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Review

# How do voltage-gated sodium channels enhance migration and invasiveness in cancer cells?☆

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ABSTRACT

Voltage-gated sodium channels are abnormally expressed in tumors, often as neonatal isoforms, while they are not expressed, or only at a low level, in the matching normal tissue. The level of their expression and their activity is related to the aggressiveness of the disease and to the formation of metastases. A vast knowledge on the regulation of their expression and functioning has been accumulated in normal excitable cells. This helped understand their regulation in cancer cells. However, how voltage-gated sodium channels impose a pro-metastatic behavior to cancer cells is much less documented. This aspect will be addressed in the review. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.



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## 1. Introduction

Voltage-gated sodium channel alpha subunits (VGSC, term that will be used throughout this article when not referring to a particular isoform) were discovered more than 60 years ago. They have been extensively characterized for the electrogenic role they play in neurons (NaV1.1, 1.2, 1.3, 1.6, 1.7, 1.8, and 1.9, respectively coded by genes *SCN1A*, *SCN2A*, *SCN3A*, *SCN8A*, *SCN9A*, *SCN10A*, *SCN11A*), skeletal muscle cells (NaV1.4, gene *SCN4A*) and cardiac muscle cells (NaV1.5, gene *SCN5A*). This role in the generation of the action potential and its propagation is now well described [1,2].

*Abbreviations:* AKAP, A kinase anchoring protein; GPCR, G protein-coupled receptors; NCX, sodium-calcium exchanger; NHE-1, type 1 Na+/H+ exchanger; PKA, protein kinase A; PKC, protein kinase C; TTX, tetrodotoxin; VGSC, voltage-gated sodium channel, pore forming α subunit

☆ This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

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Over the last two decades, an increasing number of studies have documented the expression of one or more types of these channels in non-excitable cells, where they regulate physiological functions such as phagocytosis, endocytosis, secretion, or motility [3], referred to as noncanonical roles for VGSC as reviewed recently by J.A. Black and S.G. Waxman, and more generally, cell proliferation [4], differentiation [5], as well as the organization of the cells during embryo development [4,6].

Many other studies during the same last two decades have described the anomalous expression of VGSC in cancer cells, where they were associated with increased cell motility or invasiveness, therefore increasing the risk of metastases development. Generally, comparative studies showed that they are not expressed, or only at a low level in the corresponding non-cancer cells [7].

Many studies have investigated how partner proteins regulate VGSC activity (for examples, see reviews [8–10]). However, how VGSC activity can increase the motility or invasiveness of cancer cells, therefore increasing the risk of metastases development, remains largely unknown. Some of the proteins that interact, directly or indirectly, with the channels and modulate their activity, also might be the vector through which VGSC exert their pro-invasive effect.

In this article, we will review the roles of VGSC in the motile and invasive properties of cancer cells, and the mechanisms or partner proteins proposed for these functions. We will also try to show which aspects are lacking for the understanding of their implication in the formation of metastases.

## 2. VGSC in cancer cells and normal corresponding tissues

VGSC have been discovered in a wide variety of metastatic cancers (see Table 1). They were reported in carcinoma cell lines derived from small-cell lung cancer [11], prostate cancer [12], melanoma [13], breast cancer [14], neuroblastoma [15], mesothelioma [16], non-small cell lung cancer [17], cervical cancer [18], ovarian cancer [19], and colon cancer [20]. VGSC are also present in other cancer types (gliomas [21,22] or lymphoma [23] or leukemia cells [23]). In some cancers, this is an abnormal expression of functional channels in cancer cells while their normal cognate cells are not known to have VGSC currents. This is suggested by two kinds of data: 1) electrophysiological investigations in corresponding normal cells could not detect inward sodium currents although the protein was present [17] or 2) the comparison of immunohistochemical stainings in tumor and non-tumor corresponding tissues have shown that VGSC were expressed in tumors, not in the normal epithelial biopsies [7,24]. In contrast, in the case of colon cancer, ovarian cancer, cervical cancer, glioma and leukemia, the expression of VGSC is not abnormal, but rather, the level of overexpression of VGSC in these tissues seems to be related to tumor aggressiveness. Indeed, the cognate stem cells, progenitor cells, or related normal cells (normal colon [20], normal ovary [25,26], normal cervix cells [27], glial cells [3] or leukocytes [28,29]) also express VGSC.

It is important to note that in some instances, the expressed isoform is a fully functional neonatal splice variant [24], which is active during embryonic life but is no longer expressed in the young and the adult. This is interesting regarding the possibilities to develop drugs that could be used to specifically inhibit the neonatal VGSC, leaving the adult isoform fully functional in the excitable cells. Therefore, there is a double abnormality: 1) the expression of a gene normally silenced in a tissue where it is not supposed to be expressed and 2) a neonatal isoform is produced by alternative splicing. This feature is often found in cancer cells where silenced genes become expressed again, resulting in dedifferentiation [30,31]. In the case of those VGSC that are expressed as neonatal isoform, not only the gene is transcribed in the wrong cells, but along with it, the genes of proteins that regulate developmental differential splicing [32].

## 3. Proteins that modulate the activity of voltage-gated sodium channels by direct or indirect interaction

A vast amount of knowledge is available concerning the proteins that directly or indirectly interact with, and modulate the expression and/or activity of VGSC (for reviews see [8–10]). Most of this knowledge was obtained in excitable cardiac and neuronal cells and was used as a guide for the investigations in cancer cells. Here are a few examples of the proteins with direct or indirect actions on VGSC, discovered in studies performed on excitable cells (Fig. 1).

VGSC, which consist of a main α subunit forming the channel, is associated with one or two β subunits among the four isoforms β1, β2, β3, and β4 (respectively encoded by genes *SCN1B*, *SCN2B*, *SCN3B* and *SCN4B*). These β subunits modulate the activation–inactivation properties of the channel, thus modulating the sodium current (for a review see [2]). They also exert other properties, not directly related to the regulation of the sodium flux, such as VGSC addressing to specific domains of the membrane; adhesion properties through interaction of their extracellular extremity with the extracellular matrix or *via trans* homophilic adhesion [33,34]; anchoring to the cytoskeleton through interaction with their intracellular C-terminal; and scaffolding for various other proteins (for a review see [35]). Another way β subunits could regulate sodium current density is by modulating the transcription of VGSC genes. It was reported that the intracytoplasmic domain of β2, after cleavage by protease BACE1, increased the transcription of *SCN1A* and increased the expression of NaV1.1 in mouse brain, but reduced its addressing to the membrane [36]. Subunit β4 was also reported to be a substrate of proteases BACE1 and γ-secretase in the brain of mice, and β1, β2 and β3 were substrates as well in transfected cell models [37].

VGSC have been shown to interact with many scaffolding (or adapter) proteins such as syntrophins and dystrophin [38], caveolin-3 in striated muscle [39], and ankyrin-G [40,41]. These scaffolding proteins recruit various other proteins to make multiprotein complexes in multifunctional lipid microdomains. The proteins recruited in the domains interact directly or indirectly with VGSC. Here are a few examples of the multiple partners that modulate VGSC expression, localization and activity.

Calmodulin and FHF (fibroblast growth factor homologous factor) associate with NaV1.5 C-terminal domain to form a ternary complex [42]. FHF also binds a scaffold protein (IB2) that recruits MAP kinases [43]. All these gathered proteins participate in the modulation of VGSC localization and activity by phosphorylation.

Ubiquitin ligases nedd4 and nedd4-2 bind VGSC, attach one, two, or more ubiquitin units and regulate the channel density at the membrane through internalization followed by degradation [44–47].

PKA and PKC can be recruited by another family of anchoring protein: AKAP (A kinase anchoring protein). It was shown in rat brain that NaV1.2 binds AKAP15, which recruits PKA and PKC. PKA activity on NaV1.2 reduces the sodium current and PKC activity further enhances this reduction. The platform constituted by the recruitment of PKA and PKC by AKAP near NaV1.2 allows the integration of several signaling pathways [48]. No other study reports such an association with other VGSC but the contrasting effects of phosphorylation by PKA and PKC on VGSC that are reported in different models (animal species, VGSC isoforms) could be in part due to the various members of AKAP expressed.

PKA is not always associated with AKAP but is also found associated with caveolin in caveolae (caveolin-containing lipid rafts) [49]. Since VGSC are also present in caveolae [50], the association between PKA and caveolin facilitates the regulation of VGSC presence/activity in the lipid rafts.

Fyn (a member of the src family kinases) which is present in lipid rafts [51] also interacts and phosphorylates VGSC [52,53].

Also present in lipid rafts [54], Sigma-1 receptor binds VGSC with a 4-fold symmetry (1 sigma-1 receptor per set of six transmembrane regions) [55] and regulate its activity [55,56]

**Table 1**
Occurrence of gene transcripts, protein, sodium currents, and role of VGSC in cancer cells and the corresponding non-cancer cells.

| First reported on year: | Cancer tissue — mRNA, protein | Cancer tissue — Na+ current, function | Corresponding non-cancer tissue — mRNA, protein | Corresponding non-cancer tissue — Na+ current, function |
|---|---|---|---|---|
| 1989 Small-cell lung cancer | | VGSC current (type not identified) in small-cell lung cancer cells [11] | Expression of protein (immunolabeling) in normal lung biopsies [92].Expression (mRNA detection by RT-PCR) of NaV1.1 to NaV1.3 and NaV1.5 to NaV1.8 with different profiles in two immortalized normal lung epithelial cell lines; NaV1.7 protein detected in intracellular membranes, not at plasma membrane [17]. NaV1.7 protein immuno-detected faintly in epithelium of normal lung biopsies [7] | No VGSC currents detected [17]. |
| 2007 Non-small cell lung cancer | Expression (mRNA detection by RT-PCR) of NaV1.1 to NaV1.9 with different profiles [17] | VGSC currents in different non-small cell lung cancer cell lines; VGSC current involved in increased invasive capacity [17] | | |
| 1995 Prostate cancer | | VGSC current (type not identified) in rat prostate cancer cells [12]; VGSC current involved in secretory activity [72]; major isoform identified as NaV1.7 [93] | Expression of mRNA (assessed by RT-qPCR) of all NaV1 isoforms except for NaV1.4 and NaV1.8 in normal prostate tissue and benign prostate hyperplasia [94]. Expression of VGSC protein (type not identified, immunolabeling) in normal prostate biopsies [95] | |
| 1997 Melanoma | | VGSC current (type not identified) in melanoma cells [13] | | VGSC current (type not identified) in normal human melanocytes [96] |
| 2002 Glioma | Expression (mRNA detection by RT-PCR) of NaV1.1, NaV1.2, NaV1.3, NaV1.4, and NaV1.6 in gliomas [21] | | Expression (immunolabeling of protein) of NaV1.1, NaV1.5, and NaV1.6 [3] | NaV1.1 and NaV1.6 play role in cytokines release in glial cells [3] |
| 2003 Breast cancer | | NaV1.5 current in human highly invasive breast cancer cell line, role in invasive properties, no current or role in invasiveness in less invasive cancer cell lines [14] | No or very low expression (immunolabeling of protein) in biopsies of breast tissue [24] | No current in non-cancer human breast cell line [24] |
| 2004 Lymphoma | Expression (mRNA detection by RT-PCR) of NaV1.5, NaV1.6, NaV1.7, and NaV1.9 in lymphoma cell line [23] | NaV1.5 plays a role in invasiveness [23] | | VGSC current (type not identified) in normal human lymphocytes (3 cells out of 90)[97] Phagocytosis by macrophages regulated by NaV1.5; protein localized in late endosome [28] |
| 2006 Mesothelioma | Expression (mRNA detection by RT-PCR) of NaV1.2, NaV1.6, and NaV1.7, and less for NaV1.3, NaV1.4, and NaV1.5 in mesothelioma cells along with the disappearance of the 3 potassium currents found in normal mesothelial cells [16] | Sodium current recorded in mesothelioma cells and involved in cell migration [16] | | Normal mesothelial cells do not have any VGSC current while they express 3 main potassium currents [16] |
| 2007 Cervix cancer | Expression (mRNA detection by RT-PCR) of NaV1.1, NaV1.4, NaV1.6, and NaV1.7 in cervical cancer cells [18] | Measurement of tetrodotoxin-sensitive VGSC currents in cervical cancer cells [18] | Expression (mRNA detection by RT-PCR) of NaV1.4 in normal cervix cells; [18]; proteins NaV1.6 and NaV1.7 were immuno-detected in normal cervix cells [27] | Current was not detectable in normal cervix cells [18,27] |
| 2010 Colon cancer | Expression of NaV1.5 protein (immuno-detection) in colon cancer biopsies [20] | NaV1.5 current measured in colon cancer cell lines NaV1.5 expression involved in cancer cell invasiveness [20] | NaV1.5 protein immuno-detected faintly in biopsies of normal colon [20] | |
| 2010 Ovarian cancer | Expression (mRNA detection by RT-PCR) of NaV1.1 to 1.9 in ovarian cancer cells; NaV1.5 immuno-detected in ovarian cancer cell lines and tumor biopsies [19] | Tetrodotoxin-resistant VGSC involved in cell invasive capacity [19] | VGSC protein (type not identified) expressed (western blot, immuno-staining of biopsies with pan NaV antibody) in normal corpus luteum cells of the ovary; VGSC identified as NaV member by RT-PCR [25,26]. Expression (mRNA detection by RT-PCR) of NaV1.1 to 1.9 in biopsies of normal ovary and benign ovarian tumors; NaV1.5 immuno-detected in biopsies of normal ovary and benign ovarian tumors [19] | VGSC current recorded, inhibited by tetrodotoxin; involved in physiological luteolysis of normal corpus luteum cells of the ovary [25,26] |

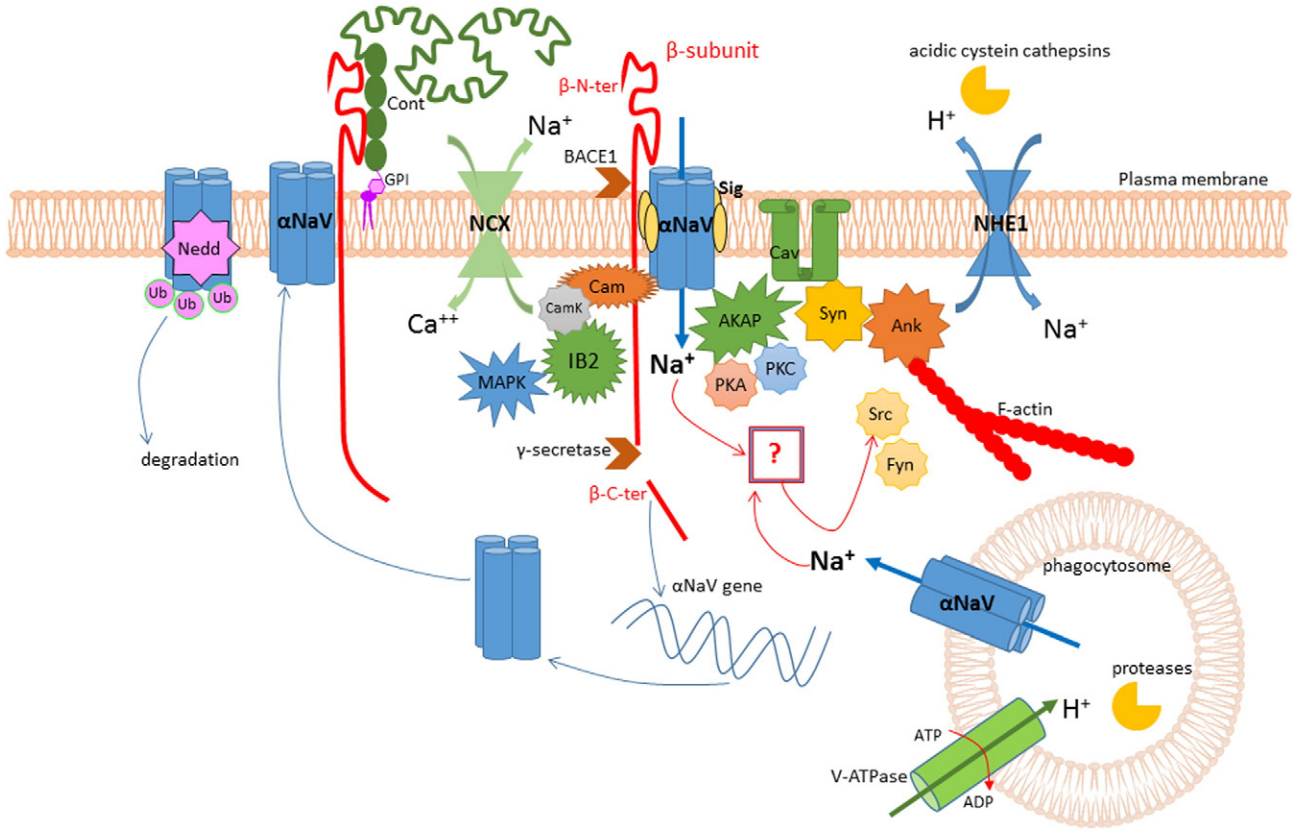


**Fig. 1.** Schematic interactions of scaffolding and regulatory proteins with VGSC at the plasma membrane and in endosomes. VGSC alpha-subunits (αNaV) interact with scaffolding proteins such as IB2 [43], AKAP [48], syntrophin (Syn) [38], ankyrin (Ank) [40,41], GPI-anchored contactin (Cont) [51,69], caveolin 3 (Cav) [39] and other proteins (sigma1 receptor: Sig [54–56]; VGSC beta subunits: β-subunit [2,33–35]) to make large multiprotein complexes. Kinases such as PKA [48,49,71], PKC [48], MAPK [43], and CamKII [68] are recruited in these complexes and modulate the activity of αNaV and also channel density at the membrane. The sodium-calcium exchanger (NCX) functioning in reverse mode was shown to be a part of this regulation through CamKII [68]. Proteases BACE1 and γ-secretase can cleave β-subunits [37]. The C-terminus of β1 subunit was shown to be a transcription factor for αNaV [36]. Ubiquitin ligase Nedd4 interacts with αNaV and transfers several ubiquitins (Ub), leading to the degradation of αNaV [44–47]. VGSC activity stimulates the invasiveness of cancer cells. It was shown that sodium entry through VGSC leads to the formation and activity of invadopodia [50,74–77], with the polymerization of actin and increase in sodium-proton exchanger type 1 (NHE1) activity [50,74], acidification of the extracellular surface of the plasma membrane making a favorable milieu for the activity of acidic cysteine cathepsins [73]. Similarly, the presence of αNaV in late endosomes of macrophages was shown to regulate endosomal acidification and phagocytosis [28]. How sodium ions are involved in the VGSC-dependent motility and invasiveness of cancer cells is not known (box with "?"). Candidate kinases are Fyn or members of the Src family kinases [51–53,69,74]. GPI: glycosylphosphatidylinositol.

Two VGSC α subunits can also interact with one another, as was recently shown; this interaction modulates the trafficking and activity of NaV1.5; the direct interaction is located at their N-terminal region [57,58].

The sodium-calcium exchanger NCX was also found to be associated with annexin-3 in lipid rafts of cardiac cells [59,60] and associated with various VGSC isoforms in intraepidermal nerve terminals [61].

Depending on the VGSC isoform and the excitable cells, many different proteins have been found to interact with VGSC (for more details, see reviews [8–10]).

It is therefore understandable that the large protein complexes comprising VGSC, in bringing multiple partners in close proximity to their multiple targets, permit the modulation of the channel activity.

It is important to stress that the association of partner proteins in membrane lipid rafts is dynamic and can be altered by post-translation modifications such as palmitoylation [62] or association with special phospholipids such as phosphatidylinositol-bis-phosphate PIP2 [63]. It is also dependent on the lipid composition of rafts as was shown in breast cancer cell line MDA-MB-435S: a protein complex comprising Orai1 and SK3 channels, which was functional within caveolin-rich lipid rafts, dissociated and became inactive upon the introduction of a synthetic analogue of ether-phosphatidylcholine [64]. It is then conceivable that modifications of dietary lipids or pharmacological intervention with synthetic lipid analogues might disrupt the pro-invasive property of VGSC. Reports on breast cancer cell line MDA-MB-231 indicate that NaV1.5 is sensitive to n-3 long chain polyunsaturated docosahexaenoic acid (DHA, 22:6n-3), which was supplemented in the culture medium. The sodium current and migration MDA-MB-231 cells, along with NaV1.5 mRNA and protein levels were reduced by DHA [65], as were invasiveness and the relation (explained below in Section 4) between the activities of NaV1.5 and NHE-1 [66].

## 4. Proteins that are, directly or indirectly, modulated by VGSC activity

In these large multiprotein complexes, the activity of the channel could in turn influence the behavior of one or several of the proteins in the scaffold (whether it is through Na + ions or through the modulation of membrane potential, or both, is not clear yet). This could be how a signal triggered by VGSC activity is transduced or propagated to produce its effects on motility or invasiveness of cancer cells.

For example (although the mechanism of regulation is not known), the activity of the channel was shown to regulate the splicing of VGSC mRNA: in a drosophila model of epilepsy, two splice variants differing in the region of the voltage sensor S4 of domain III were produced alternatively when the activity of the channel was left uncontrolled or when it was inhibited by phenytoin [67].

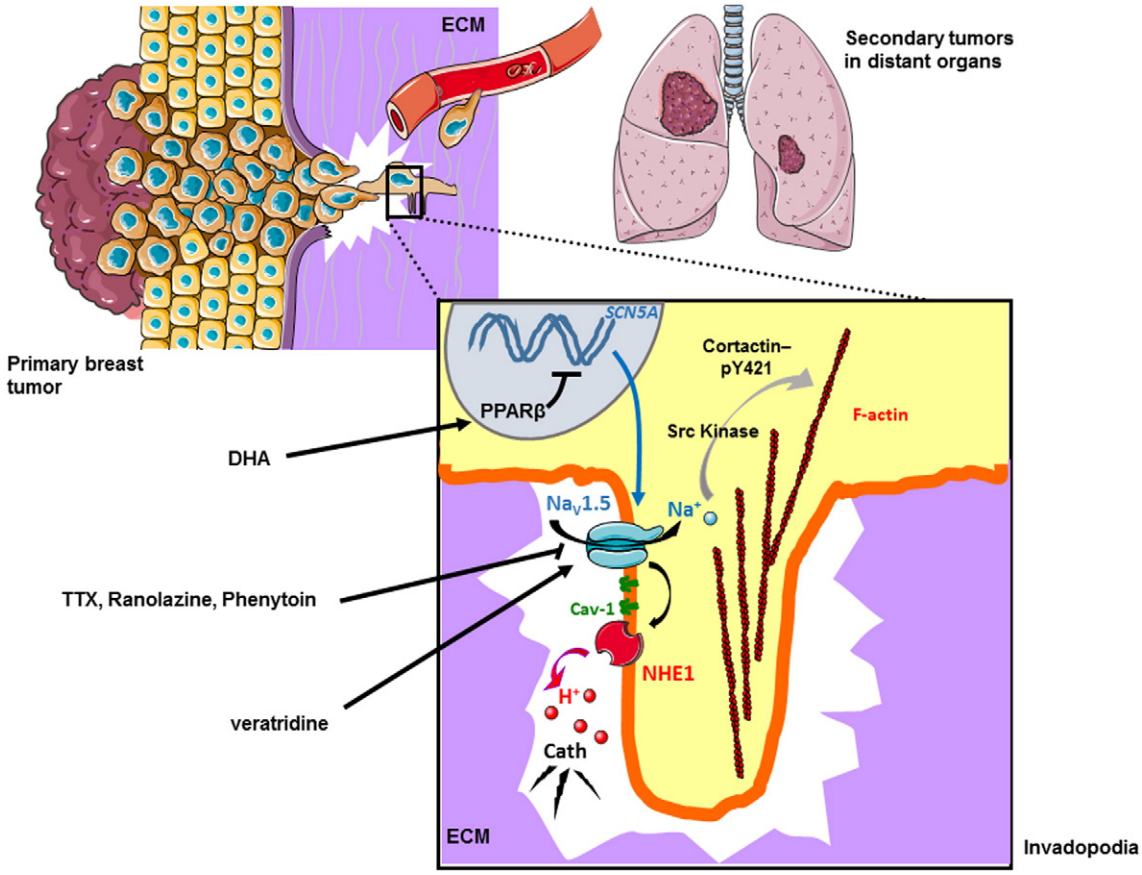


**Fig. 2.** $Na_V1.5$ promotes breast cancer cell invasiveness and metastatic progression. Invasive breast cancer cells escape from the primary tumor by degrading and migrating through the extracellular matrix (ECM), allowing them to reach the blood circulation and eventually to colonize and form secondary tumors (metastases) in distant organs, such as lungs. The proteolysis of the ECM by cancer cells is dependent on the formation and activity of protrusive structures, enriched in F-actin, called invadopodia (magnification). $Na_V1.5$ channels are abnormally expressed in highly invasive breast cancer cells [14,24], and are localized in caveolin-1 (Cav-1)-containing lipid rafts in invadopodia [50]. They are co-localized with Na+-H+ exchanger type 1 (NHE-1) and promote their activity of proton extrusion. This leads to a peri-invadopodial acidification favorable to the activity of acidic cysteine cathepsins (Cath), released by cancer cells, and to the ECM degradation [50,73]. Furthermore, $Na_V1.5$ activity sustains Src kinase activity, the phosphorylation (Y421) of the actin nucleation-promoting factor cortactin, and the polymerization of actin filaments [74]. These results suggest that $Na_V1.5$ activity in cancer cells enhances both the formation and ECM degradative activity of invadopodia. $Na_V1.5$ activity can be inhibited by sodium channels blockers such as tetrodotoxin (TTX), ranolazine and phenytoin thus reducing cancer cell invasiveness *in vitro* and metastatic colonization of organs *in vivo* [98,99]. The n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA, 22:6n-3) reduces the expression of *SCN5A* gene through the participation of the lipid-sensitive transcription factor peroxisome proliferator activated receptor (PPAR)-β, therefore leading to a decreased activity of NHE-1 and a reduced invasion [65,66].

Another example is the report of a positive feedback loop in rat and mouse cardiac ventricular myocytes, where the sodium influx through NaV1.5 leads to the activation of the kinase CaMKII (resulting from the reverse-mode sodium-calcium-driven increase in intracellular calcium concentration that allows CaMKII and NaV1.5 interaction) and the reciprocal increase in NaV1.5 activity (from its phosphorylation by CaMKII), further increasing the intracellular sodium concentration [68].

Another example showing a reciprocity between VGSC and partner proteins was given by a study in cerebellar granule neurons. NaV1.6 localization at the axonal initial segment required its association with NaV β1 subunit. Reciprocally, the role played by β1 required NaV1.6 activity and sodium ions since the highly specific inhibitor of VGSC tetrodotoxin (TTX) or the reduction of extracellular sodium concentration reduced neurite outgrowth [69]. Neurite outgrowth also required the association of β1 with ankyrin-G, fyn kinase and contactin [51,69]. How sodium ions regulate the activity of the complex is however not known.

The VGSC protein could modulate the behavior of cells through non-electrogenic mechanisms. For example, it has been shown that zebrafish embryo cardiac cell proliferation is impaired if Scn5Lab (NaV1.5 ortholog in zebrafish) is not expressed and that this observation is apparently independent of the channel function since a sodium current cannot yet be recorded at this stage of the embryo development [4,6].

Sodium-calcium exchangers (NCX) were shown to be the link between VGSC activity and cell migration. In oligodendrocyte progenitor cells NG2, GABA(A) receptors induced a depolarization that was able to activate a voltage-gated sodium channel and a non-inactivating Na+ current, which in turn allowed the reverse mode functioning of NCX-1. This led to an increased calcium concentration and NG2 cell migration [70].

Available studies providing such examples of feedback are scarce, even more in cancer cell models. How the activity of VGSC can increase the motility or invasiveness of cancer cells, therefore increasing the risk of metastases development, remains largely unknown, merely because proteins that transduce the activity of VGSC into a signaling pathway have been less extensively studied than in neurons or cardiac cells.

As already indicated above, reactivation of silenced genes in cancer proteins is a frequent phenomenon [30,31]. Since carcinoma cells abnormally express adult or neonatal VGSC, it would not be surprising to find that they also express neuronal or muscular proteins that are capable of interaction with VGSC.

A few studies exist that try to elucidate how VGSC increase migration and invasiveness of cancer cells.

An interesting study performed in breast cancer cell lines MDA-MB-231 and MCF-7 identified a positive feedback loop between PKA and VGSC (neonatal nNaV1.5) in MDA-MB-231 cells, where PKA stimulation

increased mRNA and plasma membrane protein level of nNaV1.5 and increased cell migration and invasion. Further proof of the positive feedback loop was permitted with the use of TTX, which inhibited nNaV1.5, reduced migration and invasion, and also reduced the fraction of phosphorylated, active PKA [71]. This activity-dependent positive feedback was absent in MCF-7, which do not have VGSC activity.

Processing of the extracellular matrix by proteases is a potentiating phenomenon for migration and invasion of cancer cells. Endocytic or exocytic vesicle trafficking was studied in prostate cell lines expressing (Mat-Lylu) or not (AT-2) functional VGSC. Trafficking was shown to be twice as high in the highly metastatic Mat-Lylu cells as in the other less metastatic cell line. TTX treatment or Na+-free medium reduced vesicle trafficking by half in Mat-Lylu cells, but had no reducing effect in AT-2 cells which are devoid of VGSC, clearly indicating that VGSC activity and sodium ions were involved in the enhanced endocytic and exocytic vesicle trafficking [72]. A very interesting study in a monocytic cell line, THP-1, and in primary human monocyte-derived macrophages showed that VGSC were expressed intracellularly and were functional: NaV1.6 was associated with cytoskeletal filaments and the endoplasmic reticulum; NaV1.5 was localized in late endosomes. NaV1.5 was shown to be functional, resulting in the efflux of sodium from the endosomes and in intraendosomal acidification. Inhibition of NaV1.5 by TTX prevented phagocytosis, sodium efflux and endosomal acidification [28]. In a model of breast cancer using MDA-MB-231 cell line, in contrast to what was found in prostate cell line Mat-Lylu, secretion of acidic cathepsins was not a NaV1.5-dependent phenomenon. Rather, NaV1.5 enhanced the acidification of the extracellular perimembrane environment, creating a locally favorable milieu for the activity of extracellular matrix-degrading acidic cathepsins (Fig. 2). Invasion of the extracellular matrix, perimembrane acidification and cathepsins activity were reduced in the presence of TTX or when NaV1.5 expression was reduced by small-interfering RNA. In contrast, invasion was increased when the sustained sodium current present as a window current at the membrane potential of MDA-MB-231 cells was increased by a treatment with channel opener veratridine [73]. It was then shown in the same cells that the acidification of the perimembrane extracellular environment was due to a functional link between the activity of the Na+/H+ exchanger NHE-1 and that of NaV1.5. Decreasing the expression of NaV1.5 with small interfering RNA or reducing its activity by TTX treatment reduced the activity of NHE-1. The two proteins were detected at the same location in caveolin-rich membrane lipid rafts [50]. NHE-1, NaV1.5 and caveolin-1 co-immunoprecipitated, indicating a close association, and they were found at focal sites of matrix remodeling in invadopodia of MDA-MB-231 cells. It was also found that NaV1.5 enhanced the activity of NHE-1 through an allosteric regulation mechanism (the nature of which was not elucidated), rendering it more active at less acidic intracellular pH. In parallel of its action on NHE-1, NaV1.5 activity altered cell morphology, invadopodia formation, and actin cytoskeleton and promoted the phosphorylation of actin-nucleation-promoting factor cortactin on Y421 by a member of the Src family kinase [74]. VGSC are not only involved in the activity of invadopodia in cancer cells [50,74–76] or podosomes in normal cells [77] but they participate in their formation [74,77].

To summarize, VGSC activity can increase vesicle trafficking [72], enhance the activity of acidic cathepsins that digest the extracellular matrix [73], increase the activity of NHE-1 through an allosteric mechanism which arouses its activity for less acidic intracellular pH [50], facilitate the dynamics of invadopodia formation and activity, in part through the stimulation of Src kinase and actin cytoskeleton remodeling [74]. It is also important to note that, although they are not found at their generally known plasma membrane location, intracellular VGSC can be functional in some organelles as was shown in late endosomes of macrophages, where they participate in phagocytosis [28].

The rare studies investigating the events, downstream of VGSC, that enhance migration and invasion were performed in a limited array of breast or prostate cancer cell lines. Extending these studies to other cellular models (other breast and prostate cell lines, as well as other cancer types) would be helpful to elucidate the role of sodium ions or the protein itself as a scaffolding element.

## 5. Sodium appears to be an important factor, but so is membrane potential

VGSC could exert their effects on the enhancement of migration and invasion through the increase in Na+ concentration in the cytosol, through the change in membrane potential or through non-electrogenic mechanisms.

Several studies demonstrated that sodium was an important factor in the effects generated by VGSC current on migration and invasiveness: using a sodium-free extracellular medium [14,78] or, on the contrary, using channel opener veratridine alone [73,77] and veratridine plus channel blocker TTX [73] indicated that sodium was involved in VGSC-dependent migration and invasiveness.

Sodium influx is important as it depolarizes the membrane and can therefore allow the reverse mode functioning of sodium-calcium exchangers NCX [68,70], which will increase cytosolic calcium concentration and enhance migration/invasion [79].

In an experiment aiming to show if a regulatory feedback existed between the electrical activity of a neuron and VGSC density at the membrane of rat brain neurons, the importance of sodium was demonstrated. When VGSC were active, a reduction of their density could be observed within 15–30 min. This down-regulation was lost when TTX was used to inhibit the channel or when Li+ were used to replace Na+; the down-regulating effect was independent of the protein since, under condition of inhibition by TTX, the increase in intracellular sodium (triggered by the membrane ionophore monensin, capable of transporting $Na^+$) restored the down-regulation of VGSC [80].

Sensors of intracellular sodium concentration exist such as the sodium-dependent potassium channels Slack and Slick (for a review see [81]). If one postulates that cytosolic calcium concentration is increased by the reverse functioning of NCX, which occurs when the membrane potential is more depolarized than −40 mV, Slack and Slick would be irrelevant with the issue of regulation of migration and invasion. However, intracellular calcium concentration can increase through TRP channels or Orai channels, provided that they are activated by the proper condition [82]. Since the efflux of potassium through Slack and Slick will hyperpolarize the membrane, it will increase the driving force that will allow calcium to enter the cell through TRP or Orai.

It has been reported that Na+ could activate NMDA receptors, and that Src family kinases were involved in this effect but the authors were still looking for the sensor [83].

Although G protein-coupled receptors (GPCR) do not directly interact with VGSC, they could share common downstream signaling pathways and cooperate or compete for the final effect they are expected to generate. A recent study showed that GPCR possess a binding site for one sodium ion and that this ion is important for the stabilization and activity of the receptor. This could be considered a sensor of intracellular sodium concentration [84].

Therefore, it appears that Na+ is important in the downstream effects of VGSC but how it is sensed and whether all the proteins cited above are the direct Na+ sensors remains unexplained. Furthermore, similar regulatory effects of Na+ could occur in cancer cells which do not express VGSC. For example, cells expressing other Na+-permeable channels types such as purinergic receptor P2X7 [85] could have a similar Na+-dependent regulatory mechanism of invasive properties.

Membrane potential is modified by the influx of sodium through VGSC, but also by the other permeabilities that are active in the studied cells [79,86]. In cancer cells, membrane potential is generally depolarized as compared to excitable cells [86] and at this potential, VGSC present a window current that results in a permanent influx of sodium. Whether membrane potential is important or sodium is important, they are both intertwined and displacing the membrane potential to a more positive

or more negative potential could place the VGSC at a potential where they become fully inactive and no longer let sodium enter the cell.

Membrane potential is indeed very important for the conformation of plasma membrane proteins but also for the conformation of cytosolic proteins [79] as was shown for example for the phosphoinositide phosphatase Ci-VSP in *Xenopus* oocytes, or actin cytoskeleton in endothelial cells (for a review see [79]). The membrane potential of cells located at a distant site from the tumorigenic site is influential as well, as was shown in a recent study by Chernet and Levin [87]: hyperpolarizing cells in *Xenopus* embryos at a distant site from the tumorigenic oncogene-expressing cells resulted in a reduction of tumor-like structures formation. Hyperpolarization itself was sufficient, whether it was achieved through the activity of K+ or Cl− channels. The long distance signaling between the hyperpolarized cells and oncogene-expressing cells was apparently not an electrical signaling but was in part mediated through the production of butyrate by the host microbiota, the influx of butyrate into the oncogene-expressing cells and inhibition of histone deacetylase by butyrate, resulting in tumor cell proliferation arrest.

## 6. Non electrogenic role of VGSC

VGSC being part of the multiprotein scaffold in lipid rafts, in caveolae, they are likely to influence their partners through direct interaction. The example given above in Section 4, to show that VGSC can exert their effect through a non-electrogenic mechanism, was not a study on the migration or invasion of cancer cells but on the proliferation of zebrafish embryo cardiomyocytes [4,6]. To the best of our knowledge, no such study exists for cancer cells. This should be done, and could be done to the best using loss-of-function (non-pore) mutants of VGSC, although all of which, unfortunately for the sake of research, are not fully non-permeant [88–90].

## 7. Conclusion

The discovery of VGSC in cancer cells is quite recent when compared to excitable cells. However, understanding their functioning in cancer cells progressed at a fast rate thanks to all the knowledge that was accumulated studying neurons and muscle cells. Knowing how the expression and functioning of VGSC are regulated in cancer cells is certainly very valuable because it gives multiple different approaches as how to potentially decrease their activity and therefore reduce the formation of metastases. However, since VGSC are also expressed in normal macrophages and lymphocytes, inhibiting sodium channels altogether in immune cells and cancer cells might reduce the benefit that could be expected in the inhibition of metastases formation. For this reason, identifying the downstream proteins that are regulated by VGSC activity is also very important and would help find cancer cell specific treatments, by enlarging the array of therapeutic targets. The study by House *et al.* [20], presents a novel and very efficient bioinformatics approach combining the analysis of transcriptomics and invasion experiments, which allowed identifying a large array of genes/proteins involved in colon cancer cell behavior under the functional expression of NaV1.5. This method, called "factor graph nested effects model" [91] seeks interactions (transcriptional or functional) among silenced genes/proteins and downstream effect genes/proteins. This is a very powerful approach that could valuably be reproduced in many cancer types to predict the downstream effectors of VGSC in specific tissues. The abnormal expression of VGSC in cancer cells, particularly in the form of neonatal isoforms, suggests that some of the other partners of VGSC in the multiprotein platforms are likely to be neonatal isoforms as well. This should be investigated because what was discovered in normal excitable cells might not be identical in cancer cells. Finding the effectors that are sensitive to the intracellular sodium concentration will also be important and could help broaden the therapeutic arsenal against metastases. Moreover, if sodium concentration is the key, the discovery of the sodium sensors would enlarge this particular fight against metastases to tumors that do not express VGSC but express non-voltage-gated sodium transporters or sodium channels. Last, the study of the association of partner proteins in lipid rafts could help find pharmacological or nutritional lipids that would disrupt the association and reduce the pro-metastatic property of VGSC.

## Transparency document

The Transparency document associated with this article can be found, in the online version.

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