Connexins and angiogenesis: Functional aspects, pathogenesis, and emerging therapies (Review)

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Abstract. Connexins (Cxs) play key roles in cellular communication. By facilitating metabolite exchange or interfering with distinct signaling pathways, Cxs affect cell homeostasis, proliferation, and differentiation. Variations in the activity and expression of Cxs have been linked to numerous clinical conditions including carcinomas, cardiac disorders, and wound healing. Recent discoveries on the association between Cxs and angiogenesis have sparked interest in Cx-mediated angiogenesis due to its essential functions in tissue formation, wound repair, tumor growth, and metastasis. It is now widely recognized that understanding the association between Cxs and angiogenesis may aid in the development of new targeted therapies for angiogenic diseases. The aim of the present review was to provide a comprehensive overview of Cxs and Cx-mediated angiogenesis, with a focus on therapeutic implications.

Contents

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Abbreviations: BG, bioactive glass; CL, cytoplasmic loop; CT, carboxy-terminal; ECs, endothelial cells; GJ, gap junctions; GJIC, Gap junctional intercellular communication; MSC, mesenchymal stem cells; VEGF, vascular endothelial growth factor; WH, wound healing

Key words: angiogenesis, cancer, cardiovascular disorders, connexins, gap junctions, intercellular communication, wound healing

1. Introduction

Angiogenesis plays a significant role in tissue growth, wound repair, tumor development, and metastasis. It is controlled by growth factors, pro-angiogenic cytokines, and neovascularization antagonists (1). Connexins (Cxs) are hexameric arrays of tetraspan integral membrane proteins that form gap junctions (GJs). GJs provide direct ionic and molecular communication between neighboring cells and coordinate the exchange of chemicals and electrical impulses between them.

Several studies have reported independent and GJ-dependent roles of Cxs in mediating angiogenesis in various disorders (2,3). Although there are a few reviews on the role of Cxs in various physiological processes (4-9), there is little discussion of how Cxs influence the angiogenic process involved in wound repair, tumorigeneses, and cardiovascular disorders. The purpose of the present study was to examine the current state of knowledge regarding Cx structure, nomenclature, function, and regulation, as well as the newly identified link between Cxs and angiogenesis. Major Cx-mediated angiogenesis disorders and potential therapeutic approaches were also examined.

2. Methodology

A literature search was performed to identify articles that discussed the role of connexins in angiogenesis. The MEDLINE, PubMed, Scopus, and Cochrane Library databases were searched until June 02, 2022. Individual or combined searches for the terms 'angiogenesis', 'connexin', 'Cx', and 'gap junctions' were performed. By scanning the references of the included studies, additional studies were identified. Letters to the editor and articles without abstracts were excluded.

Structure and diversity of Cxs and GJs. Each Cx has four hydrophobic transmembranes (M1-M4), two extracellular areas (E1 and E2) that bind to another Cx in the neighboring cell, and three cytoplasmic regions that correspond to the cytoplasmic loop (CL), and the amino-terminal (NT), and carboxy-terminal (CT) tail regions. The N-terminus, membrane-spanning sections, and extracellular loops are consistent throughout the structure; however, the size and structure of the CL and the CT are not. The GJ channel is composed of two hemichannels (or connexons), consisting of six transmembrane proteins (Cx subunits) connected to the

plasma membrane of each symmetric cell. When two hemichannels join to produce a cell-cell conduit, one is tilted by 30˚ with respect to the other. Homotypic GJs are formed when identical Cx subunits dock, whereas heterotypic GJs are formed when two different connexons (hemichannels) dock (10). The central cytoplasmic part and the second extracellular domain (E2) regulate the heterotypic adaptation of Cxs. Heterotypic channels have features that differ from those of homotypic channels, such as unitary conductance and gating. The permeabilities of different channels formed by different Cxs differ, allowing secondary messengers to be discriminated against (cyclic guanosine monophosphate, Ca^{2+}, or IP_3) (Fig. 1).

Functional role of Cxs. Hemichannels regulate cellular responses to a wide range of physiological, oxidative, and metabolic stressors, whereas GJs permit intercellular transmission (Fig. 1). Molecules transported through these channels are responsible for several physiological functions. Different stimuli, including variations in voltage, $Ca2^{+}$, pH, and Cx phosphorylation, can dynamically control gap junctional intercellular communication (GJIC) (10-15). Voltage sensitivity is critical for controlling the intercellular connectivity of excitable cells. Channel independence has been demonstrated in the context of cellular proliferation, attachment, motility, apoptotic processes, and signaling (2,12,16-19). It was also recently revealed by the authors' research group that Cx43 levels regulate angiogenesis in endothelial cells (ECs), irrespective of GJ function (2). Moorby and Patel conducted extensive research on the GJ-dependent and-independent functions of Cx43 and discovered that the carboxyl region of Cx43 mainly governs the independent GJ function (19). It is increasingly accepted that the effects of GJIC-independent Cxs on carcinogenesis extend beyond proliferation and migration, including angiogenesis and cell death (20-23).

Cxs and angiogenic processes. Angiogenesis plays a crucial role in tissue growth, wound healing (WH), carcinogenesis, and metastasis (1,24). It begins with the growth and development of preexisting vessels, depending on a mixture of growth factors and proangiogenic cytokines, and is regulated by various neovascularization antagonists (Fig. 2) (25,26). Cxs have been shown to affect angiogenic processes in various ways, including growth, transport, and cellular stiffness (27). The roles of Cx43, Cx37, and Cx40, which are the most prevalent Cxs engaged in angiogenic processes, are discussed in the next section. The expression of Cx43 expression influences the angiogenic potential of endothelial cells independently of the GJ interaction. Because proliferation was unchanged, it was hypothesized that the Cx43 protein may significantly alter endothelial cell relocation, thereby promoting angiogenesis (2).

Cx43. Decreased Cx43 expression can result in vascular dysfunction and impaired angiogenesis (28). Cx43 is also involved in regulating lung microvascular permeability, and its modulation is related to endothelial monolayer permeability (29,30). Salmina *et al* examined GJ-dependent neurogenesis and concluded that alterations in Cx43 expression were correlated with distinct steps in neural growth (31). Cx43 is also upregulated in ECs during hemodynamic stimulation-induced angiogenesis (32). A study of the molecular processes during human trophoblast fusion revealed that protein kinase A-dependent phosphorylation of Cx43 enhances cell fusion (33). Furthermore, decreased Cx43 expression can result in improper embryo implantation and inadequate angiogenesis (34). It has also been found that Cx43, as a negative regulator, participates in critical steps of WH, such as inflammation response, remodeling of the extracellular matrix, proliferation of epidermal/skin cells, and migration (35).

Cx37 and Cx40. Cx37 and Cx40, which are co-expressed in ECs, have overlapping functions. Cx40 can promote EC migration, vessel sprouting, and expansion, whereas Cx40 deficiency and inhibition reduce angiogenesis (36). Endothelial Cx40, according to Haefliger *et al*, affects the initial phases of angiogenesis in the retina by controlling vascularization (37). In $Cx37^{-/-}$ mice, improved recovery of the hind limb was associated with increased vasculogenesis, which resulted in greater collateral remodeling and angiogenesis (38). Furthermore, the global deletion of Cx37 in mice causes increased angiogenesis during tissue injury, aiding the recovery process after ischemic injury (39). Growth inhibition mediated by Cx37 involves CT and the pore-forming domain (14). Nitric oxide affects endothelial vasomotor activity by modulating calcium signaling (40). Cx37 and Cx40 have been shown to uniquely control post-ischemic limb perfusion, affecting the intensity of ischemic stress and, as a result, post-ischemic persistence (41). Cx37 selectively affects Ang II signaling by modulating Ang II receptor expression (42). Cx37 also suppresses the proliferation of vascular and cancer cells. Cx37-induced growth arrest or growth-permissive phenotypes depend on conformational changes in Cx37 caused by phosphorylation (43).

3. Cxs, diseases and potential therapies

Cxs are implicated in the regulation of innate epithelial immunity, wound repair, and inflammatory processes. The pathophysiology of various Cx-related diseases is determined by both the canonical and noncanonical functions of Cxs. Given the presence of several Cxs in the endothelium, it is possible that Cxs and immune-targeted therapies could be used synergistically. In various pathological conditions, such as ischemia, optic nerve damage, stroke, and spinal cord injury, communication between junctions and hemichannels leads to secondary damage through inflammatory processes (44). Cx43 enhanced brain blood flow restoration in a mouse model by regulating reparative angiogenesis during chronic cerebral hypoperfusion (45). Due to the variety of Cx-mediated communication and its effect on cellular physiology and pathology, a definitive link between Cxs, angiogenesis, and disease has not yet been identified. However, in numerous cases, an association between aberrant Cx function, angiogenesis, and disease has been observed. The following section highlights the key mechanistic and therapeutic findings.

WH. Different layers of the human epidermis express different levels of Cxs, which are associated with a number of skin diseases (Fig. 3). During the early phases of WH, Cx43 has been observed to be negatively regulated at the wound margins (46). Nitric oxide, a mediator of vasomotion, has been reported to be a strong modulator of GJ coupling in ECs (47).

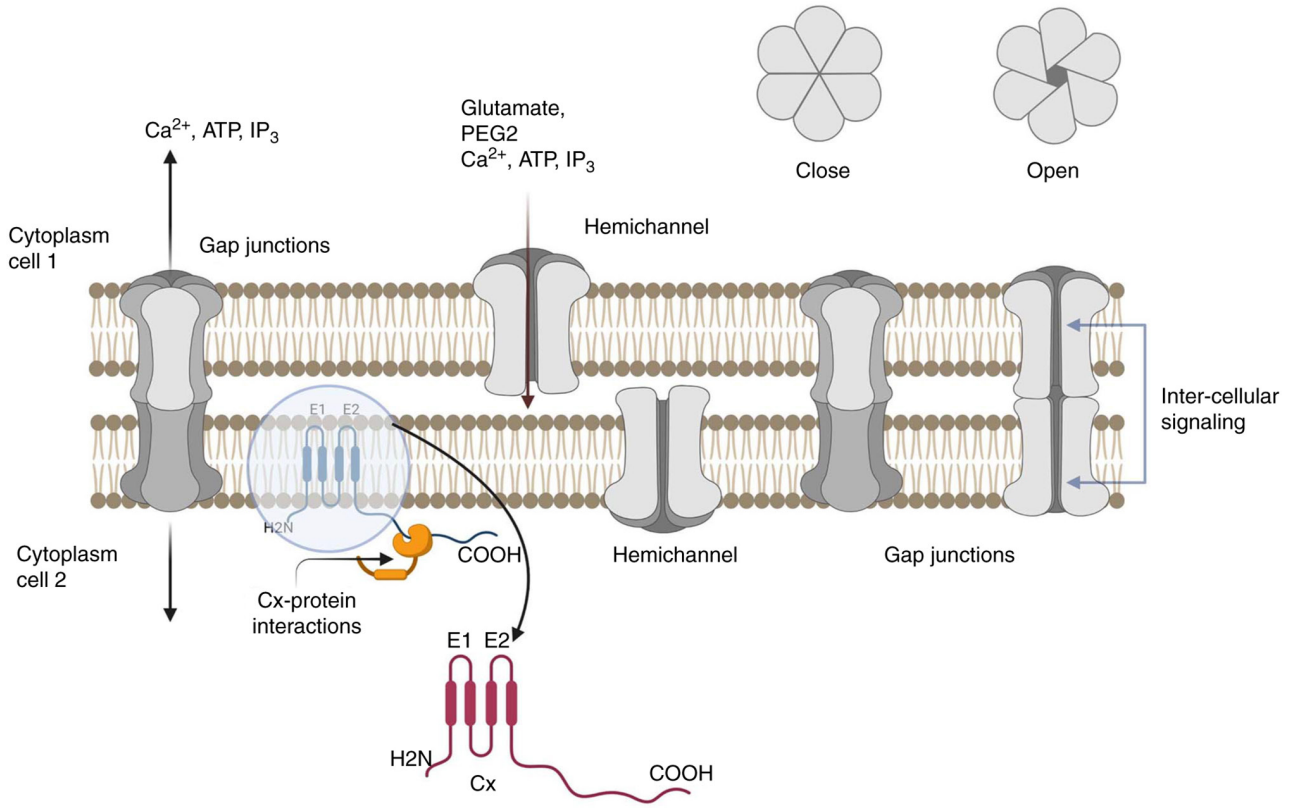

Figure 1. Schematic diagram of Cxs, hemichannels, and GJs across two neighboring cells. The cylinders in Cxs show transmembrane segments (M1-M4). Extracellular loops are shown as E1 and E2. Intracellular domains include one cytoplasmic loop and N- and C-terminals. The GJ created by linking two hemichannels rooted in the plasma membrane of each symmetric cell permits ions and molecules to transport between cells. Cxs, connexins; GJ, gap junctions.

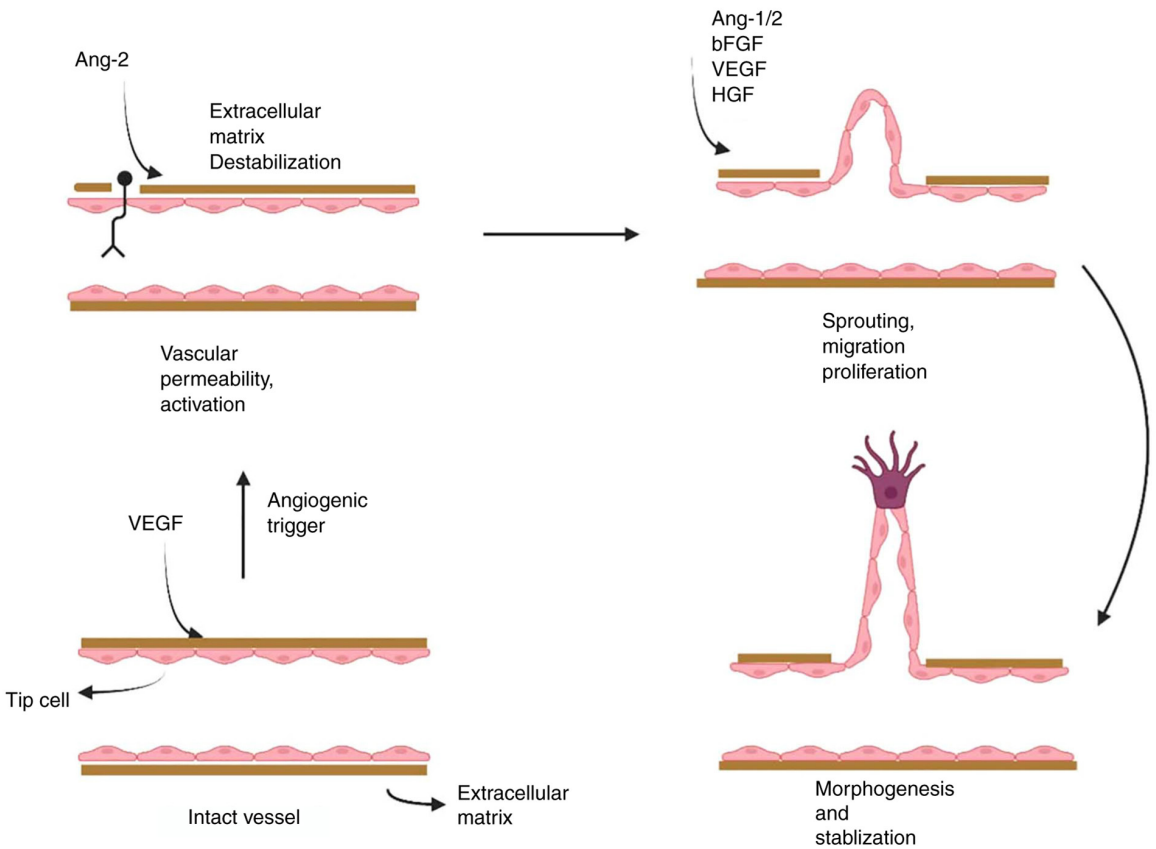

Figure 2. Key steps in angiogenesis. Stable arteries experience vascular permeability, enabling plasma proteins to extravasate. Matrix metalloproteinases break down the extracellular matrix, allowing growth factors to be released. Endothelial cells proliferate and migrate, undergo morphogenesis, and form lumen-bearing cords. VEGF, vascular endothelial growth factor.

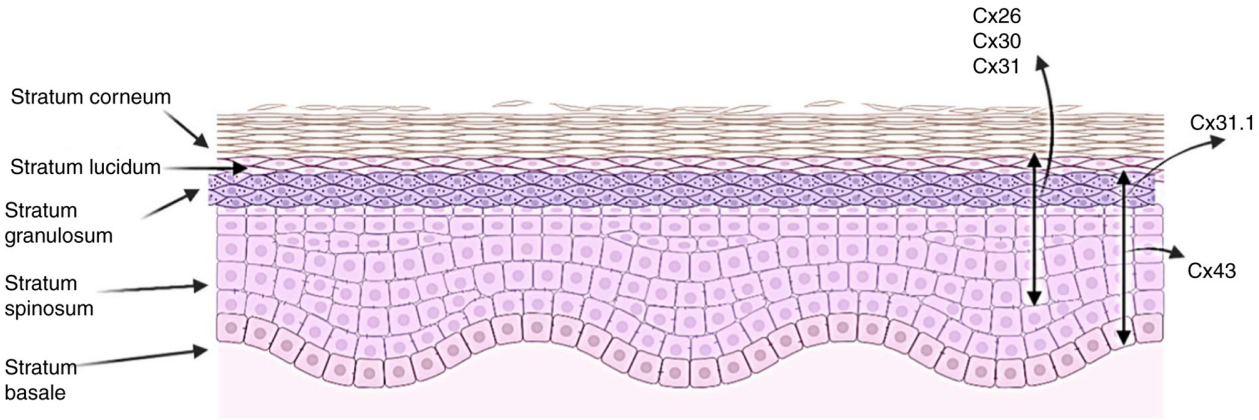

Figure 3. Representative image of key connexins in different layers of the human epidermis.

It promotes the *de novo* formation of GJ by expanding the integration of Cx40 into the plasma membrane. One of the most evident applications that demonstrates the involvement of Cxs in angiogenesis is the efficacy of bioactive glass (BG) in WH. In rats, BG stimulates GJIC, which results in increased angiogenesis and accelerates the closure of excisional wounds (48). It was recently shown that BG affects the expression of Cx43 and ROS levels, increasing WH by suppressing pyroptosis through the Cx43/ROS signaling pathway (49). Cx43 remodeling is an important event in WH that influences the cellular dynamics of keratinocytes and fibroblasts (50). It was revealed that siRNA knockdown of Cx43 in human microvascular endothelial cells reduced migration *in vitro*, as measured by a wound assay, and impaired aortic vessel sprouting *ex vivo* (16); Cx43 and the tyrosine phosphatase, SHP-2, were also revealed to mediate endothelial cell migration, revealing a novel interaction between Cx43 and SHP-2 that is required for this process (16).

Mutations in Cx26, Cx30, and Cx31 are associated with hyperproliferative skin diseases (51). Furthermore, suppression of Cx43 function affects the expression of genes associated with WH (52). Cx mutations are associated with epidermal dysplasia (15). Gain-of-function mutations alter Cx-mediated calcium signaling within the epidermis; for example, suppressing Cx43 activity in fibroblasts has been shown to increase migration and control the expression of genes associated with WH through the mitogen-activated protein kinase, specificity protein 1, activator protein 1, glycogen synthase kinase 3, and transforming growth factor pathways, contributing to rapid and scarless WH in the human gingiva (52).

Preclinical studies on peptide therapeutics, a mimetic of Cx43 CT, have reported improvements in WH (53). Cx43 has also been reported to counter-regulate caveolin-1 in controlling EC proliferation and migration, and this counterregulatory effect of Cx43 could be used in therapeutic angiogenesis (54). Morphine administration was found to inhibit angiogenesis and delay WH by upregulating Cx43, and high doses of morphine alter Cx43 expression by increasing fibronectin and actin levels through the activation of transforming growth factor signaling (55). A Cx43 mimetic peptide (TAT-Gap19) significantly upregulates matrix metalloproteinases, tenascin-C, and vascular endothelial growth factor (VEGF)-A (13).

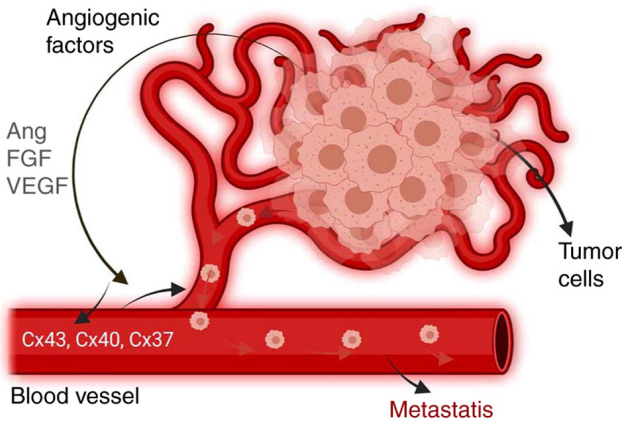

Figure 4. Angiogenesis in cancer regulates the blood supply to the tumor. The tumor secretes angiogenic factors that promote angiogenesis, and connexins play multiple dynamic roles in this process. VEGF, vascular endothelial growth factor.

Cancer. In cancer cells, intercellular communication is aberrant, and numerous studies have suggested that dysfunctional GJ and Cxs play a key role in this process (56). However, there appears to be a skewed association between Cxs and cancer, with evidence suggesting that Cxs may limit cancer cell development in certain instances while also promoting cancer cell motility, invasion, and metastatic dissemination in others (57,58). A key study revealed that inhibiting Cx37 decreases tumor angiogenesis; moreover, Cx37 and Cx40 work together to promote tumorigenesis (20). Consequently, the involvement of Cxs and GJs in cancer is more complex than previously thought.

Breast tumor cells transplanted into heterozygous Cx43 mice did not affect tumor growth, but greatly improved vascularization, indicating the role of Cx43 in vessel quiescence control and pathological tumor angiogenesis (22). The passage of tumor cells through the endothelial barrier is an important step in metastasis, in which endothelial cells adhere to the target organ by direct cell-cell communication and paracrine activation to initiate angiogenesis (Fig. 4). Cx46 regulates cancer stem cell and epithelial-to-mesenchymal transition features in breast cancer cells, suggesting that it may be useful in the development of future cancer therapeutics (59).

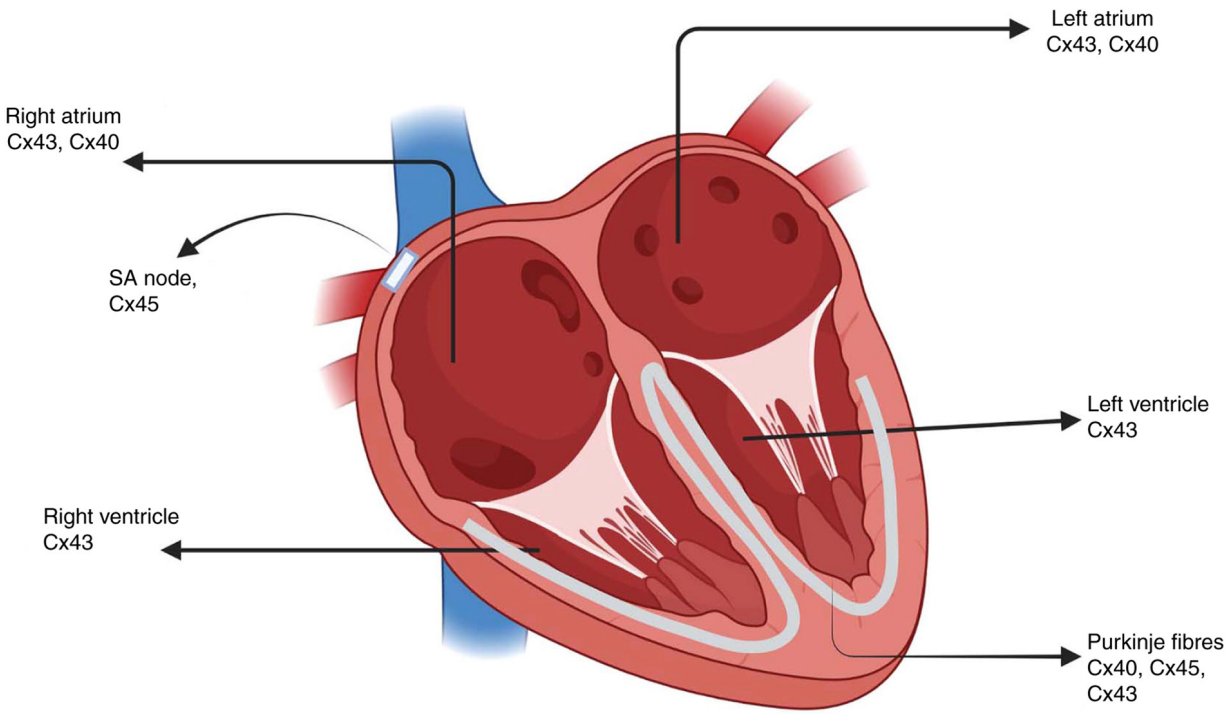

Figure 5. Key connexins expressed in the different regions of the heart.

Intercellular communication is also required for tumor cell trafficking across the lymphatic endothelium (60). Hemichannels have been reported to facilitate interactions between cancer cells and blood vessels, leading to angiogenesis. Choudhary *et al* revealed that tumors downregulate Cx43 function, allowing the endothelium to respond to angiogenic stimuli, leading to pathogenic angiogenesis (22). The roles of Cx and Notch endothelial signaling in coordinating the appropriate proliferation and angiogenesis of ECs have been identified (61). It has also been shown that GJIC inhibits tumor growth by transferring microRNAs from one EC to surrounding tumor cells, indicating a bystander role that can be exploited in cancer treatment (21).

Targeting Cx may be a promising therapeutic approach for cancer (23). Exosomes containing anti-angiogenic microRNAs released immediately through Cx channels can prevent cancer cells from promoting angiogenesis (21). Peptide-mediated inhibition of Cx40 in EC is a successful anti-angiogenesis approach that suppresses tumor angiogenesis (36). In the conditioned medium, tumor size and vessel density in Cx43-knockdown tumor cells decreased, indicating that Cx43 prevented tumor growth by decreasing angiogenesis (62).

Cardiovascular disorders. Several Cxs are co-expressed in the heart; in particular, distinct combinations of Cx40, Cx43, and Cx45 are observed in functionally specialized cardiomyocytes (Fig. 5). GJ channels in the cardiovascular system regulate vascular tone, which is essential for the coordination of cell activity, by permitting the transport of chemical messengers and energy substrates (63-65). Cxs form GJs for the transmission of precisely choreographed current flow patterns that control the synchronized beat of a healthy heart. Several pathophysiological conditions, including atherosclerosis, hypertension, hypertrophy, ischemia, and arrhythmias, have been linked to dysregulation of Cxs in the cardiovascular system in terms of expression, function, posttranslational modifications, and location. Ugwu *et al* reported a recurring somatic Cx43-gene c.121G>T mutation as a cause of cutaneous venous abnormalities (66). Although Cx43 levels are high in cardiac neural crest cells, both heterozygous and homozygous knock-in mice live long and do not exhibit symptoms of coronary heart disease (67). Similarly, point mutations in Cx43 were not found to cause the tetralogy of Fallot (68).

Treatment with granulocyte colony-stimulating factor improves arterial and capillary density and increases Cx43 expression in failing hearts (69). Through Cx43, VEGF stimulates endothelial progenitor cells and supports vascular healing (70). In ECs, ischemia/reperfusion causes reactive species to disrupt Cx/pannexin signaling mitochondrial prompt division and promote macrovesicle release (71). Cx43 and angiogenesis levels are higher in the exercised mouse heart, indicating increased remodeling (72). Long-term alienation combined with moderate environmental pressure has been associated with depressive symptoms and aberrant expression of Cx43 and Cx45 in the left ventricle (73). Notably, EC-specific molecule 1 enhances the potential of induced pluripotent stem ECs to promote angiogenesis and neovascularization (74). Su *et al* determined that preconditioning for ischemia had cardioprotective effects on arrhythmia and myocardial recovery by upregulating phosphatidylinositol 3-kinase-mediated Cx43 signaling (75). In a study on myometrial cell patch transplantation to cure myocardial infarction, angiogenesis was reported to occur in the transplanted myometrium and Cx43 expression was observed in the transplanted patches (76). Cx43 passivation from intercellular signaling and buildup at the mitochondrial inner membrane has been revealed in diabetic cardiomyocytes, demonstrating that mtCx43 is responsible for triggering aberrant contraction and disrupting electrophysiology in cardiomyocytes (77).

Heart disease caused by myocardial tissue injury and fibrosis is related to Cx43-based GJs. As a result, several Cx43 mimetic peptides have been proposed as potential therapeutics for Cx43-related degenerative disorders, some even reaching human clinical trials (78). Cx43 improves infarcted heart angiogenesis, as evidenced by higher levels of VEGF and basic fibroblast growth factor (18). The cardioprotective properties of expanded umbilical cord mesenchymal stem cells (MSC) were attributed to paracrine substances that tend to enhance angiogenesis and preserve Cx43 GJ function (75). Cx43 was found to be dispensable for the adipogenic differentiation of early-stage MSC, although it was protective against cell senescence (79). The survival and tube formation of MSCs are improved by Ang II treatment and Cx43 expression (80). TEM immunogold studies on rat heart ventricles indicated the lack of Cx26 at intercalated discs but the presence of Cx26 at various subcellular compartments (17). It was found that after a localized ischemic stroke, Cx43 regulated the angiogenesis of *Buyang Huanwu* decoction through VEGF and Ang-1 (81). Due to the increase of tissue Cx43 and proangiogenic markers, regenerative treatment using nanofiber-expanded hematopoietic stem cells has been reported to have a favorable effect on rat heart function following myocardial infarction (82).

4. Conclusions and future directions

Several studies have elucidated GJ/Cx-mediated angiogenesis. To adequately describe the *de novo* blood vessels involved in the response to tumor angiogenesis, researchers must examine changes in the expression patterns of GJIC and Cxs in pro-angiogenic stimuli in the neovasculature. Antiangiogenic therapy has been shown to increase survival in human tumors; therefore, GJ-and Cx-targeting techniques could be useful in the development of novel medicines. Chemical blockers of Cx channels, peptide mimics of short Cx sequences, such as Gap19/24/27/40, and gene therapy techniques have all been shown to be extremely effective molecular techniques for unraveling the complexity of the function of Cxs. Future research should focus on determining the specific molecular pathways underlying the significance of Cxs in various diseases and designing randomized control trials for specific therapeutic alternatives.

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Availability of data and materials

Not applicable.

Authors' contributions

ZZ, WC, YL and MZ contributed to the study concept, design, literature search and computer graphics for the figures. ZZ wrote the manuscript. XZ revised the manuscript and was in charge of the final approval of the manuscript prior to submission. Data authentication is not applicable. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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