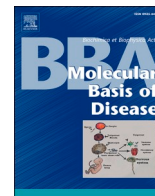




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## Harnessing the therapeutic potential of antibodies targeting connexin hemichannels

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### ABSTRACT

**Background:** Connexin hemichannels have been implicated in pathology-promoting conditions, including inflammation, numerous widespread human diseases, including cancer and diabetes, and several rare diseases linked to pathological point mutations.

**Methods:** We analysed the literature focusing on antibodies capable of modulating hemichannel function, highlighting generation methods, applications to basic biomedical research and translational potential.

**Results:** Anti-hemichannel antibodies generated over the past 3 decades targeted mostly connexin 43, with a focus on cancer treatment. A slow transition from relatively unselective polyclonal antibodies to more selective monoclonal antibodies resulted in few products with interesting characteristics that are under evaluation for clinical trials. Selection of antibodies from combinatorial phage-display libraries, has permitted to engineer a monoclonal antibody that binds to and blocks pathological hemichannels formed by connexin 26, 30 and 32.

**Conclusions:** All known antibodies that modulate connexin hemichannels target the two small extracellular loops of the connexin proteins. The extracellular region of different connexins is highly conserved, and few residues of each connexins are exposed. The search for new antibodies may develop an unprecedented potential for therapeutic applications, as it may benefit tremendously from novel whole-cell screening platforms that permit *in situ* selection of antibodies against membrane proteins in native state. The demonstrated efficacy of mAbs in reaching and modulating hemichannels *in vivo*, together with their relative specificity for connexins overlapping epitopes, should hopefully stimulate an interest for widening the scope of anti-hemichannel antibodies. There is no shortage of currently incurable diseases for which therapeutic intervention may benefit from anti-hemichannel antibodies capable of modulating hemichannel function selectively and specifically.

### 1. Connexins, connexons and intercellular gap junction channels

Twenty connexin genes are expressed both in humans (identified by a "GJ" prefix) and in mice (identified by a "Gj" prefix) (Table 1; note that two connexins, connexin 25 and 59, are human only, whereas two, connexin 23 and 33, are mouse only). All protein products of connexin genes are thought to share the same topology, with cytoplasmic N- and C-termini (NT, CT, respectively), four transmembrane domains (TM1–4) connected by two extracellular loops (referred to either as EL1 or EC1 and EL2 or EC2, respectively) and a cytoplasmic loop (CL) [1]. Connexin protomers are post-translationally oligomerized to form

hexameric assemblies, termed connexons, prior to membrane insertion either within the endoplasmic reticulum or in the trans-Golgi network [2–6]. Each connexon delineates a pore in the plasma membrane, and the coaxial arrangement of two docked connexons from adjacent plasma membranes establishes a communication pathway, termed intercellular gap junction channel (IGJC) between the cytoplasm of the two adjoined cells [7–12] (Fig. 1). Connexons may be homomeric (composed of six identical connexin subunits) or heteromeric (composed of more than one species of connexins). IGJCs may be homotypic (if connexons are identical) or heterotypic (if the two connexons are different) [13]. IGJCs are thought to cluster into plaques via long-range protein aggregation driven from interparticle interactions as well as lateral pressures

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between the junction and the surrounding glycocalyx [14,15].

## 2. Importance and role of connexin hemichannels

Unpaired connexons can establish a communication pathway between cell cytoplasm and the extracellular milieu by operating as regular plasma membrane channels, referred to as hemichannels (HCs), as detailed hereafter (see also refs. [16-20]). Thus, in a seminal article DeVries and Schwartz reported that a reduced extracellular concentration of ionized calcium ( $[Ca^{2+}]_{ex}$ ) produced a current in solitary horizontal cells isolated from catfish retinas, which could be suppressed by dopamine. Lucifer Yellow CH (LY) entered solitary horizontal cells bathed in a medium containing a reduced  $[Ca^{2+}]_{ex}$ . Adding dopamine to the extracellular solution, or increasing the  $[Ca^{2+}]_{ex}$ , blocked the influx of the dye. The authors concluded that ions and dye flowed through “hemi-gap-junction channels expressed in the surface membrane” [21].

The study of connexin HCs activity in the cell plasma membrane gained momentum from a concurrent article by Paul et al. reporting that (a) Cx46 induced nonselective channels in the oolemma that were voltage dependent and opened by large depolarizations and (b) oocytes expressing Cx46 were permeable to LY but not FITC-labelled BSA, indicating the presence of selective membrane permeabilities [22]. Trexler et al. showed that HCs comprised of rat Cx46 exhibited complex voltage gating, had a unitary conductance of ~300 pS and induced substantial ion fluxes in cells expressing Cx46 [23]. Li et al. investigated the putative opening of Cx43 HCs in Novikoff, NRK, and HeLa cells by uptake of dye (5(6)-carboxyfluorescein, 7-hydroxycoumarin-3-carboxylic acid or fura-2) from the culture medium when  $[Ca^{2+}]_{ex}$  was reduced, leading to “a hypothesis that a physical interaction [between  $Ca^{2+}$  and HCs] was involved in the process, instead of a metabolic mechanism” [24]. The pioneering studies mentioned above spurred the nowadays widespread use of dye uptake as a standard assay of HC function [25].

Years later, Muller et al. used atomic force microscopy (AFM) and revealed that the pore at the extracellular mouth of Cx26 HCs changed diameter from  $13 \pm 3 \text{ \AA}$ , in 0 mM  $[Ca^{2+}]_{ex}$  to  $5 \pm 3 \text{ \AA}$  in 0.5 mM  $[Ca^{2+}]_{ex}$

presence of mM levels of extracellular divalent cations ( $Ca^{2+}$  and  $Mg^{2+}$ ) [24,28–32]. However, also other factors modulate the open probability of HCs, including mechanical strain, changes in pH, transmembrane voltage, post translational modifications (and in particular protein phosphorylation) and cellular redox state, metabolic inhibition, nitric oxide (NO) and linoleic acid [33–42]. In particular (as mentioned above) lowering the  $[Ca^{2+}]_{ex}$  favors the opening of HCs formed by several different types of Cxs [43]. In contrast, the open probability of HCs shows a more complex, bell-shaped dependence on cytosolic  $Ca^{2+}$  concentration ( $[Ca^{2+}]_{cyt}$ ) with a peak around 500 nM [44–46]. A pivotal role for calmodulin has been proposed also for this incompletely characterized, yet fundamental control mechanism [47,48].

When open, HCs mediate the diffusive release of paracrine messengers, most importantly ATP [49–55], but also glutamate and prostaglandins, NAD(+) or glutathione [56]. Solute permeation through HCs has been scrutinized in numerous studies by experimental methods (see e.g. [20,57–61]) as well as molecular dynamics simulations [62–67]. Based on pore size estimates, the list of permeating molecules could potentially cover >35,000 members of the Human Metabolome Database<sup>1</sup> [68]. In several instances, molecules released through open HCs contribute to paracrine and autocrine signalling pathways during inflammation and cell death [69–72].

## 3. Anti-HC antibodies: an ongoing search with a remarkable pedigree

Polyclonal antibodies are generated by the immune response to an antigen involving the activation of multiple B-cells all of which target a specific epitope on that antigen; as a result, a large number of antibodies are produced with different specificities and epitope affinities [73]. As antibodies usually bind extracellular domains of proteins, the two extracellular loops of the connexin proteins (EL1/EC1 and EL2/EC2), which are exposed in unpaired HCs, became natural targets for the generation of a variety of antibodies (Fig. 2).

The search started with Meyer et al., who selected Novikoff hepatoma cells (that are readily dissociated without the use of proteases and,

**Table 1**  
Connexin genes and proteins.

#	<i>Homo sapiens</i>				<i>Mus musculus</i>			
	Gene symbol	Protein name	OMIM ID	NCBI CCDS database ID	Gene symbol	Protein name	MGI ID	NCBI CCDS database ID
1	<i>GJA1</i>	CX43	121014	CCDS5123.1	<i>Gja1</i>	Cx43	95713	CCDS23851.1
2	<i>GJA3</i>	CX46	121015	CCDS9289.1	<i>Gja3</i>	Cx46	95714	CCDS27152.1
3	<i>GJA4</i>	CX37	121012	CCDS30669.1	<i>Gja4</i>	Cx37	95715	CCDS18667.1
4	<i>GJA5</i>	CX40	121013	CCDS929.1	<i>Gja5</i>	Cx40	95716	CCDS17652.1
5	–	–	–	–	<i>Gja6</i>	Cx33	95717	CCDS30506.1
6	<i>GJA8</i>	CX50	600897	CCDS30834.1	<i>Gja8</i>	Cx50	99953	CCDS17651.1
7	<i>GJA9</i>	CX59	611923	CCDS432.1	–	–	–	–
8	<i>GJA10</i>	CX62	611924	CCDS5025.1	<i>Gja10</i>	Cx57	1339969	CCDS18015.1
9	<i>GJB1</i>	CX32	304040	CCDS14408.1	<i>Gjb1</i>	Cx32	95719	CCDS30314.1
10	<i>GJB2</i>	CX26	121011	CCDS9290.1	<i>Gjb2</i>	Cx26	95720	CCDS27153.1
11	<i>GJB3</i>	CX31	603324	CCDS384.1	<i>Gjb3</i>	Cx31	95721	CCDS18668.1
12	<i>GJB4</i>	CX30.3	605425	CCDS383.1	<i>Gjb4</i>	Cx30.3	95722	CCDS18669.1
13	<i>GJB5</i>	CX31.1	604493	CCDS382.1	<i>Gjb5</i>	Cx31.1	95723	CCDS18670.1
14	<i>GJB6</i>	CX30	604418	CCDS9291.1	<i>Gjb6</i>	Cx30	107588	CCDS27154.1
15	<i>GJB7</i>	CX25	611921	CCDS5008.1	–	–	–	–
16	<i>GJC1</i>	CX45	608655	CCDS11487.1	<i>Gjc1</i>	Cx45	95718	CCDS25504.1
17	<i>GJC2</i>	CX47	608803	CCDS1569.1	<i>Gjc2</i>	Cx47	2153060	CCDS24760.1
18	<i>GJC3</i>	CX29/30.2/31.3	611925	CCDS34697.1	<i>Gjc3</i>	Cx29	2153041	CCDS19782.1
19	<i>GJD2</i>	CX36	607058	CCDS10040.1	<i>Gjd2</i>	Cx36	1334209	CCDS16563.1
20	<i>GJD3</i>	CX31.9	607425	CCDS58547.1	<i>Gjd3</i>	Cx30.2	2384150	CCDS25369.1
21	<i>GJD4</i>	CX40.1	611922	CCDS7191.1	<i>Gjd4</i>	Cx39	2444990	CCDS29049.1
22	–	–	–	–	<i>Gje1</i>	Cx23	1923993	CCDS48505.1

Note: 40 unique genes (20 human + 20 mouse) in total. Click on the above CCDS links to access nucleotide and amino acid sequences.

[26]. Thimm et al. reported a similar effect for Cx43 HCs [27]. Currently, a vast body of data supports the notion that HCs remain predominantly closed at negative membrane potential and/or in the

<sup>1</sup> <http://www.hmdb.ca/structures/search/metabolites/mass>.

when reaggregated, form functional junctions over a time course of minutes [74]) to test (the first) affinity-purified rabbit polyclonal antibodies (EL-46, EL-186, Cx32-164-189) against a connexin 43 (Cx43) EL1 peptide (a.a. 46-76, GDEQSAFRNCNTQQPGCENVCYDKSPISHVR), a Cx43 EL2 peptide (a.a. 186-206, TCKRDPCPHQVDCFLSRPTEK) and a Cx32 EL2 peptide (a.a. 164-189, RLVKCDVYPCNTVDCFVSRPTEKTV), respectively [75]. Cells were dissociated in EDTA, allowed to recover and reaggregate for 60 min in media containing Fab fragments of the above mentioned antibodies, resulting in no cell-cell dye transfer 4 min after microinjection in 90% of the treated cell pairs (in control experiments, cell-cell dye transfer was detected within 30 s). In addition, no gap junctions were detected by freeze-fracture electron microscopy (EM) and no adherens junctions by thin section EM between cells treated with the Fab fragments that blocked cell-cell dye transfer [75].

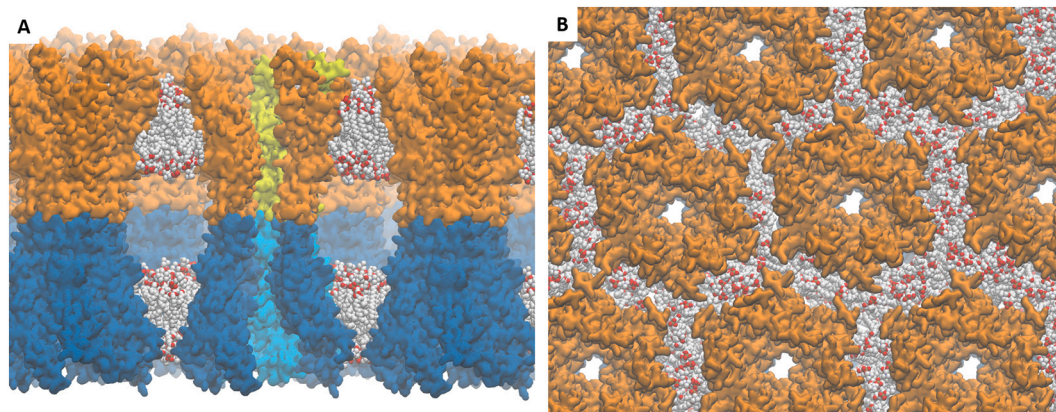
Following suit, Hofer and Dermietzel immunized rabbits against a Cx43 EL1 peptide (aa 42–54: ESAWGDEQSAFRC) and a EL2 peptide (aa: 186–206, TCKRDPCPHQVDCFLSRPTEK), aiming to generate antibodies for labelling and blocking HCs in vital cells [76]. Of the two retrieved polyclonal antibodies, respectively named aEL1-42 and aEL2-186, only the latter (which was not specific as it also recognized bands at positions 30 kDa and 32 kDa in western blots of brain homogenates) was retained for further analyses. Whole antibody as well as Fab<sub>2</sub> fragments of aEL2-186, applied to unpermeabilized cultured astrocytes, produced staining patterns that were most prominent in subconfluent cultures at membrane processes and filopodia, suggesting that HCs accumulate at the site of growing tips of noncontiguous cells. Incubation with either Fab<sub>2</sub> or complete EL2-186 antibodies led to a significant reduction of LY-labelled RGCN cells (an immortalized astrocytic cell line) in in 0 mM [Ca<sup>2+</sup>]<sub>ex</sub>, leading to the conclusion that (a) dye uptake operates through HCs in the plasma membrane and (b) HCs are accessible for blocking by external loop antibodies. In addition, LY as well as Ca<sup>2+</sup> transfer between adjacent cultured astrocytes were significantly reduced after exposure to aEL2-186 for a time interval exceeding by a factor of four the Cx43 half-life measured by pulse-chase assays [76].

Together, the experiments performed by Hofer and Dermietzel and Meyer et al., shaped the notion that, by interfering with the docking of unopposed connexons in adjacent cells, antibodies targeting the extracellular domain of connexins may act as cell adhesion inhibitors, eventually impeding the formation of new gap junction channels [77]. Then, Siller-Jackson et al. immunized rabbits against a Cx43 EL2 peptide (a.a. 185–206, YTCKRDPCPHQVDCFLSRPTEK) to generate yet another anti-Cx43 polyclonal antibody, referred to either as Cx43(E2) or Cx43-E2 [78]. HCs opening and associated release of prostaglandin E2 from MLO-Y4 cells (an osteocyte-like cell line) in response to fluid shear stress were significantly inhibited by this antibody, which recognized the

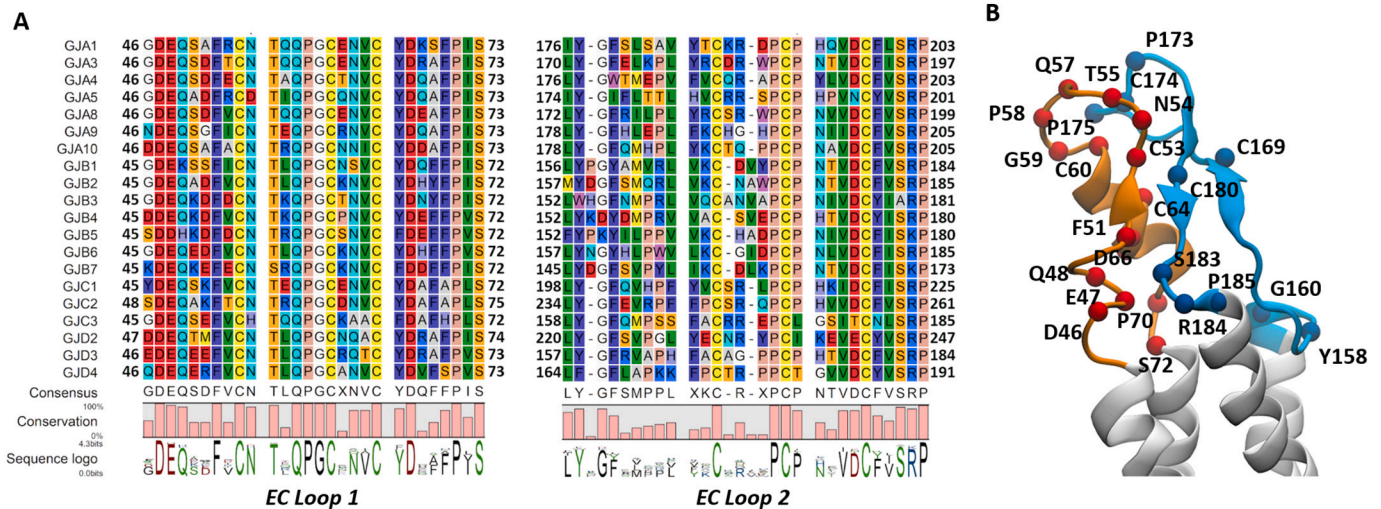
extracellular domain of Cx43, did not block pannexin or P2X<sub>7</sub> channels and also failed to detect other connexins including Cx32, Cx26, Cx50, and Cx46 (note, however, that data to support this latter claim were not shown). Scrape-loading LY transfer assays showed gap junction function was not affected by incubating MLO-Y4 cells with increasing concentrations (28, 56, and 100 µg/ml) of Cx43(E2) antibody for 4 h [78]. Subsequently, Kar et al. used the Cx43(E2) antibody to study H<sub>2</sub>O<sub>2</sub>-induced cell death of primary osteocytes and MLO-Y4 cells. There was a dose-dependent increase of HCs function by H<sub>2</sub>O<sub>2</sub>, and the antibody completely blocked dye uptake induced by H<sub>2</sub>O<sub>2</sub>, exacerbating further H<sub>2</sub>O<sub>2</sub>-induced cell death [79]. Zhou et al. showed that the opening of osteocyte Cx43 hemichannels mediated by mechanical stimulation caused the release of ATP, which exerted an inhibitory effect on breast cancer cells. This inhibition was attenuated by the Cx43(E2) antibody, suggesting that Cx43 hemichannels in osteoblast bone cells may provide an intrinsic self-defence mechanism against breast cancer metastasis [80]. Finally, Xu H. et al. studied mechanical sensing by osteocyte Cx43 by simulating unloading caused by weightlessness with a random position machine (RPM). Dye uptake assay showed that HCs opening increased after RPM for 2 h, leading to increased prostaglandin E2 release. This increase was blocked by the Cx43(E2) antibody [81]. Together, these studies highlighted the usefulness of the Cx43(E2) polyclonal antibody as a research tool in diverse contexts.

Based on the work of Meyer et al. [75] and Hofer and Dermietzel [76], Clair et al. selected an eleven amino-acid peptide in human connexin 26 (Cx26) EL1 (a.a. 42–53:VWGDEQADFVC) and another fourteen amino-acid peptide in EL2 (a.a. 172–184:AWPCNTVDCFVSR) to immunize rabbits [82]. In HeLa cells expressing Cx26, connexin 32 (Cx32) or Cx43, staining with Connexin Extracellular Loop 1 Antibody (CELAb1, an antibody raised against the EL1 peptide), labelled surface structures putatively identified as HCs. A similar labelling was also obtained with Connexin Extracellular Loop 2 Antibody (CELAb2, an antibody raised against the EL2 peptide; note, however, that also in this case data to support this second claim were not shown). The lack of specificity exhibited by both polyclonal antibodies was explained based on a. a. sequence alignment for the ELs of Cx26, Cx32 and Cx43 (see also Supplementary Table S1) [82]. Anselmi et al. and Majumder et al. used CELAbs to label ATP-releasing Cx26 and Cx30 HCs on the endolymphatic surface of non-sensory cells in the mouse organ of Corti [54,83].

When tested functionally in low [Ca<sup>2+</sup>]<sub>ex</sub>, both CELAbs inhibited equally well LY uptake in HeLa cells expressing Cx26 or Cx32, whereas inhibition was less efficient in cells expressing Cx43, suggesting unequal relationship between binding and blocking for these different connexin isoforms. No inhibition of dye transfer could be observed when monolayers of Cx26 expressing cells were microinjected with LY in the



**Fig. 1.** Structure of gap junction channels. Gap junction channels arrange in a hexagonal pattern to form a junctional plaque. A) Side view. Two connexins have been removed from one of the channels depicted in panel A. The connexins in the internal part of the pore has been coloured with brighter colours to promote visual perception of depth. B) View from the cytoplasm of one of the two cells. (Adapted from ref. [1].)



**Fig. 2.** Conservation of extracellular loops in the connexin family. A) Sequence alignment (top) and sequence logo (bottom) of the first and second extracellular loop of the human connexin family. Amino acids are coloured according to the "Rasmol colour scheme". B) Position of the alpha carbons of the residues that are conserved in at least 90% of the family (numeration is based on position in the Cx26 sequence). The two sequences are largely conserved in the family, especially the three cysteine pairs that create disulfide bridges between the two loops, and the prolines responsible for the sharp apical turns.

presence of CELAbs, indicating that the antibodies preferentially inhibited HCs but had little effects on connexin-mediated intercellular communication [82]. CELAbs demonstrated also the importance of HC signalling since they inhibited dye uptake at low  $[Ca^{2+}]_{ex}$ , and at physiological  $[Ca^{2+}]_{ex}$  during invasion of intestinal epithelial cells by *Shigella* [82], the causative agent of bacillary dysentery [84,85].

Summarizing, the experiments performed with Cx43(E2) and CELAbs showed targeting the extracellular domain of connexins with these polyclonal antibodies could label/inhibit HCs without interfering with IGJC function.

#### 4. Addressing unmet medical needs with anti-HCs monoclonal antibodies (mAbs)

Although the body's natural reaction to antigens is inherently polyclonal, mAbs can bind an antigen with a high degree of affinity and specificity and, due to this remarkable property, "have made a striking transformation from scientific tools to powerful human therapeutics" [86]. Selection of mAbs from combinatorial phage-display libraries [87–91] is a key technology that has earned the 2018 Nobel Prize for Chemistry.<sup>2</sup> As unique protein product of well-defined DNA sequences, mAbs can be generated in numerous ways by highly reproducible, scalable processes [92] that can meet the good manufacturing practice (GMP) standards required for pharmaceutical products [93]. The spectrum of potentiality of antibodies has been further expanded by the discovery of naturally occurring heavy chain antibodies (hcAbs, two polypeptide chains, 75 kDa) containing a highly stable and soluble single antigen-binding V-domain designated VHH or nanobody (15 kDa). Compared with full length antibodies, nanobodies have a smaller size, higher solubility, higher stability, and increased capability of tissue penetration *in vivo* [94]. Together, these properties are leading mAbs to become the largest and fastest growing class of therapeutic proteins [95,96]. A recent review by Caufriez et al. lists no fewer than 18 diseases linked to the opening of (connexin) HCs [97] (Table 2). In the following, we dwell in particular with two disease classes for which anti-HC mAbs with excellent therapeutic potential are available.

**Table 2**

Human diseases associated with the opening of wild type connexin HC.

Disease	Connexin	Reference
Inflammation	Cx43	[152–154]
Atherosclerosis	Cx37/Cx43	[155,156]
Diabetes	Cx43	[157,158]
Ischemia/stroke	Cx43	[159,160]
Myocardial ischemia/reperfusion	Cx43	[161]
Cardiomyopathy	Cx43/Cx45	[162]
Chronic kidney disease	Cx43	[163]
Microbial gut infection	Cx43	[164]
HIV infection of astrocytes	Cx43	[165]
Acute liver failure	Cx32/Cx43	[166]
Liver fibrosis and cirrhosis	Cx43	[167]
Nonalcoholic steatohepatitis	Cx32/Cx43	[168]
Alzheimer's disease	Cx43	[169]
Epilepsy	Cx36/Cx43	[170,171]

##### 4.1. Cancer

Cx43 is a major gap junction protein in astrocytes, and its expression is enhanced significantly in glioma-associated astrocytes, especially at the peri-tumoral region, where it is thought to promote glioma invasion [98–101]. It mediates also gap-junctional coupling between collectively invading breast cancer cells and, via HCs, adenosine nucleotide/nucleoside release into the extracellular space [102]. Thus, autocrine purinergic signalling through Cx43 HC is a critical pathway in leader cell function and collective invasion [102].

Aiming to fight cancer, Baklaushev et al. generated a mAb targeting an epitope in Cx43 EL2 (aa 173–208, QWYIYGFSLSA-VYTCRDRPCPHQVDCFLSRPTEKTI) and used it to label extracellular structures on GFAP-positive reactive astrocytes in brain sections of a rat with experimental C6 glioma [103]. The antibody was subsequently referred to as MAbE2Cx43, and shown to accumulate *in vivo* at the peritumoural invasion zone after conjugation either with radio-isotope or a fluorescent dye [104]. Deceleration of glioma growth in rats receiving intravenous administration of MAbE2Cx43 was accompanied by a significant prolongation of lifespan, and complete recovery without delayed relapses in ~20% of the animals, suggesting that "the mechanism of tumour-suppressing effects of antibodies can be related to inhibition of specific functions of Cx43 in glioma cells in the peritumoural zone" [105]. Forty-eight hours after *in vivo* delivery of PEGylated

<sup>2</sup> <https://www.nobelprize.org/prizes/chemistry/2018/winter/facts/>.

immunoliposomes based on MabE2Cx43 antibodies, fluorescent-labeled liposomal nanocontainers were detected at the periphery of the glioma [106]. Treatment with MabE2Cx43 in combination with radiotherapy was associated with mutual boosting of the therapeutic efficiencies, leading to a significant inhibition of tumour development and prolongation of rat survival median to 60 days, “presumably due to the increase in blood-brain barrier permeability for antibodies after irradiation of the brain and to additional inhibitory effect of antibodies towards radioresistant migrating glioma cells” [107]. Intravenous administration of cisplatin-loaded nanogels conjugated with MabE2Cx43 in the experimental model of glioma 101/8, ensued in a 27 day median extension the rat lifespan [108]. Together, these studies (a) convincingly demonstrated that antibodies against the extracellular domain of Cx43 can be used to directly limit glioma growth, as well as for targeted transport of diagnostic and therapeutic drugs; (b) support to the notion that cancer phenotype can be altered as a direct consequence of experimental targeting of connexins (reviewed in refs. [101, 109]).

In this vein, two human-mouse chimeric mAbs, named HMAB1 and HMAB2, that bind to Cx43 HCs, were generated by Jiang et al. according to Patent Application Publication No. US 2019/0359696 A1 (pub. Date: Nov. 28, 2019) [110]. Complementarity determining regions (CDRs) of these mAbs are provided in Tables 1 and 2 on page 14 of the patent document. HMAB1, a Cx43 HC inhibitor, falls in line with the other

antibodies described above. In contrast, HM2Ab2 was found to *activate* Cx43 HCs. In addition, HM2Ab2 inhibited osteolytic tumour growth, at odds with studies of Cx43 role in glioma (as discussed above), but in accord with the finding that osteolytic tumour growth was augmented in osteocyte-specific Cx43 knockout mice (as reported in the patent). Of note, the patent claims HMAB1 and HMAB2 can be used for treating or preventing not only breast cancer, prostate cancer, osteosarcoma or a metastasis, but also osteoporosis, osteopenia, or, osteoarthritis, multiple sclerosis, Alzheimer’s disease or Parkinson’s disease, spinal cord injury (SCI), traumatic brain injury (TBI), or stroke [110].

Involvement of Cx43 in these disabling human pathologies has been reviewed extensively [72,101,109,111–116]. Future will tell whether HMAB1 and HMAB2 are indeed endowed with such “magic bullet” properties. Clearly, therapeutic efficacy in just one of these unmet medical needs would be a major step forward for mankind.

As a closing remark for this section, we note that recent results have widened significantly the therapeutic potential of gap junctions. In particular, Gadok et al. engineered cell-derived drug-loaded lipid vesicles termed “connectosomes”, containing functional HCs and a displaying a single-domain camelid antibody on the surface. Newly formed IGJC between vesicle and target cells expressing a surface antigen recognized by the antibody, promoted the selective delivery of the chemotherapeutic doxorubicin to a target cell population. Based on the

**Table 3**  
Human connexin variants potentially targeted by inhibitory anti-HCs antibodies, and corresponding mouse models of disease.

Affected organ(s)/tissues	Type of disease/OMIM #	Gene	Cx	Mutation	Reference/mouse model (if available)						
Various organs	Oculodentodigital dysplasia (ODDD)/#164200	<i>GJA1</i>	Cx43	G138R I31M G143S G60S (Note 2)	[172]/MGI:3776013 EM:02386 (Note 1) [173]/MGI:2682016						
Heart	Atrial fibrillation, familial, 11 (ATFB11)/#614049	<i>GJA1</i>	Cx43	G38D V85I L221I	[174] [175,176]						
Skin	Palmoplantar keratoderma and congenital alopecia 1 (PPKCA1)/#104100 Erythrokeratoderma variabilis et progressiva (EKVP3)/#617525	<i>GJA1</i>	CX43	G8V A44V (Note 3) E227D (Note 3)	[177,178] [178–180] [178,180]						
						Erythrokeratoderma variabilis et progressiva 1/#133200	<i>GJB3</i>	Cx31	R42P (Note 4)	[181]	
	Ectodermal dysplasia 2, Clouston type/#129500	<i>GJB6</i>	Cx30	G11R  A88V	[135] MGI:5607781 [135] MGI:5607781 EM:07626						
Inner ear and skin	Keratitis-ichthyosis-deafness (KID) syndrome/#148210	<i>GJB2</i>	Cx26	G12R N14K D50N I30N D50Y N14Y S17F  A40V G45E D50A A88V	[182]  [183]  [184] [143,185,186]/MGI:4867482 EM:05215 [187] [188,189]/MGI:5635524 [190]						
						Lens	Cataract 14, multiple types (CTRCT14)/#601885 Cataract 1, multiple types (CRTCT1)/#116200	<i>GJA3</i>	Cx46	G143R	[191]
						<i>GJA8</i>		Cx50	G46V	[192]	
						Nervous system	Charcot-Marie-Tooth neuropathy, X-linked dominant 1 (CMTX1)/#302800	<i>GJB1</i>	Cx32	S85C D178Y F235C	[193] [32] [194]

Note 1: MGI, Mouse Genome Informatics; EM, INFRAFRONTIER/European Mouse Mutant archive.

Note 2: G60S is not listed among human variants of Cx43 (*GJA1*) in the UniProt database, <https://www.uniprot.org/uniprot/P17302>; see also ref. [195]. It is, however, a mouse model for Oculodentodigital dysplasia.

Note 3: In tissue sections of human affected skin, the E227D and A44V mutants of Cx43 (*GJA1*) do not localize to intercellular junctions and accumulate in a subcellular compartment. In addition, cells transfected with either mutant demonstrate aggregation of Cx43 in the Golgi [180].

Note 4: According to Tattersall et al. “Previous studies suggest that Cx-associated cell death may be related to abnormal ‘leaky’ HCs but we produced direct evidence against that being the major mechanism. Additionally, our immunocytochemistry showed upregulation of components of the unfolded protein response (UPR) in cells expressing the EKV-associated Cx31 mutants but not (WT)Cx31 or (66delD)Cx31” [196].

outcome of these experiments, the authors suggested that this innovative targeted targeting strategy could be used to deliver a range of membrane impermeable drugs and reagents, such as siRNA peptides, and other macromolecules, to diverse populations of target cells [117].

#### 4.2. Monogenic diseases

At least 29 dominant mutations in 8 human Cx genes (*GJA1*, *GJA3*, *GJA5*, *GJA8*, *GJB1*, *GJB2*, *GJB3*, *GJB6*) generate HCs with augmented activity that have been implicated in 11 monogenic diseases (Table 3 and Fig. 3), mostly rare, affecting facial appearance, dentition (small and carious teeth), eyes (microphthalmia, microcornea), fingers (syndactyly), inner ear (deafness), skin (various disorders), lens (cataracts), nervous system (myelination defects) and heart (atrial fibrillation). In several instances, aberrant connexin HCs cause an unbalance of  $Ca^{2+}$  signalling and/or augmented release of ATP which, acting as deregulated paracrine factor, compromises tissue homeostasis [118–121]. A limited number of mouse models, which carry some of these mutations, have been generated by different laboratories using different targeting strategies and genetic backgrounds (Table 3).

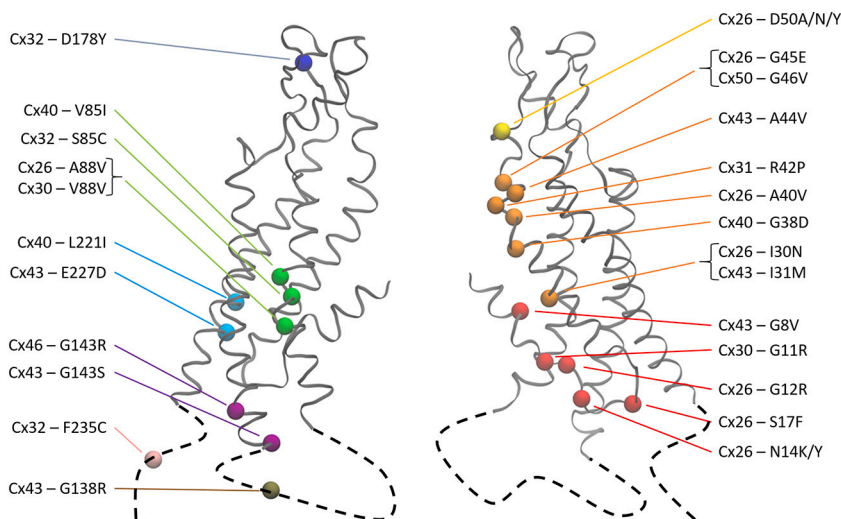
Aiming to address unmet medical need in the field of monogenic diseases, Xu L. et al. used a bait peptide, named pepEC1.1 (biotin-KEVWGDEQADFCNTL, corresponding to a.a. 41–56 of Cx26 EL1), to select human antibody fragments by “panning” a vast library expressed in phage. The selected fragments comprised a heavy chain variable domain ( $V_H$ ) and a light chain variable domain ( $V_L$ ) connected by a 7 a.a. flexible spacer to create a single-chain fragment variable (scFv) complex, whose structure was solved by X-ray crystallography (Protein Data Bank accession code 5WYM). The gene encoding the pepEC1.1-binding scFv complex, preceded by 60 nucleotides encoding a self-cleaving interleukin 2 secretion signal peptide [122], was inserted into a cloning plasmid to generate a scFv-Fc fusion protein, comprising constant hinge,  $CH_2$  and  $CH_3$  domains of human secreted IgG1 (UniProtKB – P01857). A chimeric version with constant hinge,  $CH_2$  and  $CH_3$  domains of mouse secreted IgG1 (UniProtKB – P01868) was also generated. A FreeStyle™ 293-F cell line was stably transfected with the expression vector of either scFv-Fc polypeptide. Both protein products harvested from the cell culture supernatant formed homodimers (named abEC1.1 and abEC1.1 m, respectively) with a MW of ~103 kDa through a di-body interaction between  $V_H$  and  $V_L$  [123] and disulfide bonds in the hinge region [124]. In low extracellular  $Ca^{2+}$  conditions (0.2 mM), patch clamp recordings from HeLa cells expressing Cx26 HCs yielded half maximal inhibitory concentration ( $IC_{50}$ ) of ~40 nM for abEC1.1. The inhibitory effect was incomplete, leaving a residual conductance of ~18 at saturating concentrations (<1  $\mu$ M). The dose-inhibition response

curve vs. abEC1.1 concentration was fitted with a modified Hill function  $y = \alpha [1 + (x/\gamma)^n]^{-1} + \beta$ , where  $x$  is antibody concentration (in nM). The value of the exponent ( $n = 2$ ) suggests that two abEC1.1 homodimers are required to inhibit a single HC. Experiments with intact epithelia in organotypic cochlear cultures, showed abEC1.1 did not interfere with IGJC function [125].

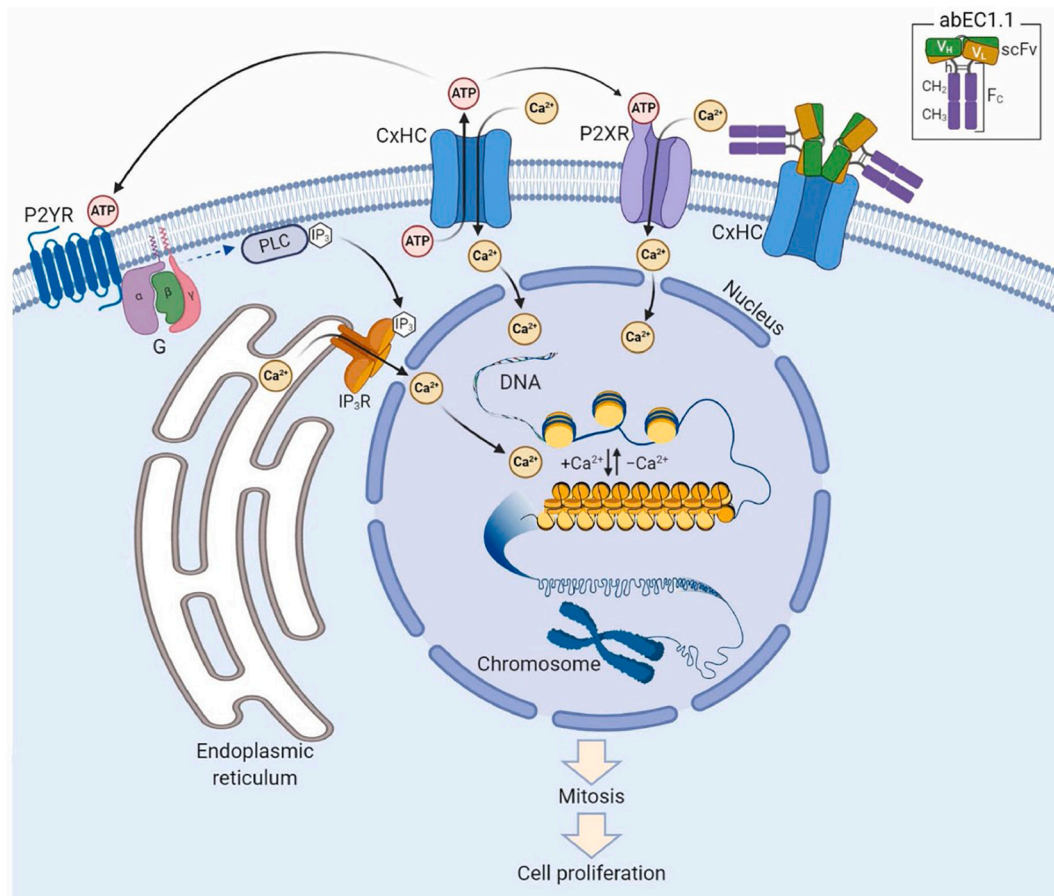
Then, Ziraldo et al. demonstrated abEC1.1 inhibited equally well Cx26, Cx30 and Cx32 HCs, when applied at <1  $\mu$ M concentration, but was ineffective on pannexin 1 channels [126]. By performing *in silico* analysis of HC-abEC1.1 interaction, critical amino acids (N54, T55, L56, Q57, P58, P175 and N176) that are conserved in the extracellular domain of Cx26, Cx30 and Cx32 were identified. Remarkably, even a single a.a. difference in the above a.a. list reduced drastically the inhibitory effects of abEC1.1 on all other tested Cx HCs (Cx30.2/31.3, Cx30.3, Cx31, Cx31.1, Cx37, Cx43 and Cx45) [126], most of which are expressed in the skin and its appendages [127–132].

Finally, Kuang et al. used abEC1.1 and abEC1.1m to treat adult mutant Cx30<sup>A88V/A88V</sup> mice, the only available animal model of Clouston syndrome (OMIM #129500), a rare orphan disease caused by either Cx30 p.G11R or Cx30 p.A88V leaky HCs (Table 3). Two weeks of antibody treatment sufficed to repress cell hyperproliferation in mouse skin and reduce hypertrophic sebaceous glands (SGs) to wild type (wt) levels. These effects were obtained whether mutant mice were treated topically, by application of an abEC1.1 cream formulation, or systemically, by intraperitoneal injection of abEC1.1m. *In vitro* experiments with mouse primary keratinocytes and HaCaT cells revealed abEC1.1 blocked  $Ca^{2+}$  influx and diminished ATP release through leaky Cx30 p.A88V HCs, suggesting these scFv-Fc mAbs gained control over leaky HCs and contributed to restoring epidermal homeostasis [133]; see also Fig. 4.

The phenotype of Cx30<sup>A88V/A88V</sup> mutant mice does not overlap perfectly with that of human carriers of the Cx30 p.A88V mutation [134–138]. Nonetheless, these mice exhibit hyperproliferation in the skin and its appendages [139], a feature shared by most Cx-related genodermatoses [119,132,140,141]. Also Cx26<sup>G45E/+</sup> mice, a model of keratitis-ichthyosis-deafness (KID) syndrome (OMIM #148210), have hyperkeratosis, scaling, skin folds, hyperplasia, acanthosis, papillomatosis and increased cell size, as well as increased HC currents in transgenic keratinocytes [142]. In addition, Cx26<sup>S17F/+</sup> mice, another model for KID syndrome [143], show altered epidermal lipid processing and  $Ca^{2+}$  distribution. Xu L. et al. proved abEC1.1 was effective *in vitro* against Cx26 p.G45E and p.D50N KID-related hyperactive HCs [125]. In addition, at least three Cx32 mutations (S85C, D178Y, F235C) causing Charcot-Marie-Tooth disease, X-linked dominant 1 (CMT1X, OMIM #302800), a demyelinating peripheral neuropathy, produce HCs with augmented activity (Table 3) and, possibly, deregulated ATP release



**Fig. 3.** Point mutations associated with hyperactive connexin HCs. Two opposing connexin of a hemichannel are shown. Mutations leading to natural variants associated with hyperactive hemichannels are represented by spheres in either of the two represented connexin proteins. The position of a given residue in the protein was determined using Cx26 as a template, and taking into account the sequence alignment of Fig. 2 and Supplementary Table S1. The cytoplasmic loop and the C-terminus are represented as dotted lines, due to the large differences in length and sequence of these two regions across the connexin family. Amino acids are coloured according their topology in the protein (NT: red, TM1: orange, EC1: yellow, TM2: green, ICL: tan, TM3: purple, EC2: blue, TM4: cyan, CT: pink).



**Fig. 4.** Schematic representation of putative antibody mechanism of action. The abEC1.1 antibody (inset) comprises a Cx-binding scFv complex (VH-linker-VL) fused to a fragment constant (Fc) composed of hinge (h), CH2 and CH3 domains of secreted IgG1. Two scFv-Fc homodimers bind the outer vestibule of the connexin hemichannel (CxHC) blocking ATP release and Ca<sup>2+</sup> influx. The ATP released by open/unblocked CxHCs diffuses in the extracellular milieu and activates G-protein coupled P2Y receptors (P2YR), triggering a canonical signal transduction cascade that leads to Ca<sup>2+</sup> release from the endoplasmic reticulum via phospholipase C (PLC), PIP<sub>2</sub> (not shown) and IP<sub>3</sub>. At the same time, Ca<sup>2+</sup> flows into the cell through ATP-gated P2X ionotropic receptors (P2XR) and open/unblocked CxHCs. Diffusion of Ca<sup>2+</sup> in the nucleus promotes transition of chromatin structure from fibrous to globular, chromosome condensation, mitosis and cell proliferation. (Adapted from ref. [133].)

[144]. The results summarized above suggest abEC1.1 might be effective in both contexts.

## 5. Concluding remarks

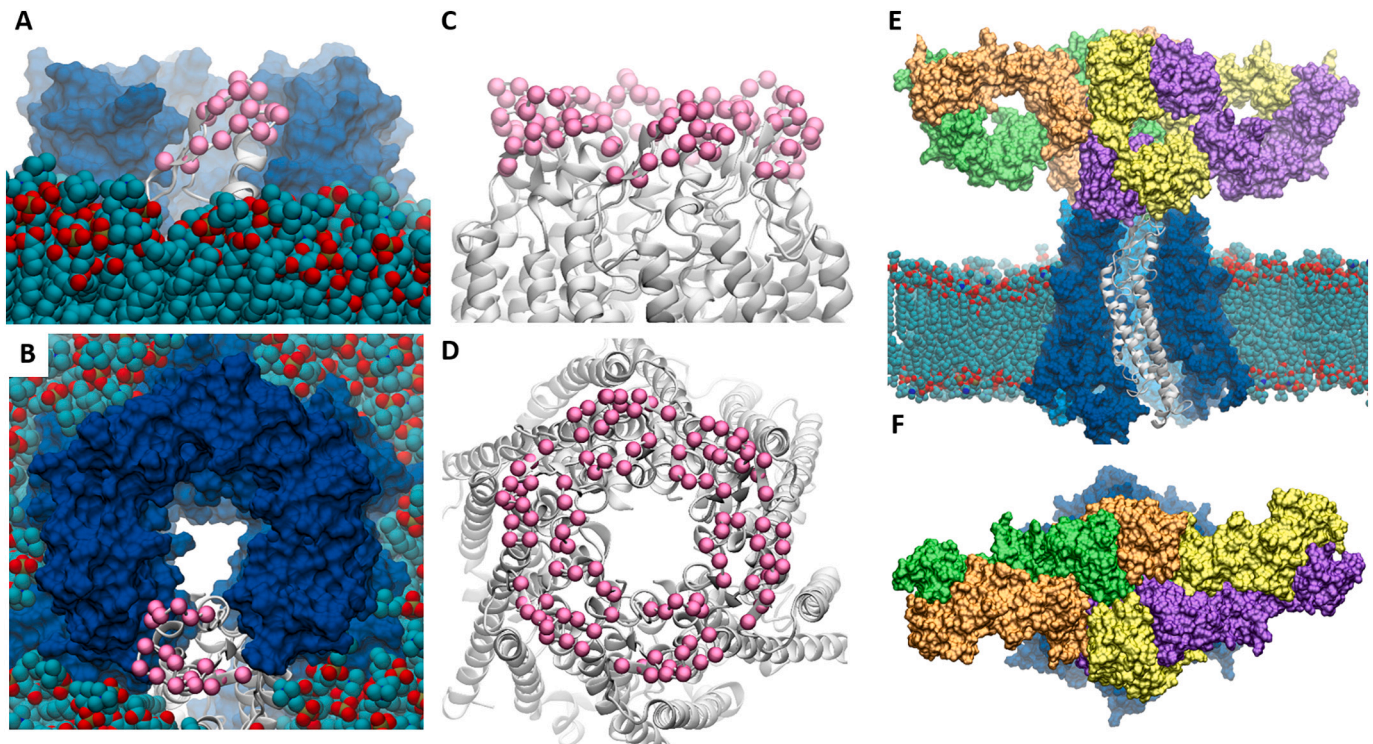
All known antibodies that modulate connexin HCs target the two small extracellular loops of the connexin proteins. Recent advances are paving the way to engineering stable antibodies against intracellular epitopes [145], however such antibodies would unlikely be able to discriminate between fully formed junctional channels and undocked HCs, and thus may produce unwanted side effects. The extracellular region of different connexins is highly conserved, especially the first extracellular loop (Fig. 2A and Supplementary Table S1). This, combined with the fact that only few residues of each connexins are exposed (Fig. 5), suggests that it will be difficult to generate an antibody that specifically recognizes (and selectively modulates the function of) a given homomeric HC. Based on the literature reviewed in this article, designing/finding an antibody for each Cx subfamily appears a more feasible task.

The molecular mechanism of action of the antibodies and the HCs is unknown in most of the cases. A model based on molecular dynamics simulation suggests abEC1.1 sterically obstructs the HC preventing the passage of most ions and possibly all other molecules [125,126]. The same model also shows that a VH-VL pair is large enough to recognize the extracellular region of three connexins at the same time, implying

that each HC can interact with two different VH-VL pair. It is reasonable to think that other antibody HC blockers share the same molecular mechanism and stoichiometry of abEC1.1, however the existence of activating antibodies such as HM2Ab2 suggests different binding modes are also possible.

Most of the antibody-development efforts, so far, have targeted Cx43 (Table 4), and the only antibody explicitly developed for other HCs is abEC1.1. Other connexin HCs have been, so far, neglected. For example, Cx46 is also important as a glioblastoma cancer stem cell regulator that is required for stem cell maintenance [146]. Furthermore, Cx46 is contained in extracellular vesicles (EVs) released from breast cancer cells overexpressing Cx46 (EVs-Cx46) that facilitate the interaction between EVs and the recipient cell, resulting in an increase in their migration and invasion properties [147]. Combinatorial antibody libraries with their huge amount of affinity binders [148] coupled with phage display technology [87] are rich sources of modulators for cell surface receptors or membrane channels. The search for new antibodies may develop and unprecedented potential for therapeutic applications, as it may benefit tremendously from novel whole-cell screening platforms that permit *in situ* selection of antibodies against membrane proteins in native state [149,150]. The demonstrated efficacy of mAbs in reaching and modulating HCs *in vivo*, together with their relative specificity for connexins overlapping EL epitopes, should hopefully stimulate an interest for widening the scope of anti-HC mAbs.

Summarizing, there is no shortage of currently incurable diseases



**Fig. 5.** Plausible epitopes for antibody recognition. Side view (A) and top view (B) of a HC embedded in the plasma membrane. A single connexin protein is shown in cartoon representation; the rest of the HC (blue) and the plasma membrane (carbon atoms in cyan, oxygen atoms in red, phosphate atoms in blue) are represented in terms of volume occupancy. Segments of the extracellular loops that are accessible and can be recognized by an antibody are shown as pink spheres corresponding to the position of their alpha carbon in (A) and (B). They comprise two short fragments in EC1 (corresponding to residues 54–59 in Cx26) and the second half of EC2 (corresponding to residues 166–178 in Cx26). Every other part of the protein is masked either by the plasma membrane or by adjacent connexins. The segments of the EC loops facing the pore are as likely to be inaccessible to an antibody, due to steric hindrance. C) and D) show how the putative accessible residues are arranged in the whole HC (side and top view respectively). Despite being two short fragments in a single connexin, they produce a large surface once the proteins are arranged in the hexamer. E) shows the putative mode of interaction of two abEC1.1 antibodies, each one of which is a scFv-Fc homodimer, with the extracellular domain of the HC. In particular, yellow and purple molecules are coupled in one diabody, while green and orange in the second one. Connexon and membrane are shown with the same colour code of panel A and B. Panel (F) is a top view of the antibody-hemichannel complex showing the complete obstruction of the channel pore (the membrane is not shown for clarity).

**Table 4**  
Anti-HCs antibodies mentioned in this article.

Name	Type	Target	Organism	Ref.	Major properties
EL-46, EL-186, Cx32-164-189	Polyclonal	Cx43 and/or Cx32	Rabbit	[75]	Blocked cell-cell dye transfer and prevent formation of gap-junction channels
aEL2-186	Polyclonal	Cx43	Rabbit	[76]	Significantly reduced $\text{Ca}^{2+}$ and LY transfer through IGJCs
Cx43-E2	Polyclonal	Cx43	Rabbit	[78–81]	Significantly inhibited HC opening
CELAB1, CELAB2	Polyclonal	Cx26, Cx32, Cx43	Rabbit	[54,82,83]	Efficiently inhibited LY uptake of Cx26 and Cx32 HCs, less efficient for Cx43 HCs
MAbE2Cx43	Monoclonal	Cx43	Mouse	[103–108]	Effective <i>in vivo</i> in animal models of glioma.
HMAb1	Monoclonal	Cx43	Humanized	[110]	HC inhibitor
HMAb2	Monoclonal	Cx43	Humanized	[110]	HC activator
abEC1.1	Monoclonal	Cx26, Cx30, Cx32	Human	[125,126,133]	Efficiently inhibited Cx26, Cx30 and Cx32 HCs. Did not interfere with fully formed gap junction channels in organotypic cochlear cultures. Effective <i>in vivo</i> in Cx30A88V mutant mice.

linked to the opening of HCs [97] (Table 2). For all of them, therapeutic intervention may in principle benefit from anti-HC mAbs capable of modulating HC function selectively and specifically. Monoclonal antibodies are particularly promising since they have the potential to overcome the lack of specificity of small molecule alternatives and have superior *in vivo* stability compared with connexin mimetic peptides, some of which also are effective modulators of HC function [151].

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## CRedit authorship contribution statement

Conceptualization, F.M., F.Z.; Funding acquisition, F.M., F.Z.; Project administration, F.M., F.Z.; Resources, F.Z.; Investigation, D.B., V.D.; Visualization, D.B., V.D.; Writing – original draft, F.M.; Writing – review & editing, D.B., V.D., F.Z., F.M.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Drs. F. Zonta and F. Mammano report a patent: “Fully human antibody specifically inhibiting connexin 26”, Inventors: Qu Z, Yang G, Mammano F, Zonta F, International application number: PCT/CN2016/109847, pending to ShanghaiTech University; and a patent: “Composition and Methods to treat Ectodermal Dysplasia 2, Clouston Type”, Inventors: Mammano F, Yang G, Zonta F, International Application No.: PCT/CN2019/088689, International Filing Date: 2019-05-28, pending to ShanghaiTech University. All other Authors have nothing to declare.

## References

- [1] F. Mammano, Inner ear connexin channels: roles in development and maintenance of cochlear function, *Cold Spring Harbor Perspectives in Medicine* 9 (2019).
- [2] L.S. Musil, D.A. Goodenough, Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques, *J. Cell Biol.* 115 (1991) 1357–1374.
- [3] G. Gaietta, T.J. Deerinck, S.R. Adams, J. Bouwer, O. Tour, D.W. Laird, G. E. Sosinsky, R.Y. Tsien, M.H. Ellisman, Multicolor and electron microscopic imaging of connexin trafficking, *Science* 296 (2002) 503–507.
- [4] T. Thomas, K. Jordan, J. Simek, Q. Shao, C. Jedezko, P. Walton, D.W. Laird, Mechanisms of Cx43 and Cx26 transport to the plasma membrane and gap junction regeneration, *J. Cell Sci.* 118 (2005) 4451–4462.
- [5] A.F. Thévenin, T.J. Kowal, J.T. Fong, R.M. Kells, C.G. Fisher, M.M. Falk, Proteins and mechanisms regulating gap-junction assembly, internalization, and degradation, *Physiology* 28 (2013) 93–116.
- [6] T. Aasen, S. Johnstone, L. Vidal-Brime, K.F.S. Lynn, M. Koval, Connexins: synthesis, post-translational modifications, and trafficking in health and disease, *International Journal of Molecular Sciences* 19 (2018).
- [7] G.A. Perkins, D.A. Goodenough, G.E. Sosinsky, Formation of the gap junction intercellular channel requires a 30 degree rotation for interdigitating two apposing connexons, *J. Mol. Biol.* 277 (1998) 171–177.
- [8] V.M. Unger, N.M. Kumar, N.B. Gilula, M. Yeager, Three-dimensional structure of a recombinant gap junction membrane channel, *Science* 283 (1999) 1176–1180.
- [9] S. Maeda, S. Nakagawa, M. Suga, E. Yamashita, A. Oshima, Y. Fujiyoshi, T. Tsukihara, Structure of the connexin 26 gap junction channel at 3.5 Å resolution, *Nature* 458 (2009) 597–602.
- [10] J.B. Myers, B.G. Haddad, S.E. O'Neill, D.S. Chorev, C.C. Yoshioka, C.V. Robinson, D.M. Zuckerman, S.L. Reichow, Structure of native lens connexin 46/50 intercellular channels by cryo-EM, *Nature* 564 (2018) 372–377.
- [11] A.K. Khan, M. Jagielnicki, W.E. McIntire, M.D. Purdy, V. Dharmarajan, P. R. Griffin, M. Yeager, A steric “ball-and-chain” mechanism for pH-mediated regulation of gap junction channels, *Cell Rep.* 31 (2020) 107482.
- [12] J.A. Flores, B.G. Haddad, K.A. Dolan, J.B. Myers, C.C. Yoshioka, J. Copperman, D. M. Zuckerman, S.L. Reichow, Connexin-46/50 in a dynamic lipid environment resolved by CryoEM at 1.9 Å, *Nature Communications* 11 (2020) 4331.
- [13] N.M. Kumar, N.B. Gilula, The gap junction communication channel, *Cell* 84 (1996) 381–388.
- [14] J.R. Abney, J. Braun, J.C. Owicki, Lateral interactions among membrane proteins. Implications for the organization of gap junctions, *Biophys. J.* 52 (1987) 441–454.
- [15] J. Braun, J.R. Abney, J.C. Owicki, How a gap junction maintains its structure, *Nature* 310 (1984) 316–318.
- [16] M.V. Bennett, J.E. Contreras, F.F. Bukauskas, J.C. Saez, New roles for astrocytes: gap junction hemichannels have something to communicate, *Trends Neurosci.* 26 (2003) 610–617.
- [17] J.C. Saez, M.A. Retamal, D. Basilio, F.F. Bukauskas, M.V. Bennett, Connexin-based gap junction hemichannels: gating mechanisms, *Biochim. Biophys. Acta* 1711 (2005) 215–224.
- [18] W.H. Evans, E. De Vuyst, L. Leybaert, The gap junction cellular internet: connexin hemichannels enter the signalling limelight, *The Biochemical Journal* 397 (2006) 1–14.
- [19] J.C. Saez, K.A. Schalper, M.A. Retamal, J.A. Orellana, K.F. Shoji, M.V. Bennett, Cell membrane permeabilization via connexin hemichannels in living and dying cells, *Exp. Cell Res.* 316 (2010) 2377–2389.
- [20] J.C. Saez, L. Leybaert, Hunting for connexin hemichannels, *FEBS Lett.* 588 (2014) 1205–1211.
- [21] S.H. DeVries, E.A. Schwartz, Hemi-gap-junction channels in solitary horizontal cells of the catfish retina, *J. Physiol.* 445 (1992) 201–230.
- [22] D.L. Paul, L. Ebihara, L.J. Takemoto, K.I. Swenson, D.A. Goodenough, Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of *Xenopus* oocytes, *J. Cell Biol.* 115 (1991) 1077–1089.
- [23] E.B. Trexler, M.V. Bennett, T.A. Bargiello, V.K. Verselis, Voltage gating and permeation in a gap junction hemichannel, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 5836–5841.
- [24] H. Li, T.F. Liu, A. Lazrak, C. Peracchia, G.S. Goldberg, P.D. Lampe, R.G. Johnson, Properties and regulation of gap junctional hemichannels in the plasma membranes of cultured cells, *J. Cell Biol.* 134 (1996) 1019–1030.
- [25] R.G. Johnson, H.C. Le, K. Evenson, S.W. Loberg, T.M. Myslajek, A. Prabhu, A. M. Manley, C. O’Shea, H. Grunenwald, M. Haddican, P.M. Fitzgerald, T. Robinson, B.A. Cisterna, J.C. Saez, T.F. Liu, D.W. Laird, J.D. Sheridan, Connexin hemichannels: methods for dye uptake and leakage, *J. Membr. Biol.* 249 (2016) 713–741.
- [26] D.J. Muller, G.M. Hand, A. Engel, G.E. Sosinsky, Conformational changes in surface structures of isolated connexin 26 gap junctions, *EMBO J.* 21 (2002) 3598–3607.
- [27] J. Thimm, A. Mechler, H. Lin, S. Rhee, R. Lal, Calcium-dependent open/closed conformations and interfacial energy maps of reconstituted hemichannels, *J. Biol. Chem.* 280 (2005) 10646–10654.
- [28] A. Pfahnl, G. Dahl, Gating of cx46 gap junction hemichannels by calcium and voltage, *Pflügers Archiv: European Journal of Physiology* 437 (1999) 345–353.
- [29] M. Kamerlings, I. Fahrenfort, K. Schultz, U. Janssen-Bienhold, T. Sjoerdsma, R. Weiler, Hemichannel-mediated inhibition in the outer retina, *Science* 292 (2001) 1178–1180.
- [30] B.C. Bennett, M.D. Purdy, K.A. Baker, C. Acharya, W.E. McIntire, R.C. Stevens, Q. Zhang, A.L. Harris, R. Abagyan, M. Yeager, An electrostatic mechanism for Ca<sup>2+</sup>-mediated regulation of gap junction channels, *Nat. Commun.* 7 (2016) 8770.
- [31] V.K. Verselis, M. Srinivas, Divalent cations regulate connexin hemichannels by modulating intrinsic voltage-dependent gating, *The Journal of General Physiology* 132 (2008) 315–327.
- [32] J.M. Gomez-Hernandez, M. de Miguel, B. Larrosa, D. Gonzalez, L.C. Barrio, Molecular basis of calcium regulation in connexin-32 hemichannels, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 16030–16035.
- [33] K. Pogoda, P. Kameritsch, M.A. Retamal, J.L. Vega, Regulation of gap junction channels and hemichannels by phosphorylation and redox changes: a revision, *BMC Cell Biol* 17 (Suppl. 1) (2016) 11.
- [34] P.P. Cherian, A.J. Siller-Jackson, S. Gu, X. Wang, L.F. Bonewald, E. Sprague, J. X. Jiang, Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin, *Mol. Biol. Cell* 16 (2005) 3100–3106.
- [35] M.A. Retamal, C.J. Cortes, L. Reuss, M.V. Bennett, J.C. Saez, S-nitrosylation and permeation through connexin 43 hemichannels in astrocytes: induction by oxidant stress and reversal by reducing agents, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 4475–4480.
- [36] M.A. Retamal, N. Froger, N. Palacios-Prado, P. Ezan, P.J. Saez, J.C. Saez, C. Giaume, Cx43 hemichannels and gap junction channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activated microglia, *J. Neurosci.* 27 (2007) 13781–13792.
- [37] M.A. Retamal, K.A. Schalper, K.F. Shoji, M.V. Bennett, J.C. Saez, Opening of connexin 43 hemichannels is increased by lowering intracellular redox potential, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 8322–8327.
- [38] M.A. Retamal, S. Yin, G.A. Altenberg, L. Reuss, Modulation of Cx46 hemichannels by nitric oxide, *American Journal of Physiology. Cell Physiology* 296 (2009) C1356–C1363.
- [39] M.A. Retamal, Connexin and pannexin hemichannels are regulated by redox potential, *Front. Physiol.* 5 (2014) 80.
- [40] M.A. Retamal, J.C. Saez, Hemichannels: from the molecule to the function, *Front. Physiol.* 5 (2014) 411.
- [41] M.A. Retamal, C.G. Leon-Paravic, M. Ezquer, F. Ezquer, R. Del Rio, A. Pupo, A. D. Martinez, C. Gonzalez, Carbon monoxide: a new player in the redox regulation of connexin hemichannels, *IUBMB Life* 67 (2015) 428–437.
- [42] V. Figueroa, P.J. Saez, J.D. Salas, D. Salas, O. Jara, A.D. Martinez, J.C. Saez, M. A. Retamal, Linoleic acid induces opening of connexin26 hemichannels through a PI3K/Akt/Ca<sup>2+</sup>-dependent pathway, *Biochim. Biophys. Acta* 1828 (2013) 1169–1179.
- [43] I. Fosciani, A. Temperan, L.F. Perez-Atencio, A. Escudero, P. Martinez-Montero, J. Molano, J.M. Gomez-Hernandez, C.L. Paino, D. Gonzalez-Nieto, L.C. Barrio, Regulation of connexin hemichannel activity by membrane potential and the extracellular calcium in health and disease, *Neuropharmacology* 75 (2013) 479–490.
- [44] A. Carrer, A. Leparulo, G. Crispino, C.D. Ciobotaru, O. Marin, F. Zonta, M. Bortolozzi, Cx32 hemichannel opening by cytosolic Ca<sup>2+</sup> is inhibited by the R220X mutation that causes Charcot-Marie-Tooth disease, *Hum. Mol. Genet.* 27 (2018) 80–94.
- [45] E. De Vuyst, E. Decrock, L. Cabooter, G.R. Dubyak, C.C. Naus, W.H. Evans, L. Leybaert, Intracellular calcium changes trigger connexin 32 hemichannel opening, *EMBO J.* 25 (2006) 34–44.
- [46] E. De Vuyst, N. Wang, E. Decrock, M. De Bock, M. Vinken, M. Van Moorhem, C. Lai, M. Culot, V. Rogiers, R. Cecchelli, C.C. Naus, W.H. Evans, L. Leybaert, Ca<sup>2+</sup> regulation of connexin 43 hemichannels in C6 glioma and glial cells, *Cell Calcium* 46 (2009) 176–187.

- [47] X. Zhang, T. Zou, Y. Liu, Y. Qi, The gating effect of calmodulin and calcium on the connexin50 hemichannel, *Biol. Chem.* 387 (2006) 595–601.
- [48] Z. Hu, M.A. Riquelme, B. Wang, V. Bugay, R. Brenner, S. Gu, J.X. Jiang, Cataract-associated connexin 46 mutation alters its interaction with calmodulin and function of hemichannels, *J. Biol. Chem.* 293 (2018) 2573–2585.
- [49] I. Toma, E. Bansal, E.J. Meer, J.J. Kang, S.L. Vargas, J. Peti-Peterdi, Connexin 40 and ATP-dependent intercellular calcium wave in renal glomerular endothelial cells, *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology* 294 (2008) R1769–R1776.
- [50] M.L. Cotrina, J.H. Lin, A. Alves-Rodrigues, S. Liu, J. Li, H. Azmi-Ghadimi, J. Kang, C.C. Naus, M. Nedergaard, Connexins regulate calcium signaling by controlling ATP release, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 15735–15740.
- [51] C.E. Stout, J.L. Costantin, C.C. Naus, A.C. Charles, Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels, *J. Biol. Chem.* 277 (2002) 10482–10488.
- [52] R.A. Pearson, N. Dale, E. Llaudet, P. Mobbs, ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation, *Neuron* 46 (2005) 731–744.
- [53] C.L. Ho, C.Y. Yang, W.J. Lin, C.H. Lin, Ecto-nucleoside triphosphate diphosphohydrolase 2 modulates local ATP-induced calcium signaling in human HaCaT keratinocytes, *PLoS One* 8 (2013), e57666.
- [54] F. Anselmi, V.H. Hernandez, G. Crispino, A. Seydel, S. Ortolano, S.D. Roper, N. Kessaris, W. Richardson, G. Rickheit, M.A. Filippov, H. Monyer, F. Mammano, ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca<sup>2+</sup> signals across the inner ear, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 18770–18775.
- [55] F. Mazzarda, A. D'Elia, R. Massari, A. De Ninno, F.R. Bertani, L. Businaro, G. Ziraldo, V. Zorzi, C. Nardin, C. Peres, F. Chiani, A. Tettey-Matey, M. Raspa, F. Scavizzi, A. Soluri, A.M. Salvatore, J. Yang, F. Mammano, Organ-on-chip model shows that ATP release through connexin hemichannels drives spontaneous Ca(2+) signaling in non-sensory cells of the greater epithelial ridge in the developing cochlea, *Lab Chip* 20 (2020) 3011–3023.
- [56] N. Wang, M. De Bock, E. Decrock, M. Bol, A. Gadicherla, M. Vinken, V. Rogiers, F. F. Bukauskas, G. Bultynck, L. Leybaert, Paracrine signaling through plasma membrane hemichannels, *Biochim. Biophys. Acta* 1828 (2013) 35–50.
- [57] E.B. Trexler, F.F. Bukauskas, J. Kronengold, T.A. Bargiello, V.K. Verselis, The first extracellular loop domain is a major determinant of charge selectivity in connexin46 channels, *Biophys. J.* 79 (2000) 3036–3051.
- [58] J.A. Orellana, E. Diaz, K.A. Schalper, A.A. Vargas, M.V. Bennett, J.C. Saez, Cation permeation through connexin 43 hemichannels is cooperative, competitive and saturable with parameters depending on the permeant species, *Biochem. Biophys. Res. Commun.* 409 (2011) 603–609.
- [59] B.S. Nielsen, J.S. Alstrom, B.J. Nicholson, M.S. Nielsen, N. MacAulay, Permeant-specific gating of connexin 30 hemichannels, *J. Biol. Chem.* 292 (2017) 19999–20009.
- [60] J.C. Saez, A.A. Vargas, D.E. Hernandez, F.C. Ortiz, C. Giaume, J.A. Orellana, Permeation of molecules through astroglial connexin 43 hemichannels is modulated by cytokines with parameters depending on the permeant species, *International Journal of Molecular Sciences* 21 (2020).
- [61] S. Ahmad, J.A. Diez, C.H. George, W.H. Evans, Synthesis and assembly of connexins in vitro into homomeric and heteromeric functional gap junction hemichannels, *The Biochemical Journal* 339 (Pt 2) (1999) 247–253.
- [62] F. Zonta, G. Polles, G. Zanotti, F. Mammano, Permeation pathway of homomeric connexin 26 and connexin 30 channels investigated by molecular dynamics, *J. Biomol. Struct. Dyn.* 29 (2012) 985–998.
- [63] F. Zonta, G. Polles, M.F. Sanasi, M. Bortolozzi, F. Mammano, The 3.5 angstrom X-ray structure of the human connexin26 gap junction channel is unlikely that of a fully open channel, *Cell Communication and Signaling: CCS* 11 (2013) 15.
- [64] F. Zonta, D. Buratto, C. Cassini, M. Bortolozzi, F. Mammano, Molecular dynamics simulations highlight structural and functional alterations in deafness-related M34T mutation of connexin 26, *Front. Physiol.* 5 (2014) 85.
- [65] F. Zonta, D. Buratto, G. Crispino, A. Carrer, F. Bruno, G. Yang, F. Mammano, S. Pantano, Cues to opening mechanisms from in silico electric field excitation of Cx26 hemichannel and in vitro mutagenesis studies in HeLa transfectants, *Front. Mol. Neurosci.* 11 (2018) 170.
- [66] T. Kwon, A.L. Harris, A. Rossi, T.A. Bargiello, Molecular dynamics simulations of the Cx26 hemichannel: evaluation of structural models with Brownian dynamics, *The Journal of General Physiology* 138 (2011) 475–493.
- [67] B.S. Nielsen, F. Zonta, T. Farkas, T. Litman, M.S. Nielsen, N. MacAulay, Structural determinants underlying permeant discrimination of the Cx43 hemichannel, *J. Biol. Chem.* 294 (2019) 16789–16803.
- [68] J.L. Esseltine, D.W. Laird, Next-generation connexin and pannexin cell biology, *Trends Cell Biol.* 26 (2016) 944–955.
- [69] E. Decrock, M. Vinken, E. De Vuyst, D.V. Krysko, K. D'Herde, T. Vanhaecke, P. Vandabeele, V. Rogiers, L. Leybaert, Connexin-related signaling in cell death: to live or let die? *Cell Death Differ.* 16 (2009) 524–536.
- [70] A. Chandrasekhar, A.K. Bera, Hemichannels: permeants and their effect on development, physiology and death, *Cell Biochem. Funct.* 30 (2012) 89–100.
- [71] M. Vinken, Connexin hemichannels: novel mediators of toxicity, *Arch. Toxicol.* 89 (2015) 143–145.
- [72] M. Dosch, J. Gerber, F. Jebbawi, G. Beldi, Mechanisms of ATP release by inflammatory cells, *International Journal of Molecular Sciences* 19 (2018).
- [73] N.S. Lipman, L.R. Jackson, L.J. Trudel, F. Weis-Garcia, Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources, *ILAR J.* 46 (2005) 258–268.
- [74] R. Johnson, M. Hammer, J. Sheridan, J.-P. Revel, Gap junction formation between reaggregated Novikoff hepatoma cells, *Proc. Natl. Acad. Sci.* 71 (1974) 4536.
- [75] R.A. Meyer, D.W. Laird, J.P. Revel, R.G. Johnson, Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies, *J. Cell Biol.* 119 (1992) 179–189.
- [76] A. Hofer, R. Dermietzel, Visualization and functional blocking of gap junction hemichannels (connexons) with antibodies against external loop domains in astrocytes, *Glia* 24 (1998) 141–154.
- [77] M.A. Riquelme, R. Kar, S. Gu, J.X. Jiang, Antibodies targeting extracellular domain of connexins for studies of hemichannels, *Neuropharmacology* 75 (2013) 525–532.
- [78] A.J. Siller-Jackson, S. Burra, S. Gu, X. Xia, L.F. Bonewald, E. Sprague, J.X. Jiang, Adaptation of connexin 43-hemichannel prostaglandin release to mechanical loading, *J. Biol. Chem.* 283 (2008) 26374–26382.
- [79] R. Kar, M.A. Riquelme, S. Werner, J.X. Jiang, Connexin 43 channels protect osteocytes against oxidative stress-induced cell death, *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research* 28 (2013) 1611–1621.
- [80] J.Z. Zhou, M.A. Riquelme, S. Gu, R. Kar, X. Gao, L. Sun, J.X. Jiang, Osteocytic connexin hemichannels suppress breast cancer growth and bone metastasis, *Oncogene* 35 (2016) 5597–5607.
- [81] H. Xu, R. Liu, D. Ning, J. Zhang, R. Yang, M.A. Riquelme, J. Li, J.X. Jiang, P. Shang, Biological responses of osteocytic connexin 43 hemichannels to simulated microgravity, *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society* 35 (2017) 1195–1202.
- [82] C. Clair, L. Combettes, F. Pierre, P. Sansonetti, G. Tran Van Nhieu, Extracellular-loop peptide antibodies reveal a predominant hemichannel organization of connexins in polarized intestinal cells, *Exp. Cell Res.* 314 (2008) 1250–1265.
- [83] P. Majumder, G. Crispino, L. Rodriguez, C.D. Ciubotaru, F. Anselmi, V. Piazza, M. Bortolozzi, F. Mammano, ATP-mediated cell-cell signaling in the organ of Corti: the role of connexin channels, *Purinergic Signal* 6 (2010) 167–187.
- [84] M. Bonnet, G. Tran Van Nhieu, How Shigella utilizes Ca(2+) jagged edge signals during invasion of epithelial cells, *Front. Cell. Infect. Microbiol.* 6 (2016) 16.
- [85] G. Tran Van Nhieu, C. Clair, R. Bruzzone, M. Mesnil, P. Sansonetti, L. Combettes, Connexin-dependent inter-cellular communication increases invasion and dissemination of Shigella in epithelial cells, *Nat. Cell Biol.* 5 (2003) 720–726.
- [86] S. Singh, N.K. Kumar, P. Dwiwedi, J. Charan, R. Kaur, P. Sidhu, V.K. Chugh, Monoclonal antibodies: a review, *Curr. Clin. Pharmacol.* 13 (2018) 85–99.
- [87] J. McCafferty, A.D. Griffiths, G. Winter, D.J. Chiswell, Phage antibodies: filamentous phage displaying antibody variable domains, *Nature* 348 (1990) 552–554.
- [88] A. Frenzel, T. Schirrmann, M. Hust, Phage display-derived human antibodies in clinical development and therapy, *mAbs* 8 (2016) 1177–1194.
- [89] R.A. Lerner, Combinatorial antibody libraries: new advances, new immunological insights, *Nat. Rev. Immunol.* 16 (2016) 498–508.
- [90] G. Chen, S.S. Sidhu, Design and generation of synthetic antibody libraries for phage display, *Methods Mol. Biol.* 1131 (2014) 113–131.
- [91] S. Miersch, Z. Li, R. Hanna, M.E. McLaughlin, M. Hornsby, T. Matsuguchi, M. Paduch, A. Saaf, J. Wells, S. Koide, A. Kossiakoff, S.S. Sidhu, Scalable high throughput selection from phage-displayed synthetic antibody libraries, *Journal of Visualized Experiments: JoVE* 51492 (2015).
- [92] P. Chames, *Antibody Engineering: Methods and Protocols*, 2nd ed., Humana Press, New York, 2012.
- [93] G.P. Bunn, *Good Manufacturing Practices for Pharmaceuticals*, 7th ed., CRC Press, Taylor & Francis Group, Boca Raton, 2019.
- [94] P. Bannas, J. Hambach, F. Koch-Nolte, Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics, *Front. Immunol.* 8 (2017) 1603.
- [95] M.S. Castelli, P. McGonigle, P.J. Hornby, The pharmacology and therapeutic applications of monoclonal antibodies, *Pharmacol. Res. Perspect.* 7 (2019), e00535.
- [96] L. Liu, Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins, *Protein & Cell* 9 (2018) 15–32.
- [97] A. Caufriez, D. Bock, C. Martin, S. Ballet, M. Vinken, Peptide-based targeting of connexins and pannexins for therapeutic purposes, *Expert Opin. Drug Discovery* (2020) 1–10.
- [98] R. Oliveira, C. Christov, J.S. Guillamo, S. de Bouard, S. Palfi, L. Venance, M. Tardy, M. Peschanski, Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas, *BMC Cell Biol.* 6 (2005) 7.
- [99] W.C. Sin, Q. Aftab, J.F. Bechberger, J.H. Leung, H. Chen, C.C. Naus, Astrocytes promote glioma invasion via the gap junction protein connexin43, *Oncogene* 35 (2016) 1504–1516.
- [100] T. Aasen, M. Mesnil, C.C. Naus, P.D. Lampe, D.W. Laird, Gap junctions and cancer: communicating for 50 years, *Nat. Rev. Cancer* 17 (2017) 74.
- [101] J. Wang, Z.Y. Yang, Y.F. Guo, J.Y. Kuang, X.W. Bian, S.C. Yu, Targeting different domains of gap junction protein to control malignant glioma, *Neuro-oncology* 20 (2018) 885–896.
- [102] A.A. Khalil, O. Ilina, A. Vasaturo, J.H. Venhuizen, M. Vullings, V. Venhuizen, A. Bilos, C.G. Figdor, P.N. Span, P. Friedl, Collective invasion induced by an autocrine purinergic loop through connexin-43 hemichannels, *The Journal of Cell Biology* 219 (2020).
- [103] V.P. Baklaushev, O.I. Gurina, G.M. Yusubalieva, N.F. Grinenko, E.B. Cytrin, I. V. Victorov, V.P. Chekhonin, Immunofluorescent analysis of connexin-43 using

- monoclonal antibodies to its extracellular domain, *Bull. Exp. Biol. Med.* 148 (2009) 725–730.
- [104] V.P. Baklaushev, G.M. Yusubalieva, E.B. Tsitrin, O.I. Gurina, N.P. Grinenko, I. V. Victorov, V.P. Chekhonin, Visualization of connexin 43-positive cells of glioma and the periglioma zone by means of intravenously injected monoclonal antibodies, *Drug Delivery* 18 (2011) 331–337.
- [105] G.M. Yusubalieva, V.P. Baklaushev, O.I. Gurina, M.V. Gulyaev, Y.A. Pirogov, V. P. Chekhonin, Antitumor effects of monoclonal antibodies to connexin 43 extracellular fragment in induced low-differentiated glioma, *Bull. Exp. Biol. Med.* 153 (2012) 163–169.
- [106] V.P. Chekhonin, V.P. Baklaushev, G.M. Yusubalieva, A.E. Belorousova, M. V. Gulyaev, E.B. Tsitrin, N.F. Grinenko, O.I. Gurina, Y.A. Pirogov, Targeted delivery of liposomal nanocontainers to the peritumoral zone of glioma by means of monoclonal antibodies against GFAP and the extracellular loop of Cx43, *Nanomedicine: Nanotechnology, Biology, and Medicine* 8 (2012) 63–70.
- [107] G.M. Yusubalieva, V.P. Baklaushev, O.I. Gurina, Y.A. Zorkina, I.L. Gubskii, G. L. Kobayakov, A.V. Golanov, S.A. Goryainov, G.E. Gorlachev, A.N. Kononov, A. A. Potapov, V.P. Chekhonin, Treatment of poorly differentiated glioma using a combination of monoclonal antibodies to extracellular connexin-43 fragment, temozolomide, and radiotherapy, *B Exp Biol Med* 157 (2014) 510–515.
- [108] V.P. Baklaushev, N.N. Nukolova, A.S. Khalansky, O.I. Gurina, G.M. Yusubalieva, N.P. Grinenko, I.L. Gubskiy, P.A. Melnikov, K. Kardashova, A.V. Kabanov, V. P. Chekhonin, Treatment of glioma by cisplatin-loaded nanogels conjugated with monoclonal antibodies against Cx43 and BSAT1, *Drug Delivery* 22 (2015) 276–285.
- [109] T. Aasen, E. Leithe, S.V. Graham, P. Kameritsch, M.D. Mayan, M. Mesnil, K. Pogoda, A. Tabernero, Connexins in cancer: bridging the gap to the clinic, *Oncogene* 38 (2019) 4429–4451.
- [110] J.X. Jiang, Z. An, N. Zhang, W. Xiong, M.A. Riquelme, S. Gu, N. Ledene Sayre, Connexin (cx) 43 Hemichannel-binding Antibodies and Uses Thereof, in *USA*, 2019, pp. 1–48.
- [111] O.F. Sanchez, A.V. Rodriguez, J.M. Velasco-Espana, L.C. Murillo, J.J. Sutachan, S. L. Albarracin, Role of connexins 30, 36, and 43 in brain tumors, neurodegenerative diseases, and neuroprotection, *Cells* 9 (2020).
- [112] D.W. Laird, P.D. Lampe, Therapeutic strategies targeting connexins, *Nat. Rev. Drug Discov.* 17 (2018) 905–921.
- [113] T. Martins-Marques, T. Ribeiro-Rodrigues, D. Batista-Almeida, T. Aasen, B. R. Kwak, H. Giro, Biological functions of connexin43 beyond intercellular communication, *Trends Cell Biol.* 29 (2019) 835–847.
- [114] L. Xing, T. Yang, S. Cui, G. Chen, Connexin hemichannels in astrocytes: role in CNS disorders, *Front. Mol. Neurosci.* 12 (2019) 23.
- [115] M. Varela-Eirin, J. Loureiro, E. Fonseca, S. Corrochano, J.R. Caeiro, M. Collado, M.D. Mayan, Cartilage regeneration and ageing: targeting cellular plasticity in osteoarthritis, *Ageing Res. Rev.* 42 (2018) 56–71.
- [116] J. Czyz, K. Szpak, Z. Madeja, The role of connexins in prostate cancer promotion and progression, *Nat Rev Urol* 9 (2012) 274–282.
- [117] A.K. Gadok, C. Zhao, A.I. Meriwether, S. Ferrati, T.G. Rowley, J. Zoldan, H.D. C. Smyth, J.C. Stachowiak, The display of single-domain antibodies on the surfaces of connectomes enables gap junction-mediated drug delivery to specific cell populations, *Biochemistry* 57 (2018) 81–90.
- [118] M.A. Retamal, E.P. Reyes, I.E. Garcia, B. Pinto, A.D. Martinez, C. Gonzalez, Diseases associated with leaky hemichannels, *Front. Cell. Neurosci.* 9 (2015) 267.
- [119] I.E. Garcia, F. Bosen, P. Mujica, A. Pupo, C. Flores-Munoz, O. Jara, C. Gonzalez, K. Willecke, A.D. Martinez, From hyperactive connexin26 hemichannels to impairments in epidermal calcium gradient and permeability barrier in the keratitis-ichthyosis-deafness syndrome, *J Invest Dermatol* 136 (2016) 574–583.
- [120] L. Leybaert, P.D. Lampe, S. Dhein, R. Kwak, P. Ferdinandy, E.C. Beyer, D. W. Laird, C.C. Naus, C.R. Green, R. Schulz, Connexins in cardiovascular and neurovascular health and disease: pharmacological implications, *Pharmacol. Rev.* 69 (2017) 396–478.
- [121] M. Srinivas, V.K. Verselis, T.W. White, Human diseases associated with connexin mutations, *Biochim. Biophys. Acta* 1860 (2018) 192–201.
- [122] T. Taniguchi, H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto, J. Hamuro, Structure and expression of a cloned cDNA for human interleukin-2, *Nature* 302 (1983) 305–310.
- [123] O. Perisic, P.A. Webb, P. Holliger, G. Winter, R.L. Williams, Crystal structure of a diabody, a bivalent antibody fragment, *Structure* 2 (1994) 1217–1226.
- [124] E. Bujak, M. Matasci, D. Neri, S. Wulhfard, Reformating of scFv antibodies into the scFv-Fc format and their downstream purification, *Methods Mol. Biol.* 1131 (2014) 315–334.
- [125] L. Xu, A. Carrer, F. Zonta, Z. Qu, P. Ma, S. Li, F. Ceriani, D. Buratto, G. Crispino, V. Zorzi, G. Ziraldo, F. Bruno, C. Nardin, C. Peres, F. Mazzarda, A.M. Salvatore, M. Raspa, F. Scavizzi, Y. Chu, S. Xie, X. Yang, J. Liao, X. Liu, W. Wang, S. Wang, G. Yang, R.A. Lerner, F. Mammano, Design and characterization of a human monoclonal antibody that modulates mutant connexin 26 hemichannels implicated in deafness and skin disorders, *Front. Mol. Neurosci.* 10 (2017) 298.
- [126] G. Ziraldo, D. Buratto, Y. Kuang, L. Xu, A. Carrer, C. Nardin, F. Chiani, A. M. Salvatore, G. Paludetti, R.A. Lerner, G. Yang, F. Zonta, F. Mammano, A human-derived monoclonal antibody targeting extracellular connexin domain selectively modulates hemichannel function, *Front. Physiol.* 10 (2019) 392.
- [127] A. Butterweck, C. Elfgang, K. Willecke, O. Traub, Differential expression of the gap junction proteins connexin45, -43, -40, -31, and -26 in mouse skin, *Eur. J. Cell Biol.* 65 (1994) 152–163.
- [128] D. Salomon, E. Masgrau, S. Vischer, S. Ullrich, E. Dupont, P. Sappino, J.H. Saurat, P. Meda, Topography of mammalian connexins in human skin, *J Invest Dermatol* 103 (1994) 240–247.
- [129] J.A. Goliger, D.L. Paul, Expression of gap junction proteins Cx26, Cx31.1, Cx37, and Cx43 in developing and mature rat epidermis, *Developmental Dynamics: An Official Publication of the American Association of Anatomists* 200 (1994) 1–13.
- [130] W.L. Di, E.L. Rugg, I.M. Leigh, D.P. Kelsell, Multiple epidermal connexins are expressed in different keratinocyte subpopulations including connexin 31, *J Invest Dermatol* 117 (2001) 958–964.
- [131] G. Mese, G. Richard, T.W. White, Gap junctions: basic structure and function, *J Invest Dermatol* 127 (2007) 2516–2524.
- [132] C. Faniku, C.S. Wright, P.E. Martin, Connexins and pannexins in the integumentary system: the skin and appendages, *Cellular and Molecular Life Sciences: CMLS* 72 (2015) 2937–2947.
- [133] Y. Kuang, V. Zorzi, D. Buratto, G. Ziraldo, F. Mazzarda, C. Peres, C. Nardin, A. M. Salvatore, F. Chiani, F. Scavizzi, M. Raspa, M. Qiang, Y. Chu, X. Shi, Y. Li, L. Liu, Y. Shi, F. Zonta, G. Yang, R.A. Lerner, F. Mammano, A potent antagonist antibody targeting connexin hemichannels alleviates Clouston syndrome symptoms in mutant mice, *EBioMedicine* 57 (2020) 102825.
- [134] J. Lamartine, G. Munhoz Essenfelder, Z. Kibar, I. Lanneluc, E. Callouet, D. Laoudj, G. Lemaître, C. Hand, S.J. Hayflick, J. Zonana, S. Antonarakis, U. Radhakrishna, D.P. Kelsell, A.L. Christianson, A. Pitaval, V. Der Kaloustian, C. Fraser, C. Blanchet-Bardon, G.A. Rouleau, G. Waksman, Mutations in GJB6 cause hidrotic ectodermal dysplasia, *Nat. Genet.* 26 (2000) 142–144.
- [135] G.M. Essenfelder, R. Bruzzone, J. Lamartine, A. Charollais, C. Blanchet-Bardon, M.T. Barbe, P. Meda, G. Waksman, Connexin30 mutations responsible for hidrotic ectodermal dysplasia cause abnormal hemichannel activity, *Hum. Mol. Genet.* 13 (2004) 1703–1714.
- [136] K. Sugiura, M. Teranishi, Y. Matsumoto, M. Akiyama, Clouston syndrome with heterozygous GJB6 mutation p.Ala88Val and GJB2 variant p.Val27Ile revealing mild sensorineural hearing loss and photophobia, *JAMA Dermatology* 149 (2013) 1350–1351.
- [137] R. Yang, Z. Hu, Q. Kong, W. Li, L. Zhang, X. Du, S. Huang, X. Xia, H. Sang, A known mutation in GJB6 in a large Chinese family with hidrotic ectodermal dysplasia, *Journal of the European Academy of Dermatology and Venereology: JEADV* 30 (2016) 1362–1365.
- [138] Y. Zhan, S. Luo, Z. Pi, G. Zhang, A recurrent mutation of GJB6 in a big Chinese family with hidrotic ectodermal dysplasia, *Hereditas* 157 (2020) 34.
- [139] F. Bosen, N. Schutz, A. Beinhauer, N. Strenzeke, T. Franz, K. Willecke, The Clouston syndrome mutation connexin30 A88V leads to hyperproliferation of sebaceous glands and hearing impairments in mice, *FEBS Lett.* 588 (2014) 1795–1801.
- [140] P.E. Martin, M. van Steensel, Connexins and skin disease: insights into the role of beta connexins in skin homeostasis, *Cell Tissue Res.* 360 (2015) 645–658.
- [141] D.W. Laird, C.C. Naus, P.D. Lampe, SnapShot: connexins and disease, *Cell* 170 (6) (2017) 1260–1260.e1.
- [142] G. Mese, C. Sellitto, L. Li, H.Z. Wang, V. Valiunas, G. Richard, P.R. Brink, T. W. White, The Cx26-G45E mutation displays increased hemichannel activity in a mouse model of the lethal form of keratitis-ichthyosis-deafness syndrome, *Mol. Biol. Cell* 22 (2011) 4776–4786.
- [143] F. Bosen, A. Celli, D. Crumrine, K. vom Dorp, P. Ebel, H. Jastrow, P. Dormann, E. Winterhager, T. Mauro, K. Willecke, Altered epidermal lipid processing and calcium distribution in the KID syndrome mouse model Cx26S17F, *FEBS Lett.* 589 (2015) (1904-1910).
- [144] M. Bortolozzi, What's the function of connexin 32 in the peripheral nervous system? *Front. Mol. Neurosci.* 11 (2018) 227.
- [145] H. Kabayama, M. Takeuchi, N. Tokushige, S.I. Muramatsu, M. Kabayama, M. Fukuda, Y. Yamada, K. Mikoshiba, An ultra-stable cytoplasmic antibody engineered for in vivo applications, *Nat. Commun.* 11 (2020) 336.
- [146] M. Hitomi, L.P. Deleyrolle, E.E. Mulkearns-Hubert, A. Jarrar, M. Li, M. Sinyuk, B. Otvos, S. Brunet, W.A. Flavahan, C.G. Hubert, W. Goan, J.S. Hale, A. G. Alvarado, A. Zhang, M. Rohaus, M. Ollivier, V. Vedam-Mai, J.M. Fortin, H.S. Futch, B. Griffith, Q. Wu, C.H. Xia, X. Gong, M.S. Ahluwalia, J.N. Rich, B.A. Reynolds, J. D. Lathia, Differential connexin function enhances self-renewal in glioblastoma, *Cell Rep.* 11 (2015) 1031–1042.
- [147] R.A. Acuna, M. Varas-Godoy, V.M. Berthoud, I.E. Alfaro, M.A. Retamal, Connexin-46 contained in extracellular vesicles enhance malignancy features in breast cancer cells, *Biomolecules* 10 (2020).
- [148] A. Frenzel, J. Kugler, S. Wilke, T. Schirrmann, M. Hust, Construction of human antibody gene libraries and selection of antibodies by phage display, *Methods Mol. Biol.* 1060 (2014) 215–243.
- [149] Z. Yang, Y. Wan, P. Tao, M. Qiang, X. Dong, C.W. Lin, G. Yang, T. Zheng, R. A. Lerner, A cell-cell interaction format for selection of high-affinity antibodies to membrane proteins, *Proc. Natl. Acad. Sci. U. S. A.* 116 (2019) 14971–14978.
- [150] E. Gallo, A. Kellil, P.E. Bayliss, A. Jegannathan, O. Egorova, L. Ploder, J.A. Adams, P. Giblin, S.S. Sidhu, In situ antibody phage display yields optimal inhibitors of integrin alpha11/beta1, *mAbs* 12 (2020), 1717265.
- [151] T. Delvaeye, M.A.J. De Smet, S. Verwaerde, E. Decrock, A. Czekaj, R. E. Vandenbroucke, K. Lemeire, A. Goncalves, W. Declercq, P. Vandenabeele, D. V. Krysko, L. Leybaert, Blocking connexin43 hemichannels protects mice against tumour necrosis factor-induced inflammatory shock, *Sci. Rep.* 9 (2019) 16623.
- [152] B.W. Calder, J. Matthew Rhett, H. Bainbridge, S.A. Fann, R.G. Gourdie, M.J. Yost, Inhibition of connexin 43 hemichannel-mediated ATP release attenuates early inflammation during the foreign body response, *Tissue Eng. A* 21 (2015) 1752–1762.
- [153] J.A. Orellana, N. Froger, P. Ezan, J.X. Jiang, M.V. Bennett, C.C. Naus, C. Giaume, J.C. Saez, ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels, *J. Neurochem.* 118 (2011) 826–840.

- [154] O.O. Mugisho, C.R. Green, D.T. Kho, J. Zhang, E.S. Graham, M.L. Acosta, I. D. Rupenthal, The inflammasome pathway is amplified and perpetuated in an autocrine manner through connexin43 hemichannel mediated ATP release, *Biochim. Biophys. Acta Gen. Subj.* 1862 (2018) 385–393.
- [155] C.W. Wong, T. Christen, I. Roth, C.E. Chadjichristos, J.P. Derouette, B.F. Foglia, M. Chanson, D.A. Goodenough, B.R. Kwak, Connexin37 protects against atherosclerosis by regulating monocyte adhesion, *Nat. Med.* 12 (2006) 950–954.
- [156] J.C. Saez, S. Contreras-Duarte, V.C. Labra, C.A. Santibanez, L.A. Mellado, C. A. Inostroza, T.F. Alvear, M.A. Retamal, V. Velarde, J.A. Orellana, Interferon-gamma and high glucose-induced opening of Cx43 hemichannels causes endothelial cell dysfunction and damage, *Biochim Biophys Acta Mol Cell Res* 2020 (1867) 118720.
- [157] C. Kuo, C.R. Green, I.D. Rupenthal, O.O. Mugisho, Connexin43 hemichannel block protects against retinal pigment epithelial cell barrier breakdown, *Acta Diabetol.* 57 (2020) 13–22.
- [158] J.C. Saez, S. Contreras-Duarte, G.I. Gomez, V.C. Labra, C.A. Santibanez, R. Gajardo-Gomez, B.C. Avendano, E.F. Diaz, T.D. Montero, V. Velarde, J. A. Orellana, Connexin 43 hemichannel activity promoted by pro-inflammatory cytokines and high glucose alters endothelial cell function, *Front. Immunol.* 9 (2018) 1899.
- [159] T. Nakase, G. Sohl, M. Theis, K. Willecke, C.C. Naus, Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes, *Am. J. Pathol.* 164 (2004) 2067–2075.
- [160] F. Molica, X.F. Figueroa, B.R. Kwak, B.E. Isakson, J.M. Gibbins, Connexins and pannexins in vascular function and disease, *International Journal of Molecular Sciences* 19 (2018).
- [161] N. Wang, E. De Vuyst, R. Ponsaerts, K. Boengler, N. Palacios-Prado, J. Wauman, C. P. Lai, M. De Bock, E. Decroock, M. Bol, M. Vinken, V. Rogiers, J. Tavernier, W. H. Evans, C.C. Naus, F.F. Bukauskas, K.R. Sipido, G. Heusch, R. Schulz, G. Bultynck, L. Leybaert, Selective inhibition of Cx43 hemichannels by Gap19 and its impact on myocardial ischemia/reperfusion injury, *Basic Res. Cardiol.* 108 (2013) 309.
- [162] D. Lu, S. Soleymani, R. Madakshire, P.A. Insel, ATP released from cardiac fibroblasts via connexin hemichannels activates profibrotic P2Y2 receptors, *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 26 (2012) 2580–2591.
- [163] G.W. Price, C.E. Chadjichristos, P. Kavvadas, S.C.W. Tang, W.H. Yiu, C.R. Green, J.A. Potter, E. Siamantouras, P.E. Squires, C.E. Hills, Blocking connexin-43 mediated hemichannel activity protects against early tubular injury in experimental chronic kidney disease, *Cell Communication and Signaling: CCS* 18 (2020) 79.
- [164] J.A. Guttman, A.E. Lin, Y. Li, J. Bechberger, C.C. Naus, A.W. Vogl, B.B. Finlay, Gap junction hemichannels contribute to the generation of diarrhoea during infectious enteric disease, *Gut* 59 (2010) 218–226.
- [165] J.A. Orellana, J.C. Saez, M.V. Bennett, J.W. Berman, S. Morgello, E.A. Eugenin, HIV increases the release of dickkopf-1 protein from human astrocytes by a Cx43 hemichannel-dependent mechanism, *J. Neurochem.* 128 (2014) 752–763.
- [166] M. Maes, S. Crespo Yanguas, J. Willebrords, J.L. Weemhoff, T.C. da Silva, E. Decroock, M. Lebofsky, I.V.A. Pereira, L. Leybaert, A. Farhood, H. Jaeschke, B. Cogliati, M. Vinken, Connexin hemichannel inhibition reduces acetaminophen-induced liver injury in mice, *Toxicol. Lett.* 278 (2017) 30–37.
- [167] S. Crespo Yanguas, T.C. da Silva, I.V.A. Pereira, J. Willebrords, M. Maes, M. Sayuri Nogueira, I. Alves de Castro, I. Leclercq, G.R. Romualdo, L.F. Barbisan, L. Leybaert, B. Cogliati, M. Vinken, TAT-Gap19 and carbenoxolone alleviate liver fibrosis in mice, *International Journal of Molecular Sciences* 19 (2018).
- [168] J. Willebrords, B. Cogliati, I.V.A. Pereira, T.C. da Silva, S. Crespo Yanguas, M. Maes, V.M. Govoni, A. Lima, D.A. Felisbino, E. Decroock, M.S. Nogueira, I.A. de Castro, I. Leclercq, L. Leybaert, R.M. Rodrigues, M. Vinken, Inhibition of connexin hemichannels alleviates non-alcoholic steatohepatitis in mice, *Sci. Rep.* 7 (2017) 8268.
- [169] C. Yi, A. Koulakoff, C. Giaume, Astroglial connexins as a therapeutic target for Alzheimer's disease, *Curr. Pharm. Des.* 23 (2017) 4958–4968.
- [170] N. Maier, M. Guldenagel, G. Sohl, H. Siegmund, K. Willecke, A. Draguhn, Reduction of high-frequency network oscillations (ripples) and pathological network discharges in hippocampal slices from connexin 36-deficient mice, *J. Physiol.* 541 (2002) 521–528.
- [171] M.C. Laura, F.P. Xochitl, S. Anne, M.V. Alberto, Analysis of connexin expression during seizures induced by 4-aminopyridine in the rat hippocampus, *J. Biomed. Sci.* 22 (2015) 69.
- [172] R. Dobrowolski, P. Sasse, J.W. Schrickel, M. Watkins, J.S. Kim, M. Rackauskas, C. Troatz, A. Ghanem, K. Tiemann, J. Degen, F.F. Bukauskas, R. Civitelli, T. Lewalter, B.K. Fleischmann, K. Willecke, The conditional connexin43G138R mouse mutant represents a new model of hereditary oculodentodigital dysplasia in humans, *Hum. Mol. Genet.* 17 (2008) 539–554.
- [173] A.M. Flenniken, L.R. Osborne, N. Anderson, N. Ciliberti, C. Fleming, J.E. Gittens, X.Q. Gong, L.B. Kelsey, C. Lounsbury, L. Moreno, B.J. Nieman, K. Peterson, D. Qu, W. Roscoe, Q. Shao, D. Tong, G.I. Veitch, I. Voronina, I. Vukobradovic, G. A. Wood, Y. Zhu, R.A. Zirngibl, J.E. Aubin, D. Bai, B.G. Bruneau, M. Grynbas, J. E. Henderson, R.M. Henkelman, C. McKerlie, J.G. Sled, W.L. Stanford, D.W. Laird, G.M. Kidder, S.L. Adamson, J. Rossant, A Gja1 missense mutation in a mouse model of oculodentodigital dysplasia, *Development* 132 (2005) 4375–4386.
- [174] D. Patel, J. Gemel, Q. Xu, A.R. Simon, X. Lin, A. Matiukas, E.C. Beyer, R. D. Veenstra, Atrial fibrillation-associated connexin40 mutants make hemichannels and synergistically form gap junction channels with novel properties, *FEBS Lett.* 588 (2014) 1458–1464.
- [175] Y.-Q. Yang, X. Liu, X.-L. Zhang, X.-H. Wang, H.-W. Tan, H.-F. Shi, W.-F. Jiang, W.-Y. Fang, Novel connexin40 missense mutations in patients with familial atrial fibrillation, *EP Europace* 12 (2010) 1421–1427.
- [176] Y. Sun, M.D. Hills, W.G. Ye, X. Tong, D. Bai, Atrial fibrillation-linked germline GJA5/connexin40 mutants showed an increased hemichannel function, *PLoS One* 9 (2014), e95125.
- [177] H. Wang, X. Cao, Z. Lin, M. Lee, X. Jia, Y. Ren, L. Dai, L. Guan, J. Zhang, X. Lin, J. Zhang, Q. Chen, C. Feng, E.Y. Zhou, J. Yin, G. Xu, Y. Yang, Exome sequencing reveals mutation in GJA1 as a cause of keratoderma-hypotrichosis-leukonychia totalis syndrome, *Hum. Mol. Genet.* 24 (2015) 243–250.
- [178] M. Srinivas, T.F. Jannace, A.G. Cocozzelli, L. Li, N. Slavi, C. Sellitto, T.W. White, Connexin43 mutations linked to skin disease have augmented hemichannel activity, *Sci. Rep.* 9 (2019) 19.
- [179] N. Umegaki-Arao, T. Sasaki, H. Fujita, S. Aoki, K. Kameyama, M. Amagai, M. Seishima, A. Kubo, Inflammatory linear verrucous epidermal nevus with a postzygotic GJA1 mutation is a mosaic erythrokeratoderma variabilis et progressiva, *J Invest Dermatol* 137 (2017) 967–970.
- [180] L.M. Boyden, B.G. Craiglow, J. Zhou, R. Hu, E.C. Loring, K.D. Morel, C.T. Lauren, R.P. Lifton, K. Bilguvar, A.S. Paller, K.A. Choate, Dominant de novo mutations in GJA1 cause erythrokeratoderma variabilis et progressiva, without features of oculodentodigital dysplasia, *J Invest Dermatol* 135 (2015) 1540–1547.
- [181] J. Chi, L. Li, M. Liu, J. Tan, C. Tang, Q. Pan, D. Wang, Z. Zhang, Pathogenic connexin-31 forms constitutively active hemichannels to promote necrotic cell death, *PLoS One* 7 (2012), e32531.
- [182] J.R. Lee, A.M. Derosa, T.W. White, Connexin mutations causing skin disease and deafness increase hemichannel activity and cell death when expressed in *Xenopus* oocytes, *J Invest Dermatol* 129 (2009) 870–878.
- [183] H. Aypek, V. Bay, G. Mese, Altered cellular localization and hemichannel activities of KID syndrome associated connexin26 I30N and D50Y mutations, *BMC Cell Biol.* 17 (2016) 5.
- [184] I.E. Garcia, J. Maripillan, O. Jara, R. Ceriani, A. Palacios-Munoz, J. Ramachandran, P. Olivero, T. Perez-Acle, C. Gonzalez, J.C. Saez, J.E. Contreras, A.D. Martinez, Keratitis-ichthyosis-deafness syndrome-associated Cx26 mutants produce nonfunctional gap junctions but hyperactive hemichannels when co-expressed with wild type Cx43, *J Invest Dermatol* 135 (2015) 1338–1347.
- [185] M. Schutz, T. Auth, A. Gehrt, F. Bosen, I. Korber, N. Strenzke, T. Moser, K. Willecke, The connexin26 S17F mouse mutant represents a model for the human hereditary keratitis-ichthyosis-deafness syndrome, *Hum. Mol. Genet.* 20 (2011) 28–39.
- [186] E. Press, K.C. Alaga, K. Barr, Q. Shao, F. Bosen, K. Willecke, D.W. Laird, Disease-linked connexin26 S17F promotes volar skin abnormalities and mild wound healing defects in mice, *Cell Death Dis.* 8 (2017), e2845.
- [187] H.A. Sanchez, R. Bienkowski, N. Slavi, M. Srinivas, V.K. Verselis, Altered inhibition of Cx26 hemichannels by pH and Zn<sup>2+</sup> in the A40V mutation associated with keratitis-ichthyosis-deafness syndrome, *J. Biol. Chem.* 289 (2014) 21519–21532.
- [188] B.C. Stong, Q. Chang, S. Ahmad, X. Lin, A novel mechanism for connexin 26 mutation linked deafness: cell death caused by leaky gap junction hemichannels, *Laryngoscope* 116 (2006) 2205–2210.
- [189] D.A. Gerido, A.M. DeRosa, G. Richard, T.W. White, Aberrant hemichannel properties of Cx26 mutations causing skin disease and deafness, *American Journal of Physiology. Cell Physiology* 293 (2007) C337–C345.
- [190] P.V. Mhaske, N.A. Levit, L. Li, H.Z. Wang, J.R. Lee, Z. Shuja, P.R. Brink, T. W. White, The human Cx26-D50A and Cx26-A88V mutations causing keratitis-ichthyosis-deafness syndrome display increased hemichannel activity, *American Journal of Physiology. Cell Physiology* 304 (2013) C1150–C1158.
- [191] Q. Ren, M.A. Riquelme, J. Xu, X. Yan, B.J. Nicholson, S. Gu, J.X. Jiang, Cataract-causing mutation of human connexin 46 impairs gap junction, but increases hemichannel function and cell death, *PLoS One* 8 (2013), e74732.
- [192] J.J. Tong, P.J. Minogue, W. Guo, T.L. Chen, E.C. Beyer, V.M. Berthoud, L. Ebihara, Different consequences of cataract-associated mutations at adjacent positions in the first extracellular boundary of connexin50, *American Journal of Physiology. Cell Physiology* 300 (2011) C1055–C1064.
- [193] C.K. Abrams, M.V. Bennett, V.K. Verselis, T.A. Bargiello, Voltage opens unopposed gap junction hemichannels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 3980–3984.
- [194] G.S. Liang, M. de Miguel, J.M. Gomez-Hernandez, J.D. Glass, S.S. Scherer, M. Mintz, L.C. Barrio, K.H. Fischbeck, Severe neuropathy with leaky connexin32 hemichannels, *Ann. Neurol.* 57 (2005) 749–754.
- [195] W.A. Paznekas, B. Karczeski, S. Vermeer, R.B. Lowry, M. Delatycki, F. Laurence, P.A. Koivisto, L. Van Maldergem, S.A. Boyadjiev, J.N. Bodurtha, E.W. Jabs, GJA1 mutations, variants, and connexin 43 dysfunction as it relates to the oculodentodigital dysplasia phenotype, *Hum. Mutat.* 30 (2009) 724–733.
- [196] D. Tattersall, C.A. Scott, C. Gray, D. Zicha, D.P. Kelsell, EKV mutant connexin 31 associated cell death is mediated by ER stress, *Hum. Mol. Genet.* 18 (2009) 4734–4745.