Developing Stable cell lines with Combinatorial Kv subtypes for Drug discovery.

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Background

Voltage gated Potassium channels (Kv) represent a large family (40 genes) of related proteins found in both excitable and non excitable cells. Neurons express multiple Kv channels however particular Kv subtypes are confined to discrete populations of cells. In addition Potassium channels form homo- and heterotetramers with different combinations of subunits. As well as giving flexibility of function for the neuron this also means they represent an attractive therapeutic target with a possibility of reduced off target effects. We have developed a model system allowing the exploration of Kv channel biology and the testing of drugs that modulate this system.

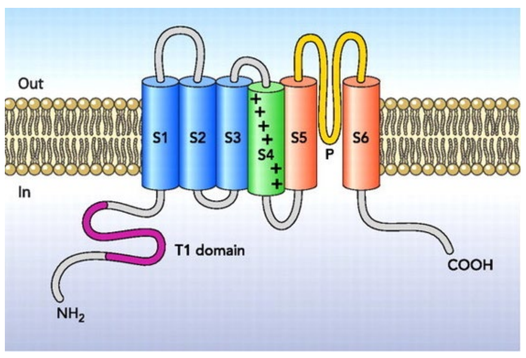
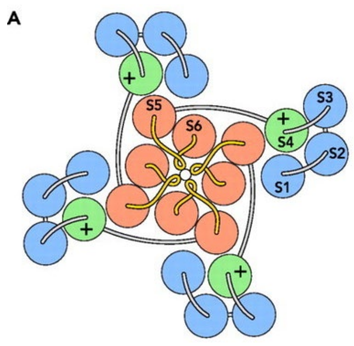
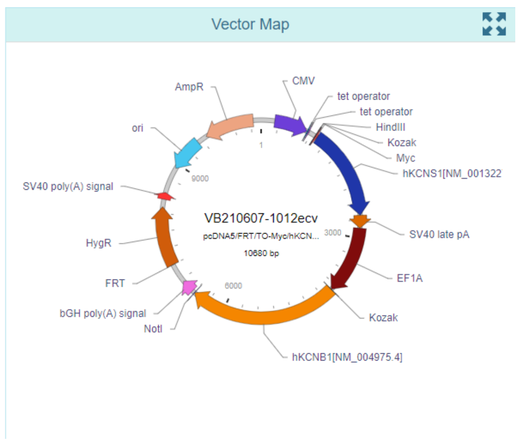


Figure 1 Right. Side view of the structure of a single Kv α-subunit consisting of six transmembrane segments (S1-S6) . Right. A top view in which four Kv subunits are assembled into a tetramer. (From Bocksteins and Snyder)



Cell System

HEK293Flp-In™ T-Rex ™ system (Invitrogen) was used to establish stable cell lines. Integration of plasmid DNA occurs at a single recombination site in the HEK293 genome by co transfecting a recombinase plasmid with the pcDNA5 based vector carrying the genes of interest (GOI). The Inducible bicistronic vector system guarantees expression of two proteins for example Kv2.1 and Kv9.1 protein in the same cell. Uninduced cells express one protein.



Features:

- pcDNA5 based vector that allows for production of a stable cell line with GOI at a single frt (Flp recombinase target) site in HEK293-Flp cells
- Kv2.1 constitutively expressed from EF1A promoter (strong mammalian promoter)
- Kv9.1 is tagged with myc and is repressed until addition of doxycyclin (or tetracyclin)

Figure 2 DNA vector (Vector Builder) for transfection and selection (hygromycin) of stable cell lines

Validation-Immunoblotting and Imunnofluorescence

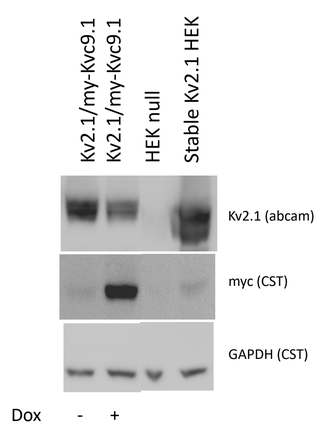


Figure 3 Stable cell lines were induced for 24 hrs with doxycycline. Cell lysate were collected from the stable cell line and western blotting was performed using antibodies to Kv2.1, GAPDH and myc as a proxy for kv9.1

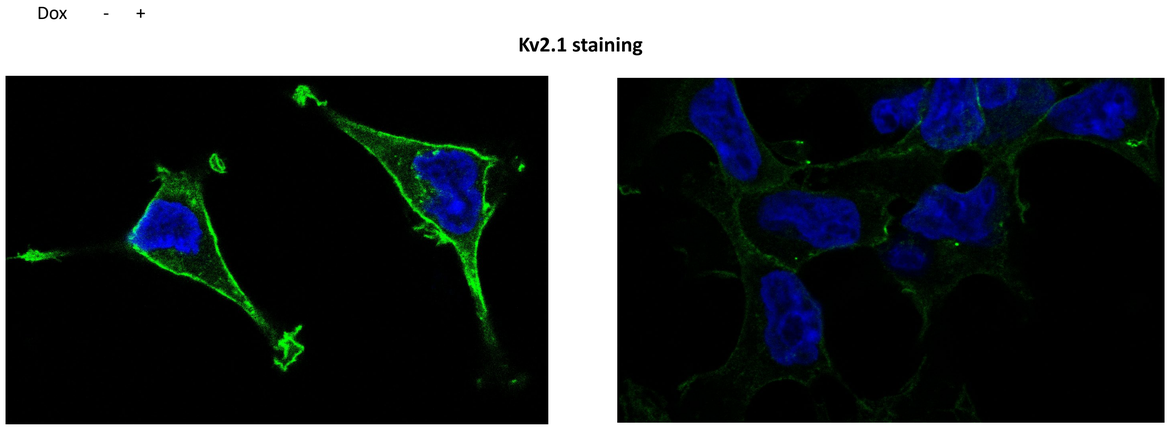


Figure 4 HEK293-Flp stable cell lines were induced for 24 hrs with doxycycline and compared to uninduced cell lines. Cells were stained with antibodies to Kv2.1 and counter stained with DAPI. Confocal images were acquired on Leica SP8 microscope (63X oil objective and 488 nm laser line)

Kv2.1/myc-Kv9.1 Cell line in a Thallium flux assay

We tested the functionality of the Kv 2.1/myc-Kv9.1 cell line in a Thallium flux assay. Potassium channels are permeable to thallium (Tl-). On stimulus thallium flows down its concentration gradient into the cells and generates a fluorescence signal in the FluxOR™ dye.

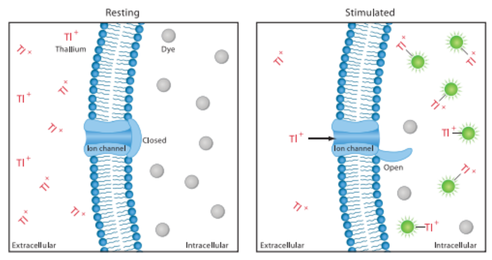
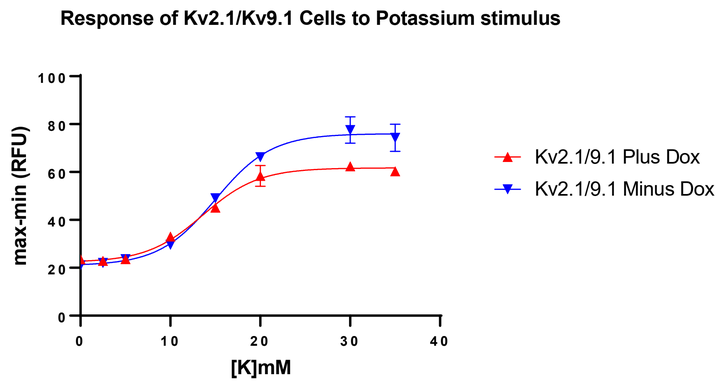


Figure 4 Thallium Flux assay. Basal Fluorescence from cells loaded with FluxOR™ dye as shown in left panel is low until potassium channels are stimulated with thallium and potassium thalium enters the cells activating the dye, as seen in right panel (From Thermofisher)

Figure 5 Thallium Flux assay. Potassium concentration dose response curve for Stable HEK293-Flp cells expressing Kv2.1 and inducible Kv9.1



Induction of Kv9.1 with doxycycline in Kv2.1 HEK293-Flp cells resulted in a reduction in K+ channel activity

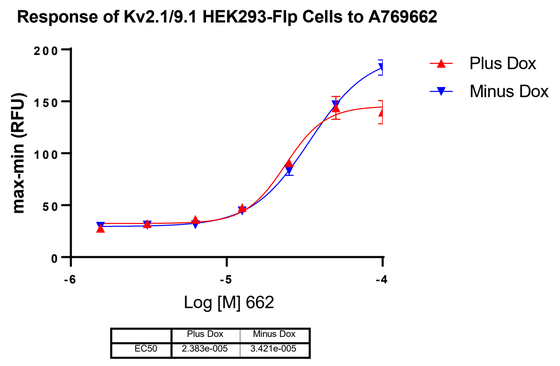


Figure 6 Thallium Flux assay. Stable HEK293-Flp cells expressing Kv2.1 and inducible Kv9.1. Response to AMPK A769662

A 769662 is a potent activator of the kinase AMPK and phosphorylates Kv2.1 (Ikematsu et al 2011) facilitating activation of Kv2.1. Kv9.1 has been shown to inhibit Kv2.1 activity although having no K+ channel activity of its own (Salinas et al 1997) Induction of Kv9.1 with doxycycline in Kv2.1 HEK293-Flp cells resulted in a reduction in the Kv2.1 response to A769662 suggesting that these cell lines recapitulate known behaviour of Kv2.1 and Kv9.1 in the literature.

Summary & Future plans

- We have established a stable cell line allowing for the constitutive expression of potassium channel Kv2.1 with the ability to co express Kv9.1 after induction with doxycycline.
- The cell line appears to behave in a way predicted from the known literature
- Applications include its use as a tool for examining the basic biology of Kv heterotetramers and as a screening cell line.
- Further work includes an examination of the behaviour of the cell line in electrophysiology

References

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M Salinas, F Duprat, C Heurteaux, J P Hugnot, M Lazdunski. J. Biol. Chem., 1997 Sep 26 , 272, 24371-9

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