Activation of mechanoreceptor Piezo1 inhibits enteric neuronal growth and migration *in vitro*

[1]Chioma Moneme, [2]Oluyinka O. Olutoye II, [1]Michał F. Sobstel, [1]Yuwen Zhang, [1]Xinyu Zhou, [1]Jacob L. Kaminer, [2]Britney A. Hsu, [1]Chengli Shen, Arabinda Mandal1, [2,3]Hui Li, [2,3]Ling Yu, [2,3]Swathi Balaji, [2,3]Sundeep G. Keswani, [1,2,3]Lily S. Cheng

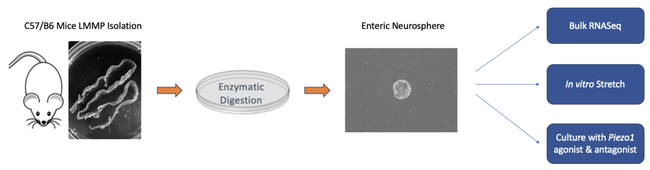
1Department of Surgery, University of Virginia, Charlottesville, VA, United States, 2Department of Surgery, Baylor College of Medicine, Houston, TX, United States, 3Department of Pediatric Surgery, Texas Children's Hospital, Houston, TX, United States

Background

- The enteric nervous system (ENS) comprises a sophisticated network of more than 500 million neurons, often called the "second brain," which coordinates many crucial gastrointestinal functions.
- Dysfunction within the ENS has been associated with various functional gastrointestinal disorders, including Hirschsprung disease, a congenital disorder involving dysfunction of the enteric nervous system (ENS) due to abnormal migration, resulting in an aganglionic distal bowel.
- Piezo1 is a large trimeric transmembrane ion channel known primarily for its role in mechanotransduction.
- Our hypothesis is that the activation of Piezo1 could inhibit the development and modify the differentiation of the developing ENS.

Aim: To determine the impact of Piezo1 agonism and antagonism on enteric neuron postnatal growth and development

Methods



Results

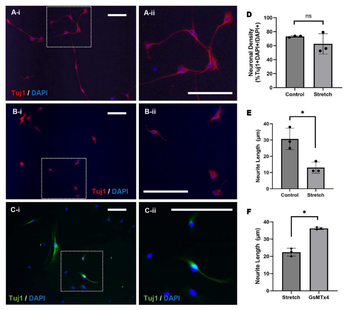


Figure 1. Stretch results in shorter neurite length and differential gene expression, whereas Piezo1 antagonism prevents neurite stunting in response to stretch. ENPC-derived neurons from adult mice were subjected to up to 5% cyclic stretch. There was a difference in morphology (A = unstretched vs B = stretched). There was no difference in cell density.

Results

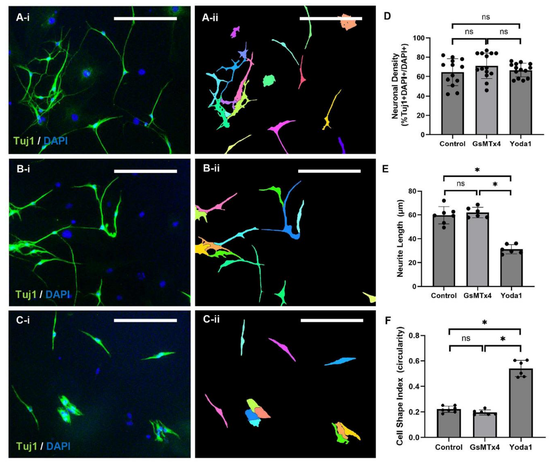


Figure 2. Piezo1 agonism also results in significantly shorter neurite lengths in vitro.
ENPC from adult mice were differentiated in vitro with vehicle alone (A), with Piezo1 antagonist GsMTx4 (B), or with Piezo1 agonist Yoda1 (C). The results are using the pan-neuronal marker. There were no significant differences in neuronal density among control, GsMTx4-treated, or Yoda1-treated ENPCs (D). In ENPC-derived neurons treated with Yoda1, neurite length was significantly shorter (E), and cell circularity was significantly higher (F) compared to both control and GsMTx4-treated cells.

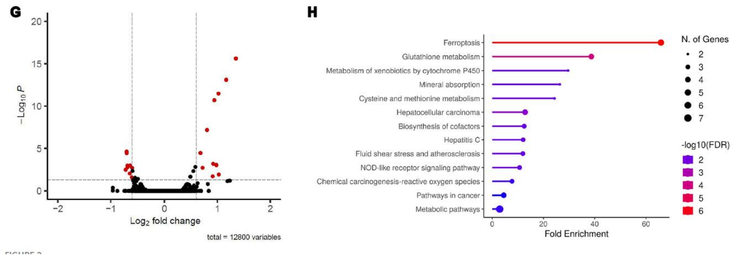


Figure 3. Bulk RNASeq showed significant differential expression in response to stretch
Twenty-seven genes were significantly differentially expressed in the stretched cells compared to the unstretched controls (G). This corresponded with a significant fold enrichment in 13 KEGG pathways (H)

Results

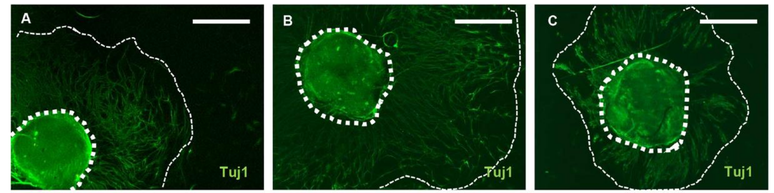


Figure 3. Piezo1 agonism leads to significantly reduced neuronal migration in vitro. Enteric neurospheres were treated with either vehicle alone (A), the Piezo1 agonist(Yoda1) (B), or the Piezo1 antagonist (GsMTx4) (C). The wavefront of neuronal migration was measured from the edge of the neurosphere. This wavefront of migration can be observed qualitatively using immunohistochemistry for the pan-neuronal marker, Tuj1, as shown in representative images of each condition.

Future Directions

- Investigate effect of Piezo1 on neuronal subtypes differentiation
- Confirm impact of Piezo1 with gene knockout mouse variants
- Assess in vivo GI functional studies in Piezo knockout variants

Conclusions

1. There are phenotypic changes occur in the ENS in response to biomechanical force that is recapitulated by *Piezo1* agonism.
2. *Piezo1* agonism leads to decreased cell migration and recovery from injury.
3. *Piezo1* may play a role in during ENS development and the pathogenesis of diseases that result from dysfunction in ENS migration.

Funding & Acknowledgements

NIH T32 to CM and NIH K08 (K08DK133673), American Pediatric Surgery Association Jay Grosfeld Scholar Award, American College of Surgeons Franklin H. Martin Research Award to LSC