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Ion channels and transporters in cancer. 3. Ion channels in the tumor cell-microenvironment cross talk

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Arcangeli A. Ion channels and transporters in cancer. 3. Ion channels in the tumor cell-microenvironment cross talk. *Am J Physiol Cell Physiol* 301: C762–C771, 2011. First published May 11, 2011; doi:10.1152/ajpcell.00113.2011.—The traditional view of cancer as a collection of proliferating cells must be reconsidered, and cancer must be viewed as a "tissue" constituted by both transformed cells and a heterogeneous microenvironment, that tumor cells construct and remodel during multistep tumorigenesis. The "tumor microenvironment" (TM) is formed by mesenchymal, endothelial, and immune cells immersed in a network of extracellular matrix (ECM) proteins and soluble factors. The TM strongly contributes to tumor progression, through long distance, cell-to-cell or cell-to-matrix signals, which influence different aspects of tumor cell behavior. Understanding the relationships among the different components of the cancer tissue is crucial to design and develop new therapeutic strategies. Ion channels are emerging as relevant players in the cross talk between tumor cells and their TM. Ion channels are expressed on tumor cells, as well as in the different cellular components of the TM. In all these cells, ion channels are in a strategic position to sense and transmit extracellular signals into the intracellular machinery. Often, this transmission is mediated by integrin adhesion receptors, which can be functional partners of ion channels since they form molecular complexes with the channel protein in the context of the plasma membrane. The same relevant role is exerted by ion transporters, which also contribute to determine two facets of the cancer tissue: hypoxia and the acidic extracellular pH. On the whole, it is conceivable to prospect the targeting of ion channels for new therapeutic strategies aimed at better controlling the malignant progression of the cancer tissue.

tumor microenvironment; tumor progression; integrins; ion transporters

RECENT STUDIES have led to ample revision of the traditional view of cancer as a disease caused by groups of transformed cells acquiring cell autonomous hyperproliferative, invasive, and limitless survival capacities. Tumors can be viewed as organs whose complexity approaches or sometimes exceeds that of normal tissues (46). Hence, the biology of tumors can be understood by studying both the individual transformed cells and the "tumor microenvironment" (TM) that tumor cells construct during multistep tumorigenesis (35, 73). The TM strongly influences the behavior and malignancy of the transformed cells; moreover, since the TM may change during tumor progression, it may differ (structurally and functionally) from the primary tumor to its metastases (54, 68, 81). When looking at cancer under this viewpoint, we must consider both carcinogenesis and tumor progression as processes involving heterotypic multicellular interactions within a newly formed tissue, the "cancer tissue" (15, 102). According to this view, the therapeutic strategies that have been concentrated so far on the targeting of tumor cells are better turned to target the "cancer tissue" as a whole, hence both tumor cells and the TM (58, 111).

In this context, deciphering the role of ion channel proteins in the cross talk between the tumor cell and the various constituents of the TM merits particular attention. This is further stressed by the fact that ion channels are highly accessible surface molecules and hence represent good drug targets (7).

Hereafter is reported a description of the TM and of its principal constituents, as well as a brief outline of those "hallmarks of cancer" (58) that rely on, and are modulated by, the interactions between tumor cells and TM. I will mainly focus on the role of integrins, the main receptors mediating cell-to-cell and cell-to-matrix adhesion. In the second part of the review, I will describe the principal steps of the TM/tumor cell cross talk, wherein ion channels and transporters are involved.

Tumor Microenvironment

The TM, also called the "tumor stroma," comprises both "nonmalignant" cells as well as the extracellular matrix (ECM), which contains, among proper matrix proteins, a multitude of growth factors and cytokines that, more or less directly, affect malignant cells (11). The cellular component of the TM consists of distinct cell types, including endothelial cells (ECs) and their precursors, pericytes, smooth muscle cells, fibroblasts, as well as cells of the innate and specific immunity (see Fig. 1). Although the stromal cells are not malignant, in that they do not bear cancerogenic mutations, they can exhibit epigenetic changes, which affect their behavior and protein expression (112). Moreover, the ECM-producing cells inside the TM are generally activated, and this leads to a defensive mechanism known as tumor desmoplasia (85). From a mere mechanistic point of view, the desmoplastic reaction tends to confine the tumor and in principle might prevent further neoplastic growth. However, desmoplasia, directly or through the creation of an hypoxic environment, may participate in several aspects of tumor progression such as angiogenesis, invasion, and metastasis (3). Moreover, current evidence suggests that tumor desmoplasia is the main determinant of chemoresistance (15). Most of the observations on the relevance of the TM stem from studies of carcinomas, in which the neoplastic epithelial cells constitute a compartment (the parenchyma) that is clearly distinct from the mesenchymal cells forming the tumor stroma. An active tumor stroma and a significant desmoplastic reaction characterizes mainly breast, prostate, and pancreatic adenocarcinomas (32, 70, 88).

Extracellular matrix. The reactive tumor stroma shows increased production of ECM products such as collagens. Type I

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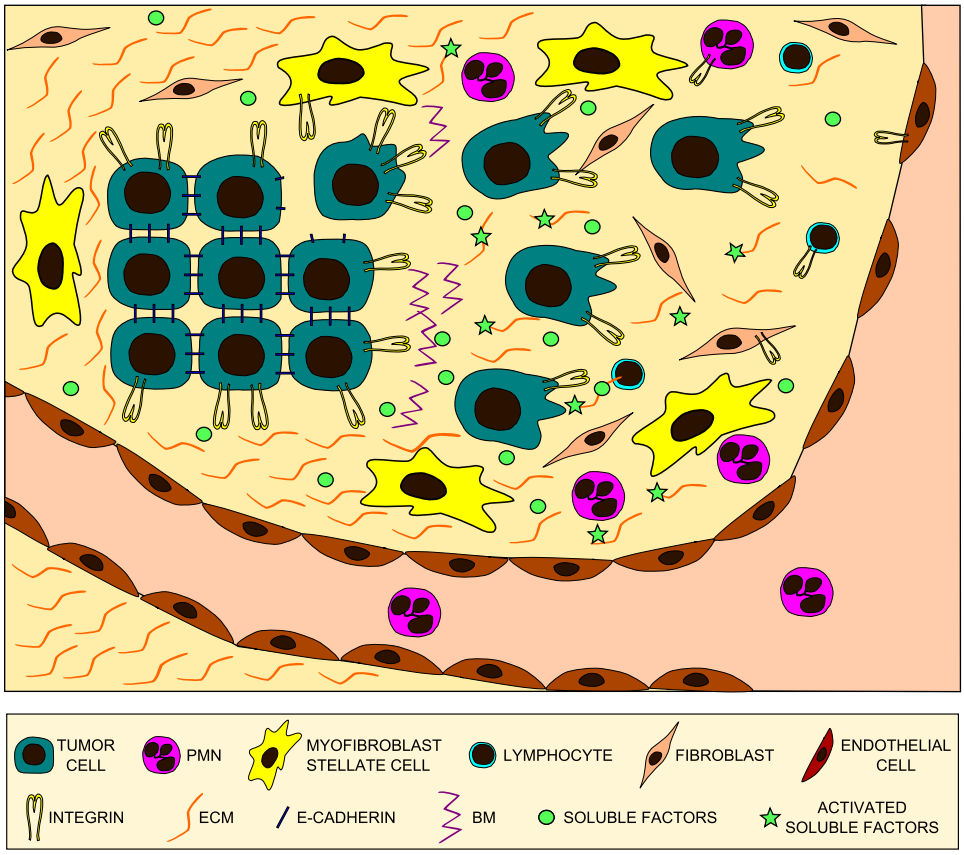

Fig. 1. Schematic representation of the cancer tissue. The scheme shows the anatomical interactions between the principal cellular elements (tumor cells, fibroblasts, mesenchymal cells, endothelial cells, cells of the innate and specific immunity) and the extracellular matrix (ECM) proteins and soluble factors present in the tumor microenvironment, which collectively contribute to form the cancer tissue. Integrins, the principal receptors mediating cross talk between parenchymal (tumor)/mesenchymal cells and the tumor microenvironment are indicated. Note that the integrins expressed on the plasma membrane of cells of the tumor microenvironment (fibroblasts, endothelial cells, neutrophils, and lymphocytes) are drawn only on one cell, for simplicity. For details see text and references.

collagen is predominant in the TM and in the desmoplastic reaction of different cancers and may have a role in tumor progression, since it determines the increased motility of several types of normal and neoplastic cells (8, 107). Fibronectin and thrombospondins are other ECM proteins that characterize the TM and can affect tumor progression by controlling cell motility as well as by dictating tumor cell differentiation programs (53, 71). The ECM of the TM is produced by both tumor and mesenchymal cells; both cell types also modulate their ECM by secreting proteases (45, 67, 72). This remodeling process can lead to the release of molecules sequestered in the ECM, such as the vascular endothelial growth factor (VEGF) (49) and many cleavage products of ECM proteins (35). These molecules further control tumor progression, controlling tumor angiogenesis and metastasis formation. A highly specialized ECM is the basement membrane (BM) characterized by a high presence of laminin. Epithelia are always associated with a BM, whose components strongly affect the physiology of normal epithelial cells (19). In epithelia-derived cancers the BM, mainly through laminin, can become an inducer of tumor progression, facilitating tumor cell growth and invasion (82). On the whole, soluble stromal "signals" can influence the growth of the primary tumor and can also contribute to create a "soil" for metastatic cells to grow (58).

Mesenchymal cells. Inside the TM, this term comprises *1*) "cancer-associated fibroblasts" (69) with similarities to the fibroblasts that create the structural foundation supporting most epithelial tissues; and *2*) myofibrobasts, or specialized mesenchymal cell types, such as the pancreatic stellate cells (PSC) (48).

Cancer fibroblasts represent reprogrammed variants of normal tissue-derived fibroblasts (108). While normal fibroblasts usually inhibit the growth of epithelial cells, irradiated and cancer-derived fibroblasts do not, but are, on the contrary, essential for proliferation, survival, and invasion of neoplastic parenchymal cells (10, 16, 119).

Among specialized mesenchymal cells, the PSCs, present in the pancreatic cancer stroma, merit special attention. PSCs have the ability to transdifferentiate from a "quiescent" to an "activated," α-smooth muscle actin producing phenotype. Activators include cytokines, growth factors, and reactive oxygen species released by inflammatory cells present in the TM. Activated PSCs, in turn, can produce autocrine factors [platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), cytokines, and proinflammatory molecules] that may affect tumor cells, supporting their growth and invasive properties (2, 17, 85).

Immune cells. Pathologists have long recognized that tumors are densely infiltrated by cells of both the innate and adaptive arms of the immune system (44), but the exact role of immune mechanisms in controlling tumor progression has long been debated (18, 56, 66). The observation that tumors can continue to grow and metastasize despite immunity has greatly puzzled scientists: it has emerged that the adaptive immune response against tumor is very weak and largely inefficient (40, 41). For example, lymphocytes infiltrating tumors have mainly an effector/memory (TEM) phenotype, but once inside the TM, these cells are either dysfunctional, or worse, display tumor-promoting properties (89, 90). The tumor cells themselves, or the surrounding TM, may contribute to downregulate TEM

responses. Also the recruitment and activation of cells of the innate immunity, such as neutrophils, that resemble that observed in infections, act to the detriment of the host, when occurring inside the cancer tissue (56).

Endothelial cells. Tumor-associated endothelial ECs can form new vessels inside the cancer tissue. The process of neo-angiogenesis, which serves to provide nutritional support to the growing tumor, can occur through sprouting from locally preexisting vessels or recruitment of bone marrow-derived endothelial progenitor cells. Neo-angiogenesis is a determinant step in tumor progression (50). The process is well orchestrated by both "soluble" (i.e., angiogenic factors and their counteracting inhibitors) and "solid-state" signals (i.e., ECM and cell adhesion proteins, mainly integrin receptors), which are transmitted in a bidirectional manner between the TM and ECs (36). We must also consider that ECs are the interface between circulating blood cells, tumor cells, and the ECM, thereby playing a central role in controlling leukocyte recruitment, tumor cell behavior, and metastasis formation.

Since recent studies are revealing distinctive gene expression profiles and cell-surface markers of tumor-associated versus normal ECs (95, 110), it is possible to develop novel therapies that exploit these differences to selectively target the ECs inside the cancer tissue (111).

Role of Integrins and of Integrin-Mediated Signaling

Integrin receptors are the main cellular receptors mediating cell adhesion and cell-to-cell interactions. They are transmembrane proteins formed by noncovalent association of α and β subunits. All subunits are type I transmembrane glycoproteins with a short cytoplasmic tail, a membrane-spanning helix, and a large multidomain extracellular portion (64). Integrin subunits can combine to form at least 24 functional heterodimers, each of which binds a specific array of ECM proteins or cell adhesion molecules. The conformation of integrins, and thus the binding to ECM, is modulated by interaction of the cytoplasmic domain with intracellular signaling and cytoskeletal proteins ("inside-to-out signaling"). Conversely, integrin activation stimulates a set of scaffolding, cytoskeletal, and regulatory proteins [such as the the focal adhesion kinase (FAK), the Src family kinases and the integrin-linked-kinase (ILK)] to associate to the cytoplasmic domain, leading to the assembly of the focal adhesion (FA) complexes, which in turn activate different downstream signaling pathways ("outside-to-in signaling") (22).

This "outside-to- in signaling" controls downstream functions such as cell migration, proliferation, survival, and differentiation. Since the list of papers describing the many facets of integrin-mediated signaling pathways is immense, I refer to those (22, 53, 94) and only limit to summarize that integrins seem to be linked to almost all of the known signaling pathways, including induction of cytosolic kinases, stimulation of the phosphoinositide metabolism, activation of Ras/mitogen-activated protein kinase (MAPK) and PKC pathways, and regulation of Rho GTPase. Moreover, integrin signaling often overlaps with that triggered by growth factor or cytokine receptors (22). The overlap and proper integration of differently arising signals makes physiological sense, because cells must integrate multiple stimuli from the ECM, growth factors, hormones, and mechanical stress to organize appropriate responses. The same integration even more occurs and determines the fate of tumor cells inside the cancer tissue.

TM as a Niche-Favoring Tumor Cell Survival and Metastasis: Role of Hypoxia and pH

Besides the direct cross talk between tumor cells and cells or molecules taking part in the TM, many of such interactions and signals may be orchestrated, and sometimes triggered, by two facets characterizing the cancer tissue, especially when a high desmoplastic reaction occurs, i.e., hypoxia and the acidic pH. Hypoxia (i.e., O_2 tension below 2.5 mmHg) represents a critical parameter modulating tumor progression being associated with a more aggressive phenotype and an increased propensity for metastases (60). Hypoxia can trigger such diverse effects since it switches on the expression of selected genes [such as those encoding VEGF, the glucose transporter 1 (Glut-1), and the carbonic anhydrase IX (CAIX)], through the activity of the hypoxia inducible transcription factor (HIF-1) (117). The increased expression of HIF-1-dependent proteins allows tumor cells to survive the harsh TM, mainly by switching on the process of neo-angiogenesis (83). Moreover, hypoxia, in association with the desmoplastic reaction, mediates radio- and chemoresistance (118), with an obvious impact on tumor malignancy (52).

Since the Warburg's works (128), it is well established that tumors are characterized by an alkaline intracellular pH and an acidic interstitial pH value. This "reversed" pH gradient across the tumor cell membrane increases as tumor progresses and strongly affects tumor progression and resistance to treatment (26). The development and maintainance of this gradient is directly due to the ability of tumor cells to secrete protons, a function obviously dictated by ion transporters (see below). The molecular mechanisms sustaining extracellular pH acidification are upregulated during tumor progression and are determined by the ECM components, as well as by hypoxia itself (26). Hence, a direct link between hypoxia and pH exists, which largely bypasses the strict regulation of hypoxia on the glycolytic pathway and hence on lactate production (116, 126).

Ion channels and Cross Talk With the Tumor Microenvironment

It is interesting, at this point, to discuss the role, if any, of ion channels (and transporters), which are expressed on either parenchymal or mesenchymal cells, in the functional interactions that occur inside the cancer tissue. I will not analyze the role of ion channels in tumor cell migration and invasiveness or in tumor angiogenesis in general terms, because these topics are objects of other reviews of this series. The main part of my discussion will focus the role of ion channels in cell-to-matrix and cell-to-cell interactions, which are functionally relevant to tumor progression. This role can be direct since ion channels can behave as adhesion receptor, or indirect, through the mediations of integrin receptors. Moreover, I will discuss the potential role of ion channels expressed by the cellular components of the TM (mainly ECs and immune cells) in orchestrating the functional interactions between TM and tumor cells. Finally, a brief survey will be presented of those ion transporters that generate and maintain the hypoxic and acidic conditions of the extracellular space inside the cancer tissue. Examples of these mechanisms are illustrated in Fig. 2.

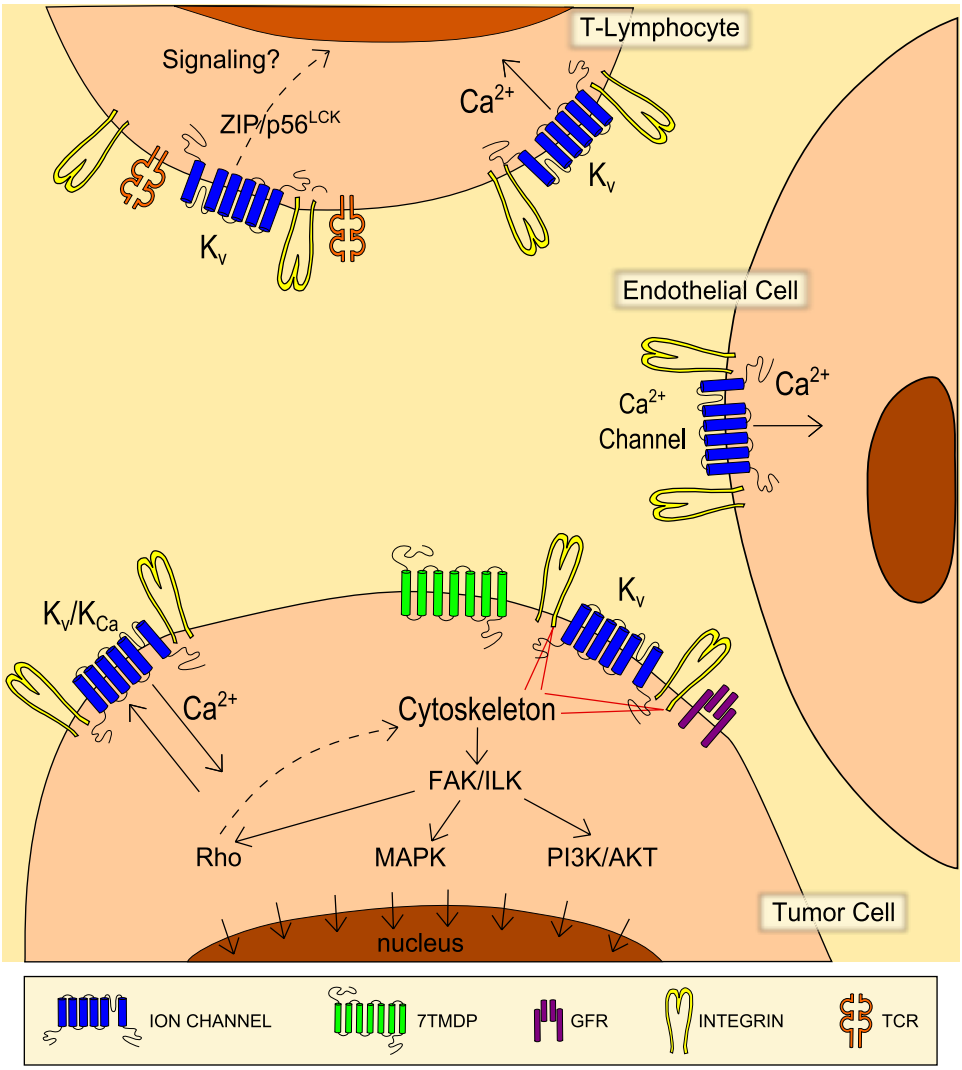

Fig. 2. Cell signaling mechanisms triggered by integrins/ion channels cross talk in cells of the cancer tissue. The three principal cell types (tumor cells, endothelial cells, and lymphocytes) in which an interaction between ion channels and integrins has been demonstrated are shown. The signaling pathways triggered by ion channels activation (elevation of intracellular Ca^{2+} or activation of intracellular signaling pathways) are highlighted. For details see text and references.

Ion Channels and ECM: Role of Integrins

The relationships between integrins and ion channels in the cell-to-cell and cell-to-matrix interactions have been detailed elsewhere (4). Here, I will only summarize the main physiological contexts in which an integrin-channel interaction has been studied and then move to a more cancer-focused scenario. The earliest indications came from studies on neuronal and leukemic cells, in which many cellular processes elicited by the engagement of adhesive proteins, such as differentiation, migration, and neurite extension, turned out to depend on ion channel activation (6, 12, 42, 62).

From a mechanistic point of view, integrin function is often described as "bidirectional." When associated with integrins, ion channel function becomes bidirectional as well; it is regulated by extracellular signals (through integrins) and in turn controls integrin activation and/or expression. This fact can link the intracellular state with that of the external environment (4). Interestingly, the same kind of complex bidirectional signaling has been observed for some ion transporters, in particular, those mediating proton fluxes (14, 92, 124), which are so relevant in the establishment of a reversed H^+ gradient that characterizes neoplastic malignancy (26). How can integrins interact with ion channels in tumor cells cells or alter tumor functions via ion channels? The bidirectional cross talk between integrins and ion channels mainly relies on cytoplasmic messengers (Ca^{2+}, protein kinases) commuting between the two proteins (4). Examples are the effects integrins may have on intracellular Ca^{2+} homeostasis. In ECs, for example, integrins regulate cell spreading and migration by controlling the level of cytosolic Ca^{2+} (79, 115). The process of T lymphocyte activation, in which β_1 integrins are largely involved, is underlied by a coordinated influx of Ca^{2+}, which is orchestrated by Ca^{2+} channels and tuned by K^+ channels (23). Another mechanism, triggered by integrins and mediated by calcium, is the transmission of mechanical forces at focal adhesion sites (37). Integrin-mediated mechanotransduction is accomplished by activation of signaling molecules, such as FAK and c-Src, in conjunction with the recruitment of multiple proteins to focal adhesions and reorganization of the cytoskeleton in vascular small muscle cells. It has been shown that Ca^{2+} entry through the L-type Cav1.2 calcium channel represents the ultimate link between integrins and the machinery transducing mechanical force (57, 131, 132). This mechanism, proven in physiological systems, might also, and perhaps even

more efficiently, integrate mechanical stress and biological responses in the leaky vessels that characterize the cancer tissue. A critical process in angiogenesis, which also takes place at the level of the tumor vasculature, is the orientation of ECs lining blood vessels that is triggered by cyclic mechanical strains. Although the role integrins in this "reorentation" process remains to be definitively proved, stretch-activated Ca^{2+} channels, in particular TRPV4 channels, are involved in this process (123).

Another mechanism of integrin/ion channels interaction that has a clear relevance in the cross talk between tumor cells and the ECM, is the fact that certain integrin and ion channel types can interact directly. In other words, the two proteins coassemble on the plasma membrane and give rise to supramolecular complexes, which constitute platforms for triggering and orchestrating downstream intracellular signals. The first evidence of the assembly between integrins and ion channels was obtained in immune cells by Levite and colleagues (80), who found the β_1 integrin subunit associated with $K_v1.3$ channels in T lymphocytes. This leads to activate the integrin adhesive properties and prompts cell migration. Shortly afterward, a physical link between $K_v1.3$ channels and β_1 integrins was described in melanoma cells (9). We found that the β_1 integrin subunit associates with another K^+ channel, hERG1 (or Kv 11.1), on the plasma membrane of tumor cells. First described in leukemia cells (31), a β_1/hERG1 plasma membrane complexes was subsequently described in other cancer cells. Two types of β_1/hERG1 have been identified so far (103). The first type is a bimolecular plasma membrane complex limited to β_1 and hERG1. Once formed, this complex recruits cytosolic signaling proteins, like FAK and PI3K, which in turn activate intracellular signaling in an integrin- and ion channel-dependent manner (30, Crociani O and Arcangelic A, unpublished observations). Such signaling can converge to regulate cancer cell motility and invasive properties (78) as well as the secretion of VEGF (Crociani O and Arcangelic A, unpublished observations) in gastrointestinal cancers. Interestingly, a direct association between FAK and another K^+ channel (Kv2.1) was recently described in rat cortical neurons (129).

The second type of β_1/hERG1 is a trimolecular complex in which a third membrane protein associates with the integrin/channel dimer. Such complex is typical of leukemia cells: in acute myeloid leukemia cells (and in human cytokine-stimulated hematopoietic precursors) the third member is the VEGF receptor 1 (Flt-1). The complex stimulates intracellular signaling through the Flt-1-dependent pathway, which eventually modulates cell survival inside the bone marrow and leukemia cell invasion into the bloodstream, with a clear negative impact on the leukemia disease (104). In acute lymphoblastic leukemias the third element is the CXCR4 chemokine receptor, and the complex stimulates signaling through ILK, toward Akt, and controls cell survival and chemoresistance (105). Altogether, it emerges that the association of integrin receptors with hERG1 is a common occurrence in cancer cells and serves to functionlly distinguish tumor hERG1 channels from those expressed in the heart (5), which do not assemble with the β_1 integrin subunit (Pillozzi S and Arcangelic A, personal communication). Such multiprotein structures serve as molecular signaling platforms that can recruit proteins and second messengers thus integrating messages originating from the tumor ECM, with functionally relevant impact on tumor malignancy.

While I will not illustrate the relationships between ion channels and cell motility in general terms, I will discuss some examples, and forthcoming hypotheses, of the relationships between integrins, ion channels, and the machinery that regulates cell migration, which have evident implications in cancer (109). For example, it is now well recognized that Ca^{2+}-dependent potassium (K_{Ca}) and voltage-dependent (K_v) channels are implicated in the regulation of cell movement, both converging on the regulation of Ca^{2+} influx, which in turn has manifold effects on cell migration (113). Hence, it is not surprising that the effects of integrins (and cell adhesion, in general) on cell migration could be mediated by the above integrin-K^+ channels cross talk. Moreover, migration is accompanied by cell shape rearrangements that require local volume alteration, a process that requires, at least in the early phases of the process, a water KCl efflux caused by coordinated activation of K^+, Cl^-, and aquaporin channels (61, 99, 114). These processes are particularly relevant from our standpoint since cell volume control is strictly linked to cell adhesion (61). However, K^+ channels can regulate the migration machinery in other ways: they can form, for example, the above-described molecular complexes with FAK and integrin subunits. Besides these examples, interesting speculations also derive from studies on $\alpha_9\beta_1$ integrins, which can regulate cell movement by activating inward rectifier K^+ channels (IRK) (39). IRK channels, along with the integrin, are physically linked to spermidine/spermine N1-acetyltransferase (29), the key enzyme in the pathway that acetylates spermine and spermidine to putrescine, thus controlling the intracellular concentration of polyamines (101). Polyamines are critical regulators of neoplastic growth (101) and also the main intracellular messengers controlling IRK activity (96). A functional network may hence be figured out, where an adequate intracellular concentration of polyamines converges to trigger a proper $\alpha_9\beta_1$-dependent cell movement through the modulation of IRK channels (125). This is another example of how the TM may affect tumor cell behavior via the modulation of an ion channel.

Ion Channels as Adhesion Molecules

The relationship between the ECM and ion channels is not always mediated by membrane receptors such as integrins, but sometimes the channel proteins directly interact with the environmental matrix, behaving themselves as adhesion molecules. The first example was that of voltage-gated Ca^{2+} channels, which, in the presynaptic membrane of neuromuscular junctions, were shown to directly bind to laminin β2 (120). Another example, with a closer impact to cancer cells, concerns voltage-gated Na^+ channels (VGSC) and their accessory β subunits. VGSC β subunits are multifunctional molecules that not only modulate Na^+ current but also mediate different effects, because they function as cell adhesion molecules inside the VGSC signaling complexes (100). Interestingly, many β subunit cell adhesive functions can occur even in the absence of pore-forming VGSC α subunits. This is particularly interesting in neoplastic cells, where VGSC are implicated in late stages of tumor progression and alterations in β subunit expression can be detected, especially during the onset of the metastatic process (20, 21). For instance, the $Na_V1.7$ channel is associated with strong metastatic potential in prostate cancer in

vitro and its activity potentiates cell migration (55, 97). These intriguing, although still fragmentary, evidences bring us to consider the possible implications of the adhesion processes involving ion channels and/or accessory units in the cellular mechanisms underlying the metastatic process.

Other intriguing results, in the context of neoplasia, have been obtained by studying Cl^-channels, especially of the CLCA (calcium-activated chloride channel regulator) protein family. CLCAs have high homology to cell adhesion proteins and have been implicated in metastatic processes, although it is still uncertain whether they form Cl^- channels or are accessory subunits (84). In ECs, CLCA2 behaves as a vascular addressin for metastatic, blood-borne, cancer cells, facilitating vascular arrest of cancer cells via adhesion to β_4 integrins and hence promoting metastatic growth. In particular, since human breast cancer cells expose β_4 integrins on their surfaces, these can recognize the lung endothelial CLCA. Lung colonization from blood-borne neoplastic cells is thereby considerably facilitated. In addition, the β_4-integrin-CLCA complex stimulates Src-dependent cell signaling through FAK and extracellular signal-regulated kinase (ERK), leading to increased proliferation and thus higher metastatic potential (1).

Ion Channels in Mesenchymal Cells of TM: Role in the Regulation of Tumor Cell Functions?

Activated ECs inside the TM are required for the triggering of neo-angiogenesis, one of the most relevant steps in tumor progression. Crucial to this process is Ca^{2+}-permeable channels and Ca^{2+}-dependent signaling (106). Alteration of ECs' Ca^{2+} channels activity (and more generally of intracellular Ca^{2+} homeostasis) are mediated by angiogenic factors [VEGF, fibroblast growth factor 2 (FGF2), etc.], which are secreted by mesenchymal or immune cells in the TM or by the tumor cells themselves (77). Carboxyamidotriazole (CAI), an inhibitor of store-operated Ca^{2+} channels, inhibits the increase in intracellular Ca^{2+} during VEGF-A induced EC proliferation (47). This effect is consistent with the fact that CAI reduces the relative vascular volume in hepatic metastases by reducing the size and volume of microvessels (87). In addition, Ca^{2+} influx might stimulate ECs, or the tumor cells themselves, to produce and release angiogenic factors, which in turn might stimulate angiogenesis in an autocrine or paracrine manner (51). Also K^+ channels are involved in this mechanism with different mechanisms: for example, KCa provide the electrochemical driving force for Ca^{2+} entry and the subsequent Ca^{2+}-dependent secretion of growth and vasodilating factors. Indeed an increased expression of KCa channels has been detected in ECs of mesenteric arteries from patients with colonic adenocarcinomas (76), as well as in capillary endothelia of metastatic brain tumors compared with those of the normal brain tissue (63). Moreover, the pharmacological blockade of K_{Ca} channels can inhibit the FGF2-induced proliferation of ECs, and, consistently, FGF2 significantly potentiates KCa channel activity (130). The role of voltage K_v in angiogenesis has emerged only recently and limited to hERG1 channels in high grade brain tumors (91) and gastrointestinal cancers (Crociani O and Arcangeli A, unpublished observations) and to EAG1 (Kv10.1) channels (43). These channels have a pro-angiogenic effect through an enhacement of VEGF secretion by tumor cells. However, this effect is independent from an increase in Ca^{2+} concentration but related to a modulation of HIF-1 activity. In the case of hERG1, such modulation has been attributed to an enhancement of intracellular signaling downstream to the β_1/hERG1 plasma membrane complex (Crociani O and Arcangeli A, unpublished observations).

Ion Channels in Innate and Specific Immune Cells

Neutrophils exploit their antimicrobial activity through the activation of the respiratory burst and the subsequent release of oxygen radicals (ROI). Essential for these processes are different types of channels, such as transient receptor potentials (TRPs), K_{Ca}, and Cl^- channels (13). Cl^- release from these cells is, at least in part, dependent on β_2 integrin-mediated adherence to fibronectin (93). Hence adhesion molecules elicit signals that activate neutrophils through the intervention of ion channels. Moreover, since ROI have a clear effect on tumor cell invasion (127), the β_2 integrin-mediated activation of Cl^- efflux could be relevant in mediating the effects of the natural immunity on tumor progression.

Another interesting network has been recently described in macrophages. Macrophages express inwardly rectifying K^+ (K_{IR}) channels, whose activity is clearly modulated by VLA4 integrin receptors and hence by cell adhesion (33). This integrin-dependent K^+ channel modulation strongly affects the Ca^{2+-}-dependent macrophage activation (34). Moreover, macrophages express P2X7 receptors (74), and it has been recently reported that the stimulation of P2X7 receptors evokes a rapid release of lysosomal cathepsin in quantities sufficient to induce ECM degradation. This effect is supposed to have a relevance in destructive joint disease (86) but conceivably could be also relevant in the remodeling of the TM ECM, which strongly contributes to malignant progression.

Which ion channels are expressed in cells of the adaptive immunity and which is their function in T lymphocytes that infiltrate the cancer tissue? For more than 25 years it has been widely recognized that a coordinated influx of Ca^{2+} is essential to trigger the activation of T lymphocytes and that a unique contingent of ion channels (including $K_v1.3$ and $K_{Ca}3.1$ K^+ channel) orchestrates the duration and intensity of the Ca^{2+} signals that control T cell activation (23, 38). $K_v1.3$ localizes on the plasma membrane of T cells, as part of a signaling complex that includes the β_1 integrin, a PDZ-domain protein called hDlg (or SAP97), an auxiliary channel subunit $K_v\beta_2$, and the adapter proteins ZIP and $p56^{lck}$ (Lck). This signaling complex collectively modulates T cell activation (23, 27). After activation, effector/memory T (TEM) cells express higher levels of $K_v1.3$ and lower levels of $K_{Ca}3.1$ compared with naïve and central memory T cells (TCM). Therefore, the balance of these channel types constitutes a specific functional marker of activated TEM lymphocytes. These observations might have relevance in the context of the cancer tissue, providing a possible explanation of the misfunctioning of tumor TEM, as well as a possible novel therapeutic target (28). In this context, it has been recently shown that the tumor necrosis factor-α (TNF-α) on the major cytokines present in the TM inhibits the CD-3-induced upregulation of Kv 1.3 in T cells (98).

Role of Ion Transporters in the Control of Extracellular pH: a Dependence on Hypoxia?

As detailed above, a potent control of intra- and extracellular pH can be considered one of the cancer hallmarks (26, 122). Recent work from S. Reshkin's research group (24–26, 59) contributed to highlight the fundamental mechanisms determining tumor extracellular acidification and its role in malignant invasion. Extracellular environment is mainly acidified by the Na^+/H^+ exchanger NHE1 and the H^+/lactate cotransporter that are typically active in cancer cells. What is more, NHE1 also regulates formation of invadopodia-cell structures that mediate tumor cell migration and invasion. The NHE1 located at invadopodia acidifies the local extracellular nanoenvironment to drive protease-dependent and -independent proteolysis of the ECM, thus permitting invasion to occur. This process of ECM digestion is also stimulated by serum deprivation, hypoxia, and epidermal growth factor (EGF). These observations provide a starting place to figure out the mechanisms by which the TM, with its low oxygen tension and acidic pH, interacts with growth factors to drive tumor progression (24, 25, 59).

Another molecule that links intratumoral hypoxia and extracellular acidification is CA-IX. CA-IX is one of the hypoxia-inducible proteins and can control tumor pH since its hydrates extracellular CO_2 to HCO_3^- and H^+ in several types of cancer. Such a facilitated CO_2 diffusion maintains a steep outward CO_2 gradient, with alkaline intarcellular and acidic extracellular pH, strongly contributing to tumor growth and invasiveness (65, 75, 123).

Conclusion

On the whole, a paradigm shift is emerging in oncology, where aberrant signals from the extracellular compartment can promote the initiation of oncogenesis even in the context of normal epithelial physiology. In other words, those neoplastic phenotypes that display a high grade malignancy do not arise in a strictly cell autonomous manner, and their manifestation cannot be understood solely through analyses of tumor cell genomes. The ability of malignant cells to negotiate most of the steps of the invasion-metastasis cascade may be acquired, at least in certain cases, through their interaction with the TM, without the requirement to undergo additional mutations beyond those that were needed for primary tumor formation.

It is now clear that a better understanding of the biology of the TM can offer potential therapeutic targets for the treatment of cancers, especially those that, because of a strong desmoplastic reaction, are potentially the most malignant and untractable cancers. Among the mechanisms that underlie the cross talk between tumor cells and the TM, ion channels are emerging as relevant players. The better deciphering of the relationships between ion channel proteins and the different components of the TM might help to identify new targets and pathways suitable for designing therapeutic strategies appropriate for blocking malignant progression.

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