Smooth Muscle Cell Piezo1 in the Mechanosensation of the Small Bowel

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INTRODUCTION

The role of mechanosensation in intestinal motility is complex and not yet thoroughly studied. Piezo1 is a recently identified mechanosensitive non-selective cation channel located in various tissue and cell types, including the layers of the small bowel. Piezo1 is present in the intestinal muscularis cells (IMCs), including the myenteric plexus located between the two muscularis layers, yet its role is currently unknown. Since the SMCs within the muscularis layer are the primary mediators of motility, Piezo1 in SMCs may play a role in maintaining peristalsis and other stretch-related functions given its mechanosensitive properties.

HYPOTHESES

1. Piezo1 in the SMCs of the intestinal muscularis plays a role in stretch-induced contractility.
2. Piezo1 in the SMCs of the muscularis is important for the maintenance of the crypt-villi axis and stem cell differentiation via mechanical signaling from the muscularis.

METHODS

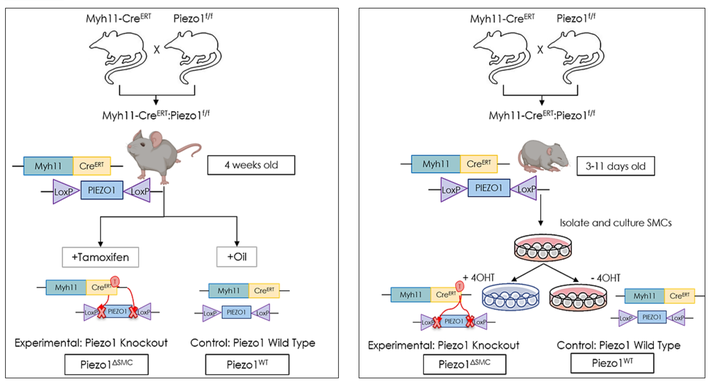

Figure 1. Diagram of methods to model Piezo1 knockout *in vivo* and *in vitro*.

- ***In vivo***: Experimental mice were given Tamoxifen (50mg/kg) via OG for 5 days. Sacrificed at 10-days and 21-days post-tam, samples obtained for DNA, RNA, protein, and histology analysis. Control mice were also subjected to distal partial SBO surgery in order to stimulate persistent stretch.
- ***In vitro***: IMCs extracted from small intestine were then treated with 4-OHT to induce Piezo1 knockout. Cells were then stretched by decreasing the temperature of the hydrogel to 33˚C. Contraction and calcium flux were analyzed using confocal video microscopy at 37˚C and 33˚C.
- ***Statistics***: Statistical analysis was done using unpaired Student's t-tests with Welch's correction through GraphPad Prism. P <0.05 was considered significant.

RESULTS (In vivo)

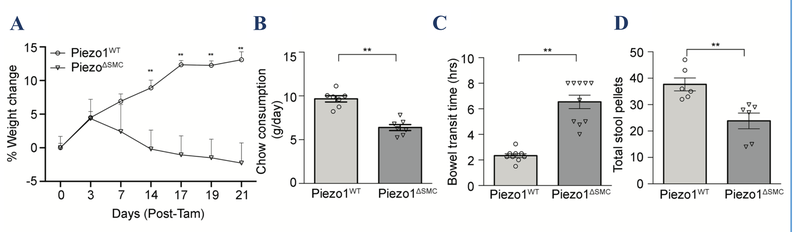

Figure 2. Piezo1$^{\Delta SMC}$ leads to (A) weight loss, (B) decreased chow consumption, (C) decreased stool output and (D) increased whole bowel transit time.

RESULTS (In vitro)

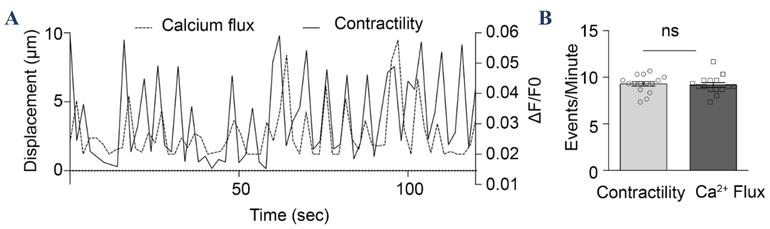

Figure 3. (A) Interchanging signals between IMC contractions (left axis) and Ca^{2+} influx (right axis). (B) Showing similar frequencies.

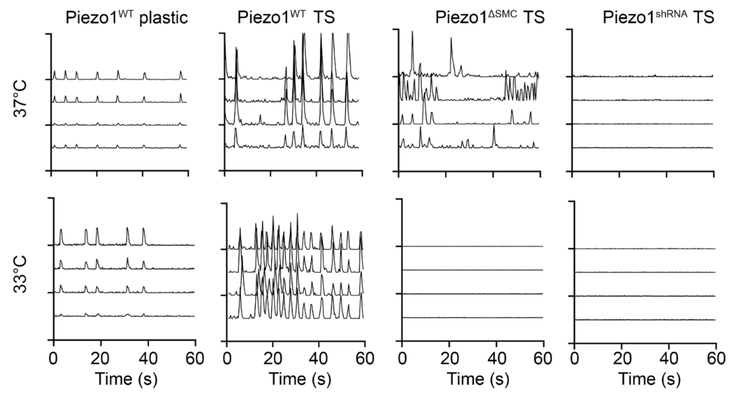

Figure 4. Representative Ca2+ tracings in response to temperature (37°C to 33°C) of IMCs on plastic and with stretch on TS scaffolds for Piezo1^{WT}, Piezo1$^{\Delta SMC}$, and Piezo1^{shRNA} and compared with a decrease in Ca2+ influx on plastic at 33°C.

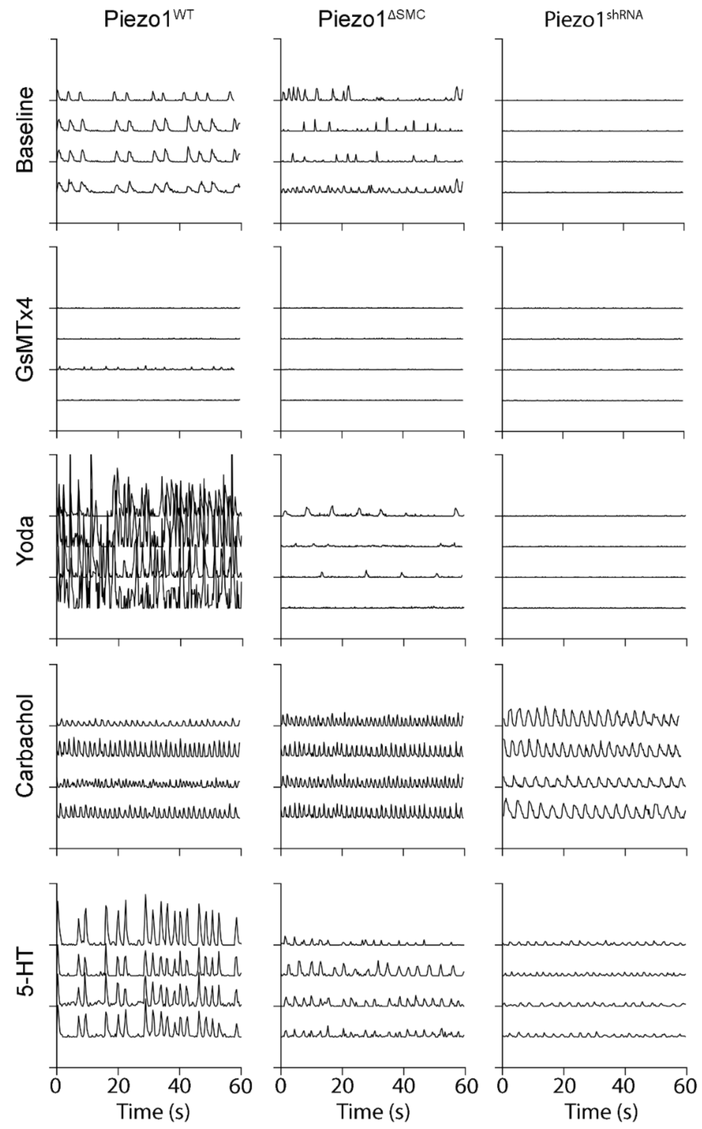

Figure 5. Representative calcium tracings of Piezo1^{WT}, Piezo1$^{\Delta SMC}$, and Piezo1^{shRNA} IMCs at with GsMTx4, Yoda, carbachol, and 5HT at 37°C.

RESULTS (IHC/IF)

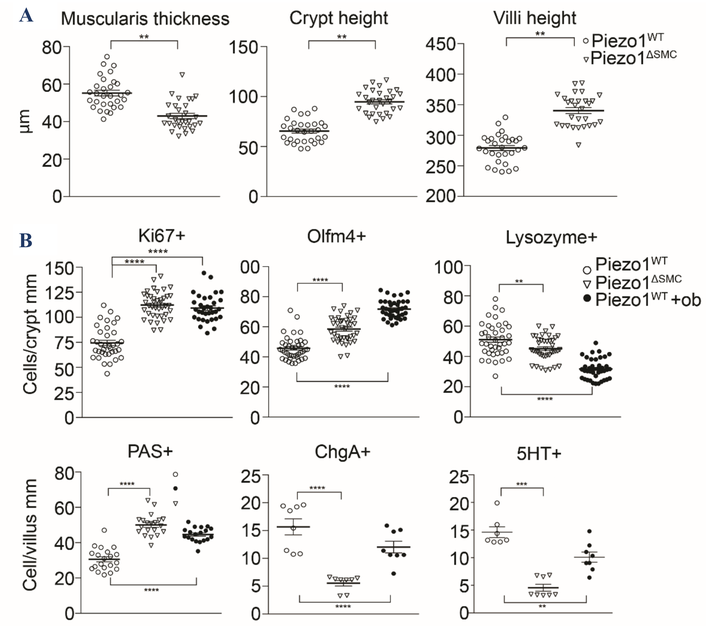

Figure 6. (A) Morphological changes of distal small bowel at 21 days post-tamoxifen treatment show decreased muscularis thickness, increased crypt height, and increased villi height Piezo1$^{\Delta SMC}$ when compared to Piezo1^{WT}. (B) Specific cell populations within the epithelium showed similar changes in Piezo1$^{\Delta SMC}$ and obstructed Piezo1^{WT} compared to Piezo1^{WT} unobstructed mice.

SUMMARY OF FINDINGS

- ***In vivo***: Piezo1 in the SMCs in the muscularis is important for:
  - Normal weight gain and bowel transit time.
  - Normal crypt and villus morphology.
  - Normal ratio of ISCs, PCs, EECs, and Goblet cells in the epithelium.
- ***In vitro***: Piezo1 in the SMCs in the muscularis is important for normal spontaneous, synchronized and rhythmic contractions.
  - Piezo1 expression in IMCs other than SMCs is important for normal baseline and stretch-induced spontaneous contractions.
  - But it is not necessary for the cholinergic and serotonergic activation of Ca2+ flux.

CONCLUSIONS

- The complex interactions that generates the electromechanical coupling of contractions within the muscularis are dependent on Piezo1 expression in the SMCs.
- SMC Piezo1 is important for the maintenance of gut motility and normal epithelial growth.

REFERENCES

1. Mazet B. Gastrointestinal motility and its enteric actors in mechanosensitivity: past and present. Pflugers Arch 2015;467:191-200.
2. Bertrand PP. Real-time measurement of serotonin release and motility in guinea-pig ileum. J Physiol 2006;577:689-704.
3. Gnanasambandam R, Bae C, Gottlieb PA, et al. Ionic Selectivity and Permeation Properties of Human PIEZO1 Channels. PLoS One 2015;10:e0125503.
4. Wang Q, Wang K, Solorzano-Vargas RS, Lin P-Y, Walthers CM, Thomas A-L, Martín MG, Dunn JCY. 2018. Bioengineered intestinal muscularis complexes with long-term spontaneous and periodic contractions. Plos One 13.

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