

epithelium prone to infection and in intestine leads to obstruction. Patients homozygous for F508del, have a tremendous variation in the severity of disease. Recent Genome-wide association studies indicate that this variation is due to presence of modifier genes, with SLC6A14 as the top modifier (Sun et al., 2012). SLC6A14 is a Na⁺/Cl⁻ dependent cationic/neutral amino-acid transporter on the surface of lung and colonic epithelium. As both transporters are expressed apically, we hypothesized that SLC6A14 would modify the fluid secretory capacity of the epithelium. So in collaboration with TCP, we generated a SLC6A14 knock-out mouse. We can measure in-vivo fluid secretion in mice, using an intestinal closed-loop assay. SLC6A14 knock-out mice exhibited a decrease in cAMP stimulated fluid secretion mediated via CFTR relative to Wt control. To explore the mechanism by which this modification occurs, we utilized a BHK heterologous expression system, overexpressing CFTR and SLC6A14. Interestingly, the functional interaction can be recapitulated in this system, suggesting that it not tissue-type dependent. Preliminary biochemical and anion-flux studies support the hypothesis that SLC6A14 does not affect the processing or stability of Wt or F508del-CFTR proteins rather it augments the activity of these channel proteins once localized to the cell surface. Future studies will focus on understanding if this augmentation is related to modification of CFTR's phosphorylation dependent gating. These results show a positive impact of SLC6A14 on CFTR channel function and fluid secretion, providing an alternative drug target for CF patients.

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Enhanced Activation of an Amino-Terminally Truncated Isoform of Voltage-Gated Proton Channel HVCN1 Enriched in Malignant B cells

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HVCN1 is the only mammalian voltage-gated proton channel. In human B lymphocytes, HVCN1 associates with the B Cell Receptor (BCR) and is required for optimal BCR signaling and redox control. HVCN1 is expressed in malignant B cells that rely on BCR signaling, such as Chronic Lymphocytic Leukemia (CLL) cells. Compared with normal B lymphocytes, HVCN1_S expression is higher in B-cell lines and in B cells from patients with Chronic Lymphocytic Leukemia. We found that HVCN1 was expressed in B cells as two protein isoforms. The shorter isoform (HVCN1_S) was enriched in B cells from a cohort of 76 CLL patients. When overexpressed in a B-cell lymphoma line, HVCN1_S responded more profoundly to PKC-dependent phosphorylation. This more potent enhanced gating response was mediated by increased phosphorylation of the same residue responsible for enhanced gating in HVCN1_L, Thr²⁹. Although B cells from CLL patients expressed both isoforms, their PMA response was comparable with that of cells heterologously expressing HVCN1_S alone, indicating that this isoform dominates. Furthermore, the association of HVCN1_S with the BCR was weaker, which resulted in its diminished internalization upon BCR stimulation. Finally, HVCN1_S conferred a proliferative and migratory advantage, as well as enhanced BCR-dependent signaling. Overall, our data show for the first time the existence of a shorter isoform of HVCN1 with enhanced gating that is specifically enriched in malignant B cells. HVCN1_S properties suggest it may contribute to the pathogenesis of BCR dependent B-cell malignancies.

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Positive KCa Channel Gating Modulators with Selectivity for KCa3.1

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Small-conductance (KCa2) and intermediate-conductance (KCa3.1) calcium-activated K⁺ channels are voltage-independent and share a common calcium/calmodulin mediated gating mechanism. Existing positive gating modulators like EBIO, NS309 or SKA-31 activate both KCa2 and KCa3.1 channels with similar potency or, as in the case of CyPPA and NS13001, selectively activate KCa2.2 and KCa2.3 channels. We recently performed a structure activity rela-

tionship (SAR) study with the aim of optimizing the benzothiazole pharmacophore of SKA-31 towards KCa3.1 selectivity and have identified SKA-121 (5-methylnaphtho[2,1-d]oxazol-2-amine), which displays 41-fold selectivity for KCa3.1 (EC50 109 nM ± 14 nM) over KCa2.3 (EC50 4.4 ± 1.6 μM). SKA-121 is 200-400 fold selective over representative KV (KV1.3, KV2.1, KV3.1 and KV11.1), NaV (NaV1.2, NaV1.4, NaV1.5 and NaV1.7) as well as CaV1.2 channels. SKA-121 is a typical positive-gating modulator, which shifts the calcium-concentration response curve of KCa3.1 to the left but also increases open probability at saturating Ca²⁺ concentrations. In order to understand why introduction of a "simple" CH₃ group in 5-position of the benzothiazole/oxazol system could achieve such a significant gain in selectivity for KCa3.1 over KCa2.3 we are currently performing site-directed mutagenesis of the calmodulin binding domain (CaMBD) guided by structural modeling. We used the RosettaLigand method to generate preliminary models of SKA-121 binding to our model of the KCa3.1 C-terminal CaMBD in complex with calmodulin. Our most energetically favorable model suggests that SKA-121 is positioned in close proximity to F301, R362, V365, M368, V369, and S372 in KCa3.1.

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Expression and Contributions of TRPM7 and KCa2.3/SK3 Channels to the Increased Migration and Invasion of Microglia in Anti-Inflammatory Activation States

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Microglia rapidly respond to CNS injury and disease and can assume a spectrum of activation states that are reflected by changes in gene expression. Classical activation (often induced by lipopolysaccharide) and alternative activation (IL4-induced) are now well studied but less is known about acquired deactivation in response to IL10. Here, we are addressing how microglial activation states affect their migration and invasion; crucial functions in the developing CNS and after injury in adults.

We found that LPS-treated rat microglia migrate very poorly; whereas, IL4- or IL10-treated cells migrated and invaded much better than resting cells. Nevertheless, there were similarities and differences in gene induction by IL4 and IL10. The lamellum of migrating microglia contains a large ring of podosomes/microscopic structures that are thought to mediate adhesion, migration and invasion. IL10 (not IL4) increased podosome expression.

Then, based on the observed enrichment of SK3 Ca²⁺-activated potassium channels in podosomes, we predicted that it regulates migration and invasion. KCNN3 (SK3) expression was similar under all three activation conditions and KCa2.3 currents were observed. A surprising finding was that, of the three SK3 inhibitors tested (apamin, tamapin, NS8593); only NS8593 reduced the migration and invasion of IL4- or IL10-activated microglia. Surprisingly, in addition to blocking SK3, we found that NS8593 blocked TRPM7 channels in microglia. We confirmed that TRPM7 (not SK3) regulates migration and invasion by using a TRPM7 inhibitor (AA-861) that does not block SK3 channels.

We conclude that TRPM7 (not SK3) contributes to the enhanced ability of microglia to migrate and invade when in anti-inflammatory states. This will be an important consideration in the current efforts to develop TRPM7 inhibitors for treating CNS injury.

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Atomic Basis for Potassium Channel Potentiation by A Novel Class of Anti-Epileptic Drugs

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Retigabine is the prototypical member of a recently discovered class of anti-epileptic drugs that act by potentiating the neuronal M-current formed by KCNQ2-5 potassium channel subunits. Retigabine interacts with the pore of KCNQ2-5 channels, requiring a Trp residue present in KCNQ2-5 to exert its effects, suggesting the notion of a hydrophobic binding pocket. We have used nonsense suppression to subtly alter the properties of this Trp residue (265) in homomeric KCNQ3 channels and identify the specific chemical interactions required for retigabine effects. Remarkably, simply changing the position of the indole nitrogen atom from Trp265 (removing its hydrogen bonding