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Review

Connexins, gap junctions and tissue invasion

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ABSTRACT

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 diminishe 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 invasiveness 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

Abbreviations: GJIC, gap junctional intercellular communication; ECM, extra cellular matrix; EMT, epithelial to mesenchymal transition; MMP, matrix metalloproteases; TIMP, tissue inhibitors of metalloproteases; MAT, mesenchymal to amoeboid transition; AMT, amoeboid to mesenchymal transition

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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-cytoplasmic 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 (detergent-resistant gap junctions) 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 (epitheloid) 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 epithelioid 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 fibrobastoid cells and their migration capacity appeare to occur independently of the formation of gap junctions [20].

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