Formation of metastases negatively impacts the survival prognosis of cancer patients. Globally, if the various steps involved in their formation are relatively well identified, the molecular mechanisms responsible for the emergence of invasive cancer cells are still incompletely resolved. Elucidating what are the mechanisms that allow cancer cells to evade from the tumor is a crucial point since it is the first step of the metastatic potential of a solid tumor. In order to be invasive, cancer cells have to undergo transformations such as down-regulation of cell-cell adhesions, modification of cell-matrix adhesions and acquisition of proteolytic properties. These transformations are accompanied by the capacity to “activate” stromal cells, which may favor the motility of the invasive cells through the extracellular matrix. Since modulation of gap junctional intercellular communication is known to be involved in cancer, we were interested to consider whether these different transformations necessary for the acquisition of invasive phenotype are related with gap junctions and their structural proteins, the connexins. In this review, emerging roles of connexins and gap junctions in the process of tissue invasion are proposed.

**1. Introduction**

Formation and growth of secondary tumors (metastases) in vital organs negatively impact the survival prognosis of cancer patients. Since more than 90% of deaths by solid tumors are the consequence of metastatic growth, it is crucial to decipher molecular mechanisms responsible for such a process in order to discover potential therapeutic targets that could prevent its development [1].

Globally, the various steps involved in the formation of metastases from the spreading of invasive cells coming out from the primitive tumor are relatively well identified and individualized. Covering the distance from the primary tumor to the final location of metastases involves the crossing of several physical barriers by the invading tumor cells. This means that these cells are not only able to migrate through the extracellular matrix around the tumor but have afterwards to cross the endothelial barriers of the blood or lymphatic vessels (intravasation) before reaching distant organs they may “colonize” after crossing for a second time the endothelial barrier (extravasation). Moreover, during their transportation in the blood circulation, these cancer cells have to resist to particular mechanical stress (blood stream and pressure) and to leucocytes that diminish their survival.

This cascade of events obviously depends on the first step which initiates the apparition of particular subpopulations of cells inside the primary tumor that acquired molecular criteria required for spreading out of the core of the tumor. Interestingly, even if this step controls so-called late stages of tumor progression which are the apparition of metastases, it becomes obvious that these invasive capacities are mostly acquired at the beginning of the carcinogenesis process and possibly even before the tumor is clinically diagnosed. Indeed, clinical cases have been described in which metastases appeared before the primary tumor was diagnosed. This emphasizes the importance of deciphering the molecular mechanisms controlling the acquisition of motility and invasive-ness by some cancer cells even if it does not predict that metastases will occur because the accomplishment of the following steps (crossing of endothelial barriers; survival to blood circulation and mechanical stress) is random.

The apparition of cancer cells able to spread out of the tumor is thus the very fundamental and initiation step that predetermines the burden of metastasis formation. Such an initial process mostly depends on the sequence of the following events which permits to the malignant cells (1) to loose, through epithelial-to-mesenchymal transition (EMT), their initial intercellular adhesion to be separated from the primary tumor, and then, (2) by the secretion of proteases, to degrade the basal lamina proteins permitting them to migrate...
through the extracellular matrix (ECM) and to invade the stroma underneath.

Among this sequence, two major events involve the modulation of cellular interactions. The first one is the physical detachment of those particular cells from their counterparts in the solid tumor and then the establishment of cooperative interactions between these subpopulations of cells and the tissue microenvironment that permits their migration out of the core of the tumor and the invasion of the surrounding stroma [2]. Therefore, interactions playing an important role, most of studies focused for many years on the implication of cell–cell recognition molecules (cadherins) or on molecules controlling cell–matrix interactions (integrins). In parallel, gap junction proteins (connexins) have also been shown to be involved in non-pathological migration processes such as those occurring during development: migration of neuron precursors to the cortex [3] or neural crest migration [4]. More recently, the role of connexins in migration was extended to cancer, during the past years, data showing that connexins could control migration and invasion of cancer cells accumulated. Therefore, in order to understand better what is the real involvement of connexins and gap junctions in the invasive process, we will review their implications in the control of adhesion, proteolysis and motility which govern the spreading of cancer cells.

2. Adhesion

The integrity of tissues and organs is maintained through two major types of interactions: direct adhesions between cells themselves and with the components of the ECM. For carcinoma cells that initially present an epithelial phenotype, the process of tissue invasion is mostly the consequence of a loss of intercellular adhesions (cadherins) which is accompanied by a decreased basolateral polarization and remodeling of cell–ECM adhesions (integrins). These adhesions are permitted by a chain of protein interactions (trans-membrane proteins, cytoplasmic molecules and cytoskeletal components) joining components of the cytoskeleton to the extracellular environment such as neighboring cells or ECM [5]. Underneath the plasma membrane, the link with the cytoskeleton is constituted by multiprotein complexes which anchor to the transmembrane proteins (cadherins for intercellular adhesion or integrins for cell–ECM adhesion). Concentration of these “links” in particular spots or regions of the cell constitutes adherens junctions (intercellular adhesion) or focal adhesion complexes (cell–ECM adhesion) whose stability depends a lot on the appropriate functionality of the transmembrane “link” which are cadherins or integrins depending on the adhesion type which is considered.

2.1. Cadherins

Cadherins are particularly known to play a pro- or anti-metastatic role during this initial phase of adhesion loss between tumor cells. They are transmembrane glycoproteins responsible for calcium-dependent homophilic adhesion between cells and belong to a multigene family whose members are differently expressed depending on the cell type.

Among them, E-cadherin is a fundamental component of adherens junctions between epithelial cells. In cancer, E-cadherin has been seen for long as an inhibitor of invasion and metastatic potentials of carcinomas. Its role was studied particularly in colon and breast cancers where invasiveness is inversely correlated with its level of expression [6–9]. It is important to note that the role of E-cadherin is not only for maintaining intercellular adhesions. Indeed, its intra-cyttoplasmic interactions with p120-, α-, β- and γ-catenins form a protein complex responsible for a particular organization of the cytoskeleton that controls the cell shape, polarization and the adhesion of the cells with their neighbors. Any modification of these protein interactions, through Wnt/APC signaling may be the starting point of an intracellular signal affecting the actin organization necessary for cell migration. Considering this aspect, it has been suggested that the inhibition of cell migration by expression of E-cadherin may be mainly due to its capacity to mediate intracellular signaling rather than the direct formation of intercellular junctions [10,11]. EMT is a necessary starting point for local invasion of carcinoma cells. The acquisition of the mesenchymal phenotype is accompanied by a switch of cadherin expression; E-cadherin expression decreases while the amount of another type (originally, neural type), N-cadherin, increases. Interestingly, in carcinomas, N-cadherin seems to have an opposite role to E-cadherin since its presence is not only associated with the apparition of mesenchymal characteristics but also to an increased cell migration capacity [12].

In epithelia, gap junctions are common at the proximity of intercellular adhesions where they mediate gap junctional intercellular communication (GJIC) which permits the direct transfer of ions and small metabolites between cytoplasms of neighboring cells. GJIC decrease and alteration of expression of the structural proteins of gap junctions, the connexins, have been frequently observed in tumor cells. In various cell types, it has been shown for many years, that the assembly of connexins in gap junctions depends on the establishment of adherens junctions mediated by E-cadherin [13–17].

So, in the context of EMT occurring during carcinoma progression, it would be interesting to estimate what are the consequences of cadherin switch in the control of gap junction assembly and mediation of GJIC. Recently a possible link between GJIC, connexins and cadherin was observed by considering rat liver epithelial cells undergoing EMT. As expected, the N-cadherin/E-cadherin switch increases the migration/invasion capacity of those cells but also modulates differently gap junction and GJIC. Before EMT, E-cadherin expression is associated with the presence of functional gap junctions. After EMT induction, the increase of N-cadherin prevents the formation of functional gap junctions. This process seems to prevent the formation of gap junction plaques by inducing the endocytosis of the responsible connexin, connexin43 (Cx43) via a non-clathrin-dependent pathway. However, whatever is the expression of E-cadherin or N-cadherin, the total amount of Cx43 in the plasma membrane is constant except in the detergent-resistant fractions [18]. Such an observation is in accordance with a previous one showing that functional gap junctions correspond to detergent-resistant (triton-insoluble fractions) gap junctions. Moreover, these functional gap junctions, which are known to be localized in lipid rafts, depend on the phosphorylated status of Cx43 since only the phosphorylated forms of Cx43 were shown to be targeted to the plasma membrane of the cells [19].

However, from these data, it is premature to conclude that N-cadherin expression negatively controls gap junction assembly and function. The story may be more complex and may depend on the cell type which is considered. For instance, human lung carcinoma cells (A549 cells) exhibit some heterogeneity with mesenchymal (fibroblastoid) and epithelial (epithelioid) phenotypes. These phenotypes are correlated with different motility capacities; fibroblastoid cells being characterized by a high motility capacity contrary to the epitheloid cells. While 65% of epitheloid cells and 48% of fibroblastoid express N-cadherin, Cx43 is localized to the membrane and form functional plaques for most epitheloid cells (>90%) contrary to a small portion of fibroblastoid cells (31%). Moreover, Cx43 is found to be expressed in the membrane of all fibroblastoid cells and their migration capacity appear to occur independently of the formation of gap junctions [20].
2.2. Integrin

The contact between cells and ECM is mainly established through integrins. These transmembrane molecules are activated when their extracellular domain is associated with particular ECM proteins (Fibronectin, vitronectin, collagen, laminin). In response to the integrin activation, many proteins are recruited to its cytoplasmic domain to form a complex under the plasma membrane. This protein complex, called focal adhesion complex (FAC), allows, on one hand, anchoring of the cell cytoskeleton with ECM, and on the other hand, a transmembrane signal transmission from outside to inside the cell and vice versa [21,22].

Very few data have shown a possible correlation between integrins and gap junctions. A study concerning human keratinocytes established a direct link between integrins and GJIC. In such cells, the cell–matrix junction via integrin α3β1 increases GJIC capacity when ECM contains laminin-5. This increase is not an up-regulation of the expression of the gap junction protein Cx43 at the mRNA or protein levels. In fact, the authors showed that when cells are cultured on laminin-5, the interaction between integrin α3β1 and laminin-5 increases GJIC by favoring the gap junction assembly of Cx43 in triton-insoluble fractions, (and thus possibly lipid rafts), of the plasma membrane. Furthermore, the activity of Rho seems necessary to increase the laminin-mediated GJIC through integrin α3β1 [23]. It seems that laminin-5/integrin interactions activate Rho which modulates gap junction assembly and thus GJIC. This suggests that cell–matrix interactions are also able to modulate cell–cell interactions such as GJIC.

In other cellular contexts, a link between another gap junction protein, Cx26, and integrins or their partners has been described. For instance, in prostate cancer cells, Cx26 expression is correlated with migration and invasion by interacting with the focal adhesion kinase (FAK) [24]. However, in breast cancer cells (MDA-MB-435 cells), Cx26 acts in an opposite way since its overexpression is correlated with a decrease in migration or invasion process. Interestingly, such a decrease is linked to diminished expression of integrin α1 [25]. These data tend to demonstrate that interactions between the integrin system and connexins may act differently on the migration capacity of cancer cells. Such a different behavior seems to depend both on the cell type and the connexin type which is expressed.

3. Proteolysis

Several reviews mentioned that connexins could be involved in the dissemination of metastatic cancer cells especially during processes such as diapedesis permitting to those cells to enter into (intravasation) or sort out (extravasation) the blood and/or lymphatic capillary networks [26,27]. Here, we will focus on the possible link existing between connexin expression and the invasion capacity itself that permits cancer cells to invade the peritumoral microenvironment and reach the capillary network. It is well known that tissue invasion is based on the capacity of those cells to degrade proteins that constitute the ECM. Recently, it became obvious that such a proteolytic phenomenon is complex and is accompanied by a variety of molecular processes which seems to depend on an “activation” of the peritumoral stroma. In this part, we will review data suggesting that connexins or gap junctions are involved respectively in the proteolytic capacity of the invasive cancer cells and in the activation of the peritumoral stroma.

3.1. Proteolytic degradation of the extracellular matrix

In Mammals, proteolytic degradation is performed by proteases belonging to five major families (cysteine-, aspartate-, threonine-, serine- and metallo-proteases). The activity of these proteases, which is closely regulated by their counteracting endogenous inhibitors, is necessary for non-pathological situations such as embryonic development or renewal of healthy tissues. They are also involved in pathological processes such as tumor invasion, with a particularly implication of some of them like serine- and metallo-proteases that we will review below as uPA/uPAR/PAI and MMP/TIMP systems. These enzymatic systems, whose members are secreted or located on the extracellular surface of the plasma membrane, participate in the degradation of the ECM during the cancer cell invasion process through a cascade of reactions leading to proenzyme activation [28].

3.1.1. uPA/uPAR/PAI system

The uPA/uPAR/PAI system is characterized by the enzyme urokinase (or uPA for “urokinase-type plasminogen activator”) which is a member of the serine protease family. The enzyme uPA is first secreted as a proenzyme, pro-uPA, before being activated by plasmin. Once activated, it binds to the membrane receptor uPAR and acts as a positive feedback by converting the inactivated plasminogen to active plasmin which also degrades, in turn, fibrin and other ECM components. The activity of uPA is negatively regulated by plasminogen activator inhibitors (PAI-1 and PAI-2), which are also its substrates that can be involved in the development of aggressive tumors. Indeed, high rates of intratumoral uPA and PAI-1 are associated with a high frequency of metastatic recurrence of breast cancer [29].

Data establishing a link between uPA system and connexins are rare. To our knowledge, such a link was revealed in human renal carcinoma cells (Caki-1 cells). In these cells, the overexpression of Cx32 is associated with a decreased expression of uPA, uPAR and PAI-1. Usually, the expression of these fibrinolytic factors is induced by hypoxic-induced factors (HIF-1a and HIF-2a) modulated by hypoxic conditions [30]. When Cx32 is overexpressed it blocks the src-dependent induction of HIF-1a and -2a and, in turn, decreases the invasive capacity of the cells.

3.1.2. MMP/TIMP system

Among the eight identified sub-classes of metalloproteases (MMPs), three are linked to the plasma membrane (MT-MMPs). Most of the other ones are secreted as pro-enzymes and are activated in a synergistic manner: the cleavage of MMP pro-domain by plasmin initiates a self-activation of MMPs in cascade (for example: MMP-3 activates pro-MMP-1 and pro-MMP-9). Moreover, despite of this type of activation, expression and activation of MMPs are regulated by a variety of other external stimuli which are present in the tumor microenvironment such as cytokines, hormones, growth factors, changes in cell–cell and cell–ECM contacts [31]. Expression and activity of MMPs are increased in most human cancers and are generally correlated with tumor local invasiveness and worse clinical prognosis.

The role of tissue inhibitors of metalloproteases (TIMPs) on the appearance of metastases is less clear. In some models, these inhibitors induce a pro-tumor action via the expression of anti-apoptotic (Bcl-3X) or pro-angiogenic (VEGF) factors [32].

The existence of a link between Cx43 and MMP expressions has been demonstrated in different types of tumor cells [33–35]. In most cases, cancer cells expressing high level of Cx43 exhibit an invasive capacity which is higher than cancer cells expressing less. Such a difference could result from a modification in expression and/or secretion of MMP-2 and -9 by Cx43. For instance, the invasion capacity of C6 glioma cells expressing Cx43, can be reversed by a synthetic inhibitor of MMPs, BB-94 [34].

Cx26 seems to have an opposite effect compared to Cx43. Indeed, it has been shown that Cx26 expression in human hepatoma cells (HepG2 cells) induces the expression of E-cadherin which results in a decrease of MMP-9 expression. Therefore,
Cx26 expression is associated with a decreased invasion capacity through matrigel in an *in vitro* assay [36].

Kalra et al., in 2006, studied three variants of Cx26 expressed in different clones of a breast cancer cell line (MDA-MB-435 cells). One clone expressed a GJIC-competent Cx26, another one a GJIC-incompetent GFP-Cx26 chimera and the third one the Cx26 mutant D66H which is localized in the Golgi apparatus. The three Cx26 variants were shown to be involved in the reduction of cell migration by altering the cellular distribution of actin filaments. Only the Cx26 mutant localized in the Golgi apparatus did not inhibit the invasion capacity of these cells as tested through matrigel. The three variants decreased the level of integrin β1 expression, decreased the activity of MMP-9, and increased the activity of the MMP-1 inhibitor (TIMP1). In consequence, Cx26 seems to be able to reverse the tumor phenotype by a GJIC-independent way [25].

### 3.2. “Activation” of the peritumoral stroma

Reaching the stroma compartment by crossing the basal membrane allows tumor cells to interact with a new microenvironment. This new microenvironment seems to be “activated” by the presence of the tumor cells, favoring their growth and invasion capacity. The activation is mediated by proteolysis of ECM which releases a large number of sequestered cytokines and/or growth factors that play a trophic role and a chemotactic function for the invasive tumor cells [37].

During this invasive phase, establishment of GJIC has been observed between tumor cells and stroma cells [38–40]. This was mostly demonstrated with glioma cells which were shown to establish heterocellular GJIC with astrocytes both *in vitro* and *in vivo*. Interestingly, some phenotypic modifications in astrocytes resulted from this direct interaction as a reactive process. For instance, the size of the astrocytes significantly appears smaller in the presence of Cx43 expressing C6 rat glioma cells compared to Cx43 non-expressing C6 cells. Secondly, these astrocytes (as stroma cells) express lower levels of GFAP compared to astrocytes cultured in the presence of Cx43 non-expressing C6 cells. These phenotypic reactions of astrocytes seem to be the consequence of the establishment of heterocellular GJIC with the glioma cells expressing Cx43. Such heterocellular GJIC could contribute to a higher susceptibility of the surrounding tissue to be invaded by tumor cells [38]. Others have also reported that Cx43 is essential for the invasive process of glioma cells in brain sections by permitting the establishment of heterocellular GJIC with the stroma cells [39]. Similarly, the high metastatic potential of prostate cancer cells has been shown to be associated to Cx43 expression and the establishment of heterocellular GJIC with fibroblasts in co-culture [40]. Activation of the peritumoral stroma may also change the endogenous GJIC capacity. Indeed, a decrease in homologous GJIC between human dermal fibroblasts (stromal cells) is observed after addition of skin tumor cells (SCL-1 cells) in the culture. Interestingly, this GJIC reduction between the stromal cells seems to be the consequence of a calcium effect instead of alteration of Cx43 expression or localization [41]. However, such a reaction of the stromal cells may not always depend on the establishment of heterocellular GJIC with tumor cells. For instance, even in lack of direct contact, human
glioma U87 cells can induce activation of cocultured astrocytes (increased GFAP and MMP-2 expressions, decreased Cx43 expression). In those conditions, MMP-2 increase is associated to the decrease of TIMP2 expression. Therefore, even without any contact with glioma cells, the activation of astrocytes may favor the invasion process of glioma cells [42].

It is interesting to note that the invasive process may be differently regulated depending on the connexin type which is expressed in tumor cells. For instance, as mentioned above, Cx32 expression in human metastatic renal cancer cells (Caki-1 cells) results in a reduction of their invasive capacity through src inactivation. It seems that src inactivation leads to a decrease in VEGF production via the abolition of STAT-3 activation. Thus, Cx32 expression in tumor cells prevents them to activate cells by stromal VEGF secretion [43].

4. Motility

There are different types of cancer cell migration and they depend on the composition and topography of ECM (Fig. 1). For example, moving of some cancer cells in particular ECMs may depend on their protease activity while it is not the case for others [44]. On a plane surface, cell migration appears to be a cyclic process exhibiting four different stages. First, the cell is polarized and produces membrane extensions to its “front”. These extensions (lamellipodia and filopodia) are stabilized by cell–matrix adhesions or focal adhesions [45]. Thereafter, the cell “body” moves in the direction of extension, using these anchored points. Finally, after disassembly of rear cell–matrix adhesions, the cell shrinks back. These steps are directly dependent on cytoskeletal rearrangements, cell–matrix adhesions and can be regulated by soluble factors which are secreted in the microenvironment [46].

Here, we will review how these different modes of cell migration and cytoskeleton rearrangements can be related respectively to gap junction and connexin expressions.

4.1. Different modes of cell migration/invasion

One type of cell migration is the “amoeboid” migration which is characterized by a round cell morphology and occurs after an epithelial to amoeboid transition (EAT). This migration is not based on the existence of focal contacts and cell–matrix interactions appear limited in this case. This amoeboid migration, or “pseudo-crawling amoeboid” movement, is characterized by cells changing their shape in order to “slide” between the collagen fibers of ECM without degrading it. In vitro, tumor cell lines using this type of migration would be essentially from lymphomatous or carcinomatous origins (mainly small cell lung carcinomas).

After EMT, carcinoma cells are “liberated” and can move individually because of the loss of function of cadherins. These cells can then perform mesenchymal migration (elongated shape with fibroblast morphology). This individual “fibroblastoid” migration is realized by the invasive tumor cell repeating the four following steps: extension/adsorption/retraction/movement, to which is added ECM degradation. This degradation, localized in ventral region of the cell which is embedded in the ECM with many focal contacts, is associated with proteases (secreted or present on outer surface of the plasma membrane) expressed at the level of invadopodial structures. These cellular movements can be studied with different techniques in the laboratory. In most cases, the so-called 2D migration is mostly observed on plane surfaces by performing a wound healing assay while the 3D migration or invasion is observed in Boyden chambers coated or not with a matrix (matrigel or specific ECM proteins). This last type of migration can be also tested in ex vivo organotypic slice invasion assay or in vivo experiments. Therefore, by using such techniques, it is possible to observe the various processes used by tumor cells to migrate or invade their environment [46–49].

By using techniques testing 2D migration, the motility process seems to be mostly associated with a reduced expression of Cx43. Indeed, in a study in which glioma cells, dissected from different regions of a canine brain tumor, were placed in a culture dish coated with different matrix proteins, an inverse relationship between Cx43 expression, GJIC and the migration rate was demonstrated [50,51]. Similarly, when Boyden chambers without degradation of matrix are used, an increased capacity of 3D migration is associated with decreased Cx43 expression and GJIC in breast cancer cells [52,53]. These data suggest that, when motility of individual cells is tested, the migration capacity of tumor cells is inversely correlated with GJIC and expression of Cx43.

The first observation associating an ex vivo invasive 3D process and connexin expression was made by using HeLa cells transfected with different connexin types (Cx32, Cx40 or Cx43). By doing so, it was observed that these three connexins facilitated HeLa cells invasion in embryonic chicken heart fragments which were cultured in semi-solid agar. In those experiments, Cx43 expression was correlated with the longer distance of invasion when compared with HeLa cells expressing the two other types connexins [54]. It seems that, in this type of ex vivo experiment, other Cx43-dependent functions appeared. For example, heterocellular GJIC between tumor cells and stromal cells seems to be essential for activating tissue invasion. This has been demonstrated also in vitro by using C6 rat glioma cells overexpressing Cx43. When these cells are cocultured with astrocytes, they exhibit an increased invasion capacity in Boyden chambers [38]. In such cocultures permitting heterocellular GJIC with the stromal cells, the Cx43-carboxy tail (Cx43-Ct) appears to be essential for the invasive process involving matrix degradation. In such conditions, higher is expressed Cx43 in C6 glioma cells and more the cells are invasive in the 3D invasion test. Interestingly, this invasion capacity does not decrease in the presence of a GJIC inhibitor but depends on the presence of the Cx43–Ct [55,56]. Indeed, this particular function of the Cx43–Ct tail seems to be important for the formation of the leading edge during cancer invasion [57]. Similarly, we have shown that Cx43 controls the invasion capacity of U251 glioblastoma cells through its localization in lipid rafts. It appeared that such a capacity could be mediated by the interaction between Cx43–Ct and caveolin-1 (Cav-1; a lipid raft marker) [51]. Finally, connexins could be involved in the invasion process through their adhesion properties. Indeed, after transfection of Cx43 or Cx32 cDNAs in C6 glioma cells, an increase in cell aggregation capacity is observed. This property might be carried by the extracellular loops of connexins and not by their communication properties. Collective cell migration chain is defined as a migration process used by carcinoma cells keeping their intercellular junctions. Such a process could be mediated by the adhesion properties of connexins. On the other side, the invasion process of C6 cells observed in vivo shows that Cx43-positive cells have larger invasion capacity inside the parenchyma than those transfected with Cx32. This means that connexin adhesive property is not sufficient to permit invasion of surrounding tissue by tumor cells [58].

Regarding Cx26 and Cx32, it appears that these connexins have an opposed effect on the behavior of tumor cells when compared to Cx43. When Cx26 is up-regulated in breast cancer cells (MCF-7 cells), cell migration and invasion through matrigel in Boyden chambers is reduced [59]. In another breast cancer cell line (MDA-MB-435 cells), Cx26 alters the distribution of the actin cytoskeleton and reduces the 3D migration and invasion [25]. In 2007, an experimental model was created for inducing the overexpression of a cytoplasmic and non-functional Cx32 in human hepatocellular carcinoma cells. From such a model, it appears that when
Cx32 is not at the plasma membrane the invasion capacity of cells is increased and consequently their metastasis behavior [60]. Therefore, it seems that the various possible functions of connexins (GJIC, hemi-channel, or scaffolding protein function) may have different effects in cell migration or invasion processes. Moreover, a recent study found that Cx31.1 may act on migration and invasion of lung cancer cells. In such a study, the overexpression of Cx31.1 in the lung cancer cells (H1299 cells) eliminates their 3D migration and invasion by inducing mesenchymal–epithelial transition (MET) [61].

4.2. Cytoskeletal remodeling

During EMT, there is a remodeling of the cytoskeleton in which cortical actin fibers are redistributed in actin stress fibers; a characteristic of the mesenchymal phenotype. While the cytoskeleton is remodeled, the accompanying disruption of intercellular adhesions mechanically leads to loss of the apical-basolateral polarity and involves Rho GTPases, Cdc42 and Rac. These three partners play a major role in pro-migratory reorganization of actin filaments and formation of focal contacts with ECM in response to activation of PI3K by extracellular chemotactic signals [62]. Rac and Cdc42 were described to be responsible for polymerization of actin microfilaments in periphery or cell anterior pole, by forming membrane sheet structures (lamellipodia) or cell micropikes (filopodia). Rac also induces formation of focal contacts with ECM along these extensions. Moreover, Rho regulates, via ROCK/Rho kinase, actin/myosin contraction and assembly of actin stress fibers, responsible for translating the cell body forward. Rho also may be involved in the organization of actin fibers for the invadopodia formation which are cortactin dependent structures [63,64]. The protomorphogenic role of the Rho family has been widely emphasized by comparative genomic analysis techniques [65].

Above, we have seen that Cx43-Ct seems to play an important role in the process of cancer cell invasion. This cytoplasmic Cx43 part interacts with many cytoskeleton proteins, including actin [66]. In particular CCN3, a protein that binds to Cx43-Ct, is known to be a direct activator of rac1 [67]. Thus, Cx43 could enhance the migration/invasion process by activating small GTPases. What is also interesting to consider is that Cx43 has been reported to interact with cortactin, a protein associated with actin, to promote invasive capacity by facilitating invadopodia formation [68,69]. In addition, some authors showed that Cx43-Ct regulates motility by interacting with proteins involved in lamellipodia formation [70].

5. Conclusion

During the embryonic development, EMT permits to epithelial cells to exhibit a mesenchymal phenotype in response to different types of stimuli. This transition results in a loss of intercellular junctions (tight junctions, adherens junctions, desmosomes and gap junctions), loss of apical-basolateral cell polarization marked by selective localization of adhesion proteins (cadherins, integrins), induction of protease expression, destruction of the basal membrane and stroma invasion [71,72]. EMT is also known to be abnormally reactivated in adults in at least two pathologies: tissue fibrosis and cancer. In cancer, EMT has been widely described to be responsible for the metastatic potential acquisition by carcinoma cells. And it has been reported in many models that tumor progression in invasive and metastatic stages is associated with cellular dedifferentiation, cell polarity alteration and acquisition of fibroblastoid morphology [73–75].

At first glance, the involvement of connexins in this EMT process appears to be contradictory. And this seems to be particularly the case for Cx43 which has been extensively studied in this context. However, when EMT is considered into different stages, some coherence can be found in the various, and apparently contradictory, roles that may be played by connexins during the invasive process. Indeed, the first step of EMT, which permits the detachment of invasive cells from the primary tumor, is the loss of cadherin-dependent cell–cell adhesions and the establishment of specific cell–matrix adhesion. The switch between E-cadherin and N-cadherin which happens during this step is often accompanied by a loss of junctional plaques associated with a loss of GJIC. Contact with ECM via integrins appears to allow maintenance of Cx43 expression associated with more or less organized junctional plaques. At this point, the function of GJIC seems to establish heterocellular GJIC between tumor cells and stromal cells which, in turn, activates the microenvironment; a crucial step for cancer cell invasion. This activation of the peritumoral stroma allows secretion and activation of stroma proteases which favor cell invasion within the ECM. At this time, when the invasive process results in reorganization of actin fibers and membrane structures of the tumor cell, the function of scaffold protein played by Cx43-Ct and/or hemichannel may favor the invasive process mediated by ECM-degradation. This assumption seems to be supported by the fact that in plane migration process without cellular degradation of the ECM, on a surface, Cx43 does not appear essential. However, when EMT transforms a motionless cell in an invasive one, Cx43 seems to undergo different types of regulation at the transcriptional, translational or post-translational levels that permit its localization in triton insoluble structures of the plasma membrane (lipid rafts). Furthermore, the involvement of Cx43 appears to be mediated through its different functions such as GJIC, hemichannel and scaffold protein, depending on time and step of the invasive process. What then are the signals controlling precisely such a complex process?

The mechanisms regulating EMT are various: many extracellular activators may induce or inhibit this process, relayed by intracellular signaling pathways (Wnt, Hedgehog, Notch and signaling pathways activated by cytokines, TGF-β, EGF, FGF, HGF/SF) and re-activation of transcription factors driving the process (proteins Snail and Slug, Twist, Zeb and NF-kappaB) [72,76].

The association between the secretion of TGF-β and Cx43 expression has already been described in different models [77]. In the context of brain, it has been shown that TGF-β increases astrocyte coupling while it decreases GJIC in the rat C6 glioma cells, via a regulation of the Cx43 P2-phosphorylated form [78]. Another example of EMT regulator has been linked to connexin. It seems that there is a signaling HGF/c-Met/connexin involved in changing liver architecture that increases the number of metastases at this level [79,80]. Similarly, a recent study showed a correlation between the transcription factor Twist, associated with the formation of metastasis [81–83] and expression of Cx43. Indeed, activation of the TWIST gene in breast cancer cells causes increased expression of Cx43 and GJIC associated with colonization of the brain [84]. Moreover, in melanoma cells, a possible link between different markers important for EMT was shown. After activation of the ET(B) receptor by treatment with ET-1 or ET-3, a decrease in the expression of E-cadherin associated with an increase of N-cadherin was observed. This involves a transcriptional mechanism since it was seen an increased expression of snail mRNA, associated with an inhibition of GJIC by Cx43 phosphorylation. This was accompanied by an increased expression of integrins (α9β3 and α2β1), MMP-2 and -9 activation of MT-MMP-1 and secretion of TIMP2. These phenomena seem to go through the activation of signaling proteins involving FAK and ERK1/2 [33,85].

In conclusion, connexins, and particularly Cx43, tend to play a pivotal role in the control of cell migration and invasion. This role seems to be the consequence of an accumulation of functions that
Cx43 may carry out simultaneously or in different times during the acquisition of the invasive process and its maintenance. Such an accumulation of functions is favored by the possibility that Cx43 acts intracellularly by interacting with various cytosolic proteins involved in the regulation of the migration machinery and secretion of proteases. But it is also favored by the capacity of Cx43 to mediate hemichannel transmembrane communication with the microenvironment or heterocellular GJIC with stromal cells.

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