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Review

Ion channels and transporters in metastasis☆

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ABSTRACT

An elaborate interplay between ion channels and transporters, components of the cytoskeleton, adhesion molecules, and signaling cascades provides the basis for each major step of the metastatic cascade. Ion channels and transporters contribute to cell motility by letting through or transporting ions essential for local Ca^{2+}, pH and – in cooperation with water permeable aquaporins – volume homeostasis. Moreover, in addition to the actual ion transport they, or their auxiliary subunits, can display non-conducting activities. They can exert kinase activity in order to phosphorylate cytoskeletal constituents or their associates. They can become part of signaling processes by permeating Ca^{2+}, by generating local pH-nanodomains or by being final downstream effectors. A number of channels and transporters are found at focal adhesions, interacting directly or indirectly with proteins of the extracellular matrix, with integrins or with components of the cytoskeleton. We also include the role of aquaporins in cell motility. They drive the outgrowth of lamellipodia/invadopodia or control the number of β1 integrins in the plasma membrane.

The multitude of interacting ion channels and transporters (called transportome) including the associated signaling events holds great potential as therapeutic target(s) for anticancer agents that are aimed at preventing metastasis. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

Contents

1. Introduction

Metastasis is based on a complex, multi-step process called the metastatic cascade [1]. In carcinomas, i.e. tumors developing from epithelial cells, the metastatic cascade typically begins with an epithelial–mesenchymal transition (EMT), another multi-step process, during which cells, originally featuring epithelial characteristics transmute into cells that display mesenchymal characteristics [2]. To acquire a mesenchymal phenotype, epithelial cells that are usually attached to a basement membrane undergo numerous biochemical and molecular changes such as the activation of transcription factors, the modified expression of specific cell-surface proteins, the expression and reorganization of cytoskeletal proteins, changes in microRNA expression and secretion of ECM (extracellular matrix) — degrading enzymes. These changes are accompanied by a loss of cell–cell contacts, an increase in cell motility (migration and invasiveness), a strengthened resistance to apoptosis, and an enhanced production of matrix components [2].

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During embryonic development, postnatal growth and wound healing, EMT occurs in an orderly way and is terminated by its reverse process called MET (mesenchymal–epithelial transition), whereas the acquisition of an invasive, eventually metastatic phenotype due to a delay or even a lack of a controlled MET represents EMT's pathological potential. EMT is accompanied by changes in the expression patterns of transcription factors such as SNAIL, TWIST or ZEBs which leads to a downregulated expression of E-cadherin, ZO1, claudins and occludins and an upregulated expression of N-cadherin, ECM components such as collagens and fibronectin, and matrix metalloproteases (MMPs) [3]. It is conceivable that ion channels and transporters are involved in EMT since their expression is as well modulated by transcription factors. For instance, the unscheduled expression of a neonatal splice variant of the voltage gated sodium channel $Na_v1.5$ ($nNa_v1.5$) clearly correlates with migration and invasion of metastatic breast cancer [4–6].

In general, a malfunctioning or oncogenic EMT can lead to metastable cellular phenotypes combining both epithelial and mesenchymal characteristics [7]. EMT includes loss of contact inhibition and the cells' ability to break out of the organized tissue structure [8–10]. The following detachment from the primary tumor requires the release of intercellular junctions that are typically mediated by cadherins or integrins [11,12]. The next step in the metastatic cascade is the cellular invasion of the surrounding stroma. For this purpose cells remodel the extracellular matrix by secreting matrix metalloproteinases and simultaneously exhibit a dynamic cell–substrate interaction in order to migrate directionally [13]. The migrating cells eventually enter the vasculature or the lymphatic system where they become circulating tumor cells as they are being carried away by the blood or lymph stream. Only a very small percentage of these circulating tumor cells survive the extracellular milieu and adhere to the endothelium at a distant site [14]. The cells then extravasate and invade the surrounding tissue in order to form a metastasis which finally develops a full-grown secondary tumor [15]. All four steps of the metastatic cascade, (i) loss of cell–cell contacts, (ii) invasion of the surrounding stroma and the vasculature, (iii) adhesion to the endothelium, and (iv) extravasation into the tissue of the target organ require the presence and/or concerted action of ion channels and/or transporters (Fig. 1, Table 1), also referred to as migration-associated transportome [16].

2. EMT and loss of cell–cell contacts

Oncogenic epithelial–mesenchymal transition (EMT) is based on a coordinated gene expression program, includes the early steps of malignant transformation and is accompanied by a gain of pro-metastatic properties such as an increased basal motility including invasion and cancer stem cell characteristics [17–19]. A loss of cell–cell contacts can be induced by ectopic expression of carbonic anhydrase IX (CAIX) which results in the redistribution of CAIX and its pH-regulatory interaction partners, the Na^+,HCO_3^- cotransporter NBC and the anion exchanger AE2, to the leading edge where they can fulfil tasks required for cell migration and invasion [20]. Thus, several ion transport proteins are involved in this process of epithelial–mesenchymal transition (EMT). Whether or not and to what extent other transport proteins associated with tumor metabolism, such as the Na^+/H^+ exchanger NHE1 or, monocarboxylate transporters (MCTs), or aquaporins contribute to EMT remains to be elucidated.

A Cl^- and HCO_3^- conducting channel, known as the cystic fibrosis transmembrane conductance regulator (CFTR), plays a role as an EMT suppressor in the human breast adenocarcinoma cell lines MCF-7 and MDA-MB-231 [21]. Both the presence and the function of CFTR decrease the metastatic potential of MCF-7 cells. Conversely, CFTR knockdown reduces the strength of cell–cell contacts by decreasing the expression of E-cadherin and occludins and – like the functional inhibition with inh172 or GlyH101 – leads to a dramatic increase in migration and invasion. On the same lines, the physical interaction of CFTR with the scaffolding protein NHERF1 (Na^+/H^+ exchanger regulatory factor1) in a CFTR–NHERF1–ezrin–actin multiprotein complex stabilizes cell–cell junctions through the tight-junction protein ZO-1 [22]. In nude mice, the growth of implanted MDA-MB-231 cells that overexpress CFTR is not reduced while the metastatic potential is clearly decreased, and in clinical breast cancer samples a low CFTR expression correlates with disease progression and poor prognosis [21]. Consistently, CFTR overexpression suppresses EMT and invasive behavior in MDA-MB-231 cells. A functional CFTR has been shown to control NFκB-mediated inflammatory signaling [23,24]. Knocking down CFTR leads to activation of NFκB, while conversely, the inhibition of NFκB countermands CFTR knockdown-induced EMT and the development of an invasive phenotype in MCF-7 cells. This indicates that CFTR acts as a tumor suppressor by tightly controlling NFκB-signaling via its ion-conducting function [21]. Studies employing a structurally functioning but at the same time transport-deficient CFTR could help clarify as to what extent purely structural protein interactions determine cell–cell adhesion and impact on malignancy. Interestingly, in colon cancer cells, CFTR and the adherens junction molecule AF-6/afadin physically interact at cell–cell contacts. When CFTR is knocked down these cells display an enhanced invasive phenotype which can be completely reversed by either AF-6/afadin overexpression or inhibition of ERK, indicating the involvement of the AF-6/MAPK pathway [25].

Calcium signaling mediated by calcium-permeable ion channels including the transient receptor potential-melastatin-like 7 (TRPM7) channel contributes to EMT in breast cancer, too [26]. Chelation of intracellular Ca^{2+} by BAPTA or EGTA reduces EGF- and hypoxia-induced EMT and inhibits the EGF-dependent activation of signal transducer and activator of transcription 3 (STAT3), probably including CaMK/NFκB signaling [27], while leaving the Akt and ERK1/2 pathways entirely unaffected. Since silencing TRPM7 inhibits only parts of the EGF-induced processes inhibited by Ca^{2+} chelation, additional Ca^{2+} channels or -transporters other than TRPM7 must be involved [26].

The Ca^{2+}-activated $K_{Ca}3.1$ channel (KCNN4, IK1) participates in the EMT of colorectal cancer [28]. Stimulation of $K_{Ca}3.1$ expression by phosphatase of regenerating liver-3 (PRL-3) is accompanied by an elevation of the cytosolic Ca^{2+}-concentration $[Ca^{2+}]_i$ through an unknown mechanism, possibly through a constitutive entry through voltage-independent Ca^{2+}-permeable channels under hyperpolarization of the membrane potential. This increase in $[Ca^{2+}]_i$ causes a constitutive autophosphorylation of CaM-kinase II leading to phosphorylation of GSK-3β [29]. In human colon adenocarcinoma cells (LoVo) transfected with PRL-3, the expression levels of phosphorylated GSK-3β and the transcription factor SNAIL are increased. The increased expression of these proteins can be repressed by both $K_{Ca}3.1$ siRNA and the specific channel blocker TRAM-34 [28]. SNAIL inhibits E-cadherin expression not only in colorectal cancer [28] but also in breast cancer cells [30] promoting the loss of cell–cell contacts characteristic of EMT [3].

EMT is accompanied by a loss of cell contact inhibition. NIH3T3-cells stably transfected with $K_v11.1$ (hERG1) channels show a loss of cell contact inhibition. In culture, these cells grow in multiple layers and at high density [31]. Interestingly, their morphology changes from fibroblast-like to spindle-shaped while both the degree of cell polarization and migration increase. Furthermore, allogeneic transplantation of hERG1-expressing cells into nude mice leads to an increased incidence of tumors [31].

3. Invading the surrounding stroma and vasculature

In addition to being involved in EMT TRPM7 contributes to a more migratory and invasive phenotype [32], so that in human breast cancers, the expression level of TRPM7 and the formation of metastases are positively correlated. Knocking down TRPM7 leads to elevated myosin light chain (MLC) — and paxillin phosphorylation including an increased number of focal adhesions [33]. The resulting increases in contractility and adhesion strength cause a significant decrease in cell motility and thus metastasis. In this process TRPM7 functions via a “dual mode of