Generating human intestinal tissue from pluripotent stem cells in vitro.

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Abstract

Here we describe a protocol for generating 3D human intestinal tissues (called organoids) in vitro from humanpluripotent stem cells (hPSCs). To generate intestinal organoids, pluripotent stem cells are first differentiated into FOXA2(+)SOX17(+) endoderm by treating the cells with activin A for 3 d. After endoderm induction, the pluripotent stem cells are patterned into CDX2(+) mid- and hindgut tissue using FGF4 and WNT3a. During this patterning step, 3D mid- or hindgut spheroids bud from the monolayer epithelium attached to the tissue culture dish. The 3D spheroids are further cultured in Matrigel along with prointestinal growth factors, and they proliferate and expand over 1-3 months to give rise to intestinal tissue, complete with intestinal mesenchyme and epithelium comprising all of the major intestinal cell types. To date, this is the only method for efficiently directing the differentiation of hPSCs into 3D human intestinal tissue in vitro.

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