Development of human intestinal organoids from iPS cell technology

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Intestinal analysis has been usually performed using established cell lines or primary cells in 2D culture. However, these culture systems can not satisfy the complexity of 3D structure and diversity of composed cell types in the intestinal epithelial tissue.

Here, we report the generation of intestinal organoids using human iPS cells (iPSCs) by sequential treatment with various cytokines and compounds. We observed that almost cells were double positive for the definitive endoderm markers SOX17 and FOXA2 at day 3 of differentiation. The expression of *CDX2*, a marker of the mid/hindgut, was upregulated at day 7 of differentiation, and floating and semi-adherent spheroids were positive for CDX2. Within several days after floating spheroids were embedded in Matrigel and incubated in intestinal growth medium, round organoids were observed at day 21. Immunocytochemical analysis revealed that these organoids consisted of monolayer cells, which were positive for intestinal markers E-cadherin (ECAD) and KLF5. In addition, RT-qPCR analysis revealed that multiple epithelial cell markers, *LGR5* (intestinal stem cells), *VIL1* (enterocytes), *MUC2* (goblet cells) and *LYZ* (paneth cells) were upregulated on day 21.

These data suggest that human iPSCs are successfully differentiated into intestinal organoids consisting of epithelial monolayers.