 Ion channels and transporters in tumour cell migration and invasion

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Cell migration is a central component of the metastatic cascade requiring a concerted action of ion channels and transporters (migration-associated transportome), cytoskeletal elements and signalling cascades. Ion transport proteins and aquaporins contribute to tumour cell migration and invasion among other things by inducing local volume changes and/or by modulating Ca$^{2+}$ and H$^+$ signalling. Targeting cell migration therapeutically bears great clinical potential, because it is a prerequisite for metastasis. Ion transport proteins appear to be attractive candidate target proteins for this purpose because they are easily accessible as membrane proteins and often overexpressed or activated in cancer. Importantly, a number of clinically widely used drugs are available whose anticipated efficacy as anti-tumour drugs, however, has now only begun to be evaluated.

1. Introduction

Tumour progression towards metastatic disease follows a well-defined sequence of events [1]. The metastatic cascade includes several critical steps that rely on the ability of tumour cells to migrate: local invasion of the affected tissue following the disruption of the basement membrane as well as intra- and extravasation of blood or lymph vessels (figure 1). Without their ability to move, tumour cells would not be able to metastasize. However, tumour cells do not necessarily act independently. They also can migrate collectively as a group [2]. Moreover, there is an intense mutual communication between tumour and stromal cells, and the tumour microenvironment that is typically characterized by local acidosis and hypoxia [3–5]. Thus, in pancreatic ductal adenocarcinoma (PDAC), reciprocal activation of stromal stellate cells and cancer cells strongly promotes tumour progression [6]. Metastases in PDAC may even contain tumour cells and pancreatic stellate cells [7]. Monitoring the collective invasion of co-cultured fibroblasts and squamous cell carcinoma cells revealed that the invading cell group is always led by fibroblasts [8]. Thus, the ability to migrate is equally important for cancer and stromal cells.

This review highlights the role of ion channels and transporters in the steps of the metastatic cascade that rely on the ability of tumour and stromal cells to migrate. Local fluctuations of the cell volume as well as pH and Ca$^{2+}$ signalling evolved as common mechanisms linking ion transport proteins to the metastatic behaviour. We refer to three recent reviews [9–11] for a broader overview of ion transport in cell motility.

2. Ionic mechanisms of cell migration

Most malignant tumours are of epithelial origin. Hence, carcinoma cells have lost their epithelial polarization during epithelial–mesenchymal transition (EMT). Mesenchymal cells can detach and move away from the epithelial layer [12]. EMT involves a coordinated gene expression programme in association with the early steps of transformation which also can include proteins involved in ion transport. Thus, the ectopic expression of carbonic anhydrase (CAIX) is accompanied by a loss of cell–cell contacts. Subsequently, CAIX, pH-regulating transport proteins (e.g. NBCe1, AE2, NHE1, MCTs) and aquaporins are
transport proteins, the 'transportome', including their signalling pathways in cell migration has only begun to be appreciated. We will not discuss the interaction of K⁺ channels with integrins as this is covered elsewhere in this issue [17].

(a) pH-dependent regulation of the cytoskeleton in tumour cell migration

Intracellular and extracellular pH homeostasis is particularly important in cancer as outlined elsewhere in this issue [4,5,18]. As the ionization state of all cellular and extracellular proteins including their function in (patho)physiological processes depends on pH, (directional) tumour cell migration is controlled by intra- and extracellular pH [19–23].

Cofilin regulates cell motility pH dependently [24]. It generates new sites of actin filament assembly by severing actin filaments and producing free barbed filament ends. This promotes dynamic actin polymerization and membrane protrusion at the cell front or at the tip of invasive structures [25]. The inhibition of cofilin activity by PI-(4,5)-P2 binding is removed upon an intracellular alkalization [26]. The local intracellular alkalization in the lamellipodium required for cofilin activation is generated by the activity of the Na⁺/H⁺ exchanger NHE1 that accumulates at the front of migrating tumour cells and fibroblasts [27–31]. NHE1 is upregulated in numerous tumours [22,32,33] and is required for motility of melanoma, breast cancer and cervix carcinoma cells [34]. Another way of pH-dependent regulation of cofilin involves cortactin. An NHE1-mediated increase in pH triggers the release of cortactin-bound cofilin. Cofilin then induces barbed end generation, thereby promoting actin polymerization [35]. Gelsolin is another actin-binding protein that is activated by an acidic pH and that controls actin assembly and disassembly [36]. Because NHE1 activity contributes to the generation of an intracellular pH gradient along the moving direction of migrating cells with more alkaline pH values in the lamellipodium [37], we assume that cofilin is more relevant for regulating actin dynamics at the leading edge, whereas gelsolin is more active at the acidic rear end. Finally, actin self-assembly and binding of myosin to actin is promoted by neutral or slightly acidic pH values [38,39]. These examples demonstrate that actin dynamics underlying the outgrowth of lamellipodia or invadopodia relies on an optimal intracellular pH environment [11,40], which, in turn, is adjusted by the activity of pH regulating H⁺/HCO₃⁻ transporters, possibly in cooperation with carbonic anhydrases or monocarboxylate transporters [13,32,41–43].

(b) Ca²⁺-dependent regulation of the cytoskeleton in tumour cell migration

The intracellular calcium concentration ([Ca²⁺]) has a great impact on the migration machinery of 'normal' (e.g. keratinocytes), tumour and stromal cells, too, because many of its elements such as myosin II [44], myosin light chain kinase [45], calpain [46], Ca²⁺/calmodulin-dependent protein kinase II [47,48], focal adhesion kinase [49] or ion channels (e.g. KᵥCa or TMEM16 channels) are Ca²⁺-sensitive. Ca²⁺ regulation of cell migration involves a tight spatial and temporal control. In addition to a global front–rear gradient with [Ca²⁺] increasing towards the rear end of migrating cells [50,51], there are also local Ca²⁺ microdomains [45,52–54]. Spatial gradients of...
Here, local Ca\(^{2+}\) in the generation of a myosin-II-dependent contractile force gradient. pH rear part [37,71]. The alkaline pH not surprising that the pericellular pH only 20 nm into the extracellular space [69]. Therefore, it is [68]. Conceptually, it is important that integrins protrude dependence of the mechanical stability of focal adhesions [67] or by a pH conformational changes that lead to an enhanced aviditity adhesion at acidic extracellular pH values is explained by [54]. Adhesion of cancer cells to the ECM is also mediated by invadopodia that share many similarities with podosomes found in 'normal' cells such as macrophages [75]. In microglial cells, the formation of invadopodia depends on the presence of extracellular Ca\(^{2+}\) whose transport into invadopodia is likely mediated by Orai1 [76].

\(\text{(e) Cell volume dynamics during cell migration}\
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As outlined above, cell migration can be viewed as a repetitive cycle of protrusion of the cell front and retraction of the rear part. The rear part often lags behind for quite some time before retracting at a much faster speed. Such shape changes are particularly prominent when cells are moving within a three-dimensional environment where tumour cells or stromal cells may extend processes as long as 100 \(\mu\)m before the cell body eventually catches up. This fast retraction of the rear part coincides with or follows an elevation of the [Ca\(^{2+}\)] [35,77] which is likely to be caused by the activation of mechanosensitive Ca\(^{2+}\) channels. Their molecular identity has not yet been conclusively determined. TRPC1 [78], TRPM7 [53] and TRPV4 [79] channels are possible candidates. In addition to triggering the Ca\(^{2+}\)-dependent mechanisms outlined above, the elevation of [Ca\(^{2+}\)] also leads to an activation of Ca\(^{2+}\)-sensitive ion channels such as K\(_{\text{Ca}}\)3.1 [80], K\(_{\text{Ca}}\)2.3 [81], CIC3 [48,82] or TMEM16A/ANO1 [83–85]. Studies of cell volume regulation revealed that cell shrinkage can be elicited by the simultaneous activation of K\(^{+}\) and Cl\(^{-}\) channels [86]. Therefore, a hydrodynamic model was postulated according to which ion channels and transporters elicit local changes of cell volume that act in concert with cytoskeletal mechanisms underlying rear end retraction and/or the protrusion of the cell front [80,87,88]. This model was confirmed experimentally by several groups [89–93]. The observation that aquaporins are essential components of the cellular migration apparatus [94] lent further strong support to this model. Aquaporins provide the route for osmotically driven water influx or efflux, leading to local cell swelling at the cell front or shrinkage at the rear part, respectively.

3. Ionic mechanisms of tumour cell invasion

Tumour cell invasion is frequently linked to invadopodia [75]. They are sites of proteolytic degradation of the ECM, and thereby facilitate migration through a three-dimensional network of matrix fibres (figure 2). Traditionally, the role of invadopodia in tumour cell invasion is reflected by the presence of several proteases (e.g. MT1MMP, MMP2). The ‘microscopic’ NHE1-mediated acidification at the cell surface of lamellipodia and invadopodia facilitates the action of
The release of cortactin-bound cofilin. Cofilin then promotes actin polymerization. Activity of matrix metalloproteinases has also been linked to activating ORAI1. (Online version in colour.) PLA2g6 supports the phosphorylation of the focal adhesion kinase (FAK) by dia. ORAI1 contributes to focal adhesion dynamics by co-localizing with PLA2g6.

Potential stable and controlling the amount of osmolytes entering the invadopodia outgrowth requires local volume increase that is mediated by water uptake through aquaporins (AQP) and possibly driven by the osmolytes glucose and Na⁺ imported by the Na⁺/glucose co-transporter 1 (SGLT1). By analogy with podosomes, Ca²⁺ influx through ORAI channels would occur and stimulate both Ca²⁺ and H⁺ extruded by NHE1 causes an extracellular acidification, thereby facilitating the interaction between integrins and collagen and promotes the activity of matrix metalloproteinases (MMP). Lamellipodia/invadopodia outgrowth requires local volume increase that is mediated by water uptake through aquaporins (AQP) and possibly driven by the osmolytes glucose and Na⁺ imported by the Na⁺/glucose co-transporter 1 (SGLT1). By analogy with podosomes, Ca²⁺ influx through ORAI channels would occur and stimulate both calpain2 (cald) to deave cortactin and calmodulin to activate Kᵥ₂.3 channels. Kᵥ₂.3 could fine-tune Na⁺ entry through the SGLT1 by keeping the membrane potential stable and controlling the amount of osmolytes entering the invadopodia. ORAI1 contributes to focal adhesion dynamics by co-localizing with PLA2g6. PLA2g6 supports the phosphorylation of the focal adhesion kinase (FAK) by activating ORAI1. (Online version in colour.)

4. Outlook and clinical perspectives

Studies from the past approximately 15 years provided proof of concept that ion channels and transporters are crucial for the metastatic behaviour of tumour cells. It is becoming increasingly clear that ion transport proteins do not act in an isolated manner on their own, but that they act in networks of functionally cooperating units [13,63,114,115]. This is reflected by the concept of the migration-associated transportome that we recently introduced [9]. It implies that ion transport signalling pathways such as pH, Ca²⁺ or cell volume are closely linked to each other. However, neither the pathophysiological significance of this crosstalk is well understood, let alone the interaction of ionic with kinase-based signalling pathways. For instance, it has not yet been investigated systematically how the altered expression of pH regulatory transporters in solid tumours affect the functional impact of other members of the migration-associated transportome and their crosstalk with growth factor signalling. Similarly, it is unknown whether transport proteins whose upregulation is mediated by hypoxia-induced HIF1α constitute ‘functional units’ involved in cell migration.

Extravasation of tumour cells in tumour-specific target organs is another step of the metastatic process for which the role of ion channels and transporters has not yet been investigated in detail. This process bears similarities to the recruitment of leucocytes from the bloodstream. It is supported by the cooperation of tumour cells with platelets and leucocytes [116]. Thus, it is reasonable to assume that tumour cells use similar ionic mechanisms to leucocytes (e.g. neutrophil granulocytes) in which Ca²⁺ influx via Orai1 plays an important role in the initial steps of recruitment [117]. Indeed, functional coupling between Kᵥ₂.3 and ORAI1 channels in lipid rafts seems to promote bone metastases of breast cancer cells [115]. Along the same lines, one could speculate that the reciprocal activation of integrins and Kᵥ₁.11.1 channels in tumour cells...
contributes to their ability to extravasate, because Kv11.1 expression in acute myeloid leukaemia cells correlates with a higher probability of relapse [118].

Many of the transport proteins (e.g. Kv10.1) are not only involved in controlling cell migration and invasion, but also in other ‘hallmarks of cancer’ such as proliferation [114,119]. Thus, drugs that target members of the migration-associated transportome are likely to elicit more responses than just the inhibition of tumour cell migration and invasion. Combined effects such as inhibition of migration and proliferation by blocking Kv3.1 channels may be desirable [120,121], whereas the combined inhibition of migration and apoptosis by Kv1.3 channel blockade would certainly not be advantageous.

Because most tumour patients die of metastases the inhibition of the mechanisms underlying metastasis offers great therapeutic potential. Targeting tumour cell migration would therefore be a good choice because it is one of the prerequisites for metastasis. Indeed, migration has been targeted in order to treat chronic inflammatory diseases [122] which is another pathological condition strongly relying on the ability of (inflammatory) cells to migrate. Transport proteins that could qualify as a potential anti-migratory target include among others NHE1, Kv3.1 channels or Kv1.3. Migration and invasion of all tumours cells studied to date relies at least partially on the activity of one of these proteins [9]. Importantly, there are also small molecule inhibitors validated in phase III clinical trials (Kv3.1 blocker senicapoc [123] and NHE1 blocker cariporide [124]) or that are already widely used clinically such as amide-linked local anaesthetics that block NHE1s. Presently it is being discussed whether the NaV-mediated metastatic behaviour of tumour cells can be targeted by using amide-linked local anaesthetics during cancer surgery [125]. Similarly, the use of the K+ sparing diuretic amiloride which is also an NHE1 blocker, can be envisaged [126]. Thus, there is an increasing number of functionally relevant ion transport proteins to be targeted. They are easily accessible because they are membrane proteins and often overexpressed or activated in cancer. Moreover, they have a long history of being drug targets in other medical fields such as cardiology, nephrology or anaesthesia. Alternatively, transport-associated proteins such as the tumour marker carbonic anhydrase IX could be targeted by specific antibodies [127].

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