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**Involvement of ion channels and transporters in carcinoma angiogenesis
and metastasis**

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Running title: Ion transport modulates tumor angiogenesis and metastasis

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ABSTRACT

Angiogenesis is a finely tuned process, which is the result of the equilibrium between pro- and anti-angiogenic factors. In solid tumor angiogenesis, the balance is highly in favor of the production of new, but poorly functional blood vessels, initially intended to provide growing tumors with nutrients and oxygen. Among the numerous proteins involved in tumor development, several types of ion channels are overexpressed in tumor cells, as well as in stromal and endothelial cells. Ion channels thus actively participate in the different hallmarks of cancer, especially in tumor angiogenesis and metastasis. Indeed, from their strategic localization in the plasma membrane, ion channels are key operators of cell signaling, as they sense and respond to environmental changes. This review aims to decipher how ion channels of different families are intricately involved in the fundamental angiogenesis and metastasis hallmarks, which lead from a nascent tumor to systemic dissemination. An overview of the possible use of ion channels as therapeutic targets will also be given, showing that ion channel inhibitors or specific antibodies may provide effective tools, in a near future, in the treatment of carcinomas.

KEYWORDS: ion channel, tumor vascularization, cell signaling, ion channel-targeted therapy

ABBREVIATIONS: HIF, hypoxia-inducible factor; CRC, colorectal carcinoma; ccRCC, clear cell renal cell carcinoma; CAIX, carbonic anhydrase IX; RVD, regulatory volume decrease; PDAC, pancreatic ductal adenocarcinoma; VEGFR, VEGF receptor; TSP, thrombospondine; MMP, matrix metalloproteinase; SOCE, store-operated calcium entry; CRAC, Ca^{2+} -release activated channel; ROCE, receptor-operated calcium entry; IP3, inositol 1,4,5-trisphosphate; EMT, epithelial-mesenchymal transition; TME, tumor microenvironment; STIM, stromal interaction molecule; MAM, mitochondrion-associated ER membrane; NMDAR, N-methyl, D-aspartate Receptor; CML, chronic myeloid leukemia; ER, endoplasmic reticulum; BiP, binding immunoglobulin protein; NSAID, non-steroid anti-inflammatory drug; GPCR, G protein-coupled receptor.

I. Introduction

Carcinoma is a solid cancer developing from epithelial cells and is the most widely spread form of human cancer. Among the phenomena leading to the development and progression of this type of cancer, one of the most important is angiogenesis. Indeed, tumor vascularization is essential for a growing tumor to obtain oxygen and nutrients needed for its ongoing growth, *in situ*. Tumor neovascularization is also a means for the tumor to grow away from its primary site towards a remote neo-colonized structure (metastasis).

In the last 10 to 15 years, many efforts have been made on the elucidation of which genes might be directly involved or affected during the different steps of tumorigenesis, described by Hanahan and Weinberg as the hallmarks of cancer (95, 96). Multiple studies demonstrated that, among these genes, plasma membrane ion channels and transporters are clearly modulated during carcinogenesis, in different types of carcinomas (9, 20, 31, 75, 78, 93, 133, 147, 201, 207). Several different roles have been ascribed to ion channels during carcinogenesis, depending on the step of the tumorization process and on the organ in which it takes place.

In this review, we do not intend to make an exhaustive list of channels involved in cancer development. Instead, we choose to focus on the involvement and/or alteration of some ion channels in angiogenesis, from the vascularization of recently formed tumors to the metastasis cascade. Indeed, neo-formation of tumors involves pH changes, which are associated with proton exchange through the plasma membrane (127, 209, 217) and water transport (17, 162). The subsequent tumor growth needs neovascularization, associated with Ca^{2+} , K^{+} and Na^{+} channels (5, 61, 201). Finally, Na^{+} , K^{+} , Ca^{+} and Cl^{-} channels are implicated in the transition to metastasis (32, 73, 75).

The main ion channels involved in tumor development processes and mentioned in this review are listed in Table 1.

II. Neoangiogenesis

A. The fate of new tumors

In the early steps of its formation, a tumor depends solely on the host blood vessels (104). However, this initial peripheral vasculature quickly disappears, leaving the tumor and its microenvironment in a hypoxic state (84, 97). Folkman (77) was the first to show that, under these conditions, the tumor growth is limited to a few cubic millimeters, unless reorganization of a new blood supply is promoted (neoangiogenesis).

The lowered local O_2 pressure (pO_2) observed near cancer cells and the resulting tumor microenvironment acidification activate hypoxia-inducible factors, especially HIF-1 (44, 58, 115). HIF-1 α is stabilized by phosphorylation (172) and dimerizes with the constitutively expressed HIF-1 β to form the transcription factor HIF-1, which in turn stimulates a set of genes involved in tumor development. Conversely, recent reports present HIF-2 as the tumorigenic form of HIF, whereas HIF-1 would be a tumor suppressor (227, 263).

Initially, the cancer cells undergo metabolic changes, among which increased glucose consumption and lactate production (the Warburg's effect) lead to acidosis of the cells. In conjunction with the higher need for glucose, several authors report a HIF-dependent expression of the GLUT-1 glucose transporter in breast or colorectal carcinoma cell (CRC) models (25, 84, 132) and a repressed mitochondrial function (175). In clear cell renal cell carcinomas (ccRCC), some isoforms of glucose transporters are equally increased, including the GLUT-5 fructose transporter (240). Moreover, in order to prevent acidosis induced by the accumulation of lactate, cancer cells increase the amount and/or activity of several families of acid-extruding plasma membrane proteins. In several carcinoma models, these HIF-1 increased proteins include: NHE1, the Na^+/H^+ exchanger (79, 170, 231), the MCT1 and MCT4 forms of monocarboxylate transporters, dedicated to lactate extrusion (62, 184, 246), the SLC4A4 (NBC) Na^+/HCO_3^- cotransporter (1, 180), involved in the bicarbonate reabsorption which contributes to intracellular pH recovery. Acid-sensing ion channels (ASIC1, 2, 3) are also up-regulated in adenoid cystic carcinomas, following the acidification of the extracellular space (257). Finally, carbonic anhydrases (especially CAIX, the main carbonic anhydrase found in hypoxic tumors (64)), dedicated to microenvironment pH modulation are also upregulated (132, 171, 180). The different ion channels involved at this stage are summarized in Figure 1. Activation of all these transport proteins eventually contribute to a modest elevation of the tumor cell intracellular pH, while the extracellular medium is acidified.

Subsequently, the newly constituted tumor will only grow larger if it manages to regenerate a constant blood supply to provide O₂ and nutrients to the tumor cells and to rid the cells of metabolites. Two main families of plasma membrane ion channels have been proposed to be involved in this tumor developmental stage and regulated by HIF. Transient receptor potential (TRP) channels contribute to calcium homeostasis and are upregulated in several cancer cell types, thus playing an important part in Ca²⁺ influx, regulatory volume decrease (RVD) and cell cycle progression (63, 254). A variety of K⁺ channels are also involved in the HIF-induced regulation of proliferation and revascularization, namely the voltage-dependent “ether-à-gogo” potassium channel (EAG1, KCNH1, Kv10.1) (31, 177), and the two-pore domain potassium channels TASK (30) and TREK (243). In pancreatic ductal adenocarcinoma (PDAC), the Ca²⁺-dependent potassium channel of intermediate conductance, IK_{Ca} (K_{Ca}3.1) is up-regulated and contributes to cell proliferation (114).

B. Angiogenic switch

Tumor angiogenesis is the key step for tumor growth, invasion and metastasis. Tumor angiogenesis mostly relies on the same processes as those involved in physiological angiogenesis (27, 45, 176). Particularly, ischemia and hypoxia are in both cases, major initiators of angiogenesis processes (“on” switches). Moreover, tumor angiogenesis and physiological angiogenesis heavily depend on VEGF secretion, as was described elsewhere and as we will describe here for tumor-depending angiogenesis. The main differences are: 1) Pathological angiogenesis is more dependent upon VEGF than physiological angiogenesis (45); and 2) Physiological angiogenesis events recede when vessel perfusion starts and upon disappearance of the stimuli that gave rise to neoangiogenesis (“off” switch), whereas tumor angiogenesis is a continuous process, fueled by the steady secretion of tumor-induced angiogenesis factors (45).

The concept of “angiogenic switch” was formulated in order to characterize angiogenesis as a balance between processes that tend to favor and processes that tend to prevent angiogenesis (94). When tumor cells enter a hypoxic state, activation of HIF leads to the subsequent activation of a multiple array of pro-angiogenic factors; thus, the regular balance of angiogenesis is lost: the angiogenic switch has been turned “ON”. The most studied HIF-regulated pro-angiogenesis factor is probably VEGF, the angiogenesis cytokine that regulates endothelial cell proliferation and blood vessel formation. Both VEGF and its receptors VEGFR1 (FLT-1) and VEGFR2

(KDR/FLK1), which are located principally on the surface of endothelial cells, are upregulated by HIF (123, 185). Fibroblast Growth Factor (FGF) is also regulated by hypoxia and may act synergistically with VEGF to amplify angiogenesis (125).

The “angiogenic switch” is turned “ON”, in several types of cancer cells, including mouse colon carcinoma, mouse melanoma and human neuroblastoma, when several types of ion channels are modulated. P2X₇R, the non-selective cationic pore, has been shown to promote tumor growth and VEGF release (4, 5, 7). TRPC channels are functionally coupled to VEGF or FGF, playing important roles in cancer development steps (76, 156). Moreover, in renal cell carcinoma (RCC), the TRPC4 Ca²⁺ channel expression is decreased, which impairs Ca²⁺ metabolism, hence preventing the production of thrombospondin-1 (TSP1), an angiogenesis inhibitor (238). Numerous studies report overexpression of most families of K⁺ channels in human cancers. In particular, the closely related voltage-dependent hERG and EAG1 K⁺ channels are both involved in the processes leading to angiogenesis. Indeed, hERG channels regulate angiogenesis in CRC by increasing the HIF-1 activated VEGF expression and secretion in a β 1-integrin-dependent manner and via a PI3K/Akt pathway (46). This regulation has been confirmed in transgenic mice expressing hERG channels and treated with azoxymethane. These mice present increased staining for VEGF in immunohistochemistry (IHC) studies and a higher total number of blood vessels, consistent with neoangiogenesis, as well as colorectal lesions, on the whole showing acceleration of the colorectal cancer phenotype (74). EAG1, but not EAG2 contributes to tumor progression, as demonstrated by xenograft tumor formation resulting from implanted MDA-MB-435S breast cancer cells, EAG1-transfected CHO cells and EAG1- or EAG2-transfected NIH-3T3 cells. Furthermore, EAG1 stimulates VEGF secretion, measured in CHO- or 3T3-transfected cells. Finally, EAG1 increases HIF-1 amount in EAG1-expressing cells, thus demonstrating that EAG1 is involved in the angiogenic switch and the subsequent neoangiogenesis (61).

The role of Ca²⁺ channels in conjunction with angiogenic switch is worth to mention. Indeed, it has been demonstrated that VEGF utilizes Ca²⁺ signaling to promote endothelial cell proliferation and subsequent neoangiogenesis, which we largely discuss in the next section. However, it is noteworthy that some Ca²⁺ channels, mostly of the TRP family, are upregulated by hypoxia in cancer cells (76). The subsequent increase in intracellular Ca²⁺ concentration is at the basis of cancer cell hallmarks such as cell proliferation or migration (76).

C. Neovascularization

Tumor vasculature is a crucial feature of carcinoma development. New blood vessels develop by sprouting (angiogenesis) or intussusception from pre-existing vessels (144, 199). They can also emerge from the assembly of endothelial precursors derived from the bone marrow (vasculogenesis) (189). Sprouting is a very well studied process, which is involved in tumor blood vessel formation. Angiogenesis is a multistep process that begins when endothelial cell VEGF receptors are stimulated by VEGF (19, 34) to give rise to new blood vessels growing towards the avascular tumor that initially secreted the stimulating cytokine. The different steps of the process are detailed below:

1. VEGF-induced blood vessel dilatation

Most research on tumor angiogenesis has focused on VEGF-A. VEGF-A signaling occurs through two VEGF receptors, VEGFR1 and VEGFR2 (19, 144). Although VEGF-A has the highest affinity for VEGFR1, the higher tyrosine kinase activity of VEGFR2 makes it the primary receptor involved in angiogenesis processes. VEGFR1, conversely, is involved in macrophage chemotaxis, as well as in tumor cell survival and invasion (136, 205, 255). Additionally, VEGFR1 may also have a negative role on angiogenesis and tumorogenesis processes, only acting as a decoy receptor (102, 142). VEGF-A binds to its receptor and this first results in blood vessel dilatation, as recently shown by *in vivo* studies of breast tumors, by sonographic examination (41). Like most mammalian cells, endothelial cells express cell-swelling activated Cl^- channels, called the Volume-Regulated Anion Channel (VRAC) (166, 167). Interestingly, receptor tyrosine kinases such as VEGFRs can serve as mechanosensors (40). As such, they are involved in the VRAC signaling pathway. Thus, one might infer that VEGF signaling in endothelial cell is mediated by VRAC Cl^- channels, as shown by the suppression of angiogenesis by VRAC inhibitors (146).

2. Disorganization of the vascular bed

Angiogenesis partly proceeds by the VEGF stimulation of endothelial cell matrix metalloproteinases (MMPs) (188). Secretion of these MMPs, more precisely MMP-2 and MMP-9 (18, 250), triggers the dissolution of the basement membrane surrounding these endothelial cells, thus provoking the destabilization of the entire blood

vessel wall and leading to vessel leak (34). Among the multiple mechanisms that cooperate for the close regulation of MMP activity, Ca^{2+} signaling is one of the most important (121). The work, led by Kato, demonstrates that under extracellular acidification, B16 mouse melanoma cell line displays increased voltage-gated Ca^{2+} channel expression and activity, resulting in an increase in intracellular Ca^{2+} concentration and in MMP-9 induction. Thus, Ca^{2+} signals and channels are potentially the most important points in the regulation of the VEGF-triggered blood vessel reorganization. However, the work conducted by Kato and colleagues addresses the other types of Ca^{2+} channels that may be involved in the complex processes going from VEGF upregulation to basement membrane disorganization. Indeed, under a hypoxic stimulus, the formation of a VEGF-VEGFR complex at the endothelial cell plasma membrane can also result in intracellular Ca^{2+} increase, due to the release of intracellular Ca^{2+} stores (Fig. 2). The depletion of these stores in turn results in Ca^{2+} influx, via plasma membrane Ca^{2+} channels which results in store-operated calcium entry or SOCE (for review, see (75)). Several Ca^{2+} channels of the transient receptor potential (TRP) superfamily appear to be involved in SOCE and help explain how SOCE contributes to VEGF-induced basement membrane disruption. Among the TRPCs (canonical), TRPC1 activation magnifies the VEGF-induced increase in endothelial cell monolayer transepithelial permeability (111, 178). Conversely, inhibition of the TRPC1 activity reduces this transepithelial permeability increase (111). TRPC4 is a necessary intermediate in the response of human pulmonary artery endothelial cells to VEGF stimulation (71). TRPC6 is also presented as a good candidate in the process of VEGF-induced vascular permeability increase. Indeed, as shown in individually perfused frog microvessels, stimulation of TRPC6 generates an increase in the hydraulic conductivity (L_p) of the vessels (186). The Ca^{2+} -release activated Ca^{2+} (CRAC) channel (composed of the plasma membrane Orai1 subunit coupled with the endoplasmic reticulum STIM subunit) is also involved in VEGF-dependent SOCE (139). Indeed, in human umbilical vein endothelial cells, inhibition or disruption of Orai1 reduces VEGF-induced calcium entry, cell migration and *in vitro* angiogenesis. The increase in transepithelial permeability may result from both in-cell and in-between-cell pore creation (43, 98). Finally, VEGF effects can also occur via receptor-operated-calcium entry (ROCE), as shown on human microvascular endothelial cells (42); TRPC3 and TRPC6 are two examples of channels involved in calcium-mediated vascular permeabilization.

In conclusion, VEGF-induced calcium-dependent events leading to the basement membrane disorganization, though resulting in the same final outcome, appear to be the consequence of several calcium entry pathways.

3. Endothelial cell proliferation, migration and organization

Breakdown of the blood vessels (i.e. dissolution of the basement membrane) leads endothelial cells to be released into the extracellular matrix (ECM) where they proliferate and organize to generate new blood vessels, growing towards the tumor that initially emitted stimulating signals. Angiogenic sprouting is a guided process in which the vasculature expands outwards from the preexisting vessel, following a gradient of angiogenic factors such as VEGF-A (85) or soluble VEGFR1 (38). In this regard, Gerhardt and colleagues demonstrated that the first cell of the column, the so-called “endothelial tip cell,” consists of a single, non-proliferative, highly polarized endothelial cell, which is the sensor of the emitted elongation signal. The cell migration along this signal (chemotaxis) paves a path for the cells that follow, the “stack cells”, which undergo high rate mitosis to form the newly elongating blood vessel. At the endothelial cell level, it is now recognized that VEGFR2 is the main actor of the angiogenic cascade. VEGF, when binding to VEGFR2, activates a signaling cascade starting from auto-phosphorylation of VEGFR2, subsequent phosphorylation and activation of phospholipase C- γ (PLC- γ), increased production of inositol 1,4,5-trisphosphate (IP3) and resulting increase in the Ca^{2+} release from endoplasmic reticulum. The subsequent rise in calcium influx by the aforementioned SOCE process involves TRPC channels, as already discussed (see chapter II.C.2) and promotes endothelial cell migration via a Ca^{2+} /calmodulin/calcineurin pathway (72). The role of SOCE was also established in bovine artery endothelial cells for the different mechanisms of angiogenesis (i.e., cell sprouting, cell proliferation and cell migration) and involves the Ca^{2+} /calmodulin/protein kinase cascade (15).

One of the fundamental roles of ion channels in endothelial Ca^{2+} signaling is the fine-tuning of the electrochemical gradient for Ca^{2+} (118). Several types of ion channels cooperate in elaborating the cell membrane potential, which regulates calcium entry into the endothelial cells (118). Among those, K^{+} channels, especially Ca^{2+} -activated K^{+} channels (251), inwardly rectifying K^{+} channels (K_{ir}) and voltage-dependent K^{+} channels, are the major classes of ion channels involved in setting the membrane potential (165), which in turn influences the driving force for the Ca^{2+} via SOCE. Indeed, blockers of K^{+} channels, as well as plasma membrane depolarization, stop the cell cycle in G1 phase, thus reducing cell proliferation (153, 165). However, among the Ca^{2+} -dependent K^{+} channels, the Ca^{2+} -dependent intermediate conductance K^{+} channel IK1 (SK4,

KCNN4) increases cell proliferation with a completely different mechanism, in a HEK overexpressing system. Indeed, the potassium current itself does not seem to mediate the effect, since a non-transporting mutant of the channel induces the same proliferation. So it seems that the IK1-induced proliferative effect is not SOCE-mediated (152). The cell membrane potential is partly regulated by Cl⁻ channels (247). Hence, volume-regulated anion channels (VRAC), Ca²⁺-activated Cl⁻ channels (CaCC), as well as cystic fibrosis transmembrane regulator (CFTR) (145) might be important regulators of SOCE, which drives the proliferation step of angiogenesis.

4. Maturation of the new blood vessel

Two steps are essential for completion of angiogenesis:

(1) A new vessel is formed when the elongating sprout lumenizes to create a tube, which will eventually be able to transport fluid and the multiple blood components: this step is called tubulogenesis (117, 143). At least two models of tubulogenesis have been described. The “cell hollowing” model (currently the most studied) evolves from a chain of single endothelial cells (117). This model involves intracellular vesicle formation, then fusion of these vesicles into vacuoles, first intracellularly, and then from cell to cell, all along the sprout, when the vacuoles get bigger. The future lumen vacuole membrane finally acquires hallmarks of an apical membrane, thus defining the apical-basolateral polarity of the neo-formed vessel. In the less studied “cord hollowing” model, which happens in a multi-cellular elongated stack, the cells first lose their initial polarity, then basolateral membrane markers are accumulated at the membrane facing the extracellular matrix, while vesicles are accumulated and get fused on the opposite membrane, hence forming an initial lumen that expands afterwards (179, 248). In the “cell hollowing” model, the formation of vacuoles is linked to the activation of intracellular chloride channels of the CLIC family. The chloride intracellular channel (CLIC) family of proteins contains six mammalian members (CLIC1-6), from which only CLIC1 and CLIC4 are expressed in endothelial cells (230). CLIC proteins can change from a soluble state to a membrane-linked state. In the latter case, when inserted in such organelles as mitochondria, Golgi membranes, nuclear membranes, etc., CLICs function as chloride channels, although very different in structure from the other known Cl⁻ channels (65, 233). In the “cell hollowing” model of tubulogenesis, CLIC4 is present in human umbilical vein endothelial cells and promotes several processes involved in angiogenesis, including lumenal formation (229). Particularly, endothelial cells

derived from transgenic mice, in which the *Clc4* gene has been invalidated, fail to proceed to the vacuole creation and extension process. Tung and colleagues show that the enlarging vacuoles acidify during their formation, involving an electrogenic vacuolar proton-ATPase (vH^+ -ATPase), and propose that CLIC4 supports this acidification by creating an electrical gradient for the proton transfer. The same article proposes that CLIC1 may partially compensate for the absence of CLIC4 in the process of tubulogenesis, although CLIC1 seems mainly committed to proliferation and migration processes rather than to morphogenesis of endothelial cells (230).

(2) The new hollow tube thus created is finally stabilized by the formation of a new basement membrane. Indeed, in physiological angiogenesis, accessory cells, such as pericytes and smooth muscle cells are recruited to the newly developed vessel, to form a new basement membrane (54). In tumor angiogenesis, however, the newly formed blood vessels are very heterogeneous in structure and function (54). Their architecture is very chaotic and disorganized, with abnormal branching, inflated cells and tortuous courses. Subsequently, the association between endothelial and mural cells is altered, becoming loose and leading to highly permeable vessels, which, paradoxically, are not adapted to a fluent and regular irrigation of the tumor, hence favoring its dissemination to other, supposedly more welcoming, locations (35). The leaky vessels allow the tumor to escape the noxious environment in which it grows (metastasis). Again, Ca^{2+} channels of the TRP family, namely TRPV4 Ca^{2+} channels, are key determinants in the balance between normal and defective vasculature (2). Indeed, tumoral endothelial cells naturally contain low levels of TRPV4. Conversely, overexpression or pharmacological activation of TRPV4 normalizes the vasculature and restores the endothelial cell layer physical and biophysical properties.

III. Metastasis

A. The tumor microenvironment (TME): role and ion channel profile

The extracellular environment of tumor cells is viewed as a complex network of stromal cells, deregulated vasculature, extracellular membrane (ECM) proteins, growth factors and cytokines, all of which generated or activated by the tumor cells, acting together to influence the growth, behavior and malignancy of the tumor

(236), and constantly evolving while the tumor grows and becomes more aggressive. The physical and chemical characteristics of the TME is cancer-type dependent, but there are a number of typical, cancer type-independent features, among which hypoxia, acidic pH, high levels of lactate and low levels of glucose (21, 82). In this context, ion channels and transporters are important specialized proteins involved in the regulation of signaling pathways associated with these properties. All the cells composing the tumor microenvironment (TME) are laden with several types of misexpressed/overexpressed ion channels, which participate in the crosstalk described by Arcangeli, between the tumor and its TME (8). These ion channels also contribute to maintain the harsh conditions that allow tumor growth (33). Concerning the vasculature, we have already discussed the channels involved in neoangiogenesis and mentioned the importance of endothelial cell Ca^{2+} homeostasis throughout this process. Mesenchymal cells (fibroblasts, myofibroblasts, immune cells) also express ion channels, particularly lymphocytes that infiltrate the tumors express K^+ channels of the $\text{K}_v1.3$ and $\text{K}_{Ca3.1}$ types, which are involved in T cell activation via Ca^{2+} signaling (124). Interestingly, tumor-associated macrophages express $\text{P2X}_7\text{R}$ non-selective cation channels and inwardly-rectifying K_{IR} K^+ channels; both families are involved in the Ca^{2+} -dependent activation of macrophages (8).

B. Role of the epithelial-mesenchymal transition (EMT) in the metastasis process

Metastasis of epithelial cancer cells is a multistep process, which involves mobilization of the cells from their original location, migration of the cells, invasion of surrounding locations, intravasation of cancer cells into newly formed tumoral blood vessels, cell survival in the blood flow and seeding to more distant new niches, where the cells start proliferating again, thus creating secondary tumors. Multiple oncogenic signals originating from the TME convert the cells into their new metastatic and invasive phenotype. These signals lead the tumor cells to acquire several biological capabilities described as hallmarks of cancer (95, 96) and mediated by an epithelial-mesenchymal transition (EMT) process, otherwise called the metastatic cascade. During this cascade, cells, which originally display epithelial cell features (i.e., adhesion to each other and to the basement membrane, high level of E-cadherin involved in the epithelial integrity (16)) are progressively transformed into mesenchymal cells (116). Briefly, during this process, polarized epithelial cells acquire a new phenotype, which includes increased resistance to death, enhanced migratory capacities and invasiveness. The complete spectrum

of signaling agents that contribute to EMT remains an open question. EMT is a complicated process and ion channels from several different families play a particularly important role in this transition (249).

1. Mobilization of the cells: involvement of ion channels

Loss of cell-cell contacts is the first step of EMT. This phenotype is associated with repression of cell junction proteins, such as E-cadherin, and correlated with stimulation of both vimentin (an intermediate filament protein) and N-cadherin (neural cadherin, normally expressed in migrating non-tumoral cells) synthesis (107, 164, 226). This process has been shown in breast cancer cells to be Ca^{2+} -dependent (52). In fact, induction of EMT is clearly related to the expression of TRPM7, the melastatin-like TRP Ca^{2+} channel. Indeed, upregulation of TRPM7 regulates the expression of the EMT marker vimentin. Conversely, downregulation of the channel prevents EMT induction in breast cancer cells. In colorectal carcinoma cells, the Ca^{2+} -activated KCNN4 ($\text{K}_{\text{Ca}3.1}$; SK4) channels have been shown to be involved in EMT promotion, probably through participation in the cell hyperpolarization, subsequent induction of Ca^{2+} entry via voltage-dependent Ca^{2+} channels and an increase in intracellular Ca^{2+} concentration (129).

Tumor cell mobilization is the next step in the metastatic cascade. This step involves migration of the cells from their initial site and invasion of the surrounding stroma and vasculature. This is a complex process requiring the coordination of multiple macromolecules involved in adhesion, cytoskeletal dynamics and digestion of the ECM. Among the extracellular elements favoring EMT, hypoxia and microenvironment acidification have been the most studied and are involved in tumor cell mobilization. As discussed earlier in this review, tumor acidity is a direct result of hypoxia (83, 237). Indeed, low pO_2 raises the rate of glycolysis, which produces elevated levels of lactate and protons in tumor cells. In order to maintain pH_i within a tight range ($\text{pH } 7.2 - 7.4$), the tumor cells upregulate H^+ extrusion, which, in turn, results in microenvironmental acidosis, down to $\text{pH } 6.5$ (50) or even to $\text{pH } 5.8$ as shown in highly metastatic MDA-MB-435S breast tumor cells (208). Extracellular acidosis increases even more due to hypoxia-induced activation of carbonic anhydrases, especially CAIX (223). Consequently, tumor cells are equipped with mechanisms that allow them to sense and react to the low pH_e to which they have been exposed. pH sensors of several types are present in the plasma membrane of mammalian tumor cells and these pH sensors are categorized into G-protein coupled receptors (GPCRs) and non-GPCR pH

343 sensors (50). GPCR signaling pathways are manifold: ERK pathway/ Ca^{2+} release (105), G_s -protein/cAMP,
 344 $\text{G}_{12/13}$ -protein/Rho, and G_q -protein/phospholipase C pathways (140). pH sensing results in reorganization of the
 345 cytoskeleton (e.g. actin rearrangement, stress fiber synthesis, etc.), thus contributing to tumor migration and
 346 eventually to tumor progression (39). Non-GPCR proteins include ion channels of several families and
 347 participate in migration and invasion phenotypes. Indeed, expression of the tumor cell cation-permeable Acid
 348 Sensing Ion Channels (ASICs) is pH-dependent. ASIC1a is associated with tumor cell migration and invasion in
 349 hepatocellular carcinoma cells (112); ASIC2a and ASIC3 have been identified in the plasma membrane of
 350 adenoid cystic carcinoma cells and proposed to be involved in the acquired invasive capabilities of the cells
 351 (257). The Ca^{2+} channel TRPV1 is also activated by low pH (259) and is correlated with tumor cell proliferation
 352 in human prostate cancer cell lines (159). Both families of channels are involved in cancer-associated pain (57,
 353 259) and plasma membrane phospholipids are proposed to be modulators of ASIC and TRPV1 activities (128).
 354 Other acid-sensitive channels comprise several members of the Ca^{2+} channel TRP family, ionotropic
 355 purinoceptors (P2X), inward rectifier K^+ channels (K_{ir}), voltage-activated K^+ channels (K_v family), L-type Ca^{2+}
 356 channels (Ca_v , CACN), hyperpolarization-activated and cyclic nucleotide-gated cation channels (HCN), gap
 357 junction channels, and Cl^- channels. They are involved in the process of tumor cell migration (217).
 358 Interestingly, the Ca^{2+} -activated, high conductance, BKCa K^+ channels are not involved in breast cancer
 359 progression, as shown by the absence of effect of iberiotoxin (an inhibitor of BKCa) in the processes of
 360 proliferation, survival, migration or invasion in several breast cancer cell lines (200).
 361 Migratory cells are polarized along their longitudinal axis, with hypoxia- and TME acidosis-dependent
 362 protrusion of lamellipodia at the cell front edge and coordinated retraction of the rear edge (213). This process is
 363 intracellular Ca^{2+} concentration-sensitive, thus involving Ca^{2+} release from intracellular stores and SOCE
 364 channels (228). It creates the amoeboid movement characteristic of migrating tumor cells which is highly
 365 dependent on ion and water flux (210). Indeed, the formation of lamellipodia is accompanied by relocalization
 366 at the leading edge of a number of plasma membrane transport proteins, among which CAIX, NHE, MCT (all
 367 involved in pH modulation), anion exchangers (such as NBC transporters or the anion exchanger AE2) and
 368 water channels (aquaporins; AQP). It is proposed that the coordinated action of all these channels and
 369 transporters makes it possible for the cells to establish the osmotic gradient that subsequently induces AQP-
 370 mediated water flux at the leading edge of the cell. Water extrusion by AQP, at the rear end of the cell, is

mediated by the combined activity of Ca^{2+} -activated K^+ channels ($\text{K}_{\text{Ca}3.1}$) and Volume-Regulated Anion Channels (VRACs) and leads to regulatory volume decrease (RVD) and shrinkage of the cells (206).

2. Invasion of surrounding TME and vasculature

Invasion of healthy tissues by tumor cells is critically dependent on the local degradation of ECM and basement membrane, which otherwise would constitute a barrier to tumor metastasis. The degradation process thus creates the escape pathway by which single tumor cells leave their primary location and colonize surrounding areas. This degradation results from the low extracellular pH produced by tumor cells and relies on the secretion or membrane expression of acid-dependent proteases (MMPs and cathepsins). Soluble (MMP2, MMP9) or membrane-bound (MMP14) MMPs are key enzymes in tumor cell invasiveness, involved in both ECM and basement membrane degradation (187, 193, 253) and involved as well in the angiogenic sprouting (18, 53). MMP activity is promoted by low pH_e localized at the front part of migrating cells. As stated above, NHE1 is one of the main actors of this extracellular acidification. While the acidification process appears as a crucial step to weaken and break the ECM barrier, the physical progression of aggressive tumor cells relies on the formation of ECM-degrading protrusions, called invadopodia, at the leading edge of the cells. NHE1 has been localized within these invadopodia (29) and this localization has been correlated with extracellular acidification and MMP expression and activity (88), thus suggesting the invadopodia as the major sites of ECM digestion and NHE1 as the main channel involved in this process.

Voltage-gated sodium channels are highly expressed in epithelial tumors. In the MDA-MB-231 breast cancer cell line, the $\text{Na}_\text{v}1.5$ Na^+ channel, by driving Na^+ influx and depolarizing the cells, greatly increases NHE1 potency to promote extracellular acidification and tumor invasion (23, 24, 86).

Calcium channels are also involved in the migration and invasion processes. Indeed, TRP channels, which mediate SOCE Ca^{2+} entry and regulate intracellular $[\text{Ca}^{2+}]$, especially TRPV2 (158) and TRPM8 (169), positively influence carcinoma invasion by upregulating MMP2 and MMP9 synthesis and production. Thus, MMP production may be an intracellular $[\text{Ca}^{2+}]$ -dependent process. Interestingly, high concentrations of ATP are observed in the TME of most hypoxic solid tumors (181). ATP acts as a signaling agent by activating plasma membrane ligand-gated receptors of the P2X family, namely P2X₇R cation channels promote migration

and invasion, as shown in T47D breast cancer cells (253). P2X₇R cation channels mediate Ca²⁺ influx in tumor cells thus contributing to the increase in intracellular [Ca²⁺]. In the breast cancer cell line MDA-MB-435S, ATP activation of P2X₇R cation channels activates Ca²⁺-dependent SK3-mediated potassium currents, thus favoring the elongation of cell protuberances, which, in turn, promote cell migration (110). In this model, the P2X₇R-promoted invasive phenotype also relies on the activation of proteolytic enzymes, essentially cathepsin B. Correlatively, pharmacological inhibition or downregulation of P2X₇R channels in PC-3M human prostate carcinoma-derived cell lines inhibit ATP-driven migration and invasion (193). This Ca²⁺-dependent process requires activation of either PI3/AKT or ERK1/2 signaling pathway. It reduces the expression of proteins involved in the maintenance of cell-cell contacts (e.g. E-cadherin and claudin-1) and correlatively increases the expression of Snail (an inhibitor of E-cadherin), interleukin 8 (IL-8, a promoter of migration and angiogenesis) and MMPs, thus contributing to the invasion phenomenon. The metastasis- and malignancy-promoting status of P2X₇R, however, is still an important field in cancer research. Indeed, recent studies have been designed to explore the effects of P2X₇R on tumor development and metastatic outcome *in vivo*. Hofman and collaborators demonstrate that colitis-associated cancers (colon adenocarcinoma, colon carcinoma) are favored by P2X₇R silencing or pharmacological inhibition (103). A different study reveals that P2X₇R restricts tumor progression by developing antitumoral immunity (4).

3. Metastasis: transit in blood vessels and extravasation

Tumor cell metastasis occurs when the cells successfully escape from their primary location, overcome the step of transit in the circulation as unattached cells and finally disseminate to their secondary sites by extravasation from the vessels and reattachment to a new ECM support.

Circulating tumor cells do not always survive long enough to metastasize. It is estimated that only 0.01% of the cells that enter the vasculature will eventually survive the harsh conditions to which they are submitted (87).

Tumor cells must resist anoikis whereby apoptosis is triggered by inadequate or loss of cell anchorage. Among the multiple signals involved in anoikis resistance, several types of ion channels have been described (137).

Indeed, to effectively resist detachment-induced apoptosis, tumor cells utilize mechanisms that eventually limit the rise in intracellular [Ca²⁺]. Ca²⁺ channels are thus downregulated, especially those acting via SOCE (130).

Depletion in other Ca^{2+} channels (TRPM2, TRPV6) also tends to reduce cell apoptosis (211). Activity of caspases and other DNA-degrading enzymes, as well as a decrease in membrane potential have been linked to loss of intracellular $[\text{K}^+]$ by K^+ channels. Cell shrinkage involved in apoptotic volume decrease (AVD) results from a loss of KCl by K^+ and Cl^- channels, with a concomitant loss of water. Thus, downregulation of K^+ (K_v , K_{Ca} , K_{ATP} , K_{IR} , $\text{K}_{2\text{P}}$) and Cl^- channels also prevents apoptosis (26). Alternatively, anoikis-resistant cells can develop regulatory volume increase (RVI) whereby Cl^- channels of the volume-regulated (VRAC) family, especially prostate cancer cell ClC-3 (138), which are involved in the maintenance of cell volume under hyperosmotic stress, are upregulated in cells that resist apoptosis. Interestingly, Ca^{2+} -activated Cl^- channels (CLCA) may be either activated or not activated in anoikis-resistant cells, as shown in mammary gland cells (67, 244), thus suggesting a different function of these channels in cancer cells.

Tumor cells are subjected to mechanical forces resulting from the blood stream, especially in narrow capillaries. Mechanical stress is the main destruction pathway that tumor cells must avoid in order to be able to form metastases. To prevent destruction, they develop integrin-involving adhesion processes and eventually get attached to the endothelial bed of the vessel in which they are traveling (81). This interaction between circulating tumor cells and endothelial cells is fundamental for the rest of the metastasis process, as it determines the site where the cells will eventually exit from the vessel, hence initiating the extravasation process. Gout and Huot have described the whole process of extravasation (87). Briefly, this process involves a first step of tumor cell adhesion to endothelial cells, utilizing specific receptors. Tumor cells rolling along the endothelial layer characterize this “light adhesion” step. A second step of firmer cell attachment then takes place, mediated by cytokines and cell adhesion molecules. Extravasation (or diapedesis) finally occurs when the cells cross the endothelial barrier through cell-cell junctions. As for several steps of the tumorigenesis process, the importance of intracellular $[\text{Ca}^{2+}]$ and of the associated Ca^{2+} channels is also demonstrated for metastasis of tumor cells. Indeed *Orai1*, the pore-forming component of the store-operated Ca^{2+} channel and its associated *STIM1* Ca^{2+} sensor are associated with cell migration, invasion and metastasis in human breast carcinoma cells (256). *In vivo* studies in a zebrafish model corroborate the importance of the *Orai1*/*STIM1* partners and of SOCE Ca^{2+} entry in the process of cell extravasation (262). Indeed, inhibition of the store-operated Ca^{2+} entry or knockdown of *Orai1* in nasopharyngeal carcinoma cells prevents extravasation of the cells from vasculature in a zebrafish hematogenous metastasis model.

Finally, diapedesis is the result of tumor cells passing in between insufficiently tight endothelial cell junctions (due to damaged vasculature). The alterations in cell shape necessary for diapedesis are most likely explained by K^+ and Cl^- fluxes and by the following water fluxes. Indeed, it has been already shown that glioma cell migration and invasion are supported by K^+ and Cl^- channels, which, by governing transmembrane water movements and the resulting cell shape, are important for glioma invasion through narrow spaces (215). Colonization of the secondary site by the tumor is not simply a matter of gaining access to this site. Tumor cells will only be able to colonize compatible target tissues. The host tissue must then adapt to be able to foster the colonizing tumor cells; this is the concept of “pre-metastatic niche” (212). Tumor cells must also adapt to their new stroma and become able to secrete all the molecules necessary for their efficient attachment and ulterior vascularization (91). Hence, there are many barriers to overcome before the tumor actually metastasizes.

IV. Sigma 1 receptor: the main organizer?

In the present review, we will skip the long history of sigma-1 receptor (Sig-1R), which has been widely studied on the functional, pharmacological, structural and mechanistic points of view for the last 30 – 40 years (11, 148, 245). Similarly, we will not open this discussion to the sigma-2 receptor, which has not been cloned yet, although it was shown to be involved in cancer development (234). Instead, we will focus on the specific role of chaperone of Sig-1R that is of major interest in the context of cancer development.

Sig-1R is a 25 kD, endoplasmic reticulum (ER)-resident, ligand-regulated protein, which is involved in many diseases, ranging from toxic substance addiction to stroke and cancer (10, 11, 148). It is mainly present in normal brain, heart and liver, but is highly expressed in tumor cell lines and human cancer tissues of various origins, including lung, colon, prostate carcinomas, sarcomas, breast tumors, brain tumors, and melanomas (10, 11, 113, 173, 241), in which it interferes with cell cycle and proliferation (10, 197). Interestingly, in breast cancer cells, Sig-1R positively correlates with the metastatic grade of the cells, i.e. highly metastatic cells contain higher levels of Sig-1R mRNA and protein than their less aggressive counterparts (10).

Sig-1R is anchored in the ER membrane, in a specific region called mitochondrion-associated ER membrane (MAM). At rest, it is in a “dormant” state, associated with a chaperone protein, called GRP78 or BiP (100). BiP

is a main regulator of ER function: it has a high Ca^{2+} -buffering capacity and serves to store Ca^{2+} , which is essential to many signaling pathways. Under specific ligand stimulation or under prolonged ER stress such as ER Ca^{2+} depletion, Sig-1R is up-regulated, dissociates from BiP and becomes able to act as a chaperone (100) after translocation to other ER areas or to the plasma membrane (101). In the plasma membrane, Sig-1R interacts with – and regulates the activity of – several ion channels (Ca^{2+} , K^+ , Na^+ , Cl^-), receptors (NMDA receptor) or kinases (Src) (148).

In the context of cancer development, as we discussed earlier in this review, many ion channels of diverse classes (voltage-gated, calcium-gated, volume activated) are involved in a number of tumor cell processes. Voltage-gated ion channels are involved in cell processes including proliferation, apoptosis, angiogenesis, adhesion to ECM and migration. Interestingly, Sig-1R interacts with most of these channels, as demonstrated in several works (12, 13, 126, 197). Patch-clamp studies and western blot assays in the presence of Sig-1R ligands (e.g. (+)-pentazocine, igmesine, and 1,3-di(2-tolyl)guanidine [DTG]) were conducted in small cell lung cancer (SCLC) derived cell lines (NCI-H146 and NCI-H209) and in T-cell leukemia-derived Jurkat cells. These studies demonstrate that ligand-mediated inhibition of Sig-1R results in cell cycle arrest in G_1 phase with no stimulation of apoptosis (197). This effect correlates with the reduction of voltage-operated K^+ currents in all these cell lines, indicating that Sig-1R-mediated cell growth arrest is the result of the negative regulation of K^+ channels (197). Cell cycle arrest also correlates with the negative modulation of Volume-Regulated Chloride Channels (VRCC), both effects leading to the overall inhibition of the Regulated Volume Decrease (RVD) process, (196), which is known to govern cell cycle progression and numerous cellular functions such as proliferation, apoptosis, migration, ... (202). The *Xenopus* oocyte system allowed direct reconstitution (in the absence of Sig-1R ligands) of the interaction that exists between Sig-1R and potassium channels (Kv1.4 and Kv1.5) and established that Sig-1R directly acts as an auxiliary subunit of a few channels to govern several cell functions (12). In order to provide further evidence of this direct interaction, I participated in a study (47) showing that in chronic myeloid leukemia (CML)-derived cells (K562) – which overexpress Sig-1R – the abnormally expressed cardiac K^+ channel hERG co-immunoprecipitated with Sig-1R. In this model, Sig-1R extinction by a specific shRNA or Sig-1R inhibition by Sig-1R ligands led to the reduction of hERG K^+ currents, whereas co-injection of both hERG and Sig-1R in *Xenopus* oocytes increased hERG current when compared to oocytes injected with

hERG alone. This modulation proceeds from the regulation of hERG channel maturation and stability, which is in adequation with the role Sig-1R as a chaperone of hERG K⁺ channel in cancer cells (47).

Recently, using the technique of atomic force microscopy, Sig-1R was shown to physically associate, in a four-fold symmetry (1 channel molecule vs. 4 Sig-1R molecules), with the voltage-gated Na⁺ channel Na_v1.5 (13), previously proven to promote invasiveness in breast cancer cells (24, 86). With the same technique, the same team demonstrated physical interaction between Sig-1R and acid-sensing ion channels (ASIC1a; (36)) and provided evidence of Sig-1R association with the heteromeric GluN1/GluN2A NMDA receptor, via specific binding of Sig1R to the GluN1 subunit (14).

These data raise the following question: how can an ER protein interact with plasma membrane ion channels of so many different families and govern so many cell functions in oncogenesis? A plausible explanation resides in the following observations: 1) Sig-1R is localized in ER, a cell area where Ca²⁺ signaling is crucial; 2) Sig1R is highly mobile in the ER membrane and Sig-1R overexpression, cellular stress or pharmacological treatment lead to dissociation of Sig-1R from its chaperone BiP and subsequent translocation to the plasma membrane (100, 149, 221), where it interacts with its client proteins (ion channels, receptors). Indeed, during cancer progression, the tumor cells undergo harsh conditions (hypoxia, low pH, starvation in nutrients) or even treatments that create cell stress and alter ER protein expression levels or generate abnormal secretion of ER-resident chaperones (157). Indeed, inasmuch as ER chaperones such as Sig-1R contribute to the correct folding of newly synthesized or conformationally distorted proteins, or to protect proteins that are prone to de misfolded, their role becomes crucial in case of cell stress. Moreover, it is worth pointing out that the BiP-Sig-1R association is tightly regulated in an ER Ca²⁺/Mn²⁺-dependent manner. Any cell signal that results in lowering ER Ca²⁺ concentration below physiological concentration (0.5 mM) leads to disassembly the two chaperones, thus increasing the activity of each (100). Under cell stress conditions, Sig-1R stabilizes the conformation of type3-IP3 receptor in the ER, thus stimulating Ca²⁺ transport from the ER to the mitochondria (100) and to the cytosol (252). Hence, Sig-1R orchestrates Ca²⁺ signaling between ER and mitochondria, by fueling the production of ATP by the mitochondria, which conversely regulates Ca²⁺ concentration in the ER (99). Sig1R also maintains proper folding and proper function of the plasma membrane proteins challenged by cell stress: it thus plays a major role in cell survival (Figure 3). Sig-1R has since then been proposed as a modulator of inter-organelle signaling (220).

Subsequently, several lines of evidence demonstrate that Sig-1R interacts with different types of proteins including ER stress sensors, cytokines, ion channels and that this interaction constitutes the mechanism whereby Sig-1R governs cancer cell behavior in response to alterations of the tumor microenvironment. Sig-1R modulates cell survival (160), apoptosis (60, 151) and cell cycle (196, 197). Recently, I participated in a study presenting evidence that Sig-1R is a key regulator of the signaling events that occur between the tumor cell and its microenvironment. Indeed, in chronic myeloid leukemia- and colon carcinoma-derived cells (resp. K562 and HCT-116 cells), we showed that extracellular matrix triggered a Sig-1R-dependent increase in cell adhesion, cell migration, cell invasiveness, as well as VEGF secretion (48) and that a hERG K⁺ channel-β1 integrin interaction mediated these effects in both cell lines. Silencing of Sig-1R in K562 and HCT-116 cells resulted in *in vivo* reduction of angiogenesis, invasion and extravasation (48). Sig-1R thus appears as a major regulator of tumor development. Further studies are necessary to assess whether Sig-1R might be envisioned as a therapeutic target and whether Sig-1R ligands might be designed as antitumoral treatments.

V. Antiangiogenic therapy and therapy resistance

Cancer is one of the deadliest pathologies, with approximately 14 million of new cases and 8 million of cancer-related deaths in 2012; negative WHO predictions expect annual cancer cases to rise from 14 million in 2012 to 22 million in the next two decades. Carcinomas (lung, liver, stomach, colorectal, breast, esophageal) are among the most common causes of cancer deaths <http://www.who.int/mediacentre/factsheets/fs297/en/>. Conventional global anti-tumoral therapies (chemotherapies) are only partly efficient because of the lack of responsiveness of some cancers and because these therapies sometimes induce systemic toxicity or resistance, ending up in treatment discontinuation. For vascularized cancers, a more precise strategy of angiogenesis inhibition has been developed, which focuses on the neutralization of the effects of angiogenic factors (FGF, VEGF) or on the inhibition of their receptors (70, 122). This strategy leads to vasculature destruction. Given the drawbacks of such therapies (89, 90), which can lead to more severe condition or even to death (194, 195), solid tumor therapies evolved towards normalization rather than destruction of the abnormal vasculature, the objective being to restore a close to normal blood flux and pO₂, to favor normal immune cell function and

finally, to allow efficient delivery of anticancer agents (106, 109, 190, 219). Thus, antiangiogenic therapies were used to treat several vascularized cancers in the last 40 years. However, too many carcinomas are still refractory to antiangiogenic-based treatments.

Hence, in parallel to existing therapeutic strategies, it is important to identify predictive biomarkers of stage and aggressiveness for the different types of tumors, as well as further targets for the development of complementary treatments to be used in combination with VEGF-targeted therapies. Carcinogenesis is a multistep process in which each step constitutes a potential angle of attack for a new therapeutic strategy development. Ion channels are implicated in the growth, migration, invasion and metastasis of tumors and therefore cancers can be included in the category of “channelopathies” (pathologies characterized by alterations in channel function). Consequently, recent studies present ion channels as potential targets for the development of future treatments. Several factors are in favor of the use of ion channels as therapeutic targets: i) cell expression of ion channels is often modified in cancers; ii) this expression varies with the type of tumor and, in a given type of tumor, with the specific step of tumor progression; iii) ion channels are accessible from the extracellular side of the cell and the potential pharmacological tools (available or yet to be designed) are multiple.

We do not intend here to review how ion channels can be targeted in each step of cancer development or in different cancers to design potential therapeutic strategies. Several excellent reviews have already been written from these standpoints (9, 49, 155, 203, 239). Rather, we will take a few examples of global cancer-associated clinical dysfunctions in which ion channels may be targeted in order to restore normal function or diminish cancer-associated disagreements and, finally, to improve patient’s condition.

Restoring a less toxic microenvironment

Concomitant with gradual accumulation of mutations in tumor cells, changes in the physical parameters of the microenvironment also contribute to reinforce the cell aggressiveness. Among these physical changes, as discussed in §II.A of this review, the microenvironment undergoes hostile conditions characterized by low pO₂, high interstitial pressure, low extracellular glucose and high extracellular lactate – all of these create high extracellular acidity. Regulating the establishment of such harsh conditions would reduce the metastatic potential of tumor cells. Cancer therapies thus include trials based on the regulation of extracellular pH. They

consist of systemic buffering using sodium bicarbonate to increase extracellular pH or take advantage of the proton transport systems involved in tumor cells (150). For the latter, inhibition of carbonic anhydrases (especially CAIX), of the Na^+/H^+ exchanger, using amiloride or EIPA are efficient but have not been developed for clinical use. Conversely, inhibition of the vacuolar H^+ -ATPase, using omeprazole (already used to suppress gastric acidity) was proven efficient as a pH normalizer in cancer treatment. Interestingly, the opposite treatment consisting in hyperacidification of tumor cells or of their microenvironment is also a promising treatment. Intracellular hyperacidification promotes tumor cell apoptosis whereas extracellular hyperacidification may provide an important tool to deliver therapeutic agents directly to the tumor site using acid-accumulating peptides (69).

Normalizing the vasculature

Carcinoma progression is associated with the production, by tumoral and stromal cells, of angiogenic factors, which participate in the development of a non-productive angiogenesis, due to blood vessel disorganization and to vessel wall high permeability. Antiangiogenic treatments, which tend to block the development of new blood vessels and harvest the tumor from oxygen and nutrients, are inadequate or even counter-productive to treat cancers. Other targets are now under scrutiny, which could participate in the emerging process known as vascular normalization, and among these targets are ion channels. Physical properties of the ECM have been shown to influence normal and malignant cell behavior. Especially, angiogenic response is influenced by the stiffness of tumor ECM (134). Ca^{2+} influx is one of the main responses of endothelial cells to the mechanical stress resulting from the growth of new vessel tips in such a stiff environment. In a recent paper, it has been shown that in a model of mouse prostate adenocarcinoma, mechanosensitive transient receptor potential vanilloid 4 (TRPV4) calcium channels regulate angiogenesis and tumor vessel maturation by the regulation of tumor endothelial cell mechanosensitivity via the reduction in basal Rho activity (2). This confirms that ion channels must be included in the set of molecules to be targeted in the design of the new tools aiming at normalizing vasculature, hence allowing better delivery of complementary antitumoral therapies. To further reinforce the biological relevance of Ca^{2+} channels as molecular targets for antitumoral treatments, we can mention that all the families of Ca^{2+} channels have recently been the focus point of researches demonstrating the involvement of these channels in pro-angiogenic processes. Many of these channels are now the object of

patents that describe the use of modulators of their function in order to treat the symptoms associated with cancer, i.e., proliferation, angiogenesis and metastasis; for review, see (163). The use of K^+ channel or pH modulators to fight cancer development has been patented as well (49, 198).

Fighting cancer-induced pain

Cancer-related pain is one of the most devastating side effects of tumor development. Indeed, hypoxia and, consequently, tumor acidosis are known to be responsible for the generation of pain (108). Cancer-related pain can also be the result of tumor metastasis especially in the case of bone dissemination. Indeed, cytokines produced by the tumor cells or by the bone microenvironment activate osteoclasts and thus contribute to the signaling of cancer-associated pain in bones (225). Anti-tumoral treatment administration can, by itself, be a factor of pain, whether the treatment is traditional chemotherapy, radiation, or surgery.

Moreover, it now seems that, except for morphine, for which the study results are not conclusive, conventional pain-reducing therapies (NSAIDs, cannabinoids, opioids, GABA-ergic drugs) may have a negative impact on cancer evolution (135). Consequently, the research of other analgesics and their targets is required. Current research on this topic focuses on ion channels associated with pain, such as ASIC (57, 258), especially ASIC3 (56), and such as TRP channels of several sub-families, especially TRPV1 (192). Indeed, both types of channels are known to be expressed in sensory neurons and to be activated by an acid load. These channels represent relevant targets for the future development of new analgesics. Treatments based on TRPV1 pharmacological inhibitors are already being clinically tested with some showing promising results (22). Voltage-gated Ca^{2+} channels and voltage-gated Na^+ channels are also under study, especially $Ca_v2.2$, $Ca_v3.2$, $Na_v1.7$, $Na_v1.8$. Specific antagonists derived from conotoxins have been developed and now seem to have good results in clinical tests (242).

This field of research is still wide open and progressing very quickly. It is interesting to note that most of the ion channels involved in the perception of pain are also implicated in one step or another of tumor development. This leaves open the possibility that a specific treatment designed against an ion channel in order to treat cancer-associated pain might also be used to prevent cancer progression. In particular, cannabinoids demonstrate antitumoral effect in several cancer types (hepatocellular carcinoma, ccRCC, etc.), which is not mediated by the regular CB1 and CB2 cannabinoid receptors (131, 235). Since cannabinoids can bind to many different ion

channel types, such as GPCR, NMDA receptors, GABA_A receptors (182), any of them can mediate the effect of cannabinoids in tumor cells, thus shedding a new light on the yet unexplained anti-oncogenic effect of cannabinoids.

In this topic, it is tempting to propose the potential development of Sig-1R ligands as treatments to fight cancer-induced pain. Indeed, as we mentioned earlier, Sig1R promotes the action of ASIC channels, which are involved with nociception (36). Sig-1R-inhibiting agents might be a way to obtain relief in this matter.

VI. Conclusion

A new concept in oncogenesis has emerged for the last 20 to 25 years, which states that ion channels and transporters regulate different aspects of the neoplastic cell physiology. A growing body of evidence now demonstrates that they govern the so-called “hallmarks of cancer”. As for neoangiogenesis, ion channels are involved at all stages, in tumor cells, as well as endothelial cells or stromal cells. Ion channels thus represent a new and promising field of research concerning the development of novel therapeutic agents. TRP channels for instance, which are highly expressed in endothelial cells and involved in thermosensation, osmoregulation and mechanoreception, are under current scrutiny for novel therapeutic strategy developments. Similarly, whereas monocarboxylate transporters (MCT1 and MCT4) have now emerged as anticancer targets, the lactate shuttle mechanism governing tumor cell pH regulation is still under study (55, 59). However, it is important to take into account that most ion channels, though overexpressed in many cancer types, still exist in healthy organs. The use of ion channel inhibitor-based therapies might thus induce many side effects, which will need to be carefully prevented or minimized. As an example, blocking hERG1 K⁺ channel prevents the angiogenic switch in colorectal cancer and, in turn, reduces metastasis (46). However, hERG1 is one of the main heart-expressed K⁺ channels, which certainly hinders the use of any of the current hERG1-targeted molecule as a cancer therapeutic agent. It might thus be important to develop non-cardiotoxic hERG1 blockers to be used as complementary anti-angiogenic treatments. The ion channel-based antitumoral therapies, though very attractive and potentially powerful, probably need more research time in order to be carefully designed for safe clinical use. It might be more fruitful to develop pharmacological treatments against Sig-1R, taking into account that this protein is

present in many tissues (CNS, lung, liver, pancreas, spleen), in membrane compartments. Targeted pharmacological action on Sig-1R at the MAM or at the plasma membrane represents a potential new axis for the development of therapies against numerous diseases including cancer.

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FIGURE LEGENDS

Fig. 1: Schematic representation of the different transport systems activated in tumor cells by hypoxia.

Hypoxia induces activation of the α subunit of HIF1 (hypoxia-induced factor), which dimerizes with HIF1 β to result in HIF1. HIF1, in turn, stimulates a set of proteins involved in membrane ion or solute transport. NHE: Na^+/H^+ exchanger; ASIC: Acid-Sensing Ion Channel; NBC: $\text{Na}^+/\text{HCO}_3^-$ cotransporter; CAIX: carbonic anhydrase type IX; GLUT: Glucose transporter; MCT: Monocarboxylate transporter.

Fig. 2: Schematic representation of Ca^{2+} signaling in endothelial cells activated by the tumor environment physical and biochemical conditions. In a hypoxic environment, acidification of the microenvironment triggers the synthesis and release of VEGF (blue circles) by both tumor and stromal (CAF) cells. VEGF binds to its specific endothelial cell receptor (VEGF-R2), which, in turn, activates Ca^{2+} entry by two main processes. Store-operated Ca^{2+} entry (SOCE) results from 1) activation of Ca^{2+} release from endoplasmic reticulum (ER), especially via inositol 1,4,5-trisphosphate (IP_3R), and subsequent replenishment of these stores by Ca^{2+} entry via TRPC1 or TRPC4 channels; 2) activation of the stimulating unit (STIM) of the STIM-ORAI complex and subsequent Ca^{2+} influx by the ORAI transporting unit. Receptor-operated Ca^{2+} entry (ROCE) results from the direct activation of TRPC3 or TRPC6 Ca^{2+} channels. The ensuing increase in intracellular $[\text{Ca}^{2+}]$ is the mainspring of mobilization, organization and permeabilization of new blood vessels.

Fig. 3: Schematic representation of plasma membrane ion channel chaperoning and Ca^{2+} signaling enhancement by Sig-1R in cancer cells (adapted after Su et al, 2010, Trends Pharmacol. Sci.31: 557-566). During cancer progression, tumor cells are submitted to stressful conditions caused by low pO_2 , low pH, and few nutrients. Under these conditions, Sig-1R (blue wide arrow) released from its ER co-chaperone BiP, activates the ER IP_3R Ca^{2+} channel, thus enhancing Ca^{2+} flux from the ER to the mitochondria and increasing the production of energy by the mitochondria. Sig-1R also stimulates the *bcl2* oncogene transcription, which reduces ROS production by the mitochondria and is in favor of cell survival. Under prolonged conditions of stress, ER chaperones are up-regulated; Sig-1R is overexpressed and translocates to the plasma membrane area where it becomes able to chaperone and activate membrane proteins such as ion channels (hERG, $\text{Na}_v1.5$, ASIC1a, ...) or enzymes (e.g. Src kinase).

Table 1. Main ion channels and solute transporters involved in tumor development and their role in these processes.

TRANSPORT PROTEIN	GENE	OFFICIAL NAME	FUNCTION IN ONCOGENESIS
Water channel			
AQP-1	<i>Aqp1</i>	Aquaporin	Angiogenesis, proliferation, migration (66, 68, 120)
Solute carriers (Na⁺/solute exchangers)			
GLUT-1	<i>Slc2a1</i>	Glucose transporter	Tumor cell glucose metabolism (6, 84, 132)
GLUT-5	<i>Slc2a5</i>	Fructose transporter	Tumor cell glucose metabolism (240, 260)
NHE1	<i>Slc9a1</i>	Na ⁺ /H ⁺ exchanger isoform 1	Cell volume and pH homeostasis (33, 170) Tumor angiogenesis (80) Metastasis (88, 218)
Bicarbonate transporters			
AE1-3	<i>Slc4a1-3</i>	Anion exchangers	pH homeostasis (222) Cell migration (222)
NBC transporters	<i>Slc4a4, 5, 7, 10</i>	Sodium-bicarbonate cotransporters	pH homeostasis (180) Tumor growth (180) Metastasis (180)
MCT family of monocarboxylate transporters			
MCT 1-4	<i>Slc16a1-4</i>	Solute carrier family 16 members 1-4	Cell migration and metastasis (184)
TRP superfamily of cation channels			
TRPA (ankyrin) family	<i>Trpa1</i>	Transient receptor potential cation channel, subfamily A	Cell migration and metastasis (63) Nociception, inflammation (22)
TRPC (canonical) family	<i>Trpc1-7</i>	Transient receptor potential cation channel, subfamily C	Cell migration and metastasis (76) Tumor angiogenesis (261)
TRPM (melastatin) family	<i>Trpm1-8</i>	Transient receptor potential cation channel, subfamily M	Cell migration and metastasis (63) Tumor angiogenesis (75)
TRPV (vanilloid) family	<i>Trpv1-6</i>	Transient receptor potential cation channel, subfamily V	Cell migration and metastasis (119) Tumor angiogenesis (75) Nociception (22)
Voltage-gated family of K⁺ channels			
Kv1.1-1.8	<i>Kcna1-7, 10</i>	Potassium channel, voltage gated, shaker related subfamily A, members 1-7	Proliferation of cancer cells (168) Apoptosis resistance (168) Immunoresponsiveness (224)
EAG1, 2	<i>Kcnh1, 5</i>	Ether-a-go-go K ⁺ channels	Proliferation of cancer cells (31) Migration of breast cancer cells (183)
hERG 1-3 or Kv11.1-11.3	<i>Kcnh2, 6, 7</i>	Ether-a-go-go-related gene K ⁺ channels	Proliferation of cancer cells (214) Crosstalk with cell adhesion molecules (183) Tumor angiogenesis (183)

Table 1. *cont'd*

Two-Pore K⁺ channel family			
K _{2p} 2.1 or TREK	<i>Kcnk2</i>	Arachidonic acid-sensitive K ⁺ channel	Cell proliferation (243)
K _{2p} 3.1 or TASK	<i>Kcnk3</i>	Acid-sensitive two-pore K ⁺ channel	Cell proliferation (127, 243) Evasion from apoptosis (127)
Calcium-activated K⁺ channels			
K _{Ca} 1.1 or BK _{Ca}	<i>Kcnn1</i>	Large conductance Ca ²⁺ -activated K ⁺ channel	Proliferation (200) Apoptosis (28)
K _{Ca} 2.1-3 or SK1-3	<i>Kcnn1-3</i>	Small conductance Ca ²⁺ -activated K ⁺ channels	Cell migration and metastasis (37, 110, 191)
K _{Ca} 3.1 or IK _{Ca} or SK4	<i>Kcnn4</i>	Intermediate conductance Ca ²⁺ -activated K ⁺ channel	Proliferation of cancer cells (141) Cell migration (141) Tumor angiogenesis (141) Evasion from immune destruction (174)
Voltage-gated Na⁺ channels			
Na _v	<i>Kcnn1</i>	Voltage-gated Na ⁺ channel	Tumor cell migration, invasion, endocytosis (32) Metastasis (32)
Voltage-gated family of Ca²⁺ channels			
Ca _v 1.1-1.4	<i>Cacna1s, c, d, f</i>	L-type Ca ²⁺ current	Tumor angiogenesis (51)
Ca _v 2.1-2.3	<i>Cacna1a, b, e</i>	P/Q-, N-, R-type Ca ²⁺ currents	Tumor angiogenesis (154)
Ca _v 3.1-3.3	<i>Cacna1g, h, i</i>	T-type Ca ²⁺ currents	Tumor angiogenesis (204)
P2X Receptor (P2XR) family of Na⁺, K⁺, Ca²⁺ ion channels			
P2X ₇ receptor	<i>P2rx7</i>	Ligand-gated cation channel	Proliferation of cancer cells (3, 103) Apoptosis resistance (3, 103)
Acid-sensing ion channel family			
ASIC1-3	<i>Asic1-3</i>	Acid-sensing ion channels	pH homeostasis (92) Cell migration and invasion (92)
Chloride intracellular channel family			
CLIC1-6	<i>Clic1-6</i>	Nuclear and intracellular organelle Cl ⁻ channels	Tumor angiogenesis (232)
Calcium release-activated calcium channels (CRAC channels)			
Orai1 (works with STIM)	<i>Orai1</i>	Calcium release-activated calcium channel protein 1	Cell migration and metastasis (161, 216) Tumor angiogenesis (139) Immunosuppression (174)





