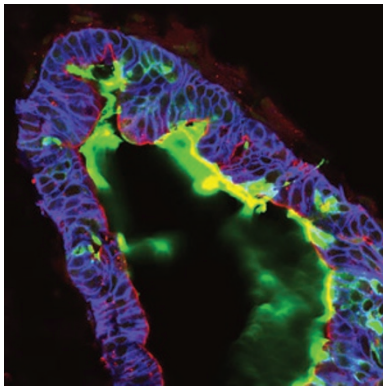
STEM CELLS

Differentiation in three dimensions

Pluripotent stem cells form intestine-like structures *in vitro*.



Intestinal organoids derived from pluripotent stem cells produce functional cells. Image courtesy of James M. Wells.

The goal of most stem cell differentiation protocols is to produce homogenous populations of the right sorts of cells: for example, hepatocytes such as those normally found in the liver. For some protocols, however, the goal is to generate a three-dimensional structure of several specialized cell types arranged in a precise architecture. Reporting in *Nature*, researchers led by James M. Wells at the Cincinnati Children's Hospital Medical Center describe a way to coax human pluripotent stem cells (PSCs) into three-dimensional 'organoids' made up of multiple kinds of cells with a similar spatial organization found in nature.

The organoids generated by Wells and his colleagues are small versions of the intestine. They contain villi-like projections and proliferating regions of cells expressing markers of intestinal stem cells. Compared to techniques that are used to produce intestinal cells from differentiating embryoid bodies, the process reported by Wells and colleagues is up to 50 fold more efficient. It also results in an epithelium that is almost entirely intestinal.

The differentiation project grew out of basic research on intestinal development. Wells and coauthor Aaron Zorn had identified several pathways involved in the process, says Wells. "We hypothesized that the same pathways could be used to promote the intestinal development of human pluripotent stem cells." The researchers first converted the PSCs into definitive endoderm, one of three fundamental categories of nonreproductive tissue and the one from which the intestine is derived.

To push the endoderm cells to form intestine tissue, the researchers manipulated several pathways they had previously identified as important. Work in several species has shown that the proteins WNT3A and FGF4 steer cells away from foregut development and toward hindgut development, and the researchers found a combination of these proteins was essential for differentiation. After a few days of exposure to these proteins, sheets of flat endoderm cells rolled up into tubes reminiscent of embryonic hindgut. Many of the tubes budded off to form spheroids. After about four more weeks in three-dimensional culture conditions known to favor intestinal growth, the spheroids developed organized layers of highly specialized cells and grew to about 40 times the original mass of the spheroids. They were still relatively spherical but highly convoluted.

The differentiation technique worked on two of the most commonly used human embryonic stem cells as well as four human induced PSC lines. Organoids could be passaged and split and grew healthily for over 140 days. What is more, several cell types in the organoids passed various tests of functionality. Paneth cells expressed lysozyme and goblet cells secreted mucins into the lumen of the organoid.

These structures will be useful in the hunt for additional pathways involved in human intestinal development, says Wells. He is also working with collaborators toward therapeutic applications. Intestinal organoids could help drug companies identify molecules that are readily absorbed by the intestine, a desirable trait for many drugs. Another project is to engraft organoids into animal models of bowel disease.

The ability to produce intestinal organoids from human PSCs may pave the way for producing other structures too. "We used studies of intestinal development as a blueprint to generate three-dimensional hindgut structures," says Wells. "A similar approach might work well for generating foregut structures such as the lung, liver and pancreas."

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