Effects of Tumor Microenvironmental Factors on Ion Channels in Breast Cancer Development

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List of abbreviations:

BC: Breast Cancer
DDR: Discoidin Domain Receptor
ECM: Extracellular Matrix
EGF: Epidermal Growth Factor
EGFR: Epidermal Growth Factor Receptor
EMT: Epithelial-to-Mesenchymal Transition
ER: Endoplasmic Reticulum
GSTO1: Glutathione S-Transferase Omega 1
HIF: Hypoxia Inducible Factor
MCU: Mitochondrial Calcium Uniporter
MMP: Metalloproteinase
ROS: Reactive Oxygen Species
SICE: Store-Independent Calcium Entry
TGF-β: Transforming Growth Factor β
TME: Tumor Microenvironment
TNBC: Triple Negative Breast Cancer
VEGF: Vascular Endothelial Growth Factor

Contents:

Abstract

In recent years, it has been shown that breast cancer consists not only of neoplastic cells, but also of significant alterations in the surrounding stroma or tumor microenvironment. These alterations are now recognized as a critical element for breast cancer development and progression, as well as potential therapeutic targets. Furthermore, there is no doubt that ion channels are deregulated in breast cancer and some of which are prognostic markers of clinical outcome. Their dysregulation is also associated with aberrant signaling pathways. The number of published data on ion channels modifications by the microenvironment has significantly increased last years. Here, we summarize the state of art on the cross-talk between the tumor microenvironment and ion channels, in particular collagen 1, EGF, TGF-β, ATP, hypoxia and pH, on the development and progression of breast cancer.

Keywords: Tumor microenvironment, ion channels, breast cancer, Collagen 1, TGF-β, EGF, ATP, hypoxia, pH

1. Introduction

While enormous progress has been made in understanding the genetics of tumors and the fundamental molecular mechanisms involved in tumor progression, it is only in recent years that we have been interested in the role of the tumor microenvironment in cancers development including breast cancer (BC) [1]. Breast tissue represents an organ whose stroma plays a very important role in the development of the mammary gland. Indeed, during mammogenesis, the growth of the milk ducts and lobules requires infiltration of the epithelial cells into the surrounding stromal tissue. This mechanism requires remodeling of the microenvironment to allow the growth and migration of epithelial cells in a "similar" manner to an infiltrating tumor. Thus, many processes related to the microenvironment are deregulated and used by cancer cells during its tumor progression. The tumor microenvironment (TME) communicates and dynamically interacts with cancer cells permanently, thus making its components key players in the cancer development and progression. The TME is a dynamic entity composed of stromal cells, including fibroblasts, adipocytes, endothelial cells and immune cells. Furthermore, it is also composed of extracellular matrix (ECM) which contains soluble factors and adhesive components, which greatly influence cancer progression [2].

The composition and the dynamic of TME in BC have been extensively reviewed [1]. However, very little data are reported in the literature concerning the relationship between ion channels and the TME in BC. Ion channels have recently been identified as "new markers" in oncological research. Studies over the last 20 years have clearly shown the contribution of these channels to the aggressiveness of cancer and more and more studies suggest them as key players in interactions between tumor cells and TME through signals' transduction of cell signaling from the TME [3-5]. This review is divided into two parts. The first deals with the effect of the different components of extra cellular matrix (collagen 1, EGF, TGF-β, ATP), hypoxia and pH on BC progression. The second part will summarize the works that explored the possible involvement of ion channels in the TME-dependent effect on BC.

2. Impact of tumor microenvironment on breast cancer hallmarks

In recent decades, several works have underlined the importance of the microenvironment in BC progression [6,7]. Indeed, several studies have highlighted the importance of bidirectional communication between tumor cells and their microenvironment in the modulation of their phenotype. The TME consists of stromal cells and ECM components. Stromal cells components include cancer associated fibroblasts [8], cancer associated adipocytes [9], immune cells [10]

and endothelial cells [11]. ECM consists of adhesion factors, including type I and VI collagens, which are overexpressed in aggressive breast tumors [12]. Soluble factors, which correspond to the "secretome" of stromal cells, include among others Transforming Growth Factor-β (TGF-β) [13,14] and Epidermal Growth Factor (EGF) [15,16]. All these factors regulate cell proliferation, survival, epithelial-to-mesenchymal transition (EMT), migration, invasion, and metastasis.

2.1 Extracellular matrix

One of the components of the breast TME is the ECM, which plays an important role in the regulation of BC progression [17,18]. ECM contains adhesive and soluble factors and among adhesives components, type 1 collagen (collagen 1) is one of important factors that regulates the tumorigenesis, EMT, migration, invasion, metastasis and response to anticancer therapies [19].

2.1.1 Collagen 1 and breast cancer cell phenotype

The collagen superfamily is the major component of this ECM, particularly type 1 collagen, which is the most abundant in several organs such as breast, skin and lung. Biophysical investigations have given an evidence for different molecular fingerprints for collagen (fiber alignment, stiffness and density) in breast carcinoma tissues when compared to normal tissues. In fact, analysis of mammographic and particularly collagen density, analyzed by second harmonic generation microcopy (SHG) has shown a relationship between collagen density and BC risk and progression [20,21]. Kakkad *et al.* have investigated the relationship between lymph node metastasis and the properties of collagen in the primary breast tumors. They demonstrated an increase in collagen density only in primary tumors associated with positive lymph node metastasis [22]. Concerning the collagen stiffness, Stowers *et al.* have recently shown using the non-malignant MCF-10A epithelial breast cells, that matrix stiffening induces a malignant phenotypic transition and thus could be involved in the acquisition of invasive and metastatic properties in normal epithelial cells [23]. Finally, Morris *et al.* have shown that, in metastatic BC cells, higher collagen density induced an alteration in cell metabolism. Such observed shift was associated to changes in gene expression profile [24].

Collagen 1 consists of three subunits, two α1 chains and one α2 chain. The combination of these 3 chains leads to a right triple helix measuring 300 nm long and 1.5 nm in diameter [25]. Amino acid sequence of the subunits consists of a Gly-X-Y triplet repeats. X and Y correspond frequently to proline and hydroxyproline respectively. In addition to its architectural function, collagen 1 also modulates the behavior of surrounding cells by interacting with them *via*

specific receptors. The most studied receptors of collagen 1 are β1-integrin heterodimers (α1β1, α2β1, α10β1 and α11β1) [26]. Several studies have underlined the importance of integrins in the regulation of cancer stemness, metastasis and drug resistance [27]. α1β1 and α2β-integrins have been reported to mediate invasion in mouse breast tumor cells [28]. Kim *et al.* have demonstrated that collagen is able to induce MMP-2 activation in BC cells and that α2β1 integrin signaling was involved in this process [29]. Collagen also activates pro-MMP-2 and estrogen-induced proliferation in human breast epithelial cells *via* α2β1 integrin and β1 integrin respectively [29,30].

Discoidin domain receptors DDR1 and DDR2 have been also reported to interact with collagen 1 [31-33] and to play a role in tumor progression [34,35]. These receptors, which harbor a tyrosine kinase activity, recognize GVMGFO sequence of collagen 1 [36] and exhibit a relatively late and prolonged activation [37]. DDR1 seems to be preferentially expressed in luminal-like breast carcinoma, whereas the basal-like one express predominantly DDR2 [38,39]. Moreover, the high level of DDR2 expression is associated with high BC grade [40]. More recently, DDR1 mutations were strongly associated with poor prognosis in estrogen receptor-positive BC [41]. However, among the basal-like cell lines, MDA-MB-231 cells are the exception since they express weakly DDR1 and do not express DDR2 [42,38]. Maquoi group was the first to show the role of the collagen/DDR1 axis as a tumor suppressor in breast carcinoma. Indeed, this group showed that the 3D collagen matrix, by activating DDR1, inhibited the proliferation and induced apoptosis in luminal-like MCF-7 and ZR75-1 BC cells [43]. In more recent works, DDR1 has been shown to activate an apoptotic signaling pathway by inducing BIK expression [44,45]. While such phenotype was not observed in MDA-MB-231 cells [43], enforced expression of DDR1 in these cells restored cell proliferation suppression and apoptosis [38]. Concerning involvement of DDR2 in BC progression, the first data have been reported by Longmore group, who demonstrated that this receptor was able to enhance invasion and metastasis by stabilizing SNAIL1 [46]. Another work has demonstrated that DDR2 in tumor cells, but also in cancer associated fibroblasts, is important for BC metastasis [47]. More recent data have shown that DDR2 controls breast tumor metastasis *via* the regulation of the matrix stiffness and integrin signal transduction in cancer associated fibroblasts. Thus, DDR2 has been proposed at a promising target for the treatment of metastatic BC [48].

2.1.2 Cytokines and growth factors

Among cytokines and growth factors, TGF-β and EGF are respectively important players in BC progression. TGF-β is secreted in the extracellular environment by several cell types,

including macrophages, T cells and monocytes. This factor is produced in a latent form until it is activated to interact with its receptors and this activation is highly controlled [49]. TGF-β has been associated to poor prognosis in patients with BC [50]. At the functional level, it has been shown recently that this factor plays a crucial role in induction of EMT and invasion in BC cells [51]. Moreover, inhibition of TGF-β has been described to sensitize triple-negative breast carcinoma to chemotherapy (TNBC) [52].

EGF is expressed by several human tissues and promotes a variety of cell phenotypes *in-vivo* and *in-vitro*[53]. The paracrine signaling of epidermal growth factor (EGF) and its associated receptor EGFR has been shown to have an important role in driving BC progression and metastasis [54,55]. While it is known to promote cell proliferation [56], EGF has been also described to have an important role in bone metastasis process [15,57]. EGF has been also reported to promote EMT in BC [58]. Therefore, overexpression of EGFR and its activation by EGF have been depicted to be predictive markers for poor clinical outcome in BC patients [59,60].

2.2 Impact of hypoxia on breast carcinoma behavior

Because of the weak vascular network associated to the exacerbated proliferation, solid tumors present often a very low oxygen level. Consequently, this generates hypoxia environment in the tumor [61] and particularly in breast carcinoma cells [62]. Transcriptomic studies on a large cohort of BC have shown the role of a family of transcription factors, HIF-1α and HIF-2α (HIF for Hypoxia Inducible Factors), which are activated under hypoxia conditions, in the regulation of the expression of key genes encoding proteins involved in the various processes of tumor progression (The Cancer Genome Atlas Network, 2012). Some of the first works have demonstrated that up-regulation of HIF-1α was related to tumors and associated metastasis in human breast carcinoma and that this factor was a poor prognosis factor [63-65]. Concerning the stroma matrix receptors which could be involved in metastasis process, a recent work has shown that the fibronectin receptor α5β1 receptor was overexpressed by activation of HIF-1α in hypoxic conditions and that this integrin heterodimer was responsible for metastasis of BC to lymph nodes and lung [66]. In other works, the level of HIF-1α expression has been associated to carcinogenesis process and an increase in proliferation rate of BC [67,68]. After demonstrating that hypoxia was able to induce vascular endothelial growth factor (VEGF) expression and to initiate angiogenesis [69], other works reported that HIF-1α expression in hypoxic conditions was responsible for this effect [70]. Drug resistance has been also associated

to HIF-1α expression in hypoxic conditions [71]. Another work has demonstrated that HIF-1α expression was necessary for drug resistance in BC stem cells [72]. Xiang *et al.* have shown recently that HIF-1α was involved in the expression of TAZ, one of the matrix stroma sensors, and its recruitment into the nucleus to induced BC stem cell phenotype in hypoxic conditions [73].

2.3 pH in mammary tissue

It has recently been well described how the acidic tumor microenvironment drives cancer progression [74]. The tightly regulated pH of cells is important to maintain a cellular homeostasis as chemical processes in the cytoplasm and in cell compartments which require an optimal pH [75,3]. The balance between the intracellular (pH_i) and extracellular pH (pH_e) is involved in regulating metabolic pathways *via* a fine-tuned balance between proton production and extrusion. The disruption of such balance in tumors is a consequence of the combination of high metabolic demands of cancer cells, in conjunction with poor perfusion and regional hypoxia. The low oxygen environment makes cells undergo a metabolic switch towards a more glycolytic phenotype, thus a higher production of protons (Warburg effect) [76]. Excessive proton production induces intracellular acidification and apoptosis [77,78]. To compensate the high proton production, cells adapt by increasing the expression and activity of net acid extruders, keeping the intracellular pH normal or slightly alkaline. As a consequence, the microenvironment becomes acidic. A reversed pH gradient is associated with cancer progression, with acidic pH_e stimulating invasion and migration [79,80,78] and the slightly alkaline pH_i promotes cell survival and increased proliferation [81,78]. This ability to sense pH changes in tumors are important for both normal stromal cells and cancer cells to survive. A study carried out by Hashim et al. has shown that BC cell lines MCF-7 and MDA-MB-231 showed a pH_e as low as ~6.8 *vs* 7.4 in normal tissue, and a normal or slightly alkaline pH_i, ~7.4-7.6 *vs* 7.2 in normal tissue [82]. The essential pH regulation in both normal tissue and in tumors are maintained by plasma membrane transporters and enzymes, including: Na^+/H^+ exchanger 1 (NHE1), Na^+/ HCO_3^- co-transporters, Na^+ driven Cl^-/HCO_3^- exchanger, the anion exchangers AE1 and AE2, monocarboxylate transporters (MCT1, MCT2, MCT3 and MCT4) and the V-ATPase [78,3]. Several of these transporters are involved in driving cancer proliferation, migration and invasion [81,83,84,74].

3. Dialogue between microenvironmental elements and ion channels: effect on breast cancer hallmarks

3.1 Collagen induced breast cancer survival and migration through K^+ channels

Although some studies have reported the effect of collagen type 1 or fibronectin on potassium and calcium channels in several cancers [85-88], the relationship between matrix proteins and ion channels has been poorly studied in BC. Ouadid-Ahidouch's team has demonstrated a role of a complex composed by Kv10.1 potassium channel, Orai1 channel and SPCA2 (Golgi ATPase), in signals transduction induced by collagen 1 in BC cells survival. In non-invasive ER^+ BC cell lines (MCF-7, T47-D), collagen 1, under free serum culture medium, promotes cell survival through the tyrosine kinase DDR1 receptor but not β1-integrin [85]. Indeed, collagen 1, by activating DDR1, activates ERK1/2 that increases Kv10.1 and Orai1 expression and activity leading to an increase in the basal calcium entry independently of the reticular stores (Store-Independent Calcium Entry: SICE) that activates the ERK pathway that, in turn, promotes the expression of the oncogene c-Myc leading therefore to cell survival. Peretti *et al.* [89] deeply demonstrated how collagen 1 favors the interaction of the complex Kv10.1/Orai1 and SPCA2. Collagen 1 increases the plasma membrane fraction of Kv10.1 and Orai1 channels, promotes their co-localization and interaction with SPCA2 in the lipid rafts. SPCA2 is indispensable to both Orai1 and Kv10.1 trafficking to plasma membrane in the presence of collagen 1. Silencing of SPCA2 induces Orai1 retention in the cytoplasmic compartment and in the Golgi for Kv10.1 [89]. Moreover, Kv10.1, Orai1, SPCA2 and DDR1 are highly expressed and co-localized in aggressive BC tissues, while in non-tumor samples, these proteins are less expressed at the plasma membrane and the expression of Kv10.1 is restricted to the Golgi [89]. Collagen 1 is also involved in BC aggressiveness. Collagen fiber alignment facilitates persistence by limiting cell protrusions, thus promoting cell migration and invasion [90], and redirect cell migration to move only in one direction [91]. Basal BC MDA-MB-231 cells possess a high metastatic capacity and they also expressed Kv10.1 channel that regulate cell migration through two mechanisms: 1) by regulating calcium entry through Orai1 channels [92] and 2) by interacting with β1-integrin and focal adhesion kinase [93]. The extracellular matrix through fibronectin and collagen 1 also positively modulates Kv10.1-dependent cell migration. The MDA-MB-231 cell line adopts a more elongated morphology and increased migration rate when growing on double coating with fibronectin and collagen 1. Several studies have also shown that fibronectin, is able to increase potassium Kv11.1 channel activity, which is part of the same subfamily as Kv10.1 in the neuroblastoma and colorectal cancers [86,94].

In BC, fibronectin could increase both the interaction and the co-localisation of Kv10.1 with β1 integrin (unpublished data).

3.1.1 Stiffness, mechano-sensitive ion channels and breast cancer

Malignant tumor extracellular matrix is often stiffer than the matrix surrounding adjacent non-malignant cells [95] and such pressures could stimulate mechanosensitive ion channels [96]. Functional expression of Piezo channels has been described in BC cell lines [97], and their relevance to BC has just been investigated recently. Piezo 1 is functional in MCF-7 BC cell line but not in MCF-10A normal mammary epithelial cell line. Pharmacological blockade of this channel reduced cellular motility of MCF-7 but not that of MCF-10A cells [97]. Moreover, BC patients with high Piezo1 mRNA levels showed a shorter overall survival when compared to those showing low Piezo1 expression levels [97]. Recently, in brain metastatic BC cell line MDA-MB-231-BrM2, Valverde's team has clearly reported that calcium influx via Piezo2 regulates cell migration by regulating the cytoskeleton organization through the RhoA-mDia pathway [98]. Lou *et al.*, by using the "Atlas database analysis", identified a decreased Piezo2 expression in BC compared with normal control tissues [99]. They also investigated the relation between Piezo2 expression level and the overall survival of patients, and they found that high expression of this channel is correlated to a favorable prognosis in BC. Moreover, the expression of Piezo2 is potentially targeted by five miRNAs and correlated with the downregulation of 109 genes enriched in Hedgehog signaling pathway, including regulated cell adhesion molecules downregulated by oncogenes [99].

The mechano-receptor TRPM7 has been shown to reduce the cytoskeletal tension through Myosin II activity in MDA-MB-231 cell line [100,101]. Silencing of TRPM7 or its pharmacological inhibition, by waixenicin A, increased cytoskeletal tension likely through reducing SOX4 expression. Moreover, the increase of matrix stiffness (in a collagen coating model) decreased both the expression of TRPM7 and SOX4. They also found that both mRNA expression of SOX4 and TRPM7 are positively correlated in primary breast tumor samples. The authors suggested SOX4 as a downstream transcriptional target of TRPM7 signaling in mesenchymal-type BC cell lines [100].

To our knowledge, only one study has reported on the involvement of voltage-activated T-type calcium channels in MCF-7 BC cell proliferation [102] in relation with matrix density. The increase of extracellular pressure up to 40 mm Hg activates T-type Ca^{2+} channel (Cav3.3) leading to calcium influx and activation of PKC-b, which in turn activates NF-kB [102].

3.2 EGF and TGF-β modulate EMT, invasion, migration and proliferation through potassium, calcium and sodium channels

3.2.1 EGF

Abnormal expression and activity of EGF and EGFR promotes EMT in cancer cells through ERK1/2 and PI3K/Akt pathways, which are involved in proliferation, metastasis and invasion [103-105]. It has previously been shown that EGF, EGFR and the phosphorylation of its tyrosine residues modulates the activity of ion channels [106], including potassium [107-109], calcium [109,110], chloride [111] and voltage-gated Na^+ channels [112]. Tyrosine kinases and phosphatases regulate the function ion channels activities that are involved in cancer proliferation, migration, invasion and apoptosis [113,114]. These pathways are associated with EMT, induced by EGF and EGFR. Furthermore, several studies have identified that EMT induces a changes in the expression and activity of different plasma membrane ion channels including potassium, calcium and sodium, which will in turn regulate tumor invasion [114].

The calcium activated potassium channel $K_{Ca}3.1$ (SK4) has been studied in several types of cancer including BC [115]. A blockage of SK4 has shown to inhibit proliferation and promote apoptosis in MDA-MB231 cells. Furthermore, it has been shown that MDA-MB-231 and MDA-MB-468 cell lines can undergo EMT mediated by EGF/basis fibroblast growth factor (bFGF), whereas MCF-7 and T47D cells are not able to undergo EMT at all [115]. In addition, in MDA-MB-231 cells (harboring the most significant mesenchymal phenotype compared to other cell lines) the mRNA expression of SK4 is upregulated, and the decrease of SK4 channel expression downregulates the expression of the mesenchymal markers Vimentin and Snail1. The authors concluded that the expression of SK4 is associated with EGF/bFGF-induced EMT and that it might drive both the EMT and migration process in TNBC cells [115].

It is well studied that remodeling of Ca^{2+} permeable channels, their expression and Ca^{2+} signaling, are linked to EMT and that they promote the expression of several proteins associated with cells transforming to a more mesenchymal phenotype [114]. The team of Monteith has deeply investigated the role of Ca^{2+} during the EMT process in BC cells [116-118]. They have observed that treatment with EGF increases EMT markers as Twist, SNAIL1 and Vimentin in MDA-MB468 cells and have identified specific channels involved in Ca^{2+} remodeling as regulators of EMT induced by EGF [117,118]. TRPM7 is involved in EGF-mediated EMT by enhancing Vimentin protein expression. Furthermore, TRPM7 silencing results in a reduction of the EGF induced STAT-3 phosphorylation, but does not alter the cytosolic Ca^{2+} response induced by EGF [117]. In another study, the same authors found not only a higher expression of EMT related markers, but also an increased expression of Orai1 and Ca^{2+} entry [118]. In this

study, they found that EGF-induced EMT in MDA-MB468 cells is associated with reduced agonist-stimulated and store-operated Ca^{2+} influx. It is known that both Orai1 and TRPC1 maintain the constitutive Ca^{2+} influx, but here it has been shown that only Orai1, and not TRPC1, is associated with EGF-mediated EMT [118]. Orai1 silencing inhibited non-stimulated Ca^{2+} influx, agonist-stimulated and store-operated Ca^{2+} influx, whereas the silencing of TRPC1 only inhibited non-stimulated Ca^{2+} influx, but in a manner dependent on Orai1 [118]. This suggests that the altered activity of Orai1 and TRPC1 plays a role in EGF-mediated EMT. In addition, TRPC1 silencing has been shown to be associated with a significant reduction in ERK1/2 signaling function, showing that TRPC1 is involved in proliferation. All these data indicate that EMT in MDA-MB-468 cells is linked to a remodeling of Ca^{2+} influx, which might be regulated by Orai1 and TRPC1 channel functions [118]. The same group has found that EMT in BC also is associated with the altered gene expression of specific Endoplasmic Reticular (ER) calcium channels and pumps [116]. The expression of the ER channels inositol 1,4,5-triphosphate receptor IP_3R1, IP_3R3 and ryanodine receptor RYR2 has been shown to be upregulated in EGF-treated MDA-MB-468 cells when compared to the non-treated cells. Under the same conditions, the ER pump SERCA2 is significantly upregulated, whereas the SERCA3 is downregulated. This suggests that EGF induced EMT in BC induces changes in the expression of ER channels and pumps and thereby storage and Ca^{2+} signaling [116].

Voltage-gated Na^+ (Na_v) channels are widely expressed in metastatic cells of different types of cancer, including BC [119-121]. $Na_v1.5$ is upregulated in BC, which promotes invasion and metastasis phenotype [122,123,119]. In a new study, it has been shown that $Na_v1.5$ channels are involved in EGF-induced EMT. EGF induces both the expression and activity of $Na_v1.5$ in MDA-MB-231 cells [122]. Furthermore, the motility of MDA-MB-231 cells is increased when induced with EGF, through the functional expression of $Na_v1.5$. It can be suggested, that $Na_v1.5$ channels are not acting alone during cell migration, when induced with EGF. As the activation of $Na_v1.5$ depolarizes the plasma membrane potential, other ion channels responding to voltage can be activated [122]. In addition, the influx of Na^+ through $Na_v1.5$ channels stimulates the activity of NHE1, resulting in a higher proton extrusion, acidifying the extracellular environment and thus activating metalloproteinases, which can promote the invasive and migratory capacity of cells [122,124,125]. Disrupting the homeostasis of ions as Na^+, Ca^{2+} and H^+ could potentially activate protein kinases and downstream pathways that affect migration, invasion or proliferation [122]. Rho family GTPase, Rac1 has been shown to be involved in migration by regulating cytoskeletal rearrangement and lamellipodia formation [126]. $Na_v1.5$-dependent plasma membrane depolarization leads to Rac1 co-localization with

phosphatidylserine and thereby activation. The activation of Rac1 in MDA-MB-231 cells results in lamellipodial protrusion formation, migration and thereby a more invasive phenotype [126].

The sodium content has shown to be higher in mammary adenocarcinomas than in normal lactating mammary epithelium [127,128], even though it is not clear if tumor functions are correlated with the extra- or intra-cellular sodium concentration [127]. Recently, it has been shown that a treatment with NaCl and pro-inflammatory interleukin 17 (IL-17) have a synergistic inflammatory effect on the growth in BC cell lines. This treatment also enhanced the production of reactive nitrogen and oxygen (RNS/ROS) species, which correlated with an upregulation of the epithelial sodium channel (ENaC) expression level in various BC [127,129,130]. Treatment with NaCl, IL-17 and knockdown of ENaC reduce RNS/ROS species production. Furthermore, the same treatment enhances expression and phosphorylation of ERK1/2 in MDA-MB-231 cells. These data suggest that ENaC plays a role in proliferation through downstream ERK1/2 signaling and in the inflammatory process in BC [127].

3.2.2 TGF-β

Transforming Growth Factor Beta, (TGF-β), is another EMT inducer *via* the canonical or the non-canonical pathways in epithelial cells [131,132]. Cell calcium entry, especially upon store-depletion, is also involved in TGF-β-induced EMT by promoting cellular migration and potentially leading to metastasis. TGF-β treatment is known to increases migration, calpain activity, expression of EMT markers (Vimentin, N-cadherin) and decreases expression of epithelial markers (E-cadherin) in NMuMG and MDA-MB-231 cells [133]. TGF-β-treatment increases store-mediated Ca^{2+} entry, *via* TRPC1 /Stim1 that activates calpain leading to migration, a loss of E-cadherin and MMP activation. Silencing of TRPC1 or Stim1, or using pharmacological inhibition of SOCE (SKF-96365) decreased TGF-β induced Ca^{2+} current, and inhibited calpain activation and cell migration [133]. Moreover, the overexpression of TRPC1 increases Ca^{2+} entry and promotes TGF-β-mediated cell migration. TGF-β affects more the activity of TRPC 1/Stim1 but neither the expression of Stim1 nor that of Orai1. TGF-β also suppresses cell proliferation in both MDA-MB-231 and MCF-7 cells by inducing cell cycle arrest at the G0/G1 phase by accumulating p21 and reducing Cyclin E expression [134]. These effects are calcium-dependent since they are altered by EGTA or by pharmacological inhibition of SOCE. Treatment MDA-MB-231 cells with TGF-β decreases both Stim1 expression and Thapsigargin-induced calcium entry. Moreover, stably Stim1 overexpressing in MDA-MB-231 cells suppresses the TGF-β-induced effects. TGF-β increases the expression of the

transcriptional inhibitory factor of Stim1 (Wilm's tumor suppressor 1, WT1). Silencing of WT1 restores the expression of Stim1 in the presence of TGF-β. Moreover, both TGF-β and Thapsigargin increased ERK1/2 phosphorylation and pharmacological inhibition of SOCE reduces the TGF-β–induced ERK phosphorylation [134]. Hu et *al.* have found that TGF-β–induced EMT through downregulating of Oct4 that up-regulates Stim1 and Orai1 expression leading to an increase in SOC entry[135].

3.2.3 ATP, *via* purinergic receptors, regulates proliferation, invasion, migration and EMT.

ATP is a nucleotide firstly known to provide energy to different biological processes in living cells. In physiological conditions, intracellular ATP rate (5-10 mM) is relatively high when compared to extracellular medium (10-100 nM) [136]. However, this balance is totally changed in case of cancer. Indeed, real time extracellular ATP concentration monitoring showed that it could reach hundreds of millimolar in the tumor microenvironment [137,138]. Cell damage as well as non-lytic pathways has been reported to explain the high extracellular ATP rate. Among these pathways, vesicular exocytosis and ATP efflux through ATP release channels and nucleotide transporters have been reported[139]. Otherwise, the involved cells are both infiltrating inflammatory cells and tumor cells in response to inflammation, hypoxia or to some therapies.

Extracellular ATP is sensed at P2 purinergic receptors. Burnstock and Kennedy [140] have proposed the distinction between P2X and P2Y receptors based on pharmacological criteria. Cloning and transduction mechanisms led to the nomenclature of P2X ionotropic ligand-gated ion channel receptors and P2Y metabotropic G protein-coupled receptors [141]. Seven subunits (named P2X1-7) assemble as homo- or hetero-trimers to form the channel of P2X receptors. ATP is the physiologic agonist of these receptors whose activation results in the alteration of cytosolic calcium concentration. On the other hand, there are eight isoforms of P2Y receptors named P2Y1 & 2; P2Y4 & 6 and P2Y11-14. They differ from each other by the nature of their agonists and by the signal transduction pathways that their activation triggers. Indeed, P2Y1 & 2; P2Y4 & 6 are coupled to a Gq protein that, in turn, activate a Phospholipase Cβ, while P2Y12-14 are coupled to a Gi protein that inhibit the Adenylyl cyclase. Among the Gq coupled subfamily, P2Y2 is the only ATP-active isoform, it is also considered UTP equally active. ADP, UTP, and UDP are respectively the preferred ligands of the P2Y1, P2Y4, and P2Y6 isoforms. Among the Gi coupled subfamily, ADP is the preferred ligand of P2Y12&13 while P2Y14 is activated by sugar nucleotides such as UDP-Glucose and UDP-galactose. P2Y11 is a special

case in that it is coupled to both a Gq and a Gs protein, thus when activated by ATP it results in an increase of intracellular calcium and cAMP concentrations [142].

The effect of ATP depends on its concentration in the extracellular medium and the panel of P2 receptors expressed in tumor and/or stromal cells. In MCF-7 BC cells, all P2Y receptors are expressed but not all the P2X isoforms. Indeed, P2X1 and P2X3 transcripts are not expressed. Moreover, the transcript profile shows a relative strong expression of P2X4 when compared to P2X2 & 5. P2X6 & 7 are not significantly expressed. When exposed to 30 µM $[ATP]_o$, MCF-7 show a 50 pA inward current which seems to be a P2X-like current. Extracellular ATP treatment does not significantly affect cell death or proliferation. However, cell migration is increased. As assessed by specific siRNA and the use of different inhibitors, ATP-induced migration results in the activation of the nucleotide on P2Y2 receptor leading to an increase in $[Ca^{2+}]_{cyt}$ and subsequent activation of MEK pathway [143].

Highly invasive MDA-MB-435s BC cell line express P2X4, P2X5, P2X6 and P2X7. However, P2X7 isoform is by far the most expressed and seems to be the only active form. Furthermore, in MDA-MB-435 cells, millimolar concentrations of ATP activate an inward current, leading to an increase in $[Ca^{2+}]_{cyt}$. P2X7R activation regulates MDA-MB-435s apoptosis, migration and invasiveness. Indeed, ATP concentrations over 3 mM results in significant cell death that is inhibited both by KN62 and A740003 P2X7R inhibitors. Additionally, millimolar ATP exposition induces elongation of "Neurite-like" MDA-MB-435s prolongations, which are hallmarks of a migratory cell profile. The ATP induces regulation cell migration through activation of SK3 potassium channels by the calcium-induced influx. P2X7R activation is also involved in MDA-MB-435s cell invasiveness in a dose dependent manner both *in-vitro* and *in-vivo* through regulating Cathepsin B active forms [144].

Recently, Maffey *et al.* [145] reported that mesenchymal stem cells from tumor microenvironment promote BC stem cell proliferation, and metastatis through purinergic signaling. Such process take place through exosomes and microvesicles that increase cytosolic calcium concentration. A significant increase in cell responsiveness is observed when exogenous ATP is added to the cells. In these cells, ATP acts through P2X ionotropic receptors as assessed by P2X inhibition experiments. Indeed, P2X7 by A438079 inhibition leads to a significant decrease in cell metabolism activity and cell growth. Moreover, ATP depletion in the extracellular medium leads to a significant decrease in BC cell invasiveness.

ATP action is modulated by other factors present in tumor microenvironment such as EGF [146]. Davis et al. have shown that EGF induces changes in the response to ATP in MDA-MB-468 BC cells. Indeed, in the presence of ATP, EGF induces a significant change in the calcium

profile and expression of EMT markers such as Vimentin. Analysis of purinergic receptors has shown that P2X5 isoform is up-regulated (4.6 folds) of in the presence of EGF. Upregulation of this isoform is confirmed (13 folds) in mesenchymal-like BC cells when compared to epithelial- like cells. Moreover, P2X5 knockdown leads to a decrease in Vimentin which remains significant despite its weak expression [146].

A synthetic presentation of the different actors previously described is reported in the **Table 1** and in **Figures 1 and 2**.

3.3 Involvement of ion channels in the adaptation of cells to live in:

3.3.1 hypoxic conditions

As depicted previously, hypoxia is an important feature of the tumour microenvironment, which promotes, e.g., BC adaptation, resistance and aggressiveness. In addition, hypoxia regulates different parameters of the tumour microenvironment like angiogenesis, extracellular matrix composition or stromal cells' functions. However, underlying mechanisms are not yet clearly defined. Among the numerous tracks currently explored, ion channels could be good candidates due to their involvement in multiple tumor processes and their membrane localisation. Indeed, a large information panel is already available regarding their role in the response to oxygen variations in the cardiovascular and neuronal system but few elements are evaluated in tumour context.

Some reports described the involvement of Ca^{2+} signalling in response of BC cells to hypoxia. It has been previously described that hypoxia could induce EMT [147]. In fact, Davis *et al.* demonstrated that Ca^{2+} chelation reduces the hypoxia-induced EMT in MDA-MB-468 cells [117]. By deciphering the putative molecular support, they analysed the role of TRPM7 channel but they could not demonstrate that this channel is involved in the modulation of the intracellular [Ca^{2+}] even though it participates to the regulation of EMT markers.

Works from the Monteith's lab brought information about other Ca^{2+} channels involved in the response of BC cells to hypoxia. In fact, TRPC1 seems to be involved in hypoxia-mediated events [148]. More precisely, hypoxia increases TRPC1 mRNA expression that regulates SNAIL and Claudin 4 expression and participates to the regulation of EGFR and STAT3 phosphorylation. In addition, TRPC1 is also involved in autophagy process through the EGFR pathway. In a recent study, the same group demonstrated that BC molecular subtypes present different Ca^{2+} channel expression profile. Accurately, they showed that Orai3 channel is more specific to luminal cell type compared to Orai1, which is mostly expressed and active in the basal cell type [149]. They further analysed Orai3's involvements in hypoxia and they

demonstrated that: i) Orai3's expression is increased in hypoxia through HIF-1α pathway; ii) this channel is not involved in the regulation of EMT markers' expression; iii) Orai3 regulates EGFR autophosphorylation without any effect on migration; iv) it participates to the regulation of hypoxia of migration and to the inflammatory/immune response gene profile.

In a similar manner, Liu and collaborators demonstrated that Orai1 is also involved in the response of hypoxia [150]. More precisely, they showed that Orai1 is involved in a Notch1 signalling pathway associated to a store-operated Ca^{2+} entry and NFAT4 to participate to the aggressiveness of TNBC cells. Intracellular Ca^{2+} transporters could also be involved in the TNBC phenotype. Indeed, it has been shown that mitochondrial calcium uniporter (MCU) could regulate the HIF-1α pathway, and subsequent expressed genes, as well as the ROS production, participating to the metastatic processes [151]. The results of this study suggest that MCU could be an actor in the response to hypoxia.

It is now clearly established that hypoxia also promotes resistance to therapy [152]. In this context, Lu *et al.* demonstrated that intracellular Ca^{2+} concentration is increased by carboplatin treatment in a TNBC model [153]. More precisely, Glutathione S-Transferase Omega 1 (GSTO1), which is regulated by HIF-1α and HIF-2α and whose expression is increased by carboplatin, interacts with RyR1 and promotes Ca^{2+} release from the internal store. The Ca^{2+} raise activates the PYK2-SRC-STAT3 pathway promoting the BC stem cell phenotype and induces consequently chemoresistance. In a similar way, it has been shown that TRPC5, which is overexpressed in Adriamycin-treated MCF-7 cells, participates to the VEGF secretion regulation through a HIF-1α pathway suggesting an involvement of this channel in the cell response to hypoxia hypoxia [154].

Regarding the potassium channels, some studies also described their involvement in BC properties in response to hypoxia. Firstly, Mu and collaborators highlighted the amplification of *KCNN9* potassium channel gene [155]. They then described an improvement of the hypoxia resistance of the cells by using *in vitro* and *in vivo* models, which is not affected by the p53 status. A second subclass of the potassium channel family has been described in the hypoxia context: the voltage-gated potassium channels. Eag1 channel, also named Kv10.1, which was among the first Kv channels highlighted in oncogenic process, has been initially involved in the hypoxia homeostasis [156]. By using Eag1-expressing CHO cells, authors demonstrated that Eag1 channel participates to the HIF-1α expression's regulation and thereby VEGF secretion and vascularisation. According to the involvement of Eag1 in the proliferation and in the motility of different BC cell models [93], Lai *et al.* analysed the putative expression relationship of this channel and HIF-1α in human samples [157]. Through their observational

study, they demonstrated that the co-expression of the 2 actors is positively correlated to node status, tumour stage and tumour size suggesting an interest to use this association like a potential biomarker. In addition, recent data obtained in our laboratory demonstrated that Eag1 channel could participate to the regulation of MDA-MB-231 cells' migration in hypoxia condition (unpublished data). At the best of our knowledge, only another study described the role of Kv3.1 and Kv3.4 in the control of BC cell migration and invasion [158].

Little information about other players of the BC cell's transportome is available. However, it has been described an increase in aquaporin 1 in HIF-1α expression in BC tissues [159]. In addition, P2X7 has been involved in the regulation of tumour cell invasion, in hypoxia context, through a signalling pathway involving RAGE, Akt, ERK1/2, NF-κB translocation and MMP-2, -9 expression [160].

Despite the fact that NaV channels are already well described in the promotion of the BC cell aggressiveness and in the response to oxygen variations in myocytes and carotid bodies, there is no study about their involvement in hypoxic breast tumours.

Ion channels and transportome actors, involved in the answer to hypoxia, are classified in the **Table 2** and **Figure 3**.

Finally, there is a very closed link between pH modification in tumour microenvironment and hypoxia. In this way, it is clear that acid-sensing ion channels and H^+ transporters could be modulated in this context but the concept has still not been demonstrated.

3.3.2 Acidic conditions

The major regulators of pH in tumor cells are the transporters and carbonic anhydrases involved in the extrusion of H^+ excess to maintain the alkaline pH_i. Whereas these transporters have often been reported to be important in tumor progression, the roles of pH sensing ion channels are less described. Here we describe the pH sensing ion channels, mediated primarily through their expression at the cell surface in BC. A synthetic presentation is available in the **Table 2** and in the **Figure 4**.

A type of ion channels, being voltage independent but affected by pH is the acid-sensitive ion channels (ASICs), where eight subunits encoded by five genes have been identified. ASICs are H^+ cation-gated channels and are activated by extracellular acid. Some types (ASIC1) are both Na^+ and Ca^{2+} permeable where other types are only Na^+ permeable [78]. ASICs are mainly expressed in the central and peripheral nervous system and belong to the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily. Despite being expressed in the nervous system, ASICs have shown to be expressed in glioma cells and BC [161,162]. As ASICs have been reported

to play an important role in acidosis-associated physiological and pathophysiological conditions [163], they may be involved in cancer progression, due to the acidic extracellular environment. ASIC1 is highly expressed in malignant BC tissue, compared to normal breast tissue and genetic alterations of ASIC1 expression correlate with the overall survival of patients [162]. Furthermore, downregulation and pharmacological inhibition of ASIC1, with amiloride or psalmotoxin, suppressed tumor growth in-*vitro* and in-*vivo*. *In vivo* studies have shown that ASIC1 also leads to metastatic activity for lung tumor nodules, compared to the control. Together, these studies show that ASIC1 is important for BC growth, invasion and metastasis. ASIC1 is expressed in some BC cell lines (MCF-7 and LM-4142), and is involved in the acidification of extracellular pH (pH_e 6.6) which leads to an increase in ROS level [164,162]. Indeed, ASIC1 activation regulates proliferation, invasiveness, migration, apoptosis and angiogenesis through ROS-AKT-NF-κB pathway [164,162]. Furthermore, the inhibition or silencing of ASIC1 suppressed acidosis-induced activation of ERK1/2, AKT and NF-κB [162]. These findings show that ASIC1 is required for ROS production in BC cells, and ROS is a central molecule in regulating downstream pathways in an acidic environment. ASICs are also responsible for Ca^{2+} entry, and activation of ASIC1 leads to a rise in intracellular calcium concentration in neurons [162]. Ca^{2+} is involved in cancer as it regulates invasion and migration signaling [118,162,165]. ROS production has been shown to be decreased by calcium chelators, indicating that ASIC1-regulation of Ca^{2+} is important for ROS generation under acidic conditions [162]. Taken together, these suggest that there is a crosslink between ASICs and Ca^{2+} influx and that ASICs can be involved in the regulation of Ca^{2+} signaling pathway.

Another type of ion channel involved in pH regulation is the voltage gated proton channel Hv1, which is highly selective for H^+, and no other cations. Hv1 is specifically expressed in highly metastatic human BC and the down-regulation of Hv1 inhibits the metastatic by reducing invasion, migration and H^+ secretion [166]. As previously described, acidic pH_e promotes degradation of ECM, which increases the secretion and activation of proteases. The proteases need the low pH_e to have optimal activity. Among many proteases,the cathepsins and the MMPs are essentially involved in degradation and remodeling of ECM [166]. The secretion and activation of some proteases are pH-regulated and MMP-9 showed reduced activity in MDA-MB-231 cells with suppressed Hv1. This indicates that secretion of protons by Hv1, ensuring the acidic pH_e, promotes invasion and metastasis by activating and secreting proteases, such as MMP-9 [166]. In addition, a knockdown of Hv1 in MDA-MB-231 cells decreases proliferation, invasiveness but also inhibited pH recovery and proton secretion, affecting cell capacity of acidifying pH_e [167]. Furthermore, the high expression of Hv1 in tumors from patients is

correlated with tumor progression and patients were more likely to have a shorter overall survival. High expression of Hv1 is associated with a poor prognosis, thus making Hv1 a prognostic factor [167]. The metastatic potential of MDA-MB-231 cells correlates with the high Hv1 expression in the plasma membrane. Taken together, the regulation of pH by Hv1 and the acidic pH_e in cancer cells affect the secretion, activity and cellular distribution of proteases, thus making the BC cells showing a more aggressive phenotype, with high proliferation, invasiveness and migration. The knockdown of Hv1 showed inhibition of tumor progression, development and metastasis, making Hv1 a molecular biomarker and target of BC therapy [167].

$Na_V1.5$ has been shown to interact with the predominant regulator of pH_i NHE1, in the caveolae to enhance the H^+ efflux, resulting in an acidification of the pericellular microenvironment ([124] (see [125])). $Na_V1.5$ and NHE1 colocalize in the plasma membrane of cancer cells and extracellular matrix assays suggest that they are both involved in the same pH-dependent invasiveness regulatory pathway [124]. The invasive properties of both channels in cancer cells, have been shown to be activated through acidic extracellular cathepsins, mainly cathepsin B and S [125]. From high-grade BC biopsies and highly invasive BC cells lines, the overexpression of $Na_V1.5$ has been associated with ECM remodeling and an increased risk of developing metastasis [168,125]. In addition, it has been shown that $Na_V1.5$ interacts with NHE1, allosterically increasing NHE1 activity in a pH range of 6.4 to 7.0, suggesting more proton extrusion at more acidic pH_i. This interaction is supposed to occur in caveolae of the invadapodia compartment, hence responsible for increased ECM degradation and invasiveness [168]. A more aggressive phenotype of the BC cells could be explained by the enhanced Src kinase activity and the phosphorylation of Y421 cortactin, involved in migration and invasion [168,169]. These data suggest that $Na_V1.5$ is regulated by pH and enhances NHE1 activity, promoting degradation of ECM and leads to invasion and migration in BC cells [168,124].

Some other ion channels sensitive to the pH are expressed in BC. CLC-2 has shown to be opened by mild acidification but is completely inhibited by strong acidification [170]. CLC-2 is highly expressed in epithelial cells, and in the colon where it has been suggested to have role in electroneutral salt absorption [171]. CLC channels are expressed in BC cells and tissue and especially CLC-3 is over expressed [172] and is related to invasion, migration and cell cycle regulation [173]. Moreover, metastatic BC cells transfected with hCLCA2 presented a reduced pH_i from 7.49 to 6.67, an acidic pH_i known to activate apoptosis. The acidification of the cytosol by hCLCA2 could explain its inhibitory effect on proliferation and cell survival, as pH has profound effects on these events [174]. In addition, mammary epithelial cells and lung

fibroblast expressing cystic fibrosis conductance regulator (CFTR) has been found to induce a drop in pH to ~6.7 to initiate apoptosis, whereas the CFTR mutant did not [175,176].

Concerning potassium channels, the two-pore domain potassium channel (K2P) family include some member that are pH sensitive [177]. Members of the subfamily the TWIK-related alkaline pH activated K^+ channels including TASK-2, TALK-1 and TALK-2, are stimulated by an extracellular alkalinization [177-179]. These channels are of interest as their genes have been found to be either altered or upregulated in BC tissue, and in some cell types are found to be required for apoptosis [177-180]. These channels are inhibited by an extracellular acidification, which may contribute to cancer cells avoiding apoptosis through these channels [3]. Another subfamily of the K2P is the TWIK-related acid sensitive (TASK) family, which is inhibited by extracellular acidification. All the genes have been found upregulated in BC tissue and the gene coding for TASK-3 (KCNK9) is recognized as a proto-oncogene and its overexpression promotes tumorigenesis [181,179,178]. Taken together, these channels (CLC and K2P) would be a potential actor to regulate downstream signaling pathways of these cancer hallmarks through an acidification of the tumor environment.

4. Conclusion and perspectives

We have summarized here the modulation of ion channels expression and/or activity by the tumor microenvironment and how this involves changes in BC development and progression.

To better understand the complex nature of BC, several cell lines were used to study the tumour microenvironment impact on BC progression: luminal-like, basal-like, proliferative, invasive, and expressing “or not” different receptors for oestrogen, progesterone or HER-2. However, the results obtained remain fragmentary and represent only an approximation of how the processes take place in patient’s tumour. Moreover, tumour heterogeneity constitutes one of the major obstacles in cancer treatment leading to recurrence of cancer. It is becoming increasingly clear that there is significant response heterogeneity in drug responses within cancer cell populations. So far, most of studies investigating the role of ion channels in cancer progression were dealing with cancer cells *in-vitro*. Up to now their modulation by the microenvironment, which is crucial for tumor progression, has not been largely studied in BC and there is little knowledge about their expression profile and their role in clonal cell populations. Consequently, experimental approaches must be improved in order to get closer to the reality of the disease. For this purpose, it would be appropriate to investigate the role of ion channels by using different models including 3D cell culture, organoids, co-culture models or microfluidic systems. Indeed, such approach could better enable to inform us about the

interactions between different cell types present in tumor environment and tumor cells and the role of ion channels in these interactions.

Channel Family	Type/Name	Involved in cancer progression by	Signaling pathway	Reference
K^+ channels	Kv10.1	Survival induced by collagen 1	DDR1, ERK phosphorylation, increased expression and membrane fraction of Kv10.1 and Orai1, increased co-localization of Kv10.1 and Orai1 through SPCA2, increased SICE, increased $[Ca^{2+}]i$, increased c-Myc expression and cell survival	[85,89]
	Kv10.1	Migration induced by fibronectin and collagen 1	Increasing co-localization and interaction between Kv10.1 and β1-integrin	Unpublished Personal data
	SK4, KCa3.1	EMT induced by EGF/bFGF	Increased vimentin and snail mRNA expression	[115]
ORAI and TRP	Orai1 & TRPC1	EMT induced by EGF	Reduced non-stimulated and agonist-stimulated, Ca^{2+} entry through Orai1 and TRPC1	[118]
	TRPC1	MDA-MB-468 cell proliferation	ERK1/2 phosphorylation, cell proliferation	[118]
	TRPM7	Maintains BC mesenchymal phenotype/response to matrix rigidity	Increased cytoskeletal tension through reducing SOX4 expression	[100]
	TRPC1	TGF-β-induced EMT	Increased store-mediated Ca^{2+} entry, activation of Calpain, loss of E-cadherin and MMP activation	[133]
	Orai1 and Stim1	TGF-β-induced EMT	Inhibition of Oct4 expression that up-regulates Stim1 and Orai1 expression leading to increase SOC Ca^{2+}	[135]
	TRPM7	EGF induced EMT	Increased Vimentin expression along with STAT3 activation	[117]

PIEZO	PIEZO1	Cellular motility	Silencing of PIEZO 1 reduces cell motility	[97]
	PIEZO2	Migration	Regulating the cytoskeleton organization through the RhoA-mDia pathway	[98]
Na^+ channels	Nav1.5	Increased migration induced by EGF	EGF increased Nav1.5 expression	[182]
	Nav1.5	Increased migration	Depolarization of the resting Vm (Na^+-dependent) that increases Rac activation	[126]
Others				
Stim	Stim1	TGF-β inhibiting Cell proliferation	Increased expression of Wilm's tumor suppressor 1 (WT1), reduction of Stim1 expression, reduction of SOCE, reduction or ERK phosphorylation, increased P21expression and reduction of cyclin E expression, Cell cycle arrest in G0-G1 phase	[134]
ER Intracellular calcium transporters	IP3R RYR SERCA	EMT induced by EGF	A high increase of RYR2 expression, A slight increase in IP_3R1 and IP_3R3 and SERCA2 expression A decrease of expression of SERCA3	[116]
P2X ionotropic ligand-gated ion channel	P2X7	Growth Proliferation Mammosphere formation Spheroid size Invasion	Stimulate invasiveness through P2X7, enhancing Ca^{2+} and Na^+ influx and K^+ efflux	[145]
	P2X5	EMT	Potentiate the EGF-induced vimentin protein expression and reduced E-Cadherin expression.	[146]

Table 1: Ion channels involved in the dialogue with microenvironment: links to matrix, EGF, TGF-β and ATP.

Channel Family	Type/Name	Involved in cancer progression by	Signaling pathway	Reference
Relation with pH homeostasis in breast cancer tumour				
Voltage gated ion channels by:				
Sodium	$Na_V1.5$	Invasion Migration	Invasiveness through NHE1, enhancing H^+ efflux –> activating ECM degradation by cathepsin Invasion and migration through, Src/Y421 activation	[168,124,125]
Proton	Hv1	Invasion Migration	Invasion and migration → regulating pH_e → secretion of cathepsin matrix metalloproteinase → ECM degradation	[166,167]
Other				
Calcium activated Chloride channel	CLCA2	Proliferation Apoptosis	Proliferation and apoptosis induced by p53 in response to DNA damage	[174]
Acid sensing ion channels	ASIC1	Proliferation Invasion Migration	Through ROS-AKT-NF-κB, ERK1/2 and Ca^{2+}	[162,164]
Relation with hypoxia in breast cancer tumour				
Ca^{2+} channels				
	TRPC1	EMT	Regulation of gene expression and of EGFR and STAT3 phosphorylation	[148]
	TRPC5	VEGF secretion	Through HIF-1α pathway	[154]
	Orai3	Hypoxia response	Regulation of EGFR autophosphorylation and Participation to the control of migration and inflammatory/immune gene profil	[149]
	Orai1	Migration Invasion Angiogenesis	Through Notch1/Orai1/SOCE/NFAT4	[150]
	MCU	Aggressiveness	Through HIF-1α pathway and subsequent genes' regulation	[151]

	RyR1	BCSC promotion Chemoresistance	Through GSTO1/RyR1/PYK2/Src/STAT3	[153]
K^+ channels				
	TASK-3 (*KCNK9*)	Hypoxia resistance	Genetic amplification promotes the resistance to drastic environment like hypoxia	[155]
	Eag1		Co-expression with HIF-1α correlate with tumor size, node status and tumor stage	[157]
	Kv3.1/Kv3.4	Migration Invasion	Increased expression in hypoxia	[158]
Other				
	Aquaporin 1		Co-expression with HIF-1α in breast cancer tissues	[159]
	P2X7	Invasion	In association with RAGE and through a Akt/Erk1/2/NF-κB translocation	[160]

Table 2: Ion channels involved in the relation between hypoxia and pH and breast cancer cells

Captions of the Figures:

Figure 1. Schematic illustration of ion channels, involved in collagen 1-induced effects in breast cancer. A) Effect of collagen and fibronectin on cell migration in the highly metastatic MDA-MB-231 breast cancer cell line. B) Effect of collagen on the survival and proliferation in the non-invasive MCF-7 breast cancer cells.

Figure 2. Ion transporters regulation by EGF and TGF-B and conferring breast cancer invasion, migration and proliferation. The sequence of events for each channel type is indicated by black arrows. See text for details. Green color indicates SERCA2 and 3 and Stim1 that are located in the ER.

Figure 3. Schematic representation of the different ion channels described in hypoxia context of breast cancer. Membrane or intracellular Ca^{2+} and K^{+} transporters are modulated by hypoxia or modulate hypoxia signalling to promote aggressiveness / metastatic potential of breast cancer cells. Function and expression modulation of the channels are involved in signalling pathways promoting angiogenesis, survival, chemoresistance, invasion, migration, EMT. Integral descriptions are included in the main text.

Figure 4. Schematic illustration of ion channels, involved in pH regulation and sensing in breast cancer.

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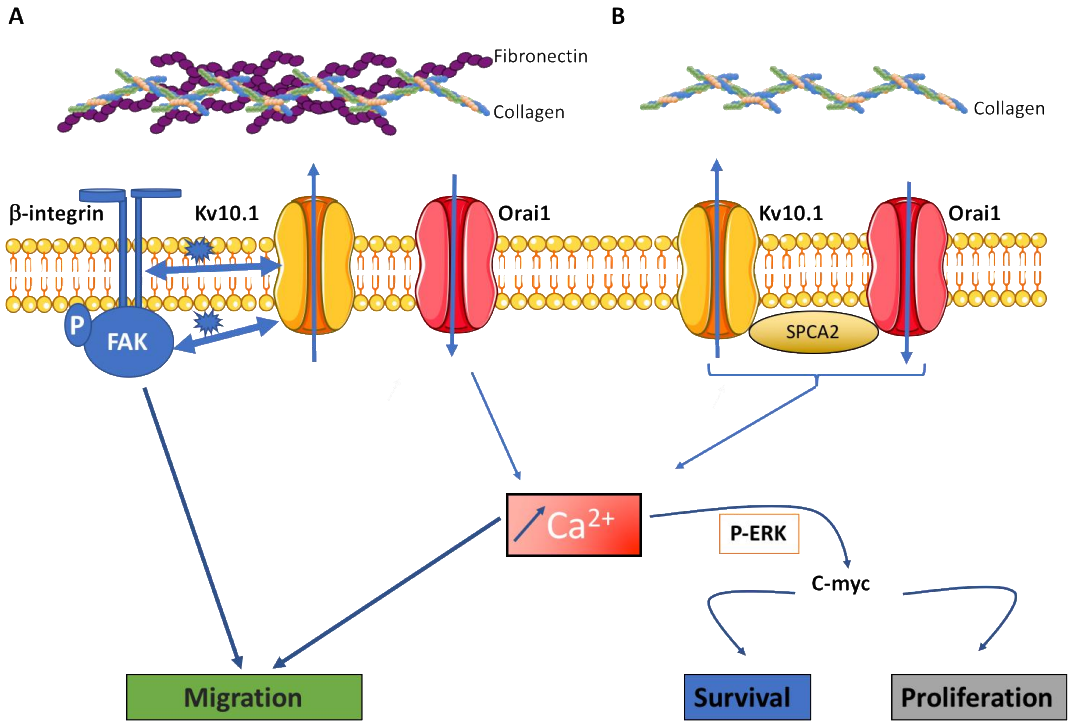

Figure 1. Schematic illustration of ion channels, involved in collagen 1 induced effects in breast cancer. A) Effect of collagen and fibronectin on cell migration in the highly metastatic MDA-MB-231breast cancer cell line. B) Effect of collagen on the survival and proliferation in the non invasive MCF-7 breast cancer cells.

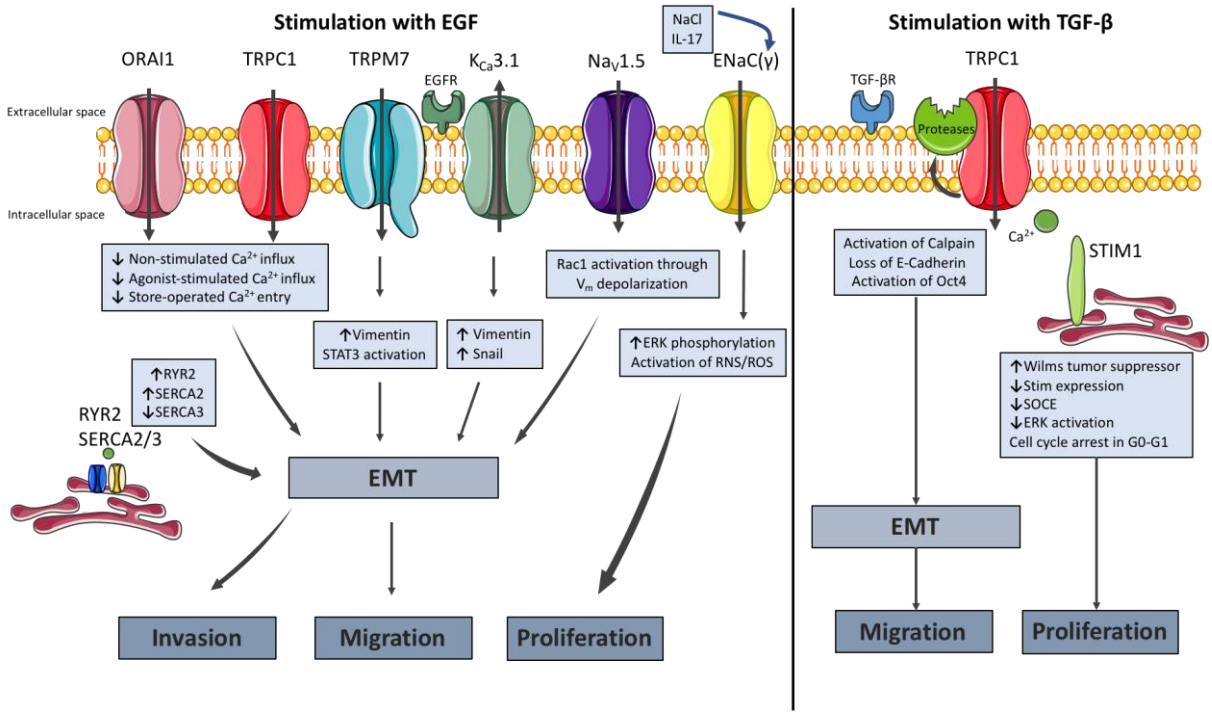

Figure 2. ion transporters regulation by EGF and TGF-B and conferring breast cancer invasion, migration, and proliferation. The sequence of events for each channel type is indicated by black arrows. See text for details. Green color indicates SERCA2 and 3 and STIM1 that are located in the ER.

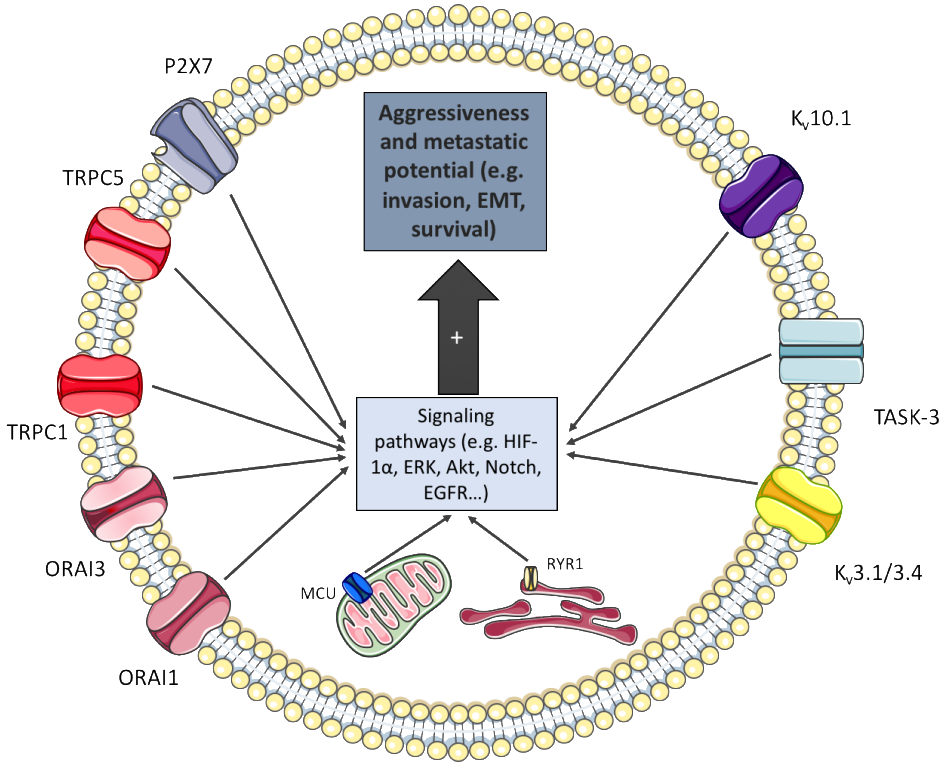

Figure 3. Schematic representation of the different ion channels described in hypoxia context of breast cancer. Membrane or intracellular Ca^{2+} and K^+ transporters are modulated by hypoxia or modulate hypoxia signalling to promote aggressiveness / metastatic potential of breast cancer cells. Function and expression modulation of the channels are involved in signalling pathways promoting angiogenesis, survival, chemoresistance, invasion, migration, EMT. Integral descriptions are included in the main text.

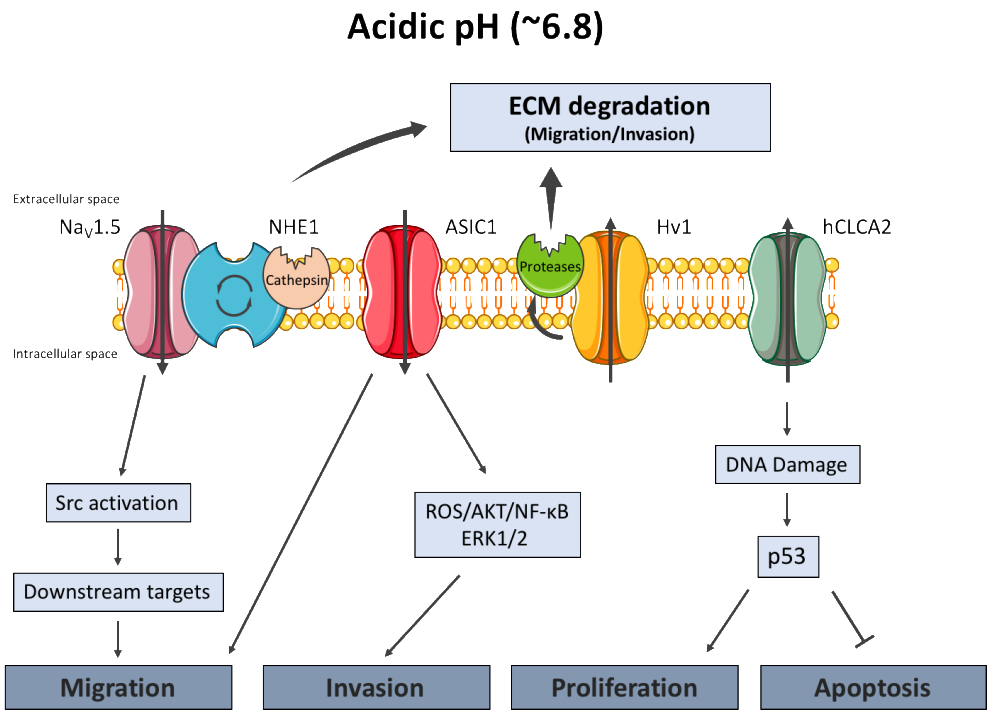

Figure 4. Schematic illustration of ion channels, involved in pH regulation and sensing in breast cancer.