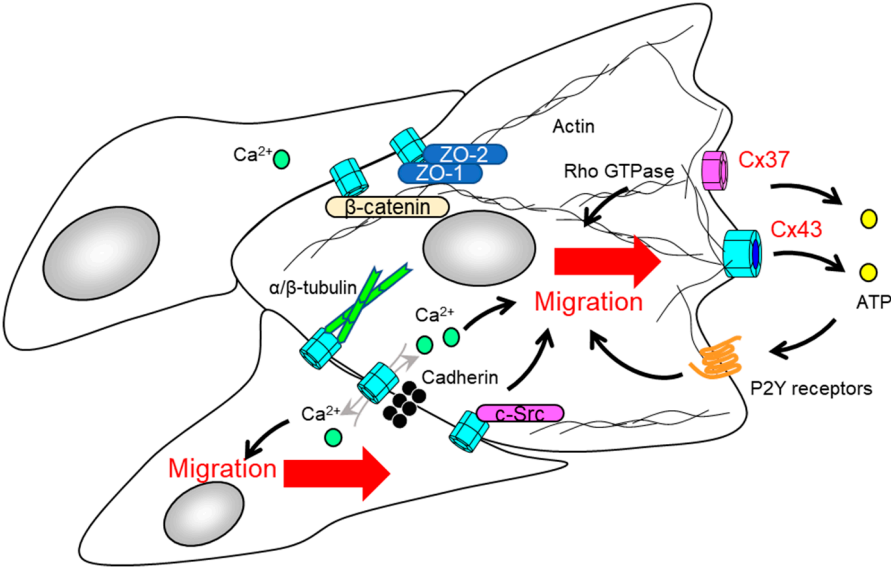192

193   **Figure 2.** Endothelial Cxs-mediated regulation of cell migration.   Extracellular ATP released by Cx-
194   hemicannels activates P2Y receptors which trigger cell migration.   GJ-mediated propagation of
195   calcium waves has been required for collective cell migration.   The interaction of Cx and GJ with
196   cytoskeletal proteins or intracellular proteins orchestrate cytoskeletal rearrangement and cell
197   migration.

198   Both GJ mediated cell-cell interaction and hemichannel function have involved in the regulation
199   of cell migration in a number of cell type (Fig.2).   Cultured adrenocortical cells have shown to exert
200   intact GJIC between cells during collective cell migration [76].   In a wound assay, Cx43 expression
201   in immortalized ECs is positively associated with cell migration and wound closure [77].   Moreover,
202   GJ-mediated propagation of calcium waves has been required for smooth muscle cell polarization
203   and migration [78], therefore, it is conceivable that GJIC in a cell cluster could play an important role
204   in coordination of the migration [79].   Extracellular ATP-induced calcium signaling has been shown
205   to modulate neuronal proliferation and migration of neuronal cells [80].   Cx hemichannels have
206   been known as a pathway of ATP release from intracellular space to extracellular space.   ATP release
207   has been observed in glioma, glioblastoma and HeLa cells being transfected with Cx26, Cx32, or Cx43
208   [81].   Macrophage also releases ATP via Cx37 resulting in cell adhesion to endothelium [22].   It has
209   been considered that ATP release via Cx hemichannels from cells may induce cell migration through
210   calcium signaling following P2Y receptors activation in neighboring cells [81].
211   Intracellular domain of Cxs protein interacts with other proteins that involve in being structural
212   stability of cell-cell junction sustained by cytoskeletal scaffolds [10].   Due to the ubiquitous distribution
213   of Cx43, many studies have been performed focusing on Cx43 and their interacting proteins.   The
214   carboxyl tail of Cx43 is indeed interacting with several cytoskeletal proteins such as F-actin, α-/β-
215   tubulins, cadherins, and cortactin) [82-85].   For example, the membrane expression of N-cadherin or
216   of ZO-1 is dominantly localized in the existing site of Cx43 protein [84].   The interaction of Cx43 with
217   the cadherin family may not only be important for the mechanics of cell migration, but also generates

218 intracellular signaling.   Interaction of Cx43 and cadherins coordinates activation of Rho GTPases
219 which are promoting cell motility and invasion [86, 87].   Moreover, Rac1 in migrating cell is
220 dominantly found in forming actin-rich structures which in conjunction with E-cadherin are considered
221 responsible for the generation of traction forces of germ cells *in vivo* [88].   Intracellular carboxyl tail of
222 Cx43 has a number of interaction partners, thereby, the cell expressed a Cx43 lacking carboxyl tail
223 impairs cell migration [89].   Cx43 deficiency causes an impaired polarization caused by a non-
224 directional alignment of the microtubule organizing center.   As a consequence, a loss of directionality
225 of cell migration and then an impaired development of coronary arteries can be observed in Cx43
226 deficient mice.   A Cx43 mutant with lack of the tubulin binding site in the carboxyl tail has shown the
227 similar phenotypic pattern with Cx43 deficient [89], suggesting that interaction between Cx43 and
228 cytoskeletal protein may coordinate cell mechanics and behavior.

229 Interestingly, Cx43 seems indeed to be important for the stability of leading processes of the
230 neuronal cells determining the migratory pathway along the glial fibers [90].   Interesting mechanism
231 has been elicited that control the localization of Cx43 in the cellular extensions of migrating neurons in
232 a way that radial migration along the glia becomes possible [90].   Additionally, Watanabe and
233 colleagues have shown that fish GJ and Cx involves in fish morphological diversity, including skin
234 pattern formation and body shape determination [91].   Their studies have indicated that Cxs in
235 pigment cells, xanthophore and melanophore, dictate aggregation and separation of cells resulting in
236 pattern formation [92].   These suggest the possibility of Cxs dependent regulation of directional cell
237 migration.

6. Cxs-mediated regulation of cellular stiffness and cell migration

239 The interaction between Cx and cytoskeletal proteins correlatively contributes to the regulation
240 of cellular stiffness which is defined as the physical property of a cell to resist deformation in the
241 response to any applied force.   A contraction force which generated by the actomyosin cytoskeleton
242 and F-actin has been inseparably connected with the regulation of cellular stiffness [93, 94].
243 Activation of the Rho-actomyosin signaling pathway enhances the formation of actin bundles, stress
244 fibers, and tensile actomyosin structures [95], all of which correlate with cellular stiffness [96, 97].
245 Thus, interplay between endothelial Cxs and Rho family has implicated in the regulation of cellular
246 stiffness.   We have found that proinflammatory stimulation increased endothelial cellular stiffness
247 associating with impaired GJ function, cytoskeletal remodeling, and focal adhesion formation [98].
248 Moreover, blockade of GJs induces the cellular stiffening associated with focal adhesion formation
249 and cytoskeletal rearrangement, and prolonges TNF-α-induced endothelial cellular stiffening [98].
250 This study has provided first evidence that endothelial GJ contributes to the regulation of endothelial
251 cellular stiffness via interaction with cytoskeletal rearrangements.

252 It has been considered that endothelial cellular stiffness may be a determinant factor of leukocyte
253 adhesion to endothelium.   In general, leukocyte senses the stiffness of extracellular substrate by
254 integrin-ligand interaction and adheres more strongly to stiff substrate [99].   ECs materially work
255 as a substrate during leukocyte adhesion and migration process.   Leukocyte integrin assumes both
256 selective and cohesive adhesion via the binding to distinct endothelial adhesion receptors such as the
257 intercellular adhesion molecule 1 (ICAM1) [93].   Integrin increases the binding avidity to ligands
258 correlated with the endothelial cellular stiffness, while integrin-focal adhesion complex generates the
259 contractile force in cell and transduces the force into a mechanosignaling [100, 101].   These
260 suggested the possible mechanism which regulates leukocyte adhesion and activation via physical
261 endothelial cellular stiffness [102].

262 In addition to leukocyte, it has been reported that ECs themselves also modulate their migration,
263 proliferation, and morphological changes in the response to extracellular substrate stiffness [103, 104].
264 Thus, it has been shown a possibility that stiffening ECs in existing vasculature is favor to recruit pro-
265 angiogenic tip cells and stalk cells at the sprouting spots (Fig.3).   Of note, VEGF-induced cytoskeletal
266 rearrangement and impaired GJ function might be supposed to increases EC stiffness.   Stiff ECs may
267 recruit endothelial progenitor cells and support the cell proliferation and elongation of stalk cells.
268 Taken together, a series of studies suggest the concept that GJ-mediated EC stiffening might facilitate

269 angiogenic process of recruited ECs by being the activator of mechanosensing and transduction
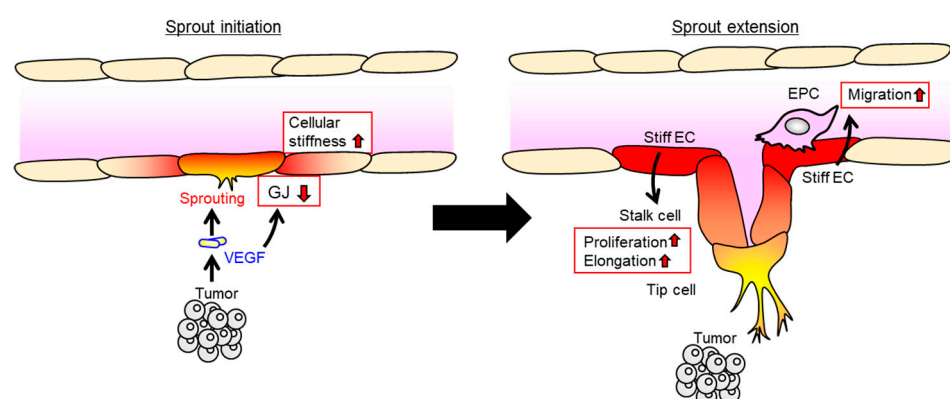270 pathway.
271

Figure 3. Potential role of endothelial cellular stiffening in angiogenesis. VEGF-induced GJ reduction increases the stiffness of ECs in sprout initiation phase. Stiff ECs provide the favorable environment for recruitment of endothelial progenitor cells, while stiff ECs support adjacent stalk cell proliferation and elongation.

7. Conclusions

We are beginning to understand that GJ and Cx in ECs might be a center for connection between biological function and cell mechanics in the context of angiogenesis. In this review, we provide an overview of the endothelial GJ function and Cxs expression found in pro-angiogenic condition and the functional role of endothelial GJ and Cxs in cell mechanics during the angiogenic process. Cell mechanics-based mechanisms hold promise the better understanding for physiological and pathological angiogenesis. Although several studies have demonstrated GJ-/Cx-dependent regulation of angiogenesis, the mechanisms are still speculative and controversial. Additionally, GJ- and Cx-mediated interactions in a number of other type cells such as vascular smooth muscle cell, pericyte, fibroblast, macrophage, and tumor cell also contribute to tumor angiogenesis through the expression of pro-angiogenic factors. Thus, further studies in the basic biology of GJ and Cx in these type cells would be required for elucidation with a particular emphasis on the interplay of angiogenesis and tumor development. We have speculated that GJ and Cx targeting approaches may be relevant to the development of the treatment of cancer patients.

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References

1. Shweiki, D.; Itin, A.; Soffer, D.; Keshet, E., Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* **1992,** 359, (6398), 843-5.

2. Poon, R. T.; Fan, S. T.; Wong, J., Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol* **2001,** 19, (4), 1207-25.

3. Gerhardt, H.; Golding, M.; Fruttiger, M.; Ruhrberg, C.; Lundkvist, A.; Abramsson, A.; Jeltsch, M.; Mitchell, C.; Alitalo, K.; Shima, D.; Betsholtz, C., VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* **2003,** 161, (6), 1163-77.

4. Jakobsson, L.; Franco, C. A.; Bentley, K.; Collins, R. T.; Ponsioen, B.; Aspalter, I. M.; Rosewell, I.; Busse, M.; Thurston, G.; Medvinsky, A.; Schulte-Merker, S.; Gerhardt, H.,

305   Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting.
306   *Nat Cell Biol* **2010,** 12, (10), 943-53.

307 5. Cao, J.; Ehling, M.; Marz, S.; Seebach, J.; Tarbashevich, K.; Sixta, T.; Pitulescu, M. E.;
308   Werner, A. C.; Flach, B.; Montanez, E.; Raz, E.; Adams, R. H.; Schnittler, H., Polarized actin
309   and VE-cadherin dynamics regulate junctional remodelling and cell migration during
310   sprouting angiogenesis. *Nat Commun* **2017,** 8, (1), 2210.

311 6. Abedi, H.; Zachary, I., Vascular endothelial growth factor stimulates tyrosine
312   phosphorylation and recruitment to new focal adhesions of focal adhesion kinase and
313   paxillin in endothelial cells. *J Biol Chem* **1997,** 272, (24), 15442-51.

314 7. Hu, J.; Qiu, J.; Zheng, Y.; Zhang, T.; Yin, T.; Xie, X.; Wang, G., AAMP Regulates Endothelial
315   Cell Migration and Angiogenesis Through RhoA/Rho Kinase Signaling. *Ann Biomed Eng*
316   **2016,** 44, (5), 1462-74.

317 8. Kumar, N. M.; Gilula, N. B., The gap junction communication channel. *Cell* **1996,** 84, (3),
318   381-8.

319 9. Saez, J. C.; Berthoud, V. M.; Branes, M. C.; Martinez, A. D.; Beyer, E. C., Plasma membrane
320   channels formed by connexins: their regulation and functions. *Physiol Rev* **2003,** 83, (4),
321   1359-400.

322 10. Harris, A. L., Connexin channel permeability to cytoplasmic molecules. *Prog Biophys Mol*
323   *Biol* **2007,** 94, (1-2), 120-43.

324 11. Chang, S. F.; Chen, L. J.; Lee, P. L.; Lee, D. Y.; Chien, S.; Chiu, J. J., Different modes of
325   endothelial-smooth muscle cell interaction elicit differential beta-catenin phosphorylations
326   and endothelial functions. *Proc Natl Acad Sci U S A* **2014,** 111, (5), 1855-60.

327 12. Chen, C. H.; Mayo, J. N.; Gourdie, R. G.; Johnstone, S. R.; Isakson, B. E.; Bearden, S. E.,
328   The connexin 43/ZO-1 complex regulates cerebral endothelial F-actin architecture and
329   migration. *Am J Physiol Cell Physiol* **2015,** 309, (9), C600-7.

330 13. Fang, J. S.; Coon, B. G.; Gillis, N.; Chen, Z.; Qiu, J.; Chittenden, T. W.; Burt, J. M.; Schwartz,
331   M. A.; Hirschi, K. K., Shear-induced Notch-Cx37-p27 axis arrests endothelial cell cycle to
332   enable arterial specification. *Nat Commun* **2017,** 8, (1), 2149.

333 14. Esmon, C. T., The interactions between inflammation and coagulation. *Br J Haematol* **2005,**
334   131, (4), 417-30.

335 15. Reglero-Real, N.; Marcos-Ramiro, B.; Millan, J., Endothelial membrane reorganization
336   during leukocyte extravasation. *Cell Mol Life Sci* **2012,** 69, (18), 3079-99.

337 16. Folkman, J.; Merler, E.; Abernathy, C.; Williams, G., Isolation of a tumor factor responsible
338   for angiogenesis. *J Exp Med* **1971,** 133, (2), 275-88.

339 17. Godo, S.; Shimokawa, H., Endothelial Functions. *Arterioscler Thromb Vasc Biol* **2017,** 37,
340   (9), e108-e114.

341 18. Larson, D. M.; Haudenschild, C. C.; Beyer, E. C., Gap junction messenger RNA expression
342   by vascular wall cells. *Circ Res* **1990,** 66, (4), 1074-80.

343 19. Yeh, H. I.; Rothery, S.; Dupont, E.; Coppen, S. R.; Severs, N. J., Individual gap junction
344   plaques contain multiple connexins in arterial endothelium. *Circ Res* **1998,** 83, (12), 1248-
345   63.

346 20. Kwak, B. R.; Mulhaupt, F.; Veillard, N.; Gros, D. B.; Mach, F., Altered pattern of vascular
347   connexin expression in atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* **2002,** 22, (2),

348   225-30.

349   21. van Rijen, H. V.; van Kempen, M. J.; Postma, S.; Jongsma, H. J., Tumour necrosis factor
350   alpha alters the expression of connexin43, connexin40, and connexin37 in human umbilical
351   vein endothelial cells. *Cytokine* **1998,** 10, (4), 258-64.

352   22. Wong, C. W.; Christen, T.; Roth, I.; Chadjichristos, C. E.; Derouette, J. P.; Foglia, B. F.;
353   Chanson, M.; Goodenough, D. A.; Kwak, B. R., Connexin37 protects against atherosclerosis
354   by regulating monocyte adhesion. *Nat Med* **2006,** 12, (8), 950-4.

355   23. Wagner, C.; de Wit, C.; Kurtz, L.; Grunberger, C.; Kurtz, A.; Schweda, F., Connexin40 is
356   essential for the pressure control of renin synthesis and secretion. *Circ Res* **2007,** 100, (4),
357   556-63.

358   24. Gabriels, J. E.; Paul, D. L., Connexin43 is highly localized to sites of disturbed flow in rat
359   aortic endothelium but connexin37 and connexin40 are more uniformly distributed. *Circ Res*
360   **1998,** 83, (6), 636-43.

361   25. Okamoto, T.; Akiyama, M.; Takeda, M.; Gabazza, E. C.; Hayashi, T.; Suzuki, K., Connexin32
362   is expressed in vascular endothelial cells and participates in gap-junction intercellular
363   communication. *Biochem Biophys Res Commun* **2009,** 382, (2), 264-8.

364   26. Inai, T.; Shibata, Y., Heterogeneous expression of endothelial connexin (Cx) 37, Cx40, and
365   Cx43 in rat large veins. *Anat Sci Int* **2009,** 84, (3), 237-45.

366   27. Van Rijen, H.; van Kempen, M. J.; Analbers, L. J.; Rook, M. B.; van Ginneken, A. C.; Gros,
367   D.; Jongsma, H. J., Gap junctions in human umbilical cord endothelial cells contain multiple
368   connexins. *Am J Physiol* **1997,** 272, (1 Pt 1), C117-30.

369   28. Okamoto, T.; Akita, N.; Kawamoto, E.; Hayashi, T.; Suzuki, K.; Shimaoka, M., Endothelial
370   connexin32 enhances angiogenesis by positively regulating tube formation and cell
371   migration. *Exp Cell Res* **2014,** 321, (2), 133-41.

372   29. Ebong, E. E.; Kim, S.; DePaola, N., Flow regulates intercellular communication in HAEC by
373   assembling functional Cx40 and Cx37 gap junctional channels. *Am J Physiol Heart Circ*
374   *Physiol* **2006,** 290, (5), H2015-23.

375   30. Okamoto, T.; Akiyama, M.; Takeda, M.; Akita, N.; Yoshida, K.; Hayashi, T.; Suzuki, K.,
376   Connexin32 protects against vascular inflammation by modulating inflammatory cytokine
377   expression by endothelial cells. *Exp Cell Res* **2011,** 317, (3), 348-55.

378   31. Bolon, M. L.; Kidder, G. M.; Simon, A. M.; Tyml, K., Lipopolysaccharide reduces electrical
379   coupling in microvascular endothelial cells by targeting connexin40 in a tyrosine-, ERK1/2-,
380   PKA-, and PKC-dependent manner. *J Cell Physiol* **2007,** 211, (1), 159-66.

381   32. Lidington, D.; Tyml, K.; Ouellette, Y., Lipopolysaccharide-induced reductions in cellular
382   coupling correlate with tyrosine phosphorylation of connexin 43. *J Cell Physiol* **2002,** 193,
383   (3), 373-9.

384   33. Baker, S. M.; Kim, N.; Gumpert, A. M.; Segretain, D.; Falk, M. M., Acute internalization of
385   gap junctions in vascular endothelial cells in response to inflammatory mediator-induced G-
386   protein coupled receptor activation. *FEBS Lett* **2008,** 582, (29), 4039-46.

387   34. O'Donnell, J. J., 3rd; Birukova, A. A.; Beyer, E. C.; Birukov, K. G., Gap junction protein
388   connexin43 exacerbates lung vascular permeability. *PLoS One* **2014,** 9, (6), e100931.

389   35. Chadjichristos, C. E.; Scheckenbach, K. E.; van Veen, T. A.; Richani Sarieddine, M. Z.; de
390   Wit, C.; Yang, Z.; Roth, I.; Bacchetta, M.; Viswambharan, H.; Foglia, B.; Dudez, T.; van

Kempen, M. J.; Coenjaerts, F. E.; Miquerol, L.; Deutsch, U.; Jongsma, H. J.; Chanson, M.; Kwak, B. R., Endothelial-specific deletion of connexin40 promotes atherosclerosis by increasing CD73-dependent leukocyte adhesion. *Circulation* **2010,** 121, (1), 123-31.

36. Derouette, J. P.; Wong, C.; Burnier, L.; Morel, S.; Sutter, E.; Galan, K.; Brisset, A. C.; Roth, I.; Chadjichristos, C. E.; Kwak, B. R., Molecular role of Cx37 in advanced atherosclerosis: a micro-array study. *Atherosclerosis* **2009,** 206, (1), 69-76.

37. Kwak, B. R.; Veillard, N.; Pelli, G.; Mulhaupt, F.; James, R. W.; Chanson, M.; Mach, F., Reduced connexin43 expression inhibits atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice. *Circulation* **2003,** 107, (7), 1033-9.

38. Haefliger, J. A.; Krattinger, N.; Martin, D.; Pedrazzini, T.; Capponi, A.; Doring, B.; Plum, A.; Charollais, A.; Willecke, K.; Meda, P., Connexin43-dependent mechanism modulates renin secretion and hypertension. *J Clin Invest* **2006,** 116, (2), 405-13.

39. Yamada, Y.; Izawa, H.; Ichihara, S.; Takatsu, F.; Ishihara, H.; Hirayama, H.; Sone, T.; Tanaka, M.; Yokota, M., Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. *N Engl J Med* **2002,** 347, (24), 1916-23.

40. Okamoto, T.; Akita, N.; Hayashi, T.; Shimaoka, M.; Suzuki, K., Endothelial connexin 32 regulates tissue factor expression induced by inflammatory stimulation and direct cell-cell interaction with activated cells. *Atherosclerosis* **2014,** 236, (2), 430-7.

41. Suarez, S.; Ballmer-Hofer, K., VEGF transiently disrupts gap junctional communication in endothelial cells. *J Cell Sci* **2001,** 114, (Pt 6), 1229-35.

42. Ferrara, N.; Gerber, H. P.; LeCouter, J., The biology of VEGF and its receptors. *Nat Med* **2003,** 9, (6), 669-76.

43. Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N., Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* **1993,** 362, (6423), 841-4.

44. Ferrara, N.; Davis-Smyth, T., The biology of vascular endothelial growth factor. *Endocr Rev* **1997,** 18, (1), 4-25.

45. Aiello, L. P.; Avery, R. L.; Arrigg, P. G.; Keyt, B. A.; Jampel, H. D.; Shah, S. T.; Pasquale, L. R.; Thieme, H.; Iwamoto, M. A.; Park, J. E.; et al., Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* **1994,** 331, (22), 1480-7.

46. Brown, L. F.; Yeo, K. T.; Berse, B.; Yeo, T. K.; Senger, D. R.; Dvorak, H. F.; van de Water, L., Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. *J Exp Med* **1992,** 176, (5), 1375-9.

47. Maniscalco, W. M.; Watkins, R. H.; Finkelstein, J. N.; Campbell, M. H., Vascular endothelial growth factor mRNA increases in alveolar epithelial cells during recovery from oxygen injury. *Am J Respir Cell Mol Biol* **1995,** 13, (4), 377-86.

48. Waltenberger, J.; Claesson-Welsh, L.; Siegbahn, A.; Shibuya, M.; Heldin, C. H., Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem* **1994,** 269, (43), 26988-95.

49. Antonetti, D. A.; Barber, A. J.; Hollinger, L. A.; Wolpert, E. B.; Gardner, T. W., Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic

434   retinopathy and tumors. *J Biol Chem* **1999,** 274, (33), 23463-7.

435   50.   Nimlamool, W.; Andrews, R. M.; Falk, M. M., Connexin43 phosphorylation by PKC and
436   MAPK signals VEGF-mediated gap junction internalization. *Mol Biol Cell* **2015,** 26, (15),
437   2755-68.

438   51.   Thuringer, D., The vascular endothelial growth factor-induced disruption of gap junctions is
439   relayed by an autocrine communication via ATP release in coronary capillary endothelium.
440   *Ann N Y Acad Sci* **2004,** 1030, 14-27.

441   52.   Pepper, M. S.; Meda, P., Basic fibroblast growth factor increases junctional communication
442   and connexin 43 expression in microvascular endothelial cells. *J Cell Physiol* **1992,** 153, (1),
443   196-205.

444   53.   Favre, C. J.; Mancuso, M.; Maas, K.; McLean, J. W.; Baluk, P.; McDonald, D. M., Expression
445   of genes involved in vascular development and angiogenesis in endothelial cells of adult lung.
446   *Am J Physiol Heart Circ Physiol* **2003,** 285, (5), H1917-38.

447   54.   Lanner, F.; Lee, K. L.; Ortega, G. C.; Sohl, M.; Li, X.; Jin, S.; Hansson, E. M.; Claesson-Welsh,
448   L.; Poellinger, L.; Lendahl, U.; Farnebo, F., Hypoxia-induced arterial differentiation requires
449   adrenomedullin and notch signaling. *Stem Cells Dev* **2013,** 22, (9), 1360-9.

450   55.   Wang, W. K.; Chen, M. C.; Leong, H. F.; Kuo, Y. L.; Kuo, C. Y.; Lee, C. H., Connexin 43
451   suppresses tumor angiogenesis by down-regulation of vascular endothelial growth factor via
452   hypoxic-induced factor-1alpha. *Int J Mol Sci* **2014,** 16, (1), 439-51.

453   56.   Pimentel, R. C.; Yamada, K. A.; Kleber, A. G.; Saffitz, J. E., Autocrine regulation of myocyte
454   Cx43 expression by VEGF. *Circ Res* **2002,** 90, (6), 671-7.

455   57.   Fan, X.; Teng, Y.; Ye, Z.; Zhou, Y.; Tan, W. S., The effect of gap junction-mediated transfer of
456   miR-200b on osteogenesis and angiogenesis in a co-culture of MSCs and HUVECs. *J Cell Sci*
457   **2018,** 131, (13).

458   58.   Huang, G. Y.; Xie, L. J.; Linask, K. L.; Zhang, C.; Zhao, X. Q.; Yang, Y.; Zhou, G. M.; Wu, Y.
459   J.; Marquez-Rosado, L.; McElhinney, D. B.; Goldmuntz, E.; Liu, C.; Lampe, P. D.; Chatterjee,
460   B.; Lo, C. W., Evaluating the role of connexin43 in congenital heart disease: Screening for
461   mutations in patients with outflow tract anomalies and the analysis of knock-in mouse
462   models. *J Cardiovasc Dis Res* **2011,** 2, (4), 206-12.

463   59.   Ya, J.; Erdtsieck-Ernste, E. B.; de Boer, P. A.; van Kempen, M. J.; Jongsma, H.; Gros, D.;
464   Moorman, A. F.; Lamers, W. H., Heart defects in connexin43-deficient mice. *Circ Res* **1998,**
465   82, (3), 360-6.

466   60.   Gu, H.; Smith, F. C.; Taffet, S. M.; Delmar, M., High incidence of cardiac malformations in
467   connexin40-deficient mice. *Circ Res* **2003,** 93, (3), 201-6.

468   61.   Simon, A. M.; McWhorter, A. R., Vascular abnormalities in mice lacking the endothelial gap
469   junction proteins connexin37 and connexin40. *Dev Biol* **2002,** 251, (2), 206-20.

470   62.   Haefliger, J. A.; Allagnat, F.; Hamard, L.; Le Gal, L.; Meda, P.; Nardelli-Haefliger, D.; Genot,
471   E.; Alonso, F., Targeting Cx40 (Connexin40) Expression or Function Reduces Angiogenesis
472   in the Developing Mouse Retina. *Arterioscler Thromb Vasc Biol* **2017,** 37, (11), 2136-2146.

473   63.   Fang, J. S.; Angelov, S. N.; Simon, A. M.; Burt, J. M., Cx37 deletion enhances vascular
474   growth and facilitates ischemic limb recovery. *Am J Physiol Heart Circ Physiol* **2011,** 301,
475   (5), H1872-81.

476   64.   Wang, H. H.; Kung, C. I.; Tseng, Y. Y.; Lin, Y. C.; Chen, C. H.; Tsai, C. H.; Yeh, H. I.,

477   Activation of endothelial cells to pathological status by down-regulation of connexin43.
478   *Cardiovasc Res* **2008,** 79, (3), 509-18.

479 65. Bacharach, E.; Itin, A.; Keshet, E., In vivo patterns of expression of urokinase and its
480   inhibitor PAI-1 suggest a concerted role in regulating physiological angiogenesis. *Proc Natl*
481   *Acad Sci U S A* **1992,** 89, (22), 10686-90.

482 66. Xu, H.; Cao, Y.; Yang, X.; Cai, P.; Kang, L.; Zhu, X.; Luo, H.; Lu, L.; Wei, L.; Bai, X.; Zhu, Y.;
483   Zhao, B. Q.; Fan, W., ADAMTS13 controls vascular remodeling by modifying VWF reactivity
484   during stroke recovery. *Blood* **2017,** 130, (1), 11-22.

485 67. Gartner, C.; Ziegelhoffer, B.; Kostelka, M.; Stepan, H.; Mohr, F. W.; Dhein, S., Knock-down
486   of endothelial connexins impairs angiogenesis. *Pharmacol Res* **2012,** 65, (3), 347-57.

487 68. Johnson, T. L.; Nerem, R. M., Endothelial connexin 37, connexin 40, and connexin 43
488   respond uniquely to substrate and shear stress. *Endothelium* **2007,** 14, (4-5), 215-26.

489 69. Kliche, K.; Jeggle, P.; Pavenstadt, H.; Oberleithner, H., Role of cellular mechanics in the
490   function and life span of vascular endothelium. *Pflugers Arch* **2011,** 462, (2), 209-17.

491 70. Eilken, H. M.; Adams, R. H., Dynamics of endothelial cell behavior in sprouting angiogenesis.
492   *Curr Opin Cell Biol* **2010,** 22, (5), 617-25.

493 71. Stapor, P.; Wang, X.; Goveia, J.; Moens, S.; Carmeliet, P., Angiogenesis revisited - role and
494   therapeutic potential of targeting endothelial metabolism. *J Cell Sci* **2014,** 127, (Pt 20), 4331-
495   41.

496 72. Simpson, K. J.; Selfors, L. M.; Bui, J.; Reynolds, A.; Leake, D.; Khvorova, A.; Brugge, J. S.,
497   Identification of genes that regulate epithelial cell migration using an siRNA screening
498   approach. *Nat Cell Biol* **2008,** 10, (9), 1027-38.

499 73. Wang, H. H.; Su, C. H.; Wu, Y. J.; Li, J. Y.; Tseng, Y. M.; Lin, Y. C.; Hsieh, C. L.; Tsai, C. H.;
500   Yeh, H. I., Reduction of connexin43 in human endothelial progenitor cells impairs the
501   angiogenic potential. *Angiogenesis* **2013,** 16, (3), 553-60.

502 74. Matsuuchi, L.; Naus, C. C., Gap junction proteins on the move: connexins, the cytoskeleton
503   and migration. *Biochim Biophys Acta* **2013,** 1828, (1), 94-108.

504 75. Kameritsch, P.; Pogoda, K.; Pohl, U., Channel-independent influence of connexin 43 on cell
505   migration. *Biochim Biophys Acta* **2012,** 1818, (8), 1993-2001.

506 76. Friedl, P.; Gilmour, D., Collective cell migration in morphogenesis, regeneration and cancer.
507   *Nat Rev Mol Cell Biol* **2009,** 10, (7), 445-57.

508 77. Kwak, B. R.; Pepper, M. S.; Gros, D. B.; Meda, P., Inhibition of endothelial wound repair by
509   dominant negative connexin inhibitors. *Mol Biol Cell* **2001,** 12, (4), 831-45.

510 78. Espinosa-Tanguma, R.; O'Neil, C.; Chrones, T.; Pickering, J. G.; Sims, S. M., Essential role
511   for calcium waves in migration of human vascular smooth muscle cells. *Am J Physiol Heart*
512   *Circ Physiol* **2011,** 301, (2), H315-23.

513 79. Defranco, B. H.; Nickel, B. M.; Baty, C. J.; Martinez, J. S.; Gay, V. L.; Sandulache, V. C.;
514   Hackam, D. J.; Murray, S. A., Migrating cells retain gap junction plaque structure and
515   function. *Cell Commun Adhes* **2008,** 15, (3), 273-88.

516 80. Weissman, T. A.; Riquelme, P. A.; Ivic, L.; Flint, A. C.; Kriegstein, A. R., Calcium waves
517   propagate through radial glial cells and modulate proliferation in the developing neocortex.
518   *Neuron* **2004,** 43, (5), 647-61.

519 81. Cotrina, M. L.; Lin, J. H.; Alves-Rodrigues, A.; Liu, S.; Li, J.; Azmi-Ghadimi, H.; Kang, J.;

520   Naus, C. C.; Nedergaard, M., Connexins regulate calcium signaling by controlling ATP
521   release. *Proc Natl Acad Sci U S A* **1998,** 95, (26), 15735-40.

522 82. Giepmans, B. N., Role of connexin43-interacting proteins at gap junctions. *Adv Cardiol* **2006,**
523   42, 41-56.

524 83. Theiss, C.; Meller, K., Microinjected anti-actin antibodies decrease gap junctional
525   intercellular commmunication in cultured astrocytes. *Exp Cell Res* **2002,** 281, (2), 197-204.

526 84. Wei, C. J.; Francis, R.; Xu, X.; Lo, C. W., Connexin43 associated with an N-cadherin-
527   containing multiprotein complex is required for gap junction formation in NIH3T3 cells. *J*
528   *Biol Chem* **2005,** 280, (20), 19925-36.

529 85. Vitale, M. L.; Akpovi, C. D.; Pelletier, R. M., Cortactin/tyrosine-phosphorylated cortactin
530   interaction with connexin 43 in mouse seminiferous tubules. *Microsc Res Tech* **2009,** 72, (11),
531   856-67.

532 86. Xu, X.; Li, W. E.; Huang, G. Y.; Meyer, R.; Chen, T.; Luo, Y.; Thomas, M. P.; Radice, G. L.; Lo,
533   C. W., Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap
534   junctions. *J Cell Biol* **2001,** 154, (1), 217-30.

535 87. Xu, X.; Francis, R.; Wei, C. J.; Linask, K. L.; Lo, C. W., Connexin 43-mediated modulation of
536   polarized cell movement and the directional migration of cardiac neural crest cells.
537   *Development* **2006,** 133, (18), 3629-39.

538 88. Kardash, E.; Reichman-Fried, M.; Maitre, J. L.; Boldajipour, B.; Papusheva, E.;
539   Messerschmidt, E. M.; Heisenberg, C. P.; Raz, E., A role for Rho GTPases and cell-cell
540   adhesion in single-cell motility in vivo. *Nat Cell Biol* **2010,** 12, (1), 47-53; sup pp 1-11.

541 89. Rhee, D. Y.; Zhao, X. Q.; Francis, R. J.; Huang, G. Y.; Mably, J. D.; Lo, C. W., Connexin 43
542   regulates epicardial cell polarity and migration in coronary vascular development.
543   *Development* **2009,** 136, (18), 3185-93.

544 90. Elias, L. A.; Wang, D. D.; Kriegstein, A. R., Gap junction adhesion is necessary for radial
545   migration in the neocortex. *Nature* **2007,** 448, (7156), 901-7.

546 91. Watanabe, M., Gap Junction in the Teleost Fish Lineage: Duplicated Connexins May
547   Contribute to Skin Pattern Formation and Body Shape Determination. *Front Cell Dev Biol*
548   **2017,** 5, 13.

549 92. Watanabe, M.; Sawada, R.; Aramaki, T.; Skerrett, I. M.; Kondo, S., The Physiological
550   Characterization of Connexin41.8 and Connexin39.4, Which Are Involved in the Striped
551   Pattern Formation of Zebrafish. *J Biol Chem* **2016,** 291, (3), 1053-63.

552 93. Schaefer, A.; Te Riet, J.; Ritz, K.; Hoogenboezem, M.; Anthony, E. C.; Mul, F. P.; de Vries, C.
553   J.; Daemen, M. J.; Figdor, C. G.; van Buul, J. D.; Hordijk, P. L., Actin-binding proteins
554   differentially regulate endothelial cell stiffness, ICAM-1 function and neutrophil
555   transmigration. *J Cell Sci* **2014,** 127, (Pt 20), 4470-82.

556 94. Stroka, K. M.; Aranda-Espinoza, H., Effects of Morphology vs. Cell-Cell Interactions on
557   Endothelial Cell Stiffness. *Cell Mol Bioeng* **2011,** 4, (1), 9-27.

558 95. Chrzanowska-Wodnicka, M.; Burridge, K., Rho-stimulated contractility drives the formation
559   of stress fibers and focal adhesions. *J Cell Biol* **1996,** 133, (6), 1403-15.

560 96. Wojciak-Stothard, B.; Potempa, S.; Eichholtz, T.; Ridley, A. J., Rho and Rac but not Cdc42
561   regulate endothelial cell permeability. *J Cell Sci* **2001,** 114, (Pt 7), 1343-55.

562 97. Wang, N.; Tolic-Norrelykke, I. M.; Chen, J.; Mijailovich, S. M.; Butler, J. P.; Fredberg, J. J.;

Stamenovic, D., Cell prestress. I. Stiffness and prestress are closely associated in adherent contractile cells. *Am J Physiol Cell Physiol* **2002**, 282, (3), C606-16.

98. Okamoto, T.; Kawamoto, E.; Takagi, Y.; Akita, N.; Hayashi, T.; Park, E. J.; Suzuki, K.; Shimaoka, M., Gap junction-mediated regulation of endothelial cellular stiffness. *Sci Rep* **2017**, 7, (1), 6134.

99. Oakes, P. W.; Patel, D. C.; Morin, N. A.; Zitterbart, D. P.; Fabry, B.; Reichner, J. S.; Tang, J. X., Neutrophil morphology and migration are affected by substrate elasticity. *Blood* **2009**, 114, (7), 1387-95.

100. Huveneers, S.; Daemen, M. J.; Hordijk, P. L., Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. *Circ Res* **2015**, 116, (5), 895-908.

101. Stroka, K. M.; Aranda-Espinoza, H., Endothelial cell substrate stiffness influences neutrophil transmigration via myosin light chain kinase-dependent cell contraction. *Blood* **2011**, 118, (6), 1632-40.

102. Schaefer, A.; Hordijk, P. L., Cell-stiffness-induced mechanosignaling - a key driver of leukocyte transendothelial migration. *J Cell Sci* **2015**, 128, (13), 2221-30.

103. Sack, K. D.; Teran, M.; Nugent, M. A., Extracellular Matrix Stiffness Controls VEGF Signaling and Processing in Endothelial Cells. *J Cell Physiol* **2016**, 231, (9), 2026-39.

104. Browning, M. B.; Guiza, V.; Russell, B.; Rivera, J.; Cereceres, S.; Hook, M.; Hahn, M. S.; Cosgriff-Hernandez, E. M., Endothelial cell response to chemical, biological, and physical cues in bioactive hydrogels. *Tissue Eng Part A* **2014**, 20, (23-24), 3130-41.