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Review Article

Connexin 43 hemichannels and related diseases

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Abstract

Connexin 43 (Cx43) protein forms hemichannels (connexons) and gap junctions, with hemichannels consisting of six Cx43 molecules and gap junctions formed by two hemichannels. While gap junctions are prevalent in organs like the heart and liver, hemichannels are found in specific cell types, such as astrocytes and osteocytes. They allow the passage of small molecules (<1.5 kDa) between the cytoplasm and extracellular matrix. Cx43 hemichannels have emerged as potential therapeutic targets in various diseases, including central nervous system disorders, bone-related diseases, diabetic complications, wound healing, and cancers. Aberrant hemichannel opening can worsen conditions by releasing inflammatory elements, such as causing gliosis in neuronal cells. Conversely, functional hemichannels may inhibit cancer cell growth and metastasis. Recent studies are revealing new mechanisms of Cx43 hemichannels, broadening their therapeutic applications and highlighting the importance of regulating their activity for improved disease outcomes.

Statement of Significance: This article provides an overview of Connexin 43 hemichannels, elucidating their crucial involvement in various diseases and outlining potential therapeutic strategies, including antibody-based interventions. Furthermore, this review lays the groundwork for advancing novel therapeutic approaches that could significantly impact patient care.

Keywords: Connexin 43; hemichannel; diseases; therapeutic targets

Introduction

Connexin research commenced in the mid-20th century, marked by the initial observations of gap junction structures in the early 1960s through electron microscopy [1]. The term "connexin" was coined based on their role in connecting cell membranes. Connexin 43 (Cx43) was first identified and characterized in the late 1980s. Researchers isolated the Cx43 protein and analyzed its sequence from various tissues, such as the heart and liver, thereby revealing its crucial role in the formation of gap junction channels with a distinctive structure [2, 3].

While various connexins have been identified to date, Cx43 is the most abundant and extensively studied connexin. Cx43 is a crucial component of the connexin protein family, playing a significant role in various physiological processes and pathologies, including cell communication and maintaining cell homeostasis. Similar to other subtypes of Cx proteins, Cx43 is a multi-pass transmembrane protein with four membrane-spanning segments and a large C-terminal cytoplasmic domain. In addition to these transmembrane domains, Cx43 possesses two extracellular loops, an N-terminal loop and a middle intracellular loop domain [4, 5]. The membrane topology of Cx43 is pivotal for its structure and function in forming channel-like configurations. Six Cx43 proteins oligomerize to form a hemichannel or connexon. These hemichannels can dock with adjacent hemichannels on neighboring cells to form gap junctions [6].

Gap junctions perform housekeeping functions and are critical for various cellular processes, such as maintaining tissue homeostasis and coordinating cellular responses [7]. They are essential for the transport of ions, metabolites, and small signaling molecules across cells, enabling synchronized responses within tissues. Substances that can pass through these channels are usually <1.5 kDa in size and include ions that maintain ionic balance and transmit electrical signals across cells, such as calcium (Ca^{2+}), potassium (K^+), and sodium (Na^+); metabolites such as glucose, lactate, glutamate, and adenosine triphosphate (ATP) aid in metabolic cooperation between cells; second messengers like cyclic AMP (cAMP) and inositol trisphosphate (IP3) facilitate the propagation of intracellular signaling cascades; small nucleotides, including RNA and DNA fragments involved in genetic regulation and signaling; reducing and oxidizing agents, such as glutathione and NAD^+/NADH, crucial for cellular redox homeostasis [8–12]. It is important to note that the permeability of these substances may vary depending on the specific cellular environment.

Originally, hemichannels were solely considered intermediates in the formation of gap junctions. However, subsequent studies revealed their independent existence and additional functions [13]. Similar to gap junctions, Cx43 hemichannels facilitate the passage of molecules smaller than 1.5 kDa, but they also possess distinct functions. These hemichannels primarily mediate communication between the inside and outside of cells, playing significant roles in autocrine and paracrine signaling and influencing a wide range of physiological and pathological states [14]. Cx43

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hemichannels are also crucial in cellular responses to physiological stress, remaining closed under normal conditions but opening under certain stimuli [15]. This opening permits the release of signaling molecules like calcium ions, glutamate, ATP, NAD^+, and prostaglandin E_2 (PGE_2), which are crucial for various physiological pathways [8–12]. In pathological scenarios, such as central nervous system (CNS) disorders, including spinal cord injuries (SCI), stroke, and neurodegenerative diseases, the substances released by these hemichannels, such as ATP and glutamate, can provoke neuroinflammation and neural damage, exacerbating dysfunction [16–19]. Similar detrimental effects are observed in conditions like osteoarthritis, wound healing, and diabetic complications in kidneys and eyes, where the opening of hemichannels and the subsequent release of harmful elements lead to inflammation and disease progression (reviewed by [20, 21]). In these instances, hemichannels appear to act as regulators of disease progression. In terms of therapy, inhibiting the aberrant opening of hemichannels has been a primary strategy. However, recent discoveries show that the opening of hemichannels can also have advantageous outcomes, through the release of an array of signaling factors with beneficial effects. For instance, this mechanism has been observed in bone osteocytes, where it modulates the bone microenvironment to hinder cancer bone metastasis [22–25]. In the eye lens, hemichannel opening transports antioxidants such as glutathione and protects lens fibers against oxidative stress [26–28]. Hence, activating hemichannels could be a beneficial therapeutic approach. These findings open new avenues for leveraging hemichannel mechanisms for disease treatment. This minireview focuses on the intricate connections and mechanisms underlying hemichannels in various diseases, underscoring recent advances in potential therapeutic applications.

Latest developments in targeting Cx43 hemichannels for therapeutic purposes

A variety of therapeutic modalities (Table 1), including mimetic peptides, small molecular inhibitors, and anti-sense oligonucleotides, have primarily been developed to inhibit or activate Cx43 expression or Cx43-forming channels, targeting several related diseases, such as skin diseases and complications caused by diabetes in kidneys and eyes [29–35].

Recent research has made significant progress in targeting Cx43 hemichannels for therapeutic purposes. This includes the expansion of selective Cx43 hemichannel inhibitors and activators that selectively inhibit or activate Cx43 hemichannels without affecting gap junctions formed by Cx43 or hemichannels formed by other connexin isoforms [19,25,51]. Improved delivery methods, such as adding internalization sequences like the HIV-derived TAT sequence, have improved peptide permeability and efficacy [52]. Areas of cardioprotective and neuroprotective applications have also seen expansion [51,53].

Interest has also grown in two important niches: the perinexus, a specialized zone adjacent to gap junctions characterized by a high concentration of undocked hemichannels, and the formation plaque, a transient structure where newly synthesized hemichannels are assembled into gap junctions or disassembled from existing junctions. These areas regulate the balance between hemichannel and gap junction activity and are considered potential therapeutic targets. Research into multilevel approaches aims to preserve Cx43 trafficking, prevent hemichannel opening, inhibit gap junction closure, and promote hemichannel integration into gap junction plaques. Modulating the perinexus to inhibit the transition of hemichannels, preserving hemichannel pools, or driving hemichannels toward integration into gap junction plaques, sets the stage for developing novel candidate pharmacological tools [53–55]. Finally, preclinical and clinical studies are underway utilizing antibody-based approaches to target Cx43 hemichannels (Fig. 1). These developments underscore the growing potential of Cx43 hemichannel-targeted therapies in addressing a range of pathological conditions. As research progresses, these approaches may lead to novel therapeutic strategies with improved specificity and reduced side effects compared to current treatments.

Cx43 hemichannel in CNS disorders

Astrocytes, the most abundant cells in the human brain, are key players in the CNS. They are involved in various critical functions, including neurotransmitter regulation, maintenance of the blood–brain barrier, and modulation of synaptic activity [58]. Abnormalities in astrocyte function have been linked to diseases such as Alzheimer's, Huntington's, and Amyotrophic Lateral Sclerosis [59]. Cx43 hemichannels in astrocytes are a focal point in neurobiological studies due to their critical influence on brain functionality and disease mechanisms. These hemichannels operate autonomously to facilitate the transfer of molecules across intracellular and extracellular spaces. The role of Cx43 hemichannels in astrocytes encompasses a range of vital physiological activities, such as the propagation of calcium waves, secretion of gliotransmitters, and management of the extracellular environment [60]. Activation of these hemichannels is tightly controlled and triggered by various factors, including mechanical stress, voltage fluctuations, and changes in extracellular ion and cytokine levels [61]. Recent research has underscored the involvement of Cx43 hemichannels in pathological scenarios, including ischemia, traumatic brain and SCI, and neurodegenerative disorders [16–18]. Improper functioning of Cx43 hemichannels in these situations can aggravate injury and inflammation, leading to severe consequences such as neuronal damage and death through the unrestrained discharge of substances like ATP and glutamate [62–64]. Specifically, Cx43 hemichannels have been found to open during ischemic events, contributing to cell death and tissue damage. Following trauma in the CNS, Cx43 hemichannels become activated, leading to the release of excitatory neurotransmitters and pro-inflammatory molecules, which contribute to secondary damage and impair recovery. In Alzheimer's and Parkinson's diseases, excessive opening of these channels can disrupt cellular homeostasis and contribute to neurodegeneration progression [51]. Considering their pivotal function in abnormal brain processes, Cx43 hemichannels in astrocytes are being investigated as potential targets for therapy. Modulating their activity presents innovative methods for treating various CNS diseases [17].

In cases of SCI, secondary damage following the original injury triggers inflammatory responses and further neural injury. This results in impaired physiological functions, notably in motor and sensory abilities. SCI leads to prolonged and excessive ATP release from areas surrounding the trauma. ATP activates purinergic receptors, contributing to inflammatory changes in astrocytes and microglial cells, and neuronal damage [65]. Cx43 expression increases in areas adjacent to traumatic lesions in the spinal cord [66,67]. Studies have shown that peritraumatic ATP release, inflammatory responses such as astrogliosis and microglia activation, and the traumatic lesion size surrounding the area after SCI are less in Cx43 knockout mice compared to Cx43 wild-type mice. Importantly, Cx43 knockout mice exhibited a quicker and more extensive recovery of motor functions following SCI compared to Cx43 wild-type mice [68]. These data suggest that ATP

Table 1. Therapeutic agents developed targeting Cx43 hemichannels/gap junctions potentially for various diseases

Name	Molecule type	Target on connexins	Mechanism of action	Disease indications
ALMB-0166	Antibody	Extracellular domain	Hemichannel blocker	Acute spinal cord injury [19,36]
ALMB-0168	Antibody	Extracellular domain	Hemichannel activator	Osteosarcoma [25,37]
Mimetic peptide 5	Peptide	Extracellular domain	Channel blocker	Diabetic retinal injury [38]
Gap19	Peptide	Intracellular loop	Channel blocker	Dry/wet macular degeneration, Diabetic retinal disease/diabetic nephropathy [39,40]
Xentry-Gap19	Cell-penetrating peptide (CPP)	Intracellular loop	Channel blocker	Dry/wet macular degeneration, Diabetic retinal disease/diabetic nephropathy [40,41]
Gap26	Peptide	Extracellular domain	Channel blocker	NA [42,43]
Gap27	Peptide	Extracellular domain	Channel blocker	NA [42,43]
Xiflam (Tonabersat)	Small molecule	NA	Channel blocker	Diabetic macular edema, Diabetic nephropathy, Geographic atrophy [40,44]
αCT1	Peptide	C-terminal domain	Channel remodeling, phosphorylation mediator	Diabetic foot ulcers, skin and eye diseases [30,45,46]
Rotigaptide (ZP123)	Peptide	NA	Channel modifier, gap junction coupling	Ischemic injury of the heart [47]
Danegaptide (ZP1609)	Peptide	NA	Channel modifier, gap junction coupling	Myocardial infarction [48,49]
Nexagon	Oligonucleotide	Cx43 DNA	Decreasing Cx43 level	Corneal/skin wounds [50]

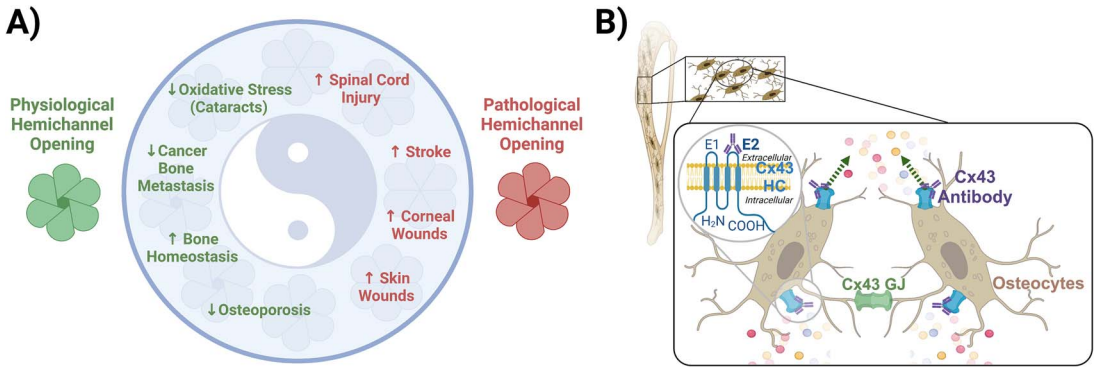

Figure 1. **Hemichannels as an antibody therapeutic target.** (**A**) Antibodies, either through the promotion of physiological or blocking of pathological hemichannel opening, are thought to be a unique approach to combat an array of diseases. (**B**) As an example, in osteocytes, the most abundant cells in bone, which express high levels of Cx43, forming both hemichannels (allowing communication between the intracellular and extracellular environments) and gap junctions (enabling direct cell-to-cell communication between adjacent osteocytes), antibodies present a unique approach to target specific hemichannels without affecting gap junctions. Immunoglobulin G (IgG) molecules, with a large molecular weight (~150 kDa) cannot access epitopes located within the tightly packed arrays of gap junctional plaques, where the intercellular space is limited (channel distance ~ 100 Å), Connexin hemichannels have two extracellular loops (E1 and E2) exposed to the extracellular environment, making them ideal targets for developing targeting antibodies. Among these loops, E2 has been shown to have the highest immune specificity [56]. Preclinical models have demonstrated the ability to utilize Cx43 hemichannel-blocking and activating antibodies targeting E2 in various disease states [19,25,57], and clinical trials are currently underway utilizing this approach in different clinical applications [36,37]. Created in BioRender. Acosta, F. (2024) BioRender.com/c15p060

release mediated by Cx43 hemichannels likely plays a significant, detrimental role in inflammatory responses, neural damage, and motor functions after SCI. Reducing the spread of secondary neural damage signals after inhibiting hemichannel opening following SCI appears to be a promising strategy for therapy.

Among various targets under therapeutic development, one peptide (Gap19) shows potential for application in the acute phase of SCI [69], as well as the use of 30-mer antisense oligodeoxynucleotide (AsODN) [70]. However, antibody therapy may offer greater specificity to hemichannels and maintain improved system stability. Recently, a monoclonal antibody (MHC1) that specifically binds and inhibits the opening of Cx43 hemichannels, without affecting gap junctions, significantly reduces secondary damages in mouse SCI models [19]. The study revealed that the antibody specifically blocked the opening of Cx43 hemichannels in both primary spinal astrocytes and astrocytes *in situ*. Additionally, antibody treatment reduced astrocyte gliosis and the size of injury lesions, while enhancing neuronal survival. Importantly, administering the antibody post-SCI markedly improved hind limb locomotion function. This research suggests that focusing on blocking the opening of Cx43 hemichannels using the antibody approach offers a potentially novel and innovative therapeutic strategy for treating SCI. Currently, a clinical trial is underway to explore the use of a humanized version of this antibody in the treatment of acute SCI [36].

Cx43 hemichannel in skin diseases

Oculodentodigital Dysplasia (ODDD) and Palmoplantar Keratoderma and Congenital Alopecia-1 (PPKCA1) are skin disorders

in humans linked to mutations in the Cx43 gene [71,72]. These diseases often involve an increase in active hemichannels in the cell membrane [73]. For instance, ODDD, a rare disease characterized by birth defects affecting the face, eyes, teeth, and limbs, can result from mutations in the Cx43 gene, leading to various pathological changes, such as disrupted transport and assembly of channels or heightened hemichannel activity [74,75]. Other than genetic diseases, Cx43 hemichannels are also involved in wound healing and other skin diseases. Wound healing is a complex process that involves various cell types and molecular pathways, typically progressing through four stages: hemostasis, inflammation, proliferation, and maturation [76]. The inflammatory stage, which follows hemostasis, recruits leukocytes to eliminate bacteria and damaged cells and is considered the most crucial stage for wound healing and scar formation [21]. Reduced or absent inflammatory responses have been associated with improved healing and minimal or no scarring during wound healing [77–79].

Hemichannels are implicated in mediating inflammation during wound conditions. Under normal conditions, they remain closed to preserve essential metabolic and ionic elements. However, in certain situations, such as wounding, hemichannels can open in response to cytokines, electrical, or chemical stimuli [80,81]. Cx43 levels in epidermal cells typically exhibit a temporary reduction, both at and around the wound edge, within the initial 24 hours post-injury [82,83]. Polymorphonuclear neutrophils and macrophages are key immune cells contributing to wound healing inflammation during wound healing. ATP, released immediately from damaged cells or continuously through Cx43 hemichannels, interacts with purinergic receptors such as P2X7 and P2X1, facilitating the recruitment of immune cells to the wound site [84,85]. ATP activation also triggers the toll-like receptor pathway in response to pathogen-associated molecular patterns or damage-associated molecular patterns. This leads to the activation of transcription factors NF-κB and MAPK pathways, thereby enhancing cytokine-mediated inflammation [86,87].

Generally, diminishing inflammation at the wound site through the suppression of Cx43 appears to be an effective strategy for enhancing wound healing. This approach potentially accelerates wound closure and minimizes scar formation. A-connexin carboxyl-terminal peptide (ACT-1), which targets the C-terminal domain of Cx43, does not affect the expression level of Cx43 [88]. However, it promotes healing rates, reduces inflammation, and decreases scar tissue formation in patients with chronic venous leg ulcers and in animal models, likely achieved through inhibiting Cx43 hemichannel opening and enhancing gap junction functions [29,33,89]. Treatments that prevent the upregulation of the Cx43 gene are also advantageous for wound healing. Administration of a Cx43 antisense gel to wound sites right after injury accelerates the reduction of Cx43 levels in the epidermis. Additionally, the knockdown of Cx43 using short interfering RNAs (siRNA) enhances wound healing and cell growth [90,91].

Cx43 hemichannel in bone tissues

Bone tissue is primarily composed of three major cell types: osteocytes, osteoblasts, and osteoclasts. Osteoblasts are vital for bone formation and remodeling, processes that are fundamental for the upkeep and health of the skeletal system. Meanwhile, osteoclasts play an essential role in bone resorption, an integral process for bone maintenance, remodeling, and injury repair. Osteocytes, the most prevalent bone cell type, accounting for 90–95% of all bone cells, are extensively networked through elongated dendritic processes. Osteocytes orchestrate bone remodeling and homeostasis by modulating the activities of osteoblasts and osteoclasts and influencing bone matrix properties [92–94]. Studies utilizing animal models with osteoblast- and osteocyte-specific Cx43 knockouts [95–97] have demonstrated that Cx43 is instrumental in bone cell proliferation, survival, and differentiation. Dysfunctional Cx43 hemichannels in osteocytes have been linked to adverse effects on bone formation, remodeling, and the viability of osteocytes [98]. Furthermore, Cx43 hemichannels play a pivotal role in the transition from osteoblasts to osteocytes and are involved in regulating the differentiation of osteoclasts [99].

PGE_2 significantly influences various physiological and pathological processes through its signaling. When mechanically stimulated, Cx43 hemichannels serve as a direct portal for releasing prostaglandins E_2 (PGE_2) [57,100,101], a crucial bioactive lipid synthesized by cyclooxygenase 2 (COX-2). The released PGE_2 from opened hemichannels of osteocytes mediates the anabolic action of mechanical loading by promoting bone formation [57]. The released PGE_2, through autocrine effects acting on EP2 and EP4 receptors, leads to an increase in β-catenin and a decrease in sclerostin expression within osteocytes. Elevated β-catenin levels in osteocytes enhance the expression of Cx43, the formation of gap junctions, mechanosensitivity, and osteocyte survival [102,103]. The reduction in sclerostin secretion fosters osteoblast activity and simultaneously restrains osteoclast activity [101].

In addition to its role in mediating the anabolic function of mechanical loading on the bone, in conditions like osteoarthritis, PGE_2 elevates inflammatory cytokine levels, exacerbating cartilage deterioration and joint inflammation [104]. It also hinders the formation of proteoglycans and collagen in cartilage, enhances the expression of matrix metalloproteinases, which break down cartilage, and has a direct effect on cartilage integrity [105]. Additionally, PGE_2 increases the sensitivity of nociceptors in the joint, making them more reactive to pain stimuli. This leads to the speculation that targeting Cx43 hemichannel blockage, thus reducing the release of PGE_2, can also be applied in bone tissues.

Activation of Cx43 hemichannels by bisphosphonates, drugs known for protecting bone health, maintains the viability of osteoblasts and osteocytes. It was also shown that hemichannel permeability, rather than gap junctions, is vital for the cAMP-mediated anti-apoptotic impact of parathyroid hormone on osteoblasts [95,106–108]. Additionally, Cx43 hemichannels, through parathyroid-related protein, drive lactation-induced osteocyte acidification and perilacunar-canalicular remodeling [109]. Interestingly, a critical relationship between major players in the anabolic function of mechanical loading on bone, such as Piezo1, Cx43, and Panx1 hemichannels, has also been explored [110]. In this study, the Piezol channel activated by fluid shear stress is required for the activation of Cx43 hemichannels and Pannexin1 channels, and the influx of Ca^{2+} plays a critical role in the activation of hemichannels. With ongoing studies employing various molecular and genetic tools aimed at conclusively determining the role of Cx43 hemichannels in bone formation and remodeling, and bone cell functions, the potential of Cx43 hemichannels as a new therapeutic target for treating bone loss has increasingly been recognized, as indicated by a recent review [101]. This evolving understanding opens promising avenues for treatments that could more effectively address bone health issues.

Cx43 hemichannel in malignant bone cancers

Although the activation of Cx hemichannels under pathological conditions is typically viewed as negative, contributing to disease progression, their activation in osteocytes within bone

malignancies may have a positive impact, potentially inhibiting tumor cell migration and growth. When hemichannels in bone cells (osteocytes or osteoblasts) open, ATP is released, contributing to the killing of tumor cells [23, 24]. This occurs through the hemichannel-activation mechanisms that improve the tumor microenvironment and promote the immune system's response. For instance, ATP binds to and activates the purinergic receptor P2X7 on dendritic cells, subsequently triggering the activation of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome pathway. This pathway involves Caspase-1, which converts Pro-IL1β into IL1β and releases it into the extracellular environment. The released IL1β then acts as a primer, recruiting other cytokines to activate immune cells like CD8+ T lymphocytes, which are crucial for killing tumor cells [111]; [112,113]. However, the action of IL1β has two sides: it helps anti-tumor efforts, while IL-1β generated by tumor-infiltrating macrophages promotes tumor growth and metastasis within the tumor microenvironment [114].

ATP released by opened Cx43 hemichannels also directly inhibits tumor migration and growth. In the tumor environment of breast cancer bone metastasis, ATP binds to the purinergic receptor P2Y11 in osteocytes, causing a reduction of the P2Y11 through the process of internalization. This results in lower mRNA and protein expression of the C-X-C chemokine receptor type 4 (CXCR4) receptor, which reduces the signaling between CXCR4 and its ligand CXCL12 in the downstream pathway. The reduced signaling leads to the suppression of both the migration and growth of breast cancer cells in bone tissue [22–24]. P2Y11 is another purinergic receptor that plays an important role in cell migration and growth. Research indicates that the activation of the P2Y11 receptor in hepatocellular carcinoma cells enhances the migration of these cancer cells [115]. P2Y11 also plays a role in the ATP-mediated anti-cancer process in prostate cancer cells [116]. CXCR4 is a receptor that binds to the chemokine CXCL12 (also known as stromal cell-derived factor 1, SDF-1), playing a crucial role in various biological processes, including the activation of downstream PI3K-AKT pathways promoting tumor metastasis and growth [117,118]. CXCR4 is prominently expressed in different types of tumors and is linked with the chemotaxis, invasion, and proliferation of tumor cells [119–124]. Knockdown or knockout of CXCR4 expression significantly reduces cell proliferation, growth, migration, and invasion [125,126]. The migration of cancer cells was inhibited using P2Y11 antagonists or P2Y11 siRNA, and this also attenuated the inhibitory effect of ATP analogs on breast cancer cell migration. Similarly, knocking down CXCR4 with siRNA inhibited cancer cell migration and abolished the inhibitory effect of ATP analogs on breast cancer cell migration [22]. Additionally, ATP analogs directly inhibit the migration of breast cancer cells both *in vitro* and *in vivo*, and they also prevent the growth of cancer cells in the tibia [22]. This research reveals a novel mechanism wherein continuous extracellular ATP, released by the opening of osteocyte Cx43 hemichannels, plays a crucial role in suppressing breast cancer cell migration and bone metastasis. The suppressive role of ATP is achieved through its binding to the purinergic receptor P2Y11R, subsequently leading to the downregulation of the CXCR4 function in tumor cells.

It is important to note that ATP is unstable and hydrolyzed by ecto-ATPases into other metabolic products, such as ADP, AMP, and adenosine. In contrast to the effect of ATP, adenosine can promote cancer growth. Thus, it is unsafe to use ATP directly to treat cancer [127]. However, recent pre-clinical studies show that the Cx43-M2 antibody, which activates Cx43 hemichannels in osteocytes, reduces breast cancer and osteosarcoma cell growth and improves survival rates by increasing the population and activation of tumor-infiltrating immune-promoting effector T lymphocytes while reducing immune-suppressive regulatory T cells. This is achieved through the facilitation of ATP release and purinergic signaling, transforming the cancer microenvironment from a supportive to a suppressive state [25]. This transformation changes the cancer microenvironment from a supportive to a suppressive state [25]. It is likely that the levels of eATP released and its byproducts may have more predominant anti-cancer rather than pro-cancer roles. Potential therapeutic strategies for cancer applications using antibodies targeting Cx43 hemichannels are currently in clinical trials by AlaMab Therapeutics Inc [37].

Summary and conclusion

Cx43 hemichannels are implicated in inflammation associated with various other diseases, including secondary complications in the kidneys and eyes caused by diabetes [20]. However, these aspects are not addressed in this minireview. Cx43 hemichannel activation leads to the release of factors that exacerbate inflammation, such as activating the NLRP3 pathway. This exacerbation can worsen or prolong disease progression, positioning hemichannels as negative regulators in these diseases. Therapeutically, restraining overactive hemichannels has been a key focus. Meanwhile, recent research indicates that activating hemichannels in osteocytes promotes the release of substances like ATP, which can inhibit cancer cell proliferation and bone metastasis. This anti-tumor effect stems from stimulating tumor-destroying immune cells and directly interacting with purinergic receptors on cancer cells. Consequently, stimulating hemichannels might offer a novel therapeutic strategy.

Overall, Cx43 hemichannel blockers or activators represent a promising future therapeutic option in treating various diseases. However, several aspects, primarily regarding the detailed mechanisms underlying the activation and inhibition of Cx43 hemichannels, require further elucidation. These include understanding the specific conditions under which hemichannels open during both pathological states and in response to therapeutic molecules. It is also important to explore how inflammation-prone molecules interact with downstream receptors, whether there are other receptor-mediated pathways that stimulate inflammation or other biological events, and whether molecules other than ATP, glutamate, and PGE_2 are involved in disease progression. Additionally, research should investigate whether other diseases are influenced by Cx43 hemichannels, any interactions of Cx43 with other connexins or molecules in these diseases, and the potential of different or modified therapeutic modalities, such as mimetic peptides, nucleotides, and antibodies, for improved efficacy.

Author contributions

Yanfeng Zhang (Conceptualization [equal], Formal analysis [equal], Investigation [equal], Writing—original draft [equal], Writing—review & editing [equal]), Francis ca Acosta (Conceptualization [equal], Investigation [equal], Validation [equal], Writing—review & editing [equal]), and Jean Jiang (Conceptualization [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Writing—original draft [equal], Writing—review & editing [equal])

Conflict of interest

Y.Z. is an employee of AlaMab Therapeutics Inc.

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Data availability

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Ethics and Consent Statement

Consent was not required.

Animal Research Statement

Not applicable.

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