Tank binding kinase-1 interacts with the cytoplasmic C-terminus of the Kv3.3 potassium channel

Yalan Zhang[1], Luis Varela[3], Klara Szigeti-Buck[3], Manavi Chatterjee, Tamas L. Horvath[3] and Leonard K. Kaczmarek[1,2]

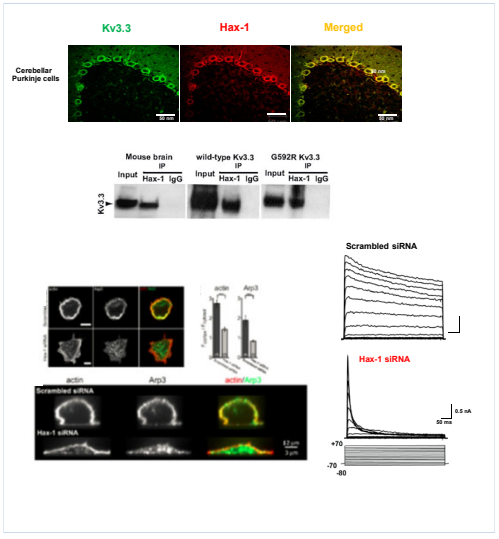
Depts of [1]Pharmacology, [2]Cellular and Molecular Physiology, and [3]Comparative Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520

Abstract

Spinocerebellar Ataxia type 13 (SCA13), a condition that lead to cerebellar degeneration, is caused by mutations in the *KCNC3* gene, which encodes the voltage-dependent potassium channel Kv3.3. These channels are expressed at particularly high levels in Purkinje cells in the cerebellum, and directly bind Hax-1, a cell survival protein required for survival of the cerebellum. To investigate how KCNC3 mutations lead to neurodegeneration, we generated mice bearing the G592R Kv3.3 mutation using CRISPR Cas9 gene editing. G592R Kv3.3 channels are fully functional as potassium channels but have abnormal interactions with cytoplasmic signaling pathways and lead to cerebellar degeneration in humans. A screen for protein kinases activated by this mutation revealed that the activity of Tank-Binding protein 1 (TBK1), an enzyme that plays a key role in the formation of multivesicular bodies, autophagy and mitophagy, is much higher in the cerebellum of G592R Kv3.3 knock-in mice than in that of wild type animals. Total levels of TBK1 and of phosphorylated S6 protein were not altered by the mutation, and no differences in TBK1 activation were detected in the forebrain of mutant and wild type animals. We found that TBK1 can be coimmunoprecipitated with Kv3.3 channels and that this binding is significantly enhanced by the G592R mutation. To determine sites on the Kv3.3 channel required for interaction with TBK1, we carried out a series of gene truncations C-terminus of Kv3.3 and determined that a stretch of amino acids in the center of the cytoplasmic C-terminus, including polyproline rich-region that contains G592R is required for tight association of TBK1 with the channel. Using transfected cell lines, we found that G592R Kv3.3 channels increases TBK1 activation basally and that depolarization of the mutant channels further stimulates TBK1 activity over that produced by depolarization of wild type channels. By immunoelectron microscopy and western blotting, we find that the enhanced activation of TBK1 in G592R Kv3.3 knock-in mice is associated with increased numbers of intracellular multivesicular bodies containing Hax-1, increased levels of CD63, a molecular marker for multivesicular bodies, and increased LAMP2 expression. No change was found in levels of LC3B, a protein associated with autophagy. Our findings suggest that Kv3.3 channels both physically and functionally interact with TBK1, and that this protein complex regulate the trafficking of Kv3.3 together with the survival protein Hax-1 into multivesicular bodies and lysosomes for degradation.

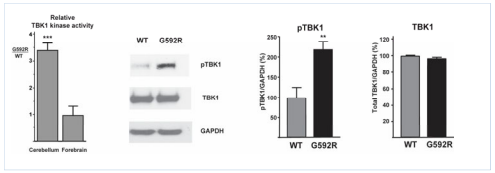
Background

Kv3.3 K^+ channels bind the cell survival protein Hax-1 to prevent rapid inactivation

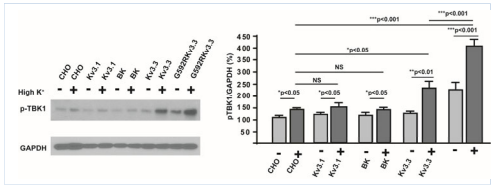


Results

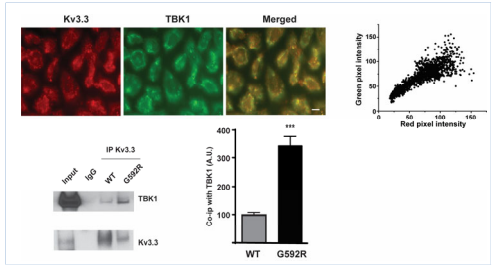
TBK1 activity is elevated in the cerebellum of spinocerebellar ataxia mutant Kv3.3 mice



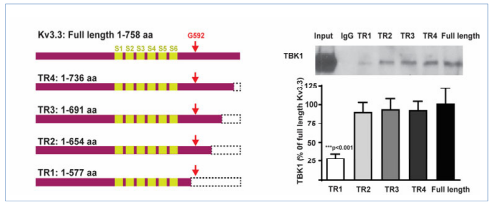
TBK1 activity is activated by depolarization of cells expressing Kv3.3 channels



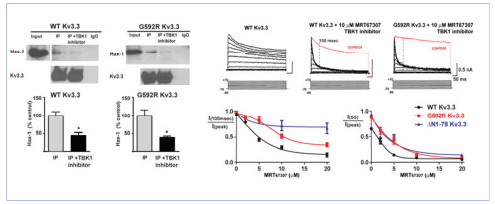
Kv3.3 potassium channels interact with TBK1



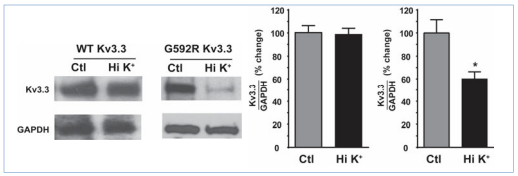
Kv3.3 C-terminal aa 577-654 are required for TBK1 binding



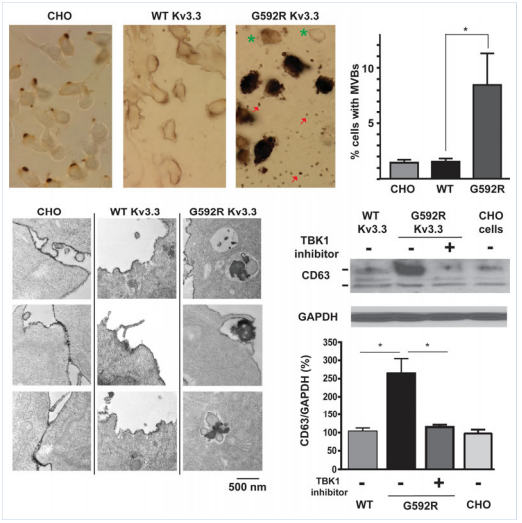
TBK1 activity keeps Hax-1 bound to Kv3.3 channels to prevent inactivation



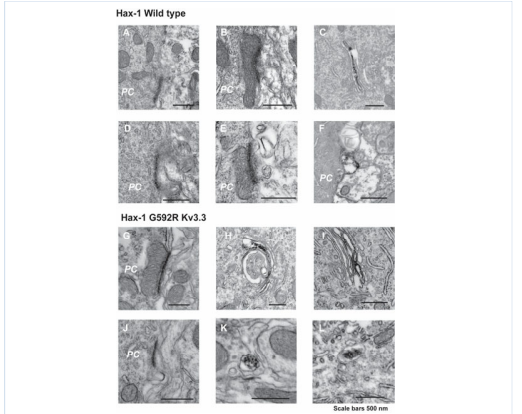
Prolonged depolarization causes loss of G592R Kv3.3 channels, but not wild type channels



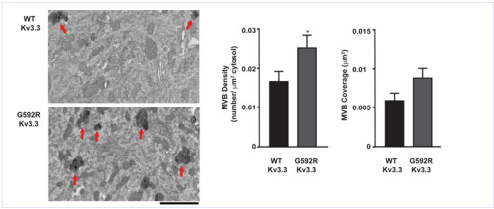
Mutant Kv3.3 channels traffic Hax-1 into multivesicular bodies in Kv3.3 expressing cells



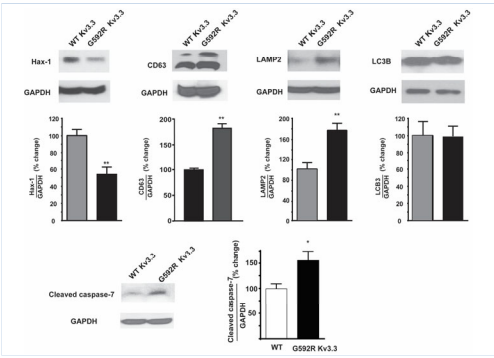
Mutant Kv3.3 channels traffic Hax-1 into multivesicular bodies in Purkinje neurons



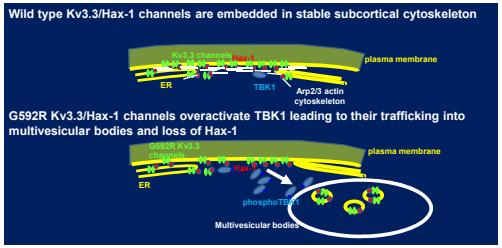
Multivesicular body distribution density is increased in G592R Kv3.3 knock in mice



Hax-1 expression level is reduced in mutant knock in mice



Working Model



Conclusions

1. Activation of TBK1 is greater in cells and mice that express the G592R Kv3.3 channel mutation.
2. More multiple vesicular bodies are in the mutant G592R expressing cells.
3. Loss of the Hax-1 may contribute to the neurodegeneration caused by SCA13.

Supported by NIH R01DC001919 (LKK), National Ataxia Foundation, NIDA Keck Center.