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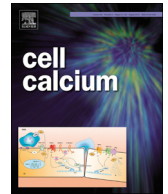
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# Emerging roles for multifunctional ion channel auxiliary subunits in cancer

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## ABSTRACT

Several superfamilies of plasma membrane channels which regulate transmembrane ion flux have also been shown to regulate a multitude of cellular processes, including proliferation and migration. Ion channels are typically multimeric complexes consisting of conducting subunits and auxiliary, non-conducting subunits. Auxiliary subunits modulate the function of conducting subunits and have putative non-conducting roles, further expanding the repertoire of cellular processes governed by ion channel complexes to processes such as trans-cellular adhesion and gene transcription. Given this expansive influence of ion channels on cellular behaviour it is perhaps no surprise that aberrant ion channel expression is a common occurrence in cancer. This review will focus on the conducting and non-conducting roles of the auxiliary subunits of various  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Cl}^-$  channels and the burgeoning evidence linking such auxiliary subunits to cancer. Several subunits are upregulated (e.g.  $\text{Ca}_v\beta$ ,  $\text{Ca}_v\gamma$ ) and downregulated (e.g.  $\text{K}_v\beta$ ) in cancer, while other subunits have been functionally implicated as oncogenes (e.g.  $\text{Na}_v\beta_1$ ,  $\text{Ca}_v\alpha_2\delta_1$ ) and tumour suppressor genes (e.g.  $\text{CLCA2}$ ,  $\text{KCNE2}$ ,  $\text{BK}_{\gamma_1}$ ) based on *in vivo* studies. The strengthening link between ion channel auxiliary subunits and cancer has exposed these subunits as potential biomarkers and therapeutic targets. However further mechanistic understanding is required into how these subunits contribute to tumour progression before their therapeutic potential can be fully realised.

## 1. Introduction

Ion channels are heteromeric membrane protein complexes which permit transmembrane ion conduction. Several ion channels, e.g.  $\text{K}^+$  channels and voltage-gated  $\text{Na}^+$  channels (VGSCs), are notable for regulating membrane potential in excitable cells [1], but an expanding repertoire of other cellular processes, such as proliferation, differentiation [2], cell volume control and migration [3,4], are also known to be influenced by ion channels. Owing to their extensive impact on cellular function, it is no surprise that ion channel dysregulation is a common characteristic in cancer [5]. Ion channels are often multimeric, with ion-conducting subunits accompanied by non-conducting auxiliary subunits [6]. Auxiliary subunit-mediated modulation of the conducting subunit is well established but increasing evidence has unveiled a multitude of non-conducting roles for these proteins as well [7–14]. An emerging field has focused on investigating auxiliary subunits in cancer, which, like the conducting subunits, are often aberrantly expressed and could represent novel therapeutic targets. In this review,

we dissect the conducting and non-conducting roles of the auxiliary subunits of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Cl}^-$  channels and the growing evidence supporting a link to cancer.

## 2. $\text{Ca}^{2+}$ channels

$\text{Ca}^{2+}$  channels regulate a multitude of cellular processes; accordingly, much research has focused on various  $\text{Ca}^{2+}$  channels in cancer, including voltage-gated  $\text{Ca}^{2+}$  channels (VGCCs) [15], STIM and Orai [16], and TRP channels [17]. In terms of  $\text{Ca}^{2+}$  channel auxiliary subunits however, only VGCC auxiliary subunits have received notable attention thus far. VGCCs are transmembrane complexes responsible for the inward  $\text{Ca}^{2+}$  current seen in excitable cells following depolarisation, however VGCCs are also expressed in other non-excitable cell types, e.g. osteoblasts and osteoclasts [18,19]. VGCCs are composed of a  $\text{Ca}^{2+}$ -conducting  $\alpha_1$  subunit ( $\text{Ca}_v1-3.x$ ) associated with multiple auxiliary subunits ( $\alpha_2\delta_{1-4}$ ,  $\beta_{1-4}$ ,  $\gamma_{1-8}$ ), with the exception of  $\text{Ca}_v3.x$ , which can form a T-type  $\text{Ca}^{2+}$  channel in the absence of an associated

**Abbreviations:** BK, large-conductance calcium-activated potassium channel; CaCC, calcium-activated chloride channel; CAM, cell-adhesion molecule; CLC, voltage-gated chloride channel; CLCA, chloride channel accessory; DREAM, downstream regulatory element antagonistic modulator; GIRK, G-protein inwardly rectifying potassium channel; KChIP, potassium channel interacting protein; Kir, inwardly-rectifying potassium channel; SUR, sulfonylurea receptor; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel; VGSC, voltage-gated sodium channel

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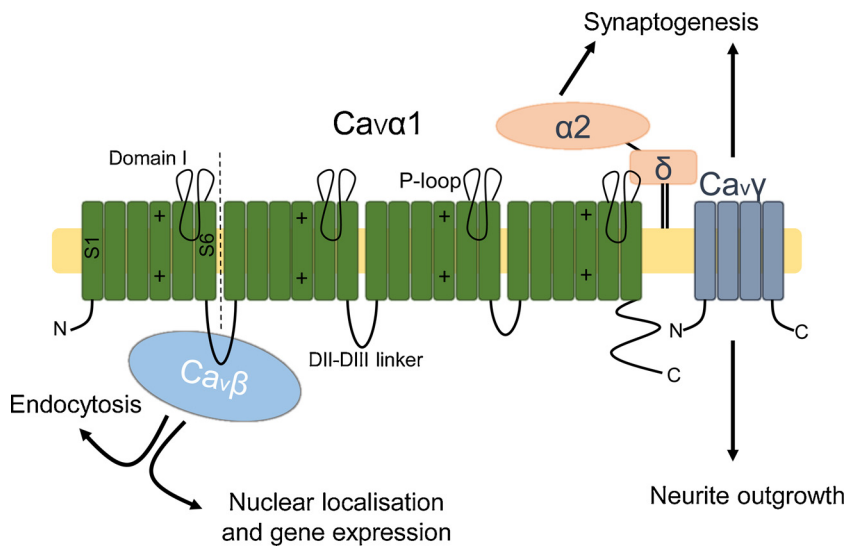
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**Fig. 1.** Voltage-gated  $\text{Ca}^{2+}$  channel auxiliary subunits. Voltage-gated  $\text{Ca}^{2+}$  channels (VGCCs) are composed of a conducting  $\alpha_1$  subunit accompanied and functionally modulated by  $\text{Ca}_v\beta$ ,  $\alpha_2\delta$  and  $\text{Ca}_v\gamma$  subunits [20].  $\alpha_1$  consists of four domains (domains I–IV), each consisting of six segments (S1–S6). The voltage-sensing domain is found within S4 of each domain and the pore consists of the P-loop found between S5–6 of each domain.  $\text{Ca}_v\beta$  modulates  $\text{Ca}^{2+}$  influx via binding the DI–DII linker of  $\alpha_1$ .  $\text{Ca}_v\beta$ s are also involved in regulating gene expression and endocytosis [22,36–38,40,44].  $\alpha_2\delta$  subunits are extracellular proteins that remain associated to the membrane via a GPI-anchor [54].  $\alpha_2\delta$  subunits are involved in synaptogenesis [65].  $\text{Ca}_v\gamma$  subunits are four-pass transmembrane proteins also involved in cervical ganglion neurite outgrowth and synaptogenesis [108,109].

auxiliary subunit (Fig. 1) [20]. A  $\text{Ca}_v1/2$  subunit is joined at the membrane by an  $\alpha_2\delta$ -,  $\beta$ -, and potentially a  $\gamma$ -subunit, although  $\gamma$ -subunits are not always precipitated with  $\text{Ca}_v\alpha$  [21].  $\text{Ca}_v\alpha_1$  subunits have an oncogenic influence in cancer [15]. Research into  $\text{Ca}_v$  auxiliary subunits in cancer is a growing field, but it appears  $\text{Ca}_v$  auxiliary subunits have both oncogenic and tumour-suppressive effects.

### 2.1. $\text{Ca}_v\beta$

The VGCC  $\beta$ -subunits are cytoplasmic proteins that interact with the  $\alpha_1$  DI–DII intracellular linker region [22–24].  $\beta$ -subunit binding enhances membrane expression of  $\alpha_1$  subunits [25,26], however the mechanism by which this occurs has not yet been elucidated. It is thought that  $\beta$ -subunit binding prevents ER retention and the subsequent degradation of  $\text{Ca}_v2.2$ , resulting in a higher proportion of  $\text{Ca}_v2.2$  at the plasma membrane [25,27]. However, membrane targeting of the DI–DII linker of  $\text{Ca}_v2.2$  via an inserted palmitoylation motif still results in ER retention and degradation, leading to the hypothesis that  $\text{Ca}_v\beta$  subunits are required for correct folding, and thus membrane insertion, of functional  $\alpha_1$  subunits [28]. The impact on electrophysiological properties of  $\alpha_1$  subunits by  $\text{Ca}_v\beta$ s is complex. In general,  $\text{Ca}_v\beta$ s increase current density and regulate activation/inactivation kinetics. For instance, disruption of the  $\text{Ca}_v\beta_3$ – $\text{Ca}_v2.2$  interaction by a small molecule inhibitor results in a decrease in current density and a depolarised shift in the voltage threshold of activation and inactivation [29]. In comparison,  $\text{Ca}_v\beta_2$  enhances the current density more than  $\text{Ca}_v\beta_3$ , potentially through increased membrane expression as  $\text{Ca}_v\beta_{2a}$ , unlike  $\text{Ca}_v\beta_3$ , contains a palmitoylation site [30]. Additionally, forced membrane localisation of  $\text{Ca}_v\beta_3$  using the N-terminal Lyn sequence enhanced the current density relative to WT-  $\text{Ca}_v\beta_3$  [30]. The complexity arises in the differential sensitivity to  $\text{PIP}_2$ -mediated modulation of different  $\text{Ca}_v\beta$ s [30,31], competition for  $\alpha_1$ -binding between  $\text{Ca}_v\beta$  subunits [32], the spectrum of functionally-distinct  $\text{Ca}_v\beta$  splice variants [33,34], and the opposing impacts on  $\alpha_1$ -function by the different domains within the  $\text{Ca}_v\beta$  protein [35].

$\text{Ca}_v\beta$ s are functional independent of direct  $\alpha_1$  association. All  $\text{Ca}_v\beta$ s demonstrate nucleus localisation,  $\text{Ca}_v\beta_4$  particularly within nucleoli, and gene expression regulation [36–39]. All  $\text{Ca}_v\beta$ s also contain a Src homology 3 domain capable of regulating endocytosis via interaction with dynamin and can interact with small GTPases [40,41].  $\text{Ca}_v\beta$ s show subunit-specific function as well, for instance  $\text{Ca}_v\beta_1$  is expressed in muscle progenitor cells (MPCs) earlier than  $\text{Ca}_v1.1$ , where it regulates proliferation and directly suppresses myogenin expression. Accordingly,  $\text{Ca}_v\beta_1$  knockout mice demonstrate impaired muscle development [36,42]. Similarly,  $\text{Ca}_v\beta_2$  is required for ventricle cell proliferation and

heart development in zebrafish, although pharmacological VGCC inhibition caused a similar phenotype, suggesting  $\text{Ca}_v\beta_2$  may be functioning in an  $\alpha_1$ -dependent manner [43].  $\text{Ca}_v\beta_2$  is also required for depolarisation-induced c-Fos and meCP2 activation, which intriguingly was shown to be independent of  $\text{Ca}^{2+}$  influx [37].  $\text{Ca}_v\beta_4$  regulates cell proliferation *in vitro* [44], downregulates Wnt signalling via sequestration of the Wnt pathway effector TCF4 [39], and regulates gene expression via various interacting partners [45,46]. Interestingly, the nuclear localisation of  $\text{Ca}_v\beta_4$  was inhibited when co-expressed with  $\text{Ca}_v1.1$  and only upon depolarisation and the presence of extracellular  $\text{Ca}^{2+}$  did  $\text{Ca}_v\beta_4$  interact with its nuclear signalling partner, B56 $\delta$  [45].

Owing to its role in driving cellular functions such as proliferation and migration, it is perhaps no surprise that  $\text{Ca}_v\alpha_1$  expression is increased in various cancers [47–49]. However, much research has also been dedicated to evaluating the involvement of  $\text{Ca}_v$  auxiliary subunits in cancer.  $\text{Ca}_v\beta_1$  expression is upregulated in colon cancer [50],  $\text{Ca}_v\beta_2$  mutations are seen in bladder cancer [51] and increased  $\text{Ca}_v\beta_3$  expression is observed in patients with recurrent non-small cell lung tumours compared to recurrence-free patients [52]. Furthermore, expression of  $\text{Ca}_v\beta_1$  and  $\text{Ca}_v\beta_3$  are included in proposed high-risk gene signatures that correlate with decreased patient survival in colon and recurring non-small cell lung cancer [50,52]. However, the aforementioned studies are largely limited to statistical observations based on tissue sequencing data that identified altered  $\text{Ca}_v\beta$  RNA expression as a high-risk prognostic marker [50–52]. Chen et al. (2016) offered additional pathophysiological justification for increased  $\text{Ca}_v\beta_2$  expression in cancer, by observing an enrichment in mutations of genes, including *CACNB2* which encodes  $\text{Ca}_v\beta_2$ , involved in NCAM-mediated neurite outgrowth [51].

### 2.2. $\alpha_2\delta$

The  $\text{Ca}_v \alpha_2\delta$  subunit has a unique structure compared to other auxiliary subunits. The translated polypeptide is proteolytically cleaved into two separate proteins,  $\alpha_2$  and  $\delta$ , which remain coupled by a disulphide bond [53]. The  $\alpha_2$  segment is extracellular while the  $\delta$ -subunit remains associated with the membrane via a GPI-anchor [54].  $\alpha_2\delta$  and  $\text{Ca}_v\beta$  subunits can both induce surface expression of  $\alpha_1$ , but also function synergistically to maximise  $\alpha_1$  surface expression and  $\text{Ca}^{2+}$  current [26,55,56]. Preventing proteolytic cleavage of the  $\alpha_2\delta_1$  pro-protein reduces both  $\text{Ca}_v2.2$  surface expression and presynaptic  $\text{Ca}^{2+}$  influx in hippocampal neurons [57] and site-directed mutagenesis of either cysteine residue involved in the disulphide interaction, which results in a dissociation of  $\alpha_2$ , reduces the whole-cell  $\text{Ca}^{2+}$  current [53]. Similarly, digestion of the GPI anchor of  $\alpha_2\delta_3$ , by prokaryotic

phosphatidylinositol-phospholipase C, results in a release of the  $\alpha_2\delta$  from the membrane and a decreased  $\text{Ca}^{2+}$  current [54]. Both these results suggest an intact  $\alpha_2\delta$  subunit is required at the membrane to induce and sustain the  $\alpha_2\delta$ -mediated regulation of  $\alpha_1$  subunits. In addition to its role in trafficking,  $\alpha_2\delta$  has been proposed to stabilise  $\alpha_1$  at the membrane by reducing internalisation and in targeting  $\alpha_1$  to detergent-resistant membranes [54,58]. Phenotypes of  $\alpha_2\delta$  knockout mice have been very informative, both  $\alpha_2\delta_1$  and  $\alpha_2\delta_3$  have thus been implicated in neuropathic pain, with  $\alpha_2\delta_1$ -overexpressing mice demonstrating hyperalgesia [59] and  $\alpha_2\delta_3$ -knockout mice demonstrating an enhanced insensitivity to pain [60]. Mice deficient in  $\alpha_2\delta_2$ , the isoform found overwhelmingly in cerebellar Purkinje neurons, present with seizures and ataxia [61]. Gabapentin, used in the treatment of epilepsy and neuropathic pain, preferentially binds to  $\alpha_2\delta_{1/2}$  and lowers  $\alpha_2\delta$  surface expression, demonstrating that the  $\alpha_2\delta$  auxiliary subunit is a druggable target [62–64]. All  $\alpha_2\delta$  subunits are involved in synaptogenesis, but potentially through different mechanisms [65].  $\alpha_2\delta_1$  promotes cortical synaptogenesis, independently of  $\text{Ca}^{2+}$  influx, through binding to secreted astrocytic thrombospondin in the postsynaptic membrane and promoting actin remodelling via Rac-1 [66], whereas loss of  $\alpha_2\delta_4$  causes impaired retinal synaptogenesis, which correlates with a decrease in presynaptic  $\text{Ca}_v1.4$  [67,68].

More is known about the involvement of  $\alpha_2\delta$  subunits in cancer compared to the other  $\text{Ca}_v$  auxiliary subunits. Increased  $\alpha_2\delta_1$  expression occurs in both ovarian and hepatocellular tumour-initiating cells and correlates with decreased overall survival and a shorter progression-free survival in clinical ovarian samples [69–71]. Zhao et al. developed a monoclonal antibody against  $\alpha_2\delta_1$ , 1B50-1 [71]. Sorting of a 1B50-1-positive subpopulation of Hep-11 cells, a hepatocellular carcinoma (HCC) cell line, resulted in a subset of cells that initiated tumour formation in all implanted mice, whereas the 1B50-1-negative subpopulation failed to form any tumours. Furthermore, 62/86 of HCC samples were 1B50-1-positive compared to 0/6 normal tissue samples. *in vivo* experimentation demonstrated that administering 1B50-1 reduced tumour volume following implantation of two HCC cell lines and increased survival, especially when co-administered with doxorubicin, compared to doxorubicin or 1B50-1 alone. Lastly, *in vitro* work in the same study demonstrated  $\alpha_2\delta_1$  to be involved in maintaining cell viability and spheroid formation, via increasing  $\text{Ca}^{2+}$  influx through L-type and N-type  $\text{Ca}^{2+}$  channels and MAPK signalling [71]. In non-small cell lung cancer cells,  $\alpha_2\delta_1$  expression confers radioresistance *in vitro*, by enhancing the DNA repair response, and chemoresistance *in vivo*, potentially through MAPK signalling [72,73]. In addition, various miRNAs that are downregulated in cancer target  $\alpha_2\delta_1$  expression, including hsa-miR-208a-3p and hsa-miR-1207-5p in medulloblastoma [74], and miR-107 in chronic myeloid leukaemia (CML) [75]. Overexpressing miR-107 promotes differentiation in CML cell lines, which is reversed when expression of  $\alpha_2\delta_1$  is restored [75].

The involvement of  $\alpha_2\delta_2$  in cancer is complex, as  $\alpha_2\delta_2$  can be both oncogenic and tumour suppressive [76,77].  $\alpha_2\delta_2$  was initially identified as a potential tumour suppressor gene as it is encoded by *CACNA2D2*, which is absent in the 3p21.3 chromosomal deletion commonly observed in lung and breast cancer [78]. Similarly, *CACNA2D2* is deleted in cervical carcinoma [79], is commonly methylated in head and neck squamous cell carcinoma [80], is downregulated in lung squamous cell carcinoma via miR-205 [81], and its expression correlates with improved survival in patients with lung adenocarcinoma [82]. Functionally, *in vitro* experiments using various non-small cell lung cancer cell lines have demonstrated that overexpression of  $\alpha_2\delta_2$  induces apoptosis via mitochondrial cytochrome-c release and subsequent caspase activation [77]. In contrast,  $\alpha_2\delta_2$  overexpression occurs in prostate tumours [76] and in insulin-secreting pancreatic adenomas, where elevated intracellular  $\text{Ca}^{2+}$  is known to stimulate  $\beta$ -cell proliferation [83]. Furthermore,  $\alpha_2\delta_2$  overexpression in prostate cancer cells induces tumorigenesis and angiogenesis in mice, which is treatable by administering the  $\alpha_2\delta_2$  inhibitor, gabapentin [76].

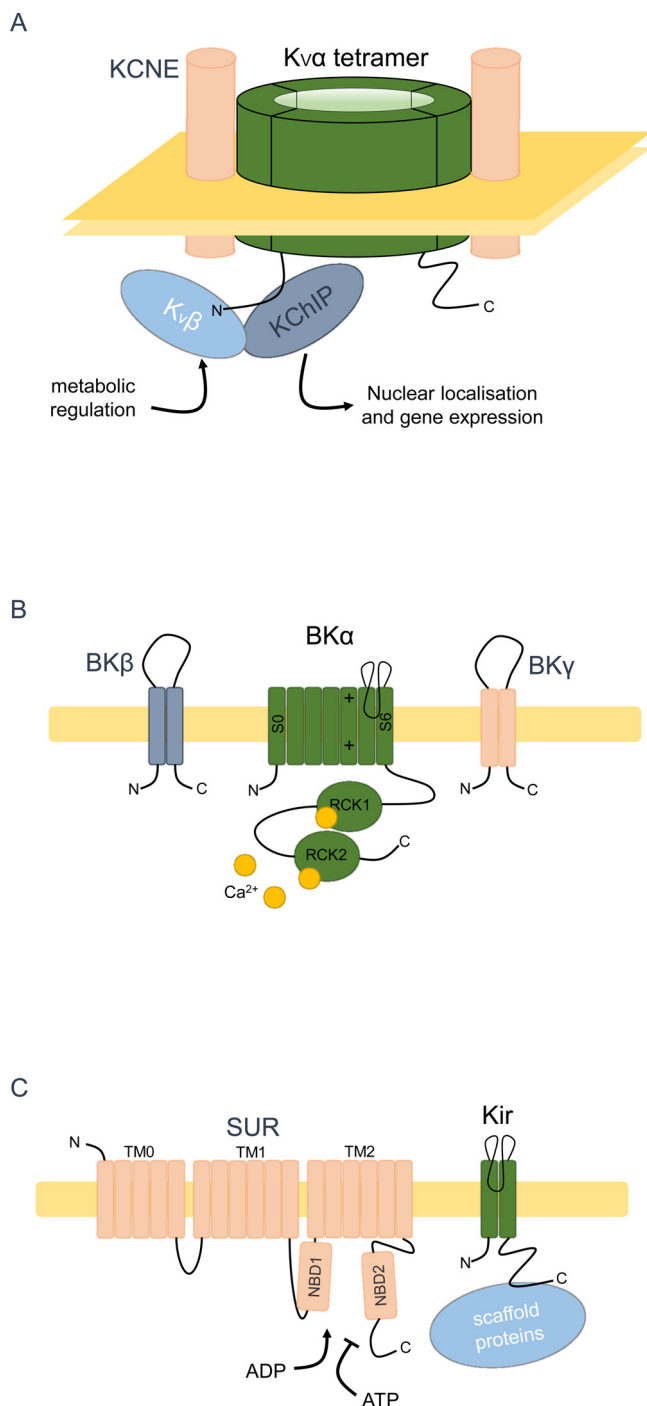
Conversely,  $\alpha_2\delta_3$  is considered a tumour suppressor gene, as downregulation or deletion is seen in nasopharyngeal cancer [84], breast cancer [85], oesophageal squamous cell carcinoma [86,87], gastric cancer [88,89], lung cancer [90] and cholangiocarcinoma [91]. Mice implanted with cancer cells overexpressing  $\alpha_2\delta_3$  show a decreased tumour volume, compared to implanted control cells, in nasopharyngeal cancer [84], oesophageal cancer [87] and glioma [92] models. The consensus mechanism points towards an inhibition of motility and invasion by  $\alpha_2\delta_3$ , and induction of apoptosis through an increase in intracellular  $\text{Ca}^{2+}$ , leading to mitochondria-induced apoptosis [84,87,92].

### 2.3. $\text{Ca}_v\gamma$

The interaction between  $\text{Ca}_v\gamma$ -subunits and  $\alpha_1$  subunits is less well understood.  $\text{Ca}_v\gamma$ -subunits were originally identified following immunoprecipitation of the skeletal muscle 1,4-dihydropyridine (DHP) receptor (later known as L-type VGCCs), which yielded  $\gamma_1$  as a binding partner [93,94]. Following the discovery of  $\text{Ca}_v\gamma_1$ , seven more  $\text{Ca}_v\gamma$ -subunits were identified by homology studies [95–98].  $\text{Ca}_v\gamma_2$  and  $\text{Ca}_v\gamma_3$  have been shown to associate with  $\text{Ca}_v2.1$  [99],  $\text{Ca}_v\gamma_{2-4}$  to  $\text{Ca}_v2.2$  [99] and  $\text{Ca}_v\gamma_6$  to  $\text{Ca}_v3.1$  [100]. Using cryo-electron microscopy, the  $\gamma$ -subunit was predicted to interact with the  $\text{Ca}_v1.1$  voltage-sensing domain (S4) of domain IV [24]. However, the  $\alpha_1$ - $\gamma$  coupling remains contentious as more recent efforts failed to precipitate a  $\text{Ca}_v\gamma$ -subunit with  $\text{Ca}_v2$ . Further,  $\text{Ca}_v\gamma_2$  can regulate  $\text{Ca}_v2.2$  indirectly, suggesting a direct coupling may not be necessary for  $\text{Ca}_v\gamma$ -induced channel modulation [21,101].  $\text{Ca}_v\gamma$ -subunit mRNA is expressed in skeletal muscle ( $\gamma_{1,6,7}$ ) and brain ( $\gamma_{2,8}$ ) as well as other tissues such as kidney, liver, colon, testis and lung [98]. Functionally,  $\text{Ca}_v\gamma$ -subunits negatively regulate VGCC-mediated  $\text{Ca}^{2+}$  influx by decreasing channel expression and current amplitude [102], hyperpolarising the voltage threshold of inactivation, accelerating channel inactivation [103], and increasing the time taken for recovery from inactivation [96].  $\text{Ca}_v\gamma$ -induced regulation of  $\text{Ca}^{2+}$  influx observed at the cellular level is supported by the *Star-gazer* mouse mutant, which lacks  $\text{Ca}_v\gamma_2$  and presents with ataxia and absence seizures [104]. Interestingly, a subclass of  $\text{Ca}_v\gamma$ -subunits,  $\gamma_{2/3/4/5/8}$  (known as transmembrane AMPA receptor regulatory proteins [TARPs]), which localise to the brain [105], interact with ionotropic AMPA receptors and induce membrane localisation [106,107]. Other functions of  $\gamma$ -subunits include  $\text{Ca}_v\gamma_7$ -induced neurite outgrowth in superior cervical ganglion neurons [108] and  $\text{Ca}_v\gamma_2$ -induced synaptogenesis [109].

Aberrant  $\text{Ca}_v\gamma$  expression is seen in various cancers, including increased  $\text{Ca}_v\gamma_1$  in early progressing human epidermal growth factor-positive (HER2+) metastatic breast cancer [110], increased  $\text{Ca}_v\gamma_4$  in bladder squamous cell carcinoma [111] and increased  $\text{Ca}_v\gamma_7$  in leiomyoma via downregulation of miR-197 [112]. Furthermore, a prediction algorithm using a dataset of 1.7 million cancer mutations identified  $\text{Ca}_v\gamma_3$  as a putative oncogene [113]. Similar to  $\text{Ca}_v\beta$ , the functional role of  $\text{Ca}_v\gamma$  in cancer is not yet clear. However, a  $\text{Ca}_v\gamma_4$  mutation appears in a cluster of mutations involved in MAPK signalling [111], suggesting a possible role in regulation of mitogenesis.

In summary, although  $\text{Ca}_v\alpha_1$  subunits have an oncogenic role [15], it is not yet clear whether  $\text{Ca}_v$  auxiliary subunits function through  $\text{Ca}_v\alpha_1$  or have secondary functions in cancer, or both. Given that  $\text{Ca}_v\beta$  and  $\text{Ca}_v\gamma$  are both oncogenic but have antagonistic effects on  $\alpha_1$  function, and  $\text{Ca}_v\alpha_2\delta$  can be oncogenic or tumour suppressive, it would seem that the involvement of auxiliary subunit-mediated  $\text{Ca}^{2+}$  influx in cancer is tumour type/stage-specific, dependent on the expression profile of other subunits, or subordinate to a secondary function of the auxiliary subunit.  $\text{Ca}_v$  auxiliary subunits have functions, potentially  $\alpha_1$ -independent, that could contribute to oncogenesis and tumour progression. All  $\text{Ca}_v\beta$ s regulate gene expression and interact with small GTPases [36–38,40,41,44].  $\text{Ca}_v\beta_1$  and  $\text{Ca}_v\beta_2$  are also essential for maintaining proliferation and cellular plasticity during development



**Fig. 2.**  $K^+$  channel auxiliary subunits. (A) Voltage-gated  $K^+$  channels (VGKCs). The conducting subunit,  $K_v\alpha$  forms tetramers within the membrane that are accompanied and functionally modulated by four  $K_v\beta$ s (for  $K_v1$ ), four RChIPs (for  $K_v4$ ) or two KCNEs ( $K_v7.1$ ) [119–122]. The function of  $K_v\beta$  is modulated by pyridine nucleotides [143]. RChIPs are involved in regulating gene expression [173]. (B) Large conductance  $Ca^{2+}$ -activated  $K^+$  (BK) channels. BK channels consist of a  $K^+$ -conducting, seven-pass (S0–S6) membrane protein subunit ( $BK\alpha/Slo$ ) accompanied and modulated by dual-pass  $BK\beta$  and  $BK\gamma$  [182,187]. S0 of  $BK\alpha$  is required for interaction with  $BK\beta$ , S4 is involved in voltage-sensing, the pore region is formed by the linker of S5–6 and an enlarged C-terminus containing two RCK (regulator of conductance of  $K^+$ ) domains sense intracellular  $Ca^{2+}$  [330]. (C) Inwardly rectifying  $K^+$  ( $K_{ir}$ ) channels. Tetrameric  $K_{ir}6$  subunits, containing the  $K^+$ -conducting pore, are functionally regulated at the membrane by 17-pass SUR subunits (1:1 stoichiometry), which confer ATP-sensitivity onto  $K_{ir}6$  via NBDs (nucleotide binding domains) [196].  $K_{ir}1-4$  can be bound and modulated by various C-terminal binding proteins [331].

[36,43]. The TARP family of  $Ca_v\gamma$ s induce AMPA receptor membrane trafficking [107], a receptor with an emerging involvement in cancer [114,115], and  $Ca_v\gamma_4$  and  $Ca_v\gamma_7$  induce transcellular adhesion and neurite outgrowth respectively [108,109].  $\alpha_2\delta_1$  is also involved in transcellular adhesion [66]. Furthermore, increased  $Ca^{2+}$  conductance potentially underpins both the oncogenic function of  $\alpha_2\delta_1$  and  $\alpha_2\delta_2$  [71,83] and the tumour suppressive function of  $\alpha_2\delta_2$  and  $\alpha_2\delta_3$  [77,92].

### 3. $K^+$ channels

$K^+$  channels represent an extensive superfamily of channels, many of which have been implicated in regulating key elements of tumour progression [116–118]. Here, we focus on the function and involvement in cancer of the auxiliary subunits of the voltage-gated  $K^+$  channel (VGKC), BK channel and  $K_{ir}$  channel complexes (Fig. 2A–C). VGKC  $\alpha$ -subunits represent a diverse family of forty  $K^+$ -conducting proteins,  $K_v1-12.x$ , which conduct an outward  $K^+$  current in response to depolarisation of the membrane potential. Three classes of VGKC auxiliary subunits have been identified:  $K_v\beta_{1-3}$ , RChIP1–4, and KCNE1–5 which canonically interact with  $K_v1$ ,  $K_v4$ , and  $K_v7.1$  respectively [119–122], although  $K_v\beta$ s and KCNEs interact with other VGKC  $\alpha$ -subunits and  $K_v\beta$ s also interact with TRPV1 and  $K_2P2.1$  [123–126]. The activity of  $K_v1$  [116,127],  $K_v4$  [128], and  $K_v7.1$  [129] is upregulated in various cancers. However, the expression pattern of VGKC auxiliary subunits in cancer is more complex.

#### 3.1. $K_v\beta$

$K_v\beta$  subunits are cytoplasmic proteins, which form homo- or heterotetramers [130] that are involved in trafficking of  $K_v1$  and  $K_v4.3$  to the cell surface [131–133]. Additionally,  $K_v\beta_2$  is involved in targeted axonal trafficking of  $K_v1.2$  and  $K_v\beta_1$  differentially regulates the  $K_v$  composition in ventricular myocytes [134,135].  $K_v\beta_1$  and  $K_v\beta_3$  modulate VGKC  $\alpha$ -subunits via an N-terminal ball domain, which permits rapid inactivation of delayed-rectifying  $K_v1$   $\alpha$ -subunits [136,137].  $K_v\beta_1$  also slows deactivation, accelerates slow inactivation and hyperpolarises activation of  $K_v1.2$  [138].  $K_v\beta_2$  lacks the ability to inactivate delayed-rectifying  $K_v1$  channels, but does hyperpolarise channel activation [139].  $K_v\beta_1$  and  $K_v\beta_2$  are both expressed in developing rat heart and skeletal muscle and during induced myogenesis of L6E9 cells [140]. Furthermore, deletion of  $K_v\beta_1$  results in aberrant cardiac electrical activity and cardiac hypertrophy in female mice [141].  $K_v\beta_2$  deletion leads to reduced  $K_v1.5$  surface expression in coronary arterial myocytes and a reduction in total skeletal muscle volume, potentially mediated through downregulation of Pax7 and upregulation of NEDD4 [133,142]. Interestingly,  $K_v\beta$ s are part of the aldo-keto reductase (AKR) superfamily owing to their C-terminal AKR domain. The AKR domain allows for binding and functional modulation by pyridine nucleotides (NAD and NADP).  $NADP^+$  inhibits  $K_v\beta_1$ - and  $K_v\beta_3$ -mediated inactivation of  $K_v1.5$  as well as inhibiting  $K_v\beta_2$ -mediated hyperpolarisation of  $K_v1.5$  activation [143,144].

Evidence suggests that  $K_v\beta$ s are downregulated in cancer.  $K_v\beta_1$  is downregulated in malignant thyroid carcinomas relative to benign thyroid adenomas [145,146]. The gene encoding  $K_v\beta_2$  is the most significant site of methylation in non-functional (non-hormone secreting) pituitary adenoma compared to functional (hormone-secreting) adenomas and is one of the genes ablated in the common 1p36.3 chromosome deletion seen in neuroblastoma [147,148]. Methylation of the promoter of the gene encoding  $K_v\beta_3$  is seen in oral squamous cell cancers relative to adjacent normal tissue [149]. Together, these data suggest  $K_v\beta$ s are tumour suppressor genes, but in depth *in vitro* and *in vivo* characterisation of  $K_v\beta$  in cancer is still currently lacking.

#### 3.2. KCNE

KCNEs are single-pass transmembrane proteins that interact

primarily with  $K_v7$ ; two KCNEs interact with tetrameric  $K_v7$  [150]. *In vitro* studies document a range of effects of KCNEs on  $K_v7.1$ . For example, KCNE1 and KCNE3 both increase surface expression and current density, while KCNE4 and KCNE5 have no effect on current density [151]. KCNE2 and KCNE3 interaction with  $K_v7.1$  produces voltage-insensitive channels and all KCNEs depolarise the activation voltage of  $K_v7$ , with KCNE4 and KCNE5 depolarising activation to a non-physiological membrane potential [151].  $K_v7.1$  has a well-established role in cardiac rhythm and in regulating osmotic and salt transport across gastrointestinal, cochlear and renal epithelia; this is reflected in *Kcne1* knockout mice demonstrating atypical QT intervals, hair cell degeneration, impaired renal fluid, glucose and electrolyte uptake, and faecal  $Na^+$  and  $K^+$  wasting [152–155]. Furthermore, mutations in *KCNE1* underlie Long QT Syndrome 5 and Jervis and Lange-Nielsen syndrome, a disorder characterised by deafness and cardiac arrhythmia [156,157].

With regard to cancer, KCNE1–3 are expressed in uterine cancer cell lines, in which they influence proliferation [158] and a 5-fold and 3-fold upregulation of KCNE3 and KCNE4 respectively has been reported in glioblastoma datasets [159]. Paradoxical to the upregulation of KCNE1 in uterine cancer cell lines, KCNE1 overexpression in an astrogloma cell line (U87-MG) induces apoptosis and *KCNE1* is one of the four genes deleted in the 21q22.12 microdeletion which causes a predisposition to acute myelogenous leukaemia [160,161]. The apoptotic influence of KCNE1 in U87-MG cells is proposed to occur through canonical  $K^+$  efflux through  $K_v7.1$ , inducing decreased cytoplasmic  $K^+$ , a known apoptotic trigger [160,162], whereas KCNE1 induces uterine cancer cell proliferation via modulation of HERG channels [158,163]. HERG channels induce proliferation in a range of cell lines and HERG channel inhibition decreases MAPK phosphorylation and c-fos expression in MDA-MB-435S cells [164]. Out of all the  $K_v$  auxiliary subunits however, KCNE2 has the most established link to cancer. KCNE2 downregulation is observed in gastric cancer tissue and gastric cancer cell lines, correlates with gastritis cystica profunda development (pre-neoplastic condition characterised by large gastric cysts) and is a risk factor in gastric cancer stratification [165–167]. Furthermore, *Kcne2* knockout mice display a 6-fold increase in stomach size, an upregulation of Ki67 and Cyclin D1 in gastric mucosa, an increase in the metaplastic marker TFF2, pyloric adenomas and neoplastic invasion compared to wild-type mice [168]. Overexpression of KCNE2 in the SGC7901 gastric cancer cell line reduces proliferation and significantly reduces xenograft tumour volume compared to parental SGC7901 cells [167].

KCNE2- $K_v7.1$  complexes, in the apical membrane of non-excitabile gastric parietal cells, are essential for maintaining acidification of the stomach, as KCNE2 transforms  $K_v7.1$  to a constitutively open channel that is potentiated by extracellular  $H^+$  [169]. Luminal  $K^+$  released by KCNE2- $K_v7.1$  is then recycled back into the parietal cell, in exchange for  $H^+$ , via the  $H^+/K^+$  ATPase, resulting in gastric acidification [169,170]. *Kcne1* knockout mice demonstrate reduced  $H^+$  secretion, reduced gastric acidification, gastric hyperplasia and atypical  $K_v7.1$  localisation [170]. However, it is not yet known whether KCNE2 downregulation contributes to gastric cancer progression through a failure to acidify the lumen of the stomach or via its role in regulating tumour cell proliferation.

### 3.3. KChIP

$Ca^{2+}$ -sensing  $K_v$  channel interacting proteins (KChIPs) are involved in  $K_v4$  channel modulation. KChIPs increase surface channel density, hyperpolarise the voltage of activation, slow inactivation and accelerate the recovery from inactivation [119,171]. KChIPs were identified by a yeast 2-hybrid screen searching for interaction partners with  $K_v4.2/3$  N-termini [119]. Interestingly, KChIP3 was already known as calsenilin/downstream regulatory element antagonistic modulator (DREAM). KChIP3/DREAM plays a key role in differentiation and apoptosis independently of  $K^+$  channels [172]. DREAM binds upstream genetic

elements (DRE sites) as a tetramer and represses transcription of the downstream gene until upon  $Ca^{2+}$  stimulation, DREAM tetramers dissociate from DNA allowing gene transcription [173]. Despite KChIP3 being the first  $Ca^{2+}$ -sensing transcriptional repressor identified, the other KChIPs are also capable of DRE-site binding [174]. DREAM expression is required for maintenance of human embryonic stem cell pluripotency; DREAM knockdown by siRNA results in an increase in apoptosis and spontaneous differentiation [172]. Potentially independent of its nuclear role, DREAM expression induces  $Ca^{2+}$ -mediated apoptosis possibly through sequestration of hexokinase I from mitochondria [175,176]. Additionally, DREAM expression induces process outgrowth in pheochromocytoma PC12 cells by RhoA inactivation and induces thrombus formation in anucleate platelets via PI3K stimulation [177,178]. There is currently limited evidence of a role for KChIPs in cancer. However, one study identified KChIP4 gene disruption in a renal cancer cell chromosomal break [179]. In addition, KChIP1 upregulation and KChIP3 downregulation have been shown in glioblastoma multiforme, with KChIP2 upregulation correlating with decreased survival for glioblastoma patients [180]. The involvement of KChIP3/DREAM in regulating differentiation, apoptosis, transcellular adhesion and process outgrowth suggests cancer-expressed or down-regulated KChIPs could be a worthwhile subject of further study.

### 3.4. BK channels

Large conductance  $Ca^{2+}$ -activated  $K^+$  (BK) channels are seven membrane-pass  $K^+$  channels that conduct a particularly large outward  $K^+$  current synergistically in response to membrane depolarisation and a rise in intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) [181]. BK channels can be stimulated by depolarisation or increased  $[Ca^{2+}]_i$  alone, however the required membrane potential ( $V_{1/2} = 168$  mV at  $[Ca^{2+}]_i = 0$ ) or  $[Ca^{2+}]_i$  ( $EC_{50} \geq 10$   $\mu$ M at resting membrane potential) are out of physiological range [182]. BK channels are expressed in most tissues and are involved in a range of functions, such as learning and memory [183], pain modulation [184] and blood pressure regulation [185]. BK channels are upregulated in glioblastoma primary cells and promote proliferation and invasion [117,186]. BK channel function is modulated by two groups of auxiliary subunits-  $BK\beta_{1,4}$  and  $BK\gamma_{1,4}$ , both double-pass membrane proteins.  $BK\beta_1$  and  $BK\beta_2$  increase  $Ca^{2+}$  sensitivity [187],  $BK\beta_2$  hyperpolarises and accelerates channel activation [188],  $BK\beta_3$  depolarises channel activation [188] and  $BK\beta_4$  hyperpolarises channel activation whilst simultaneously inhibiting channel opening at low  $[Ca^{2+}]_i$  but enhancing activation at high  $[Ca^{2+}]_i$  [189].  $BK\gamma$  subunits hyperpolarise BK channel activation [190].  $BK\gamma_1$  hyperpolarises channel activation to such an extent ( $-140$  mV in LNCaP prostate cancer cells) that BK channels open without the need for increased  $[Ca^{2+}]_i$  at resting membrane potentials [182].

Despite the extensive involvement of BK channels in a range of physiological processes, the link between BK channel auxiliary subunits and cancer is still very tentative, with thus far only  $BK\gamma_1$  implicated. There are conflicting reports on the involvement of  $BK\gamma_1$  (also known as LRRC26 and CAPC) in cancer.  $BK\gamma_1$  is upregulated in the MDA-MB-456 breast cancer cell line and in metastatic secondary breast cancer tumours compared to the primary tumour of a single patient [191].  $BK\gamma_1$  is also upregulated in many breast and prostate cancer cell lines and breast, prostate, colon and pancreatic samples [192,193]. However,  $BK\gamma_1$  is frequently methylated in triple-negative breast cancer specimens and cell lines and siRNA knockdown of  $BK\gamma_1$  in the triple-negative HCC70 breast cancer cell line enhances anchorage-independent growth, invasion, migration, and NF- $\kappa$ B activity [194]. Similarly, knockdown of  $BK\gamma_1$  expression enhances anchorage-independent growth in LNCaP cells and overexpression of  $BK\gamma_1$  in the triple-negative MDA-MB-231 breast cancer cell line downregulates NF- $\kappa$ B activity and inhibits tumourigenesis and metastasis in nude mice [195]. Furthermore,  $BK\gamma_1$  expression is lowest in poorly differentiated and highly invasive prostate and breast cancer lines [195]. Thus,  $BK\gamma_1$  appears to have

oncogenic and tumour-suppressive function depending on the cancer type. At this stage, the mechanism by which BK $\gamma_1$  performs these functions in cancer cells is unclear. BK channels may thus perform multiple functions in cancer cells, dependent on, or independent of, BK $\gamma_1$ .

### 3.5. $K_{ir}$ channels

Inwardly-rectifying  $K^+$  ( $K_{ir}$ ) channels are double pass membrane proteins which form tetramers in the membrane [196].  $K_{ir}$  channels lack a voltage sensor domain.  $I_{K_{ir}}$  is instead dictated by the electrochemical gradient and an increasing intracellular blocking of the pore when the membrane potential ( $E_m$ ) >  $E_K$ , resulting in an inward  $I_K$  when  $E_m$  <  $E_K$  and an outward  $I_K$  when  $E_m$  >  $E_K$ , which is progressively blocked as  $E_m$  rises [197].  $K_{ir}$  channels are therefore important for maintenance of the hyperpolarised resting membrane potential and regulating activity in excitable cells, such as vascular smooth muscle [198], central neurons [199] and cardiomyocytes [200]. Subfamilies of  $K_{ir}$  channels exist that are ATP-sensitive ( $K_{ATP}$  channels;  $K_{ir}6.x$ ) and G-protein gated (G-protein inwardly rectifying  $K^+$  channels- GIRKs;  $K_{ir}3.x$ ) [201,202].  $K_{ATP}$  channels are inhibited by ATP/stimulated by ADP. They function as metabolic sensors, for instance in smooth muscle where  $K_{ATP}$  channels regulate vascular tone [203]. GIRKs facilitate G-protein-mediated inhibitory neurotransmitter signalling, such as GABA signalling [204,205].

Certain  $K_{ir}$  channels are regulated by auxiliary subunits.  $K_{ir}6$  binds sulfonylurea receptors (SUR) 1 or 2 in an octameric conformation (tetrameric  $K_{ir}6$  plus tetrameric SUR) to form a  $K_{ATP}$  channel [196]. Channel assembly is required before  $K_{ATP}$  is released from the endoplasmic reticulum [206]. SUR subunits impart differential sensitivity to ADP/ATP and are the binding target of sulfonylureas, a common form of treatment for type 2 diabetes mellitus [207,208]. SUR1 is overexpressed in cerebral metastases where it decreases vascular permeability [209]. Resveratrol binds to and inhibits SUR1, inducing apoptosis in HEK293 cells, suggesting a potential pro-survival function of SUR1 [210]. SUR2B expression is present in leiomyoma and metastatic breast cancer cells and glibenclamide, a sulfonylurea targeting SUR proteins, inhibits proliferation in these cells [211,212]. SUR2 expression, along with  $K_{ir}6.2$ , is upregulated in cervical cancer biopsies [213]. In addition, the effectiveness of glibenclamide at inhibiting proliferation correlates with the  $K_{ir}6.2$  expression of the cell line tested, suggesting proliferation is dependent on SUR and  $K_{ir}6.2$  activity [213]. Glibenclamide also inhibits proliferation in MDA-MB-231 breast cancer cells, inducing G0/G1 cell cycle arrest through an upregulation of P27 and reduction of cyclin E [212]. Treatment of MDA-MB-231 cells with the  $K_{ATP}$  channel opener, minoxidil, conversely induces proliferation, suggesting  $K^+$  influx underlies  $K_{ATP}$ -regulated proliferation [212]. Glibenclamide treatment also prevents tumour growth *in vivo* in Sprague-Dawley rats treated with N-nitroso-N-methylurea [214]. Furthermore, in insulinoma, a pancreatic  $\beta$ -cell cancer characterised by insulin release, which is regulated by  $K_{ATP}$  channels, SUR1 expression is increased [215]. In summary, SUR subunits appear to play an oncogenic role in a  $K_{ir}$ -dependent manner.

## 4. $Na^+$ channels

There is a growing body of evidence supporting a role for  $Na^+$  channels in regulating various aspects of cancer progression [216,217]. With regard to auxiliary subunits, however, only those of the VGSC have been characterised to date and will therefore be the focus of this section (Fig. 3).

### 4.1. Voltage-gated $Na^+$ channels

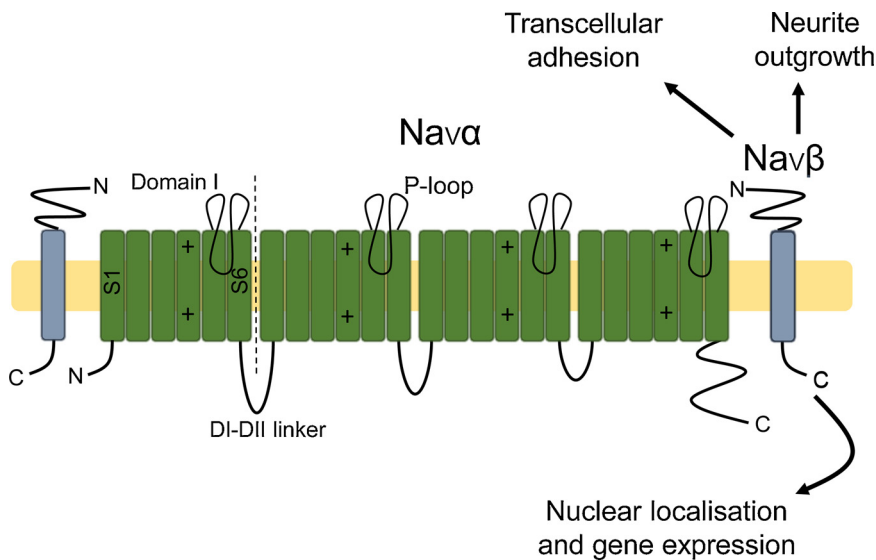
VGSCs conduct an inward  $Na^+$  current in response to membrane depolarisation [218]. VGSCs are composed of a pore-forming  $\alpha$ -subunit

( $Na_v1.1$ – $1.9$ ) and auxiliary  $\beta$ -subunits ( $Na_v\beta_1$ – $Na_v\beta_4$ ).  $Na_v\beta$ s are single pass transmembrane glycoproteins that bind  $Na_v\alpha$  covalently, in the case of  $Na_v\beta_2$  and  $Na_v\beta_4$  [219,220], or non-covalently, in the case of  $Na_v\beta_1$  and  $Na_v\beta_3$  [221–223].  $I_{Na}$  is responsible for propagation of action potentials and mutations in  $Na_v\beta$ s underlie certain types of epilepsy [224] and cardiac arrhythmia [225].  $Na_v\beta_{1-3}$  traffic  $Na_v\alpha$  to the cell surface [226–228] and all  $Na_v\beta$ s increase  $I_{Na}$  [229–231].  $Na_v\beta$ s induce other changes in  $Na_v\alpha$  gating kinetics, including accelerated recovery from inactivation [232,233] and accelerated inactivation [230,234].  $Na_v\beta$ s can both positively and negatively shift the voltage of activation [235,236] and inactivation [222,226], possibly dependent on endogenous expression of  $Na_v$  subunits and other  $Na_v$ -interacting proteins in the experimental system used.  $Na_v\beta$ s are also cell adhesion molecules, owing to the presence of an extracellular immunoglobulin loop [237–240], which permits  $Na_v\beta$ -mediated neurite outgrowth [241–244].  $Na_v\beta_1$  plays an important role in regulating neuronal migration in CNS development, particularly in the cerebellum [14,245], and  $Na_v\beta_2$  promotes dendritic expansion during hippocampal development via a  $Na_v\alpha$ -independent mechanism [243].  $Na_v\beta$  subunits are also substrates for proteolytic processing by secretases [246,247] and evidence suggests that the cleaved intracellular domain of  $Na_v\beta_2$  shuttles to the nucleus to regulate expression of  $\alpha$ -subunit genes [248].

Emerging evidence suggests that  $Na_v\beta$ s play diverse functional roles in cancer.  $Na_v\beta_1$  is upregulated in breast cancer samples and is more highly expressed in strongly metastatic, compared to weakly metastatic, prostate cancer cell lines [249,250]. Overexpression of  $Na_v\beta_1$  in the MDA-MB-231 breast cancer cell line promotes primary tumour growth and metastasis to multiple organs when grafted into mice, compared to parental MDA-MB-231 cells [249]. The  $Na_v\beta_1$ -induced increase in primary and secondary tumour growth was accompanied by a decrease in apoptotic cleaved caspase-3 staining, no change in proliferative Ki67 staining, and an increase in endothelial CD31 staining, suggesting increased apoptotic resistance and vascularisation underlie the oncogenic influence of  $Na_v\beta_1$  [249]. *In vitro*, MDA-MB-231- $Na_v\beta_1$  cells demonstrate increased cell-cell adhesion, VGSC-mediated  $Na^+$  current and neurite-like process outgrowth, which is reversible by inhibiting  $I_{Na}$  [249,251]. Interestingly, MDA-MB-231- $Na_v\beta_1$  cells show decreased *in vitro* motility and proliferation compared to MDA-MB-231 cells and knockdown of endogenous  $Na_v\beta_1$  in the MCF-7 breast cancer cell line increases cell migration [251]. Similarly,  $Na_v\beta_1$  is also expressed in cervical cancer cells where it inhibits motility [252]. Furthermore, treatment of mouse melanoma B16F10 cells with the anti-cancer polymethoxyflavone, casticin, inhibits cell migration and invasion and causes a concomitant genomic upregulation of *SCN1B* (encoding for  $Na_v\beta_1$ ) [253].  $Na_v\beta_1$  therefore appears to have a negative influence on cell behaviour *in vitro* and potentially induces tumour growth and metastasis through an increase in apoptotic resistance and transcellular adhesion.

$Na_v\beta_2$  also appears to be oncogenic.  $Na_v\beta_2$  expression is increased in strongly metastatic prostate cancer cell lines relative to weakly metastatic cell lines [254]. Perineural invasion is common in invasive prostate cancer, and LNCaP prostate cancer cells overexpressing  $Na_v\beta_2$  demonstrate an increased association with *ex vivo* murine spinal cord axons and an increase in migration, invasion and growth [254,255]. Despite the invasion-promoting behaviour of  $Na_v\beta_2$  *in vitro*, overexpression of  $Na_v\beta_2$  in LNCaP cells inhibits tumour growth, compared to LNCaP cells, when implanted into mice, suggesting the functional contribution of  $Na_v\beta_2$  might be site or stage-specific during cancer progression [255].

Unlike  $Na_v\beta_1$  and  $Na_v\beta_2$ ,  $Na_v\beta_3$  and  $Na_v\beta_4$  are considered tumour-suppressive. *SCN3B* (encoding for  $Na_v\beta_3$ ) expression is strongly upregulated by p53 following DNA damage and  $Na_v\beta_3$  expression induces apoptosis and suppresses colony formation in osteosarcoma and glioblastoma cell lines [256].  $Na_v\beta_4$  expression is downregulated in thyroid and high-grade breast cancer and is associated with favourable survival [231,257]. Downregulation of  $Na_v\beta_4$  in MDA-MB-231 breast cancer



**Fig. 3.** Voltage-gated  $\text{Na}^+$  channel auxiliary subunits. Voltage-gated  $\text{Na}^+$  channels (VGSCs) contain a conducting  $\text{Na}_v\alpha$  subunit and auxiliary  $\text{Na}_v\beta$  subunits.  $\text{Na}_v\alpha$  consists of four domains (domains I–IV), each containing six segments (S1–S6). The voltage-sensing domain is found within S4 of each domain and the pore consists of the P-loop found between S5–6 of each domain.  $\text{Na}_v\beta$ s function as cell adhesion molecules via an extracellular immunoglobulin domain [238,239,332].  $\text{Na}_v\beta$ s also induce neurite outgrowth and migration [245] and the intracellular domain of  $\text{Na}_v\beta_2$  has putative transcription regulation function [248].

cells with shRNA increases primary tumour growth and metastasis in xenograft mice models, relative to MDA-MB-231 cells overexpressing  $\text{Na}_v\beta_4$  [231]. Furthermore, loss of  $\text{Na}_v\beta_4$  increases  $\text{Na}_v\alpha$ -independent RhoA-mediated cancer cell migration and invasion [231].  $\text{Na}_v\beta_4$  also suppresses invasion in cervical cancer cells [252].  $\text{Na}_v\beta$ s are structurally very similar and generally have a broadly comparable effect increasing  $I_{\text{Na}}$ , so it is intriguing that  $\text{Na}_v\beta_1$  and  $\text{Na}_v\beta_2$  are oncogenic, whereas  $\text{Na}_v\beta_3$  and  $\text{Na}_v\beta_4$  are tumour-suppressive. Additionally, both  $\text{Na}_v\beta_1$  and  $\text{Na}_v\beta_4$  were investigated using the same breast cancer cell, MDA-MB-231, so the endogenous VGSC subunit expression accompanying the  $\text{Na}_v\beta$ -subunit is comparable [231,249]. Both  $\text{Na}_v\beta_1$  and  $\text{Na}_v\beta_4$  inhibit cell migration *in vitro* and induce neurite outgrowth in developing neurons, thus it is unclear where the functional discrepancy between the two proteins lies [231,241,251,258].

## 5. $\text{Cl}^-$ channels

$\text{Cl}^-$  channels are a family of relatively poorly understood proteins that facilitate transmembrane  $\text{Cl}^-$  transport.  $\text{Cl}^-$  concentration is highest intracellularly and  $E_{\text{Cl}} \sim -30$  to  $-60$  mV, so channels conduct an outward  $\text{Cl}^-$  current at resting membrane potentials that can reverse on depolarisation, although inwardly and outwardly rectifying  $\text{Cl}^-$  channels have been identified [13].  $\text{Cl}^-$  channels are involved in regulating a range of bodily functions, including renal salt retention [259], synaptic inhibition [260], skeletal muscle contraction [261], smooth muscle tone [262] and sperm motility [263]. Various subfamilies of  $\text{Cl}^-$  exist, but only the voltage-gated  $\text{Cl}^-$  channel (CLC) and  $\text{Ca}^{2+}$ -sensitive  $\text{Cl}^-$  channel (CaCC) subfamilies possess auxiliary subunits with a robust link to cancer (Fig. 4A, B).

### 5.1. Voltage-gated $\text{Cl}^-$ channels

CLCs represent a range of cell surface  $\text{Cl}^-$  channels (CLC-1,2,K) and intracellular  $\text{Cl}^-$  exchangers (CLC-3–7). Some CLCs are regulated by auxiliary subunits; CLC-2 by GlialCAM [264,265], CLC-7 by Ostm1 [266], and CLC-K by Barttin [267]. GlialCAM targets CLC-2 to cell-cell junctions, increases  $\text{Cl}^-$  current ( $I_{\text{Cl}}$ ), accelerates  $I_{\text{Cl}}$  activation, and abolishes CLC-2 inward rectification and pH sensitivity [264]. GlialCAM also functions as a cell adhesion molecule via an extracellular immunoglobulin domain [268,269]. CLC-7 is an intracellular, electrogenic  $\text{H}^+/\text{Cl}^-$  exchanger involved in lysosomal acidification [270]. Interestingly, CLC-7 regulates the trafficking and expression of its auxiliary subunit, Ostm1 [266,271]. Nevertheless, Ostm1 is required to activate CLC-7 function [270]. Barttin traffics CLC-K to the cell surface, resulting

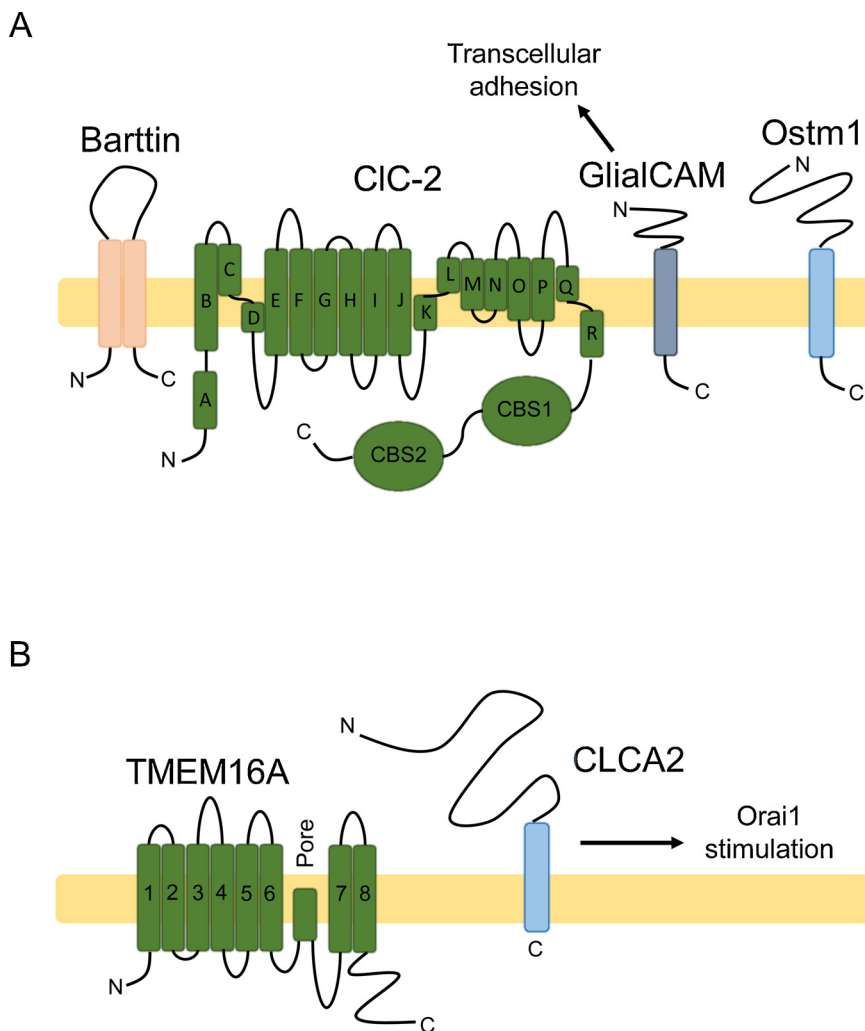
in increased  $I_{\text{Cl}}$ , and abolishes the voltage-dependence of CLC-K [272–274]. Mutations in the gene encoding Barttin are the cause of Bartter syndrome type IV, characterised by hypokalaemia, blood alkalosis and hypotension [275,276]. Knockin mice with the disease-causing Barttin mutation R8L present with reduced plasma membrane Barttin-CLC-K complexes and transepithelial  $\text{Cl}^-$  transport is impaired in the loop of Henle [277].

GlialCAM (also called HepaCAM) was identified as a putative tumour suppressor gene that is silenced in hepatocellular carcinoma [278]. GlialCAM downregulation is observed in liver, bladder, prostate, kidney, breast, uterus, colon, stomach, and rectal cancer biopsies [269,278–282]. Functionally, when GlialCAM is expressed in the liver carcinoma cell line HepG2, cell motility and adhesion are increased, colony formation is reduced, and proliferation is reduced [278]. Similarly, when expressed in MCF-7 breast cancer cells, GlialCAM increases cell motility and adhesion, decreases proliferation, and induces p53-mediated cellular senescence [279,283]. GlialCAM inhibits proliferation and  $\beta$ -catenin signalling in bladder carcinoma cells [284,285]. Furthermore, in renal carcinoma cells, GlialCAM decreases proliferation, induces cell cycle arrest, and stimulates c-Myc degradation [286]. GlialCAM expression is also sufficient for reducing Notch-mediated invasion and migration in prostate cancer cells [282]. Lastly, GlialCAM stabilises connexin-43 at cell-cell gap junctions [287], connexin-43 being a potential tumour suppressor itself [288,289]. In summary, GlialCAM has a strong anti-proliferative influence when expressed in cancer cells, which could underpin its role as a tumour suppressor.

### 5.2. $\text{Ca}^{2+}$ -sensitive $\text{Cl}^-$ channels

Four single membrane-pass auxiliary subunits of CaCCs have been identified (known as  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channel regulator or  $\text{Cl}^-$  channel accessory [CLCA]1–4) [290,291]. Interestingly, the molecular identities of the conducting subunits were only discovered later and termed Best1–4 and TMEM16 [292–295]. CaCCs demonstrate voltage-dependence at steady-state, which is abolished following an increase in  $[\text{Ca}^{2+}]_i$  [296]. Increased  $[\text{Ca}^{2+}]_i$  also increases  $I_{\text{Cl}}$  and accelerates current onset [296]. CaCCs are expressed in epithelia and excitable tissues, where they regulate excitability [297], smooth muscle contraction [298] and fluid secretion [299]. Expression of CLCA1 and CLCA2 in HEK293 cells induces an enlarged and outwardly-rectifying  $I_{\text{CaCC}}$  [290,300]. More recent work has demonstrated that the secreted N-terminus of CLCA1, produced following autoproteolysis, is sufficient to stabilise TMEM16A at the membrane, increasing  $I_{\text{CaCC}}$  [301–303]. CLCA1 contains an intrinsic metalloprotease domain in the N-terminus





**Fig. 4.**  $\text{Cl}^-$  channel auxiliary subunits. (A) CLCs are a subfamily of voltage-sensitive  $\text{Cl}^-$  channels and transporters found at the plasma membrane and internal membranes [13]. Barttin modulates CIC-K, GlialCAM modulates CIC-2 and Ostm1 modulates the intracellular CIC-7 transporter [264,266,267]. CLCs are composed of eighteen helical domains and two C-terminal cystathionine- $\beta$ -synthase (CBS) domains which facilitate dimerization [333]. Depicted is the plasma membrane CIC-2 which interacts with single-pass GlialCAM, the only CIC auxiliary subunit implicated in cancer [264]. GlialCAM can also function as a cell adhesion molecule [268]. (B) Two separate CaCC conducting subunits exist- TMEM16 and Bestrophin. Depicted is eight-pass TMEM16A which is modulated directly by secreted CLCA1 and indirectly by single-pass CLCA2 [303,305]. CLCA2 stimulates  $\text{Ca}^{2+}$  store replenishment by interacting with Orai1 and STIM1 [305].

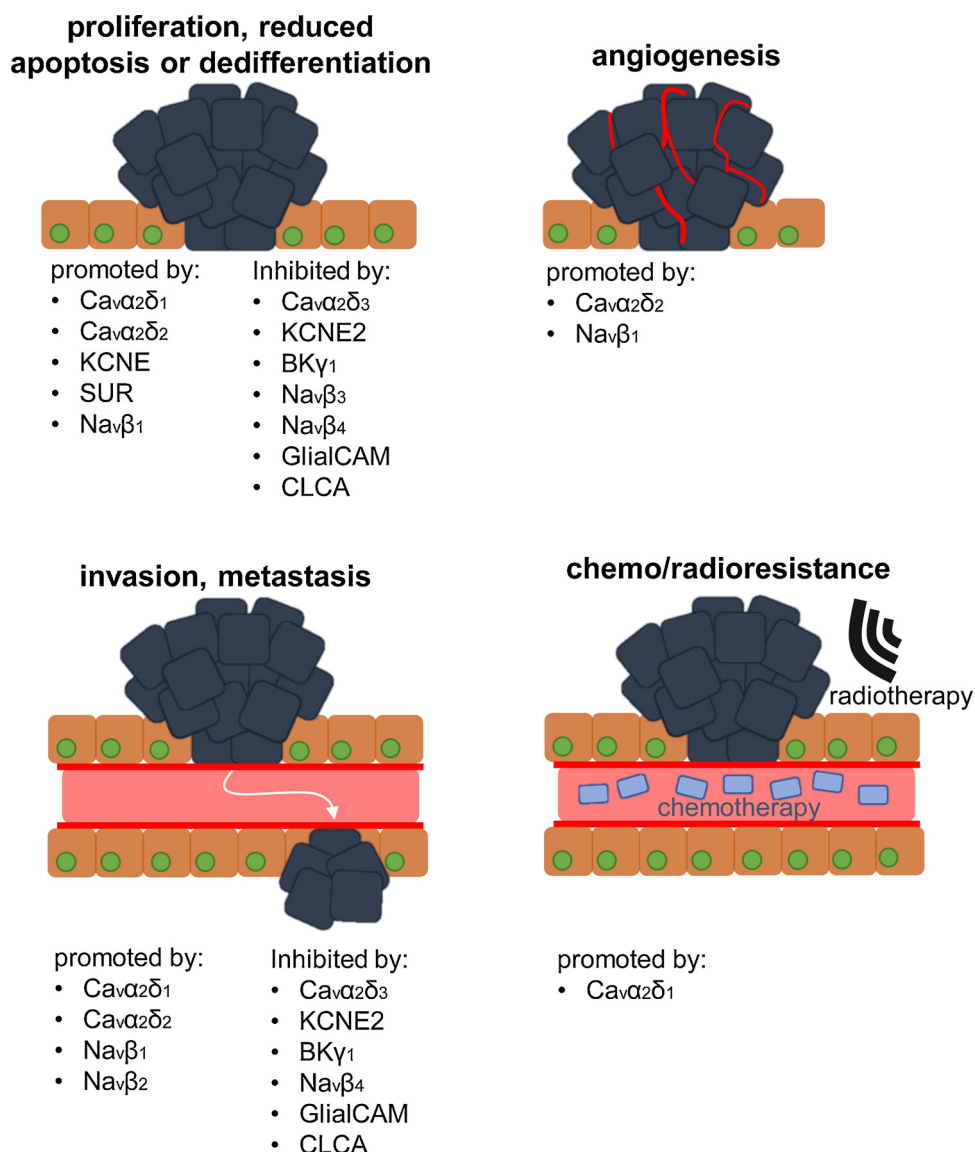
that is thought to be responsible for autoproteolysis and regulating mucus turnover in the colon [304]. Despite CLCA2 enlarging  $I_{\text{CaCC}}$ , CLCA2 does not interact directly with TMEM16 or Best1 [305]. Instead, CLCA2 interacts directly with store-operated  $\text{Ca}^{2+}$  channels, Orai1 and STIM-1, stimulating ER  $\text{Ca}^{2+}$  replenishment following cytosolic depletion [305].

CLCAs have a well-documented tumour-suppressive role [306–308]. CLCA1 is downregulated in colorectal and pancreatic cancer specimens [306,309–311]. CLCA1 knockdown induces proliferation and inhibits differentiation of caco-2 colorectal cancer cells [311]. Furthermore, CLCA1 overexpression inhibits Wnt signalling and colorectal tumour growth and metastasis *in vivo* [306]. CLCA2 expression is also decreased in high-grade nasopharyngeal, colorectal, lymphoid and breast cancer specimens compared to low grade samples [307,312–314]. Expression of CLCA2 decreases nasopharyngeal and breast tumorigenesis *in vivo* [307,312,315]. Similarly, CLCA2 depletion increases the number of circulating prostate tumour cells in mice [316]. At a cellular level, CLCA2 inhibits Wnt signalling [317], decreases invasion [315], inhibits proliferation [312], induces transcellular adhesion [316], inhibits epithelial-to-mesenchymal transition [312,316], induces differentiation [316,318], inhibits focal adhesion kinase [312,319] and induces p53-mediated cellular senescence [320]. The ability of CLCA2 to inhibit cancer cell migration appears to be  $I_{\text{Cl}}$  independent, as inhibiting  $I_{\text{Cl}}$  has a further anti-migratory effect in cells expressing CLCA2 as well as having an anti-migratory effect in cells not expressing CLCA2 [312]. Ramena et al. observed CLCA2 at cell-cell junctions, interacting with EVA1/ZO-1 or  $\beta$ -catenin [317]. Sequestration of  $\beta$ -catenin at the

plasma membrane was therefore suggested as a mechanism for CLCA2-induced inhibition of epithelial-to-mesenchymal transition. CLCA4 expression is decreased in bladder, hepatocellular and breast cancer specimens compared to adjacent normal tissue [308,321,322]. CLCA4 expression also decreases tumourigenicity in mice [321]. Furthermore, CLCA4 depletion induces epithelial-to-mesenchymal transition via PI3K/Akt signalling [308,322]. Despite the abundance of evidence implicating CLCAs as tumour suppressor genes, CLCAs have also been implicated in induction of lung colonization *in vivo* via adhesive interactions between endothelial CLCA and  $\beta_4$  integrin expressed on circulating cancer cells [323,324]. Similarly, increased CLCA2 expression is seen in circulating lung adenocarcinoma cells and ovarian cancer cell aggregates [325,326], suggesting CLCAs may potentially be tumour suppressors on the one hand, and metastasis-promoting on the other.

## 6. Conclusion

Many ion channel auxiliary subunits are upregulated, e.g.  $\text{Ca}_v\beta_3$ , or downregulated, e.g.  $\text{K}_v\beta_3$ , in tumours and thus may represent novel cancer biomarkers. *in vitro* and *in vivo* experimentation has further implicated various auxiliary subunits in tumour formation and progression, such as  $\text{Na}_v\beta_1$  and  $\alpha_2\delta_1$  (Fig. 5). However, others, e.g. CLCAs,  $\text{Na}_v\beta_{3/4}$ , may function as tumour suppressors. Clearly, it is important from a treatment perspective to understand the mechanistic function of ion channel auxiliary subunits, including the extent that they contribute to cancer progression through potentiating ion conductance or via non-conducting signalling. For example,  $\alpha_2\delta_1$ - and  $\alpha_2\delta_2$ -induced  $\text{Ca}^{2+}$



**Fig. 5.** Involvement of ion channel auxiliary subunits in different stages of tumour progression. A number of different ion channel auxiliary subunits are up- or down-regulated in cancer cells promoting proliferation, reducing apoptosis and differentiation. Other auxiliary subunits have been shown to regulate angiogenesis, invasion, and metastasis, thus promoting tumour progression. Finally, ion channel auxiliary subunits may also play a role in chemo/radioresistance, underscoring the potential importance of these proteins in relation to therapeutic intervention.

influx may promote hepatocellular carcinoma cell sphere formation and pancreatic adenoma proliferation respectively [71,83]. Other examples include Nav $\alpha$ -dependent, Nav $\beta$ 1-mediated process outgrowth and the extent of glibenclamide-induced inhibition of SUR2-mediated cancer cell proliferation correlating with the mRNA expression of Kir6.2 [213,249]. Validating the contribution of ion conductance to the oncogenic function of these auxiliary subunits would provide a potential therapeutic target, as many ion channel inhibitors are already in clinical use and could be repurposed [327–329]. On the other hand, numerous auxiliary subunits many regulate cancer progression via non-conducting roles, e.g. regulation of transcription, proliferation and differentiation by Cav $\beta$ 1 and KChIP3 [36,172]. Various auxiliary subunits also function as adhesion molecules in cancer cells, e.g. GlialCAM, CLCAs and Nav $\beta$ s [254,278,316]. Further work is required to fully delineate the diverse functional contributions of these subunits to carcinogenesis, tumour progression and metastasis, and understand their potential as novel therapeutic targets.

#### Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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