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An emerging role for voltage-gated Na⁺ channels in cellular migration: Regulation of central nervous system development and potentiation of invasive cancers

William J. Brackenbury¹, Mustafa B. A. Djamgoz², and Lori L. Isom¹

¹Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109-0632

²*Neuroscience Solutions to Cancer* Research Group, Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK

Abstract

Voltage-gated Na⁺ channels (VGSCs) exist as macromolecular complexes containing a pore-forming α subunit and one or more β subunits. The VGSC α subunit gene family consists of ten members, which have distinct tissue-specific and developmental expression profiles. So far, four β subunits ($\beta 1$ – $\beta 4$) and one splice variant of $\beta 1$ ($\beta 1A$, also called $\beta 1B$) have been identified. VGSC β subunits are multifunctional, serving as modulators of channel activity, regulators of channel cell surface expression, and as members of the immunoglobulin superfamily, cell adhesion molecules (CAMs). β subunits are substrates of β -amyloid precursor protein-cleaving enzyme (BACE1) and γ -secretase, yielding intracellular domains (ICDs) that may further modulate cellular activity *via* transcription. Recent evidence shows that $\beta 1$ regulates migration and pathfinding in the developing postnatal central nervous system (CNS) *in vivo*. The α and β subunits, together with other components of the VGSC signaling complex, may have dynamic interactive roles depending on cell/tissue type, developmental stage, and pathophysiology. In addition to excitable cells like nerve and muscle, VGSC α and β subunits are functionally expressed in cells that are traditionally considered to be non-excitable, including glia, vascular endothelial cells, and cancer cells. In particular, the α subunits are upregulated in line with metastatic potential, and are proposed to enhance cellular migration and invasion. In contrast to the α subunits, $\beta 1$ is more highly expressed in weakly metastatic cancer cells, and evidence suggests that its expression enhances cellular adhesion. Thus, novel roles are emerging for VGSC α and β subunits in regulating migration during normal postnatal development of the CNS as well as during cancer metastasis.

Keywords

Cancer; Development; Migration; Signaling; Voltage-gated Na⁺ channel

Introduction

Voltage-gated Na⁺ channels (VGSCs) are heteromeric, polytopic membrane proteins that consist of a single pore-forming α subunit in association with one or more β subunits (Catterall 1992; Catterall 2000). The mammalian α subunit gene family contains ten members, Na_v1.1 through Na_v1.9 and Na_x, encoded by genes *SCN1A-SCN11A* (Table 1A)

(Goldin and others 2000). The different gene products exhibit unique tissue/subcellular distributions and subtle but potentially significant electrophysiological and pharmacological variability (e.g. Clare and others 2000). Additional functional diversity is achieved by alternative mRNA splicing (reviewed in Diss and others 2004). VGSCs are widely expressed in membranes of excitable cells, including neurons, neuroendocrine cells, and skeletal and cardiac myocytes, where they initiate and propagate action potentials (Catterall 1992; Hille 1992). VGSCs have also been identified in traditionally "non-excitable" cells, including glia and human vascular endothelial cells, where their role is less well defined (Barres and others 1990; Gautron and others 1992; Gosling and others 1998).

So far, four mammalian VGSC β subunits have been identified: $\beta 1$ through $\beta 4$, encoded by genes *SCN1B-SCN4B* (Table 1B). $\beta 1$ can also be expressed as the splice variant $\beta 1A$ (also called $\beta 1B$), containing a novel C-terminus encoded by a retained intron, in a developmentally regulated manner (Kazen-Gillespie and others 2000; Qin and others 2003). $\beta 1$, $\beta 1A/B$, and $\beta 3$ are non-covalently associated with α subunits (Isom and others 1992; Morgan and others 2000). In contrast, $\beta 2$ and $\beta 4$ are disulfide-linked to α (Isom and others 1995; Yu and others 2003). VGSC β subunits are multifunctional molecules. β subunits accelerate channel kinetics, shift voltage-dependence, and increase channel cell surface expression when expressed *in vitro* (Isom and others 1992). They are unique among ion channel auxiliary subunits in that they can also function as immunoglobulin superfamily cell adhesion molecules (IGSF-CAMs), promoting adhesion *in vitro*, both in the presence and absence of α subunits (Isom and Catterall 1996; Isom and others 1994; Malhotra and others 2000; McEwen and others 2004). In addition, $\beta 1$ interacts with other signaling proteins, including the CAMs contactin, neurofascin, NrCAM, VGSC $\beta 2$, and cadherin, the extracellular matrix molecule tenascin, receptor protein tyrosine phosphatase β (RPTP β), ankyrin β and ankyrin γ (Figure 1) (Malhotra and others 2000; Malhotra and others 2002; McEwen and Isom 2004; Meadows and Isom 2005; Ratcliffe and others 2000; Xiao and others 1999).

$\beta 1$ modulates electrical excitability *in vivo*: *Scn1b* null mice are ataxic, display spontaneous generalized seizures, and exhibit prolonged QT and RR intervals and slowed cardiac action potentials (Chen and others 2004; Lopez-Santiago and others 2007). Mutations in *SCN1B* result in human brain disease, including generalized epilepsy with febrile seizures plus (GEFS+), and temporal lobe epilepsy (TLE) (Audenaert and others 2003; Meadows and others 2002; Scheffer and others 2007; Wallace and others 2002; Wallace and others 1998). Deletion of $\beta 2$ (*Scn2b*) in mice results in reduced tetrodotoxin (TTX)-sensitive VGSC α subunit cell surface functional expression in CNS and peripheral nervous system (PNS) neurons under basal conditions (Chen and others 2002; Lopez-Santiago and others 2006). *Scn2b* expression is increased in response to peripheral nerve injury (Pertin and others 2005). Consistent with this, *Scn2b* null mice exhibit attenuated mechanical allodynia-like behavior in the spared nerve injury model of neuropathic pain as well as reduced sensitivity in the late phase of the formalin test (Lopez-Santiago and others 2006; Pertin and others 2005). Interestingly, the absence of $\beta 2$ in *Scn2b* null mice is neuroprotective in the experimental allergic encephalomyelitis (EAE) model of Multiple Sclerosis (MS), presumably by preventing VGSC α subunit upregulation in response to demyelination (O'Malley and Isom 2006; Waxman 2008a; Waxman 2008b).

Regulated signaling *via* complexes of VGSC α and β subunits can control a variety of cellular processes in both neurons as well as cell types that are traditionally thought of as non-excitable, including some types of cancer cells (Chioni and others 2006; Fraser and others 2005; Isom 2001). In this review, we will present the emerging evidence that VGSC α and β subunits play novel roles in cellular migration, focusing upon neuronal development and cancer invasion. These two processes share a number of important characteristics and it

has been noted that cancer invasion may be a deregulated process derived from the normal physiological invasion required for development of neuronal wiring during embryogenesis (Liotta and Clair 2000). Furthermore, several aggressive cancers have been found to have 'neuronal' characteristics (Onganer and others 2005).

VGSCs in the developing central nervous system

Differential, tissue-specific expression profiles for VGSC α subunit genes during development are well described (e.g. Schaller and Caldwell 2003; Yu and Catterall 2003). Most notably, *SCN3A* is highly expressed in the fetal CNS, and is subsequently replaced by *SCN1A*, *SCN2A* and *SCN8A* in early postnatal development (Beckh and others 1989; Brysch and others 1991; Gong and others 1999; Schaller and Caldwell 2000). In addition, Na_v1.6 protein replaces Na_v1.2 protein at the axon initial segment and maturing nodes of Ranvier during myelination in CNS neurons (Boiko and others 2001; Boiko and others 2003; Kaplan and others 2001; Westenbroek and others 1989). Developmentally controlled alternative splicing takes place in domain 1:segment 3 (D1:S3) of *SCN2A* and *SCN3A*, resulting in the replacement of a neutral residue in the neonatal splice variant protein by a negatively charged aspartate in the adult form (Diss and others 2004; Gustafson and others 1993; Lu and Brown 1998; Sarao and others 1991). Similar D1:S3 alternative splicing has been reported for *SCN5A*, *SCN8A*, and *SCN9A*, although it is not clear whether this process is developmentally regulated for these orthologous genes (Belcher and others 1995; Fraser and others 2005; Onkal and others 2008; Plummer and others 1998). In the case of *SCN5A*, the 'neonatal' splice variant (*nSCN5A*) encodes a protein that contains a positively charged lysine in place of the aspartate, which results in a depolarizing shift in the voltage-dependence of activation and slower kinetics *in vitro*, likely due to the charge reversal adjacent to the S4 voltage sensor (Onkal and others 2008). A different alternative splicing event occurs in exon 18 of *SCN8A*: in fetal brain and non-neuronal tissues, expression of splice variant 18N produces a truncated, non-functional form of the channel, and is replaced later in development by the functional 'adult' variant 18A (Plummer and others 1997).

Aberrant expression of VGSC gene products and/or reversion to an earlier developmental expression profile has been observed in several CNS pathophysiologicals. For example, in both rodent EAE and human MS, there is an upregulation and redistribution of Na_v1.2 and Na_v1.6 protein in response to axonal demyelination (Craner and others 2003; Craner and others 2004). In addition, sensory neuron specific *SCN10A* mRNA and Na_v1.8 protein are upregulated in cerebellar Purkinje neurons in both a rat demyelinating model and human MS (Black and others 2000; Black and others 1999b). A different situation has been reported in the PNS, where axonal injury causes upregulation of Na_v1.3 protein in dorsal root ganglion (DRG) neurons (Black and others 1999a). Na_v1.3 protein expression is also increased in subpopulations of hippocampal neurons in both *Scn1a* and *Scn1b* null mice, suggesting that Na_v1.3 upregulation in response to altered excitability may be common to the PNS and CNS (Chen and others 2004; Yu and others 2006).

Electrical activity is required for normal morphological development of axons, dendritic spines, and synaptic connections in the mammalian retinogeniculate pathway (Casagrande and Condo 1988; Dubin and others 1986; Kalil and others 1986; Riccio and Matthews 1985; Sretavan and others 1988). The highly specific VGSC blocker TTX reduces the growth of dendritic spines in pyramidal cells in the visual cortex of P21 rats (Riccio and Matthews 1985). In kitten retinal ganglion cells, action potential blockade with TTX inhibits the growth of developing axon terminals and disrupts segregation of retinal synaptic inputs onto cells in the lateral geniculate nucleus (LGN), resulting in a general reduction in the pace of maturation of retinogeniculate synapses in the developing LGN (Casagrande and Condo 1988; Dubin and others 1986; Kalil and others 1986). Although electrical activity appears

important for normal development of the visual system, it is not clear if expression/activity of specific VGSC genes is required for CNS development in general. In support of this hypothesis, *Scn2a* null mice die perinatally with severe brainstem defects, *Scn1a* null mice die at postnatal day 15 (P15), and *Scn8a* null mice die between P21–28, suggesting that Na_v1.1, Na_v1.2, and Na_v1.6 all play critical roles in early postnatal CNS development (Harris and Pollard 1986; Planells-Cases and others 2000; Yu and others 2006). In the case of Na_v1.1, haploinsufficiency still results in a severe phenotype, supporting the idea that VGSC gene family members may not compensate for each other *in vivo*. Future studies should ascertain the extent of involvement of different VGSC α subunits in various aspects of activity-dependent postnatal CNS development, in what is clearly a complex, neuron-specific process.

Scn1b mRNA and β 1 protein are expressed in CNS neurons from P1, and *Scn1b* null mice die by P20, indicating a critical role for β 1 at this early postnatal developmental stage (Chen and others 2004; Sashihara and others 1995; Sutkowski and Catterall 1990). Given that β 1 interacts with a variety of molecules involved in CNS development and axonal pathfinding in addition to α (Malhotra and others 2000; Malhotra and others 2002; McEwen and Isom 2004; Meadows and Isom 2005; Ratcliffe and others 2000; Xiao and others 1999), regulated signaling through complexes of VGSC α and β subunits may be required for normal early postnatal development of the CNS.

VGSC β subunits and cell adhesion

Increasing evidence suggests that, in addition to regulating electrical excitability, voltage-gated ion channels participate in numerous ‘non-conducting’ signaling mechanisms (reviewed in Kaczmarek 2006). Thus far, the best-characterized non-conducting role of VGSCs is in cell adhesion, via the β subunits. β 1 and β 2 interact with tenascin-C and tenascin-R, influencing cell migration, and participate in homophilic cell adhesion, resulting in cellular aggregation and ankyrin recruitment (Malhotra and others 2000; Malhotra and others 2002; Srinivasan and others 1998; Xiao and others 1999). In addition, β 1 interacts heterophilically with N-cadherin, contactin, neurofascin-155, neurofascin-186, NrCAM and VGSC β 2 (Kazarinova-Noyes and others 2001; Malhotra and others 2004; McEwen and Isom 2004; McEwen and others 2004). Interactions between β 1 and contactin, neurofascin-186, or β 2 result in increased VGSC expression in the plasma membrane *in vitro*, suggesting that adhesion may modulate excitability (Kazarinova-Noyes and others 2001; McEwen and Isom 2004; McEwen and others 2004). β 1 promotes neurite outgrowth in acutely dissociated cerebellar granule neurons (CGNs) in culture via *trans*-homophilic cell adhesive interactions, and this effect is blocked in neurons isolated from *Scn1b* null mice (Davis and others 2004). Furthermore, β 1 functions as a CAM *in vivo*, and is required for normal early postnatal CNS development. For example, in the cerebellum of P14 *Scn1b* null mice, the migration of axons through the molecular layer is disrupted, resulting in accumulation of CGNs in the external germinal layer (Figure 2, panel I) (Brackenbury and others 2008). In addition, the absence of β 1 results in defasciculation of axons in the developing corticospinal tract (Figure 2, panel II) (Brackenbury and others 2008).

IGSF-CAMs, including β 1, are known to localize to lipid rafts (Brackenbury and others 2008; Kasahara and others 2002; Niethammer and others 2002; Olive and others 1995; Schafer and others 2004; Wong and others 2005). Consistent with this, β 1 contains a putative palmitoylation site, a common feature of lipid raft-associated proteins (McEwen and others 2004). The mechanism underlying β 1-mediated neurite outgrowth involves signaling through the lipid raft-associated non-receptor tyrosine kinase fyn, and requires the presence of the glycoposphatidylinositol (GPI)-anchored CAM contactin (Brackenbury and others 2008). Similarly, NCAM-mediated neurite outgrowth is regulated by fyn kinase

(Beggs and others 1994; Kolkova and others 2000). A second non-raft associated signaling route *via* the fibroblast growth factor receptor (FGFR) regulates NCAM-mediated, but not β 1-mediated neurite outgrowth, suggesting a divergence in the signal transduction pathways mediated by these two IGSF-CAMs (Brackenbury and others 2008; Maness and Schachner 2007; Niethammer and others 2002; Sanchez-Heras and others 2006).

Recently, *SCN1B*, *SCN2B* and *SCN4B* mRNAs have been shown to be expressed in human prostate cancer (PCa) and breast cancer (BCa) cell lines (Chioni and others 2006; Diss and others 2007). Most of this work has been done on BCa, where *SCN1B* is the most abundantly expressed β subunit gene. *SCN1B* mRNA and β 1 protein levels are significantly higher in the weakly metastatic MCF-7 cell line compared to strongly metastatic MDA-MB-231 cells (Chioni and others 2006). Furthermore, in MCF-7 cells, downregulation of β 1 with siRNA decreased adhesion and increased migration (Chioni and others 2006). Therefore, in BCa cells *in vitro*, β 1 may control migration *via* cell adhesive interactions. In addition, *SCN3B*, which is not expressed in BCa cells (Chioni and others 2006), contains two response elements to the tumor suppressor p53, and may be involved in p53-dependent apoptosis (Adachi and others 2004), suggesting that absence of *SCN3B* expression may be an indicator of oncogenesis.

Clearly, the function of β 1 as a CAM is important for regulating migration, both in normal neuronal development and in invasive BCa. Further work will be required to ascertain whether downregulation of *SCN1B* and/or *SCN3B* in line with oncogenesis and/or increased metastasis may be a global phenomenon in other cancers, and to evaluate the underlying mechanism in the context of oncofetal expression of neuronal genes.

Potential role of VGSC β subunits as transcription factors

All four VGSC β subunit proteins are substrates for sequential cleavage by the β -site amyloid precursor protein-cleaving enzyme 1 (BACE1) and γ -secretase (Wong and others 2005). In addition, β 2 is cleaved by the α -secretase enzyme ADAM10 (Kim and others 2005). Processing of β subunits by BACE1/ α -secretase at cleavage sites juxtaposed to the extracellular face of the transmembrane region results in ectodomain shedding and yields membrane-bound C-terminal fragments (CTFs) (Figure 1) (Kim and others 2005; Wong and others 2005). In the case of β 1, the shed soluble ectodomain may then act as an adhesion ligand *in vitro*, and promote neurite outgrowth (Davis and others 2004; Malhotra and others 2000). The β -CTFs are further processed by γ -secretase at intracellular sites adjacent to the transmembrane region, yielding small (~12 kDa) intracellular domains (ICDs; Figure 1) (Kim and others 2005; Wong and others 2005). Pharmacological inhibition of β 2 cleavage by γ -secretase reduces cell-cell adhesion and migration (Kim and others 2005). Similarly, β 4 processing by BACE1 increases neurite outgrowth (Miyazaki and others 2007). These results suggest a functional role for the cleaved β subunit ICDs in the signaling mechanism(s) mediating adhesion, neurite extension and migration.

The β 2 ICD released by sequential BACE1 and γ -secretase cleavage localizes to the nucleus and increases *SCN1A* mRNA and $\text{Na}_v1.1$ protein levels, suggesting that the cleaved β 2 ICD may function as a transcription regulator (Kim and others 2007). Conversely, downregulation of β 1 expression in MCF-7 cells using siRNA results in upregulation of *nSCN5A* mRNA and 'neonatal' $\text{Na}_v1.5$ ($\text{nNa}_v1.5$) protein (Chioni and others 2006). Similarly, *Scn1b* null mice have increased *Scn5a* mRNA and $\text{Na}_v1.5$ protein expression in cardiomyocytes (Lopez-Santiago and others 2007). Therefore, the β subunit ICDs may function directly or indirectly to regulate transcription of genes including those encoding the VGSC α subunits. In particular, β 1 may be a novel widespread regulator of *SCN5A*.

expression. Further work should clarify how the β subunit ICDs regulate gene expression, and ascertain the extent of this phenomenon.

Involvement of VGSCs in metastatic cell behaviors of cancer cells

Metastasis, the spreading of cancer cells from a primary neoplasm to form tumors at secondary sites, is of major clinical importance, since this, rather than the primary tumors, is the main cause of cancer-related deaths (Stetler-Stevenson and others 1993). As a result, much research has focused on understanding the mechanisms underlying metastasis with a view to creating novel prognostic markers and therapies with improved accuracy and efficiency (Pantel and Brakenhoff 2004; Schwirzke and others 1999; Weigelt and others 2005). Increasing evidence suggests that strong parallels exist between neuronal development and cancer metastasis (Liotta and Clair 2000). Indeed, physiological invasive and migratory processes integral to neuronal pathfinding in the embryo may be deregulated in the case of pathophysiological cancer invasion (Liotta and others 1991). For example, the receptor for advanced glycation end products (RAGE) and its polypeptide ligand amphoterin, which colocalize to the leading edge of neurites promoting outgrowth, also increase growth and metastases of implanted and spontaneous tumors in mice (Taguchi and others 2000). Furthermore, in the case of PCa, a subpopulation of cells may undergo neuroendocrine differentiation, and become androgen-independent, highly aggressive, and dependent on growth factor signaling (Abrahamsson 1999; di Sant'Agnese 1992; Kim and others 2002). Given that many embryonic genes are re-expressed in cancer cells (Monk and Holding 2001), it is likely that acquisition of a neuronal phenotype in invasive cancer cells may be an oncofetal phenomenon. Therefore, regulatory processes involved in normal embryonic and/or early postnatal neuronal development may be re-expressed in cancer cells, providing them with a means to migrate and invade during metastasis.

Functional VGSCs are widely expressed in cells from a range of human cancers, including BCa (Fraser and others 2005; Roger and others 2003), PCa (Grimes and others 1995; Laniado and others 1997), lymphoma (Fraser and others 2004), lung cancer (Blandino and others 1995; Onganer and Djamgoz 2005; Roger and others 2007), mesothelioma (Fulgenzi and others 2006), neuroblastoma (Ou and others 2005), melanoma (Allen and others 1997) and cervical cancer (Diaz and others 2007). In addition, VGSC α subunit protein is upregulated in line with metastasis *in vivo*, in BCa, PCa, and small-cell lung cancer (SCLC) biopsy tissues (Abdul and Hoosein 2002; Diss and others 2005; Fraser and others 2005; Onganer and others 2005).

In PCa cells, the predominant VGSC α subunit gene, *SCN9A*, is upregulated ~1,000-fold at the mRNA level in the strongly metastatic rat Mat-LyLu and human PC-3 cells, compared with the corresponding weakly metastatic AT-2 and LNCaP cells (Diss and others 2001). In BCa cells, mRNA of the most highly expressed α subunit gene, *SCN5A*, is upregulated 1800-fold in metastatic MDA-MB-231 cells, compared with weakly metastatic MCF-7 cells (Fraser and others 2005). Interestingly, in MDA-MB-231 cells, the DI:S3 5'-splice variant (*nSCN5A*) is predominant (Chioni and others 2005; Fraser and others 2005). Furthermore, nNa_v1.5 protein is more highly expressed in the plasma membrane of MDA-MB-231 than MCF-7 cells (Chioni and others 2005; Fraser and others 2005). A similar DI:S3 5'-splice variant of *SCN5A* has also been described in rat brain tissue and human nB1 neuroblastoma cells (Ou and others 2005; Wang and others 2008).

The VGSC blocker TTX suppresses a variety of *in vitro* cell behaviors associated with the metastatic cascade in cancer cells (Table 2). VGSC-enhanced behaviors include invasion (Bennett and others 2004; Grimes and others 1995; Laniado and others 1997; Roger and others 2003; Smith and others 1998), transwell migration, (Brackenbury and Djamgoz 2006;

Fraser and others 2005; Roger and others 2003), galvanotaxis (Djamgoz and others 2001), morphological development and process extension (Fraser and others 1999), endocytic membrane activity (Mycielska and others 2003; Onganer and Djamgoz 2005), vesicular patterning (Krasowska and others 2004), lateral motility (Fraser and others 2003), reduced adhesion (Palmer and others 2008), nitric oxide production (Williams and Djamgoz 2005) and gene expression (Mycielska and others 2005). Specific functional downregulation of $\text{nNa}_v1.5$ using RNAi or an antibody (NESO-pAb) revealed that $\text{nNa}_v1.5$ is primarily responsible for the VGSC-dependent enhancement of migration and invasion of MDA-MB-231 cells (Brackenbury and others 2007). Importantly, transient overexpression of $\text{Na}_v1.4$ in weakly metastatic LNCaP cells increases invasion, and can be reversed by TTX, suggesting that VGSC expression is necessary and sufficient to increase their invasive potential (Bennett and others 2004). Finally, the anticonvulsants phenytoin and carbamazepine, which target VGSCs, inhibit secretion of prostate-specific antigen (PSA) and interleukin-6 by PCa cells *in vitro* (Abdul and Hoosein 2001).

The mechanism(s) responsible for VGSC α subunit upregulation in metastatic cancer cells is not understood. However, serum factors are known to play an important role in this process (Ding and Djamgoz 2004). In Mat-LyLu cells, epidermal growth factor (EGF) increases Na^+ current density and enhances migration partially *via* VGSC activity (Ding and others 2008). Similarly, EGF enhances migration, endocytosis and invasion of PC-3M cells *via* enhanced *SCN9A*/ $\text{Na}_v1.7$ functional expression (Onganer and Djamgoz 2007). In addition, nerve growth factor (NGF) increases Na^+ current density in Mat-LyLu cells, although NGF-dependent migration occurs independent of VGSC activity (Brackenbury and Djamgoz 2007). Given that growth factors including NGF play important roles during CNS development, (e.g. von Bartheld 1998), growth factor-dependent regulation of VGSC activity in cancer cells further demonstrates the strong parallel between neuronal development and cancer. Furthermore, the developmental regulator of VGSC expression, REST, has been identified as a candidate oncogene (Armisen and others 2002; Westbrook and others 2005). Steady-state VGSC functional expression in Mat-LyLu cells is maintained by a positive feedback mechanism involving both increased *SCN9A* transcription and protein kinase A-dependent VGSC trafficking to the plasma membrane, resulting in increased VGSC-dependent cellular migration (Brackenbury and Djamgoz 2006). Given that in MDA-MB-231 cells, the $\beta1$ subunit is downregulated, and $\text{nNa}_v1.5$ is upregulated, $\beta1$ itself may regulate α subunit expression/activity, either *via* transcription, or indirectly, *via* a CAM-dependent signaling mechanism (Chioni and others 2006). Thus, a complex and dynamic role is emerging for both the VGSC α and β subunits in metastatic cancer progression (Figure 3). Further work is required to elucidate the mode and extent of involvement of the β subunits as novel CAMs in VGSC-dependent metastatic behaviors.

Multifunctional roles of VGSC macromolecular signaling complexes in CNS development and cancer progression: concluding remarks

The possibility of VGSC α and β subunits functioning as macromolecular complexes in conjunction with other signaling proteins has been proposed previously, and fits in well with emerging studies identifying various non-conducting roles of voltage-gated channels (Isom 2001; Kaczmarek 2006; Meadows and Isom 2005). The canonical VGSC macromolecular signaling complex would likely comprise at minimum the α subunit together with one or more β subunits, and other interacting partners would vary with cell/tissue type and/or subcellular domain. For example, at the growth cone of migrating CGNs, the VGSC complex is proposed to localize to a lipid raft domain with $\beta1$, contactin, and fyn kinase (Figure 4) (Brackenbury and others 2008). Additional components might include cytoskeletal proteins, e.g. ankyrins, and secretases, e.g. BACE1 (Kim and others 2005; Malhotra and others 2000; Malhotra and others 2002; Malhotra and others 2004; Wong and

others 2005). In this scenario, the VGSC signaling complex would promote neurite extension in response to $\beta 1$ *trans*-adhesive interactions with $\beta 1$ subunits expressed by adjacent neurons or glia. However, many questions still remain: How does signaling through this complex result in remodeling of the growth cone? How would this signal transduction cascade promote migration and pathfinding *in vivo*? What is the involvement of VGSC-mediated changes in electrical excitability, if any, on expression/activity of this complex?

Clearly, signaling through VGSC complexes is important for normal CNS development. In addition, aberrant expression/activity of VGSC α and β subunits may be involved in aspects of the cancer process. An important emerging parallel is that VGSC signaling complexes appear to regulate migration of both neurons and cancer cells. The challenge is now to identify the molecular identity of the VGSC macromolecular complexes present under different physiological conditions *in vivo*, e.g. at different developmental stages, in different cells/tissues. Next, it will be essential to extend this information to understanding how VGSC signaling complexes may operate in pathophysiological situations, including abnormal migration in the developing CNS and metastatic cancer.

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Figure 1.

Basic functional architecture of the $\beta 1$ subunit. Amino acid residues responsible for interaction with α subunit (McCormick and others 1998; Spampanato and others 2004), generalized epilepsy with febrile seizures plus (GEFS+) and temporal lobe epilepsy (TLE) (Audenaert and others 2003; Meadows and others 2002; Scheffer and others 2007; Wallace and others 2002), site of intron retention (Kazen-Gillespie and others 2000; Qin and others 2003), putative palmitoylation site (McEwen and others 2004), ankyrin interaction site (Malhotra and others 2002) and tyrosine phosphorylation site (Malhotra and others 2004) are shown. Regions identified for N-glycosylation (ψ) (McCormick and others 1998), the Ig loop (Isom and Catterall 1996), BACE1 and α/γ -secretase cleavage (Wong and others 2005), RPTP β interaction (Ratcliffe and others 2000), and possible fyn kinase interaction (Brackenbury and others 2008; Malhotra and others 2001) are also marked. It should be noted that the C-terminal domain of $\beta 1$ is also critical for α - β subunit interactions (Spampanato and others 2004). Figure was produced using Science Slides 2006 software.

I. Cerebellum

II. Corticospinal Tract

Figure 2.

Scn1b null mice exhibit CNS pathfinding errors.

I. $\beta 1$ modulates axonal migration in the postnatal developing cerebellum *in vivo*. (A) Schematic outline of left side of cerebellum in the coronal plane. Black boxes B–D show locations of high-magnification images in (B) – (D). CENT6–9, central lobe, lobules 6–9; COPY, copula pyramidis; PRIM, paramedian lobule; sec, secondary fissure; prepyramidal fissure. Inset, parasagittal diagram indicating rostrocaudal location of coronal section. Right-hand plates (i) and (ii): low-magnification images (10X) of TAG-1 immunolabeling (green) on coronal cerebellar sections from *Scn1b* wildtype, and *Scn1b* null P14 littermates, respectively. Scale bar: 100 μ m. (B) – (D) High-magnification (100X) Z-series projections of the same (i) *Scn1b* wildtype, and (ii) *Scn1b* null cerebellar sections, at the locations

defined in (A). Scale bar: 20 μ m. Three mice of each genotype were examined, with similar results.

II. Loss of β 1 results in axonal pathfinding abnormalities in the corticospinal tract. (A) – (G) Consecutive coronal sections across the pyramidal decussation in a *Scn1b* wildtype brain. (H) – (N) Consecutive coronal sections across the pyramidal decussation in a *Scn1b* null brain. (O) – (R) Example sections from further *Scn1b* null mice. Arrows in (H) – (L) and (O) – (R): defasciculation across pyramidal decussation. Arrowheads in (I) – (K) and (O) – (R): mislocalization of axons lateral to dorsal column. Arrowheads in (M), (N): axons deviating from dorsal column after pyramidal decussation. v, ventral pyramid; d, dorsal column. Scale bar, 250 μ m. Six *Scn1b* null mice were examined. All 6 showed similar CST abnormalities compared to 7 wildtype mice. Figure reproduced with permission (Brackenbury and others 2008).

Figure 3.

Proposed model for VGSC α and β subunit involvement in metastatic cancer progression. β 1 is expressed in transformed weakly metastatic cancer cells, contributing to their adhesiveness within the proliferating tumor *in situ* (Chioni and others 2006). In response to signaling interactions between cancer cells and the local tumor microenvironment, VGSC α subunit expression is proposed to be upregulated, and β 1 expression downregulated (Brackenbury and Djamgoz 2007; Chioni and others 2006; Ding and others 2008; Ding and Djamgoz 2004; Onganer and Djamgoz 2007). Reduction of β 1 is proposed to reduce the cells' adhesiveness, increasing migration (Chioni and others 2006). The metastatic cells' migration and invasion is further potentiated by VGSC α subunit activity (Brackenbury and others 2007; Brackenbury and Djamgoz 2006; Smith and others 1998). Figure was produced using Science Slides 2006 software.

Figure 4.

A VGSC macromolecular signaling complex in migrating neurons. A proposed *trans*-adhesive interaction between $\beta 1$ on an adjacent neuronal or glial cell and the VGSC macromolecular signaling complex on the cerebellar granule neuron, comprising the α subunit, $\beta 1$ and contactin, initiates a signaling cascade through fyn kinase leading to neurite outgrowth and migration (Brackenbury and others 2008). In addition, cleavage of $\beta 1$ by BACE1 and γ -secretase is proposed to release the $\beta 1$ intracellular domain (ICD), which may enhance transcription of VGSC α subunit(s) (Kim and others 2007; Wong and others 2005). The system may be further fine-tuned by signaling mechanism(s) resulting from Na^+ influx through the VGSC α subunit (e.g. Brackenbury and Djamgoz 2006). Figure was produced using Science Slides 2006 software.

Table 1

VGSC isoforms and tissue expression.

(A) The α subunits.

Protein	Gene symbol (human)	Tissue location
Na _v 1.1	<i>SCN1A</i>	CNS, PNS, heart
Na _v 1.2	<i>SCN2A</i>	CNS, PNS
Na _v 1.3	<i>SCN3A</i>	CNS, PNS
Na _v 1.4	<i>SCN4A</i>	Skeletal muscle
Na _v 1.5	<i>SCN5A</i>	Uninnervated skeletal muscle, heart, brain
Na _v 1.6	<i>SCN8A</i>	CNS, PNS, heart
Na _v 1.7	<i>SCN9A</i>	PNS, neuroendocrine cells, sensory neurons
Na _v 1.8	<i>SCN10A</i>	sensory neurons
Na _v 1.9	<i>SCN11A</i>	sensory neurons
Na _x	<i>SCN6A, SCN7A</i>	heart, uterus, skeletal muscle, astrocytes, DRG

(B) The β subunits.

Protein	Gene symbol (human)	Tissue location
β 1	<i>SCN1B</i>	Heart, skeletal muscle, CNS, glia, PNS
β 1A (β 1B)	<i>SCN1B</i>	Heart, skeletal muscle, adrenal gland, PNS
β 2	<i>SCN2B</i>	CNS, PNS, heart, glia
β 3	<i>SCN3B</i>	CNS, adrenal gland, kidney, PNS
β 4	<i>SCN4B</i>	Heart, skeletal muscle, CNS, PNS

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; DRG, dorsal root ganglia.

Table 2

Metastatic cell behaviors potentiated by VGSC activity.

Cellular activity	Cancer/cell line tested	Reference(s)
Process extension	PCa (Mat-LyLu)	Fraser et al. (1999)
Galvanotaxis	PCa (Mat-LyLu); BCa (MDA-MB-231)	Djamgoz et al. (2001); Fraser et al. (2005)
Lateral motility	PCa (Mat-LyLu); BCa (MDA-MB-231); mesothelioma	Fraser et al. (2003); Fraser et al. (2005); Fulgenzi et al. (2006)
Transwell migration	PCa (Mat-LyLu, PC-3M); BCa (MCF-7, MDA-MB-231)	Roger et al. (2003); Fraser et al. (2005); Brackenbury et al. (2006); Onganer and Djamgoz (2007)
Endocytic membrane activity	PCa (Mat-LyLu); BCa (MDA-MB-231); SCLC (H69, H209, H510)	Mycielska et al. (2003); Fraser et al. (2005); Onganer and Djamgoz (2005)
Vesicular patterning	PCa (Mat-LyLu)	Krasowska et al. (2004)
Detachment	PCa (Mat-LyLu, PC-3M); BCa (MCF-7)	Palmer et al. (2008); Chioni et al. (2006)
Gene expression	PCa (Mat-LyLu, PC-3M)	Mycielska et al. (2005); Brackenbury and Djamgoz (2006)
Matrigel invasion	PCa (Mat-LyLu, PC-3, LNCaP C4 and C4-2); BCa (MDA-MB-231); lymphoma (Jurkat); non-SCLC (H23, H460, Calu-1)	Grimes et al. (1995); Laniado et al. (1997); Smith et al. (1998); Bennett et al. (2004); Roger et al. (2003); Fraser et al. (2004); Fraser et al. (2005); Roger et al. (2007)