Dual roles of voltage-gated sodium channels in development and cancer

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Abbreviations: BACE1, β-secretase; CAM, cell adhesion molecule; CGN, cerebellar granule neuron; CNS, central nervous system; DI:S3, domain I segment 3; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GEFS+, genetic epilepsy with febrile seizures plus; MEF, mouse embryonic fibroblast; MS, multiple sclerosis; NGF, nerve growth factor; NHE1, Na^+/H^+ exchanger 1; PKA, protein kinase A; PNS, peripheral nervous system; RPTPβ, receptor tyrosine phosphatase β; TLE, temporal lobe epilepsy; VEGF, vascular endothelial growth factor; VGSC, voltage-gated Na^+ channel; V_m, membrane potential.

Abstract

Voltage-gated Na^+ channels (VGSCs) are heteromeric protein complexes containing pore-forming α subunits together with non-pore-forming β subunits. There are nine α subunits, $Na_v1.1$-$Na_v1.9$, and four β subunits, $\beta1$-$\beta4$. The β subunits are multifunctional, modulating channel activity, cell surface expression, and are members of the immunoglobulin superfamily of cell adhesion molecules. VGSCs are classically responsible for action potential initiation and conduction in electrically excitable cells, including neurons and muscle cells. In addition, through the $\beta1$ subunit, VGSCs regulate neurite outgrowth and pathfinding in the developing central nervous system. Reciprocal signalling through $Na_v1.6$ and $\beta1$ collectively regulates Na^+ current, electrical excitability and neurite outgrowth in cerebellar granule neurons. Thus, α and β subunits may have diverse interacting roles dependent on cell/tissue type. VGSCs are also expressed in non-excitable cells, including cells derived from a number of types of cancer. In cancer cells, VGSC α and β subunits regulate cellular morphology, migration, invasion and metastasis. VGSC expression associates with poor prognosis in several studies. It is hypothesised that VGSCs are up-regulated in metastatic tumours, favouring an invasive phenotype. Thus, VGSCs may have utility as prognostic markers, and/or as novel therapeutic targets for reducing/preventing metastatic disease burden. VGSCs appear to regulate a number of key cellular processes, both during normal postnatal development of the CNS and during cancer metastasis, by a combination of conducting (i.e. *via* Na^+ current) and non-conducting mechanisms.

Introduction

Voltage-gated Na^+ channels (VGSCs) are heteromeric membrane protein complexes containing pore-forming α subunits in association with non-pore-forming β subunits (Figure 1) (Catterall, 2000). The β subunits regulate channel gating and are also cell adhesion molecules (CAMs) (Brackenbury and Isom, 2011). The classical role of VGSCs is the initiation and conduction of action potentials in electrically excitable cells, e.g. neurons (Hille, 1992). However, VGSCs are also expressed in a number of “non-excitable” cells, including

fibroblasts, glia, immune cells, and cancer cells, where their role is less well understood (Brackenbury *et al.*, 2008b). Clearly, in both excitable and non-excitable cells, VGSCs regulate a number of key cellular processes, by a combination of conducting (i.e. *via* Na^+ current) and non-conducting mechanisms. The purpose of this article is to provide an up-to-date review of the current evidence suggesting a dual role for VGSCs in regulating cellular migration during central nervous system development and cancer progression.

Structure and function of VGSCs

The pore-forming α subunit consists of four homologous domains, each with six transmembrane segments. The pore is formed from the membrane dipping loop between the 5th and 6th transmembrane segments of each domain (Figure 1) (Catterall, 2000). There are nine α subunits, $Na_v1.1$-$Na_v1.9$, encoded by *SCN1A-SCN11A* (Catterall, 2000). The different α subunits have unique, but often overlapping, tissue-specific expression patterns (Table 1A) (Goldin *et al.*, 2000). There is considerable electrophysiological and pharmacological diversity between α subunits, which may, in part explain their tissue specificity (Catterall, 2000). Alternative splicing of α subunits provides additional functional, developmental, and tissue-specific variability (Diss *et al.*, 2004). Four genes (*SCN1B-SCN4B*) encode five different β subunits, $\beta1$, and its splice variant $\beta1B$, and $\beta2$-4 (Table 1B) (Brackenbury and Isom, 2011). With the exception of $\beta1B$, the β subunits are type 1 topology transmembrane proteins, with a small intracellular C-terminus, and an extracellular N-terminus containing an immunoglobulin loop (Figure 2) (Gilchrist *et al.*, 2013, Isom *et al.*, 1992, Namadurai *et al.*, 2014). $\beta1B$ is a splice variant of $\beta1$, which, through the retention of exon 3A, transcribes an early stop codon and does not contain the transmembrane region of $\beta1$ (Kazen-Gillespie *et al.*, 2000, Qin *et al.*, 2003). $\beta1$ and $\beta3$ are non-covalently linked to α subunits, whereas $\beta2$ and $\beta4$ are covalently linked (Isom *et al.*, 1992, Isom *et al.*, 1995, Morgan *et al.*, 2000, Yu *et al.*, 2003).

Classically, the β subunits modulate the biophysical properties of the α subunit. For example, $\beta1$ and $\beta2$ increase current density, accelerate inactivation, and hyperpolarize the voltage

dependence of inactivation in heterologous cells (Isom *et al.*, 1992, Isom *et al.*, 1995). In contrast, β3 depolarizes the voltage dependence of activation and inactivation of $Na_v1.3$ in HEK-293 cells (Cusdin *et al.*, 2010), and increases Na^+ current density by enhancing trafficking of $Na_v1.5$ to the plasma membrane (Ishikawa *et al.*, 2013). β4 hyperpolarizes the voltage-dependence of activation of $Na_v1.2$ in tsA-201 cells (Qu *et al.*, 2001, Yu *et al.*, 2003). In addition, the intracellular domain of β4 has been proposed to act as an open-channel blocker in cerebellar Purkinje neurones (Grieco *et al.*, 2005). However, there have been inconsistent reports on the type and magnitude of alteration of the Na^+ current by individual β subunits, which may be dependent on the cell line/type used (Meadows and Isom, 2005, Moran *et al.*, 2003). This variability may be due to differences in the endogenous levels of α subunits and β subunits and different glycosylation states. For example, β1 and β3 have recently been shown to alter glycosylation of $Na_v1.7$ in HEK293 cells (Laedermann *et al.*, 2013). Interestingly, the β subunits may also regulate other classes of ion channels. For example, β1 regulates A-type K^+ currents in isolated cortical neurons (Marionneau *et al.*, 2012), and modifies $K_V4.3$ gating in cardiomyocytes (Deschenes *et al.*, 2008, Deschenes and Tomaselli, 2002). β1 has also been shown to modulate the biophysical properties of $K_V1.1$, $K_V1.2$, $K_V1.3$, $K_V1.6$, and $K_V7.2$ (Nguyen *et al.*, 2012).

In addition to regulating Na^+/K^+ current, the presence of the immunoglobulin loop means that the β subunits are also CAMs. β1 can interact both homophilically and heterophilically with a number of extracellular proteins and other CAMs, including β2, contactin, neurofascin-186, NrCAM, N-cadherin, and tenascin-R (Figure 2) (Kazarinova-Noyes *et al.*, 2001, Malhotra *et al.*, 2000, McEwen and Isom, 2004, Ratcliffe *et al.*, 2001, Xiao *et al.*, 1999). β2 also interacts with tenascin-C and tenascin-R (Srinivasan *et al.*, 1998). In Chinese hamster lung cells, phosphorylation of the intracellular Y181 residue on β1 abolishes recruitment of $ankyrin_G$ and $ankyrin_B$ (Malhotra *et al.*, 2002). Further, phosphorylation of Y181 regulates subcellular localization of β1 to the intercalated disks in cardiomyocytes (Malhotra *et al.*, 2004). β3 shows significant homology to β1 however, when expressed in *Drosophila* S2 cells, β3 does not participate in *trans*-homophilic adhesion, nor does it interact with β1 or contactin in Chinese

hamster lung cells, but does interact with neurofascin-186 (McEwen *et al.*, 2009, McEwen and Isom, 2004). In contrast, a recent study has shown that in HEK-293 cells, the immunoglobulin domain of β3 can indeed participate in trans-homophilic binding, and can interact heterophilically with β1 (Yereddi *et al.*, 2013). Clearly, further work is required to resolve these conflicting observations, and β3-mediated adhesive interactions may be dependent on species/cell type.

VGSCs in central nervous system development

Electrical activity is required for axonal and dendritic development and synaptogenesis in the retinogeniculate pathway and visual cortex (Casagrande and Condo, 1988, Riccio and Matthews, 1985). Similarly, deletion of $Na_v1.1$, $Na_v1.2$, or $Na_v1.6$ in mice results in central nervous system (CNS) defects and premature lethality (Harris and Pollard, 1986, Planells-Cases *et al.*, 2000, Yu *et al.*, 2006). Thus, α subunit expression and activity appear to be critical for normal CNS development. Fine-tuning of electrical activity *via* VGSC α subunit expression is tightly regulated during development. For example, $Na_v1.3$ is expressed during foetal development and is replaced by $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ postnatally (Beckh *et al.*, 1989, Schaller and Caldwell, 2000). Later in postnatal development, $Na_v1.6$ replaces $Na_v1.2$ at the axon initial segment, and nodes of Ranvier following myelination (Boiko *et al.*, 2001, Boiko *et al.*, 2003, Kaplan *et al.*, 2001). Interestingly however, α subunits may also play a non-conducting role (independent of Na^+ current) in regulating tissue development. For example, $Na_v1.5$ expression is required for normal heart development in zebrafish (Chopra *et al.*, 2010). Further developmental regulation of VGSCs is achieved by alternative splicing. Alternative splicing in domain I segment 3 (DI:S3) occurs in a number of the α subunits, and is developmentally regulated for $Na_v1.2$, $Na_v1.3$ and $Na_v1.5$ (Diss *et al.*, 2004).

β subunit expression is also developmentally regulated. During CNS development, the *SCN1B* splice variant β1B is predominantly expressed embryonically (Kazen-Gillespie *et al.*, 2000, Patino *et al.*, 2011). In contrast, β1 expression increases from birth, peaking at postnatal day 14 in mice (Kazen-Gillespie *et al.*, 2000). Finally, the developmentally regulated

expression profile of VGSCs is disrupted in CNS diseases. For example, in multiple sclerosis (MS), $Na_v1.2$, $Na_v1.6$ and $Na_v1.8$ are up-regulated in CNS neurons in response to demyelination (Black *et al.*, 2000, Craner *et al.*, 2004). Interestingly, *Scn2b* deletion is neuroprotective in the experimental allergic encephalomyelitis MS model in mice, possibly by reducing α subunit up-regulation (O'Malley *et al.*, 2009).

The β subunits also play critical roles in CNS development. *Scn1b* null mice are ataxic and display spontaneous generalized seizures (Chen *et al.*, 2004). Mutations in *SCN1B* result in genetic epilepsy with febrile seizures plus (GEFS+; OMIM 604233; reviewed in (Patino and Isom, 2010). In cerebellar granule neurons (CGNs), β1 promotes neurite outgrowth *via trans*-homophilic adhesion (Davis *et al.*, 2004). β1-mediated neurite outgrowth also requires fyn kinase and contactin (Brackenbury *et al.*, 2008a). In addition, β1 is required for neuronal pathfinding and fasciculation in the postnatally developing CNS (Brackenbury *et al.*, 2008a, Brackenbury *et al.*, 2013). β1B can also promote neurite outgrowth (Patino *et al.*, 2011). β1 is required for normal localization of $Na_v1.6$ to the axon initial segment in CGNs and the resultant inward Na^+ current is required for β1 mediated neurite outgrowth suggesting a specific reciprocal relationship between these two subunits (Figure 3A) (Brackenbury *et al.*, 2010).

Scn2b null mice appear normal in neurological tests, although they display increased seizure susceptibility, and altered sensitivity to pain stimuli (Chen *et al.*, 2002, Lopez-Santiago *et al.*, 2006). Electrical activity is reduced in the optic nerve of *Scn2b* null mice, and Na^+ current is reduced in hippocampal and dorsal root ganglion neurons, compared to wildtype animals (Chen *et al.*, 2002, Lopez-Santiago *et al.*, 2006). *Scn3b* null mice have altered cardiac function but show no abnormalities in the CNS (Hakim *et al.*, 2008). It is possible that β1 may compensate for the lack of β3 allowing for an apparently normal neurological phenotype. Overexpression of β4 in Neuro2a cells increases neurite outgrowth, dendrite formation, and filopodia-like protrusions (Oyama *et al.*, 2006), suggesting that, like β1, β4 may regulate migration and pathfinding *in vivo*.

The β subunits may play a role in downstream signalling pathways and gene transcription. The β subunits are substrates for proteolytic processing by α, β and γ-secretases (Kim *et al.*, 2005, Wong *et al.*, 2005). Sequential cleavage of β2 by β-secretase (BACE1) and γ-secretase release the β2 intracellular domain, which is proposed to translocate to the nucleus and regulate expression of $Na_v1.1$ (Kim *et al.*, 2007, Kim *et al.*, 2005). Secretase-mediated cleavage of β1 regulates neurite outgrowth, suggesting that proteolytic processing of β subunits may be an essential step in transducing the adhesion signal to promote migration (Brackenbury and Isom, 2011).

In summary, VGSC α and β subunit expression is temporally regulated during CNS development. Regulated expression of specific subtypes is critical for maintaining electrical excitability and activity-dependent synaptic connections on the one hand, and adhesive interactions, neurite outgrowth, fasciculation and migration on the other. Several studies point towards a potential causal relationship between altered VGSC expression, developmental aberrations, and CNS pathophysiologies, which requires further investigation.

VGSCs and cancer

α subunits

VGSC α subunits are widely expressed in a range of different types of cancer, including breast cancer, cervical cancer, colon cancer, glioma, leukaemia, lung cancer, lymphoma, melanoma, mesothelioma, neuroblastoma, ovarian cancer, prostate cancer (Table 1A) (Brackenbury, 2012). Although the majority of evidence is based on studies using cell lines cultured *in vitro*, a number of reports have now confirmed that α subunit expression occurs in tumours *in vivo*, e.g. (Fraser *et al.*, 2005, Gao *et al.*, 2010, Hernandez-Plata *et al.*, 2012, House *et al.*, 2010). In several cancers where multiple α subunits have been detected, one α subunit has been identified as most highly expressed, e.g. $Na_v1.5$ is predominant in breast cancer (Fraser *et al.*, 2005), whereas $Na_v1.7$ is predominant in prostate cancer (Diss *et al.*, 2004). Interestingly, $Na_v1.5$ and $Na_v1.7$ have been shown to be mainly expressed in their

neonatal DI:S3 splice forms in several cancers (Brackenbury, 2012). However, this splicing pattern is not conserved across all the tumour types studied, e.g., the neonatal DI:S3 splice form is absent in colon cancer cells, and the adult variant is expressed instead (House *et al.*, 2010). There also appears to be a cancer type-specific relationship between α subunit expression and metastatic propensity. For example, $Na_v1.5$ is more highly expressed in strongly metastatic MDA-MB-231 breast cancer cells than weakly metastatic MCF-7 cells, and elevated $Na_v1.5$ expression in tumours correlates with increased risk of recurrence, metastasis and reduced overall survival (Fraser *et al.*, 2005, Yang *et al.*, 2012). A similar pattern has been shown for α subunit expression in colon, prostate and ovarian cancers. However, there is an inverse correlation between α subunit expression and clinical grade in glioma, and no relationship has been found in lung cancer cell lines (reviewed in (Brackenbury, 2012).

The mechanisms by which VGSCs are up-regulated in cancer cells are not well understood. Several studies suggest that growth factors may play a role (Fraser *et al.*, 2014). Epidermal growth factor (EGF) and nerve growth factor (NGF) both increase Na^+ current in prostate cancer cells, the latter *via* activation of protein kinase A (PKA) (Brackenbury and Djamgoz, 2007, Ding *et al.*, 2008). Similarly, EGF signalling *via* the extracellular signal-regulated kinase (ERK)1/2 pathway increases expression of $Na_v1.7$ and Na^+ current (Campbell *et al.*, 2013). In breast cancer cells, oestrogen increases Na^+ current, suggesting that steroid hormones may also regulate VGSC expression/activity (Fraser *et al.*, 2010). Further fine-tuning of VGSC expression in cancer cells is achieved through positive feedback auto-regulation. In both metastatic breast and prostate cancer cells, Na^+ current activates PKA, which in turn, promotes functional expression of $Na_v1.5$ and $Na_v1.7$, respectively (Brackenbury and Djamgoz, 2006, Chioni *et al.*, 2010).

In vitro, the α subunits have been shown to enhance various cellular behaviours associated with metastasis, including endocytosis (Mycielska *et al.*, 2003), galvanotaxis (Djamgoz *et al.*, 2001), gene expression (Mycielska *et al.*, 2005), invasion (Grimes *et al.*, 1995), migration (Fraser *et al.*, 2003), and process outgrowth (Fraser *et al.*, 1999) (Table 2). Conflicting reports

suggest that α subunits may, or may not, also regulate proliferation (Abdul and Hoosein, 2002, Fraser *et al.*, 2000, Roger *et al.*, 2003). These discrepancies may be due to the differing specificity of the various pharmacological approaches used in different studies. Several studies have indicated that specific α subunits contribute to the invasive capacity of different cancer cell types. For example, the neonatal DI:S3 splice variant of $Na_v1.5$ enhances migration and invasion of metastatic breast cancer cells (Brackenbury *et al.*, 2007). In contrast, $Na_v1.6$ enhances invasion of cervical cancer cells (Hernandez-Plata *et al.*, 2012), and $Na_v1.6$ and $Na_v1.7$ enhance invasion and endocytosis in prostate cancer cells (Nakajima *et al.*, 2009). Nonetheless, expression of any subtype may be sufficient to promote invasion. For example, overexpression of $Na_v1.4$ increases the invasiveness of LNCaP prostate cancer cells (Bennett *et al.*, 2004).

The fact that α subunits appear to be up-regulated in cancer cells and promote metastasis-like behaviour suggests that they may be useful therapeutic targets. Indeed, the VGSC-inhibiting drugs phenytoin and ranolazine have both recently been shown to inhibit metastasis in xenograft mouse models of breast cancer (Driffort *et al.*, 2014, Nelson *et al.*, 2015). In support of this, several other VGSC-targeting antiepileptic drugs, including phenytoin, carbamazepine and riluzole, have been shown to inhibit secretory activity, cellular migration, proliferation and invasion in cell lines from several different cancers (Abdul and Hoosein, 2001, Abdul and Hoosein, 2002, Fraser *et al.*, 2003, Yang *et al.*, 2012). Given that the membrane potential (V_m) of cancer cells is relatively depolarised compared with terminally differentiated cells (Yang and Brackenbury, 2013), it is likely that the majority of VGSCs are in the inactivated state. Therefore, the persistent Na^+ current, which is typically a few per cent of the transient current, is likely to be predominant and may prove to be an important therapeutic target (Yang *et al.*, 2012).

Several theories have been proposed to explain how Na^+ flux through VGSCs contributes to invasion and metastasis. In breast cancer cells, $Na_v1.5$-mediated Na^+ influx has been shown to increase H^+ efflux through the Na^+/H^+ exchanger (NHE1), causing intracellular alkalinisation and extracellular perimembrane acidification, thus enhancing the activity of pH-

dependent cathepsin proteases and invadopodia formation (Brisson *et al.*, 2013, Gillet *et al.*, 2009). An additional possibility is that VGSCs may regulate gene expression (Brackenbury and Djamgoz, 2006). In colon cancer cells, $Na_v1.5$ has been proposed to be a key regulator of a network of invasion-promoting genes (House *et al.*, 2010). However, the intermediate steps between Na^+ current and gene transcription remain to be elucidated. A third possibility is that VGSCs may regulate the intracellular Ca^{2+} level. For example, activation of VGSCs present on intracellular membranes in macrophages and melanoma cells causes Na^+ release from cationic stores, followed by Na^+ uptake by mitochondria, and Ca^{2+} release, which then increases podosome and invadopodia formation, and enhanced invasiveness (Carrithers *et al.*, 2009). Finally, a significant number of somatic mutations have been identified in *SCN5A* in tumours (Figure 4A), which span all functional domains (Figure 1). Further work is required to establish whether and how these mutations may confer a functional advantage on the VGSC to promote invasive behaviour.

β subunits

VGSC β subunits have been detected in prostate, breast, lung, and cervical cancers (Table 1B) (Brackenbury, 2012). Subtype-specific expression varies across cancer types: β3 is present in prostate and lung cancer cells, but is absent in breast and cervical cancer cells. In contrast, β1 is predominant in breast, prostate, and cervical cancer cells (Chioni *et al.*, 2009, Diss *et al.*, 2008, Hernandez-Plata *et al.*, 2012). Similar to *SCN5A*, a number of somatic mutations have been identified in *SCN1B* in tumours (Figure 4B), in both the immunoglobulin and cytoplasmic domains (Figure 2). β1 and β2 expression levels correlate with metastatic potential in prostate cancer (Diss *et al.*, 2008, Jansson *et al.*, 2012). However, this pattern is not reflected in breast cancer (Chioni *et al.*, 2009, Nelson *et al.*, 2014). Thus, β subunit expression may vary across cancer type and grade, dependent on specific functional specialisations and heterotypic interactions.

In breast cancer cell lines cultured *in vitro*, β1 enhances cell-cell and cell-substrate adhesion, and retards migration in wound healing and transwell assays (Chioni *et al.*, 2009). In an

orthotopic mouse model of breast cancer, β1 overexpression increases tumour growth and metastasis (Nelson *et al.*, 2014). β1 overexpression also increases vascular endothelial growth factor (VEGF) secretion and angiogenesis, and reduces apoptosis. Interestingly, β1 promotes neurite-like process outgrowth from breast cancer cells via *trans*-homophilic adhesion, thus recapitulating its functional role in neurons (Figure 3A) (Davis *et al.*, 2004, Nelson *et al.*, 2014). As in neurons, β1-mediate process outgrowth in breast cancer cells requires fyn kinase activity and Na^+ current (Figure 3B) (Brackenbury *et al.*, 2010, Brackenbury *et al.*, 2008a). Thus, it appears that β1 plays parallel roles in regulating neuronal migration during CNS development, on the one hand, and cancer cell invasion during metastasis, on the other. Therefore, targeting the adhesive function of β1 may provide a novel approach to anti-cancer therapy (Brackenbury and Isom, 2008).

In LNCaP prostate cancer cells, over-expression of β2 induces a bipolar morphology and increases overall length with a concurrent reduction in volume (Jansson *et al.*, 2012). These changes could allow for greater invasion and motility. In agreement with this, β2 over-expressing cells have increased migratory capability compared to control cells in a wound healing assay (Jansson *et al.*, 2012). β2 over-expressing cells plated on various substrates preferentially adhere to vitronectin and Matrigel over fibronectin, suggesting that β2 may selectively increase adhesion dependent on the surrounding tissue/substrate (Jansson *et al.*, 2012). In contrast, β2 over-expression reduces tumour take and growth following subcutaneous implantation of LNCaP cells into nude mice (Jansson *et al.*, 2012). Thus, β2 may enhance invasion and metastasis whilst also reducing the ability of tumours to form localized masses. In support of this notion, β2 over-expression increases invasion and growth on laminin, and enhances association between prostate cancer cells and nerve axons in organotypic cultures (Jansson *et al.*, 2014). Therefore, β2 may permit association between prostate cancer cells and neural matrices, enhancing perineural invasion, thus enabling glandular egress and subsequent metastatic dissemination.

In contrast to β1 and β2, β3 may function as a tumour suppressor. *SCN3B* (encoding β3) contains two functional p53 response elements, suggesting that it may be directly regulated by the tumour suppressor p53 (Adachi *et al.*, 2004). In addition, *Scn3b* is up-regulated in wildtype mouse embryo fibroblasts (MEFs), but not *p53* null MEFs following treatment with adriamycin (Adachi *et al.*, 2004). Furthermore, β3 suppresses colony formation, and in association with various anticancer agents, β3 promotes apoptosis in a p53-dependent manner (Adachi *et al.*, 2004).

Less is known about the expression/function of β4 in cancers. Interestingly, a strong down-regulation of β4 has been reported in primary cultures of cervical cancer cells relative to cells from noncancerous cervix (Hernandez-Plata *et al.*, 2012). A similar pattern of expression has also been reported in prostate cancer cell lines (Diss *et al.*, 2008). However, β4 expression is increased in cervical cancer biopsies compared to noncancerous cervix. This difference in relative expression levels between biopsies and primary cell cultures may be due to the adhesive function differing *in vivo* as opposed to *in vitro* (Hernandez-Plata *et al.*, 2012). Further work is required to investigate this possibility.

In summary, VGSCs are up-regulated in a number of different types of cancer. Increasing evidence suggests that α and β subunits both play an important role in promoting various aspects of cancer progression and metastasis (Figure 5). The role(s) played by specific subtypes appears to be complex, and may be dependent on tumour type. A common theme is that α subunits regulate invasion *via* Na^+ current, whereas β subunits regulate adhesion interactions. The next step is to establish the extent and distribution of expression of VGSCs across tumour types, and the precise involvement of different α and β subunits, with the goal of harnessing their therapeutic potential.

Conclusions

VGSCs function as macromolecular signalling complexes in which Na^+ current through the α subunit pore is coupled with non-conducting signalling *via* the β subunits. For example, in

migrating neurons, complexes of $Na_v1.6$, $\beta1$, fyn and contactin are proposed to localise to the growth come, regulating neurite outgrowth and migration (Brackenbury *et al.*, 2010, Brackenbury *et al.*, 2008a). Similarly, complexes of $Na_v1.5$ and $\beta1$ may occur in breast cancer cells, regulating morphological changes and metastasis (Nelson *et al.*, 2014, Yang *et al.*, 2012). Additional complexity may be provided by further interactions with the cytoskeleton, e.g. *via* ankyrin, and secretases (Brackenbury and Isom, 2011, Kim *et al.*, 2005, Malhotra *et al.*, 2000). The challenge now is to understand how signalling through these complexes gives rise to morphological changes and cellular motility, and how variations in the composition of the complex might relate to cell/tissue type, functional specialisation and subcellular domain. An important observation is that VGSC α and β subunits appear to play multifunctional and parallel roles (1) in excitable cells, e.g. CNS neurons, and (2) in metastatic cancer cells, regulating Na^+ current, migration and invasion in both systems. It is therefore essential to better understand the identity, function and composition of these complexes during development and in pathophysiological situations. An intriguing possibility is that VGSCs may be useful prognostic markers, and/or novel therapeutic targets for reducing/preventing metastasis.

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Table 1. Tissue and cancer expression of VGSCs.

(A) α subunits (Brackenbury, 2012, Goldin *et al.*, 2000).

Protein	Gene	Tissue location	Cancer type
$Na_v1.1$	*SCN1A*	CNS, PNS, heart	Ovarian
$Na_v1.2$	*SCN2A*	CNS, PNS	Cervical, mesothelioma, ovarian, prostate
$Na_v1.3$	*SCN3A*	CNS, PNS	Ovarian, prostate, small cell lung cancer
$Na_v1.4$	*SCN4A*	Skeletal muscle	Cervical, ovarian, prostate
$Na_v1.5$	*SCN5A*	Uninnervated skeletal muscle, heart, brain	Breast, colon, lymphoma, neuroblastoma, non-small cell lung cancer, ovarian, small cell lung cancer
$Na_v1.6$	*SCN8A*	CNS, PNS, heart	Breast, cervical, lymphoma, melanoma, mesothelioma, non-small cell lung cancer, prostate, small cell lung cancer
$Na_v1.7$	*SCN9A*	PNS, neuroendocrine cells, sensory neurons	Breast, cervical, lymphoma, mesothelioma, non-small cell lung cancer, ovarian, prostate
$Na_v1.8$	*SCN10A*	sensory neurons	Prostate
$Na_v1.9$	*SCN11A*	sensory neurons	Lymphoma, small-cell lung cancer

(B) β subunits (Brackenbury, 2012, Brackenbury and Isom, 2011).

Protein	Gene	Tissue location	Cancer type
$\beta1$	*SCN1B*	Heart, skeletal muscle, adrenal gland, CNS, glia, PNS	Breast, cervical, non-small cell lung cancer, prostate
$\beta2$	*SCN2B*	CNS, PNS, heart, glia	Breast, cervical, non-small cell lung cancer, prostate
$\beta3$	*SCN3B*	CNS, adrenal gland, kidney, PNS	Non-small cell lung cancer, prostate
$\beta4$	*SCN4B*	Heart, skeletal muscle, CNS, PNS	Breast, cervical, non-small cell lung cancer, prostate

Table 2. Metastatic cell behaviours regulated by VGSCs (Brackenbury, 2012).

Cellular activity	Cancer	Subunit(s) implicated
Process outgrowth	Breast, prostate	$Na_v1.5$, $Na_v1.7$, $\beta1$
Galvanotaxis	Breast, prostate	$Na_v1.5$, $Na_v1.7$
Lateral motility (wound healing)	Breast, mesothelioma, prostate	$Na_v1.5$, $Na_v1.7$, $\beta1$, $\beta2$
Transwell migration	Breast, prostate	$Na_v1.5$, $Na_v1.7$
Endocytic membrane activity	Breast, prostate, small cell lung cancer	$Na_v1.5$, $Na_v1.7$
Vesicular patterning	Breast, prostate	$Na_v1.7$
Adhesion	Breast, prostate	$Na_v1.5$, $Na_v1.7$, $\beta1$, $\beta2$
Gene expression	Breast, colon, prostate	$Na_v1.5$, $Na_v1.7$, $\beta1$
Invasion	Breast, cervical, colon, lymphoma, melanoma, non-small cell lung cancer, prostate	$Na_v1.5$, $Na_v1.6$, $Na_v1.7$, $\beta1$, $\beta2$

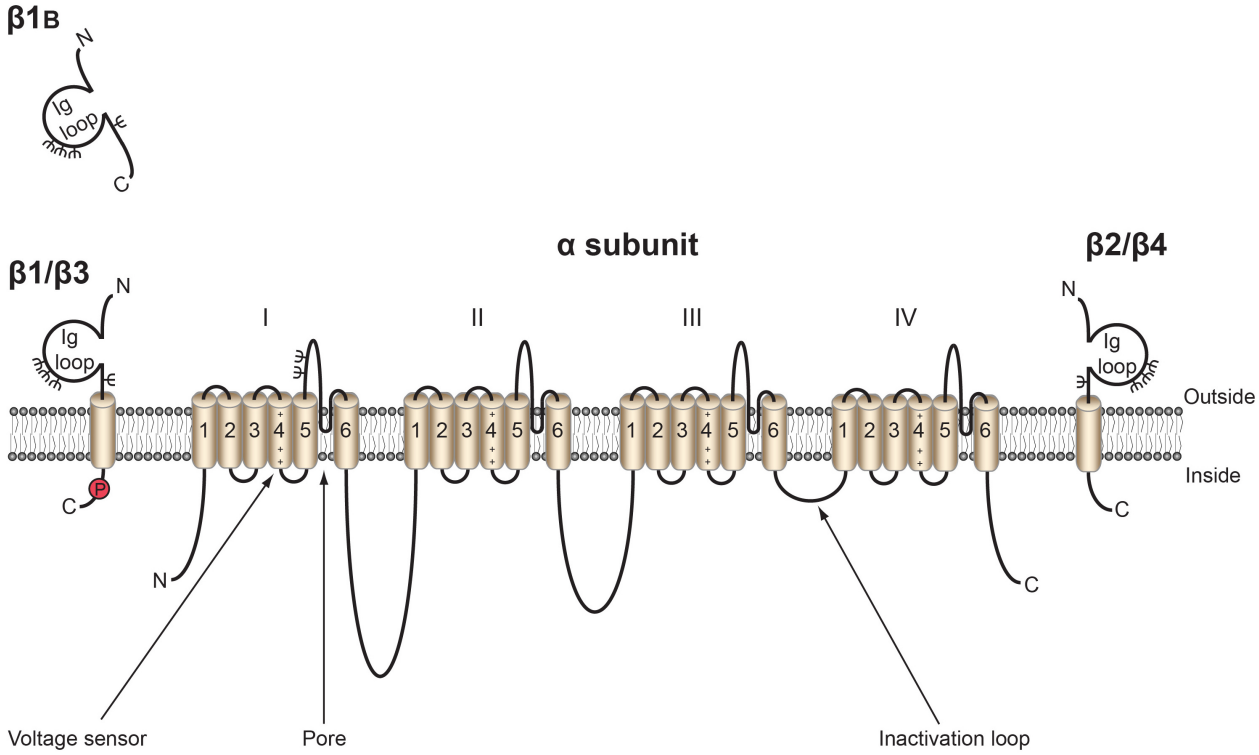

Figure 1. Topology of VGSCs. VGSCs contain a pore-forming α subunit that has four homologous domains, each containing six transmembrane segments. The voltage sensor is in segment 4 (Catterall, 2000). The β subunits contain an extracellular immunoglobulin (Ig) loop, transmembrane domain, and an intracellular C-terminal domain, with the exception of β1B, which lacks a transmembrane domain, and is thus a soluble protein (Patino *et al.*, 2011). Red P, tyrosine phosphorylation site in β1 C-terminus (Malhotra *et al.*, 2004); ψ, glycosylation sites. Figure as originally published in Brackenbury WJ and Isom LL (2011) Na^+ Channel β Subunits: Overachievers of the Ion Channel Family. Front. Pharmacol. 2:53. doi: 10.3389/fphar.2011.00053.

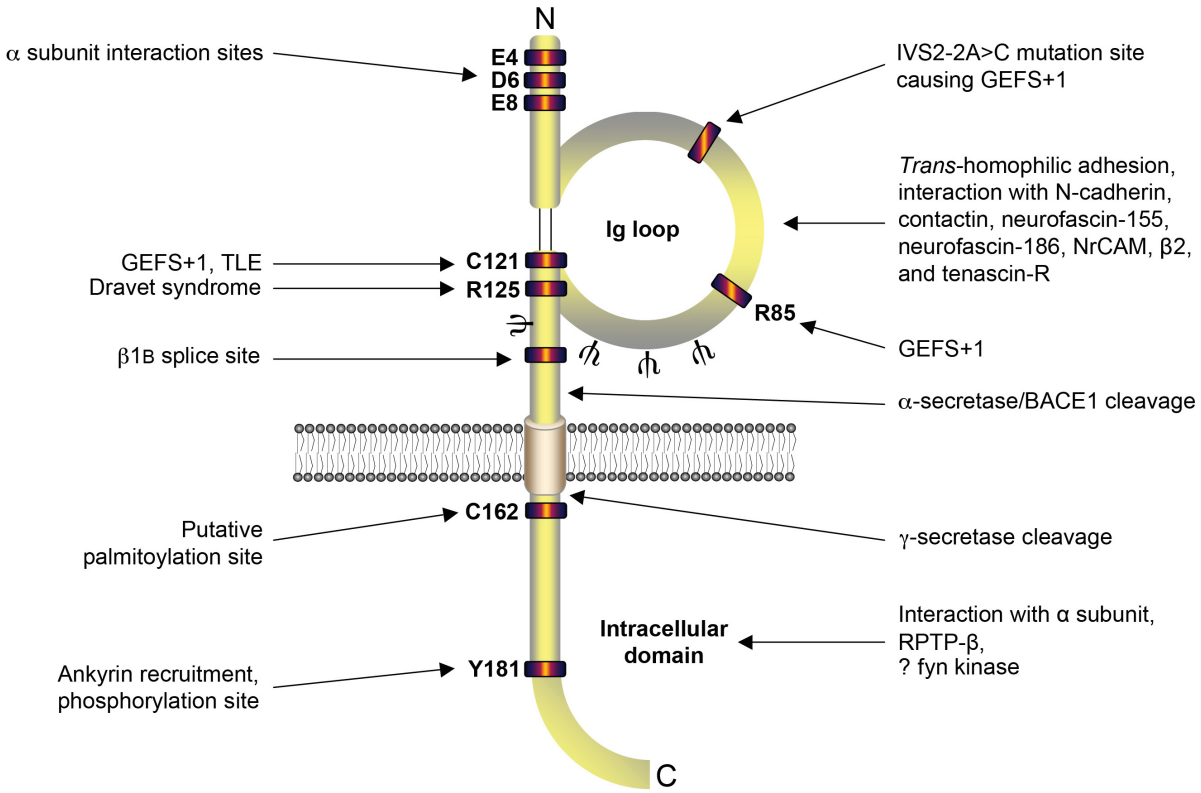

Figure 2. Functional map of β1. The extracellular immunoglobulin loop interacts with other cell adhesion molecules and extracellular matrix proteins (Kazarinova-Noyes *et al.*, 2001, Malhotra *et al.*, 2000, McEwen and Isom, 2004, Ratcliffe *et al.*, 2001, Xiao *et al.*, 1999). A number of mutations at the indicated sites in and adjacent to the immunoglobulin loop have been identified as responsible for causing temporal lobe epilepsy (TLE) and genetic epilepsy with febrile seizures plus (GEFS+) (Patino and Isom, 2010). Other sites indicated: alternative splice site, putative palmitoylation site, secretase cleavage sites, glycosylation sites (ψ), sites for interaction with ankyrin, receptor tyrosine phosphatase β (RPTPβ), and fyn kinase (Brackenbury *et al.*, 2008a, Malhotra *et al.*, 2002, Malhotra *et al.*, 2004, Patino *et al.*, 2011, Wong *et al.*, 2005). Figure as originally published in Brackenbury WJ and Isom LL (2011) Na^+ Channel β Subunits: Overachievers of the Ion Channel Family. Front. Pharmacol. 2:53. doi: 10.3389/fphar.2011.00053.

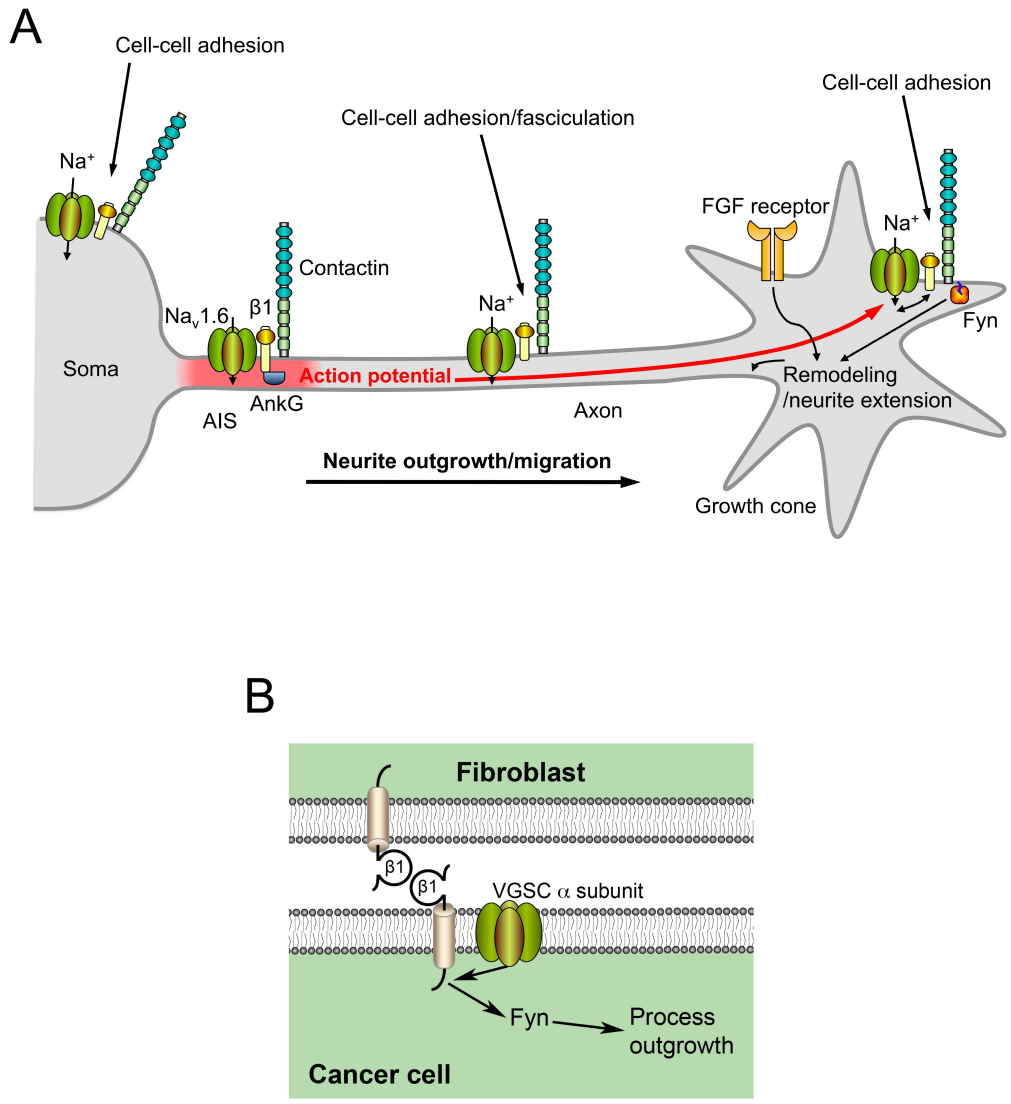

Figure 3. Functional reciprocity between α and β subunits regulating neurite outgrowth and migration during CNS development and metastasis. (A) β1 is required for localization of $Na_v1.6$ to the axon initial segment and high frequency action potential firing. The electrical activity and resultant membrane depolarisation promotes β1-mediated neurite outgrowth towards the growth cone. (B) A similar mechanism is proposed for β1-mediated process outgrowth in breast cancer cells. β1 from an adjacent fibroblast or cancer cell interacts with β1 on the cancer cell, initiating a signalling cascade that requires Na^+ current and fyn kinase. Figure panels reproduced with permission (Brackenbury *et al.*, 2010, Nelson *et al.*, 2014).

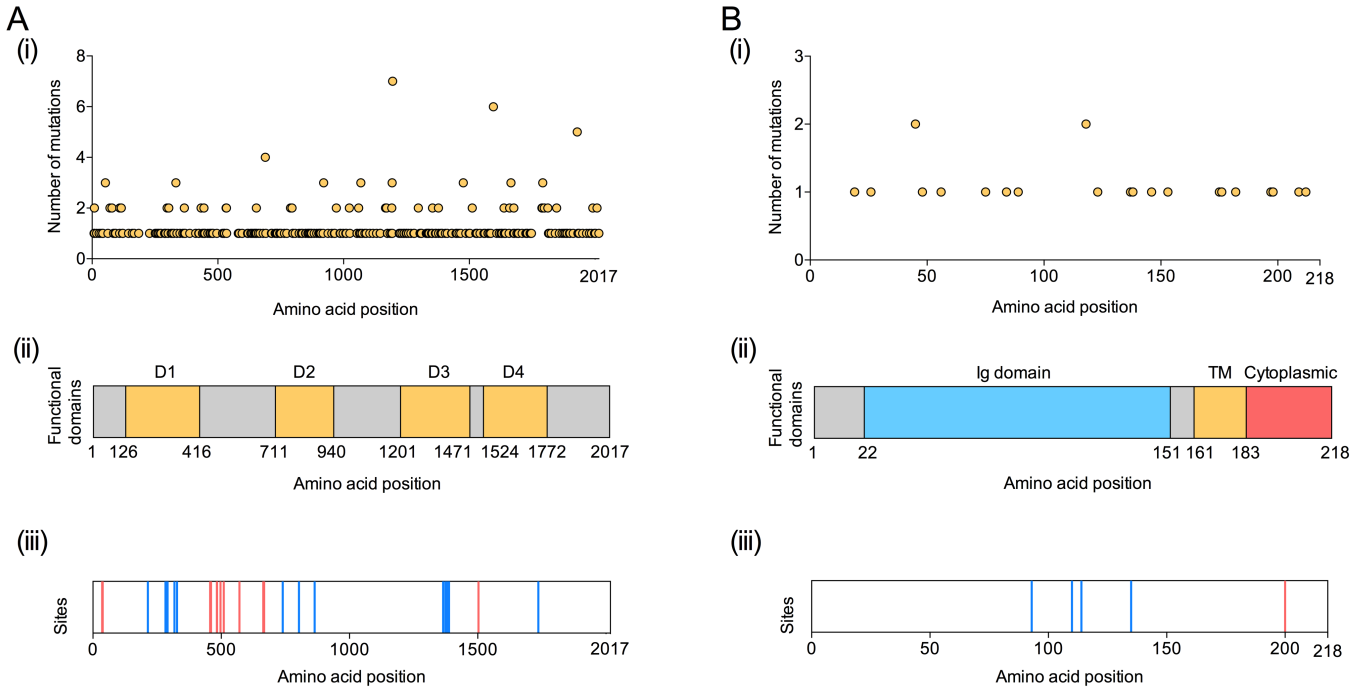

Figure 4. $Na_v1.5$ and β1 mutations in cancer. (A) $Na_v1.5$: (i) Number of mutations reported in the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) for each amino acid position on x-axis. (ii) Location of Domains 1-4 (yellow) (Catterall, 2000). (iii) Putative phosphorylation (red) and glycosylation sites (blue). (B) β1: (i) Number of mutations reported in the COSMIC database for each amino acid position. (ii) Location of immunoglobulin (Ig) domain (blue), transmembrane (TM) domain (yellow) and cytoplasmic domain (red) (Brackenbury and Isom, 2011). (iii) Phosphorylation (red) and glycosylation sites (blue).

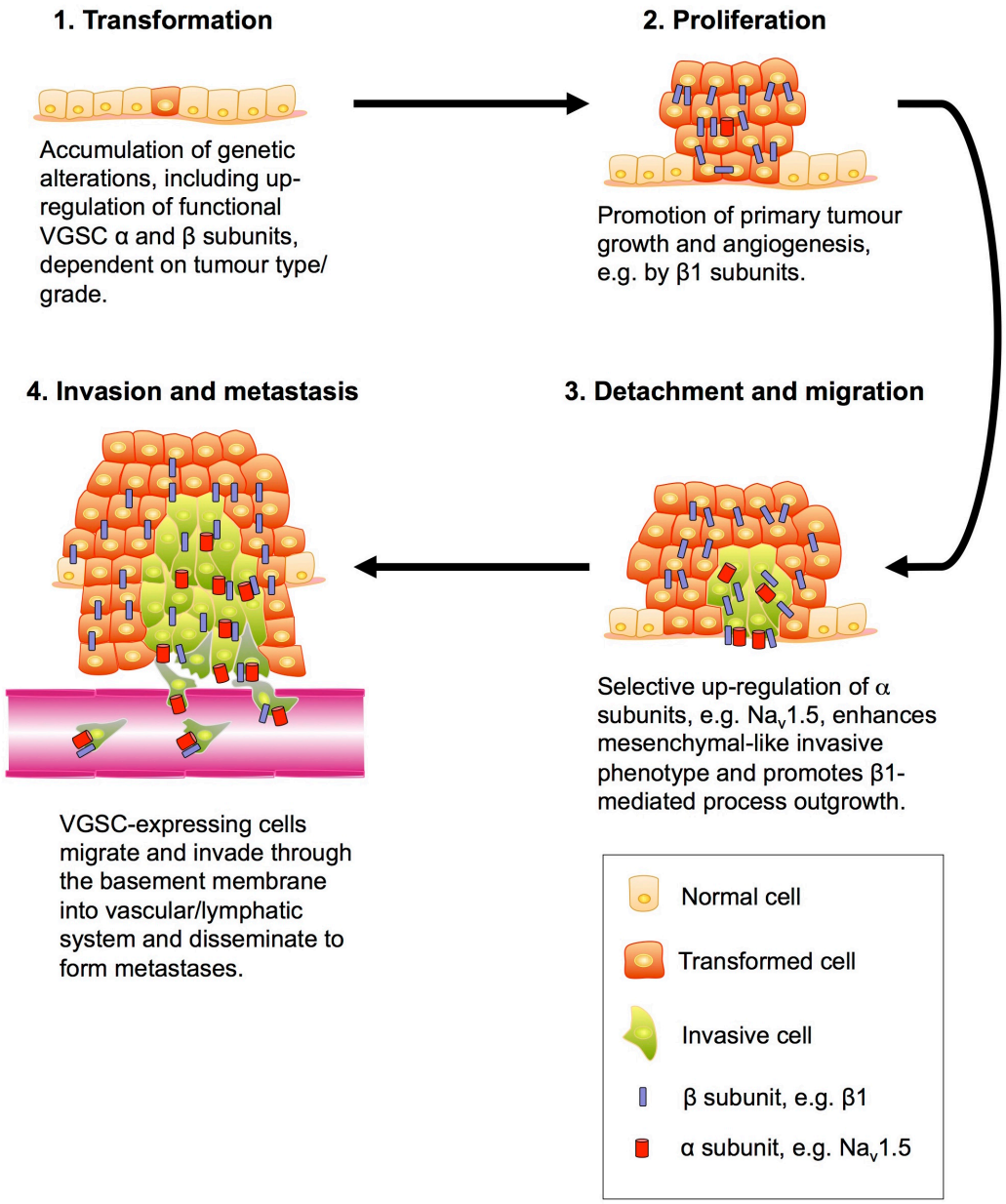

Figure 5. A model for VGSC involvement in cancer progression. β subunits are expressed in proliferating primary tumours, contributing to adhesion (Chioni *et al.*, 2009), and in the case of β1, promoting angiogenesis and resistance to apoptosis (Nelson *et al.*, 2014). Up-regulation of α subunits, e.g. $Na_v1.5$, promotes a mesenchymal-like phenotype (Brisson *et al.*, 2013, Nelson *et al.*, 2014), activation of proteases (Gillet *et al.*, 2009) and local invasion from the primary tumour (Brackenbury *et al.*, 2007, Fraser *et al.*, 2005, Roger *et al.*, 2003). VGSC-expressing cells subsequently intravasate and metastasise to distant sites (Fraser *et al.*, 2005, Jansson *et al.*, 2012, Nelson *et al.*, 2014). Figure adapted with permission (Brackenbury *et al.*, 2008b)