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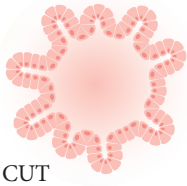
STEM CELLS

Colonic organoids for drug testing and colorectal disease modelling



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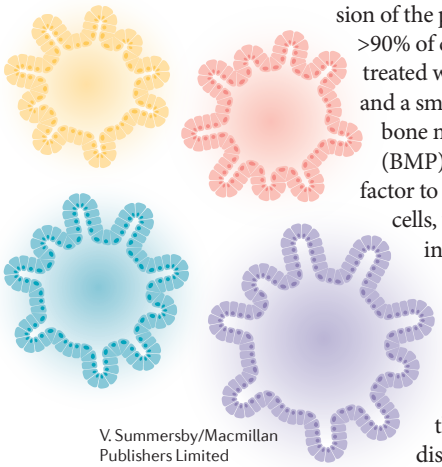
The possibility of obtaining gastric and small intestine organoids from human pluripotent stem cells (PSCs) has greatly facilitated the study of gastrointestinal development and disease. However, it has been considerably more difficult to derive distal gut tissues from human PSCs. Two studies, published in *Nature Medicine* and *Cell Stem Cell*, now report the efficient differentiation of human colonic organoids from PSCs.

Crespo *et al.* developed a step-wise strategy for the progressive generation, from human ESCs, of definitive endoderm, hindgut endoderm (which gives rise to the distal large intestine) and then colonic organoids, which involves the modulation of signalling pathways that regulate normal mouse embryonic development. To induce posterior fate in the hindgut endoderm, and therefore specifically obtain colonic tissue, they identified a chemical inhibitor of glycogen synthase kinase 3β (GSK3β), which functions downstream of WNT. Modulation of WNT signalling using this inhibitor led to expression of the posterior marker CDX2 in >90% of cells. Hindgut cells were treated with the GSK3β inhibitor and a small-molecule inhibitor of bone morphogenetic protein (BMP) and epidermal growth factor to obtain colonic epithelial cells, which were embedded in matrigel beads to form embryonic-like spheroids that progressively caveated into fully convoluted organoids. Gene expression profiling showed that these organoids are distinct from small intestine organoids and express high levels of colonic markers. Moreover, colonic organoids formed typical crypt-like structures and contained many of the cell types that are found in colonic crypts, including transit-amplifying cells, goblet cells and endocrine cells.



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Crespo *et al.* used this method to test the effects of drugs that could potentially be used for the treatment of familial adenomatous polyposis (FAP), which is an inherited form of colorectal cancer that is caused by mutations in the adenomatous polyposis coli (*APC*) gene. They derived induced PSCs from two patients with FAP, which carried either of two different *APC* mutations that result in an early stop codon, and generated colonic organoids from these and wild-type lines. Colonic epithelial cells derived from both FAP lines showed increased proliferation, which is consistent with the early stages of the FAP pathogenic phenotype. When testing compounds for their capacity to reduce cell proliferation, they found that ribosome-binding geneticin restored normal cell proliferation in FAP organoids, which could be rescued by ribosome read-through, but did not affect wild-type organoids. By contrast, XAV939 and rapamycin (both previously reported to ameliorate colorectal cancer phenotypes) reduced cell proliferation in both wild-type and FAP organoids, suggesting that they could harm healthy colonic crypts.

In another study, Múnera *et al.* developed a method for generating large intestinal organoids by first identifying markers that distinguish different domains in the embryonic gut tube. Analysing public expression databases, they identified the CUT class homeobox gene SATB2 as a conserved marker for the presumptive large intestinal epithelium, and found that BMP signalling is required for SATB2 expression. Using gut tube cultures that were derived from human PSCs, they established that BMP functions downstream of Hedgehog signalling to promote posterior fate by regulating HOX genes. Importantly, the authors showed that BMP treatment is sufficient to confer regional identity in long-term cultures of organoids, and that posterior identity was maintained following orthotopic transplantation in mice — transplanted colonic organoids developed into mature tissues that resembled the large intestine, expressed the SATB2 marker and contained crypts with regional cell subtypes. Múnera *et al.* also confirmed that the expression profiles of human colonic organoids grown *in vivo* are similar to those of cells of the human large intestine.

Together, these studies identify key pathways and markers for obtaining colonic organoids. They highlight their potential for the study of colon development, for drug testing and for modelling disease, which has important therapeutic implications given the prevalence of diseases that affect the large intestine, such as colon cancer, polyposis syndromes, colitis and irritable bowel syndrome.

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ORIGINAL ARTICLES Crespo, M. *et al.* Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nature Medicine* http://dx.doi.org/10.1038/nm.4355 (2017) | Múnera, J. O. *et al.* Differentiation of human pluripotent stem cells into colonic organoids via transient activation of BMP signaling. *Cell Stem Cell* http://dx.doi.org/10.1016/j.stem.2017.05.020 (2017)