

Review

Targeting $K_{ca}3.1$ Channels in Cancer

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$K_{ca}3.1$ channel • Cancer progression • Channel structure • Channel imaging

Abstract

The $K_{ca}3.1$ channels, previously designated as IK1 or SK4 channels and encoded by the *KCNN4* gene, are activated by a rise of the intracellular Ca^{2+} concentration. These K^+ channels are widely expressed in many organs and involved in many pathologies. In particular, $K_{ca}3.1$ channels have been studied intensively in the context of cancer. They are not only a marker and a valid prognostic tool for cancer patients, but have an important share in driving cancer progression. Their function is required for many characteristic features of the aggressive cancer cell behavior such as migration, invasion and metastasis as well as proliferation and therapy resistance. In the context of cancer, another property of $K_{ca}3.1$ is now emerging. These channels can be a target for novel small molecule-based imaging probes, as it has been validated in case of fluorescently labeled senicapoc-derivatives. The aim of this review is (i) to give an overview on the role of $K_{ca}3.1$ channels in cancer progression and in shaping the cancer microenvironment, (ii) discuss the potential of using $K_{ca}3.1$ targeting drugs for cancer imaging, (iii) and highlight the possibility of combining molecular dynamics simulations to image inhibitor binding to $K_{ca}3.1$ channels in order to provide a deeper understanding of $K_{ca}3.1$ channel pharmacology. Altogether, $K_{ca}3.1$ is an attractive therapeutic target so that senicapoc, originally developed for the treatment of sickle cell anemia, should be repurposed for the treatment of cancer patients.

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Introduction

The $K_{ca}3.1$ channel (also known as IKCa, SK4 channel), encoded by the *KCNN4* gene, belongs to the family of Ca^{2+} -activated K^+ channels, that constitute a heterogeneous family of K^+ channels with different biophysical and pharmacological properties, gated by Ca^{2+} [1]. It is a homotetrameric transmembrane protein whose subunits have six transmembrane domains, cytosolic N- and C-termini and a pore region between the 5th and 6th transmembrane domains [2]. Its activation by intracellular Ca^{2+} (100-500nM) is mediated by

calmodulin (CaM) binding to its domain (CaMBD) which is located in the membrane-proximal region of the C-terminus of the channel protein [3, 4]. In non-excitable cells $K_{Ca}3.1$ -mediated K^+ efflux results in a hyperpolarization of the cell membrane potential, which – in a positive feedback – can enhance the electrical driving force for Ca^{2+} ions to further enter the cell. The hyperpolarization and the increase of the intracellular Ca^{2+} concentration could be seen as an indirect read-out of $K_{Ca}3.1$ channel activity [5, 6].

$K_{Ca}3.1$ gating is also influenced by other molecular factors and interactions with cell signaling pathways. One example is the regulation by cAMP-dependent protein kinase (PKA) [7]. Phosphorylation of S334 reduces CaM binding to the channel and consequently the $K_{Ca}3.1$ mediated current. Physiologically, this may be triggered by activation of the adenosine A2a receptor. This decreases the open probability in wild-type but not S334A mutant $K_{Ca}3.1$ channels [8]. Studies on heterologously expressed canine $K_{Ca}3.1$ (cIK1) channels cloned from MDCK cells also showed an impact of protein kinase C (PKC) on the channel activity. Stimulation of PKC leads to an activation of the channel while mutating the PKC phosphorylation sites (T101, S178, T329) alters the regulation of the channel by PKC [9]. The channel is also activated by a nucleoside diphosphate Kinase-B (NDPK-B)-dependent phosphorylation of the histidine residue H358 [10].

$K_{Ca}3.1$ channels are widely expressed in many organs. Their expression has been demonstrated in human enteric, sensory and sympathetic neurons. In the CNS, however, they are mainly localized in microglia in which they are involved in their activation. Inhibition of $K_{Ca}3.1$ channels elicits neuroprotective effects [11]. $K_{Ca}3.1$ channels are also expressed in secretory epithelial cells of the gastro-intestinal tract as well as in exocrine glands such as the pancreas [12, 13]. In addition, the channel plays a role in cell migration, proliferation, activation and cytokine release of essentially all blood cells [14, 15]. According to their wide spread distribution, $K_{Ca}3.1$ channels play a role in many (inflammatory) pathologies, such as neurological disorders (e.g. ischemic stroke and multiple sclerosis), but also in vascular diseases such as atherosclerosis and restenosis [16-18].

Moreover, $K_{Ca}3.1$ channels have been studied intensively in the context of cancer, which is in the focus of this review. Their elevated expression in cancer cells has been linked to cancer progression and can be a valid marker and prognostic tool. Altered functions of the $K_{Ca}3.1$ channel are also proposed to promote oncogenic functions, suggesting the channel as a possible therapeutic target [19]. This aspect will be discussed in more detail in the following sections.

$K_{Ca}3.1$ channel functions in cancer

$K_{Ca}3.1$ expression in cancer

Based on qRT-PCR results $K_{Ca}3.1$ mRNA expression is 2-fold higher in clear cell renal cell carcinoma (ccRCC) than in the healthy tissue. The $K_{Ca}3.1$ high subgroup has a reduced progression-free survival [20]. The same findings were also demonstrated in papillary thyroid cancer where $K_{Ca}3.1$ mRNA levels are higher than in normal thyroid tissues. The high expression is associated with a larger tumor size, lymph node metastasis and advanced disease stage [21]. In human prostate cancer $K_{Ca}3.1$ channels are expressed grade-dependently [22]. Our group investigated why $K_{Ca}3.1$ channels are overexpressed in non-small cell lung cancer. Like in the above-mentioned cancer entities a $K_{Ca}3.1$ high phenotype also predicts a poor survival of non-small cell lung cancer patients. A genome-wide DNA methylation analysis revealed that $K_{Ca}3.1$ overexpression is due to hypomethylation of its promoter [5]. In cervical cancer tissue the elevated $K_{Ca}3.1$ expression (also correlated to the histological grade) has been proposed to result from a positive feedback. Increased $K_{Ca}3.1$ channel activity hyperpolarizes the cell membrane potential and leads to increased Ca^{2+} influx, which in turn activates the transcription factor AP-1 and post-translational histone acetylation to further drive the expression of the channel [23]. The correlation between histone acetylation and $K_{Ca}3.1$ channel expression was also shown in breast and

prostate cancer cells lines (YMB-1 and PC-3, respectively). Accordingly, vorinostat, a histone deacetylase inhibitor, downregulates K_{ca} 3.1 transcription [24].

Altered mRNA expression of K_{ca} 3.1 channels in cancer has also been investigated with tissue microarrays [25]. Thus, characterization of a set of 10 K^+ channels including K_{ca} 3.1 in a lung cancer microarray has shown its up-regulation and linkage to tumor aggressiveness. In addition to K_{ca} 3.1 channels, three other K^+ channels, namely K_{2p} 1.1, K_{2p} 12.1, K_{2p} 5.1 and an accessory subunit of K_{ca} 1.1 channels, *KCNMB3*, are upregulated, too [26]. Likewise, the expression of K_{ca} 3.1 channels is increased in tumor cells isolated from micro-dissected samples of pancreatic ductal adenocarcinoma (PDAC) and associated with unfavorable overall survival [27, 28]. Finally, in serous ovarian carcinoma patients the high expression of K_{ca} 3.1 and S100A14 in micorarrays also has predictive value since it is associated with a high incidence of recurrence [29].

Role of K_{ca} 3.1 channels in cancer

K_{ca} 3.1 channels are not only a marker and a valid prognostic tool for cancer patients. They also have an important share in driving cancer progression because their function is required for many traits of the aggressive cancer cell behavior such as migration, invasion and metastasis, as well as proliferation and therapy resistance.

K_{ca} 3.1 is a promoter of cell cycle progression and cell proliferation. Its indirect role in driving Ca^{2+} influx into the cell allows the passing of G0/G1 and G1/S checkpoints which are regulated by the intracellular Ca^{2+} concentration [30]. Thus, blocking K_{ca} 3.1 channels in the breast cancer cell line MCF-7 with clotrimazole depolarizes the cell membrane potential, decreases the intracellular Ca^{2+} concentration and inhibits cell proliferation. The cells accumulate in the G1 phase and the number of cells in the S phase of the cell cycle is reduced [31]. Likewise, treating murine breast cancer cells (MMTV-PyMT) with the K_{ca} 3.1 blocker TRAM-34 inhibits cell cycle progression. The specificity of the pharmacological blockade of K_{ca} 3.1 channels was underpinned by the observation that the channel blockers mimic K_{ca} 3.1^{-/-} breast cancer cell behavior [32]. Similar results were obtained in chronic lymphocytic leukemia (CCL) [33] and glioblastoma cells [34]. The latter study showed that the G2/M check-point is also regulated by K_{ca} 3.1 function via the activity of cdc25C phosphatase: activation of the channel with SKA-31 increases cdc2 phosphorylation [34].

These *in vitro* results are confirmed in *in vivo* studies. The volume of the cancer mass, resulting from the injection of human endometrial cancer cells (HEC-1-A) into nude mice, is decreased when mice are treated with TRAM-34 or clotrimazole [35]. The same has been shown in xenografted mice bearing intrahepatic cholangiocarcinoma Huh28 tumor cells or non-small cell lung cancer cells (A549-3R) that were treated with senicapoc [5, 36]. PET imaging also confirmed the inhibition of proliferation of lung cancer cells *in vivo* [5].

K_{ca} 3.1 channels seem to cooperate with different Ca^{2+} permeable channels. In the PDAC cell lines BxPC-3 and MiaPaCa-2 K_{ca} 3.1-regulated Ca^{2+} influx appears to occur via CRAC channels [37]. In prostate cancer cells the same mechanism is sustained by TRPV6 channels. Down-regulation of TRPV6 channels with siRNA decreases Ca^{2+} influx in response to K_{ca} 3.1 activation [38]. TRPC1 and K_{ca} 3.1 cooperate within lipid raft micro-domains in human breast cancer cells (MCF-7) where knockdown of TRPC1 suppresses Ca^{2+} influx induced by K_{ca} 3.1 activation with 1-EBIO [39]. In this context TRPC3 is yet another cooperation partner of K_{ca} 3.1 channels in pancreatic stellate cells [40].

The link between K_{ca} 3.1 expression and patient survival suggests that K_{ca} 3.1 channels are contributing to steps of the metastatic cascade, because almost all cancer patients die of the sequelae of metastasis. Several mechanisms by which K_{ca} 3.1 channels promote metastasis have been identified. Cell migration is one of the key processes of the metastatic cascade. Notably, K_{ca} 3.1 is one of the very first channels for which a role in cell migration was shown in transformed renal epithelial cells [41]. In the meantime, this initial observation has been reproduced and confirmed in many cancer cells. Examples include pancreatic, hepatocellular or non-small cell lung cancer cells to name only a few [5, 42-44]. Moreover, the inhibition of the channel with TRAM-34 also reduces glioblastoma infiltration *in vivo*.

Brain slices from SCID mice in which GL-15 glioblastoma cells were injected into the brain, show a reduction of the tumor-infiltrated area in TRAM-34 treated mice [45]. One of the common signaling events by which K_{ca} 3.1 channels affect cell migration is the modulation of the intracellular Ca^{2+} concentration. In addition, they have an impact on the (local) regulation of the cell volume of migrating tumor cells [15].

K_{ca} 3.1 channels also regulate cancer cell apoptosis. This function has been linked to their expression in the inner mitochondrial membrane of the cell [46]. The localization of the channel in the inner mitochondrial membrane has been demonstrated in colon carcinoma and human cervix adenocarcinoma cells (HeLa) cells by means of Western blot and patch-clamp recordings [47, 48]. In melanoma cells (A-375) K_{ca} 3.1 channels play a key role in sensitizing them to TRAIL-induced apoptosis. Treating the cells both with TRAIL and TRAM-34 stimulates mitochondrial release of cytochrome c and starts the caspase cascade. TRAM-34 induces a hyperpolarization of the mitochondrial membrane potential and enhanced Bax translocation to the inner mitochondrial membrane [49]. Induction of the intrinsic apoptotic pathway in D54-MG glioma cells with staurosporine triggers a K^+ efflux through the membrane via K_{ca} 3.1 channels which is followed by caspase 3 activation and apoptotic volume decrease (AVD) [50]. Finally, blocking K_{ca} 3.1 in hepatocellular carcinoma HepG2 cells induces apoptosis due to an increase of the intracellular ROS production and translocation of p53 protein from the cytoplasm into the nuclei [43].

Therapy resistance is a serious clinical problem in oncology which has not yet been overcome and which requires the development of new concepts. There is increasing evidence that ion channel modulation can help to overcome therapy resistance [51]. This also applies to K_{ca} 3.1 channels. In human epidermoid cancer cells resistant to cisplatin, K_{ca} 3.1 plays a crucial role in regulating sensitivity and resistance to cisplatin due to a control of proliferation and regulation of cell volume. Thus, K_{ca} 3.1 activation with 1-EBIO can promote apoptosis in cisplatin-resistant cells [52]. The same effect is observed in colorectal cell lines resistant to cisplatin (HCT-116 and HCT-8); application of K_{ca} 3.1 activator SKA-31 and inhibition of K_v 11.1 by E4031, have a synergistic effect with cisplatin and inhibit proliferation and activate apoptosis. K_{ca} 3.1 activators promote cisplatin uptake, and this effect further increases when K_{ca} 3.1 expression rises upon K_v 11.1 inhibition [53]. This is yet another example showing that K_{ca} 3.1 channels do not only act by themselves, but are rather as part of a network of functionally interacting channel proteins. The effect of K_{ca} 3.1 channels on drug resistance appears to depend both on the drug and on the tumor. Malignant glioma cells (GL261) are sensitized to temozolomide upon K_{ca} 3.1 inhibition. The combined treatment of TRAM-34 and temozolomide reduces cancer cell infiltration and migration more efficiently than the single drug [54]. Furthermore, in breast cancer overexpression of K_{ca} 3.1 enhances resistance to chemotherapeutic antimetabolites like gemcitabine. It does so by upregulating BCL2-related protein A1 (BCL2A1) which then suppresses apoptosis via RAS-MAPK and PI3K-AKT signaling. BCL2A1 is a member of the anti-apoptotic BCL-2 family that has been shown to confer resistance to anticancer drugs [55].

The role of K_{ca} 3.1 channels in the cancer microenvironment

So far, we have discussed the role of K_{ca} 3.1 channels in tumor cells. However, tumor cells do not act by themselves but are supported by cells of the tumor microenvironment located in the tumor stroma [56]. There is increasing evidence that ion channels expressed in tumor stroma cells such as immune cells, cancer-associated fibroblasts and endothelial cells play a crucial role in microenvironment remodeling and contribute to tumor progression [57, 58]. This also applies to K_{ca} 3.1 channels. In this context, immune cells belong to the best studied cells of the tumor microenvironment: The high K^+ concentration found in the cancer microenvironment leads to an increased intracellular K^+ concentration that can suppress T cell function by impairing Akt and PI3K pathways. Overexpression of K_v 1.3 and K_{ca} 3.1 in T cells enhance K^+ release and thereby restore T cell function and decrease cancer growth [59, 60]. K_{ca} 3.1 channels also regulate the response of Natural Killer (NK) cells against tumors, in particular in adherent NK (A-NK) cells where K_{ca} 3.1 channels are up-regulated. Blocking the

channel with TRAM-34 increases the degranulation, cytotoxicity and proliferation of A-NK cells. Moreover, TRAM-34 increases the ability of A-NK cells to reduce tumor growth [61]. In colorectal cancer, tumor-associated macrophages (TAMs) promote cancer progression through the release of cytokines such as IL-6 and IL-8. These effects can be attenuated by blocking $K_{Ca}3.1$ channels with TRAM-34, confirming the role of the channel in cytokine secretion and cancer invasion [62]. These examples clearly show that the final result of blocking $K_{Ca}3.1$ channels on tumor immunity is difficult to predict and will most likely depend on the individual tumor type. We refer to a recent review on the role of ion channels in PDAC where we extensively discuss the exciting question of ion channel function in tumor immunity [63].

In PDAC pancreatic stellate cells play an important role. Once activated they favor proliferation and metastasis of pancreatic cancer cells [64]. $K_{Ca}3.1$ is functionally expressed in pancreatic stellate cells and TRAM-34 treatment or $K_{Ca}3.1$ knockout inhibits their migration and chemotaxis. This is due to a decrease of the intracellular Ca^{2+} concentration which in turn leads to diminished calpain activity [39].

So far, there is no direct evidence for $K_{Ca}3.1$ -dependent tumor angiogenesis. However, it is well known that the channel promotes non-cancerous angiogenesis. When human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells 1 (HMEC-1) are stimulated with angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), $K_{Ca}3.1$ channels are upregulated and their blockade suppresses angiogenesis *in vivo* [65]. Likewise, proliferation of vascular smooth muscle cells depends on $K_{Ca}3.1$ channel activity [66]. Therefore, it is plausible to assume that $K_{Ca}3.1$ inhibition will also impair tumour angiogenesis.

There is one aspect of the tumor microenvironment which has not yet been properly addressed in the context of ion channel function (see [67] for review). The microenvironment of solid tumors is usually quite acidic because of a metabolic shift of cancer cells that elicit an accumulation of metabolic acids in the tissue. Thus, the intracellular pH of the cells within the tumor microenvironment is also more acidic than in the healthy tissue. Many ion channels including $K_{Ca}3.1$ are pH-sensitive. In C6 rat glioma cells an intracellular acidification in the range of pH_i 6.4–5.4 reduces channel open probability [68]. One could speculate in light of these findings that the upregulation of $K_{Ca}3.1$ channels in solid tumors is a compensatory mechanism in order to overcome the effect of reduced channel activity in an acidic environment. Hypoxia is another characteristic feature of the tumor microenvironment. It has been demonstrated in melanoma cell lines that hypoxia and hypoxia mimetics cause an upregulation of $K_{Ca}3.1$ channels [69, 70].

Imaging $K_{Ca}3.1$ channels in cancer cells

Several pharmacological modulators have been developed to either activate or inhibit $K_{Ca}3.1$ channel activity. Recent reviews give an overview about these compounds which are employed to modulate the function of the channel and thereby achieve a therapeutic effect when used clinically [71]. Channel activity itself can only be imaged indirectly by assessing the impact of the channel on the cell membrane potential or on the intracellular Ca^{2+} concentration. In addition, small molecules and channel targeting antibodies can be employed for imaging of the presence of $K_{Ca}3.1$ channels both *in vitro* and *in vivo*. Since $K_{Ca}3.1$ channel expression has predictive potential with respect to prognosis and patient survival in different tumor entities, imaging probes targeting the expression of this ion channel could serve as important diagnostic tools [72, 73].

Biological structures can be labeled, among others, by positron emission tomography (PET) tracers, antibodies and fluorescent dyes. A broad range of PET tracers has been developed to target proteins offering a distinct binding site. Thus, PET tracers are available to image ligand-gated ion channels such as nicotinic acetylcholine receptor subtypes [74] and NMDA receptors. Addressing specifically the ifenprodil binding site allows the selective

imaging of NMDA receptors containing the GluN2B subunit [75-78]. However, PET tracers for imaging of voltage- or Ca^{2+} -activated ion channels such as the K_{ca} 3.1 channel have not been developed so far.

Due to their high specificity, antibodies can be employed to image selectively almost all proteins. For example, the voltage-gated K^+ channel K_v 11.1 (hERG1), which is aberrantly expressed in human tumors, can be visualized using an anti- K_v 11.1 monoclonal antibody. The expression of the K_v 11.1 channel in primary pancreatic ductal adenocarcinoma cells (PDAC) was investigated with this antibody *in vitro* and *in vivo* [79]. The fluorescent dye-coupled antibody mAb62-Cy5.5 was used to label the K_v 10.1 voltage-gated K^+ channel of tumors *in vitro* and *in vivo* [80].

Antibody-based indirect immunofluorescence is one possibility to image K_{ca} 3.1 channels *in vitro*. Most antibodies available so far (e. g. AV35098 from Sigma-Aldrich) require fixation and permeabilization of (cancer) cells [5]. The above mentioned anti- K_{ca} 3.1 antibody binds from the cytosolic side to closed K_{ca} 3.1 channels, which is the prevalent channel conformation in resting, non-activated cells. Imaging of K_{ca} 3.1 channels using the antibody-based indirect immunofluorescence is expensive and time-consuming due to long incubation times. Moreover, usually immobilization and permeabilization of the tumor cells are required for imaging with antibodies.

Alternatively to large and expensive antibodies, various fluorescently labeled small molecule probes have been developed to image ion channels, e.g. NMDA receptors [81, 82] and K_v 11.1 channels [83]. Some important features have to be considered when employing small molecule-based imaging probes: The pharmacophoric structural element interacting with the target channel must bind tightly to its binding site at the channel. Moreover, additional functional groups have to be present allowing chemical modification and coupling of the fluorescent dye via a linker. In particular, the linker length has to be carefully considered, since it can have an impact on the activity of the probe. Using poly(oxyethylene) linkers with different length will reduce the lipophilicity of the complete molecule. Additionally, the fluorescent dye must have a suitable excitation and emission profile for the available microscope recording system and a high intensity of its emitted light (high quantum yield). Furthermore, the excitation and emission wavelengths of the dye have to be outside of the region, where cells exhibit a high degree of autofluorescence and light scattering [82].

An interesting strategy for visualization of ion channels is the use of dyes with a turn-on mechanism which will lead to a reduction of background signals. Such a probe has been developed for imaging of K_v 11.1 channels. The probe contains the 2,7-dichlorofluorescein fluorophore, which is quenched intramolecularly by the basic amino moiety (electron donor) within the incorporated piperazine ring. Once the fluorescent probe binds to the target, the conformation changes, the fluorophore is no longer quenched and a fluorescence signal appears [82]. Based on this concept, other off-on imaging probes have been developed, which are partly based on solvatochromism or molecular rotation [84].

Fluorescently labeled small molecules targeting the K_{ca} 3.1 channel can also be used for imaging the ion channel [74]. For this purpose, senicapoc (1), a highly potent and selective inhibitor of K_{ca} 3.1 channels, was connected with various BODIPY dyes via linkers of different length (Fig. 1). Both dimethylpyrrole-based BODIPY senicapoc conjugates 2 and 3 allow the visualization of K_{ca} 3.1 channels after an incubation period of only 10 min. Staining of K_{ca} 3.1 channels with the fluorescently labeled senicapoc-derivatives and the indirect immunofluorescence lead to the same typical punctate pattern [85, 86] (Fig. 2). The simple, fast and efficient staining protocol with small molecules can be performed with fixed cells without permeabilization. Moreover, the BODIPY-labeled senicapoc-derivative 2 with an ethoxy linker showed promising results in living cancer cells as well. It was postulated that BODIPY-labeled senicapoc derivatives 2 and 3 and anti- K_{ca} 3.1 antibodies label different populations of the K_{ca} 3.1 channel, which prevents co-staining with both methods. The anti- K_{ca} 3.1 antibody (Sigma #AV35098) labels the closed conformation of the channel, whereas the fluorescently labeled senicapoc derivatives 2 and 3 have to enter the open channel and thus bind to open K_{ca} 3.1 channels [87] (see below for a more detailed discussion).

Fig. 1. Senicapoc (1), a potent inhibitor of the K_{ca} 3.1 channel and senicapoc BODIPY conjugated fluorescence dyes 2 and 3.

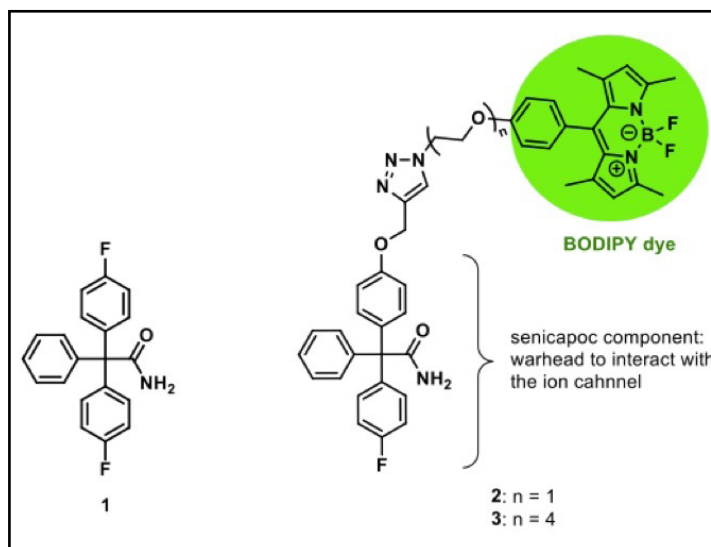
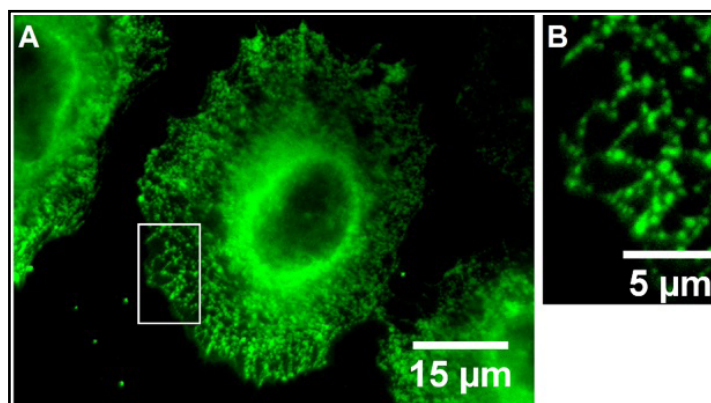


Fig. 2. A: A549-3R cells stained with the BODIPY-labeled senicapoc-derivative 2. B: Magnification of A (white box) [87].



Altogether, the BODIPY-labeled senicapoc derivatives 2 and 3 could serve as interesting new imaging tools for the diagnosis and/or monitoring of cancer patients, since they allow high throughput screening by fast and selective visualization of K_{ca} 3.1 channels.

Modelling of the K_{ca} 3.1 channel in complex with the BODIPY probe

The availability of cryo-EM structures of the K_{ca} 3.1 channel provides more in-depth insights into the activation mechanism and senicapoc binding. Lee and McKinnon determined three different cryo-EM structures in a closed state and two different activated states at a resolution of 3.4 to 3.5 Å [88]. Four subunits form a tetramer with each subunit contributing two helices (S5 and S6) for building the K_{ca} 3.1 channel pore. These two helices are surrounded by four other helices (S1 to S4) that are embedded in the membrane. Helix S6 is followed by three other helices, whereas two of them (HA & HB) are nearly parallel to the membrane and helix three (HC) is extending to the K_{ca} 3.1 channel pore. The helices HA and HB are the binding sites for the calmodulin C-lobe. The reported structures in the absence of Ca^{2+} represent the closed state (pdb 6cnm), which is in accordance with the requirement of Ca^{2+} ions for opening K_{ca} 3.1 channels. In this closed conformation, the Val282 residues of each subunit are only less than 1 Å apart and represent the constricted channel gate. A mutation of Val282 to Gly leads to a channel that conducts current even in the absence of Ca^{2+} [89].

In the presence of Ca^{2+} , four calmodulins can be identified as being bound to each of the tetramer subunits. Upon activation, the calmodulin N-lobe binds to an additional small helix (S45) that lies between the helices S4 and S5. This binding moves the S45 helix outwards and rearranges the S6 helix which in turn expands the channel gate between the Val282 residues to a radius of 3.5\AA . This can be seen in the fully open bound state II (pdb 6cno). The bound state I (pdb6cnn) is in between the closed and the fully opened conformation (see Fig. 3).

Mutational studies revealed that the compound class of triarylmethanes (TRAMs and senicapoc) is binding in the pore region near Thr250/Val275. Mutation of these residues leads to a loss of activity of TRAM derivatives [90]. These residues in the upper part of the inner pore were also identified by an earlier homology model as being important residues for binding of TRAMs and senicapoc in this region of the K_{ca} 3.1 channel [91]. BODIPY-labeled senicapoc derivatives are not able to bind to the channel when cells have been pretreated with unlabeled senicapoc [86]. This indicates a competition in the binding site in the inner pore region of the channel between senicapoc and the new probe.

Therefore, the derivatives 2 and 3 were modelled into the inner pore of the K_{ca} 3.1 channel to get more insights into the binding mode [85] and the required linker length [87]. The K_{ca} 3.1 Cryo-EM structure (pdb 6cn0) [88] in the bound state (II) with bound calmodulin was the starting point for these analyses. Fig. 4 shows the modelling results described by Brömmel et al. [85, 87]. It can be seen that both BODIPY-labeled senicapoc derivatives can bind in the inner pore of the K_{ca} 3.1 channel. The senicapoc-moiety can be placed in the upper part of the inner pore region as already described. The linker fits nicely into the smallest part of the inner pore (the channel gate), and the dye is placed outside the lower part of the pore. While the linker of the compound 1 matches perfectly the length of the pore, the linker of the compound 2 is longer and thus buried deeper in the pore.

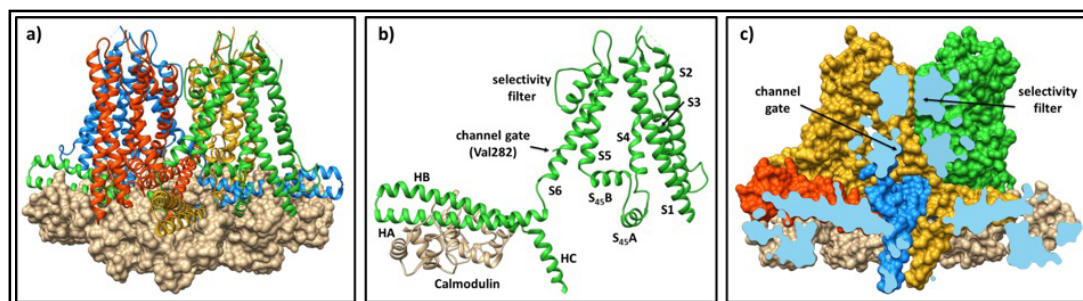


Fig. 3. Important K_{ca} 3.1 channel features (pdb 6cno[REF]). a) A tetrameric K_{ca} 3.1 channel (each subunit is coloured differently) with bound calmodulin (khaki). b) Overview about the important feature of one subunit: Helix S5/S6 form the ion channel pore surrounded by membrane-embedded helices S1-S4. Helices HA/HB build the binding site for calmodulin which is important for activation. The channel gate regulates ion conductance, and the selectivity filter is responsible for ion specificity. c) Surface representation with clipped surface showing the inner channel pore.

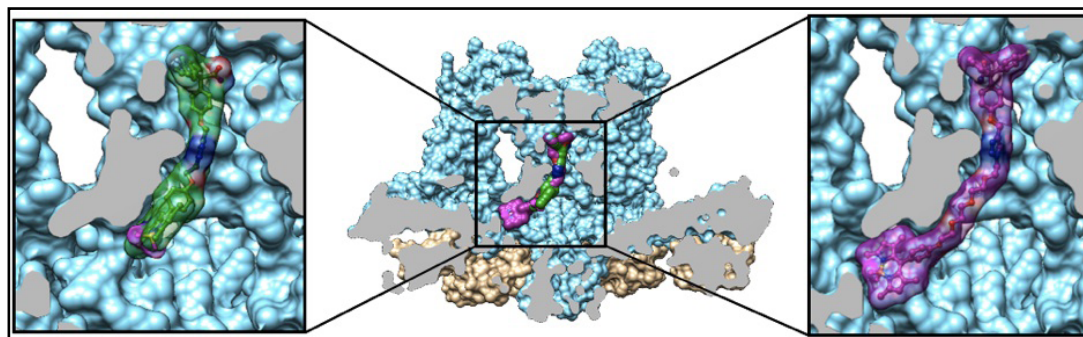


Fig. 4. The K_{ca} 3.1 channel (pdb 6cn0) with modelled BODIPY-labeled senicapoc derivatives 1 (green) and 2 (magenta).

This modelling also provides an explanation for the finding that the BODIPY-labeled senicapoc derivatives 2 and 3 and the antibodies cannot be used for co-staining [87]. It can be expected that the antibody (Sigma # AV35098) binds to the closed channel state, which is the prevalent conformation when cells are not activated and the intracellular Ca²⁺ concentration is at the low resting level. (So far, the binding sites of other K_{ca}3.1 channel antibodies have not yet been modeled.) Based on the modelling results, the linker of the BODIPY-labeled senicapoc spans through the channel gate which is only possible in the open state because it is too small in a closed state. Thus, the binding of compound 2 and 3 keeps the K_{ca}3.1 channel presumably in an open conformation, where the antibody cannot bind.

Conclusion and Outlook

The above cited work clearly shows that K_{ca}3.1 channels are highly attractive targets in oncology. There is an ever increasing number of publications showing its functional contribution to many aspects of cancer pathophysiology. Because of its wide-spread expression in tumor cells as well as in tumor stroma cells and tumor-associated immune cells, it is a complex quest to predict the therapeutic effect of K_{ca}3.1 channel modulation. Clearly, more *in vivo* studies using orthotopic or transgenic mouse models are required. Published work justifies optimism [32]. K_{ca}3.1 inhibition with senicapoc has been shown to be safe in humans. This was demonstrated in a phase III clinical trial for sickle cell disease and in a phase II clinical trial for allergic asthma [13, 92]. This clearly calls for repurposing senicapoc for the treatment of other K_{ca}3.1 driven pathologies like cancer, Alzheimer's disease (clinical trial planned for 2021) or stroke [93, 94, 95].

In addition, this short review aims to stress another property of K_{ca}3.1 channels. They are also a target for novel small molecule-based imaging probes. So far, this has been validated for fluorescently-labeled senicapoc-derivatives *in vitro*. However, it is to be expected that it will be possible to use senicapoc also as a PET tracer by modifying it accordingly. Finally, the ability to image inhibitor binding to K_{ca}3.1 channels and combine it with molecular dynamics simulations will provide a deeper understanding of K_{ca}3.1 channel pharmacology.

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Disclosure Statement

The authors declare no conflict of interest.

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