Gut contractile organoids: a novel model system to study the cellular synchronization in gastrointestinal motility

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【Introduction】

Gastrointestinal (GI) motility is important for digestion, absorption, and excretion. Previous studies have shown that this movement is regulated by smooth muscle cells (SMCs), enteric nervous system (ENS), and interstitial cells of Cajal (ICCs) known as a pacemaker. However, how these three types of cells cooperate remains unclear. One reason is that the GI tract is a multilayered structure hampering analyses at the cellular level. Therefore, I attempted to analyze GI motility using chicken embryos, which have a stiff tissue and are easy to manipulate. The biggest problem in the analysis using chickens has been the absence of available antibodies against the chicken c-Kit protein, a marker of ICCs. Therefore, I first raised a new antibody against the chicken c-Kit protein, and verified its usefulness. Next, to analyze GI motility at the cellular level, I developed a novel in vitro model which is specific to GI motility and then proceeded with the analysis.

【Materials and Methods】

Anti-c-Kit antibody: The polypeptide at the position of 716–728 amino acids of the chicken c-Kit protein was synthesized and used for immunization of a rabbit. The purified antiserum was used as anti-c-Kit antibody. To confirm the specificity of this antibody against the chicken c-Kit protein, I validated by Western blotting analysis, immunocytochemistry with c-Kit cDNA-transfected cells, and immunohistochemistry with c-Kit-expressing tissue.

In vitro model: From an E15 hindgut in chicken embryonic GI tract, by removing the serosa and intestinal epithelium, only muscle layer was collected. 5.0×10^5 cells from muscle layer were cultured with Matrigel. For analysis of this model, I used Ca^{2+} indicator GCaMP6s and gap junction inhibitors.

【Results and Discussion】

(1) Anti-c-Kit antibody: Correct signals were detected by this newly raised anti-c-Kit antibody in Western blotting analysis and immunostaining with c-Kit-overexpressing cells and tissue. Therefore, I conclude that this new antibody can detect the c-Kit protein in chickens. Using this antibody, morphology of ICCs in hindgut was also visualized. ICCs with bipolarity and multipolarity were observed, which is consistent with mammals. (2) In vitro model: To determine optimal conditions for culturing gut muscle layer-derived cells, I tried 3 kinds of FBS-free media on Matrigel. When cultured with Neurobasal medium, spheroids of cells were formed which exhibited rhythmic contractions. By immunostaining anti-c-Kit and αSMA antibodies as markers for ICCs and SMCs, I found that ICCs were located internally whereas SMCs were peripherally. Based on these characteristics, I designated this spheroid as a gut contractile organoid, serving as a novel model of GI motility. Visualization of intracellular Ca^{2+} signals by GCaMP6s revealed that the organoids exhibited Ca^{2+} oscillations with the same rhythm as the contraction. Ca^{2+} signals were synchronized among organoid-constituting cells. This synchronization was unaffected by gap junction inhibitors, indicating that there is a different mechanism other than gap junction-mediated signaling.

Interestingly, when multiple organoids with independent contraction rhythm fused to form a larger organoid, the rhythm became synchronized in fusing organoids. These findings revealed interactions between cells and organoids for coordinated contractions. To further investigate the mechanism underlying the synchronization between multiple organoids, 3-well hydrogels were prepared that prevented organoidal fusions. I have found that the rhythm of contraction and Ca^{2+} oscillations of separated organoids became synchronized when SMCs bridged separated organoids. The synchronization between organoids observed in this study recapitulates the local contractions of the GI motility along the circumferential axis of the gut.

【Conclusion】

I established the gut contractile organoid as a novel model of GI motility. The organoid serves as a powerful tool for investigating GI motility at the cellular revel.