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Non-canonical roles of Connexin-43 beyond gap junction channels in tissue homeostasis and disease

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Abstract

Connexin 43 (Cx43) is the most abundantly expressed member of the connexin family and has long been regarded chiefly as the building block of gap junction channels that couple the cytoplasm of neighbouring cells. This account is correct but incomplete. Work over the past two decades has shown that Cx43 carries out several functions that do not require intercellular channel formation, and some that do not require a channel at all. Its long cytoplasmic C-terminal tail serves as a signalling and scaffolding platform, binding cytoskeletal and regulatory proteins and influencing cell migration, adhesion, proliferation and gene transcription. Internal translation of GJA1 yields truncated isoforms, notably GJA1-20k and GJA1-11k, which traffic to mitochondria and the nucleus and hold distinct functions of their own. Undocked hemichannels permit the release of ATP, glutamate and prostaglandin E2, supporting bone mechanotransduction under physiological conditions but promoting inflammation and tissue injury in disease. The present review gathers the available evidence on these channel-independent, or non-canonical, activities of Cx43 and arranges it by molecular mechanism and by organ system, covering heart, brain, bone, kidney, skin and tumours. Therapeutic implications are also considered, the aim being not to silence Cx43 entirely but to regulate a particular pool, domain or isoform selectively while preserving its channel functions.

Keywords: *Connexin-43, Gap junction, Hemichannel, C-terminal tail, GJA1-20k, Mitochondria, Non-canonical function, Tissue homeostasis*

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1. Introduction

Gap junctions were characterized even before the identification of the proteins that constitute them, and long-term the biology of connexin was considered through the prism of intercellular communication. Gap junctions are a by-product of connexin hemichannels in adjacent cells docking together to form continuous aqueous channels that allow the direct exchange of ions, second messengers and small metabolites between neighbouring cells. Connexin-43 (Cx43), which is an encoded protein of GJA1, is the most widely expressed and widely studied among 21 isoforms of connexins. It is abundant in the heart, brain, vasculature, bone, and skin as well

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as in many epithelial tissues and has incomparable functions in electrical conduction, metabolic coupling, tissue homeostasis, and coordinated cellular activities (Zhu, 2022). Therefore, Cx43 has been traditionally considered the key facilitator of the gap junctional intercellular communication under physiological and pathological circumstances.

This classical perspective has however changed significantly in the last 20 years. There is an increased amount of evidence proving that Cx43 controls many cellular processes even without being a gap junction channel. Experimental work has demonstrated that Cx43 mutants that may not form functional gap junctions can still have an effect on cell proliferation, migration, differentiation and survival. In addition, the Cx43 cytoplasmic C-terminal domain is a dynamic signalling platform, having interactions with cytoskeletal proteins, kinases and transcriptional regulators. The localization of Cx43 to mitochondria and nucleus, combined with the identification of internally expressed isoforms, notably GJA1-20k, have also broadened its repertoire of functions to include membrane-based communication (Ribeiro-Rodrigues *et al.*, 2017; Leithe *et al.*, 2018). These results have demonstrated Cx43 to be a versatile signalling protein with biological functions far beyond traditional gap junction coupling.

In spite of these developments, the state of current knowledge is still polarized and each of the non-canonical functions is commonly studied separately and its molecular connections with other functions are not well coordinated. Moreover, numerous pathological processes such as cardiovascular disease, cancer, neurodegeneration, wound healing disorders, skeletal diseases seem to have a complex interaction between the channel-dependent and channel-independent functions of Cx43. It is thus critical to differentiate these mechanisms in order to comprehend the pathogenesis of disease as well as in order to establish therapeutic approaches that would preferentially affect only a few of the functions of Cx43 without affecting other physiological functions of Cx43.

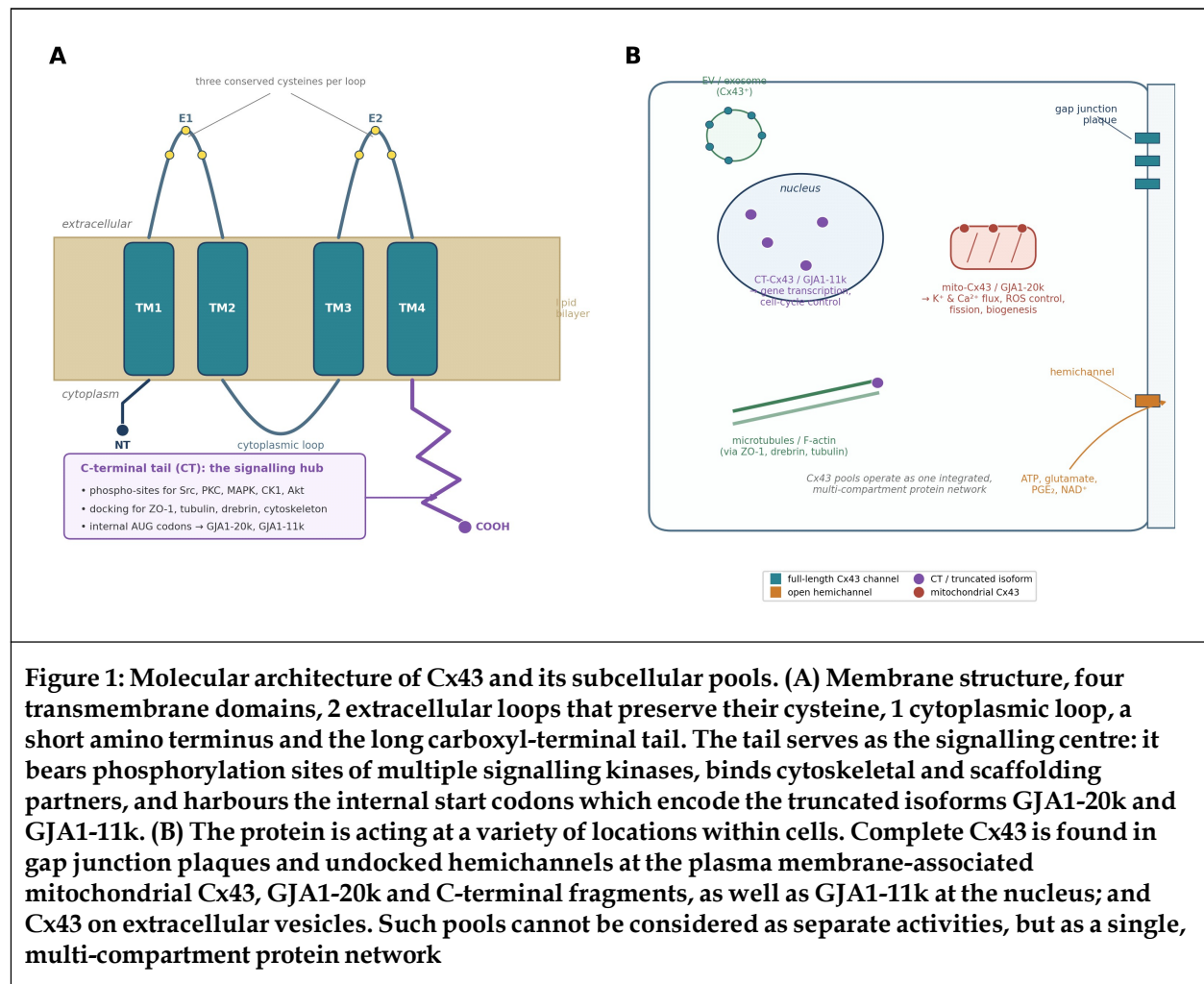
This review critically discusses the newly evolving landscape of non-canonical functions of Connexin-43 with special reference to the molecular processes involved in regulating its channel-independent functions, such as C-terminal signalling, hemichannel biology, mitochondrial and nuclear localization, and the roles of GJA1-derived protein isoforms. This review will consolidate the evidence in many different tissues and disease models to enable a global perspective on how these different mechanisms play a role in tissue homeostasis and pathology and it will be important in identifying them as potential new targets of precision therapeutic intervention.

2. Molecular architecture and life cycle: The structural basis for moonlighting

Cx43 is a tetra-span membrane protein. The lipid bilayer is crossed by four hydrophobic segments linked by two extracellular loops and one cytoplasmic loop, where the amino and carboxyl ends are all located on the cytoplasmic side. Each of the extracellular loops contains three conserved cysteines which are disulphide bonded and required to dock two hemichannels to create a complete gap junction channel. Connexin subunits assemble into a hemichannel, or connexon and it is this modular structure that enables a single protein to be used either as half of an intercellular channel, or as a free membrane pore in its own right (Figure 1A).

The amino end is short and helps to gate channels and lining of pore. The terminal distinguishing Cx43 is at its carboxyl end. It is lengthy, mostly unstructured in solitude, and overcrowded with locations of post-translational modification or protein association. This tail is a major cause of connexin isoform variations, as the transmembrane and loop domains are more similar, and it is the location of most non-canonical activity.

The Cx43 life cycle is organised by phosphorylation. The protein has a rapid half-life (about one to a few hours) and travels rapidly to the plasma membrane, via the Golgi and endoplasmic reticulum, into gap junction plaques and subsequent internalisation and degradation. Each step is programmed to have its unique serine residues modified by kinases that consist of casein kinase 1, protein kinase C, mitogen-activated protein kinase, Src and Akt, generating the characteristic electrophoretic isoforms and determining whether Cx43 is trafficked forward, assembled into plaques, opens or closes its gates or is degraded (Solan and Lampe, 2007; Solan and Lampe, 2009). Since the same residues that regulate channel behaviour, also regulate protein binding, phosphorylation is a switch between the canonical and non-canonical lives of the molecule.



Another enigma is the GJA1 transcript itself. There is only one coding exon of the gene, and thus, no alternative splicing can be done, but the messenger RNA is translated using multiple internal start codons besides the initial one. This *in vivo* translation produces a series of amino-terminally truncated isoforms which retain successively smaller fragments of the C-terminus. The most common of these is the 20k Da protein, GJA1-20k, which starts at an internal methionine and increases dramatically in response to ischaemic and metabolic stress (Shimura and Shaw and 2022). The nucleus has been described to have a smaller species 11 kDa -GJA1-11k (Epifantseva *et al.*, 2020). These fragments do not have the transmembrane parts to construct a channel, making their functions squarely beyond the canonical structure (Figure 1B).

3. The C-terminal interactome: A signalling centre and scaffolding

When the C-terminus contains the non-canonical function, its binding partners will be the mechanism. Mass spectrometry and focussed biochemistry have compiled a sizeable interactome around the Cx43 tail, and the common motif is a connection between the protein and the cytoskeleton. The tail directly attaches to alpha- and beta-tubulin, such that microtubules protrude towards Cx43 at the membrane, and this protein interacts with the actin cytoskeleton via adaptor proteins, primarily zonula occludens-1 (ZO-1) and drebrin (Giepmans *et al.*, 2001; Ambrosi *et al.*, 2016). NMR studies have revealed that these partners are non-overlapping, in separate locations on the tail, and this results in Cx43 being able to bind several of them simultaneously and serve as an organising centre in local cytoskeletal structure. These interactions too are phosphorylated; phosphorylation of certain tyrosine and serine residues by Src and other kinases relaxes or constrains binding of partners, using the scaffold to bound the cell signalling state.

3.1. Cytoskeletal cell migration and channel-independence

Among the most obvious evidences that Cx43 is not just a cell coupling factor, the studies on migration are included. Cx43 can be necessary to have cells move appropriately despite the inactivation of the channel and

the effect is localized to the cytoplasmic tail. Cx43 can impact directional movement by modulating the dynamics of microtubules and by contributing to receptor and small-GTPase signalling that guides the leading edge (Kameritsch *et al.*, 2012). This is very important in development: radial migration of neurons in the cortex and the migration of neural crest cells requires CX43, and a construct that lacks the C-terminal tail does not rescue migration despite being able to mediate channels. This protein serves as more of an adhesion and guidance molecule than a conduit, and the difference is not purely academic since it is different parts of Cx43 that a therapeutic would need to interact with.

3.2. *Excitations of the isoforms and tail length*

Internal translation brings the interactome to the cytoplasm and beyond. Since GJA1-20k is mainly a C-terminal protein minus the channel-forming domains, it is still able to bind tubulin and actin and is deprived of the channel activity. In its form it functions as a trafficking chaperone, stabilising the microtubules and directing forward movement of full length Cx43 to the membrane. GJA1-11k is even shorter and located in the nucleus where it is identified to slow down cell cycle progression. The presence of these endogenous fragments redefines much work in the past where researchers had to assemble certain parts of the C-terminus by hand: what was perceived as an artificial manipulation will reveal itself to be roughly similar to what species the cell practices in vivo.

3.3. *Non-junctional transfer: Extracellular vesicles and tunnelling nanotubes*

Material exchange between cells occurs via a variety of pathways in addition to the gap junction, Cx43 is implicated in many of these. The protein itself is packed onto extracellular vesicles where the protein can carry functional hemichannels over the vesicle surface, and the protein can also localise to tunnelling nanotubes which are bridges between cells over longer distances than a junction can span. Those Cx43 aid in cargo transport and with tunnelling nanotubes it is involved in the transport of intact organelles, such as mitochondria, between cells (Gervasi *et al.*, 2025). Some groups interpret this as having been repurposed throughout evolution, with Cx43 being used as a general communication protein, but not a specific junctional channel; and the vesicle and nanotube pathways are a subset of that broader function. These vesicle-bearing hemichannels have been implicated in intercellular transmission of inflammatory signals in inflamed tissue, which relates this delivery mechanism to the disease mechanisms referenced below. Table 1

Table 1: Non-canonical functions of Cx43 organised by structural element, molecular mechanism, key partners and principal cellular outcome			
Structural element	Mechanism	Key partners / mediators	Principal outcome
C-terminal tail (scaffold)	Direct and adaptor-mediated cytoskeletal binding	Tubulin, ZO-1, drebrin, F-actin	Cytoskeletal organisation; directional cell migration
C-terminal tail (signalling)	Docking and phospho-regulation of kinase pathways	c-Src, PKC, MAPK, CK1, Akt	Control of proliferation, adhesion and differentiation
C-terminal fragment/GJA1-11k	Nuclear localisation and transcriptional influence	Importin-β, transcriptional regulators, miR-125b/p53	Growth suppression; cell-cycle arrest; gene regulation
GJA1-20k isoform	Microtubule stabilisation and mitochondrial targeting	Actin, tubulin, outer mitochondrial membrane	Cx43 trafficking; mitochondrial fission and biogenesis
Mitochondrial Cx43	Ion flux and redox modulation at inner membrane	mitoK(ATP), Ca ²⁺ handling, respiratory chain	Preconditioning; oxidative-stress tolerance
Undocked hemichannel	Regulated efflux of small molecules	ATP, glutamate, PGE ₂ , NAD ⁺ ; P2X7	Paracrine signalling; when aberrant, inflammation
Cx43 on extracellular vesicles	Vesicle-borne intercellular transfer	Exosomal cargo, functional hemichannels	Distant signalling; propagation of inflammatory cues

summarizes the principal channel-independent activities of Cx43 grouped by the domain or isoform responsible.

4. Hemichannels: single-membrane conduits with context-dependent output

A connexon is a hemichannel prior to docking with a partner. The long-standing belief was that these unpaired channels remained closed at the cell surface, only opening temporarily, due to an open pore to the extracellular space posing a threat to a cell attempting to maintain its ionic gradients. It has changed that opinion. The conditionally opened undocked Cx43 hemichannels permit the passage of small molecules (below about 1 to 1.2 kDa) between the cytoplasm and the outside, including ATP, glutamate, NAD and prostaglandin E2 (PGE2) (Zhang *et al.*, 2024). The effects of that discharge will all depend on the amount unlocked, the duration of the release and in which cell. In one environment, the identical channel mediates normal tissue signalling whereas in another, it mediates damage (Figure 2).

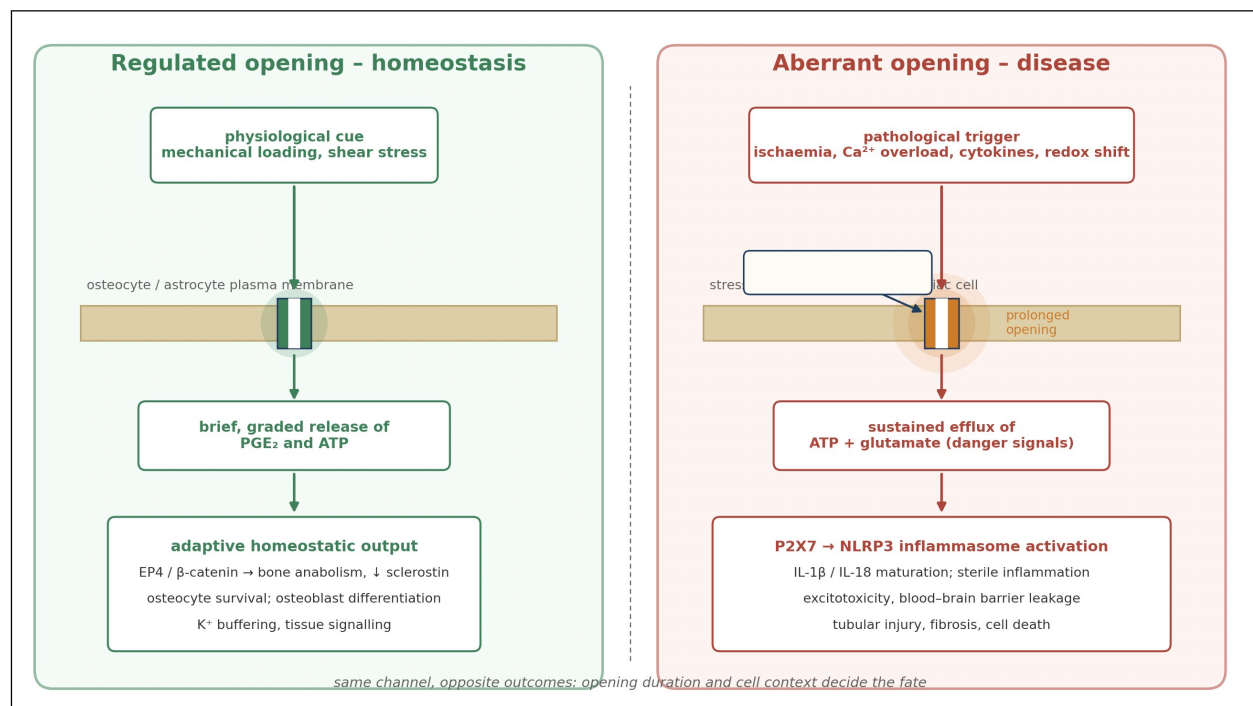


Figure 2: The Cx43 hemichannel as a context-dependent conduit. On the left, a physiological cue such as mechanical loading or shear stress triggers brief, graded hemichannel opening in osteocytes and astrocytes, releasing PGE2 and ATP in amounts that support homeostatic outputs including bone anabolism, cell survival and ionic buffering. On the right, a pathological trigger such as ischaemia, calcium overload, cytokine exposure or a redox shift causes prolonged opening and sustained efflux of ATP and glutamate; these danger signals engage P2X7 and the NLRP3 inflammasome, producing sterile inflammation, excitotoxicity, barrier leakage, tubular injury and cell death. The same channel therefore produces opposite outcomes depending on how long it opens and in which cell. Hemichannel-selective blockers (Gap19, Peptide5, TAT-Gap19, **boldine**) act on the aberrant fraction

4.1. Regulated opening in homeostasis: Bone as the clearest case

Bone provides the most well-examined instance of hemichannels performing a beneficial physiologic service. The skeleton has mechanosensors (osteocytes, the mineralised matrix-buried cells) and Cx43 is the most common connexin in these cells. When an osteocyte is subjected to mechanical load or fluid shear, Cx43 hemichannels in the osteocyte temporarily open and extracellularly release PGE2, a bone building factor, along with ATP (Zhao *et al.*, 2022; Zhao *et al.*, 2023). The magnitude of release feeds back the magnitude of the mechanical stimulus, and the PGE2 which leaks out feeds on the same and adjacent cells via the EP4 receptor to facilitate bone formation, increase beta-catenin signalling and decrease sclerostin. Mice with a mutant that exclusively disrupts hemichannels but leaves gap junctions intact elucidate the causal pathway: the response of loading to anabolic is suppressed when hemichannels are not functional. More recent evidence has further implicated this signal in the marrow where osteocyte hemichannel-generated PGE2 biases mesenchymal

progenitors to the bone-forming pathway and not fat, again via EP4 and ERK signalling. It is brief, graded and self-limiting release, and it comes off as adaptive, not harmful.

4.2. Aberrant opening in disease: From danger signal to inflammation

Inversion of the picture occurs when the hemichannels become too open or remain open too long. Sustained hemichannel opening is facilitated by ischaemia, calcium overload, redox state shift and exposure to inflammatory cytokines, and the ensuing ATP and glutamate leakage is a danger signal as opposed to the measured messenger. Extracellular ATP triggers purinergic receptors, namely P2X7, which priming and activating the NLRP3 inflammasome as well as the maturation of interleukin-1 beta and interleukin-18. A feed-forward loop where the activity of NLRP3 is primed and activated by ATP and P2X7 signalling, dependent on aberrant Cx43 hemichannel activity in tubular epithelial cells, orchestrates tubular injury via inflammasome activity in the kidney (Roger *et al.*, 2023). The reasoning is the same in the nervous system in another tissue. Astrocytic Cx43 hemichannels can increase blood-brain barrier leakage following injury and irradiation and exacerbate seizures by changing barrier permeability, which can be inhibited by hemichannel-selective blockers like TAT-Gap19 (Schumacher *et al.*, 2024; Song *et al.*, 2024). The cause of the affinity between the bone and the disease illustrations is the context and not the channel; the purpose of therapy as it will be explained later is to bridge the gap between the aberrant fraction and the useful one without destroying it.

5. Mitochondrial Cx43 and GJA1-20k: Metabolic guardianship

Part of the most vivid non-canonical biology of Cx43 occurs off of the cell surface. A pool of Cx43 is present in mitochondria which was originally identified in cardiac and vascular cells but has also been found in the central nervous system. Mitochondrial Cx43 is targeted to the organelle through assistance of chaperones, outer-membrane import machinery, and after targeting, it mediates potassium and calcium homeostasis, respiration and reactive oxygen species generation. These actions relate Cx43 with ischaemic preconditioning, the effect where a short-term sublethal stressors render the heart less susceptible to a subsequent, more prolonged ischaemic injury. Mitochondrial Cx43 is necessary to provide protection during preconditioning and phosphorylation of the protein by casein kinase 1 is essential to do so and this puts a defining modification event above a clinically significant outcome (Hirschhäuser *et al.*, 2021).

This has been made even sharper by GJA1-20k isoform. Ischaemic and reperfusion stress leads to endogenous GJA1-20k that localizes to mitochondria and is linked to the outer membrane. GJA1-20k in mouse hearts enhances mitochondrial biogenesis and reduces membrane potential, respiration and reactive oxygen species production, placing the organelle in a protective, quiescent state, reminiscent of preconditioning and minimizing post-ischemic infarct size (Basheer *et al.*, 2018). The fragment also facilitates mitochondrial fission and the localization of mitochondria in the cell and hemichannel-linked Cx43 has also been independently linked to mitochondrial ATP production and oxidative stress resistance (Zhang *et al.*, 2022). Lower GJA1-20k, in its turn, disrupts Cx43 trafficking and mitochondrial activity and is associated with arrhythmia and heart failure. Collectively, these results suggest GJA1-20k is an endogenous stress-response protein and therapeutic candidate, a unique role on behalf of a fragment transcribed by a messenger RNA of an ion channel. An additional dimension is provided by the central nervous system. In addition to being found in neural mitochondria, Cx43 is involved in the process of whole mitochondria being transferred between cells by which stressed neurons can receive healthy organelles donated by astrocytes. This transfer aids neuroprotection and tissue homeostasis in normal and pathological conditions and is far beyond anything the classical model of gap junction could have thought of Gervasi *et al.* (2025).

6. Nuclear Cx43 and transcriptional control

When a gap junction protein was first proposed to regulate genes, this idea was received with scepticism, and the initial evidence was indirect. The expression of the isolated C-terminal tail of Cx43 into cardiomyocytes and HeLa cells targeted it to the nucleus and to the cytoplasm and decreased proliferation, which indicated, but not proved, that the fragment may target gene expression and growth (Dang *et al.*, 2003). The case has been proceduralized in later work. Cx43 has been found to localise to the nuclear envelope of multiple cell types and in cardiac tissue, arriving there by an importin-beta-dependent pathway, and being present there alters the cellular transcriptome, seemingly due to an interaction with transcriptional

regulators (Martins-Marques *et al.*, 2023). The Cx43 carboxyl tail was demonstrated in a developmental context to bind basic transcription factor 3, translocate to the nucleus and directly regulate N-cadherin expression during neural crest migration, which is as direct an indication of transcriptional control by a connexin as the field has produced (Kotini *et al.*, 2018).

Here the truncated isoforms reoccur. GJA1-11k localises to the nucleus and suppresses cell cycle progression, and its natural expression in internal translation provides a natural provider of the growth suppressive fragments that previous experiments had produced artificially. The C-terminus has additionally been implicated in microRNA-dependent control of the tumour suppressor p53 in breast tissue and has extended the spectrum of transcriptional and post-transcriptional pathways on which it can act. In all these studies a similar picture can be seen where Cx43 or part of it is lost out of the membrane, into the nucleus and biases transcription towards slower growth, with this effect becoming significant when the protein is lost in cancer.

7. Non-canonical Cx43 across tissues in health and disease

The mechanisms mentioned up to this point are not independent of each other; each tissue utilizes a specific combination of them. This part has organ-biology readings and most therapeutic programmes are presented in that manner too. Figure 3 is a map that plots the dominant mechanism not included in the canon in each of the systems and the modulator being studied in each.

7.1. Heart

Cx43 is most concentrated in the heart and here the stakes of its regulation are greatest. In addition to creating the gap junctions in the intercalated discs that coordinate the action potential of ventricles, cardiac Cx43 is also found to form a broader protein network, dubbed connexome, in which mechanical junctions, gap junctions and sodium channel complex interact. The responses of organelles, which mediate ischaemic preconditioning and tolerance to reperfusion injury, are regulated by Mitochondrial Cx43 and GJA1-20k, as mentioned above. Cx43 is dephosphorylated and is no longer localized to the disc, becoming relocated to the lateral membrane, which disrupts conduction and predisposes to arrhythmia and GJA1-20k loss only exacerbates the situation with impaired trafficking and mitochondrial dysfunction. These attributes render a number of individual fragments of Cx43 biology, as opposed to the channel itself, pertinent to cardioprotection.

7.2. Brain and central nervous system

Astrocytes couple to huge networks via Cx43 and apply that association to load potassium and allocate metabolites, thus the canonical channel still plays a significant role in the brain. The top most layer is the non-canonical activities. ATP and glutamate are released by astrocytic hemichannels to mediate neuroinflammation and regulate the blood-brain barrier and aberrant opening of these channels leads to the leakage of the barrier after irradiation and the severity of seizures in epilepsy models (Schumacher *et al.*, 2024; Song *et al.*, 2024). Mitochondrial transfer between astrocytes and neurons is also facilitated by Cx43, which is a protective exchange in metabolic stress. There is a shift in favour of the destructive side of hemichannel signalling in neurodegenerative and post-ischaemic states and hence the interest of hemichannel-selective inhibitors as CNS therapeutics.

7.3. Kidney

The expression of Cx43 is enhanced in and around the renal tubules in chronic kidney disease, and the non-canonical pathway involved is hemichannel-mediated inflammation. The release of ATP in tubular epithelial cells due to aberrant hemichannel activity initiates the NLRP3 inflammasome, using P2X7, and produces chemokines and cytokines that are increased in patients and perpetuate an inflammatory cycle. The deletion of Cx43 or pharmacological inhibition of the tubule, specifically, with Peptide5 lowers the inflammatory readouts and tubular damage, which identifies the hemichannel as a target to delay disease progression.

7.4. Skin and wound healing

Skin demonstrates an example of how, instead of eliminating Cx43, tuning it can yield a better result. Cx43 is not only expressed in epidermis and dermis but a carefully controlled decrease in its concentration at wound margins is also a component of normal healing, and the continued high level of Cx43 is a sign of chronic non-

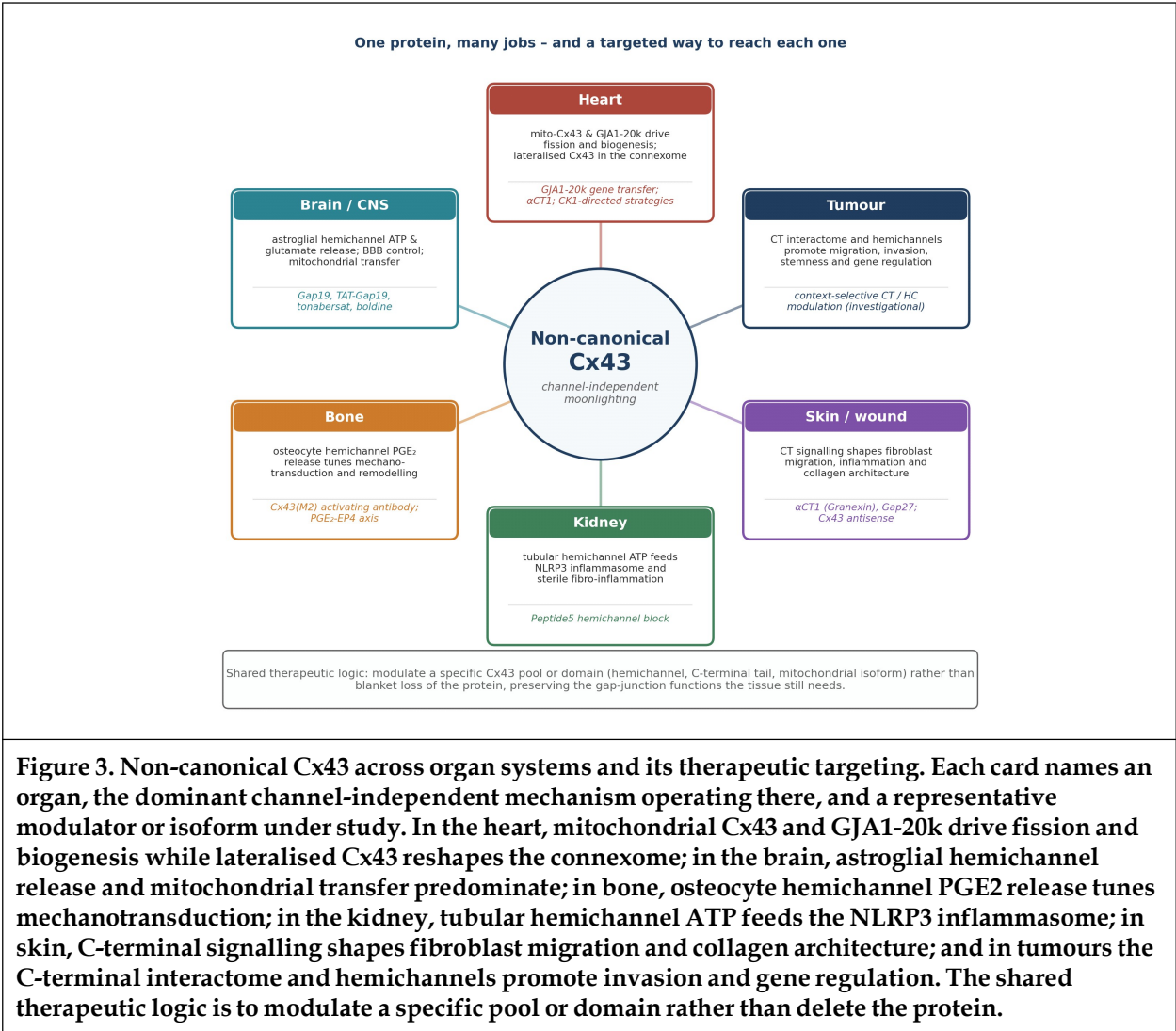
healing wounds (Montgomery *et al.*, 2018). Downregulation of Cx43 genetically or using antisense accelerates re-epithelialisation and reduces scarring. This principle has been applied to the clinic using the peptide alphaCT1 that mimics the extreme C-terminus of Cx43 and binds ZO-1: it does not cause a reduction in the total Cx43, but changes its behaviour, and in controlled trials of surgical wounds it has improved scar appearance, and histology has shown a collagen architecture more reminiscent of unwounded skin (Grek *et al.*, 2017). It seems to operate in part by the action of the peptide on the directionality of migration of fibroblasts, a channel-independent motility of the tail, and has been tested in chronic diabetic and venous ulcers.

7.5. Cancer

Cx43 can be used as a growth suppressor, which is complicated by cancer. The channel-independent activities of Cx43 proved, in most circumstances, to promote tumour progression as opposed to inhibit it. Migration, invasion and stem-like behaviour of tumour cells have been linked to signalling via the C-terminal tail and Cx43-mediated activity in the microenvironment and Cx43 can induce tumour invasiveness even in the primary tumour where Cx43 inhibits tumour growth (Paunikar and Tamagnone, 2024). Another axis is the effect of the tail, which is nuclear and transcriptional because when the C-terminal fragment which acts as a brake on proliferation is lost, a brake on proliferation is removed. What it means is that Cx43 is neither clean oncogene nor clean tumour suppressor; it acts depending on stage, tissue and which of its many functions are dominant and this is exactly why it is important to decompose the non-canonical functions of Cx43 in oncology.

7.6. Vessels and other epithelia

Cx43 is situated across vascular wall, in endothelium and smooth muscle, and its non-canonical activities are



important there as well. Endothelial hemichannels release ATP, which is one of the feeds to inflammatory signalling, a response that is involved in early host response to implanted devices, and Cx43 affects endothelial proliferation and migration in the course of vascular repair in such a manner that they echo other such cytoskeletal and signalling functions. The endothelial and the astrocytic pools of Cx43 work synergistically in the brain at the neurovascular unit, where hemichannel activity contributes to establishing barriers permeability. Related behaviour has been described with epithelia outside of the kidney, whereby hemichannel-mediated release of ATP has been described in urothelium and other secretory organs. The similarity is that regardless of where Cx43 is expressed, it is likely to have channel-independent outputs also, and the experimentation of disaggregation of the two is now a common demand to understand its contribution in any particular organ.

8. Therapeutic targeting: Toward pool- and domain-selective modulation

Blunt instruments started the clinical history of connexin drugs. Broad gap junction blockers close channels and strike hemichannels, other connexins and in many cases unrelated targets and are not able to discriminate the coupling that a tissue requires and release that is aberrant. The shift towards non-canonical biology has been accompanied by an equal shift towards selectivity, and the new generation of agents is designed to act upon a particular pool, a particular domain or isoform.

The field is dominated by mimetic peptides. Shorter loops derived of the extracellular loops, like Gap26 and Gap27, and the cytoplasmic loop, like Gap19 and its membrane-permeable form TAT-Gap19 can selectively favour the closure of hemichannels rather than the abolition of gap junctions, which is the difference that counts in inflammation and central nervous system injury. An extracellular sequence blocker, peptide5, inhibits ATP release via hemichannels and has been effective in renal tubular inflammation. The carboxyl-terminal mimetic α CT1 is yet another modification, which involves ZO-1 and the carboxyl-terminal self-interactions in reorganising Cx43 at the membrane and in modulating fibroblast behaviour, and it has undergone wound-healing and scar testing (Montgomery *et al.*, 2018; Grek *et al.*, 2017). On the isoform front, the production of GJA1-20k protein recapitulates preconditioning-like protection in the heart and is a gene-based, not a small-molecule, approach. Minor molecules and wildlife products, such as the alkaloid boldine, provide additional hemichannel-focused possibilities to neuroinflammatory disease. Table 2 describes the key agents, their molecular targets, disease(s) that they have been tested and their development.

Table 2: Representative agents that target non-canonical Cx43 functions, with molecular target, mode of action, principal disease context and approximate stage of development

Agent	Molecular target	Mode of action	Disease context	Stage
α CT1 (Granexin)	C-terminal tail/ZO-1	Reorganises Cx43; alters fibroblast migration and scar collagen	Surgical scarring; diabetic and venous ulcers	Clinical (Phase II-III)
Gap27/Gap26	Extracellular loops	Favour hemichannel and channel closure	Wound healing; vascular and endothelial injury	Preclinical/early clinical
Gap19/TAT-Gap19	Cytoplasmic loop	Selective hemichannel block, sparing gap junctions	CNS inflammation; epilepsy; barrier leakage	Preclinical
Peptide5	Extracellular loop 2	Blocks hemichannel ATP release; interrupts NLRP3 loop	Chronic kidney disease; retinal and CNS injury	Preclinical
GJA1-20k (gene transfer)	Endogenous truncated isoform	Restores mitochondrial biogenesis and preconditioning state	Ischaemia-reperfusion; arrhythmia; heart failure	Preclinical
Boldine	Hemichannel (small molecule)	Curbs aberrant hemichannel opening	Neuroinflammatory and metabolic disease	Preclinical
Cx43 antisense/siRNA	Cx43 expression (local)	Transient knockdown at wound edge	Chronic non-healing wounds	Preclinical/early clinical

9. Future directions

Future studies need to consider overcoming some of the major difficulties that presently prevent the clinical exploitation of Connexin-43-targeted therapies. The development of functional selectivity is a significant goal, because peptides with selective responses to hemichannel and gap junction activities in vitro have to exhibit a similar selectivity, stability, and long-lasting effectiveness in vivo. Even though some peptides like alphaCT1 have shown potential in terms of therapeutic application, there are still significant barriers of increasing their delivery and bioavailability over time. The other essential aspect relates to how the internally translated GJA1 isoforms, specifically GJA1-20k and GJA1-11k, whose specific biological roles indicate that controlling their expression can be a new form of therap. Moreover, the mechanisms by which post-translation changes in Cx43 are coupled to its functions in the nucleus and mitochondria are not well understood and much of the existing data is obtained with help of experimental models and not human tissues. Lastly, the Cx43 context-dependent roles, particularly in cancer, underscore the importance of therapeutic methods that are accurate, taking into account tissue type, disease stage and subcellular localization. Further progress will require the introduction of advanced imaging methods, isoform-specific reagents and genetically engineered in vivo systems that can selectively interrogate specific Cx43 functions but not interfere with its other physiological functions (Paunikar and Tamagnone, 2024).

10. Conclusion

Connexins-43 (Cx43) has been identified as being much more than a rigidity element of the gap junctions. Although its canonical function in intercellular communication is still essential in maintaining tissue homeostasis, emerging evidence suggests that Cx43 has multiple additional functions via its cytoplasmic domains, hemichannels, mitochondrial and nuclear distribution and internally expressed isoforms. These non-canonical activities control a wide range of biological activities, such as bone remodelling, cardioprotection, neuroinflammation, kidney injury, wound healing, and tumour progression, underscoring their extensive physiological and pathological impacts. This appreciation of Cx43 having different actions in different locations depending on the cellular location, interacting partners and post-translational modifications has fundamentally changed the way we see the biology of Cx43. Therefore, treatment modalities have changed with global modulation of gap junction communication to selective modulation of distinct domains of Cx43, subcellular pools or signalling pathways. This type of precision approaches has the potential to increase therapeutic efficacy whilst not disrupting the necessary physiological roles of Cx43. Further molecular, cellular and translational research efforts will be necessary to comprehensively characterize these context-specific mechanisms and enable the creation of the next generation Cx43-targeted therapies to treat a diverse range of human diseases.

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