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REVIEW

Modulation of gap junction channels and hemichannels by growth factors

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Gap junction hemichannels and cell–cell channels have roles in coordinating numerous cellular processes, due to their permeability to extra and intracellular signaling molecules. Another mechanism of cellular coordination is provided by a vast array of growth factors that interact with relatively selective cell membrane receptors. These receptors can affect cellular transduction pathways, including alteration of intracellular concentration of free Ca^{2+} and free radicals and activation of protein kinases or phosphatases. Connexin and pannexin based channels constitute recently described targets of growth factor signal transduction pathways, but little is known regarding the effects of growth factor signaling on pannexin based channels. The effects of growth factors on these two channel types seem to depend on the cell type, cell stage and connexin and pannexin isoform expressed. The functional state of hemichannels and gap junction channels are affected in opposite directions by FGF-1 *via* protein kinase-dependent mechanisms. These changes are largely explained by channels insertion in or withdrawal from the cell membrane, but changes in open probability might also occur due to changes in phosphorylation and redox state of channel subunits. The functional consequence of variation in cell–cell communication *via* these membrane channels is implicated in disease as well as normal cellular responses.

1. Introduction

Cells within an organism communicate with each other in a number of ways. They may release hormones, neurotransmitters and other molecules that act on distant cells (as in the endocrine system) or nearby (paracrine actions, where there may also be autocrine actions on the secreting cells). In addition, cell–cell communication at chemical synpases is mediated by secreted molecules. These types of intercellular communication require the presence of specific receptors in responding cells. Usually, activation of metabotropic or ionotropic receptors is initiated by ligand binding. The former type leads to the generation of second messengers and possibly a cascade of events generating cell-specific responses, and the latter type allows permeation of ions that may have signaling functions in addition to carrying charge.

Intercellular communication can also occur without the release of substances to the extracellular space. Direct communication between contacting cells can be mediated by specialized plasma membrane structures termed gap junctions,[1] which contain intercellular channels that directly connect the cytoplasms of adjacent cells. Each gap junction channel is composed of two hemichannels also called connexons. Intercellular communication through gap junctions allows for cell groups to share ions, metabolites and second messengers. Thus, gap junctions permit a coordinated response to a wide range of stimuli, even when some cells in a coupled population lack receptors for a particular extracellular signal. In addition, gap junction proteins can form undocked hemichannels in non-junctional cell membrane, enabling communication between cytoplasm and extracellular milieu. In this case, open hemichannels become routes for autocrine/paracrine interactions through the diffusional transport of small signaling molecules.

For more detailed information regarding structure and functions of gap junction channels and hemichannels, readers are referred to more comprehensive reviews published elsewhere.[2–7]

Although hemichannels are formed of the same subunits as gap junction channels and can have similar electrical properties and permeability, mounting evidence indicates that the two

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types of channels can be differentially modulated by different stimuli, such as nitric oxide, $[Ca^{2+}]_i$, oxidative stress, pro-inflammatory cytokines and growth factors.[8–14] However, the molecular mechanisms and biological implications of this difference remain largely unknown.

Growth factors constitute a heterogeneous group of soluble polypeptides produced by many cell types. They act by binding to relatively specific cell membrane receptors and in many cases activate tyrosine kinase-mediated intracellular signaling. Growth factors often induce mitogenic effects and participate in many physiological processes, such as cell differentiation during development and tissue renewal/repair, as well as pathological processes including malignant transformation, proliferation and migration of cancer cells, angiogenesis and inflammation.[15,16]

This review summarizes general structural and functional features of vertebrate gap junction channels and hemichannels and discusses the evidence regarding their modulation by growth factors.

2. Gap junction channels

Gap junctions are plasma membrane specializations that contain clusters of intercellular channels characterized by the close apposition of the plasma membranes of adjacent cells, leaving a virtual gap of 2–4 nm.[3] These structures were initially observed through electron microscopy and lanthanum tracing, and identified as heptalaminar intercellular junctions distinct from tight junctions.[1] Later on, molecular and structural studies revealed that each intercellular channel is formed by the serial docking of two hemichannels, each of which is a hexamer of protein subunits termed connexins (Cxs)[17,18] in vertebrates, and innexins in invertebrates.[19] Three glycoproteins homologs to the innexins are found in mammals, and these mammalian homologs and the invertebrate forms were then termed pannexins (Panxs), since they were thought to be expressed by cells of all phyla of multicellular organisms.[20–24] Formation of Panx gap junctions has been demonstrated in vertebrate cells exogenously expressing Panxs,[20,21,25,26] and it has been proposed that Panx3 gap junctions between osteoblasts endogenously expressing Panx3 mediate propagation of Ca waves.[27] This recent finding suggests that further studies are required to demonstrate whether all endogenously expressed Panxs can or cannot form gap junctions. It also remains to be determined whether Cx gap junctions can co-exist with Panx gap junctions.

Cxs, Panxs and innexins have the same membrane topology: four transmembrane domains (TM1-TM4), two extracellular loops (E1, E2), one intracellular loop (IL) and intracellular amino- and carboxyl-termini. In all Cxs, each extracellular loop contains three conserved cysteine residues, except for Cx23 in which E1 and E2 contain only two. These cysteine residues form intramolecular disulfide bonds.[26] The recently reported crystal structure of gap junction channels formed by human Cx26 confirmed that hemichannel docking occurs *via* non-covalent interactions between the extracellular loops of apposing hexamers.[28] Gap junctions formed by two identical hemichannels are termed homotypic, while those formed by hemichannels of different molecular composition are heterotypic. In turn, hemichannels of uniform Cx composition are homomeric, whereas those assembled from different Cx subtypes are heteromeric.[3]

Gap junction channels formed by different Cxs show differing unitary conductances and perm-selectivity properties depending on the size, shape and net charge of the permeating molecules.[29–32] Compared to the ion channels of electrically excitable cells, gap junction channels are much less selective, and allow intercellular transfer of larger solutes. All studied gap junction channels formed by Cxs have a pore diameter of $\sim$1.4–1.8 nm.[28,33] However, size and charge selectivity do exist. Several studies show that gap junction channels made of different protein subunits have different permeabilities to second messengers or metabolites.[2] In addition, the functional and regulatory properties of gap junction channels (see below) also vary depending on subunit composition.

Transfer of ions and small molecules through gap junction channels is regulated by changes in transmembrane potential, variations in cytosolic pH, changes in $[Ca^{2+}]_i$ and covalent modifications of the protein subunits.[3,34,35] Thus, gap junction channels may facilitate the intercellular exchange of ions, metabolites (*e.g.*, ATP, ADP, glucose, glutathione and glutamate) and second messengers (*e.g.*, cAMP and IP_3),[35–40] depending on subunit composition and functional state.[2] In a recent study, permeability differences were demonstrated by simultaneously evaluating electrical coupling and flux of fluorescent tracers of different size and charge.[41] In homomeric gap junctions formed of different Cxs, permeability to negatively charged probes is in decreasing order: Cx43 > Cx45 > Cx26 > Cx40. On the other hand, these channels showed fewer permeability differences to positively charged molecules. Twenty one different Cxs are expressed in humans, and a large number of different homomeric and heteromeric gap junction channels remain to be evaluated.

The contribution of gap junction channels to intercellular transfer and to particular cell functions has been widely studied by using gap junction channel blockers, such as long chain alcohols (*e.g.*, octanol and heptanol), licorice-derived gap junction blockers (*e.g.*, 18-α-glycyrrhetinic acid, 18-β-glycyrrhetinic acid and carbenoxolone), chloride channel blockers (*e.g.*, flufenamic and niflumic acids) and peptides with the same sequence of the extracellular loops of Cxs.[3,5,32,42] Gap junctional communication is essential for many physiological events including synchronization of electrical and metabolic responses during ontogeny and adulthood, and Cx mutations appear to be the basis of several human diseases.[3,43–47]

3. Connexins and pannexins

The human and rodent genomes have 20–21 different Cx genes, most of which lack introns in their coding sequence.[48,49] Cxs are synthesized in the rough endoplasmic reticulum (rER) and most of them seem to oligomerize into hemichannels in the ER/trans Golgi network.[3,6] Several covalent modifications have been detected in Cxs including acylation, phosphorylation and ubiquitinylation.[3,50,51] Phosphorylation has been implicated in Cx trafficking, channel function, assembly and internalization of gap junction plaques.[52–56] Most Cxs studied up until now show a life time of only a few hours[56] and, thus, intercellular communication can be affected by changes in

the amount of channels present at cell–cell interfaces. Connexins in lens gap junctions on the other hand might last a life time.

The distribution of Cx subtypes is tissue-specific, and most cell types express more than one Cx subtype. Some Cxs are ubiquitously expressed; others have a more restricted distribution. For example, Cx26 is abundantly expressed in hepatocytes, keratinocytes and cochlear supporting cells while Cx43 is present in many cell types including cardiomyocytes, astrocytes, keratinocytes, uterine myocytes, endothelial cells and epithelial cells from different organs.[3,6,49] The repertoire of Cxs expressed by each cell type presumably subserves cell specific requirements.

Pannexins are highly conserved transmembrane proteins encoded by three different genes, PANXs 1, 2, and 3, in the human and rodent genomes.[22] Panx proteins have no sequence homology with connexins, but they are related to the invertebrate gap junction proteins, called innexins.[23] Panx1 is the most widely expressed subtype.[23] The membrane topology of pannexins is similar to that of connexins. Unlike connexins, pannexins are glycosylated. In NRK cells, glycosylation occurs in asparagine 254 of Panx1 and asparagine 71 of Panx3. A single amino acid substitution of these residues by glutamines reduces the amount of protein at the plasma membrane and induces their accumulation in intracellular compartments, suggesting the role of glycosylation in targeting the protein to the plasma membrane.[57,58] However, it might also play a relevant role in stabilizing the protein in the cell membrane. The turnover rates of Panx1 and Panx3 are slower than that of Cx43.[57] The degradation pathways for pannexins remain unknown.

Members of the connexin and pannexin families, such as Cx43 and Panx1, are endogenously co-expressed in many cell types.[6,22,24,58] There is no indication of formation of heteromeric channels made of both Cx and Panx.

4. Cx hemichannels

Ultrastructural studies have revealed that Cx hemichannels are cylindrical, ~7.5 nm in height and with an outer diameter of ~7 nm.[28,59,60] The pore mouth diameter varies among hemichannels formed by different Cxs [*e.g.*, ~1.4 nm for Cx26 hemichannels[61,62] and ~2.5 nm for Cx43 hemichannels[60]]. This finding suggests that different homomeric hemichannels may differ in perm-selectivity. Along the same line, differences in perm-selectivity of heteromeric hemichannels and homomeric hemichannels have been demonstrated.[2,42]

Most Cx hemichannels show a low open probability in cells cultured under resting conditions, as it probably should be for the cells to stay alive. Controlling factors could be low open probability at negative membrane potentials and millimolar concentrations of extracellular divalent cations.[9,63–67] Because of their low open probability, Cx hemichannels can participate in several physiological processes without compromising cell viability. They are involved in paracrine interactions between neurons and glia,[3] and in the regulation of cell volume.[68] Cx43 hemichannels also participate in the transduction of alendronate-induced survival signals in osteoblastic cells,[69] as well as in the proliferation of fibroblasts[70] and neural retinal progenitor cells.[71] In addition, hemichannels formed by Cx43 and Cx36 have a protective effect in hypoxic preconditioning of cultured rat astrocytes[72] and neurons,[73] respectively.

Opening of Cx hemichannels is increased at inside positive potentials, by mechanical stimulation, and by reduction of extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$).[5,11,13,67] Pharmacological agents such as bisphosphonates,[69] quinine or quinidine[43,74] and membrane permeant sulfhydryl group reducing agents[67] increase the opening of Cx43 hemichannels in the presence of physiological extracellular concentrations of divalent cations. In contrast, lanthanides, Cx mimetic peptides and classic gap junction blockers decrease the open probability of Cx hemichannels.[3,5,11] Endogenous extracellular ligands can also increase the open probability in the presence of extracellular divalent cations and at resting membrane potentials.[13,14,75,76]

The mechanisms governing hemichannel opening in response to these different stimuli are poorly understood. Nevertheless, by analogy with other ion channels they might involve changes in the number of hemichannels at the cell surface, hemichannel open probability and/or single hemichannel conductance/permeability.

Increased hemichannel activity in the presence of extracellular divalent cations also occurs under several pathological conditions, including Shigella infection,[77] chemical metabolic inhibition,[8,9,78–80] oxygen and glucose deprivation[81], cytokine-mediated inflammation,[10,76,82] oxidative stress[83] and some inherited diseases associated with Cx mutations.[64,84–88] Consistent with the regulation of hemichannels by growth factors, it is known that the activity of Cx43 hemichannels is reduced by protein kinases of intracellular pathways activated by growth factors, such as PKC or MAP kinase, and is increased by dephosphorylation.[89–92]

Hemichannel opening can greatly increase membrane permeability, thus facilitating transmembrane diffusion of ions (*e.g.*, Na^+ and Ca^{2+}) and small molecules (*e.g.*, ATP, NAD^+, ascorbic acid, glutamate, prostaglandin E_2 and glutathione) down their concentration gradients.[11,12,70,71,93–99] Prolonged or frequent openings of Cx hemichannels can increase the cell susceptibility to harmful conditions and can accelerate cell death through multiple mechanisms, including collapse of electrochemical gradients, loss of metabolic substrates and cofactors, as well as influx of toxic signals.[8,9,78,83,100] What determines the participation of Cx hemichannels in different cell responses is likely to depend not only on the nature, intensity and duration of the activating stimuli, but also on the permeability properties of the affected hemichannels and the state of the cells.

5. Modulation of gap junction channels by growth factors

Most growth factors act through high affinity binding to dimeric transmembrane receptors with intrinsic protein kinase activity in their intracellular domains. Binding to their receptors activates intracellular serine/threonine kinases (*e.g.* MAPKs and PI3K), tyrosine kinases (*e.g.* Src), second messenger pathways (*e.g.* IP_3 and Ca^{2+}) and transcription factors (*e.g.* STATs), leading to a great variety of cell responses, frequently associated with cell growth, survival, motility, and differentiation.[100–104] A number of growth factors have been shown to affect the functional state

of Cx gap junction channels (Table 1). Future studies using reconstituted systems will help clarify the mechanisms.

a. Epidermal growth factor (EGF)

The first report of EGF effect on gap junctional communication showed a rapid (<2 min), reversible and concentration-dependent reduction in intercellular transfer of fluorescent dyes (Lucifer yellow and lissamine rhodamine B-labeled glutamic acid) and transjunctional conductance in NRK and BALB/c 3T3 cells.[105] The effect occurred with nanomolar EGF concentrations and lasted for up to 30 min after treatment. The permeability of the nonjunctional membrane of isolated cells was not significantly affected, suggesting that hemichannel activity remained either unchanged or it was reduced.

Shortly thereafter, it was shown that application of 10 ng/ml of EGF for 24 h reduced gap junctional communication and increased cell proliferation in cultured human keratinocytes.[106] The EGF induced responses were not associated with rapid changes in $[Ca^{2+}]_i$ or increased PKC activity. The EGF effect on gap junction intercellular communication was then demonstrated in different cell types expressing Cx43.[76,107–113] In most cases, EGF reduced the intercellular communication *via* gap junctions, which was correlated with Cx43 phosphorylation on serine residues 255, 279, and 282,[111,112,114] indicating the involvement of serine/threonine kinases. It was proposed that ERK1/2 (mitogen-activated protein kinases; MAPK) mediated this effect, because the cytoplasmic C-terminal tail of Cx43 contains several consensus sequence motifs for ERK phosphorylation. Moreover, the EGF-induced gap junctional uncoupling was prevented by PD98059, an ERK1/2 inhibitor.[111,115] BMK1/ERK5, another MAPK family member, was also shown to participate in EGF-induced uncoupling through phosphorylation of Cx43 at serine residue 255.[113] However, in some cell types such as the K7 human kidney epithelial cell line, EGF increased gap junctional communication.[115] In these cells, EGF induced rapid (15 min) Cx43 phosphorylation, which was not temporally associated with the increase in gap junctional communication that occurred after 2–3 h.[116] In addition, EGF increased the levels of Cx43 transcripts and protein in granulosa cells,[117] as well as in cultured porcine preantral follicles,[118] but reduced them in rat cortical astrocytes.[119] The mechanisms underlying EGF-induced reduction in gap junctional communication are not completely elucidated. Nevertheless, changes in gap junction channel open probability,[112] permeability,[120] Cx43 levels and distribution,[115,117–119] and turnover[121] have been implicated (Table 1).

The expression of Cx32 in cultured hepatocytes is enhanced by EGF.[122] In addition, in isolated rat liver plasma membranes, EGF induced Cx32 phosphorylation mainly on tyrosine residues.[123] EGF treatment for 24 h reduced the total Cx40 immunoreactivity and its plasma membrane localization in the human placental choriocarcinoma cell line, JAR[124] (Table 1).

EGF also affects the functional state of Cx hemichannels. In cultured rat astrocytes pre-loaded with calcein-AM, EGF in combination with FGF-2 induced a rapid (10 min) and sustained (48–96 h) decrease in hemichannel-mediated release of the dye induced by exposure to an extracellular solution with low concentration of divalent cations.[76] This effect was not associated with changes in total levels of Cx43 or shifts in electrophoretic mobility that would reveal alterations in its state of phosphorylation. The effect was inhibited by U0126, a MAPK inhibitor, and reverted by IL-1β, a pro-inflammatory cytokine known to activate MAP kinases.[125] Treatment with either U0126 or IL-1β enhanced hemichannel activity exposed to a divalent cation-free solution. This phenomenon was not observed in control tissue. These results suggest a role for the MAPK pathway in the EGF-induced modulation of hemichannels.[76]

b. Fibroblast growth factors (FGFs)

Pioneering studies in the early 90's revealed close proximity of FGF-2 and Cx43 in both rat glial cells and intercalated discs of myocardiocytes, by immunofluorescence and immunoelectron microscopy, suggesting a possible regulatory/modulatory function for FGF-2 on Cx43 gap junctions.[126,127] Then, FGF-2 was shown to induce a biphasic effect on gap junction intercellular communication in cardiac fibroblasts, reducing it during the first 30 min (short term effect), but increasing it after longer periods (>6 h)[128] (long term effect). The late increase in dye coupling induced by FGF-2 was associated with a concentration-dependent increase in Cx43 mRNA and protein levels. In neonatal rat cardiomyocytes, short-term treatment with FGF-2 also induced a reduction in intercellular communication that was associated with increased Cx43 phosphorylation on serine residues.[129] Nevertheless, the FGF-2-induced effect was blocked by genistein, a tyrosine kinase inhibitor, but not by the MAPK inhibitor, PD98059.[129] Although a fraction of Cx43 was immunoprecipitated with anti-phosphotyrosine-specific antibodies in FGF-2-treated myocytes, Cx43 phosphorylation on tyrosine residues was not detected, suggesting interaction and coprecipitation of Cx43 with phosphotyrosine-containing protein(s) rather than a direct action of a tyrosine kinase on Cx43.[129] However, in cardiomyocytes treated with FGF-2 immunoprecipitation of Cx43 using specific anti-Cx43 antibodies, PKCε, but not PKCα, coprecipitated with Cx43, a result that is consistent with the amino acid analysis that revealed phosphorylation only on serine residues.[130] Accordingly, FGF-2-induced Cx43 phosphorylation and intercellular dye transfer changes were inhibited by chelerythrine, a PKC antagonist.[129] Moreover, overexpression of dominant-negative forms of PKCε decreased Cx43 phosphorylation in cardiomyocytes.[130] Therefore, the FGF-2-induced reduction in gap junctional communication between cardiomyocytes was likely due to PKCε-mediated phosphorylation of Cx43.

In addition to its effect on intercellular communication in cardiac cells, FGF-2 induced a time-dependent increase in intercellular transfer of Lucifer yellow in bovine microvascular endothelial cells, which reached its maximum after 8–9 h of treatment.[131] The increase in dye coupling was associated with increased Cx43 mRNA and protein levels, and was inhibited by an FGF-2 neutralizing antibody.

Blood brain barrier (BBB) formation and function depend, in part, on astrocyte-derived molecules acting on the endothelium.[132] Among soluble factors released by astrocytes, FGF-2 has been demonstrated to mimic astrocyte activity on immortalized endothelial cells (ECs).[133] However, information on gap junctions (GJs) is conflicting. Shivers and collaborators[134] reported that gap junctions are up-regulated *in vitro* in non-central nervous

Table 1 Summary of most if not all published works on the effect of different growth factors on the gap junctional intercellular communication (GJIC), hemichannel (HC) activity, phosphorylation state of connexin, levels of connexin protein and/or mRNA in different cell types. NA = not affected. Numbers in the right column indicate the corresponding reference(s)

Growth factor	Cell type	Concentration	Effects	Time course	References
EGF	Rat kidney cell line (NRK), Balb/c 3T3	16 nM	↓ GJIC	10 min	105
	T51B (rat liver epithelial cells)	25 ng/ml	↓ GJIC	25 min	108
	Jar (human choriocarcinoma cells)	10 ng/ml	↓ Cx40 protein	24 h	124
	Human kidney epithelial cells	100 ng/ml	↑ Cx43 protein ↑ GJIC	2–7 h	181
	Human keratinocytes	10 ng/ml	↓ GJIC	24 h	106
	T51B (rat liver epithelial cells)	20 and 25 ng/ml	↓ GJIC ↑ Cx43 phosphorylation	10–180 min	107,108
	WB Cells (rat liver cell line)	10 ng/ml	↓ GJIC	10 min	109
	HUVEC (young)	16 nM	↓ GJIC	1 h	110
	HUVEC (senescent)	16 nM	= GJIC	1 h	110
	T51B (rat liver epithelial cells)	25 ng/ml	↓ GJIC	30 min	114
	Cx43 knockout mouse cell line expressing exogenous Cx43-wt	100 ng/ml	↓ GJIC	30 min	111,112
	Cx43 knockout mouse cell line expressing exogenous Cx43-wt	100 ng/ml	↓ GJIC	20 min	
	HEK293 cells transiently transfected with Cx43	100 ng/ml	↓ GJIC	20 min	113
	IAR6.1 (rat liver epithelial cell line)	100 ng/ml	↓ GJIC	1h	115
	K7 (human kidney epithelial cell line)	1–100 ng/ml	↑ GJIC	4 h	116
	Immature rabbit granulosa cells	10 ng/ml	↑ Cx43 protein ↑ Cx43 mRNA	48 h	117
	Cultured porcine preantral follicles	50 and 500 ng/ml	↑ Cx43 protein	8 days	118
	Cultured rat cortical astrocytes	20 ng/ml	↓ Cx43 protein ↓ Cx43 mRNA	48 h	119
	Primary cultures of rat hepatocytes	10 ng/ml	Maintenance of Cx32	4 weeks	122
	EGF receptor and isolated liver gap junctions	10 μM	↑ Cx32 phosphorylation	5 min	123
FGF-β1	Rat Schwann cells	100 ng/ml	↓ GJIC	15 min	182
EGF and bFGF	Cortical neonatal rat astrocytes	5 and 10 ng/ml	↓ HC activity	10 min	76
G-CSF	Primary rat cardiomyocytes	10 ng/ml	↑ GJIC	24 h	166
FGF-2	HeLa cells transfected with Cxs	20 ng/ml	↑ HC activity	7 h	13
	Spinal astrocytes	10 ng/ml	↓ GJIC ↑ Cx43 protein ↑ Px1 HC activity	2 and 7 h	14
	Primary cultures of chick lens epithelial cells	15 ng/ml	↑ GJIC	48 h	138,167
	Rat cardiac fibroblasts	5 ng/ml	↑ Cx43 mRNA ↑ Cx43 protein ↑ GJIC	6 h	128
bFGF	Mouse osteoblastic MC3T3-E1 cells	0.3 nM	↓ Cx43 mRNA ↓ Cx43 protein ↓ GJIC	24 h	139
	Cortical and striatal astrocytes	10 ng/ml	↓ Cx43 mRNA ↓ Cx43 protein ↓ GJIC	48 h	183
	HeLa and C6 cells transfected with Cxs	10 ng/ml	↓ GJIC ↑ HC activity	6 h	12
	Mouse osteoblastic MC3T3-E1 cells	5 ng/ml	↑ Cx43 phosphorylation	30 min	140
	Cardiac fibroblasts	5–10 ng/ml	↑ Cx43 mRNA ↑ Cx43 protein	6 h	128
	Cardiomyocytes	10 ng/ml	↓ GJIC	30 min	129,130
	Cardiomyocytes	10 ng/ml	↓ GJIC ↑ Cx43 phosphorylation	15 min	

Table 1 (*continued*)

Growth factor	Cell type	Concentration	Effects	Time course	References
	Bovine microvascular endothelial cells	3 ng/ml	↑ Cx43 ↑ GJIC	10–12 h	131
	Cortical progenitor cells	10–30 ng/ml	↑ Cx43 mRNA ↑ Cx43 protein	4 h	136
	Cultured midbrain dopaminergic neurons	10 ng/ml	↑ Cx43 mRNA ↑ Cx43 protein ↑ GJIC	24 h	137
	Mouse osteoblastic MC3T3-E1 cells	2.5–50 ng/ml	↑ Transcriptional activity cDNA rat Cx43	16 h	141
FGF-9	Astroglial cells	10 ng/ml	↓ Cx43 protein ↓ Cx43 mRNA ↓ GJIC	48 h	142
FGF-5	Astroglial cells	10 ng/ml	↓ Cx43 protein ↓ Cx43 mRNA ↓ GJIC	48 h	142
HGF	Mouse keratinocyte cell line	0.3 nM	↓ Cx43 protein ↓ GJIC	1 h	163
	Rat Hepatocytes	20 ng/ml	↓ Cx32 protein ↓ GJIC	3–12 h	164
HGF + TGFβ1	Rat hepatocytes	10 and 20 ng/ml	NA GJIC	12 h	164
IGF	Astrocytes	30 ng/ml	↑ Cx43 protein ↑ GJIC	24 h	160
	Rabbit lens epithelial cells (N/N1003A)	25 ng/ml	↓ Cx43 protein ↓ GJIC	20, 30 min	158
	Vascular smooth muscle cells	100 ng/ml	↑ Cx43 protein ↑ Cx43 mRNA	24 h	161
	Primary cultures of chick lens epithelial cells	15 ng/ml	↑ GJIC	48 h	138
NGF	PC12 transfected cells	30 ng/ml	↑ GJIC	15 min	157
	Ovarian thecal cells	100 ng/ml	↓ GJIC ↑ Cx43 phosphorylation	10–30 min	156
	PC12 Cells transfected with Cx32, Cx43	50 ng/ml	↑ HC activity	48 h	75
PDGF	Rat kidney cell line (NRK), BalbC 3T3 cells	0.6 nM	↓ GJIC	10 min	105
	NIH 3T3 cells	0.6 nM	NA GJIC	10 min	105
	Primary human smooth muscle cells	0.5–0.7 nM	↓ GJIC	1 h	184
	T51B (rat liver epithelial cells)	10 ng/ml	↓ GJIC ↑ Cx43 phosphorylation	15–20 min	144,148
	Mouse fibroblast cell line (C3H/10T1/2)	10 ng/ml	↓ GJIC	40 min	143
	Mouse fibroblasts cell line (3T3 A31)	10 ng/ml	↓ GJIC	10–20 min	145
	Rat mesangial cells	10 ng/ml	↓ GJIC	15–30 min	146
	Rat mesangial cells	50–100 ng/ml	↑ Cx43 protein	48 h	151
TGF-β	Rat kidney cell line (NRK)	0.1 nM	↓ GJIC	10 min	105
	BalbC 3T3 cells	0.1 nM	↑ GJIC	8 h	105
	Cardiac myocyte	100 ng/ml	↑ Cx43 protein	1 h	154
	Human keratinocytes	1 ng/ml	↓ GJIC	24 h	106
TGF-β3	Primary cultures of chick lens epithelial cells	4 ng/ml	↑ GJIC	48 h	167
	Rat folliculostellate cells	10 ng/ml	↑ GJIC	24 h	185
	Cortical and striatal astrocytes	10 ng/ml	↓ GJIC	48 h	183

Table 1 (*continued*)

Growth factor	Cell type	Concentration	Effects	Time course	References
BMP2, BMP4 and BMP7	Primary cultures of chick lens epithelial cells	10–50 ng/ml	↑ GJIC	6–48 h	186
BMP2 and BMP4	Embrional mouse cell line (P19)	5 and 10 ng/ml	↑ Cx43 protein ↑ GJIC	8–16 days	170
VEGF	Human umbilical vein endothelial cells (HUVEC)	50 ng/ml	↓ GJIC	15 min	152
	Endothelial cell line (Ea.hy926)	50 ng/ml	↓ GJIC ↑ Cx43 protein phosphorylation	15–30 min	152
	Cardiac myocyte	100 ng/ml	↑ Cx43 protein	1 h	154
	Rat left ventricular capillary cells	50 ng/ml	↓ GJIC	15, 30 min	153
GGF-2	Schwann cells	5 ng/ml	↑ Cx32	12 h	165

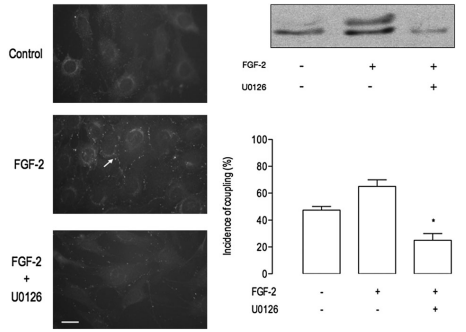

Fig. 1 FGF-2 up-regulates the levels of Cx43 and intercellular communication *via* gap junction in endothelial cells. RBE-4 cells, cell line derived from rat brain microcirculation (kindly provided by Dr P. O. Couraoud, Laboratoire d'Immuno-Pharmacologie Moléculaire, Institut Cochin de Genetique Moléculaire, Paris, France) were cultured in MEM/F10 medium without exogenous FGF-2, with 1 ng/ml FGF-2 for 3 h or the ERK1/2 inhibitor U0126 (10 μM in DMSO) applied 1.5 h before FGF-2. Left panels show the immunoreactivity (arrow, middle panel) of Cx43 in each condition. Bar: 20 μm. Top right panel, Western blot analysis of Cx43 in total homogenates of cells under each condition. Graph showing incidence of dye coupling (Lucifer yellow). Each bar represents the mean value ± SE of 3 independent experiments.

system endothelial cells by astrocyte-conditioned medium, while Tao-Cheng and collaborators[135] reported that gap junctions are diminished in brain endothelium *in vivo* as well as *in vitro*. Moreover, FGF-2 released by astrocytes enhances dye coupling between RBE-4 cells, a cell line derived from rat brain microcirculation. The effect mimicked by FGF-2 added to the cell culture and was associated with increased Cx43 levels, formation of large gap junction plaques and more frequent dye coupling, as compared to control cells (Fig. 1).

FGF-2 applied for 4 h also increased Cx43 levels and intercellular dye coupling in rat embryonic (E16) cortical brain cells. This effect was inhibited by MTA, an inhibitor of FGF-2 receptor tyrosine kinase.[136] The addition of FGF-2 to primary rat embryonic (E14) midbrain cell cultures for 24 h increased cell survival and dye coupling, as well as transcript and protein levels for Cx43 (but not for Cxs 26, 32 or 45). The increased cell survival induced by FGF-2 was inhibited by oleamide, a gap junction blocker, suggesting a direct role of increased intercellular communication in the transmission of cell survival signals.[137] In addition, treatment of serum-free cultures of chicken embryonic lens epithelial cells with FGF-2 or FGF-1 for 24 h induced a concentration-dependent and reversible increase in gap junction intercellular communication.[138] The effect on gap junctions required ERK activation, but was not accompanied by changes in Cx protein levels. The latter effect of FGFs has been linked to the intralenticular gradient of gap junction-dependent intercellular communication required for maintenance of lens transparency.[138]

Conversely, FGF-2 applied from 8–24 h induced a reduction in Cx43 protein levels and intercellular dye transfer in MC3T3-E1 osteoblastic cells.[139] Short-term (≤ 30 min) treatment of these cells with FGF-2 induced a transient association of Cx43 with

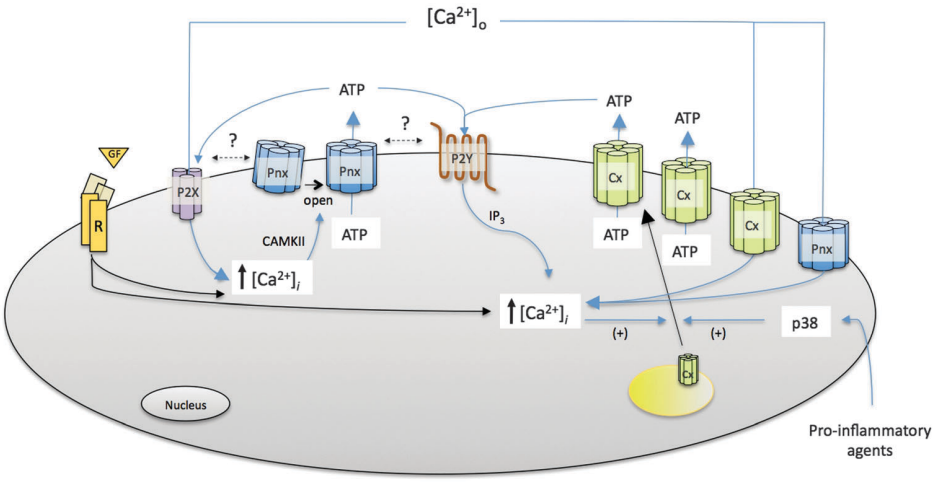

Fig. 2 Scheme of the effect of growth factors and pro-inflammatory agents on the intracellular free Ca^{2+} concentration and the role of P2 receptors and hemichannels. Binding of a growth factor (GF) to its receptor (R) leads to a rise in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$ that enhances opening of pannexin (Pnx) hemichannels possibly through calcium/calmodulin kinase II activation. Open Pnx hemichannels permit the release of intracellular ATP to the extracellular milieu where they encounter P2 receptors (P2X and/or P2Y receptors), which contribute to further increase the $[Ca^{2+}]_i$. The latter leads to an increase in surface levels of connexin (Cx) hemichannels through which more ATP is released that further activates P2 receptors. The increase in surface Cx is probably due to insertion of preformed Cx hemichannels present in intracellular vesicles. Pro-inflammatory agents *via* activation of p38 kinase can also increase the levels of Cx hemichannels present at the cellular surface. Both Pnx and Cx hemichannels are permeable to Ca^{2+}, which contributes to increase the $[Ca^{2+}]_i$.

activated PKC-δ at the plasma membrane, and a significant increase in Cx43 phosphorylation at serine 368.[140] It is interesting to note that Cx43 could modulate the response of osteoblasts to FGF-2, since overexpression of Cx43 increased the transcriptional response of osteocalcin in MC3T3-E1 osteoblasts, which is an effect that also depended on PKC-δ activation. In addition, application of FGF-2 induced the phosphorylation and translocation of PKC-δ to the nucleus in these cells.[141]

FGF-2 also reduced dye coupling as well as Cx43 transcripts and protein levels in cortical and striatal (but not mesencephalic) astroglial cells.[142] These results confirm the cell type-specific nature of the FGF-2-induced effects on gap junction-mediated intercellular communication. More recently, it was reported that stimulation of Cx43-transfected rat C6 glioma cells with FGF-2 for 6 h reduces dye coupling, and that the effect was partially inhibited by genistein.[12] In addition, treatment of Cx43-transfected HeLa cells or primary mouse spinal astrocytes with FGF-1, in conjunction with heparin, reduced the intercellular transfer of Lucifer yellow.[13,14] While reduction of intercellular communication after 7 h of FGF-1 treatment was not associated with variations in total Cx43 levels[13] in HeLa-Cx43 cells, spinal astrocytes showed a 40% reduction in Cx43 levels under identical experimental conditions.[14] Interestingly, the effect of FGF-1 was prevented by inhibitors of vesicular release, as well as by blockers of $P2X_7$ receptors or Panx hemichannels (Fig. 2). Moreover, reductions in dye coupling induced by FGF-1 in spinal astrocytes were sensitive to apyrase and mimicked by exogenous ATP.[14] Thus, it was proposed that FGF-1 induces vesicular ATP release, followed by activation of Panx hemichannels that allow further ATP release. Since the reduction in dye coupling induced by FGF-1 was sensitive to apyrase and was also mimicked by exogenous ATP,[14] it was suggested that the FGF-1 effect in spinal astrocytes was mediated by extracellular ATP (Fig. 2).

FGF-5 and FGF-9 induce downregulation of astroglial gap junctions and functional coupling in specific brain regions. FGF-5 specifically affects mesencephalic astroglial cells without altering cortical or striatal astroglial coupling, while FGF-9 reduces gap junctional coupling in all three brain regions.[142] Thus, members of the fibroblast growth factor family affect gap junction intercellular communication. However, the responses are complex and concentration-, time- and cell type-dependent. Moreover, the molecular mechanisms leading to these responses are not completely understood.

FGF-2 enhances the release of ATP after exposure of rat C6 glioma cells transfected with Cx43 to a solution lacking divalent cations.[12] Under the same experimental conditions, FGF-2 reduces the release of ATP in Cx43-expressing HeLa cells, but stimulates it in HeLa cells transfected with Cx26 or Cx43 truncated at amino acid 239. The effect of FGF-2 was evident 6 h after stimulation and was not affected by genistein. Since the FGF-2-induced effect was not associated with changes in Cx solubility in Triton X-100, it was suggested that FGF-2 did not affect the cellular distribution of Cxs.[12]

Data from our group showed that incubation of Cx43-transfected HeLa cells with FGF-1 for 4 to 14 h increased rate of hemichannel-mediated ethidium (Etd) bromide uptake, and current events in the presence of physiological concentrations of extracellular divalent cations (1.8 mM Ca^{2+} and 1 mM Mg^{2+}).[13] The FGF-1-induced increase in the hemichannel activity correlated with Cx43 levels in cells expressing Cx43 fused to green fluorescent protein (GFP). Treatment with FGF-1 induced only unitary events of around 220 pS that were fully blocked by lanthanum ions,[13] properties that are characteristic of Cx43 hemichannels.[7,10,65,67] This finding ruled out the

involvement of plasma membrane channels/hemichannels other than Cx43 hemichannels in FGF-1-induced cell permeabilization. The FGF-1 effect was maximal at 7 h of FGF-1 incubation, and was accompanied by a proportional increase in Cx43 levels at the plasma membrane, with no detectable variation in total Cx43 levels. Thus, redistribution of Cx43 might explain the increase in Cx43 hemichannel activity. Similarly, FGF-1 increased the rate of Etd uptake and hemichannel levels at the plasma membrane in HeLa cells transfected with Cx45, but had no effect on HeLa cells expressing Cxs with a short C-terminus (*e.g.*, Cx43 truncated at amino acid 257 or wild type Cx26). In addition, the FGF-1 effect was prevented by intracellular BAPTA, a Ca^{2+} chelator, and mimicked by a Ca^{2+} ionophore in responsive cells, indicating that rises in $[Ca^{2+}]_i$ were required for the FGF-1-induced effect on Cx hemichannels.[13] The increase in the Etd uptake rate induced by FGF-1 or a Ca^{2+} ionophore in HeLa-Cx43 cells was also reduced by SB202190, a p38 MAPK inhibitor. This indicates the involvement of this kinase in the observed hemichannel responses.[13] However, in Cx43-transfected HeLa cells previously exposed to a solution lacking divalent cations, FGF-1 reduced the Etd uptake rate, an effect that is most probably related to the rapid (<15 min) but transient activation of the MAPK-dependent pathway and possible Cx43 phosphorylation that occurred immediatly after FGF-1 addition.

It was recently reported that FGF-1 increased the hemichannel-mediated dye uptake (Lucifer yellow and Etd) in primary rat spinal astrocytes. The effect showed temporal variations, being predominantly mediated by Panx hemichannels after 2 h of FGF-1 incubation, and by Panx and Cx hemichannels after 7 h.[14] The effect was mediated by the activation of type 1 FGF receptor protein kinase (FGFR1), and was partially dependent on extracellular ATP mediated signaling, possibly through $P2X_7$ purinergic receptors. As in other examples, Cx43 gap junction channels and hemichannels were regulated in opposite ways by FGF-1 (Fig. 2).

c. Platelet-derived growth factor (PDGF)

The effect of PDGF on gap junctional communication has been studied in several Cx43-expressing cell types.[105,143–146] In contrast to EGF and FGFs, PDGFs reduced intercellular communication mediated by gap junctions in all cell types studied. In the mouse fibroblast cell line, C3H/10T1/2, PDGF induced a rapid (<40 min), transient and concentration-dependent reduction in gap junctional communication.[143] This response was associated with Cx43 phosphorylation on amino acid residues other than tyrosine. Accordingly, genistein did not affect the PDGF-induced inhibition of gap junctional intercellular communication.[143]

T51B cells are a rat liver epithelial cell line lacking endogenous PDGF receptors. After transfection of these cells with a retrovirus encoding wild type human PDGFRβ, PDGF caused a rapid (15–20 min), complete but transient interruption of cell communication.[144] This response was associated with increased Cx43 phosphorylation and reduced Cx43 levels. Interestingly, treatment with the antimalarial drug primaquine, a lysosomotropic amine that acts as an inhibitor of lysosomal degradation, increased the relative amount of slowly migrating (and presumably more phosphorylated) forms of Cx43, such as Cx43-P3 and other bands of even higher molecular weight.[144] However, primaquine has been shown to inhibit intracellular protein transport in cells in culture, both at the secretory and recycling levels; its mechanism of action remains uncertain.[147]

PDGF-induced Cx43 phosphorylation and decrease in dye coupling, effects that correlated with activation of MAPK; prevention of MAPK activation by PD98059 abolished the PDGF-induced effects.[148] Because the PDGF-induced increase in Cx43 phosphorylation and decrease in dye coupling were also completely blocked by inhibitors of PKC such as calphostin C, it was suggested that PKC is required for MAPK activation, but PKC does not directly phosphorylate Cx43.[148] Pretreatment of T51B cells expressing human PDGFRβ for 30 minutes with hydrogen peroxide (a potent MAPK activator, which by itself does not affect Cx43 phosphorylation or gap junction communication) abolished the PDGF-induced Cx43 phosphorylation and reduction in dye coupling. However, simultaneous treatment with hydrogen peroxide and PDGF eliminated only the decrease in gap junctional communication, but not the changes in Cx43 phosphorylation.[149] It is worth noting that PDGF did not affect dye coupling in cells expressing a PDGFR mutant lacking binding sites for phosphatidylinositol 3-kinase (PI3K), GTPase-activating protein, SHP-2 and phospholipase C-γ1. Restoration of either SHP-2 or PLCγ1 binding sites recovered the inhibitory effects of PDGF on gap junctional communication.[150] Thus, activation of both PKC and MAPK were required for the PDGF-induced effects. The decrease in gap junctional communication not only involved MAPK activation, but required complex interaction of additional signaling components.

3T3 A31 fibroblasts exposed to PDGF exhibited a 50% decrease in intercellular dye transfer, without detectable changes in Cx43 levels or distribution.[145] PDGF had no effect on dye coupling of cells transfected with Cx43 truncated at amino acid 256 with a myc tag appended to its C-terminus. This could indicate that signals responsible for the reduction in gap junctional communication interact with a Cx43 target site located within amino acids 257 to 382.[145]

In mesangial cells, PDGF induced a transient and relatively rapid (15 min) reduction in dye coupling without detectable alterations in Cx43 distribution. This PDGF effect was associated with Cx43 tyrosine phosphorylation, mimicked by a tyrosine phosphatase inhibitor and prevented by the PI3K inhibitor LY294002.[146] Treating these cells with PDGF-BB (a homodimer of PDGF B-chain) in the presence of 3-isobutyl-1-methylxanthine (a nonspecific phosphodiesterase inhibitor) dramatically increased the total levels and surface localization of Cx43. Such effects were associated with increased dye coupling.[151] To our knowledge, the functional relevance of this unexpected potentiation between PDGF and cAMP levels remains unknown. In addition, it remains unknown if PDGF affects Cx hemichannels.

d. Vascular endothelial growth factor (VEGF)

Similar to other growth factors, VEGF induced a fast (<15 min) and reversible reduction in dye coupling evaluated with Lucifer yellow in human umbilical vein endothelial cells (HUVEC) and Ea.hy926 cells.[152] The effect was mediated by VEGFR-2, required the activation of Src and MAPK, and was associated with Cx43 phosphorylation, apparently at serine/threonine residues, without

changes in total Cx43 levels. VEGF also abolished gap junctional communication within 30 min in primary cultures of rat ventricular capillary endothelial cells.[153] In this experimental system, the effect on the intercellular transfer of Lucifer yellow was accompanied by tyrosine phosphorylation and a marked decrease of Cx43 at appositional membranes, as well as increased reactivity at intracellular compartments. Tyrosine phosphorylation of Cx43 was prevented by wortmannin (a PI3K inhibitor), but not by Ro31-8220 (a PKC inhibitor). This implies the participation of a PI3K-dependent pathway in the VEGF-induced effect on gap junctional communication.[153]

VEGF has been implicated in the stretch-induced increase in Cx43 levels and conduction velocity observed in neonatal rat cardiomyocytes cultured in native type I collagen. It has been proposed that VEGF is released from cardiac myocytes subjected to stretch, because anti-VEGF antibodies inhibit the stretch-induced responses, and stretch-conditioned media upregulate Cx43 in non-stretched cardiomyocytes.[154] Interestingly, exogenous VEGF did not affect Cx43 levels in neonatal rat ventricular myocytes grown on fibronectin or denatured collagen. This suggests a role for extracellular matrix components in the VEGF-induced effect on gap junctions,[155] which is probably due to the availability of VEGF receptor. To our knowledge, the effect of VEGF on Cx hemichannels has not been reported.

e. Nerve growth factor (NGF)

NGF disrupts the intercellular transfer of calcein in isolated bovine ovarian thecal cells, which endogenously express Cx43; this effect required the activation of receptor tyrosine kinase, TrkA.[156] The NGF-induced reduction in the intercellular dye transfer was associated with a relatively rapid (10–30 min) increase in Cx43 phosphorylation at serine (but not tyrosine) residues. EGF, neurotrophins or brain-derived neurotrophic factors did not affect dye transfer in these cells, indicating a specific NGF-induced effect. However, shorter (<30 min) and longer (96 hours) incubations with NGF induced opposite effects on dye coupling between PC12 cells (a cancer cell line derived from a rat pheochromocytoma) retrovirally transduced to express Cx32 or Cx43.[157] The NGF-induced effect on gap junctional communication required the presence of wild type TrkA. This effect was paralleled by Cx43 ERK1/2 phosphorylation, and was prevented by pretreatment with the MEK1/2 inhibitor U0126. Notably, the change in Cx43 phosphorylation was not observed in PC12 cells expressing TrkA receptors mutated at Tyr499 and Tyr794 which show a marked reduction in MAPK signaling. These results suggest that a competent Ras-MAPK signaling pathway downstream of TrkA in target cells was necessary for the NGF-induced effects.[157]

Treatment with NGF for 24 to 48 h enhances neurite outgrowth in PC12 cells expressing Cx32 or Cx43.[157] The effect is mediated by ATP efflux through hemichannels and paracrine/autocrine activation of purinergic receptors.[75] The NGF-induced increase in ATP release and propidium iodide uptake in the presence of physiological extracellular concentrations of divalent cations required the expression of a functional Cx, because these effects were not observed in PC12 cells expressing EGFP or Cx43 mutants unable to form functional hemichannels.[75] Since NGFs affect gap junction channels within even shorter periods of time (see above), it remains to be determined if hemichannels are simultaneously regulated in the opposite direction.

f. Insulin like growth factors (IGFs)

In N/N1003A rabbit lens epithelial cells, treatment with 25 ng/ml IGF-1 for 20–30 min reduced the relative amount of gap junction like structures by $\sim$50%, as well as dye coupling (measured with Lucifer yellow) without affecting total Cx43 levels.[158] The effect was accompanied by considerably increased Cx43 and PKC-γ co-immunoprecipitation (possibly due to increased PKC-γ membrane translocation) and increased Cx43 serine phosphorylation. The effect of IGF-1 in these cells was mimicked by diacylglycerol, suggesting its involvement in the activation pathway. Moreover, IGF-1 or TPA increased the interaction between Cx43, PKC-γ and caveloin-1 in lipid raft domains associated with reduced gap junction intercellular communication.[159]

On the other hand, application of 30 ng/ml recombinant human IGF-1 for 24 h increased Cx43 levels and intercellular dye transfer (Lucifer yellow, scrape loading) in cortical rat astrocytes, without obvious changes in Cx43 cellular distribution.[160] Application of IGF-1 at higher concentrations (150 ng/ml) or for shorter time intervals (30 min or 8 h) had no effect on dye transfer in these cells.

IGF-1 was recently shown to dose-dependently enhance Cx43 mRNA levels in saphenous vein vascular smooth muscle cells, an effect that was blocked by the MAPK inhibitor PD98059.[161] Finally, treatment of serum-free primary cultures of embryonic lens epithelial cells for 2 days with 5–50 ng/ml IGF-1 did not affect gap junction-mediated intercellular transfer of Lucifer yellow.[138,162] To our knowledge, no information regarding modulation of functional hemichannels by IGFs has been reported.

g. Hepatocyte growth factor (HGF)

In the mouse keratinocyte cell line MK, HGF (3×10^{-10} M) induced a relatively rapid (5–10 min) and longstanding (up to 20 h) reduction in the intercellular transfer of Lucifer yellow.[163] The effect was maximal after 1 h of HGF stimulation and was accompanied by a similar reduction in total Cx43 levels. Moreover, application of 20 ng/ml of HGF for 3–12 h induced a progressive and time-dependent reduction in the intercellular transfer of Lucifer yellow in cultured rat hepatocyte doublets.[164] Reduction in intercellular communication was accompanied by a marked decrease in Cx32 levels located at or close to the cell membrane of hepatocytes. The HGF effects were inhibited by co-application of genistein or TGF-β1. To our knowledge, there are no reports addressing the effects of HGF on hemichannels.

h. Other growth factors and cytokines: glial growth factors, granulocytic colony stimulating factor, interleukins and TGF-β

Glial growth factors. In Schwann cells, Cx32 expression is up-regulated by treatment with the neuregulin-1 isoform, glial growth factor-2 (GGF2), without a corresponding increase in electrical coupling,[165] possibly due to the expression of other Cxs. In addition, GGF2 stimulated the proliferation of Schwann cell cultures obtained from normal mice, but not in cultures obtained from Cx32 KO that were electrically coupled to an extent similar to that of WT cells. Therefore, it was proposed that neuregulin-1 induces Schwann cell proliferation through a

mechanism mediated by Cx32, but independent from electrical coupling, presumably mediated by another Cx. However, possible changes in hemichannel activity were not evaluated and remain to be studied.

Granulocytic colony stimulating factor (G-CSF). Application of 10 ng/ml G-CSF for 24 h increased Cx43 levels and phosphorylation, and prevented the reduction in gap junctional communication induced by hypoxia in primary rat myocardiocytes.[166] The effect was associated with the activation of the Wnt signaling pathway, increased myocardiocyte survival after hypoxia and a reduction in the occurrence of ventricular arrhythmias after myocardial infarction by left coronary artery ligation in rats.

Transforming growth factor β (TGF-β). TGF-β1 or β2 up-regulates dye coupling in lens.[167] Long-term exposure to TGF-β1 or 2 increases the intercellular transfer of Lucifer yellow in serum-free primary cultures of chick lens epithelial cells (dissociated cell-derived monolayer cultures [DCDMLs]) by increasing the functional cell–cell coupling mediated by Cx45.6 and/or Cx56, the chicken orthologs of Cx50 and Cx46, respectively.[167] The TGF-β-induced effect was independent of bone morphogenic proteins (BMPs), and it was not inhibited by U0126, inhibitor of MAP kinases 1/2. Interestingly, the increase in intercellular communication was not observed after simultaneous exposure of these cultures to TGF-β and FGF revealing a cross-talk between the transduction pathways activated by these two growth factors. Moreover, the decrease in intercellular communication appears to be due to inhibition of gap junction assembly, which can be prevented by inhibiting p38 MAP kinase.[167] It is also worth mentioning that elevated levels of TGF-β in the lens were associated with lens dysfunction and cataract formation.

BMPs correspond to a heterogeneous group of multifunctional peptide factors belonging to the TGF-β superfamily.[168,169] They are involved in bone formation, embryonic development by controlling cellular differentiation, proliferation and apoptosis. They are also involved in diverse steps of neoplastic growth and dissemination.[168,169]

BMP2 or BMP4 inhibited differentiation of P19 cells, and maintained high degrees of Cx43 expression and gap junctional communication.[170] In addition, treatment of chick lens DCDML cells with 10–50 ng/ml of BMP-2, 4 or 7 for more than 6 h increased intercellular dye transfer by 2–3 fold.[167] In contrast to FGF-1 and 2, the effect of BMPs on gap junctional communication was independent of activation of the MAPK cascade in these cells, thus suggesting a different mechanism. In addition, endogenous BMP signaling was required for the FGF-1- and 2-mediated increase in intercellular communication in these cells.

6. Conclusions and perspectives

Different growth factors induce distinct and even opposite effects on gap junction channels and hemichannels, which can result from their differences in structure and microenvironment (*e.g.*, different molecular partners). The effect of growth factors on gap junction channels and hemichannels is complex. Many growth factor-induced effects are cell type-dependent, which can be explained, at least in part, by differences in the engaged signal transduction pathways,[171] the presence of co-stimulators/facilitators such as extracellular proteoglycans,[172–174] transactivation of other membrane receptors or ion channels,[171,175–178] distinct roles of downstream effectors in different cell types, and cross-talk with other signaling pathways.[171]

The effect of most growth factors on Cx-based channels is mediated by intracellular kinases, such as MAPK, PKC and Src, and is associated with Cx phosphorylation. Several reports show that growth factors reduce gap junction intercellular communication, which is an effect closely associated with the phosphorylation state of the Cx subunits, and which is linked to cell proliferation control.[179,180] The functional effects of growth factors on Cx-based channels are often associated with modifications in Cx levels. However, they may also occur through variations in cellular distribution, channel open probability, and channel conductance/permeability.

Several Cx genes have been implicated in human hereditary disorders, such as cataracts (Cx50 and Cx46; the lens), x-linked Charcot Marie Tooth disease (Cx32; Schwann cells of peripheral nerves), oculodentodigital dysplasia (Cx43; bone, skin and cardiovascular development) and inherited deafness (Cx26, Cx30 and Cx31; cochlea supporting epithelium), which depending on the mutation can be nonsyndromic or syndromic (*i.e.* associated with mild or profound skin disorders).[48] The fact that growth factors are involved in the physiology and pathology of these human tissues opens the possibility that Cxs are one of their targets. However, the existence of this link needs to be demonstrated. A critical point that has not been taken in consideration in all studies gathered in this review is the cell density. This issue could be particularly relevant since the effect of growth factors on Cx-based channels expressed by proliferating and quiescent cells could be under the control of different transduction pathways. Up to now, FGF-1 has been demonstrated to reduce gap junctional communication and increase hemichannel activity.[12–14] However, it might be too early to conclude that hemichannels and gap junction channels are regulated in opposite directions by different growth factors.

Exploration of the cellular consequences of the modulation of gap junction channels and hemichannels by growth factors has only begun. Future studies will help elucidate the importance of these effects.

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