Potassium and Calcium Channel Complexes as Novel Targets for Cancer Research

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Running title: Ion channel complexes in cancer

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Abstract

The intracellular Ca^{2+} concentration is mainly controlled by Ca^{2+} channels. These channels form complexes with K^+ channels, which function to amplify Ca^{2+} flux. In cancer cells, voltage-gated/voltage-dependent Ca^{2+} channels and non-voltage-gated/voltage-independent Ca^{2+} channels have been reported to interact with K^+ channels such as Ca^{2+}-activated K^+ channels and voltage-gated K^+ channels. These channels are activated by an increase in cytosolic Ca^{2+} concentration or by membrane depolarisation, which induces membrane hyperpolarisation, increasing the driving force for Ca^{2+} flux. These complexes, composed of K^+ and Ca^{2+} channels, are regulated by several molecules including lipids (ether-lipids and cholesterol), proteins (e.g., STIM), receptors (e.g., S1R/SIGMAR1) and peptides (e.g., LL-37), and can be targeted by monoclonal antibodies, making them novel targets for cancer research.

Keywords: Ca^{2+} channels; K^+ channels; SIGMAR1; STIM; LL-37; Lipids; Cancer

Abbreviations

AQP5	Aquaporin 5
AMP	Antimicrobial peptide
ARC	Arachidonic acid-regulated Ca^{2+} channels
BCR	B cell receptor
BiP	Binding immunoglobulin protein
BKCa	Big conductance calcium-activated potassium channel
CaM	Calmodulin
CaV	Voltage-gated/voltage-dependent Ca^{2+} channel
CLL	Chronic lymphocytic leukaemia
CRC	Colorectal cancer
DHA	Docosahexaenoic acid
DRM	Detergent-resistant membrane
EAG1	Ether-à-go-go K^+ channel 1
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
ENaC	Epithelial Na channel
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
FAK	Focal adhesion kinase
FRET	Fluorescence resonance energy transfer
hERG	Human ether-à-go-go-related gene
HCC	Hepatocellular carcinoma

IKCa	Intermediate conductance calcium-activated potassium channel
KCa	Calcium-activated potassium channel
KCNE2	Potassium voltage-gated channel subfamily E member 2
KCNE3	Potassium voltage-gated channel subfamily E member 3
KCNH1	Potassium voltage-gated channel subfamily H member 1
KCNH2	Potassium voltage-gated channel subfamily H member 2
KCNQ1	Potassium voltage-gated channel subfamily Q member 1
KCNN4	Potassium calcium-activated channel subfamily N member 4
Kv	Voltage-gated potassium channel
LL-37	Cathelicidin antimicrobial peptides
LB	Lymphocyte B
mAb56	Monoclonal antibody 56
mAb62	Monoclonal antibody 62
Ohmline	1-O-hexadecyl-2-O-methyl-*sn*-glycero-3-lactose
PIP2	Phosphatidylinositol bisphosphate
PRL-3	Phosphatase of regenerating liver-3
PI3K	Phosphoinositide 3-kinase
PUFA	Polyunsaturated fatty acid
SICE	Store-independent calcium entry
SIGMAR1	Sigma-1 receptor
S1R	Sigma-1 receptor
SOCE	Store-operated calcium entry
SPCA2	Secretory pathway Ca^{2+} ATPase 2
SK3	Small conductance calcium-activated potassium channel type 3
SKCa	Small conductance calcium-activated potassium channel

STIM1	Stromal interacting molecule 1
mSTIM1	Membrane stromal interaction molecule 1
$STIM1_{PM}$	Stromal interacting molecule 1 plasma membrane
TGFβ	Transforming growth factor β
TNM	Tumour/node/metastasis
TRAAK	TWIK-related arachidonic acid-stimulated K+ channel
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
TREK-1	TWIK1-related K^+ channel
TRP	Transient receptor potential
TRPC1	Transient receptor potential canonical 1
TRPV2	Transient receptor potential cation channel subfamily V member 2
TWIK	Two pore domain weak inward rectifying K^+

1. Introduction

The intracellular Ca^{2+} concentration (cytosolic and within intracellular organelles) is mainly controlled by ion channels and transporters. Ca^{2+} channels are protein molecules that span the cell membrane, allowing the passage of Ca^{2+} from one side of the membrane to the other. Other ion channels like K^+ channels act in cooperation to amplify this Ca^{2+} flux. The formation of such ion channel complexes in cancer cells represents the gain of a new biological function that did not exist for the individual channel. This suggests that, in order to evolve, the cancer cell could take advantage of the association between K^+ and Ca^{2+} channels as complexes. Voltage-gated/voltage-dependent Ca^{2+} channels (CaV) and non-voltage-gated/voltage-independent Ca^{2+} channels (Transient receptor potential, TRP, Orai and their partners, including stromal interacting molecule [STIM]) have been reported to control various biological functions of tumour cells, including proliferation and migration/invasion, and have been proposed as drug targets to inhibit cancer progression (Bong and Monteith, 2018; Buchanan and McCloskey, 2016; Déliot and Constantin, 2015; Mignen *et al.*, 2017). In addition, various K^+ channels, including voltage-gated K^+ channels (Kv) and Ca^{2+}-activated K^+ channels (KCa), have been reported to act as Ca^{2+} channel partners, acting as amplifiers of Ca^{2+} entry. Among these K^+ channels, Kv10.1 (also known as ether-à-go-go K^+ channel 1 [EAG1] and member 1 of the K^+ voltage-gated channel subfamily H (EAG-related) [KCNH1]), Kv11.1 (also known as hERG or KCNH2), the big conductance BKCa (also known as KCa1.1), the intermediate conductance IKCa (also known as KCa3.1 or SK4) and the small conductance SK3 (also known as KCa2.3) channels were found to form functional complexes with Ca^{2+} channels. Indeed, following activation by an increase in cytosolic Ca^{2+} concentration for KCa channels or by membrane depolarisation for Kv channels, these K^+ channels induce membrane hyperpolarisation, increasing the driving force for Ca^{2+} entry. In

addition, these channel complexes are regulated by lipids, receptors and peptides and can be targeted by monoclonal antibodies, making them novel targets for cancer research (Figure 1).

2. Calcium and potassium channel complexes

A few years ago, we reviewed the role of Ca^{2+} and K^+ complexes in cancer, as well as their role in controlling constitutive Ca^{2+} entry (Gueguinou *et al.*, 2014; Mignen *et al.*, 2017). Since these reviews were published, it was revealed that these complexes are not limited to two ion channels, and multicomplexes of ion channels can involve more than two channels. Indeed, in colon cancer cells, Gueguinou *et al.* (2016) detailed the role of SK3 and Ca^{2+} channels in colon cancer cell migration. In these complexes, Orai1 and TRPC1 were found to be associated with SK3, which localised in nanodomains only after phosphorylation of reticular STIM1. These complexes not only control constitutive Ca^{2+} entry, but also store-operated Ca^{2+} entry (SOCE), which is activated by the depletion of endoplasmic reticulum (ER) Ca^{2+} stores and store-independent Ca^{2+} entry (SICE). Indeed, ion channels form a complex triggered by STIM1 and regulating a singular mode for SK3 in regulating Orai1/TRPC1-dependent SOCE. Interestingly, we found that epidermal growth factor (EGF) activated these complexes, SOCE and cell migration, and that antiepidermal growth factor receptor (EGFR) monoclonal antibodies act on EGFR to modulate SOCE activated by these complexes, leading to induction or a reduction in cancer cell migration (depending on the antibody tested). More recently, the role of Orai1 and TRPC1 channels in the constitutive Ca^{2+} entry into B lymphocytes was demonstrated (Debant *et al.*, 2019; Garaud *et al.*, 2018). This Ca^{2+} influx is regulated by the pool of STIM1 located at the plasma membrane (mSTIM1, also named $STIM1_{PM}$), which is increased in B cells of patients with chronic lymphocytic leukaemia (CLL) with a pejorative clinical score and high lymphoproliferation rate. This constitutive Ca^{2+} entry represents an innovative therapeutic target for cancer, which

is completely independent from SOCE entry supported by Orai1 channels and activated by B cell receptor (BCR) engagement in B cells.

K_V10.1 is a voltage-gated K^+ channel belonging to the superfamily of KCNH channels. These channels have well-known roles in cardiac physiology, cell proliferation and neuronal excitability. The mRNA expression of this channel is mostly restricted to the brain, but expression has also been found in the testis and adrenal gland. Under physiological conditions, protein expression is strictly detected in the brain, as specified in the human atlas project (Uhlen *et al.*, 2015). However, K_V10.1 has been found to be expressed in gastric and colorectal cancers and in oesophageal squamous cell carcinomas (Ding *et al.*, 2008, 2007a, 2007b). In these cancers, K_V10.1 mRNA and protein were detected in over 70% of tumour samples and in adenomas, but not in adjacent matched tissues, suggesting a link between K_V10.1 expression and cancer onset or development. Interestingly, K_V10.1, which was initially described as a voltage-gated channel, was also found to be inhibited by Ca^{2+} signalling via a calmodulin (CaM)-dependent mechanism (Schonherr, 2000). Marques-Carvalho *et al.* (2016) showed that the C lobe of CaM binds to the cytoplasmic BDC2 fragments of K_V10.1. In this complex, the channel adopts an unusual conformation leading to its deactivation. Recent research by Ouadid-Ahidouch's team described the functional role of a complex composed of the Kv10.1 K^+ channel, the Orai1 Ca^{2+} channel and the secretory pathway Ca^{2+} ATPase (SPCA2). Their cooperation in this complex was found to promote collagen I-induced breast cancer cell survival and proliferation (Badaoui *et al.*, 2018; Peretti *et al.*, 2019), as SPCA2 enhances membrane expression of both Kv10.1 and Orai1, leading to SICE.

3. **Proteins associated with potassium and calcium channels**

Numerous dysregulated signalling pathways are involved in cancer progression, and those

involving plasma membrane proteins are good drug candidates in terms of their accessibility. Among the plasma membrane proteins that interact with Ca^{2+} channels and are deregulated in cancer, STIM1 appears to be a promising target. In fact, in the large body of available literature, STIM1 is known to be primarily located in the ER membrane and acts as a Ca^{2+} sensor, linking store depletion to store-operated Ca^{2+} channels. Many recent reviews have reported the mechanistic processes involved in STIM1 activation and its role in SOCE, as well as its deregulation and involvement in cancer (for review: Nelson and Roe, 2018; Qiu and Lewis, 2019). However, STIM1 was initially identified as a protein located at the plasma membrane that is involved in rhabdoid tumour growth suppression and myoblastic cell division regulation (Manji *et al.*, 2000; Williams *et al.*, 2002). In fact, $STIM1_{PM}$ was first thought to be a tumour suppressor protein. If the constitutive presence of STIM1 at the plasma membrane is no longer demonstrated, the role of $STIM1_{PM}$ is far from being elucidated. The role of $STIM1_{PM}$ in the activation of store-independent arachidonic acid-regulated Ca^{2+} (ARC) channels, supported by Orai3, has been known for more than 10 years (Shuttleworth *et al.*, 2007; Thompson *et al.*, 2013). This store-independent Ca^{2+} entry was later described as Ca^{2+} influx favouring prostate cancer cell proliferation (Dubois *et al.*, 2014). As previously mentioned, recent work highlighted the role of $STIM1_{PM}$ in the regulation of constitutive Ca^{2+} entry of lymphocyte B (LB). An increase in $STIM1_{PM}$ together with enhanced constitutive Ca^{2+} entry is observed in patients with a poor prognosis and high LB doubling time (Debant *et al.*, 2019). Further studies should explore the possible enhanced expression of proteins that interact with K^+ or Ca^{2+} channels in cancer cells to uncover new potential therapeutic targets.

Dysregulated signalling pathways, originating from mutation or from the interaction of cancer cells with their microenvironment, represent a driving force for cancer progression. Cell adhesion to the extracellular matrix (ECM) is primarily triggered by the binding of ECM

components to integrins. These transmembrane proteins form clusters at focal adhesion sites, which promote substrate-cell anchoring through the binding of mediator proteins to actin filaments. This process further activates signalling pathways to promote growth, survival or invasion initiated by focal adhesion kinase and Src protein activation (Cooper and Giancotti, 2019). A growing number of studies demonstrate the involvement of K^+ channels in integrin signalling macrocomplexes in various cancers. In particular, hERG (also known as KCNH2 or Kv11.1) plays a key role in cell adhesion to the ECM, integrin activates hERG current and increases the density of hERG channels at the plasma membrane (Fiore *et al.*, 2013; Pillozzi *et al.*, 2007). Activation of these channels stimulates focal adhesion kinase (FAK)-dependent growth, angiogenesis and survival in leukaemia and colorectal cancer (CRC) via the PI3K/AKT pathway. The subsequent formation of the hERG/integrin complex stimulates cytoskeleton reorganisation and cell migration (Becchetti *et al.*, 2019). Interestingly, the association between hERG and β1-integrin occurs in cancer cells but not in the heart. Triggering this specific interaction may therefore represent an interesting strategy to unlock hERG signalling complexes in cancer cells.

Another example of the tight regulation of signalling pathways by ion channels is illustrated by the interplay between KCNQ1 and the Wnt/β-catenin pathway. The function of this channel in epithelial physiology has been largely described (Jespersen *et al.*, 2005). In association with the β subunit of the KCNE family, especially KCNE3 and KCNE2, KCNQ1 regulates the transepithelial transport of electrolytes, solutes and water (Heitzmann and Warth, 2008). Surprisingly, KCNQ1 was recently identified as a tumour suppressor gene in mouse and human CRC (Than *et al.*, 2014). In this study, the authors found that KCNQ1 knockout mice exhibit enhanced intestinal tumour multiplicity (number of tumours) and progression. Also, the loss of KCNQ1 expression in human CRC liver metastases has been observed to be associated with poor prognosis. In line with these results, another study

demonstrated that the loss of KCNQ1 protein expression is a strong prognostic factor for an increased likelihood of recurrence and reduced survival in patients with stages II and III colon cancer (den Uil *et al.,* 2016). Both of these reports confirmed the function of KCNQ1 as a tumour suppressor in CRC. However, the molecular mechanism underlying this role of KCNQ1 remained unknown until the identification of KCNQ1 as a component of the Wnt pathway (Rapetti-Mauss *et al.,* 2017). In fact, KCNQ1 physically associates with β-catenin and E-cadherin at the plasma membrane to stabilise the adherens junctions (AJ) complex and control β-catenin localisation. This association promotes epithelial integrity by preventing the epithelial-to-mesenchymal transition (EMT) and repressing Wnt signalling activity. Moreover, the same study showed that KCNQ1 expression itself is repressed by Wnt/β-catenin pathway activation through a direct interaction between the TCF-4/β-catenin transcription complex and the promotor region of KCNQ1. This bidirectional interaction between KCNQ1 and β-catenin highlights the function of this channel as a fine regulator of the Wnt signalling pathway. Recently, the physical interaction between KCNQ1 and β-catenin was observed in hepatocellular carcinoma (HCC) (Fan *et al.,* 2018). The authors found that KCNQ1 expression is downregulated in HCC, and in line with the observations in CRC, patients with reduced KCNQ1 expression have lower overall survival. Furthermore, in HCC, the expression of KCNQ1 suppresses Wnt/β-catenin signalling pathway activity by interacting with β-catenin at the plasma membrane. These data suggest that KCNQ1, by sequestering β-catenin at the AJ, restricts the activation of the Wnt signalling pathway and acts as a tumour suppressor in numerous epithelial cancers. The mechanism seems to be conserved, suggesting a key role of KCNQ1 in epithelial homeostasis.

4. Role of calcium and potassium channel complexes in epithelial-to-mesenchymal transition

Changes in plasma membrane ion channel expression have been reported during the EMT process, which converts epithelial cells to a mesenchymal-like phenotype and increases cancer cell invasion and migration (Azimi and Monteith, 2016). Several studies have reported a critical role of Ca^{2+} as a key signalling transduction pathway regulating the induction of EMT. Major inducers of EMT, such as TGF-β (Cheng *et al.*, 2016; Schaar *et al.*, 2016), hypoxia and EGF (Davis *et al.*, 2014), lead to a transient increase in cytosolic Ca^{2+} concentration. In breast cancer, some Ca^{2+} channels have been identified to be involved in EMT, such as TRPC1/STIM1 (Schaar *et al.*, 2016), TRPM7 (Davis *et al.*, 2014) and Orai1/STIM1 (Hu *et al.*, 2011). In colon cancer, the KCNN4 channel, which was found to be induced in tumour tissues compared to normal tissues (Ibrahim *et al.*, 2019), participates in EMT induced by phosphatase of regenerating liver-3 (PRL-3). Moreover, *KCNN4* expression is positively correlated with the tumour/node/metastasis (TNM) stage of colorectal cancer (Lai *et al.*, 2013). Recently, we identified a new signalling pathway involving a positive feedback loop between the EMT transcription factor Zeb1 and the SK3 channel, which leads to the amplification of Ca^{2+} entry and cellular migration (Figiel *et al.*, 2019). Cytosolic Ca^{2+} is known to be involved in the expression of several EMT-associated genes. Indeed, intracellular Ca^{2+} chelation or blocking Ca^{2+} influx have been reported to reduce the expression of vimentin, Twist, Snail and N-cadherin (Davis *et al.*, 2014; Lai *et al.*, 2013; Schaar *et al.*, 2016).

5. Regulation of potassium and calcium complexes

5.1 Regulation by lipids

To be fully activated, such complexes were shown to be integrated into cholesterol-enriched nanodomains, also known as lipid-rafts. This was demonstrated for Orai1-SK3 complexes in breast cancer cells (Chantome *et al.*, 2013; Gueguinou *et al.*, 2017) and for Orai1-TRPC1-

SK3 complexes in colon cancer cells (Gueguinou *et al.,* 2016). This suggests that the formation of ion channel complexes in cancer cells represents the gain of a new biological function, which only occurs when the complex is integrated into nanodomains. These channels may interact physically, as observed between SK3 and Orai1 channels (Chantome *et al.,* 2013; Gueguinou *et al.,* 2017), or may colocalise without physical interaction. These interactions between channels should be favoured by their localisation in caveolae, and probably also by the presence of specific lipids like cholesterol in nanodomains. Several mechanisms have been proposed to explain the regulation of ion channels and interactions between channels by lipids (Figure 2). One possible mechanism could involve a change in the biophysical properties of the membrane, as exemplified by the effect of cholesterol on membrane fluidity and bilayer thickness (Lundbaek *et al.,* 1996; Schagina *et al.*, 1992, 1989). More recently, the Piezo1 mechanosensitive cation channel was found to be regulated by fatty acids following a change in the membrane bending stiffness (Romero *et al.,* 2019). In this study, margaric acid, a saturated fatty acid, was found to inhibit Piezo1 currents by increasing membrane bending stiffness, whereas polyunsaturated fatty acids (PUFA; arachidonic acid, eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) were found to decrease it (Romero *et al.,* 2019). Other mechanosensitive channels such as TREK-1/TRAAK channels have been reported to be activated by inverted-conical-shaped lipids, and these lipids were found to experimentally induce convex deformation of the plasma membrane (Maingret *et al.,* 2000). We found that the SK3 channel is inhibited by the synthetic ether-lipid 1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose (Ohmline), and we hypothesise that Ohmline interacts with cholesterol in nanodomains by removing the cholesterol OH moieties away from their main binding sites. This interaction would force new rearrangements with other lipid groups, leading to reorganisation of the lipid phases and consequently, SK3 channel inhibition (Herrera *et al.,* 2017). In addition, the SK3 channel was

found within cholesterol-enriched nanodomains, and MβCD was sufficient to abrogate the SK3 current, suggesting that the SK3 channel is activated by cholesterol (Gueguinou *et al.*, 2017; Herrera *et al.,* 2017). Another mechanism that could explain the regulation of ion channels by lipids is the involvement of specific lipid-channel interactions, as exemplified by the interaction between cholesterol and the KirBac1.1 channel (Singh *et al.,* 2009) or fatty acids with the IKCa channel (Hamilton *et al.,* 2003; Kacik *et al.,* 2014). Indeed, arachidonic acid was found to inhibit IKCa through a direct interaction with the pore-lining amino acids Thr(250) and Val(275) in IKCa (Hamilton *et al.,* 2003). In addition, the inhibition of IKCa by 14,15-epoxyeicotrienoic acids, 20-hydroxyeicosatetraeonic acid and omega-3 fatty acids (DHA) depend on the presence of electron double bonds and hydrophobicity of the 10 carbons preceding the carboxyl head of the molecules (Kacik *et al.,* 2014). Interestingly, Tian *et al.* detailed the atomic principles of the activation action of DHA on BKCa, and found that the carboxylate group of DHA and the OH group of Y318 of BKCa form an ion-dipole bond (Tian *et al.,* 2016). Elinder and Liin reviewed the molecular sites of action and the molecular mechanism underlying the effects of PUFAs on voltage-gated ion channels (Elinder and Liin, 2017). PUFAs were found to act on five different sites: the intracellular cavity, the extracellular entrance to the pore, the interface between the channel protein and the extracellular leaflet of the lipid bilayer, the voltage-sensor domain, and the interface between the extracellular leaflet of the lipid bilayer and the pore domain (Elinder and Liin, 2017). K_V10.1 was found to be modulated by cholesterol in neurons (Jimenez-Garduno *et al.*, 2014). Specifically, in plasma membranes isolated from mouse neuronal tissue, K_V10.1 was found to be split into a non-detergent resistant membrane (DRM) fraction and a DRM fraction, associated with cholesterol and sphingolipid-rich domains, cytoskeleton integrity (actin) and CaM/Ca^{2+} binding. Cytoskeleton integrity and cholesterol concentrations appear to act as stabilising factors for K_V10.1 currents, which are increased when there are changes to the

latter factors. In a well-conducted study using Xenopus oocytes, Zakany and colleagues aimed to investigate whether the effects of cholesterol on K^+ channels, using $K_V1.3$ as a reference channel, were mediated by the voltage sensor domains in the pore or whether it directly targeted the pore domain itself. They concluded that cholesterol modulated $K_V10.1$ in its pore domain (Zakany *et al.,* 2018). The interaction between ion channels and proteins localised in nanodomains enriched with cholesterol and sphingolipids such as caveolin (Alioua *et al.,* 2008; Garg *et al.,* 2009) is another mechanism that may explain the regulation of ion channels by lipids. Finally, lipids can modulate membrane insertion of ion channels, as exemplified by PIP2, which promotes ENaC insertion (Pochynyuk *et al.,* 2006) in addition to ion channel expression, as demonstrated by the modulation of aquaporin AQP5 expression by DHA and EPA PUFAs (Lopes *et al.,* 2018).

5.2 Regulation by peptides

The integration of nonlipid molecules into the cell membrane may lead to an altered membrane structure, which may, in turn, indirectly change the activity of multiple signalling pathways. The multifunctional LL-37 peptide activates multiple membrane-associated proteins, transmembrane receptors of different classes and ion channels, triggering a variety of signal transduction pathways (Verjans *et al.,* 2016).

The integration of peptides into the cellular membrane of target cells of both bacterial and eukaryotic origin, leading to disruption of the membrane structure, is a common characteristic of antimicrobial peptides, including LL-37. Several antimicrobial peptides (AMPs) form pores, leading to ion leakage. The resulting changes in membrane potential activate voltage-gated Ca^{2+} channels, leading to the induction of apoptosis (Sharma *et al.,* 2016; Soletti *et al.,* 2010). Such mechanisms have also been observed for LL-37 (Säll *et al.,* 2013), leading to the hypothesis that it could be used in anticancer therapy. However, the mechanisms of action of

this peptide are more complex, as it has been reported to both promote and suppress cancer depending on the cancer type (Piktel *et al.*, 2016). In breast cancer, LL-37 promotes a metastatic phenotype (Weber *et al.*, 2009). Consistent with this, LL-37 was also found to induce migration in breast cancer cell lines by activating the TRPV2 Ca^{2+} channel, which is recruited to pseudopodia through PI3K/AKT signalling. Entry of Ca^{2+} occurs through TRPV2, together with an efflux of K^{+} through the BKCa channel (Gambade *et al.*, 2016). Although there is currently no evidence of a physical complex between TRPV2 and BKCa, their colocalisation in pseudopodia supports their functional association. Signalling by LL-37 does not appear to require its binding to a specific receptor in a conventional receptor-ligand interaction, as its all-D enantiomer shows identical activities. Instead, LL-37 was found to bind specifically to the surface of pseudopodia and caveolae, structures rich in cholesterol and known to harbour receptors activated by LL-37 (Gueguinou *et al.*, 2015; Simons and Toomre, 2000). Binding of LL-37 to these receptors results in a strong decrease in cell membrane fluidity, which can modify the kinetics and thus the activity of the transmembrane receptors (Yamamoto and Ando, 2015). Taken together, these findings suggest that binding of LL-37 to the membrane interface may activate AKT signalling pathways and, consequently, Ca^{2+} signalling in an indirect manner. Binding studies of LL-37 model membranes have demonstrated that it adopts the conformation of an amphipathic helix, with the hydrophobic site inserted within the membrane interface (Sood *et al.*, 2008). In these model studies, however, cholesterol was shown to attenuate its attachment (Sood and Kinnunen, 2008). This apparent contradiction to findings in breast cancer cells would be resolved if LL-37 was found to be associated with nonlipid structures as well as the lipid bilayer. A recent investigation revealed that surface glycans, more specifically sulphated structures, were required to permit binding of LL-37 to the cell surface and, consequently, induce Ca^{2+} entry and cell migration. Syndecan-4, a proteoglycan associated with breast

cancer and cell mobility, was identified to play critical roles in mediating cell surface binding and the activities of LL-37 (Habes *et al.,* 2019). This suggests that syndecan-4 may serve as a "guide" for LL-37 to support its attachment to lipid raft domains. In conclusion, the activation of Ca^{2+} signalling and cell migration appears to involve the association of proteins, glycans and lipid structures, indicating a more complex cooperation of different classes of biomolecules than previously anticipated.

5.3. Regulation by the sigma-1 receptor chaperone

The sigma-1 receptor (SIGMAR1, S1R) is a poorly characterised ER chaperone protein. In its resting state, S1R is coupled to binding immunoglobulin protein (BiP), another ER residing protein. Under conditions of ER stress, S1R dissociates from BiP and acts as an interorganelle signalling modulator (for review: Su *et al.*, 2010; Tsai *et al.*, 2009). In the central nervous system, S1R promotes cell survival in many diseases including stroke and other neurodegenerative diseases (e.g., amyotrophic lateral sclerosis and Alzheimer's disease) (Fukunaga *et al.,* 2015; Kourrich *et al.,* 2013; Penke *et al.,* 2018). The S1R-dependent function can be mediated by protein–protein interactions with several protein superfamilies, including ion channels (Balasuriya *et al.,* 2012, 2014). Emerging studies have revealed the presence of S1R in cancer cells. Interestingly, S1R plays a central role in the formation of ion channel complexes in cancer cells. In particular, S1R binds hERG α subunits and enhances hERG trafficking to the plasma membrane in K562 leukaemia cells and transfected HEK293 cells, leading to increased current density (Balasuriya *et al.,* 2014; Crottès *et al.,* 2011, 2013). In myeloid leukaemia and CRC, the rapid association between hERG and the β1 subunit of integrin upon ECM stimulation requires S1R (see paragraph "Proteins associated with potassium and calcium channels"). Silencing of this chaperone abolishes both ECM-induced stimulation of hERG and the PI3/AKT pathway downstream of integrin stimulation.

Consequently, S1R inhibition reduces migration, angiogenesis and metastasis *in vitro* and *in vivo* in zebra fish and mouse models (Crottès *et al.,* 2016). S1R also controls Ca^{2+} homeostasis by regulating the SK3/Orai1 association in CRC and breast cancer (see see paragraph "Calcium and potassium channel complexes"). In fact, coimmunoprecipitation and FRET assays demonstrated that S1R binds SK3. S1R silencing was found to abrogate SK3-dependent SOCE and migration by forcing both SK3 and Orai1 out of caveolae lipid nanodomains. Interestingly, the sigma ligand igmesine mimicked these effects on Ca^{2+} influx and migration by dissociating Orai1 from SK3, the former being excluded from lipid caveolae nanodomains in MDA-MB435s cancer cells. Notably, S1R is overexpressed in human CRC and breast cancer samples, and is associated with higher grade tumours in CRC and reduced overall survival in breast cancer patients (Gueguinou *et al.,* 2017).

Together, these data suggest that S1R participates in the formation of ion channel complexes in cancer tissues and may represent a promising candidate to target ion channel-dependent signalling in cancers (Soriani and Rapetti-Mauss, 2017).

5.4. Regulation by antibodies

Monoclonal antibodies (mAbs) are currently recognised as a precision strategy to generate highly selective biologic inhibitors against cell surface-reachable antigens, which have been validated in numerous clinical trials. This is clearly highlighted by recent developments on the modulation of immune checkpoints, which rose to prominence as a means to treat a number of cancers. It is possible to distinguish a specific antigen from its nearest homologues. The therapeutic potential of ion channels and their modulators has been extensively reviewed elsewhere (Haustrate *et al.,* 2019; Hutchings *et al.,* 2019). To date, however, there are very few approved and/or marketed mAbs dedicated to ion channel blockade or activation. Due to its localisation at the plasma membrane, therapeutic targeting

of K_V10.1 with mAbs was first developed by Luis Pardo's team for several cancer models including breast, ovarian and pancreatic cancers and glioma (Gomez-Varela *et al.,* 2007; Napp *et al.,* 2016; Pardo and Stuhmer, 2008). mAb56, an IgGκ2b that targets K_V10.1, was the first mAb able to inhibit an ion channel current in cells. This antibody is highly selective and does not bind to human K_v10.2. mAb56 does not affect the K_v11.1 current that regulates cardiac repolarisation. Another an IgGκ2b, mAb62, was developed to visualise tumour cells without affecting their currents and to deliver therapeutics to the tumour. mAb62 labelled with a Cy5.5 maleimide monoreactive dye has been reported to accumulate at breast MDA-MB-435s engrafted tumour sites of immunodeficient mice, with a peak intensity observed 48 h after injection (excitation at 670 nm). mAb62 conjugated to a prodrug-activating enzyme β-D-galactosidase enabled the detection of activity *in vivo* at the tumour area.

It is worth noting that K_V10.1 is quickly internalised by endocytosis and recycled after its surface localisation (Kohl *et al.,* 2011); therefore, a classic fully human mAb could be inefficient in terms of pharmacokinetic optimisation, so other formats such as antibody drug conjugates (Joubert *et al.,* 2017) or bispecific antibodies should be developed and tested. Regarding bispecific antibodies, Hartung and colleagues developed a bispecific antibody comprising a single-chain antibody against an extracellular region of Kv10.1 (scFv62) and fused it to the human-soluble tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), leading to a strategy that selectively induced apoptosis of Kv10.1-positive prostate tumour cells (Hartung *et al.,* 2011). As previously mentioned, protein complexes of Orai1 and K^+ channels (Gueguinou *et al.,* 2014, 2015; Mignen *et al.,* 2017) contribute to Ca^{2+} influx, which could be deregulated in cancer cells. mAbs against human Orai1, inhibiting SOCE, have been developed by Amgen and Novo Nordisk and proposed as a novel therapeutic approach for the treatment of autoimmunity (Cox *et al.,* 2013; Lin *et al.,* 2013). Despite the number of studies suggesting changes in Orai1 expression in cancer cells and the

involvement of this protein in oncogenic and metastatic processes (for review: Chalmers and Monteith, 2018; Kappel *et al.,* 2019), mAbs targeting Orai1 have not yet been evaluated as a potential therapeutic option to treat cancer. Recent work by Debant *et al.* clearly suggests that $STIM1_{PM}$ supports new innovative therapeutic perspectives, such as targeting $STIM1_{PM}$ for the treatment of CLL (Debant *et al.,* 2019). mAbs to STIM1 may be associated with existing therapies that target BCR pathways, as combining an anti-STIM1 mAb with rituximab significantly reduces *in vitro* CLL B cell viability.

Conclusion

This review highlights new roles of Ca^{2+} and K^{+} channel complexes in cancer and the potential use of modulators of these channels as a novel avenue for research in the treatment or prevention of cancer.

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Figure legends

Figure 1. The intracellular Ca^{2+} concentration is primarily controlled by Ca^{2+} channels that form complexes with K^+ channels, which act as amplifiers of Ca^{2+} flux through Ca^{2+} channels. In cancer cells, voltage-gated/voltage-dependent Ca^{2+} channels (CaV) and non-voltage-gated/voltage-independent Ca^{2+} channels have been reported to interact with Ca^{2+}-activated K^+ channels (KCa). Following activation by an increase in cytosolic Ca^{2+} concentration, these channels induce membrane hyperpolarisation, increasing the driving force for Ca^{2+} flux. In addition, the entry of Ca^{2+} is regulated by various molecules including lipids (e.g., ether-lipids), proteins (e.g., STIM), receptors with SIGMAR1 and peptides (e.g., LL-37), and are the target of monoclonal antibodies, making these channels promising novel targets for cancer research.

Figure 2. Mechanisms by which lipids could favour the formation and activity of ion channel complexes. Lipids may act by (a) changing the biophysical properties of the membrane (Gueguinou *et al.*, 2017; Herrera *et al.*, 2017; Lundbaek *et al.*, 1996; Maingret *et al.*, 2000; Romero *et al.*, 2019; Schagina *et al.*, 1989, 1992), (b) inducing specific lipid-channel interactions (Elinder and Liin, 2017; Hamilton *et al.*, 2003; Jimenez-Garduno *et al.*, 2014; Kacik *et al.*, 2014; Singh *et al.*, 2009; Tian *et al.*, 2016; Zakany *et al.*, 2018), (c) promoting interactions between ion channels and proteins such as caveolin (Alioua *et al.*, 2008; Garg *et al.*, 2009), (d) modulating ion channel expression (Lopes *et al.*, 2018), and (e) modulating membrane insertion of ion channels (Pochynyuk *et al.*, 2006).