Review

Connexins in migration during development and cancer

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Abstract

Connexins, the gap junction proteins, through their multitude of actions are implicated in a variety of cell processes during animal development and cancer. They allow direct or paracrine/autocrine cell communication through their channel and hemi-channel functions. They enable adhesion and interact with a plethora of signalling molecules. Here, we review the common themes in developmental and pathological processes and we focus in their involvement in cell migration in four different systems: neurons, astrocytes, neural crest and cancer.

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Gap junctions overview

Cell co-operation is concomitant with the organisation found in multicellular systems. Throughout animal life, from embryonic development to tissue homeostasis and in pathophysiological conditions, as in cancer metastasis, cells rely on the exchange of information between collaborating individuals in order to coordinate their action. One way in which cells achieve this coordination is through direct cell–cell communication.

In metazoans, direct cell communication is achieved by gap junctions, cell membrane junctional structures that display a 20–30 angstroms “gap” between adjacent cells, as first observed in electronic micrographs from mouse heart and liver (Revel and Karnovsky, 1967). Gap junctions are characterised as channels, which allow the intercellular exchange of small molecules and ions between neighbouring cells. Electron micrographs revealed the presence of gap junctions in numerous metazoan cell types, such as in astrocytes, neurons and ependymal cells in the brain of various vertebrates (Brightman and Reese, 1969), in smooth muscle cells (Uehara and Burnstock, 1970) and in embryonic amphibian cells (Decker and Friend, 1974).

In chordates the proteins that are involved in the formation of gap junctions are called connexins (Goodenough, 1974; Nicholson et al., 1981). The participation of connexins in animal development is evident by (a) their presence in various developmental stages and organisms, and (b) their requirement in early development, since loss-of connexin expression leads to dramatic and often lethal developmental phenotypes (Kruger et al., 2000; Kumai et al., 2000; Reauame et al., 1995). Thus, cell-cell communication is established early during embryogenesis. In mouse embryos, six different connexin genes have been transcribed by the eight-cell stage (Davies et al., 1996). In Xenopus, five different connexins are reported in early development, from fertilised egg till early tailbud stage (Landesman et al., 2003). Furthermore, specific connexins are linked with distinct morphogenetic processes. For instance, connexin43 (Cx43) gap junction protein plays an essential role in morphogenesis of the embryonic chick face (McConnell et al., 2001), Cx43.4 is required for left-right axis formation in zebrafish Kupffer’s vesicle (Hatler et al., 2009) and Cx45 is crucial for mouse heart development (Alcoléa et al., 1999). In cancer, the role of connexins is shown to be both enhancing (Ezumi et al., 2008; Ito et al., 2000; Plante et al., 2010) and inhibitory (Eghbali et al., 1991; Hellmann et al., 1999; Hirsch et al., 1996; Zhu et al., 1991) depending on the stage of disease and the tissue involved. For a review on the role of connexins in cancer see (Naus and Laird, 2010).

Structure and protein domains

In a cell, a hexamer of connexins is required to form a cylindrical shaped structure, which is called connexon, and corresponds to half of a complete channel, also known as “hemi-channel” (Fig. 1A). The hexagonal structure of connexons was revealed by freeze fracture electron microscopy (Peracchia, 1973a, 1973b). Alignment and docking of two connexon counterparts belonging to adjacent cells is required to form a complete gap junction channel (Fig. 1A). The binding between connexons occurs
through non-covalent bonds of the cysteine residues of the extracellular loops (Yeager and Nicholson, 1996). Gap junctions often assemble in a more complex multi-gap junctional structure along the adjoining cell membranes, called the gap junction plaque (Musil and Goodenough, 1991).

Depending on the connexin profile of the connexons, the gap junction channels are defined as homomeric or heteromeric and homotypic or heterotypic. Homomeric/homotypic are channels that are composed of the same connexin type, while heteromeric channels are composed of two different connexins and heterotypic channels are formed by two different connexons. Not all connexins can interact with one another to generate heterotypic or heteromeric channels, since the compatibility between different connexin types is a selective process (Elfgang et al., 1995; White et al., 1995).

All connexins share a conserved tertiary structure that can be divided into the following motifs: four trans-membrane domains, two extracellular loops with 3 Cysteine residues on each one, a cytosolic loop and a cytosolic N-terminus and C-terminus (Fig. 1B) (Unger et al., 1999a, 1999b).

Connexin activities

Channel functions

Gap junction channel

Initially, connexin activity was solely attributed to the channel function of gap junctions. The presence of connexins in a tissue was synonymous to have gap junction intercellular communication (GJIC). In this model, gap junctions serve as direct links between adjoining cells, enabling tissues to function as a syncytium.

Indeed, in early development of Xenopus embryonic stages, most blastomeres are communicating through gap junctions (Landesman et al., 2000). During development, after several divisions, this communication is restricted to more localised areas and this localised communication has been suggested to control the compartmentalisation of the embryo (Bruzzone et al., 1996). and this localised communication has been suggested to control divisions, this communication is restricted to more localised areas (Landesman et al., 2000).

During development, after several most blastomeres are communicating through gap junction channel (right). (B) Connexin Structural Domains. Connexin family of proteins share distinct structural motifs: cytoplasmic N-terminus, four trans-membrane domains, two extra-cellular loops with three cysteine residues on each one, one intra-cellular loop, cytoplasmic c-terminus.

Further evidence for connexin channel activity is demonstrated in studies related to heart development. In the developing heart, gap junctions are essential for conduction of electrical impulses of the heart and for synchronous beating (Simon et al., 1998). The spatiotemporal expression of heart connexins (Cx30.2, Cx40, Cx43 and Cx45) is tightly regulated in all vertebrates and connexin inhibition or misexpression results in perturbation leading to heart arrhythmias (Kirchhoff et al., 1998; Van der Velden et al., 1998). In a recent study, Cx40, the predominant connexin in the atrial myocardium has been shown critical for the conduction properties of the atrium (Benes et al., 2014).

The channel activity of gap junctions is normally studied by the passage of fluorescent dyes that are membrane impermeable. The role of gap junctions as channels has been revealed by their inhibition with various blocking agents (Davidson et al., 1986; Harks et al., 2001; Srinivas and Spray, 2003; Xia and Nawy, 2003). However, these blocking reagents cannot discriminate among the different connexins and their specificity is unclear. In a recent study, a more refined inhibition of the channel function became possible through a single point mutation at a threonine amino acid of the third trans-membrane helix of a-type and b-type connexins (Beahm et al., 2006). This point mutation can lead to a closed gap junction channel, without affecting other connexin related activities (as discussed below). Use of targeted channel inhibition of specific connexins could reveal their distinct roles in development and cancer.

Hemi-channel

Connexins can function in a paracrine and autocrine manner through their hemi-channel activity. This is the activity of the open unpaired connexon with exchange of large membrane impermeable molecules between the cytoplasm and the extracellular environment. In physiological conditions hemi-channel activity is limited (Buvinic et al., 2009; Cherian et al., 2005; Solan and Lampe, 2009), while uncontrolled hyperactivity could lead to cell death (Contreras et al., 2002). In response to stress factors or specific physiological signals (Buvinic et al., 2009; Cherian et al., 2005) opening of hemi-channels can lead to autocrine-paracrine cell signalling. Thus, tight regulation of hemi-channel opening is essential. It is proposed that ATP release through hemi-channels leads to activation of purinergic receptors (P2Y, P2X7) and consequently to the control of Ca2+ influx (Scemes et al., 2003; Weisssman et al., 2004). The role of ATP

Fig. 1. Connexins are the structural basis of Gap Junction. (A) Connexins are depicted as blue ellipses (left); six connexins are required to form a connexon, also known as hemi-channel (middle). Two connexons align and form the gap junction channel (right). (B) Connexin Structural Domains. Connexin family of proteins share distinct structural motifs: cytoplasmic N-terminus, four trans-membrane domains, two extra-cellular loops with three cysteine residues on each one, one intra-cellular loop, cytoplasmic c-terminus.
release and hemic-channel activity is also shown in astrocytes, glial and glioma cells (De Vuyst et al., 2009; Stout et al., 2002). Release of other metabolic molecules is detected through hemic-channels, such as glutamate and NAD+ (Bruzzone et al., 2001; Ye et al., 2003). Hemic-channel function can be studied by use of blocking reagents. It is, however, challenging to specifically perturb the hemic-channel function without interfering with the full gap-junction channel activity. Novel tools that will be able to discriminate between the two distinct connexin channel activities would enlighten the specific connexin roles in physiological and pathological conditions.

Non-channel functions

Adhesion (C-C)

In addition to their function as channels, gap junctions have been implicated in cell-cell adhesion. This function is independent of the channel activity and is attributed to the cysteine residues of the extracellular loops. Firstly, exogenous connexin expression increases the adhesive competence of glioma cells (Lin et al., 2002). Secondly, a point mutation in one of the extracellular cysteine residues results in reduced cell-cell adhesion, as revealed by a marked decrease in a cell aggregation assay (Lin et al., 2002). It is important to note that these cysteine point mutations lead to reduced gap junction plaques in C6 glioma cells (Lin et al., 2002) and can cause trafficking defects resulting in less connexin in the cell membranes of granulosa cells from mouse ovaries (Tong et al., 2009). Further evidence for gap junction mediated adhesion is shown in Cx40–Cx43 chimeras (Haubrich et al., 1996) that enable gap junction formation, but not channel permeability. Expression of these chimeras increases cell-cell adhesion in glioma cells (Lin et al., 2002). The role for gap junction mediated adhesion is linked to cell migration as discussed below (Elias et al., 2007).

Interaction with signalling molecules (C-terminus)

The carboxy-tail is the most divergent domain with varying sizes among the connexins (i.e. Cx43 has a much larger cytoplasmic tail in comparison to Cx26) and has been proposed to interact with a variety of molecules. The C-terminal of Cx43 has been implicated in gene regulation and growth control by direct binding of NOV/CCN3 factors (Brigstock, 2003; Fu et al., 2004). CCN proteins are involved in wound healing, angiogenesis and chondrogenesis (Brigstock, 2003) and are known to have anti-proliferative roles. In Cx43 transfected C6 glioma cells, CCN proteins were found to be upregulated in comparison to control C6 glioma cells in which Cx43 was expressed at low levels (Gupta et al., 2001). In human glioma cells, induced expression of Cx43 leads to a reduction in cell proliferation (Crespin et al., 2010). When, on the other hand, only the Cx43 C-tail was delivered into cells an anti-proliferative function was observed. Apart from that, connexins can bind to a repertoire of other molecules that belong to the cell junctional complexes and to the cytoskeleton. The C-tail of Cx43 has been implicated in the interaction of a variety of tight and adherens junctional molecules. Among them it binds to ZO-1 (B. N. Giepmans et al., 2001a; Giepmans and Moolenaar, 1998; Sorgen et al., 2004), interacts with cadherins and catenins, such as N-cadherin (Wei et al., 2005; Xu et al., 2001), p120 (Wei et al., 2005; Xu et al., 2001), β-catenin (Ai et al., 2000; Wei et al., 2005; Xu et al., 2001). The interplay between Cx43 and junctional components links Cx43 with the equivalent signalling pathways. Moreover, Cx43 C-terminal can bind directly to α-tubulin and β-tubulin (B. N. Giepmans et al., 2001a; B. N. G. Giepmans et al., 2001b; Kang et al., 2009; Saidi Brikci-Nigassa et al., 2012) and co-localises with actin and actin binding proteins (Xu et al., 2006). Cx30 interacts with the actin microfilaments and microtubules (Qu et al., 2009). Through these interactions connexins are able to regulate the cytoskeletal machinery. It is still unclear how these interactions are related with the biological process controlled by connexins.

Examples Of connexins in cell migration

a. Connexins in neuronal migration

During corticogenesis, newborn neurons originating from radial glia migrate to the appropriate lamina of the cortical plate (Fig. 2A). In this process radial glia act not only as neural stem cells, but also as scaffolds for the migration of newborn neurons from the ventricular zone, through the intermediate zone till they reach and colonise the cortical plate. Connexins are expressed during migration throughout cortical development (Cina et al., 2007; Dermietzel et al., 1989). More specifically, Cx43 and Cx26 are present at the contacts between migrating neurons and radial glia (Elias et al., 2007; Nadarajah et al., 1997).

Correlation between connexins and proper lamina formation was evident in Cx43KO mice (Fushiki et al., 2003), where a delay in migration of BrDU-labelled cells was reported in the cortex. Most neurons were located at the intermediate zone and fewer at the cortical plate as compared to their littermate control mice. Further evidence for a migration defect came from mice with a conditional deletion of Cx43 driven by Cre expression under the human GFAP promoter. These Cx43-depleted neurons are accumulated in the subventricular/ventricular zones, a phenotype suggestive of a failure in neuronal migration (Wiencken-Barger et al., 2007). In an ex vivo model, neural cells from Cx43 null neurospheres display a lower outgrowth index, reminiscent of reduced migration (Scemes et al., 2003).

All these studies demonstrate that connexins are key regulators of neuronal migration. The question is which of the connexin activities is required for this type of migration. Recent studies demonstrate that the channel activity of connexins is dispensable...
for neuronal migration, while other connexin activities are essential. Initial evidence for that was given with the use of short hairpin RNA knockdown experiments targeting the endogenous Cx26 and Cx43 in the developing rat cortex (Elias et al., 2007). Consistently with previous finding (Fushiki et al., 2003), neurons expressing Cx43shRNA or Cx26shRNA fail to migrate to the cortical plate and remain at the intermediate zone. For this migration, neither the channel activity of gap junctions nor the C-terminal tail are required, since a dominant negative channel or a C-terminally truncated construct could rescue the phenotype exerted by shRNAs. Interestingly, cell adhesion mediated by the extracellular loops of gap junctions is essential for neuronal migration guided by radial glia. However, use of these connexin mutants can lead to lower connexin levels at the cell membrane (Tong et al., 2009), that can cause reduced migration due to trafficking defects, apart from cell-cell adhesion perturbation.

The requirement for gap junction mediated adhesion is further demonstrated in a follow-up study, in which interneurons of marginal zone were electroporated with Cx43shRNA or Cx26shRNA (Elias et al., 2010). Normally, interneurons migrate tangentially until they reach the cortical plate and then switch their mode of migration to radial. Strikingly, interneurons expressing Cx43shRNA or Cx26shRNA fail to migrate to the cortical plate and consequently they do not invade the cortical plate (Elias et al., 2010). For this process the gap junction adhesion is essential, similar to the radial migration of pyramidal neurons (Elias et al., 2007). Additionally, the C-tail is required for this switch in migratory style, since a C-terminally truncated construct could not rescue the migratory defect. Using a conditional Cx43 knockout mouse driven by nestin promoter in order to target radial glia further shows the importance of the C-tail in neuronal migration. Radial migration of pyramidal neurons towards the cortical plate is inhibited and a C-terminally truncated Cx43 is unable to rescue neuronal migration (Cina et al., 2009).

Taken together, it is apparent that both GJ mediated adhesion and connexin C-terminal tail are required for proper neuronal migration. But, how these connexin activities affect physiological cell processes? The functions of classical adhesion molecules and actin cytoskeleton are tightly linked and their interplay is involved in controlling acquisition of cell polarity and directional migration (Francis et al., 2011; Theveneau et al., 2010). Connexins are novel adhesion molecules that are known to interact with actin and actin binding proteins (Xu et al., 2006). A positive correlation between actin and connexins along with an involvement in acquisition of cell polarity (Elias et al., 2007) is observed. Actin accumulation coincides with connexin puncta at the neuron-glia interface and pyramidal neurons expressing Cx43shRNA or Cx26shRNA display fewer actin puncta. Furthermore, connexin inhibition leads to polarity defects (Elias et al., 2007). In wild-type neocortex, pyramidal neurons travel along the radial glia by extension of multiple processes, one of which prevails and corresponds to the direction of migration. More Cx43 and fewer Cx26 puncta localise at the leading process, while their depletion by shRNA, leads to a phenotype with multiple processes, none of which is dominant, which suggest that gap junction adhesion is

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**Fig. 2.** Examples of Connexins required for cell migration. (a) Control neurons form a dominant process that allows them to migrate along radial glia (glia shown in grey). Cx-depleted cells display multiple disoriented processes. (b) Cx-depleted astrocytes form multiple processes in comparison to control. In a scratch assay control astrocytes extend large processes towards the wound, while Cx-depleted form shorter processes. (c) Migrating neural crest cells are highly polarised, forming large lamellipodia towards the direction of migration, whereas Cx-depleted CNCs show multiple protrusions in all directions. (d) Metastatic cancer cells are able to intravasate and extravasate by extension of invasive processes. Cx-depleted cancer cells are more rounded and fail to invade tissues.
involved in stabilising the leading process (Elias et al., 2007). Therefore, the phenotype of migrating interneurons that fail to change direction of migration (Elias et al., 2010) could also be explained as a defect in the leading processes and consequently loss-of-directional movement. In summary, gap junction adhesion and connexin C-tail regulate directional neuronal migration through stabilisation of leading processes.

b. Connexins in astrocytes

Astrocytes, specialised glial cells of the nervous system, undergo migration in two instances during development: (a) as differentiating newborn astrocytes in the developing central nervous system in order to reach their final sites, and (b) in the adult tissue under brain scar formation as migrating astrocytes in order to reach the lesion (Fawcett and Asher, 1999). Connexins have been implicated in both of these processes (Fig. 2B).

In embryonic organotypic slice brain cultures from Cx43KO mice, an abnormal distribution of astrocytes is indicative of a mobility defect (Perez Velazquez et al., 1996). Astrocytes from Cx43 null cultures are detected in the centre of the cultures till the edges of the outgrowth, while in normal cultures astrocytes are concentrated only at the edges of outgrowth (Perez Velazquez et al., 1996). A conditional knockout mouse expressing Cre recombinase driven by GFAP promoter and a floxed Cx43 gene shows an altered distribution and lamination of glia and neurons and disorganisation of the ventricular and subventricular zones of the cortex (Wiencken-Barger et al., 2007). These phenotypes are reminiscent of cell migration defects both in neurons and glia.

The effect of the connexin mediated channel function on cell migration was shown in astrocytes from subventricular zone (SVZ) of neonatal rat explants (Marins et al., 2009). Use of GJ blocking reagents (carbenoxole, flufenamic acid) inhibits cell-cell communication and cell migration in neural cells from SVZ explants. Analysis of cell motility features (directionality, distance travelled and speed) showed that the decrease in cell migration is the result of marked low cell motility in GJIC-inhibited neural cells in comparison to control. Interestingly, this effect is reversible and the motility defect caused by GJIC inhibition can be rescued with the removal of the blocking agents (Marins et al., 2009). Additionally, the connexin channel activity regulates the morphology and density of astrocytes derived from neural progenitors in embryonic neurospheres (Duval et al., 2002).

An opposing role of Cx43 channel activity is shown in vitro by a scratch wound assay, that is similar to brain scar formation (Homkajorn et al., 2010). In this study a migratory defect found in Cx43 depleted astrocytes was independent of connexin channel activity, since the gap junction blocker carbenoxole did not affect the rate of migration. Interestingly, Cx43 depleted cells display shorter processes extending into the wound area, a phenomenon indicative of a polarity defect (Homkajorn et al., 2010). However, it has been shown that Cx43 downregulation in astrocytes did not affect general cell morphology (cell length, cell roundness and total cell area) or the organisation of global actin (Olk et al., 2010). Interestingly, morphometric analysis showed an increase in the extending cell processes in Cx43-depleted astrocytes. Additionally, in these cells the levels of cytoskeletal molecules (actin, GAP, tropomyosin, microtubule associated protein RP/EBl, tansgelin) were increased, as shown by mass spectrometry and verified by western blot (Olk et al., 2010). These results suggest that Cx43 downregulation affected the cortical domains of the cell cytoskeleton.

The increase in multiple cell protrusion lead to increase in cell migration in a transwell chamber assay and a concomitant decrease in cell-cell adhesion, as shown with a confluent culture test (Olk et al., 2010). These results are contradictory to previous findings, where connexin downregulation results in a reduction of astrocytic migratory potential (Homkajorn et al., 2010). The presence of multiple cell processes in Cx43 depleted astrocytes (Olk et al., 2010) is consistent with the phenotype of Cx43shRNA expressing neurons (Elias et al., 2007). However, whether this astrocytic phenotype could account for a polarity defect and a consequent failure in migration in vivo remains unclear. It is important to note that extensive protrusions in single cells are beneficial for in vitro migration. However, in vivo multiple protrusions, without acquisition of polarity are not sufficient to promote forward motion and migration (Elias et al., 2007).

c. Connexins in neural crest development

Neural Crest Cells arise in early development of vertebrate embryos from the neuroepithelium and migrate extensively through the embryo in order to reach target locations, where they differentiate in various cell types (Hall, 2008; Theveneau and Mayor, 2011). A role for connexins in neural crest development is indicated by (a) the presence of Cx43 in neural crest and (b) defects associated with Cx43 misexpression in neural crest derivative tissues (Fig. 2C). Cx43 is expressed in presumptive neural crest and neural crest region as revealed by in-situ hybridisation from mouse embryos (Ruangvoravat and Lo, 1992). Transgenic mice overexpressing Cx43 or Cx43 knock-out mice display conotruncal heart malformations and outflow tract obstructions. In this heart region (conotruncus) neural crest derivatives are known to be essential during development (Huang et al., 1998a, 1998b).

Neural crest cells derived from neural tube explants from the hindbrain neural folds of mouse embryos at stage E8.5 correspond to a subpopulation that gives rise to cardiac neural crest (Waldo et al., 1999). The migratory behaviour of cardiac neural crest is correlated to the expression levels or dosage of Cx43, with higher Cx43 expression leading to increased cell migration. Such a differential expression of Cx43 can be achieved by transgenic mouse overexpressing Cx43 under CMV promoter (Huang et al., 1998a: Waldo et al., 1999) or Cx43-depleted homozygote or heterozygote mice (Waldo et al., 1999; Xu et al., 2001). Analysis of Cx43 deficient neural crest cells (CNCs) revealed a reduction in the directionality, but no change in the speed or the persistence of cell movement (Xu et al., 2001). However, when Cx43 depleted CNCs migrate on higher fibronectin concentrations in vitro, they show a decreased speed and directionality, while CNCs overexpressing Cx43 display enhanced cell motility, exhibiting high directionality and high speed (Xu et al., 2006).

Interestingly, the migration defect exerted by Cx43 depleted cells is not a consequence of decreased proliferation, as confirmed by BrdU labelling (Huang et al., 1998a). These observations open the question of which Cx43 activity is required in CNC migration. Dye coupling experiments demonstrate that CNCs exhibit gap junction channel activity and cell coupling decreases along with Cx43 levels (Huang et al., 1998b). Furthermore, use of the gap junction blocker oleamide decreases GJ communication and NC migration (Waldo et al., 1999), correlating channel activity of Cx43 with neural crest migration. Gap junction intercellular communication (GJIC) is also reduced in neural crest cells deficient in N-cadherin or Wnt1, implicating these molecules with connexin gating (Xu et al., 2001).

Neural crest cells are distinctly polarised forming extensive lamellipodia protrusions at the cell front, towards the direction of migration, and retracting processes at the rear of the cell (Theveneau et al., 2010; Xu et al., 2006). Conversely, in CNCs from Cx43 KO mice cell protrusive activity was increased as revealed by analysis of extension and retraction of cell processes. The areas of positive and negative flow were distributed nearly symmetrically
around the entire cell periphery of Cx43 depleted cells, indicating reduced cell polarity and directional movement (Xu et al., 2006). Cx43 is known to directly bind to microtubules (B. N. G. Giepmans et al., 2001a) and the effect on directional motion was reported to be a result of the MTOC failure to reorient as observed in mouse embryonic fibroblasts (Francis et al., 2011). A Cx43 deletion mutant lacking the binding domain of tubulin is unable to rescue the polarity defect. In addition to the direct interaction between Cx43 and microtubules, recent studies demonstrate a link between connexins and actin (Xu et al., 2006). CNEs exhibit actin stress fibres that are aligned in a parallel arrangement along the main cell body axis, while Cx43 depleted CNCs show actin bundles encircling the cell cortex in a polygonal array. Furthermore, Cx43 localises with a variety of actin binding proteins on these cells (Xu et al., 2006). Together these observations indicate marked changes in the organisation of the cell cytoskeleton with Cx43 deficiency and a concomitant cell polarity defect. 

In summary, Cx43 is essential in CNC migration through control of cell cytoskeleton and regulation of polarity, thus allowing efficient forward motion. CNCs display cell-cell communication and its inhibition correlates with migration defect. However, the link between channel activity and cell migration remains unresolved.

d. Connexins in cancer metastasis: friend or foe?

Cancer is a complex multi-stage disease that implicates a plethora of molecules and involves a variety of cellular activities. Since connexins have been established as coordinators and positive regulators of tissue homeostasis (Alexander and Goldberg, 2003; Niessen et al., 2000; Plum et al., 2000), their role in the incidence of cancer has been investigated in a variety of tissues and stages of the disease. Initially, connexin downregulation or GJIC inhibition was linked to the onset of neoplasia and tumorigenesis in various studies (Jameson et al., 1998; Saunders et al., 2001). This hypothesis was strengthened by the fact that connexins have anti-proliferative action by regulating the cell cycle (Stein et al., 1992; Y. W. Zhang et al., 2003b). However, the notion that complete loss of connexins or GJIC leads to tumorigenesis had to be modified due to recent evidence. Connexin downregulation or loss-of the GJIC between cells that normally communicate (keratinocyte-melanocyte (Hsu et al., 2000) or astrocyte–astrocyte (W. Zhang et al., 2003a)) is followed by acquisition of novel connexin interactions and formation of functional GJs between cell types that do not communicate in the healthy tissue (melanocyte-fibroblast (Hsu et al., 2000), astrocyte-endothelial cell (W. Zhang et al., 2003a)). Therefore, tumorigenesis coincides with an alteration in the connexin profile or altered GJIC (loss of interactions with the parental tissue and acquisition of new relations).

Even if connexin downregulation is required for the initial onset of tumorigenesis, connexins’ role as enhancers of cell-cell adhesion and cell motility attracted the attention in relation to cancer metastasis. Primarily, connexin expression is detected in metastatic cells and tissues. For instance, it has been shown that even though the expression of Cx26 and Cx43 was reduced in the early stages of mouse skin carcinogenesis, Cx26 was highly expressed on the plasma membranes of cells invading the lymph nodes (Kamibayashi et al., 1995). Similar observations were made for breast cancer (Kanzuga-Koda et al., 2006) and prostate cancer (Tate et al., 2006). Furthermore, a clear relation was found between low Cx26 expression in the non-cancerous tissue in prostatectomy sections and risk of development of metastasis (Bijnensdorp et al., 2012).Clinicopathologic survey revealed association of high Cx26 expression with less differentiated histology and venous invasion (Ezumi et al., 2008).

The effect of connexin channel activity is controversial in cancer metastasis with studies proposing a positive relationship with channel function and metastatic potential and other studies a negative correlation. Cx26 may contribute to the metastasis of melanoma by facilitating communication between melanoma cells and their surrounding endothelial cells (Saito-Katsuragi et al., 2007). Hetero-cellular GJIC between breast tumour cells and endothelial cells may be an important regulatory step during metastasis (Pollmann et al., 2005). In prostate cancer heterotypic contacts between migrating prostatic cancer cells and normal fibroblasts can strongly stimulate their migration during invasion. However, this effect does not correlate with the gap junctional coupling between cancer cells and normal fibroblasts (Tate et al., 2006). In a recent study, a dominant negative Cx43 mutant deficient in channel formation exhibits a dual pattern of regulation in metastatic melanoma cells with a decrease in anchorage-independent growth and an increase in invasive potential (Zucker et al., 2013). Moreover, in glioma cell populations (W. Zhang et al., 2003a), the overexpression of gap junction proteins and the intercellular communication between tumour and non-tumour glia cells play important roles in the facilitation of gloma cell invasion. Decrease of cell invasion and migration (of highly metastatic cells L2 expressing Cx43siRNA) is demonstrated in an in vitro invasion matrigel and migration assay (chambers w/o matrix) (Ogawa et al., 2012). In vivo, fewer metastatic nodules were observed in the mice inoculated with L2 cells transfected with Cx43 siRNA compared with controls.

From these studies it is apparent that connexins have promoting activity in relation to cancer cell invasion and migration. In the quest of the specific connexin functions in relation to cancer metastasis certain recent studies shed some light. An adhesion defect was detected in multiple myeloma cells (MMs) when exposed to the blocking reagent 18 α-glyceryrhetinic acid (Zhang et al., 2014), a connexin inhibitor and GJ blocker. MMs adhere to bone marrow stem cells to facilitate their migration. Cx43 is highly expressed in MMs and its inhibition with the blocker 18 α-glyceryrhetinic acid decreases their adhesion and migratory capabilities (Zhang et al., 2014). Further evidence for connexins mediating adhesion is shown in breast cancer cells (Elzarrad et al., 2008). Breast cancer cells overexpressing Cx43 exhibit increased adhesion with the pulmonary endothelium, while cells expressing dominant-negative Cx43 adhere to endothelial cells to a lesser extent. Furthermore, upregulation of Cx43 is observed in tumour cell-endothelial cell contact areas (Elzarrad et al., 2008).

Apart from mediating adhesion between metastatic and endothelial cells, connexins facilitate cancer cell intravasation and extravasation (Fig. 2D). Studies of brain metastasis in zebrafish and chick embryos (Stoletov et al., 2013) using 4T-1 cells, a mouse breast cancer cell line that metastasises to the brain and other organs (Pulaski and Ostrand-Rosenberg, 2001), show that 4T-1 cells express Cx43 (Elzarrad et al., 2008; Stoletov et al., 2013) and are able to invade the surrounding tissue after they extravasate out of the brain vasculature. Conversely, 4T-1 cells expressing Cx43shRNA display significantly lower extravasation capabilities. Interestingly, 3-D imaging and morphometric cell shape analysis from chick brains revealed that extravasated metastatic cancer cells display an elongated invasive shape. In contrast, Cx43 depleted cells exhibit a marked decrease in invasive potential that coincides with a more rounded phenotype, indicative of a polarity defect (Stoletov et al., 2013).

The channel activity seems to be critical for cell-cell communication between metastatic cells and healthy cells from the invaded tissues. Perturbation of connexin channels coincides with reduced migration and invasion in cancer cells. Many of these studies detect cell communication by inhibition of gap junction channels with blocking reagents. However, it remains unclear.
whether these reagents perturb other connexin functions. A more refined inhibition of specific connexin activities is thus required to untangle the complex functions of connexins in cancer cell migration and invasion.

Conclusion and perspectives

In summary, connexins are multifaceted molecules that have major roles in animal development and cancer. Their multiple functions are attributed to specific structural domains: connexins (a) establish direct cell-cell communication enabling small signalling molecules to be transmitted between cells, (b) mediate cell-cell adhesion through the highly conserved cysteine residues, (c) interact directly with the cell cytoskeleton (C-terminal domain), (d) exhibit paracrine -autocrine action through their hemichannel activity and (e) are involved in a variety of signalling pathways (C-terminus).

Among different migratory cell types (neurons, astrocytes, neural crest cells and metastatic cancer cells), connexins demonstrate a critical role in cell migration through a variety of cell activities. Although gap junction channels are functional in neuronal cells and astrocytes, this activity is not required for cell migration. In neural crest cells the role of gap junction channels is not yet understood. In migratory cancer cells, connexin channel activity is required for hetero-cellular interactions. A novel function of connexins is the direct cell-cell adhesion mediated through the cysteine residues of the extracellular loops and is critical for neuronal migration. In the other cell types, cell-cell adhesions are affected when connexin is perturbed. Whether this cell-cell adhesion is linked to connexin-connexin interactions or also to other cell junctional components remains unresolved for these cell types. Moreover, connexin interaction with the cytoskeletal machinery implicates connexins with control of cell shape and polarity, thus affecting forward directional movement.

In order to dissect the distinct connexin activities in relation to specific cell processes, as in cell migration several issues need to be addressed. The role of each connexin needs to be studied, to determine whether they share common activities or they control different cellular processes. In addition, the identification of the signalling pathways controlled by each connexin needs to be analysed further, and how they are linked to the different connexin domains. Answering this and other questions will help to fully unravel the activities of connexins in migrating cells in animal development and cancer.

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References


