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Voltage-gated sodium channels and metastatic disease

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Abbreviations

CAM, cell adhesion molecule; DHT, dihydrotestosterone; DMBA, 7,12-dimethylbenz(a) anthracene; EGF, epidermal growth factor; ER, estrogen receptor α ERK, extracellular signal-regulated kinase; FHF1B, fibroblast growth factor-homologous factor 1B; HER2, human epidermal growth factor 2; Ig, immunoglobulin; NF, neurofascin; NGF, nerve growth factor; PKA, protein kinase A; PKC, protein kinase C; PR, progesterone receptor; RPTPβ, receptor protein tyrosine phosphatase β; TTX, tetrodotoxin; VEGF, vascular endothelial growth factor; VGSC, voltage-gated Na^+ channel.

Abstract

Voltage-gated Na^+ channels (VGSCs) are macromolecular protein complexes containing a pore-forming α subunit and smaller non-pore-forming β subunits. VGSCs are expressed in metastatic cells from a number of cancers. In these cells, Na^+ current carried by α subunits enhances migration, invasion and metastasis *in vivo*. In contrast, the β subunits mediate cellular adhesion and process extension. The prevailing hypothesis is that VGSCs are up-regulated in cancer, in general favoring an invasive/metastatic phenotype, although the mechanisms are still not fully clear. Expression of the $Na_v1.5$ α subunit associates with poor prognosis in clinical breast cancer specimens, suggesting that VGSCs may have utility as prognostic markers for cancer progression. Furthermore, repurposing existing VGSC-blocking therapeutic drugs may provide a new strategy to improve outcomes in patients suffering from metastatic disease, which is the major cause of cancer-related deaths, and for which there is currently no cure.

Introduction

Voltage-gated Na^+ channels (VGSCs) are comprised of a pore-forming α subunit typically in association with one or more smaller β subunits (Figure 1).[1] The β subunits regulate channel expression and gating, and are immunoglobulin (Ig) superfamily cell adhesion molecules (CAMs).[2] VGSCs are classically responsible for action potential initiation and conduction in excitable cells.[3] Both the α and β subunits have been shown to interact with a range of other signaling molecules (Figure 1), enabling fine-tuning of channel activity on the one hand, and allowing

VGSCs to participate in non-conducting signaling on the other.[4] VGSCs are also expressed in a range of cell types that are considered "non-excitable", including glia, fibroblasts, immune cells, and metastatic cancer cells.[5] The past 15 years have seen a rapid expansion in published studies documenting the expression VGSCs across a broadening number of cancers, their role in regulating cellular migration and invasion, and, importantly, their potential utility as diagnostic and therapeutic targets. In particular, several recent studies have started to define a mechanistic role for VGSCs in regulating migration and invasion. The purpose of this review is to assimilate the current body of evidence ascribing a malignant role for VGSCs during metastasis (the spread of tumor cells from primary to distant sites), and consider the clinical implications.

VGSC expression in cancer

The VGSC α subunit family contains nine members, Nav1.1-Na_v1.9, encoded by genes *SCN1A*-*SCN11A* (Table 1A).[6] There are four β subunits, β1-β4, encoded by genes *SCN1B*-*SCN4B*.[2] VGSC expression has been predominantly reported to date in carcinomas (cancers of epithelial origin). The α subunits have been identified in cells from the following carcinomas: breast cancer,[7, 8] cervical cancer,[9, 10] colon cancer,[11] melanoma[12, 13] mesothelioma,[14] neuroblastoma,[15] non-small cell lung cancer,[16] ovarian cancer,[17] prostate cancer,[18-21] and small-cell lung cancer[22, 23] (Table 1A). α subunits are also expressed in gliomas,[24, 25] lymphoma[26] and leukemia cells,[27] the latter suggesting that VGSCs may be present in hematological malignancies, in addition to solid tumors. Although the majority of reports published to date have

focused on cell lines, a number of studies now show that α subunits are also expressed *in vivo,* in patient biopsy material.[8-11, 17, 28-30]

In excitable cells/tissues, different α subunits have subtly individual, but often largely overlapping tissue distributions, which are proposed to permit functional specializations as a result of subtle variations in electrophysiological properties.[31] In cancer cells, there appears to be a variable pattern of expression of different α subunits, such that a number of cancers express multiple α subunits, but not all subtypes are expressed in all cancers (Table 1A). In several cancers expressing multiple α subunits, a predominant α subunit has been identified. For example, in lymphoma and breast cancer cells the most highly expressed α subunit is $Na_v1.5$ (gene: *SCN5A*),[8, 26] whereas in prostate cancer cells the predominant α subunit is $Na_v1.7$ (gene: *SCN9A*).[18]

Alternative mRNA splicing enables further functional variation among α subunits.[32] An important developmentally regulated splicing event occurs in exon 6, encoding the domain I segment 3 (DI:S3) region, such that the 5' "neonatal" variant is expressed at birth, whereas the 3' "adult" variant is expressed later in postnatal development.[33] In lymphoma, neuroblastoma, breast and prostate cancer cells, *SCN5A* and *SCN9A* are mainly expressed in their DI:S3 5' neonatal splice forms.[8, 15, 26, 28] In contrast, the adult *SCN5A* variant is expressed in colon cancer cells, and the neonatal variant is absent.[11] In certain cancers, the presence of α subunits may therefore be an example of oncofetal gene expression, where embryonic genes are pathologically re-expressed during oncogenesis.[34]

In several cancers, α subunit mRNA and protein expression correlates with metastatic potential. For example, in breast cancer, the neonatal *SCN5A* splice variant is expressed ~1800-fold higher in metastatic MDA-MB-231 cells than weakly metastatic MCF-7 cells. Na^+ currents are detectable in MDA-MB-231 cells, but not in weakly metastatic MCF-7 cells.[7, 8] The expression of neonatal *SCN5A* mRNA in breast cancer biopsies correlates with occurrence of lymph node metastasis.[8] Furthermore, *SCN5A* mRNA is elevated in breast tumors from patients who had a recurrence, or died within five years, and associates with increased odds of developing metastasis.[30] A similar pattern has been observed in prostate cancer cells, where VGSC expression increases in line with metastatic potential in the LNCaP progression model.[35] In agreement with this, the predominant α subunit, *SCN9A*, is elevated in prostate cancer biopsies compared to non-cancerous prostate samples,[28] and is more highly expressed in strongly metastatic PC-3 and Mat-LyLu cells than weakly metastatic LNCaP and AT-2 cells.[18] Na^+ currents have been detected only in the metastatic prostate cancer cell lines.[19, 21] The positive correlation between α subunit expression and metastatic potential has also been reported for colon[11] and ovarian cancers.[17] However, in gliomas, the mRNA level of α subunits is inversely correlated with malignancy grade.[25] There appears to be no association between α subunit mRNA expression and metastatic potential of small cell or non-small cell lung cancer cell lines.[16, 22] Therefore, the relationship between α subunit expression, tumor grade and metastatic potential may be cancer type-specific.

The expression of β subunits in cancer cells has been less extensively studied. β subunits are expressed in prostate,[36, 37] breast,[38] non-small cell lung,[16] and cervical cancers[10] (Table 1B). As with the α subunits, the β subunit expression profile appears

to vary between cancers. For example, β3 is present in prostate and non-small cell lung cancer cells,[16, 36] but is absent in breast and cervical cancer cells.[10, 38] However, β1 is the most abundant β subunit in breast, prostate, and cervical cancer cells.[10, 36, 38] Interestingly, β1 appears to be inversely correlated with *SCN5A* and metastatic potential in breast cancer cells: *SCN1B* mRNA (encoding β1) is significantly higher in weakly metastatic MCF-7 cells than in metastatic MDA-MB-231 cells.[38] This suggests that β subunits may be performing certain functions in cancer cells independent of the pore-forming α subunits. In contrast, a recent study has shown that β2 expression increases in line with metastatic potential in the LNCaP prostate cancer progression model.[37] Therefore, as with α subunits, different β subunits may be expressed at varying levels in different cancer types, and may perform distinct functions.

Functional role

VGSC α subunits potentiate a number of cellular behaviors associated with metastasis (Table 2). In breast, prostate and lung cancer cell lines, the VGSC pore blocker tetrodotoxin (TTX) inhibits behaviors including process outgrowth/extension,[39] galvanotaxis,[8, 40] migration,[8, 14, 17, 41-44] endocytosis,[8, 22, 45] vesicular patterning,[46, 47] detachment from substrate,[48] gene expression,[43, 49] and invasion.[7, 8, 10-12, 16, 17, 19, 21, 26] TTX does not inhibit proliferation of cancer cells,[7, 8, 10, 16, 17, 41] suggesting that VGSCs may be involved mainly in metastatic progression, rather than tumorigenesis.[50] However, recent evidence has shown that VGSCs also regulate angiogenic properties of endothelial cells, including vascular endothelial growth factor (VEGF)-induced proliferation, tubular differentiation, and adhesion.[51] Therefore, the exact functional

contribution of VGSCs to the cancer process may depend on the cell type, fate and state of the tumor.

Silencing *SCN5A* with siRNA reduces *in vitro* invasion of MDA-MB-231 breast cancer cells.[52] Further, specifically targeting the neonatal splice variant of *SCN5A* reduces the migration and invasion of MDA-MB-231 cells, suggesting that the neonatal form itself may be responsible for VGSC-dependent potentiation of metastatic cell behavior in breast cancer cells.[42] Similarly, siRNA targeting *SCN8A* (encoding $Na_v1.6$) and *SCN9A* reduces invasion and endocytic activity in PC-3 metastatic prostate cancer cells.[53] Finally, the $Na_v1.6$-specific toxin Cn2 inhibits the invasion of cervical cancer cells.[10] Thus, in different cancers, different α subunits appear to promote metastatic cell behaviors. However, it is not yet clear whether expression of a specific α subunit in a particular cancer provides a specific functional advantage, or is related to the natural history of the disease. The fact that any α subunit is present in a cancer cell may be more important than which α subunit it is. For example, overexpression of $Na_v1.4$ in weakly metastatic LNCaP prostate cancer cells is necessary and sufficient to increase their invasiveness, even though the predominant α subunit expressed in metastatic prostate cancer cells is $Na_v1.7$.[18, 35]

Several studies have indicated that a number of therapeutically relevant small molecule VGSC blockers can also inhibit cell behaviors associated with metastasis. For example, the anticonvulsants phenytoin and carbamazepine inhibit secretion of prostate-specific antigen and interleukin-6 by prostate cancer cells.[54] Phenytoin and the local anesthetic lidocaine also inhibit endocytic activity in small cell lung cancer cells.[22] In addition, phenytoin suppresses migration of prostate cancer cells.[41]

Furthermore, we have recently shown that therapeutically relevant concentrations of phenytoin inhibit Na^+ current, migration and invasion in metastatic breast cancer cells.[30]

Persistent Na^+ current

Cancer cells typically have a relatively depolarized membrane potential compared to terminally differentiated cells, e.g. epithelia, neurons.[55] For example, the resting membrane potential of a typical neuron may be around -65 mV,[56] whereas we and others have shown that metastatic MDA-MB-231 breast cancer cells have membrane potentials between ~ -15 to -30 mV.[7, 8, 30] Following depolarization, VGSCs open and rapidly inactivate within a few milliseconds, remaining inactivated until the membrane repolarizes.[3] Therefore, in cancer cells with depolarized resting potentials, the majority of VGSCs will be inactivated. However, several VGSCs, including $Na_v1.5$, do not inactivate completely, and a steady-state Na^+ current persists, which is typically a few percent of the peak transient current.[57, 58] We recently proposed that the persistent Na^+ current is likely to be predominant in cancer cells expressing VGSCs, and this component of the Na^+ current may specifically potentiate the cells' migration and invasion.[30]

Mechanisms of action

The obvious question is: how does Na^+ influx through VGSCs potentiate metastatic behaviors, including invasion? Over the years, there has been much speculation in the

literature (reviewed in [50]). Three models are considered below, based on recently published experimental data.

1. Regulation of pH. In MDA-MB-231 cells, Na^+ influx through $Na_v1.5$ results in intracellular alkalinization, and extracellular acidification adjacent to the plasma membrane.[52] The Na^+/H^+ exchanger NHE1, which is an important regulator of H^+ efflux, is co-expressed with $Na_v1.5$ in lipid rafts contained within the caveolae of MDA-MB-231 cells.[59] Na^+ influx through $Na_v1.5$ increases H^+ efflux through NHE1, thus enhancing pH-dependent extracellular matrix degradation and invasion (Figure 2).[59] However, the precise mechanism by which $Na_v1.5$ activity enhances NHE1 is not yet clear. The resultant ($Na_v1.5$/NHE1-dependent) perimembrane acidification is proposed to favor the proteolytic activity of cysteine cathepsins B and S, the function of which has been shown to depend, at least in part, on VGSC function.[52] A similar mechanism has been identified by which $Na_v1.5$ expressed on intracellular endosomal membranes of primed macrophages acts as a charge sink, permitting Na^+ efflux from the endosome, resulting in H^+ influx, likely via the vesicular ATPase, and subsequent endosomal acidification.[60]

2. Regulation of gene expression. Several studies have shown that VGSCs regulate gene expression, both in excitable cells, e.g. neurons and cardiomyocytes, and in cancer cells.[38, 43, 49, 61, 62] *In silico* factor graph nested effects modeling of gene expression in colon cancer cell lines has revealed a novel network of gene interactions that are implicated in cancer invasion.[11] Strikingly, *SCN5A* is a key regulator of this invasion gene network, suggesting that VGSCs, in particular $Na_v1.5$, may function as early entry points in signaling mechanisms regulating invasion. Downstream gene

ontology categories include Wnt signaling, cell migration, ectoderm development, response to biotic stimulus, steroid metabolic process, and cell cycle regulation (Figure 3).[11] These data suggest that, at least in colon cancer, $Na_v1.5$ may regulate invasion by mechanism(s) in addition to/instead of H^+ efflux.[59] The challenge now is to understand how Na^+ current mediated by $Na_v1.5$ may regulate transcription in cancer cells, or, indeed, whether the effect is mediated by mechanism(s) independent of ion conduction.

3. Regulation of intracellular Ca^{2+}. In excitable cells, Na^+ current carried by VGSCs can result in an increase in intracellular Ca^{2+} level, e.g. by activating voltage-gated Ca^{2+} channels.[63, 64] Similar VGSC-dependent elevation of intracellular Ca^{2+} has been reported in non-excitable cells. For example, Na^+ current carried by $Na_v1.5$ is essential for the sustained Ca^{2+} entry into $CD4^+$ T cells that occurs during positive selection.[65] In addition to being expressed at the plasma membrane, α subunits are also present on internal membranes of cancer cells and macrophages.[12, 30, 60] In THP-1 macrophages and HTB-66 melanoma cells, $Na_v1.6$ is expressed on vesicular structures adjacent to podosomes.[12] Agonist-mediated activation of VGSCs in these cells causes Na^+ release from cationic intracellular stores, followed by rapid Na^+ uptake by anionic mitochondria, and subsequent Ca^{2+} release into the cytosol.[12] It is proposed that this Ca^{2+} release then enhances podosome/invadopodia formation, leading to increased invasion.[12] However, it is not yet clear how VGSCs present on vesicular membranes are gated, and/or whether they interact with VGSCs present at the plasma membrane. In vascular endothelial cells, VGSC-mediated Na^+ influx is required for VEGF-induced membrane depolarization and elevation of intracellular Ca^{2+}, which in turn activates PKC and extracellular signal-regulated kinase (ERK)1/2,

potentiating angiogenic functions including proliferation, differentiation and adhesion.[51] In contrast to cancer cells and macrophages, the Ca^{2+} rise in endothelial cells occurs as a result of Ca^{2+} influx through reverse mode operation of the Na^{+}/Ca^{2+} exchanger NCX.[66]

In summary, several models have recently emerged, supported by experimental data, suggesting that VGSC α subunits may enhance cellular invasion by a variety of mechanisms. Further studies will no doubt contribute further data in order to clarify whether the mechanisms discussed above are widely applicable to all cancers in which VGSCs are expressed, or whether different VGSCs perform distinct functions in different cells in different cancers.

The role of β subunits

In breast cancer, β1 is highly expressed in weakly metastatic MCF-7 cells, where it enhances adhesion and retards transwell migration.[38] β1 is expressed at a much lower level in strongly metastatic MDA-MB-231 cells, and stable overexpression of β1 in this line increases cell-cell adhesion, induces process outgrowth, and reduces migration in wound healing assays.[38] β2 appears to play a slightly different role in prostate cancer cells. Overexpression of β2 in LNCaP cells increases adhesion, process outgrowth, migration and invasion[37] However, the same study reported that β2-over-expressing LNCaP cells have a reduced tumor take and smaller tumor volume when subcutaneously injected into nude mice. Thus, not only do β1 and β2 play subtly different functional roles in different cancer cells, these subunits may function differently in tumors *in vivo*, compared to cells in culture. This highlights the

critical importance of studying the functional role of VGSCs in cancer *in vivo*, in addition to using *in vitro* models. Nonetheless, given that β subunits are Ig family cell adhesion molecules, it is plausible to speculate that their major contribution to the metastatic behaviors of cancer cells may be through regulated adhesion/detachment. Of special note is that β subunits play a critical role in regulating adhesion and migration in excitable cells, where they are normally expressed.[2] For example, β1 enhances neurite outgrowth, neuronal pathfinding and fasciculation during early postnatal central nervous system development.[67, 68] β1-mediated neurite outgrowth in cerebellar granule neurons requires fyn kinase, the cell adhesion molecule contactin, and is dependent on Na^+ current carried by $Na_v1.6$[2, 68] (Figure 4A,B). In turn, $Na_v1.6$-dependent resurgent Na^+ current and action potential firing are dependent on the presence of β1[56] (Figure 4B). Thus, in neurons, there is a functional relationship between α and β subunits, such that both are required for neurite outgrowth, migration and electrical excitability. This is an interesting contrast with β1 and $Na_v1.5$ in breast cancer cells, which appear to play opposing, antagonistic roles in regulating adhesion and migration.[38]

β subunits regulate the mRNA levels of α subunits, including $Na_v1.5$, and other β subunits, in neurons and cardiomyocytes.[61, 69-71] Down-regulation of *SCN1B* in MCF-7 cells results in an increase in the mRNA level of the neonatal *SCN5A* splice variant,[38] raising the possibility that β1 may regulate gene transcription, and supporting the notion that β1 and $Na_v1.5$ may function antagonistically in breast cancer cells.[30] However, their expression may not be mutually exclusive, and both subunits colocalize in lamellipodia.[30]

Less is known about the expression/function of the other β subunits (β3 and β4) in cancer cells. However, *SCN3B* contains two response elements to the tumor suppressor p53, and DNA damaging agents and p53 over-expression up-regulate *SCN3B* mRNA in cancer cell lines, suggesting that β3 may be involved in p53-dependent apoptosis.[72] The function of β4 in cancer cells has not been investigated. However, a recent study has shown that, together with $Na_v1.5$, β4 is required for $CD4^+$ T cell development,[65] suggesting that β4 may play an as yet unexplored role(s) in non-excitable cells.

In summary, VGSC β subunits appear to play a role in regulating a number of cell behaviors associated with metastasis, including adhesion, migration and gene expression. Importantly, some of these functions oppose the effects of α subunits[38] (Table 3). Further work is required to delineate the respective functions of α and β subunits in cancer cells. A major challenge will be to establish whether or not β subunits are functioning (e.g. to regulate adhesion and migration) independently of α subunits.

Regulation of expression

How are VGSCs up-regulated in cancer cells? VGSCs are macromolecular signaling complexes and their activity can be regulated at multiple levels by a vast array of different interacting partners and mechanisms, which have been studied extensively in excitable cells, e.g. neurons and cardiomyocytes (Figure 1; reviewed in [2, 73]). However, the mechanisms by which expression of VGSC α and β subunits are regulated in cancer cells are not yet well understood. Serum has been shown to

modulate expression and activity of α subunits in Mat-LyLu prostate cancer cells.[74] Subsequent studies have shown that several components of serum have various effects on VGSC function. For example, epidermal growth factor (EGF) increases $Na_v1.7$ expression, Na^+ current and VGSC-dependent migration and invasion of Mat-LyLu and PC-3M prostate cancer cells.[44, 75] In addition, nerve growth factor (NGF) increases Na^+ current in Mat-LyLu cells via protein kinase A (PKA) activation.[76] Although NGF also increases migration in these cells, its effect is independent of VGSC activity,[76] suggesting that different growth factors may regulate different pools of VGSCs, which may in turn have different downstream effects on metastatic cell behavior.

Steroid hormones have also been shown to regulate VGSC expression and activity in cancer cells. In MDA-MB-231 breast cancer cells, the estrogen β-estradiol increases Na^+ current via the G-protein-coupled estrogen receptor GPR30 and PKA.[77] In addition, *SCN1B* expression is negatively regulated by the androgen dihydrotestosterone (DHT) in LNCaP and PC-3 prostate cancer cells.[36] However, there is no association between *SCN5A* expression and estrogen receptor α (ER), progesterone receptor (PR), or human epidermal growth factor 2 (HER2) status in clinical breast cancer specimens,[30] suggesting that any relationship between serum factors and VGSC expression *in vivo* may be highly complex.

Finally, α subunit expression and activity in metastatic cancer cells appears to be auto-regulated via a positive feedback mechanism. In Mat-LyLu cells, Na^+ current activates PKA, which in turn up-regulates *SCN9A* mRNA levels, and promotes externalization of α subunits to the plasma membrane.[43] Similarly, in MDA-MB-231

breast cancer cells, expression of the neonatal $Na_v1.5$ splice variant is maintained by positive feedback involving PKA.[78] In both cases, inhibition of Na^+ current with TTX effectively collapses the positive feedback loop, suppressing VGSC-dependent migration.[43, 78] In summary, expression and function of VGSC α and β subunits in cancer cells is regulated by serum components, including steroid hormones and growth factors.

Therapeutic potential

The observation that VGSCs play a pro-invasive role in metastatic cells, together with patient data associating α subunit expression with poor clinical outcome, suggests that VGSCs may be important diagnostic and/or therapeutic targets in the metastatic setting. Given that α subunits are regulated by activity-dependent positive feedback, pharmacological blockade of channel conductance may be an ideal intervention to inhibit VGSC-dependent metastatic cell behaviors. Indeed, a recent study shows that local injection of TTX into subcutaneous tumors of Mat-LyLu cells in the Copenhagen rat significantly reduces lung metastases and improves overall survival.[79] However, the toxicity of TTX would preclude its use as a systemic anti-metastatic treatment.

We have proposed that VGSC-blocking drugs FDA-approved for treating other conditions, e.g. anticonvulsants, antiarrhythmics, local anesthetics, and tricyclic antidepressants, may warrant investigation for repurposing to metastatic disease.[30] In support of this, several of these agents inhibit metastatic cell behaviors *in vitro* models.[8, 22, 30, 41, 54] Application of local anesthetics during radical prostatectomy

surgery associates with significantly reduced recurrence and metastasis.[80] In addition, a recent retrospective case-control analysis using the General Practice Research Database suggests that use of tricyclic antidepressants may have potential for prevention of glioma and colorectal cancer.[81] Furthermore, riluzole, which is both a VGSC blocker and metabotropic glutamate receptor inhibitor, reduces breast cancer tumor volume in mice, and suppresses metabolic activity of tumors in patients with resectable stage III and IV melanoma.[82, 83] Finally, a mexiletine analog (RS100642) reduces oxidative stress associated with tumor burden, and increases overall survival in the experimentally induced 7,12-dimethylbenz(a) anthracene (DMBA) rat breast cancer model [84]. In summary, several studies suggest that VGSC α subunits may be useful therapeutic targets in cancer.

Conclusion and perspectives

A growing body of evidence suggests that VGSCs play an important pathological role during cancer progression towards metastasis. However, the role of individual α and β subunits appears to be complex. Nonetheless, a common theme is that Na^+ current carried by α subunits favors an invasive phenotype, whereas β subunits may regulate adhesion. Future work is required to establish how widely VGSCs are expressed across different types of cancer, and the extent of contribution(s) of different α and β subunits to disease progression. The ultimate goal should be to definitively evaluate the potential for both α and β subunits as diagnostic and therapeutic targets. In respect of the former, repurposing FDA-approved channel blockers may be a cost-effective intervention in metastatic disease, which is the major cause of cancer-related deaths [85, 86], and treatment of which is still largely limited to palliation [87].

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Conflict of interest

The author declares no conflicts of interest.

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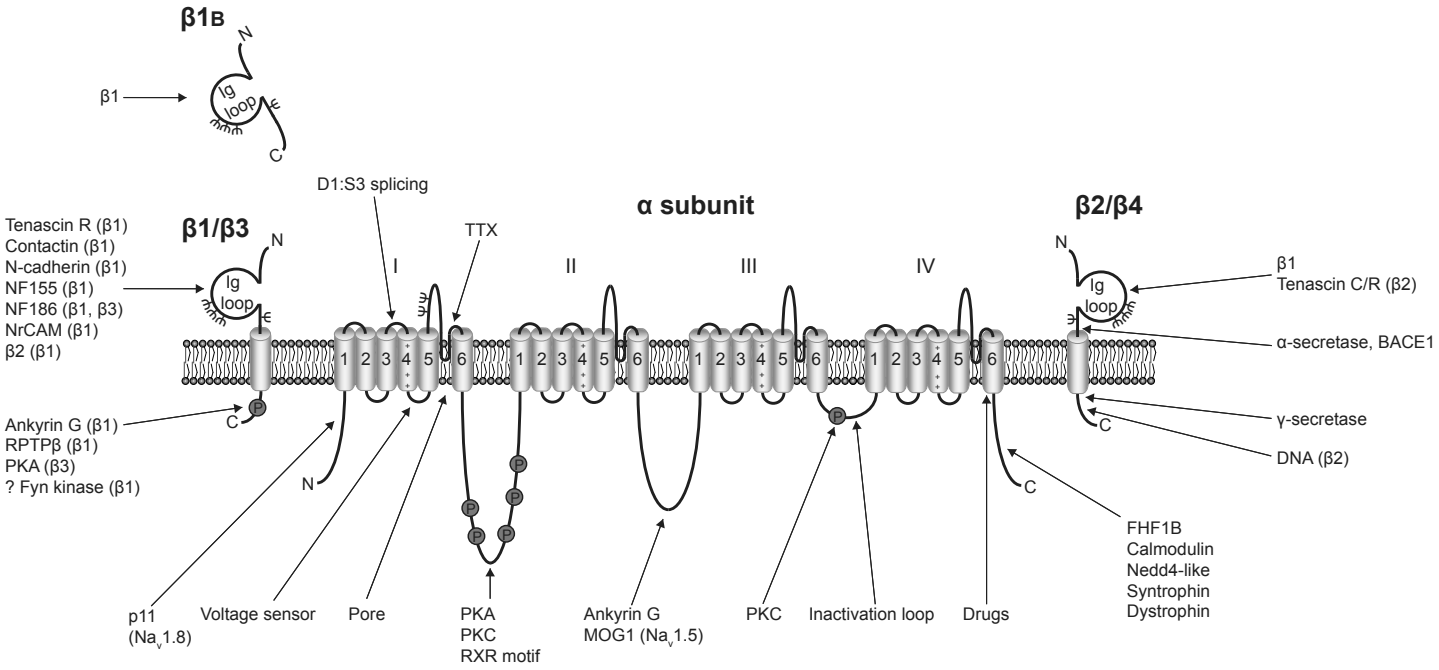

Figure 1. Multifunctional interactions of VGSCs. Basic topology of the pore-forming α subunit is shown, consisting of four homologous domains each containing six transmembrane segments. Segment 4 contains the voltage sensor.[6] The smaller β subunits contain an extracellular immunoglobulin (Ig) loop, transmembrane domain, and an intracellular C-terminal domain.[88] β1 and β3 are non-covalently linked to the α subunit, whereas β2 and β4 are covalently linked through disulfide bonds.[2] The alternative splice variant, β1B, lacks a transmembrane domain.[89] α subunits interact with a number of other signaling molecules, including p11,[90] protein kinase A (PKA),[91] protein kinase C (PKC),[92] ankyrin G,[93] MOG1,[94] fibroblast growth factor-homologous factor 1B (FHF1B),[95] calmodulin,[96] NEDD4,[97, 98] syntrophin,[99] and dystrophin.[100] Several of the β subunits interact with other cell adhesion molecules and regulatory proteins, including tenascin C,[101] tenascin R,[101, 102] contactin,[103] N-cadherin,[104] neurofascin (NF)155,[105] NF186,[105] NrCAM,[105] ankyrin G,[104] receptor protein tyrosine phosphatase β (RPTPβ),[106] PKA,[107] and fyn kinase.[68] The β subunits are substrates for proteolytic cleavage by α-secretase, BACE1, and γ-secretase.[108, 109] The intracellular domain of β2 is proposed to regulate gene expression in the nucleus.[71] ψ, glycosylation sites. Figure was produced using Science Slides 2006 software.

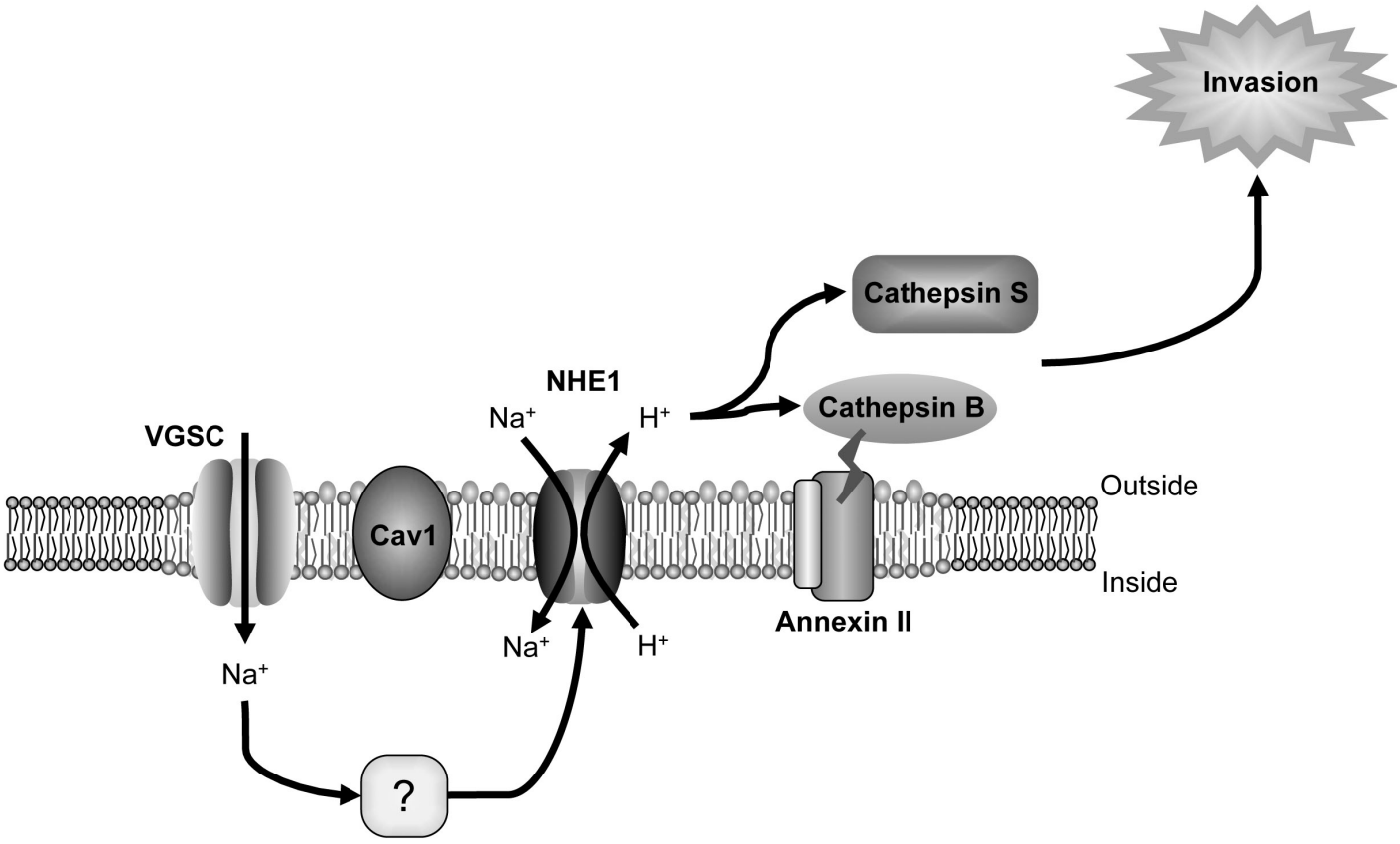

Figure 2. α subunit involvement in pH-dependent cellular invasion. Na^+ influx through $Na_v1.5$ is proposed to activate the Na^+/H^+ exchanger NHE1, co-expressed with $Na_v1.5$ in lipid rafts contained within the caveolae of invasive breast cancer cells.[59] Increased NHE1 activity results in increased H^+ efflux, which in turn enhances proteolytic activity of cysteine cathepsins B and S, which degrade the extracellular matrix, permitting invasion.[52, 59] The mechanism by which $Na_v1.5$ enhances NHE1 is not yet clear. Figure was produced using Science Slides 2006 software.

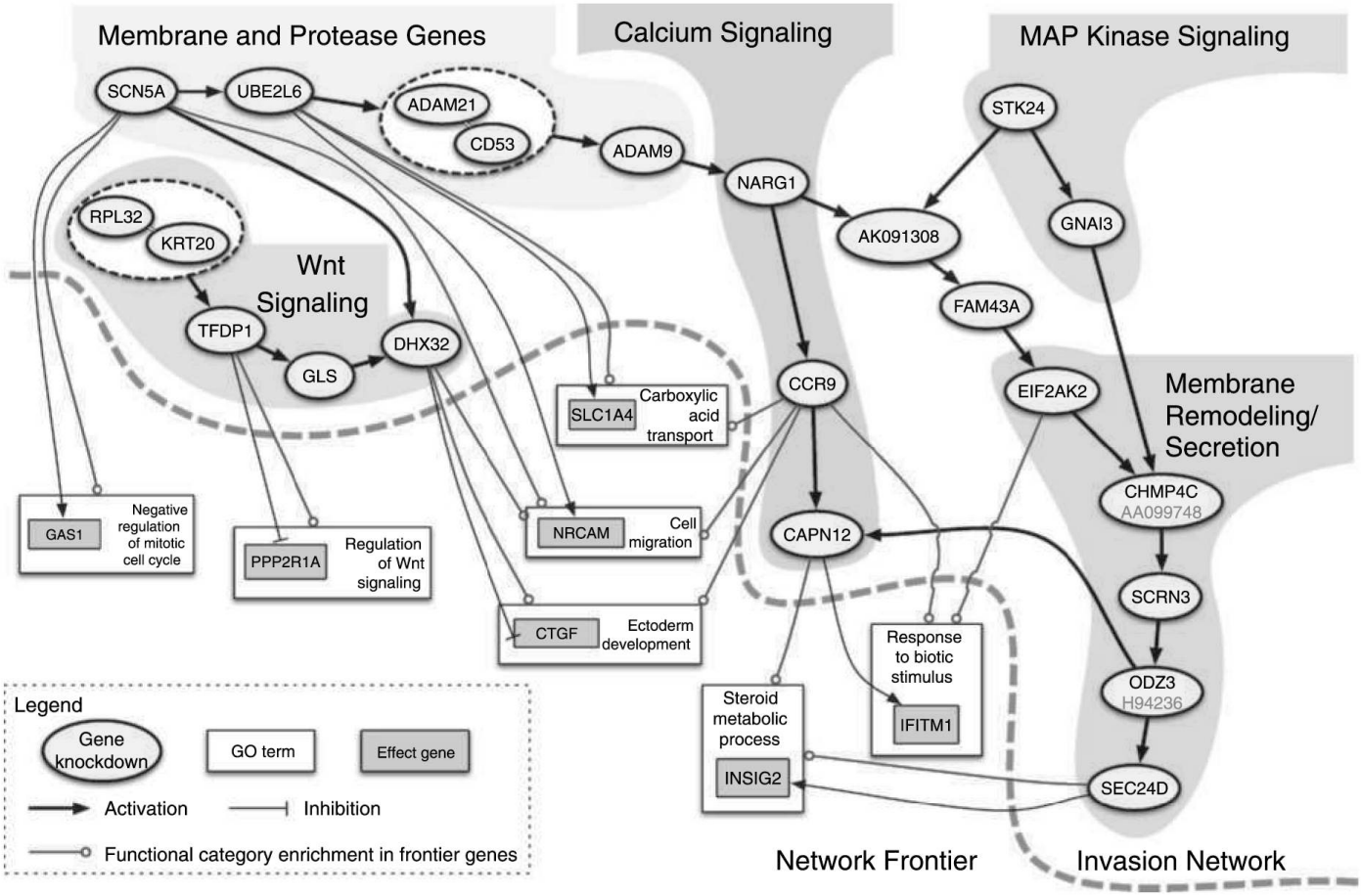

Figure 3. $Na_v1.5$-regulated gene transcriptional network controlling invasion. Oval nodes represent regulatory genes, gray boxes represent effector genes, and white boxes represent gene ontology categories. Arrows indicate activation, and tees show repression. Reprinted by permission from the American Association for Cancer Research: House CD *et al,* Voltage-gated Na^+ channel *SCN5A* is a key regulator of a gene transcriptional network that controls colon cancer invasion, *Cancer Research*, Sep 1, 2010, 70, 17, 6957-67, 10.1158/0008-5472.CAN-10-1169.

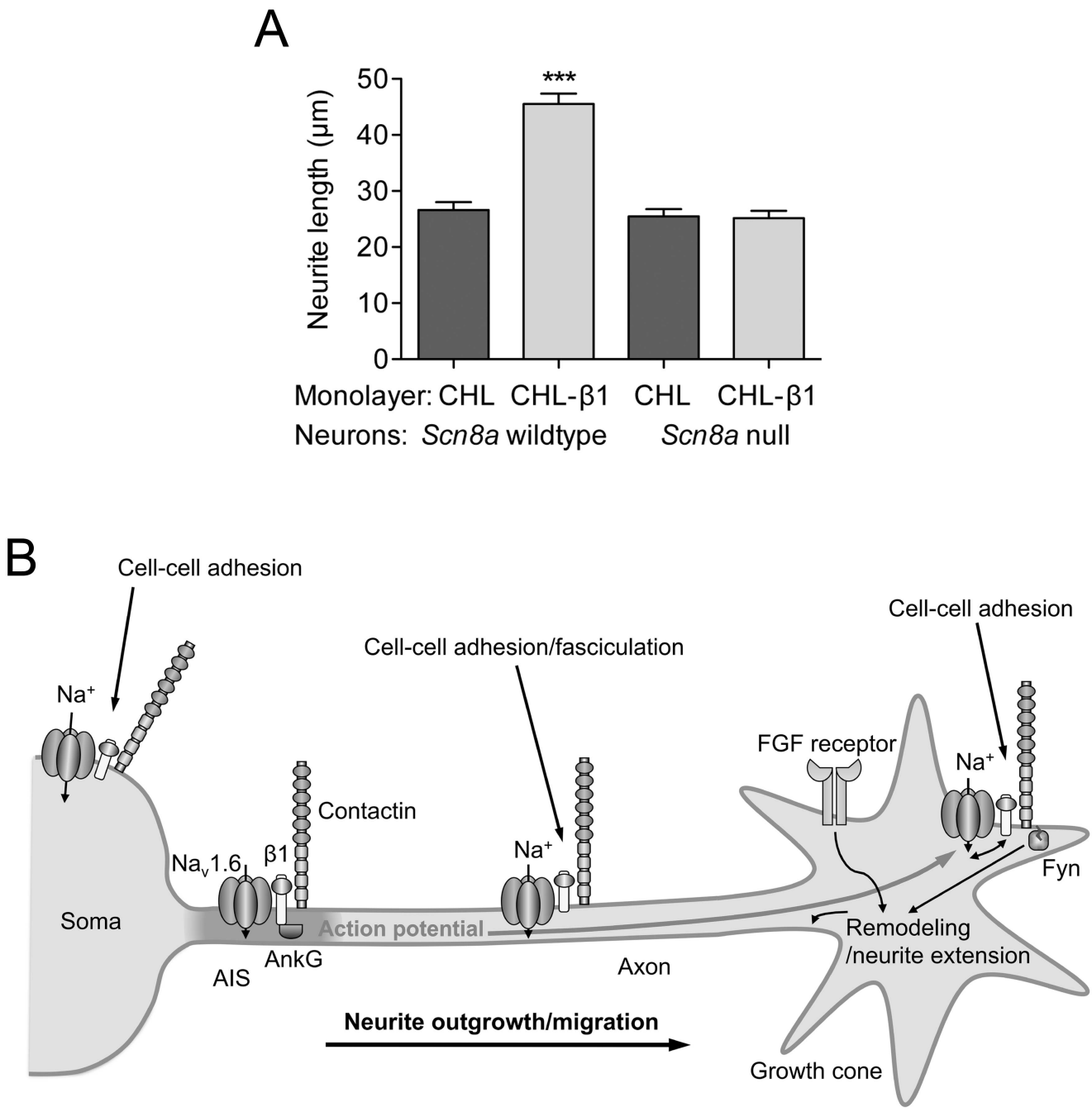

Figure 4. Neurite outgrowth and excitability regulated by β1 and $Na_v1.6$. (A) β1-mediated neurite outgrowth is inhibited by the *Scn8a* null mutation. Neurite lengths of wild-type and *Scn8a* null cerebellar granule neurons grown on monolayers of control β1-expressing fibroblasts. Data are mean and SEM. ***P < 0.001. (B) Proposed model for Na^+ current reciprocal involvement in β1-mediated neurite outgrowth. Complexes containing $Na_v1.6$, β1, and contactin are present throughout the neuronal membrane in the soma, neurite and growth cone. Na^+ influx is required for β1-mediated neurite extension and migration. VGSC complexes along the neurite participate in cell-cell adhesion and fasciculation. β1 is also required for $Na_v1.6$ expression at the axon initial segment, and subsequent high-frequency action potential firing. Electrical activity may further promote β1-mediated neurite outgrowth at or near the growth cone. Figure reproduced with permission.[56]

Table 1. VGSC subtype expression in cancer.

(A) α subunits.

Protein	Gene	TTX sensitivity	Cancer type	References
$Na_v1.1$	*SCN1A*	Sensitive	Ovarian	[17]
$Na_v1.2$	*SCN2A*	Sensitive	Cervical, mesothelioma, ovarian, prostate	[9, 14, 17, 18]
$Na_v1.3$	*SCN3A*	Sensitive	Ovarian, prostate, small cell lung cancer	[17, 18, 22]
$Na_v1.4$	*SCN4A*	Sensitive	Cervical, ovarian, prostate	[9, 17, 110]
$Na_v1.5$	*SCN5A*	Resistant	Breast[1], colon[1], lymphoma[1], neuroblastoma[1], non-small cell lung cancer, ovarian, small cell lung cancer	[8, 11, 15-17, 22, 26]
$Na_v1.6$	*SCN8A*	Sensitive	Breast, cervical, lymphoma, melanoma, mesothelioma, non-small cell lung cancer, prostate, small cell lung cancer	[8-10, 12, 14, 16, 18, 22, 26]
$Na_v1.7$	*SCN9A*	Sensitive	Breast, cervical, lymphoma, mesothelioma, non-small cell lung cancer, ovarian, prostate[1]	[8, 9, 14, 16-18, 26]
$Na_v1.8$	*SCN10A*	Resistant	-	-
$Na_v1.9$	*SCN11A*	Resistant	Lymphoma, small-cell lung cancer	[22, 26]

(B) β subunits.

Protein	Gene	Cancer type	References
β1	*SCN1B*	Breast[1], cervical[1], non-small cell lung cancer, prostate[1]	[10, 16, 36, 38]
β2	*SCN2B*	Breast, cervical, non-small cell lung cancer, prostate	[10, 16, 36-38]
β3	*SCN3B*	Non-small cell lung cancer, prostate	[16, 36]
β4	*SCN4B*	Breast, cervical, non-small cell lung cancer, prostate	[10, 16, 36, 38]

[1]Cancers in which the indicated subunit has been reported as predominant.

Table 2. Metastatic cell behaviors regulated by VGSCs.

Cellular activity	Cancer	Subunit(s) implicated	Reference(s)
Process extension	Breast, prostate	$Na_v1.7$, β1	[38, 39]
Galvanotaxis	Breast, prostate	$Na_v1.5$, $Na_v1.7$	[8, 40]
Lateral motility	Breast, mesothelioma, prostate	$Na_v1.5$, $Na_v1.7$, β1, β2	[14, 30, 37, 38, 41]
Transwell migration	Breast, prostate	$Na_v1.5$, $Na_v1.7$	[8, 17, 42-44]
Endocytic membrane activity	Breast, prostate, small cell lung cancer	$Na_v1.5$, $Na_v1.7$	[8, 22, 45]
Vesicular patterning	Breast, prostate	$Na_v1.7$	[46, 47]
Adhesion	Breast, prostate	$Na_v1.5$, $Na_v1.7$, β1, β2	[37, 38, 48]
Gene expression	Breast, colon, prostate	$Na_v1.5$, $Na_v1.7$, β1	[11, 38, 43, 49]
Invasion	Breast, cervical, colon, lymphoma, melanoma, non-small cell lung cancer, prostate	$Na_v1.5$, $Na_v1.6$, $Na_v1.7$, β2	[7, 8, 10-12, 16, 17, 19-21, 26, 37, 42]

Table 3. Complementary functions of VGSC α and β subunits in neurons and cancer cells.

Role	α subunit	β subunit
In neurons	Action potential initiation/conduction.[3] Activity-dependent axon guidance, dendrite growth, synapse formation, and neurite outgrowth during development.[5]	Modulate Na^+ current, electrical activity.[111] Enhance cell-cell adhesion.[112] Enhance neurite outgrowth, migration, pathfinding, fasciculation during development.[68] Reciprocal regulation of α subunit expression.[56]
In cancer cells	Highly expressed in strongly metastatic cells.[50] Enhance metastatic behaviors including invasion, migration.[5] Activity-dependent regulation of α subunit expression by positive feedback.[43, 78]	Highly expressed in weakly metastatic cells. Modulate Na^+ current.[37, 38] Enhance adhesion.[37, 38] Enhance process extension.[37, 38] Regulate migration.[37, 38] Regulate α subunit mRNA expression.[38]