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Regulation of connexin signaling by the epigenetic machinery

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Abstract

Connexins and their channels are involved in the control of all aspects of the cellular life cycle, ranging from cell growth to cell death, by mediating extracellular, intercellular and intracellular communication. These multifaceted aspects of connexin-related cellular signaling obviously require strict regulation. While connexin channel activity is mainly directed by posttranslational modifications, connexin expression as such is managed by classical *cis/trans* mechanisms. Over the past few years, it has become clear that connexin production is equally dictated by epigenetic actions. This paper provides an overview of the role of major determinants of the epigenome, including DNA methylation, histone acetylation and microRNA species, in connexin expression.

Keywords

connexin; hemichannel; gap junction; DNA methylation; histone acetylation; microRNA

1. Introduction

Connexins have been first described about 50 years ago and currently this protein family fosters 21 members in human [1,2]. They are all named based on their molecular weight as predicted by cDNA sequencing and share a common architecture consisting of 4 transmembrane regions, 2 extracellular loops, 1 cytosolic loop, 1 cytosolic aminoterminal tail and 1 cytosolic carboxyterminal tail [3,4]. Throughout the years, connexins have been identified as major goalkeepers of tissue homeostasis by acting at 3 communication levels. First, at the intracellular level, connexins can physically interact with regulators of the cellular life cycle, such as cyclins or B-cell lymphoma 2 proteins, or can affect their expression. Second, connexins form hexameric structures called hemichannels that create pores at the membrane plasma, providing a pathway for extracellular communication. Hemichannels link the cytosol of individual cells and their extracellular environment, and convey small (*i.e.* less than 1 kilodalton) and hydrophilic substances, such as second messengers and ions. Third, 2 hemichannels of adjacent cells can dock and generate a gap junction that mediates direct intercellular trafficking of permeants similar to those involved in hemichannel signaling [5]. Inherent to their role as critical determinants of all aspects of tissue homeostasis, connexins and their channels are also frequently involved in disease. In

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fact, although controversial, it seems that hemichannels, unlike their full channel gap junction counterparts, preferentially open up in pathological circumstances, including cell death and inflammation [3,6].

The activity and thus the opening of connexin-based channels are regulated by a plethora of mechanisms. Short-term control, so-called gating, mainly relies on posttranslational modifications of connexin proteins. Connexins can undergo several of such modifications, including glycosylation, *N*-acetylation, ubiquitination, lipidation, hydroxylation, methylation, deamidation, *S*-nitrosylation, sumoylation and phosphorylation [2,7]. The latter, typically occurring at their carboxyterminal tail, has been best studied thus far and may have various effects depending on the identity of the connexin and kinase as well as the cellular context [2]. Long-term control of hemichannel and gap junction activity involves regulation of connexin expression. The architecture of most connexin genes is simple, consisting of a first exon that harbors the 5′-untranslated region (UTR), which is separated by an intron of varying length from a second exon, bearing the complete coding sequence and the 3′-UTR. Connexin gene promoters display binding affinity for several general transcription factors, including activator protein 1, yin yang 1 and specificity protein 1. Furthermore, a number of tissue-specific transcription factors control connexin gene transcription, like hepatocyte nuclear factor 1α in liver [8,9].

In the last decade, epigenetic mechanisms, including DNA methylation, histone acetylation and microRNA-related control, have also joined in as master regulators of connexin expression. These mechanisms will be discussed in detail in the following sections. Specific attention will be paid to microRNA-related control, which has become particularly studied in the connexin field in recent years.

2. DNA methylation

Hypermethylation of gene promoters, catalyzed by DNA methyltransferase (DNMT) enzymes, is typically linked to transcriptional silencing [10]. The negative correlation between DNA methylation and gene expression is mediated by methylated DNA-binding proteins that concentrate at hypermethylated CpG dinucleotides and that recruit transcriptional suppressors [11].

The role of DNA methylation in the control of connexin expression has been mainly studied in a pathological context. In a rat model of *D*-galactose-induced presbycusis, gradual downregulation of Cx26 expression was seen in cochlear tissue, which was associated with increased methylation of its gene promoter [12]. Along the same line, Cx32 and Cx43 mRNA levels progressively decrease in human gastric mucosa during *Helicobacter pylori* infection, an event that goes hand in hand with hypermethylation of their gene promoters [13]. It should be mentioned that the latter is probably not due to infection *per se*, but rather to tumor progression. Indeed, methylation-driven suppression of connexin expression has been most extensively demonstrated for carcinogenic events. Abrogation of expression of Cx26, Cx32, Cx36, Cx43 and Cx45 has been associated with the accumulation of methylated CpG dinucleotides in the corresponding connexin gene promoters in a variety of human malignant cells, including lung cancer cells [14,15], renal carcinoma cells [16-18],

esophageal cancer cells [19], breast cancer cells [20], nasopharyngeal cancer cells [21], colon cancer cells [22,23] and glioma cells [24] as well as in other species [25-29]. Accordingly, hypomethylating agents that act by inhibiting DNMTs, such as the drugs 5-azacytidine and 5-aza-2-deoxycytidine (*i.e.* decitabine), have been found to upregulate connexin expression in many of those cancer cells (Table 1), which often results in enhanced gap junction activity [16,21], although this occurs in a cell type-dependent and connexin-specific fashion [16,19,20,25,30]. However, methylation of connexin gene promoters, including Cx30, Cx36 and Cx37, during cancer is not always accompanied by their downregulated expression [23]. In physiological conditions, such as during murine embryogenesis, decitabine was even found to suppress the production of Cx31, Cx43 and Cx45 in mouse embryos [31].

Although the mechanism of DNA methylation causing connexin gene silencing is clear, the true triggers of this epigenetic process remain more elusive. Decreased expression of connexins, *in casu* Cx26, in liver cancer has been casually linked to elevated DNMT1 mRNA levels [26]. Furthermore, aberrant binding of transcription factors to methylated connexin gene promoters could underlie poor connexin expression in cancer cells. In this respect, decreased Cx43 gene transcription in human non-small cell lung cancer cells is accompanied by DNA methylation and correlates with reduced binding of activator protein 1 to the its gene promoter [32]. Furthermore, methylated CpG dinucleotides are preferentially located in the specificity protein 1 *cis*-acting elements of the Cx26 gene promoter and the Cx32 gene promoter in human breast cancer cells [33] and rat liver cancer cells [25], respectively.

3. Histone modifications

The vast majority of data that show the involvement of histone modifications in connexin expression comes from studies using modifiers of these particular epigenetic mechanisms. This is specifically the case for reversible histone acetylation, which is catalyzed by histone acetyltransferases and that usually is paralleled by transcriptional activation through chromatin decondensation, while the opposite reaction, driven by histone deacetylase (HDAC) enzymes, frequently underlies suppression of gene expression [34].

Inhibitors of HDAC enzymes have been demonstrated to increase connexin production [35-47], often associated with induction of gap junction opening [35-41,44,48-50] in various experimental settings (Table 2). As seems to hold for DNMT inhibitors, the effects of HDAC inhibitors are dictated by the nature of the connexin species and epigenetic modifier as well as the cellular environment. In this respect, the prototypical class I and II HDAC inhibitor trichostatin A positively affects Cx36 expression in mouse pancreatic cell lines, but not in mouse fibroblasts, neuronal cells and pituitary cells [30]. On the other hand, sodium butyrate and 4-phenylbutyrate, but not trichostatin A, increase Cx43 protein levels in human nasopharyngeal tumor cells [51]. Also, suberoylanilide hydroxamic acid leaves Cx43 and Cx45 unaffected, but upregulates Cx32 and Cx37 expression and simultaneously reduces Cx40 protein amounts in cardiodystrophic mice [52]. HDAC inhibitors hereby directly affect connexin gene transcription. Indeed, trichostatin A-mediated induction of Cx43 in human prostate cancer cells relies on the recruitment of p300/cyclic adenosine monophosphate

response element-binding protein, a transcriptional coactivator displaying histone acetyltransferase activity, and the transcription factors activator protein 1 and specificity protein 1 to the Cx43 gene promoter. This is associated with hyperacetylation of histone H4 surrounding binding sequences of both transcription factors [37]. Likewise, suberoylanilide hydroxamic acid triggers accumulation of acetylated histones H3 and H4 in the Cx43 gene locus, leading to its enhanced expression in human peritoneal mesothelial cells [41]. Cx36 expression in pancreatic cells is controlled, at least in part, by the RE-1 silencing transcription factor, a transcriptional repressor consisting of 2 independently acting HDAC-recruiting repression domains [53,54]. Active Cx36 production in these cells is featured by the presence of trimethylated lysine 4 residues in histone H4 near its gene promoter, an epigenetic marker of actively transcribed genes, and is inducible by trichostatin A [30].

A number of reports have documented the identity of the HDAC enzymes that are involved in the regulation of connexin expression. Specific deletion of HDAC1 and exposure to trichostatin A diminish Cx43 mRNA levels in mouse embryonic stem cells. In fact, loss of HDAC1 increases trimethylation of lysine 9 residues in histone H3 surrounding the Cx43 gene promoter region, an epigenetic signature of silenced genes, and only slightly reduces histone H3 and H4 acetylation. This indicates that the Cx43 gene requires both HDAC1 presence and activity for its transcription, but histones H3 and H4 are merely minor targets in this regulatory process [55]. In line with this finding, silencing of HDAC1 production substantially decreases expression of Cx43 in murine induced pluripotent stem cells [56]. By contrast, small interfering RNA-mediated knockdown of HDAC1 during differentiation of rat bone mesenchymal stem cells into cardiomyocytes increases Cx43 expression [57]. Upon transfection, the breast cancer metastasis suppressor 1 protein localizes in the cell nucleus, and restores gap junction activity in human breast cancer cells [58-60] and in melanoma cells [61]. In the former case, this coincides with elevated Cx43 mRNA levels and concomitant Cx32 gene transcription [58,60]. It has been further found that breast cancer metastasis suppressor 1 interacts with the large mammalian Sin3 HDAC complex, which contains both HDAC1 and HDAC2, but also forms smaller complexes with HDAC1 [62]. The mammalian Sin3 HDAC complex is also involved in the repression of Cx43 expression in human telomerase-immortalized myometrial cells by progesterone. The latter hereby binds to the Cx43 gene promoter through a protein-protein interaction with activator protein 1 [63]. MC1568, a selective class II HDAC inhibitor, promotes expression of Cx37 and Cx43 in lung artery endothelial cells of pulmonary arterial hypertension patients. This results from inhibition of HDAC4 and HDAC5, both which regulate the activity of the transcription factor myocyte enhancer factor 2, known to control expression of Cx37 and Cx40. This is further substantiated by the observation that experimental suppression of HDAC4 and HDAC5 upregulates production of both connexins in these cells [45].

It should be mentioned that HDAC inhibitors can act at levels of connexin expression other than the transcriptional one. Thus, trichostatin A enhances gap junction opening in cultured rat hepatocytes, a finding associated with differential effects on Cx26, Cx32 and Cx43 protein contents, but not with alterations in the corresponding mRNA amounts [49]. 4-phenylbutryate increases gap junction activity in rat corpora smooth muscle cell cultures, with no effect on Cx43 protein levels and even a decline in Cx43 mRNA transcript number. This could point to stabilization of the existing Cx43 pool or alterations in functional

channel amounts [64]. In addition, HDAC inhibitors can interfere with posttranslational connexin control, as they both increase [36,39-41] and decrease [38] the abundance of phosphorylated Cx43 isoforms in different cell types. Sodium butyrate prevents tumor promoter-mediated inhibition of gap junction activity *via* extracellular signal-regulated kinase 1/2 inactivation, while trichostatin A restores gap junctional communication and induces Cx43 hyperphosphorylation by preventing p38 mitogen-activated protein kinase in cultured rat liver epithelial cells [65]. HDAC inhibitors may also affect subcellular localization of connexin proteins both *in vitro* [49,50] and *in vivo* [52]. Curiously, the interaction between histone acetylation and connexins can also occur in the opposite direction. In this regard, transfection of metastatic human pulmonary giant cells carcinoma cells with the gene encoding Cx43 increases acetylation of histones H3 and H4 in the promoter of the follistatin-like 1 gene, which in turns affects invasive and metastatic potential [66].

4. MiRNA-related control

In the last few years, microRNA (miRNA) species have emerged as critical posttranscriptional regulators of connexin expression. Following their synthesis in the cell nucleus and processing in the cytoplasm, miRNAs bind to complementary sequences in target mRNA molecules and either suppress their translation or cleave mRNAs as such [67].

A plethora of miRNAs have been reported to directly bind to the 3′-UTR region of Cx43 mRNA and thereby to suppress its translation (Table 3). This type of regulation has been studied both in a physiological and a pathological context. Regarding the former, miR-206 production is upregulated upon perinatal skeletal muscle development in mice *in vivo* and both miR-1 and miR-206 downregulate Cx43 expression during myoblast fusion *in vitro* [68,69]. Mice that overexpress miR-206 show decreased Cx43 expression and impaired bone formation [70]. Similarly, Cx43 levels increase during differentiation of bone cells, a process counteracted by miR-23a [71]. Of note, miRNAs may be involved in establishing gender-specific differences in connexin production. This has been shown for miR-1, which regulates Cx43, being expressed to a higher extent in female rat cardiomyocytes compared to male counterparts [72].

MicroRNAs can also act as positive regulators of connexin expression. In this light, miR-145 upregulates Cx43 production upon differentiation of human corneal epithelial progenitor cells [73]. Likewise, miR-208a seems to promote cardiac Cx40 expression [74]. Furthermore, microRNAs can indirectly affect connexin production. Thus, miR-103/107 directly targets the expression of receptor M type protein tyrosine phosphatase in limbal derived corneal epithelial cells, which in turns affects Cx43-based gap junctions [75]. Similarly, myocardin downregulates Cx43 expression *via* miR-1 upregulation in bladder capacity during development [76]. Also, miR-200 regulates production zinc finger E-box binding homeobox proteins 1 and 2, which transcriptionally repress Cx43 expression in human myometrial cells [77]. From the pathological perspective, miRNAs underlie modifications in connexin production during the onset and progression of several diseases, in particular cardiac pathologies. MiR-1 gained quite some attention in this respect. Its overexpression slows down conduction and depolarizes the cytoplasmic membrane [78],

resulting in atrioventricular block in rodents [79]. This is due, at least in part, to the direct negative impact of miR-1 on cardiac Cx43 production [79,80]. Furthermore, hypertrophic stimulation of cardiomyocytes induces miR-1 downregulation both *in vitro* and *in vivo*, subsequently modifying the expression of Cx43, which in turn is phosphorylated by the hypertrophic stress-induced mitogen-activated protein kinase and as such displaced from the gap junction configuration [80,81]. Aberrant production or processing of miR-1 accompanied by altered Cx43 levels has also been observed in viral myocarditis in mouse [82] and in myotonic dystrophy in human [83]. In addition, miR-1 as well as miR-206 are downregulated in patients suffering from tetralogy of Fallot [84]. Other miRNAs have also been related to cardiac tachycardia and/or arrhythmias, such as miR-130a [85] and miR-19a/b [86], respectively. Several studies have documented roles for miRNAs as tumor suppressors or promoters. MiR-125b [87] and miR221/222 [88] promote cell cycling and/or invasion in glioma cell cultures, thereby suppressing Cx43 expression. Likewise, miR-20a is highly expressed in human cancer cells and negatively affects Cx43 production [89]. Both miR-200a [90] and miR-206 [91] also modify Cx43 expression during carcinogenesis. More recently, it was found that miR-206 and miR-1 diminish Cx43 levels during experimentally induced alkali burn injury in mouse cornea [92] and in chronic neuropathy in rat sciatic nerves [93], respectively.

It should be mentioned that the interaction between miRNAs and connexins can occur in both directions. Indeed, forced expression of Cx43 in glioma cell cultures antagonizes miR-125b-mediated cell growth [87]. On the other hand, silencing of Cx43 production reverses the protective effect of miR-206 downregulation in alkali-burned cornea [92]. Interestingly, besides acting as regulators of the production of their building stones, miRNAs can also permeate gap junctions. In this regard, miR-5096 is conveyed *via* gap junctions between glioma cells and as such exerts proinvasive effects [94]. Antiproliferative miR-124-3p travels through Cx43-based gap junctions in glioblastoma cells [95]. Gap junctions also transfer miR-210 in cocultures of mesenchymal stem cells and cardiomyocytes [96] as well as miR-142 and miR-223 between macrophages and hepatocellular carcinoma cells [97].

5. Conclusions and perspectives

Connexins and their channels control all facets of the cellular life cycle by acting at multiple communication platforms [5]. A strict and well-coordinated regulation is compulsory their appropriate expression and functioning. Considerable efforts have yet been focused throughout the years on the elucidation of the *cis/trans* machinery that drives connexin gene transcription [8,9]. A large body of evidence also points to the involvement of epigenetic phenomena in this process, including DNA methylation and histone acetylation at the pretranscriptional level and miRNAs at the posttranscriptional level. These mechanisms may also act in concert while controling connexin expression. This has been recently exemplified for miR-1298, which is regulated by DNA methylation and that directly binds to Cx43 mRNA in vascular smooth muscle cells, resulting in its reduced expression and gap junction channel activity [98]. As a matter of fact, a major challenge lies ahead in deciphering the global epigenetic codes that determine connexin expression, including other histone modifications, such as histone methylation, which also emerge as regulators of connexin

production [99]. Such research is often complicated by the observation the methylation and acetylation not only affect connexin genes, but also their proteins. In this respect, HDAC3, HDAC4, HDAC5 and p300/cyclic adenosine monophosphate response element-binding protein colocalizes with Cx43 in cardiac tissue of dystrophic mice. They control Cx43 protein acetylation, which in turn determines its interaction with other junctional proteins, such as N-cadherin, and association with gap junctions [46]. Furthermore, bioinformatic analysis showed that specific Cx26 gene mutations known to be associated with human disease can directly trigger loss or gain of posttranslational Cx26 methylation [100].

Epigenetic modifiers are indispensable tools during further research of the role of the epigenome in connexin expression. In addition to the conventional and widely used inhibitors of DNMT and HDAC enzymes, such as decitabine and trichostatin A, respectively, several dietary compounds have been characterized as epigenetic modifiers that affect connexin expression. Epigallocatechin-3-gallate, a major constituent of green tea, decreases DNA methylation in the Cx32 gene promoter and increases its protein levels in human renal carcinoma cells [18]. Sulforaphane, an organosulfur HDAC inhibitor present in cruciferous vegetables, upregulates Cx43 protein amounts and induces gap junction opening in human cancer cells by affecting the phosphorylation status [43]. Resveratrol, which acts on histone acetylation and that is found in grapes and red wine, opens gap junctions in human glioblastoma cells [101] rat liver epithelial cells [102-104]. Like sulforaphane, this occurs independently of changes in Cx43 mRNA levels [103] and is allied with altered Cx43 phosphorylation [101-103].

Overall, the interplay between epigenetic mechanisms and their modifiers on the one hand, and connexin expression and signaling on the other hand has been predominantly studied in pathological scenarios. Typically, the epigenetic machinery during cancer triggers the silencing of tumor suppressor genes, including those coding for connexins [105,106]. Connexins have indeed repeatedly been demonstrated to possess potent antitumor properties by inducing cell cycle arrests, differentiation and apoptosis in neoplastic cells [5,106,107]. Upregulation of connexin expression using epigenetic modifiers may therefore represent an attractive anticancer therapy [106,108]. In addition, HDAC inhibitors have lately gained attention for the potential treatment of cardiac diseases, thereby also affecting connexins. In cardiodystrophic mice, Cx40 protein production is increased, while Cx43 shows lateralization. Administration of suberoylanilide hydroxamic acid to these animals restores normal Cx40 protein amounts and reestablishes the physiological Cx43 distribution pattern [52]. Likewise, trichostatin A reverses atrial arrhythmia inducibility and fibrosis in cardiac hypertrophy by normalizing Cx40 production [47]. It can be anticipated that further exploration of the effects of epigenetic modifiers on connexin production and channel activity in the upcoming years will open promising perspectives for the therapy of many other diseases.

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List of abbreviations

4-PB	4-phenylbutyrate
AZA	5-azacytidine
Cx	connexin
DAC	decitabine
DNMT(i)	DNA methyltransferase (inhibitor)
EG	epigallocatechin-3-gallate
HDAC(i)	histone deacetylase (inhibitor)
HMBA	hexamethylene bisacetamide
J-1	5-(4-dimethylaminobenzoyl)aminovaleric acid hydroxamide
miRNA	microRNA
NaB	sodium butyrate
SAHA	suberoylanilide hydroxamic acid
SR	sulforaphane
TSA	trichostatin A
UTR	untranslated region

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Highlights

- Connexin expression is controlled by DNA methylation, histone acetylation and microRNAs.

- The role of epigenetics in the regulation of connexin expression has been mainly studied *in vitro*.

- Epigenetically modifying connexin expression might be a potential clinical therapy for several pathologies.

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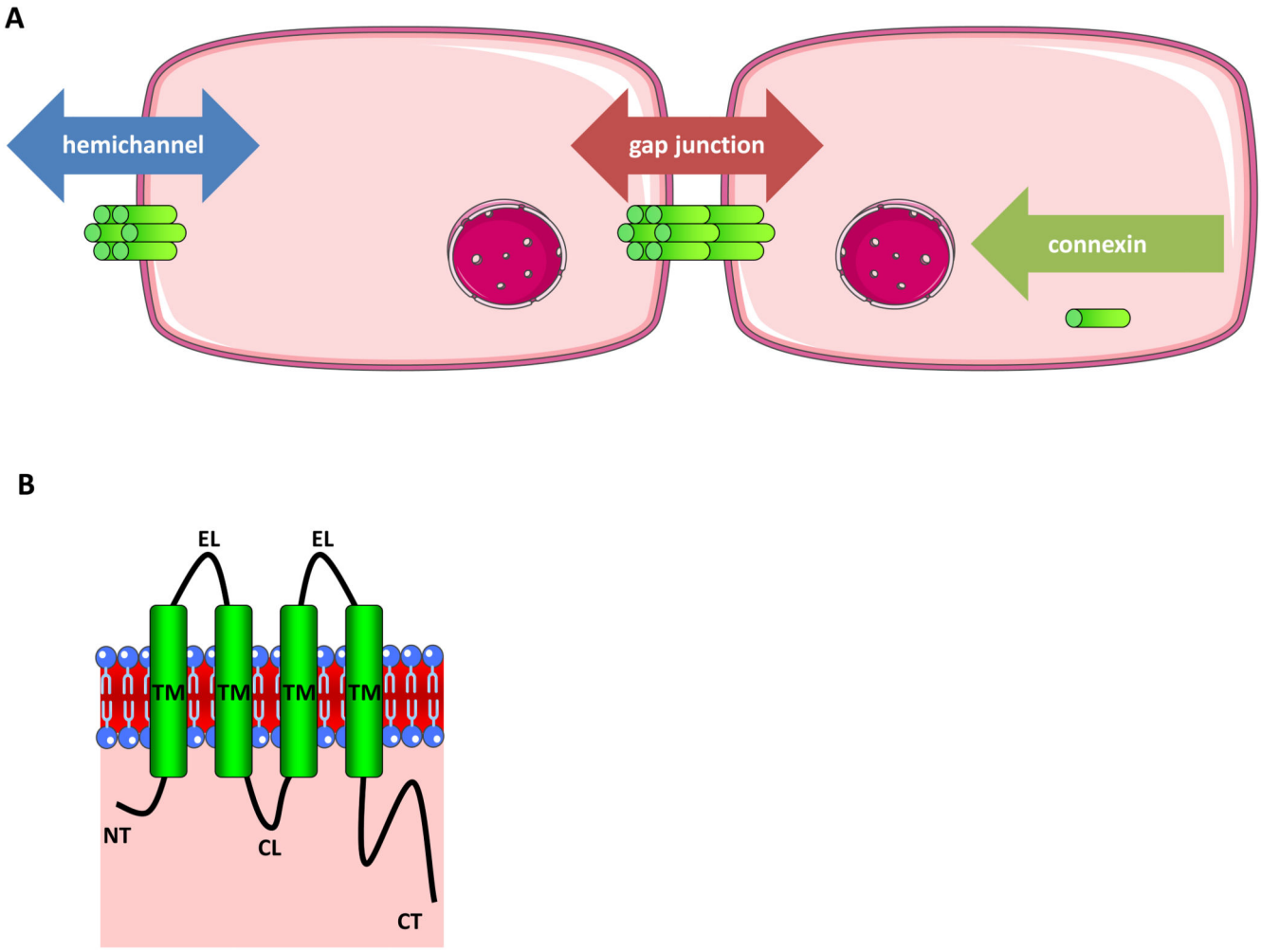

Figure 1.

A. *Architecture of connexin channels and aspects of connexin signaling.* Gap junctions are formed by the interaction between 2 hemichannels of adjacent cells and mediate intercellular communication (red arrow). Hemichannels are built up by 6 connexin proteins and support extracellular communication (blue arrow). Connexins as such may be involved in intacellular communication (green arrow).

B. *Topology of connexin proteins*. Connexins all consist of 4 transmembrane domains (TM), 2 extracellular loops (EL), 1 cytosolic loop (CL), 1 carboxyterminal cytosolic (CT) and aminoterminal (NT) tail.

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Table 1

Effects of DNA methyltransferase inhibitors on connexin expression.

Model	DNMTi	Upregulation	Downregulation	No effect	Reference
Human colon cancer cells	DAC	Cx43 **			[23]
Human esophageal cancer cells	DAC			Cx26 **/Cx43 **	[19]
Human lung carcinoma cells	DAC	Cx26 **			[14]
Human renal carcinoma cells	DAC	Cx32 *,**			[16,109]
	EG	Cx32 *			[18]
Human proximal tubular cells	DAC			Cx32 *,**	[16]
Human breast cancer cells	DAC			Cx26 **	[20]
	DAC	Cx26 **			[33]
Human cervical carcinoma cells	DAC	Cx43 *			[110]
Human nasopharyngeal cancer cells	DAC	Cx43 *,**			[21]
Rat liver epithelial cells	DAC	Cx43 **		Cx32 **	[25]
Mouse pancreatic cancer cells	AZA			Cx36 **	[30]
Mouse pituitary corticotrophic cells	AZA			Cx36 **	[30]
Mouse neuronal cells	AZA			Cx36 **	[30]
Mouse fibroblasts	AZA			Cx36 **	[30]
Mouse embryonic cells	DAC		Cx31 **/Cx43 **/Cx45 **		[31]

* protein level;

** mRNA level; AZA, 5-azacytidine; Cx, connexin; DAC, decitabine; DNMTi, DNA methyltransferase inhibitor; EG, epigallocatechin-3-gallate.

Table 2

Effects of histone deacetylase inhibitors on connexin expression.

Model	HDACi	Upregulation	Downregulation	No effect	Reference
Human glioblastoma cells	NaB			Cx43 *	[111]
	4-PB	Cx43 *			[36]
Human pancreatic cancer cells	4-PB	Cx43 *			[42]
	SR	Cx43 *			[43]
Human prostate carcinoma cells	TSA	Cx43 *,**			[37]
Human prostate epithelial cells	TSA	Cx43 *,**			[37]
Human cervical carcinoma cells	4-B	Cx43 *			[44]
Human nasopharyngeal tumor cells	4-PB/NaB	Cx43 *			[51]
	TSA			Cx43 *	[51]
Human peritoneal mesothelial cells	HMBA	Cx43 *,**			[39,40]
	SAHA	Cx43 *,**			[41]
Human lung artery endothelial cells	MC1568	Cx37 **/Cx40 **			[45]
Human embryonic kidney cells	4-PB	Cx43 *			[44]
Human neural progenitor cells	4-PB/TSA	Cx43 *			[38]
Human liver cancer cells	TSA		Cx43 **	Cx26 **/Cx32 **	[112]
Rat glioma cells	NaB			Cx43 *	[111]
	4-PB	Cx43 *			[35]
Rat colon cancer cells	NaB			Cx43 **	[113]
Rat transformed epithelial cells	SAHA	Cx43 *,**			[41]
Rat hepatocytes	TSA	Cx32 */Cx43 *	Cx26 *		[49]
	J-1	Cx32 *	Cx26 */Cx43 *		[50]
Rat corpora smooth muscle cells	4-PB		Cx43 **	Cx43 *	[64]
Mouse pancreatic cancer cells	TSA	Cx36 **			[30]
Mouse pituitary corticotrophic cells	TSA			Cx36 **	[30]
Mouse neuronal cells	TSA			Cx36 **	[30]
Mouse fibroblasts	TSA			Cx36 **	[30]
Mouse embryonic cells	TSA		Cx43 **		[55]
Cardiodystrophic mdx mice	SAHA	Cx32 */Cx37 *	Cx40 *	Cx43 */Cx45 *	[52]
HopX cardial hypetrophic mice	TSA	Cx40 *		Cx43 **	[47]

* protein level;

** mRNA level; 4-PB, 4-phenylbutyrate; Cx, connexin; HDACi, histone deacetylase inhibitor; HMBA, hexamethylene bisacetamide; J-1, 5-(4-dimethylaminobenzoyl)aminovaleric acid hydroxamide; NaB, sodium butyrate; SAHA, suberoylanilide hydroxamic acid; SR, sulforaphane; TSA, trichostatin A.

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Table 3

MicroRNA species experimentally shown to directly bind the 3′-UTR of Cx43 mRNA in different cell types.

MiRNA species	Cell type	Reference
MiR-1	Human breast cancer cells	[90]
	Rat myocardial cells	[80,82]
MiR-19a/b	Human embryonic kidney cells	[86]
MiR-20a	Human prostate cancer cells	[89]
MiR-23a	Human osteosarcoma cells	[71]
	Human breast cancer cells	[90]
MiR-125b	Human embryonic kidney cells	[87]
MiR-130a	Mouse embryonic fibroblasts	[85]
	Mouse cardiomyocyte tumor cells	[85]
MiR-186	Human breast cancer cells	[90]
MiR-200a	Human breast cancer cells	[90]
MiR-206	Human breast cancer cells	[90,91]
	Rat vascular smooth muscle cells	[92]
	Mouse corneal cells	[92]
	Mouse osteoblasts	[70]
	Mouse myoblasts	[68,69]
MiR-218	Human nasopharyngeal carcinoma	[114]
	Human breast cancer cells	[114]
	Human cervical cancer cells	[114]
MiR-222	Human glioblastoma cells	[88]
	Rat vascular smooth muscle cells	[92]
MiR-381	Human breast cancer cells	[90]
MiR-1298	Rat vascular smooth muscle cells	[98]

miRNA, microRNA.