



Connexin 43 in the function and homeostasis of osteocytes: a narrative review

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Background and Objective: Connexin 43 (Cx43) is the main gap junction (GJ) protein and hemichannel protein in bone tissue. It is involved in the formation of hemichannels and GJs and establishes channels that can communicate directly to exchange substances and signals, affecting the structure and function of osteocytes. CX43 is very important for the normal development of bone tissue and the establishment and balance of bone reconstruction. However, the molecular mechanisms by which CX43 regulates osteoblast function and homeostasis have been less well studied, and this article provides a review of research in this area.

Methods: We searched the PubMed, EMBASE, Cochrane Library, and Web of Science databases for studies published up to June 2023 using the keywords Connexin 43/Cx43 and Osteocytes. Screening of literatures according to inclusion and exclusion guidelines and summarized the results.

Key Content and Findings: Osteocytes, osteoblasts, and osteoclasts all express Cx43 and form an overall network through the interaction between GJs. Cx43 is not only involved in the mechanical response of bone tissue but also in the regulation of signal transduction, which could provide new molecular markers and novel targets for the treatment of certain bone diseases.

Conclusions: Cx43 is expressed in osteoblasts, osteoclasts, and osteoclasts and plays an important role in regulating the function, signal transduction, and mechanotransduction of osteocytes. This review offers a new contribution to the literature by summarizing the relationship between Cx43, a key protein of bone tissue, and osteoblasts.

Keywords: Osteocytes; Connexin 43 (Cx43); mechanical stimulation; signal transduction

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Introduction

Bone tissue consists of three main cell types: osteocytes, osteoblasts, and osteoclasts. Osteocytes are the most abundant cell type in bone and play an important role in mediating the mechanical sensitivity of bone and maintaining homeostasis by regulating the activities of osteoclasts and osteoblasts (1,2). They are embedded inside the bone mineral matrix, and long dendritic structures of osteocytes form a network connecting neighboring osteocytes and other cells, such as osteoblasts and osteoclasts, on the bone surface (3). Mechanical stress induces the formation and remodeling of bone tissue via cell-to-cell contact to achieve bone tissue reconstruction (4). The mechanisms of intercellular communication include chemical signal transmission, synaptic transmission, and gap junction (GJ) communication. Among them, the GJ channels play an important role in the process of converting mechanical signals into biological effects (5,6).

In bones, intercellular information transmission is mainly accomplished by GJ channels. The GJ channel is a membrane protein structure commonly existing between adjacent cells, which consists of a GJ protein called connexin (Cx) (6). Through the GJ channels, the cells in bone permit the exchange of small molecules, such as ions, metabolites, and signal molecules (7). The GJ channel composed of connexin 43 (Cx43) usually allows molecules of <1.2 kDa to pass through and typically limits larger molecules including proteins and nucleic acids from passing through (8,9). The basic structure of the GJ is the unit protein, where six connexins aggregate and surround each other to form a hydrophilic pore channel with a diameter of about 1.5 to 2 nm. This structure is called a “hemichannel”, two of which penetrate through the adjacent cell membrane (10). The “hemichannels” are anchored and docked with each other to form a full channel, namely the GJ. The openings at both ends of the GJ channel are located in the cytoplasm of two adjacent cells, which will be direct channels for cell-to-cell material exchange. The type of Cx determines the dimension of the GJ channel. GJ-mediated intercellular signal transduction plays an important role in tissue growth and development, maintaining the stability of the intracellular environment, cell differentiation, and tumorigenesis (11,12). Recently, Cx43 was found to be the most abundantly expressed Cx in human bone and cartilage and plays an important role in maintaining the functional homeostasis of cartilage. In addition to the classic GJ intercellular communication

(GJIC), there are also barrier-free interstitial junctions in the membrane that act as direct conduits between the cytoplasm and the extracellular environment (13,14). The ability of GJIC to transmit biophysics to the entire network of bone cells and osteoblasts suggests that GJIC may be an important mechanism by which the mechanical forces are transmitted through bone and subsequently lead to bone formation. In addition to bone formation, GJIC also regulates bone absorption (5,15). Cx43 is also critical for bone homeostasis, regulating bone cell function by actively participating in the regulation of signals, gene expression, cell survival, and the ability to respond to cues such as mechanical stress (16). Cx43 and macrophage functions are closely intertwined, including interactions with neighboring cells, microenvironment, migration to sites of inflammation, antigen presentation, and immune regulation (17). Furthermore, numerous studies have found frequent aberrant expression of Cx43 in myocardial infarction, diabetes, and tumors (18-24). In this study, we review the mechanism of Cx43's role in the mechanical stimulation and signal transduction of osteocytes to provide theoretical support for new molecular markers and novel targets for the treatment of bone diseases. Numerous studies have confirmed the role of Cx43 in osteoblast function and homeostasis. A better understanding of the pathways by which Cx43 regulates bone tissue remodelling may assist in the development of therapeutic approaches aimed at treating disorders of the human skeletal system. We present this article in accordance with the Narrative Review reporting checklist (available at <https://aoj.amegroups.com/article/view/10.21037/aoj-23-65/rc>).

Methods

We performed a narrative review of the literature in the PubMed, EMBASE, Cochrane Library, and Web of Science databases to find manuscripts published through June 2023 on the role of Cx43 in the function and homeostasis of osteocytes. The following search terms were used in this review: “connexin 43” or “Cx43” and “Osteocytes” [Mesh]. Two authors searched the databases independently, and a third reviewer mediated any disagreements in the results of the two screeners. Peer-reviewed, published literature, including narrative review papers, was included. Studies, editorials, letters to the editor, and abstracts were to be excluded when they involved animals. The reference lists of included papers were searched manually and included if they met the inclusion criteria. The search strategy is

Table 1 Search strategy summary

Items	Specification
Date of search	June 2023
Databases and other sources searched	PubMed, EMBASE, Cochrane Library, and Web of Science
Search terms used	“Connexin 43” OR “Cx43” AND “Osteocytes” [Mesh]
Timeframe	From 1970 to June 2023
Inclusion and exclusion criteria	Inclusion criteria: (I) English-language papers; (II) studies evaluating connexin 43 and osteocytes function and homeostasis; (III) peer-reviewed, published literature, including narrative review papers. Exclusion criteria: (I) publications with duplications or studies with overlapping data from the same author; (II) abstracts, case reports, proceedings, letters, reviews, and meta-analyses; (III) incomplete outcome data
Selection process	Two authors searched the database independently. A third reviewer mediated any disagreements between the two researchers

summarized in *Table 1*.

Discussion

Osteocyte Cx43 and mechanical stimulation

Osteocyte Cx43 and mechanical loading

The location of osteocytes in bone makes them a prime candidate for mechanosensory cells in bone tissue. The application of mechanical strain in bone cells leads to the redistribution of Cx43, the assembly of GJ channels, and the formation of other functional GJs (25–27). The expression and localization of Cx43 and the formation of interstitial connections in bone cells can be regulated by mechanical stimulation. The interstitial junction remains important in the response of bone cells to mechanical stimulation and the subsequent increase in osteoblast differentiation. Previous studies have shown that hemichannels formed by Cx43 are highly formed in bone cells and mechanically sensitive (28,29). Cx43 plays an indispensable role in maintaining the bone homeostasis and activity of mature osteocytes (5,30,31).

Bone tissue is remodeled under mechanical stress. The most abundant bone cells are considered stress receptors. Previous studies have shown that antibodies produced using the Cx43 prostaglandin E2 (PGE2) ring as an antigen can block dye absorption observed after mechanical stimulation (32,33). Studies have also indicated that the Cx43 hemichannel is the main mechanosensory channel expressed in bone cells (34). Cx43 is highly expressed in osteocytes, which are ideally positioned to sense and transmit mechanical force-induced signals in bone and osteoblasts. The expression of Cx43 is enhanced by loading

in cultured osteoblasts and osteocytes *in vivo* (35). Cx43 involvement in controlling osteocyte function and ultimate bone mass is complex and varies depending on the effect. For example, the loss of Cx43 differentially modulates the response of bone to mechanical load on the surface of the periosteum and endosteum (36,37). Inhibition of osteoblast Cx43 channels promotes unloading-induced bone loss, which mainly occurs in the cortical regions; in addition, hemichannels protect osteoblasts from apoptosis and promote periosteal bone remodeling, while GJs regulate intracortical osteoclast activity in response to unloading (38,39).

However, the loss of Cx43 not only reduces the anabolic effects of mechanical load but also blunts the effects of bone loss due to mechanical load and even aging (40,41). This means that the signals transmitted by Cx43 may be anabolic or catabolic, depending on the environment, such as aging, mechanical loading or unloading, or even location (4,42). In addition, the survival effect of a lack of mechanical stimulation in normal off-bed conditions may account for the increased apoptosis of osteocytes observed in osteoblasts/osteocytes or mice lacking Cx43 in their osteocytes. Several studies have shown that through mechanical channel stimulation, the channel composed of Cx43 can produce interstitial junction communication, and fluid flow can increase Cx43 expression, phosphorylation, and plasma membrane localization (43–45).

In vitro, osteoblasts and osteocytes can respond to mechanical disturbances and/or mechanical stresses caused by fluid flow by generating calcium oscillations that can propagate from cell to cell in a Cx43-dependent manner (45,46). The Cx43-dependent calcium oscillation between

osteocytes and osteoblasts can replicate *in vitro* in complete bone in response to mechanical stresses on the cells (47,48). Osteocytes sense mechanical stimuli and convert them into chemical signals that are transmitted to other bone cells and the extracellular environment through interstitial connections between adjacent cells or hemichannels between cells (49). In bone cells, Cx43 is the most abundant Cx responsible for the formation of interstitial junctions and hemichannels, thus playing a key role in mediating mechanical transduction. Mechanical stimulation affects the expression and cell distribution of Cx43 in expressed bone cells. The strain experienced at the tissue level under normal physiological load is insufficient to produce bone anabolic effects at the cellular level, suggesting that these forces are amplified as they propagate through the skeleton. The Piezoelectric effect and flow potential are two mechanisms of mechanical conduction (6,41,50).

In vitro studies have shown that GJIC makes bone cell networks sensitive to a variety of extracellular signals, including load induction signals. In addition, mechanical signals detected by bone cells can be communicated to osteoblasts through GJIC (51,52). Mechanical stimulation increases the expression and phosphorylation of Cx43 and Cx43-mediated GJIC. In addition to the role of GJs in the response of bone cells to mechanical loading, emerging evidence suggests that the Cx43-semicircle channel may also be a key component in osteogenic signal transduction in response to mechanical stimuli (53,54). Murine long bone osteocyte-Y4 (MLO-Y4) cells in a low-density culture showed enhanced hemichannel activity in response to fluid flow, which was attenuated by the inhibition of hemichannel activity or Cx43 expression (55). Cx43 has also been shown to play an important role in the ability of bone cells to induce biochemical responses to mechanical stress (56).

Osteocyte Cx43 and fluid shear stress

Osteocytes are the main mechanical sensory cells in bone, and fluid flow is the main type of mechanical stimulation to osteocytes (57,58). The Cx43 hemichannel in bone cells is highly sensitive to mechanical load (59). GJIC is also important for cellular mechanical transduction. Fluid shear stress and mechanical strain increase GJIC between bone cells (54,60). In this study, the loss of Cx43 in osteocytes and osteoblasts was shown to result in delayed osteoblast differentiation, reduced bone formation, and impaired mechanical properties (34). Cx43 plays an important role in restoring normal bone structure and mechanical

strength by regulating β -catenin signal transduction during fracture healing (61). Fluid flow is thought to represent major biophysical signals in mechanical transduction, and several biological effects have been described following the application of fluid flow to osteoblasts (62). Shear stress caused by mechanical load promotes nutrient and bone regulator exchange and initiates biochemical reactions. Osteocytes are well positioned in the skeleton and can sense the magnitude of mechanical strain, which is critical to the bone's adaptive response to load. Experimental studies have shown that osteocytes are sensitive to pressure applied to intact bone tissue and cell culture (63,64).

In bone cells, glycocalyx degradation has been shown to result in decreased PGE2 release after fluid shear stress (65). Mechanical loads play a crucial role in maintaining normal bone structure and function in bone tissue. Over the past decade or so, osteocytes have been recognized as modulating the biological responses of other osteocytes to mechanical loads (66). Previous studies have shown that bone cells respond to mechanical loading as well as the release of molecules such as PGE2 and nitric oxide (NO) (67-69). Since bone cells are surrounded by fluid-filled space, they are constantly subjected to the shear stress of fluid flow (70). The application of flow shear stress on osteoblastic MLO-Y4 cells increases the number and length of dendrites (71). Recently, mechanical loads in the form of fluid flow shear stress (FFSS) have been shown to mimic the flow of bone fluid in the lacrimal duct network of bone cells, thereby preventing glucocorticoid-induced apoptosis (72). The shear stress induced by fluid flow stimulates intercellular communication mediated by intercellular connectivity in osteoblast-like MLO-Y4 cells and increases the expression of Cx43 (73). This stimulation is further enhanced during the post-flow period. The report showed that fluid flow increases the release of PGE2 in osteoblast-like MLO-Y4 cells and that PGE2 is involved in the stimulation of intercellular communication by shear stress induced by fluid flow. These observations suggest that PGE2 is a factor that increases the interstitial junction activity of bone cells in response to shear stress after fluid flow (74).

Moreover, we recently demonstrated that FFSS induces the rapid opening of the hemichannel, which in turn mediates the release of PGE2 in osteolytic MLO-Y4 cells (74,75). A recent study showed that adenosine triphosphate (ATP) and PGE2 release oscillating fluid induced OCY454 osteocytes (76). In response to high and low shear stresses, Cx43 phosphorylation and reduced functional intercellular coupling also occur. Interestingly,

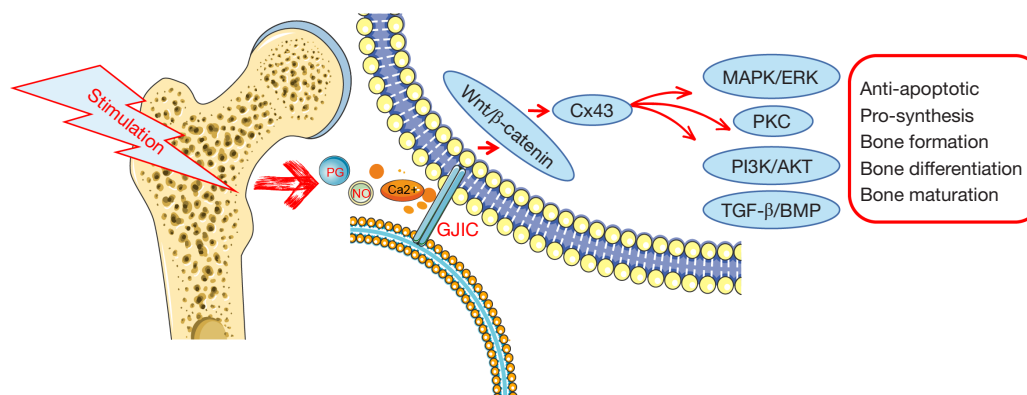


Figure 1 Cx43 affects osteoblast differentiation via signaling pathways. GJIC, gap junction intercellular communication; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated kinase; PKC, protein kinase C; TGF- β , transforming growth factor- β ; BMP, bone morphogenetic protein.

the low shear stress increases the expression of Cx43 messenger RNA (mRNA). In contrast, the expression of Cx43 mRNA decreases under high shear stress, so the gap connections could be rearranged or formed according to shear stress (77). Fluid shear stress promotes the opening of mechanically-sensitive hemichannels in osteolytic MLO-Y4 cells, leading to the release of regulatory factors such as PGE2 and ATP (55). A study has shown that the shear stress reduces the expression of Cx43 protein in MLO-Y4 bone cells and reduced GJIC (77).

The expression of Cx43 decreases with the increase of shear stress. However, differences in Cx43 and Cx45 mRNA expression were observed between low shear strength and high shear strength. Cx43 mRNA increases under low shear stress but there is no change in Cx45 expression; meanwhile, Cx43 mRNA decreases under high shear stress, which is also accompanied by an increase in the mRNA expression of Cx45. These findings suggest that Cx43 may produce a compensatory response due to the inhibition of Cx43, and the composition of GJ can be altered in response to specific mechanical stimulation. Taken together, these studies suggest that Cx43 and GJIC are critical for the anabolic response of bone to mechanical load. The formation of gap connections can be regulated by fluid flow but there is also evidence that hemichannels play an important role in conducting mechanical loads. MLO-Y4 cells are cultured at very low densities, thus minimizing the contribution of GJIC and increasing its hemichannel activity in response to fluid flow (51). In bone cells, fluid shear stress induces the release of extracellular mechanical signaling molecules required for bone homeostasis, such as PGE2, NO, ATP,

and vascular endothelial growth factor (VEGF) (78).

Osteocyte Cx43 and gravity

Several papers have reported on the expression of Cx43 in bone cells in response to gravity. A study in our laboratory has shown that the expression of Cx43 is reduced after low gravity and supergravity treatment provided by parabolic flight (79). Another study has also shown that intercellular communication significantly decreases after 24 hours of multi-dimensional gravity in MLO-Y4 cells (80). A Cx43 deficiency mouse model demonstrated that the bone is insensitive to hindlimb suspension (HLS) modeling of weightlessness (41,81,82). Similar results were shown after injecting botulinum toxin A (BtxA) to simulate mechanical unloading (83). Two studies reported that the conditioned deletion of Cx43 in osteoblasts and osteocytes partially resisted cortical bone loss caused by mechanical unloading (38,81), suggesting that Cx43 was involved in the skeletal response to weightless conditions.

Osteocyte Cx43 and signal transduction

Cx43 is involved in the intercellular transmission of second messengers and enhances the signaling pathways activated by these second messengers in adjacent cells, thus increasing the activation of signaling pathways downstream of these intercellular connections (Figure 1). The second mechanism through which Cx43 affects bone is its role in transmitting mechanical load signals between bone cells. Mechanical forces induce the formation of intercellular connections, and the ability of intercellular communication is consistent both *in vitro* and *in vivo* (Table 2). Previous experimental studies

Table 2 Brief synopsis of the articles on relevant subject

Subject	Authors (brief title)	Results
Osteoblasts and Cx43	Lilian I. Plotkin (27) (Cx43 and mechanotransduction in bone)	Cx43 participates in mechanotransduction
Cx43 and bone	Lilian I. Plotkin (11) (Cx43 and bone: not just a gap junction protein)	Cx43 is responsible for signal transduction
Gap junctions and hemichannels in osteocytes	Alayna E. Loisel (6) (Gap junction and hemichannel functions in osteocytes)	Cx43 can mediate mechanical stimulation through gap junctions and hemichannels in bone cells, leading to bone formation or absorption
CX43 affects bone homeostasis	Atum M. Buo (39) (Gap Junctional Regulation of Signal Transduction in Bone Cells)	Cx43 gap junctions and hemichannels regulate various molecular mechanisms in bone cells for cellular signaling pathways, gene expression, mechanical transduction, and cell survival
GJIC regulates the formation, differentiation, survival, and apoptosis of osteoblasts	Nidhi Batra (7) (Gap Junctions and Hemichannels in Signal Transmission, Function, and Development of Bone)	The role of interstitial connections and hemichannels in regulating signal transduction, function and development of bone cells
Gap junction and bone development	Joseph P. Stains (14) (Gap junctions in skeletal development and function)	Connexins play an important role in bone form and function
Cx43 and osteoblasts	Joseph P. Stains (16) (Molecular mechanisms of osteoblast/osteocyte regulation by connexin43)	Cx43 regulates anabolic and catabolic responses in osteoblasts and osteocyte biology

Cx43, Connexin 43; GJIC, gap junction intercellular communication.

have shown that bone cells respond to fluid shear stress and release signaling molecules such as prostaglandins, NO, calcium ion (Ca^{2+}), and other second messengers (84-87). It has also been shown that fluid-induced shear stress is an important biophysical signal in bone mechanical transduction. In osteoblasts and osteoblastic cell lines, the magnitude of fluid shear stress expected to occur in bone tissue disrupts connective communication and rearranges connective whites and to some extent the synthetic shear stresses of specific connective proteins. This disconnection from the osteocyte network due to fluid shear stress can provide a portion of the signal, thereby disconnecting the cell or the rest of the network to initiate focal bone remodeling (77).

In summary, there is growing evidence that Cx43 is a key component of intracellular mechanisms responsible for signaling in bone in response to pharmacological, hormonal, and mechanical stimuli. Under HO-induced oxidative stress, mitochondrial Cx43 (mtCx43) enhances mitochondrial ATP production to protect cells from oxidative stress by participating in the maintenance of the proton gradient between the membrane space and the matrix (88). This mechanism may be the result of small molecule release and/or the activation of other signaling pathways (6). In addition to their role in GJ channels or hemichannels, release proteins (including Cx43) regulate

cellular behavior by interacting with intercellular signaling molecules (7). In addition to the Cx43 hemichannel, the role of the ATP receptor (ionic P2X7 receptor) in bone cell mechanical transduction and signaling has been proposed (89). The activation of P2X7 receptors (P2X7Rs) has been shown to mediate the ATP-induced release of certain cell types, and P2X7R deficiency eliminates PGE2 release from osteoblasts in response to fluid shear stress (90). Mechanical signal transduction represents another aspect of osteocyte regulation in which Cx plays a crucial role. GJs and hemichannels formed by Cx43 can facilitate communication connections between cells. Osteocytes can affect the activities of osteoblasts and osteoclasts through interstitial junctions (91), which are considered to be important components of extracellular signal transduction, integration, and amplification in bone. The signals transmitted by Cx43 may be anabolic or catabolic, depending on the environment, such as aging, mechanical loading or unloading, or even location (43). In osteocytes, the hemichannel is open and the intracellular signaling pathway that affects osteoblastic function is also regulated by Cx43 (6).

Multiple *in vitro* structural and functional studies have shown that the Cx43 c-terminal plays an important role in the signaling and function of bone cells. Importantly, the over-expression of Cx43 requires the c-terminal for

signaling and transcription in bone cells but lacks the c-terminal to mimic the effect of Cx43 (92). It exhibits an antagonistic expression for signaling through the extracellular regulated kinase (ERK) and protein kinase C (PKC) pathways and osteoblast genes. This requirement for the c-terminal is consistent with the fact that PKC forms a complex with Cx43 through its c-terminal domain and, upon activation, transits from Cx43 to the nucleus interacting with runt-related transcription factor 2 (Runx2) (93). The researchers found that apoptotic osteoblasts trigger receptor activator of nuclear factor- κ B ligand (RANKL) production *in vivo* by surviving neighboring (i.e., bystander) osteoblasts; however, the mechanism is unclear. They subsequently found that the apoptosis of MLOY-4 osteoblasts in a new multiscale fluidic device (the Macro-micro-nano, or M μ n) resulted in increased osteoblast RANKL expression in adjacent compartments (94). In addition, titanium particles can increase Cx43 expression in osteoblasts, and osteoblasts may be involved in regulating osteoclast function through Cx43 during the OPP process.

The wingless/integrated (Wnt)/ β -catenin signaling pathway is an important signal regulation system *in vivo*. The Wnt signaling pathway is involved in the adaptive response of bone to stress stimulation. Following stress stimulation, bone cells can induce the rapid activation of their internal Wnt signaling pathway and affect bone reconstruction (95). Both *in vivo* and *in vitro* mechanics experiments support the Wnt/ β -catenin signal transduction pathway as a normal response to mechanical stimulation. Wnt/ β -catenin signal transduction can increase the sensitivity of bone cells to bone formation, resulting in increased bone mass and density. Biological force can induce β -catenin in bone cells to accumulate in the cytoplasm and migrate toward the nucleus, regulating gene transcription and expression. Studies have shown that fluid stimulation can release PGE2 through the β -catenin signaling pathway (72,96). Also, previous research indicates that the protein associated with the Wnt signaling pathway in osteocyte-like cells after pulsatile fluid action are all up-regulated, including Wnt-3a, secreted frizzled-related protein 4 (sFRP-4), low density lipoprotein receptor-related protein (Lrp)-5, Lrp-6, and β -catenin. In this experiment, the expression of the Wnt classic target gene Cx43 was significantly reduced under the action of Wnt antagonists. This confirms that the Wnt signaling pathway is involved in the role of Cx43 in bone reconstruction under biomechanical stimulation (95). The Wnt/Lrp5 signaling in bone cells are closely related to bone formation (97).

Lrp5/6 is currently one of the most studied cell membrane receptors that bind to the Wnt protein. Studies have shown that mechanical load can increase the gene expression of Wnt3a and Lrp5, while unloading can inhibit the Wnt/ β -catenin signaling pathway (98-100). As a key factor of the Wnt/ β -catenin signaling pathway, studies have shown that the expression of β -catenin and nuclear transfer are regulated by mechanical stress (97,101). Cyclin D1, lymphoid enhancer-binding factor 1 (Lef1), and Cx43 are the target genes of the Wnt/ β -catenin signal pathway (99). Lef1 can form a complex with T cell-specific transcription factor (Tcf) to initiate the transcription and expression of downstream genes (102,103). The expression of Cx43 is regulated by β -catenin, and the knockout of β -catenin will lead to decreased Cx43 protein expression (104).

In terms of bone formation, the Wnt/ β -catenin signaling pathway can promote the proliferation of osteoblasts by regulating the expression of LRP5 (105), induce the differentiation of bone marrow mesenchymal stem cells (BMSCs) into osteoblasts by regulating bone morphogenetic protein (BMP) (106), and promote the mineralization of extracellular matrix (ECM) by promoting the mRNA expression of the extracellular matrix phosphorylated glycoprotein (MEPE) (107). In terms of bone metabolism, this pathway can act on the osteoprotegerin (OPG)/RANKL/ receptor activator of nuclear factor κ B (RANK) pathway, inhibiting bone cells from expressing the OPG gene and thereby inhibiting osteoclast differentiation (108). Dickkopf-related protein 1 (DKK-1) is a negative regulator of the Wnt pathway, which can inhibit osteoblast differentiation and promote osteoclast differentiation and maturation during bone metabolism (109). The Wnt signaling pathway can not only promote the differentiation and proliferation of osteoblasts but also inhibit the development and maturation of osteoclasts, and plays an important role in the metabolic process of the skeletal system (110). Therefore, intercellular communication and signal complex association with interstitial connection channels may be interwoven functions not only by the charge and size of interstitial connection selective permeability but also by the locally recruited signal complex to determine the biological activity of Cx43. Cx43 is also directly involved in the regulation of osteoblast differentiation. It has been reported that Cx43 knockdown significantly inhibited osteoblast markers such as alkaline phosphatase, type I collagen, and fibroblast growth factor 23, resulting in a significant attenuation of osteoblast mineralization. In a bone wear model, osteolytic titanium (Ti) inhibited

β -connexin expression by inducing Cx42 expression, which in turn promoted bone formation. Other studies found that overexpression of Cx43 induced alkaline phosphatase expression through elevated GJ conductance and promoted the proliferation and differentiation of UMR cells (111-113).

Conclusions and future prospects

The expression of Cx43 and its channel function are particularly important in the normal physiological activities of bone tissue cells. Despite their relatively high abundance, even in the case of non-osteoblasts, osteoblasts are probably among the least studied cells in all vertebrate biology. In osteocytes, one of the important mechanical sensing molecules is Cx43, which forms interstitial junctions and hemichannels and is involved in mechanical signal transduction between adjacent cells and between cells and the extracellular environment (107-109). Further, Cx43 has been shown to be closely associated with other common skeletal developmental disorders. Cx43 deficiency was found to further enhance estrogen deficiency-induced osteoporosis, suggesting that Cx43 protects against estrogen-induced trabecular bone loss (73). Glucocorticoids, considered to be an important etiological factor in osteoporosis, have been shown to inhibit Cx43 expression in osteoblasts through activation of the AKt/mTOR signaling pathway (114). In terms of resistance to bone aging function, it was found that young skeletally mature mice deficient in Cx43 phenocopied osteoblast apoptosis and reduced bone strength, similar to the aged mouse phenotype (115). These findings suggested that Cx43 plays an important biological function in both normal bone development and skeletal disease. Furthermore, we focused on Cx43 non-skeletal biological functions. Furthermore, we have focused on Cx43 non-skeletal biological functions, with Cx43's role in cardiovascular disease being the most talked about most in addition to skeletal disease. Numerous studies have shown that deletion or phosphorylation of Cx43 expression in cardiovascular disease is associated with loss of cardioprotection (116,117). In the future, further investigation of the mechanisms and targets of Cx43 regulation of osteoblasts appears to be of great therapeutic value. In addition, pharmacological agents that antagonize or enhance Cx43 will also be further used and validated. For example, a series of peptides including alpha connexin carboxyl terminus 1 have been developed to target the specific activities and functions of Cx43, which will be further validated *in vitro* and *in vivo*.

In summary, osteocytes, osteoblasts, and osteoclasts all express Cx43 and form an overall network through the interaction between GJs. Cx43 plays a vital role in regulating bone cell function, signal transduction, and mechanical conduction. Clarifying the specific molecular mechanism of Cx43 in regulating bone homeostasis and responding to mechanical stimuli and signals can provide new molecular markers for certain bone diseases and novel treatment targets. Modulation of the immune microenvironment is increasingly becoming a favorable target for bone, cartilage, and soft tissue regeneration. The relationship between Cx43 and the bone microenvironment and its intrinsic mechanisms hold promise for new therapeutic opportunities to enhance bone, cartilage, and soft tissue regeneration through modulation of the immune microenvironment.

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Footnote

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