SnapShot: Growing Organoids from Stem Cells

Cell

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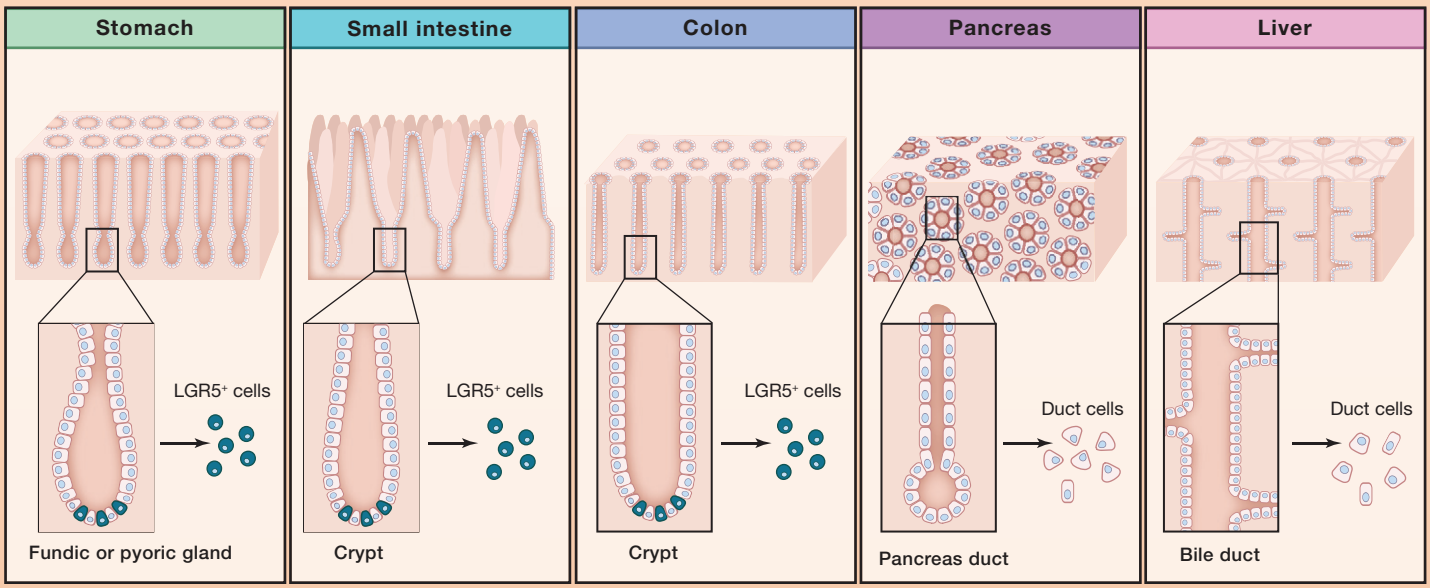

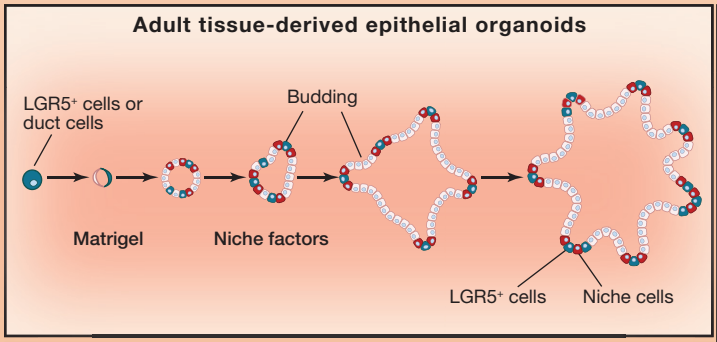

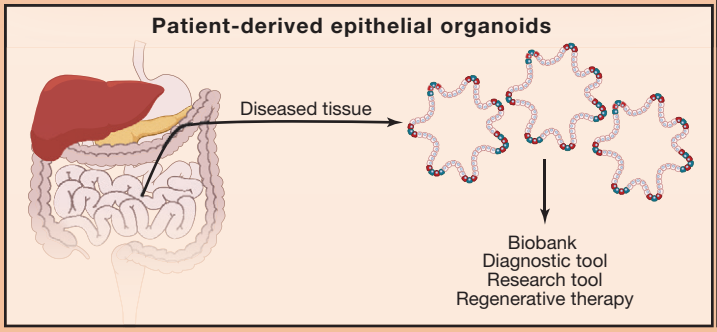

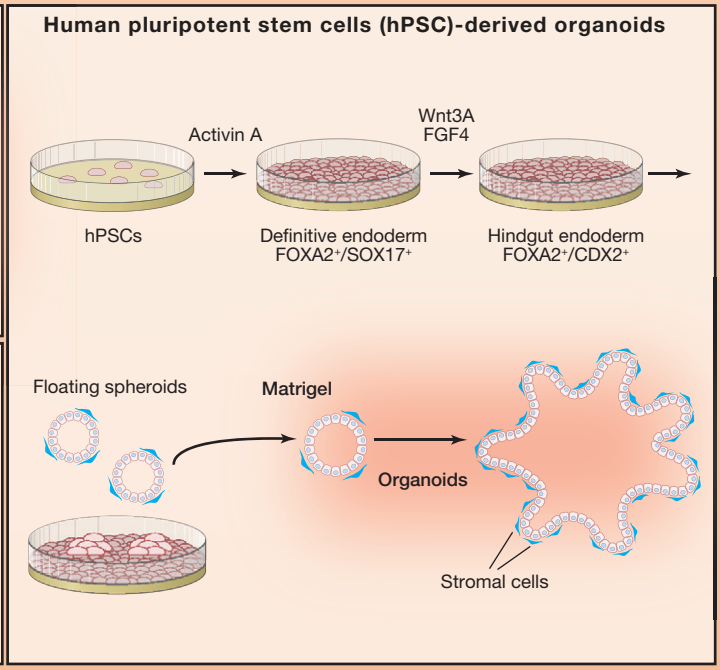

Type of tissue	Source	Stem cell culture condition (niche factors)	Differentiation culture condition
Stomach	Adult mouse	EGF, Noggin, R-spondin, Wnt-3A, FGF10	EGF, R-spondin
	Adult human	EGF, Noggin, R-spondin, Wnt-3A, FGF10	EGF, R-spondin
	hPSC	EGF	EGF
Small intestine	Adult mouse	EGF, Noggin, R-spondin	EGF, Noggin, R-spondin
	Adult human	EGF, Noggin, R-spondin, Wnt-3A TGF-b inhibitor, p38 inhibitor	EGF, Noggin, R-spondin, TGF-b inhibitor
	hPSC	EGF	EGF
Colon	Adult mouse	EGF, Noggin, R-spondin, Wnt-3A	EGF, Noggin, R-spondin
	Adult human	EGF, Noggin, R-Spondin, Wnt-3A, TGF-b inhibitor, p38 inhibitor	EGF, Noggin, R-spondin, TGF-b inhibitor
Pancreas	Adult mouse	EGF, Noggin, R-spondin, Wnt-3A, FGF10, Nicotinamide	EGF, Noggin, R-spondin, Wnt-3A
	Adult human	EGF, Noggin, R-spondin, Wnt-3A, FGF10, TGF-b inhibition, Nicotinamide	Not reported
Liver	Adult mouse	EGF, Noggin, R-spondin, Wnt-3A, FGF10, HGF, Nicotinamide	EGF, Noggin, FGF10, TGF-b inhibition, Notch inhibition
	Adult human	EGF, Noggin, R-spondin, Wnt-3A, FGF10, HGF, Nicotinamide, TGF-b inhibitor, Forskolin	EGF, Noggin, FGF10, TGF-b inhibition, Notch inhibition, BMP7

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Tissue stem cells require unique niche microenvironments. In the presence of specific combinations of niche factors, mouse and human epithelial tissues from stomach, small intestine, colon, pancreas duct, and liver bile duct efficiently form stereotypic organoids. The platform of epithelial organoids can also be employed for in vitro generation of digestive tissue from human pluripotent stem cells. Organoids hold great promise for basic and translational research.

Adult Tissue-Derived Epithelial Organoids

"Mini-gut" organoids are stereotypic tissue-like structures derived from digestive organs. The mini-gut organoid culture system was originally established from mouse small intestinal crypts (Sato et al., 2009). The technology was subsequently adapted for other digestive epithelial tissues, such as the epithelium of stomach, colon, pancreas ducts, and liver bile ducts (Barker et al., 2010; Boj et al., 2015; Huch et al., 2013; Sato et al., 2011a). Lgr5+ gut epithelial stem cells or Lgr5− duct epithelium initially form symmetric cyst structures, which then generate "budding structures." The budding structures resemble intestinal crypts (intestine) or regenerating duct epithelial structures (liver, pancreas) consisting of Lgr5+ stem cells flanked by differentiated daughter cells. In small intestinal organoids, Lgr5+ stem cells receive niche signals from the neighboring differentiated Paneth cells for their self-renewal (Sato et al., 2011b).

Single epithelial cells can form organoids in 7–10 days, which can be dissociated into single cells to reinitiate organoid formation. Mini-gut organoids can be indefinitely propagated through such passaging in the presence of optimal niche factors.

In the mini-gut culture system, the culture condition for each tissue stem cell recapitulates niche microenvironments from which the stem cells originate. Adult tissue-derived epithelial organoids require generic niche factors such as laminin-rich extracellular matrix (Matrigel), EGF, Noggin, and R-spondin. Wnt ligand is required for organoids that secrete insufficient levels of autocrine or paracrine Wnt ligands. Each tissue stem cell type often requires additional tissue-specific niche factors, such as TGF-β inhibitor, FGF10, and/or HGF, as summarized in the table. Mouse small intestinal organoids self-renew and produce all types of epithelial cells in the standard culture condition, while the other organoid types require withdrawal of certain niche factors in order to differentiate.

Human Pluripotent Stem-Cell-Derived Organoids

Human pluripotent stem cells (hPSCs) differentiate into definitive endoderm upon activin A stimulation. The hPSC-derived definitive endoderm can further differentiate into hindgut endoderm with Wnt3A and FGF4 or into posterior foregut with Wnt-3A, FGF4, Noggin, and Retinoic acid. The hindgut epithelium is transferred to mini-gut organoid culture conditions to form intestinal organoids (Spence et al., 2011). Unlike Lgr5 stem-cell-derived organoids, hPSC-derived organoids harbor niche factor-producing mesenchymal cells that mitigate niche factor requirements. Although hPSC-derived intestinal organoids display fetal intestine-like immature properties, they undergo complete differentiation upon transplantation into the kidney subcapsule of mice.

hPSC-derived posterior foregut epithelium undergoes antral specification in the presence of EGF, Noggin, and retinoic acid. The antrum epithelium forms human gastric organoids (hGOs) upon transfer into Matrigel with high concentrations of EGF. hGOs possess all types of differentiated antral epithelial cells (McCracken et al., 2014). These hPSC-derived small intestinal or gastric organoids may provide insights into the generation of other foregut tissue organoids, such as gastric fundus, lungs, and pancreas.

Translational Application of Mini-Gut Organoids

The organoid culture system is applicable to basic and translational research. Established organoids can be engrafted onto injured intestinal mucosa. Furthermore, liver ductal organoids differentiate into functional hepatocytes upon transplantation. The expanded human liver organoids show robust chromosomal stability and very low rates of single base changes, making the organoids a safe cell source for regenerative medicine (Huch et al., 2015).

Mini-gut organoids have been applied for disease modeling of genetic diseases, such as cystic fibrosis, a1-antitrypsin deficiency, and Alagille syndrome (Huch et al., 2015; Schwank et al., 2013). These organoids phenocopy clinical traits. Interestingly, mutations in CFTR gene in organoids from cystic fibrosis confer resistance against cAMP-dependent ballooning of organoids, which offers a simple functional diagnostic tool. Furthermore, CRISPR-Cas9-based gene correction can restore the cAMP responsiveness of organoids, raising a possibility of organoid-based gene therapy (Schwank et al., 2013).

The mini-gut culture system can be applied to cancer tissue, such as stomach, colorectal, pancreas, and prostate cancer. During tumorigenesis, niche factors often become dispensable, leading to a less stringent culture condition for cancer organoids as compared to wild-type organoids. Established cancer organoids can be xeno-transplanted to recapitulate histopathology of the parental tumor from which they are derived. Cancer organoids reflect genetic lesions and gene expression patterns, opening up a possibility of in vitro drug testing for the prediction of clinical treatment response in patients. Biobanking of organoids derived from diseased tissues will help to unravel the pathogenesis of disease and the development of new diagnostic tools and new drugs.

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