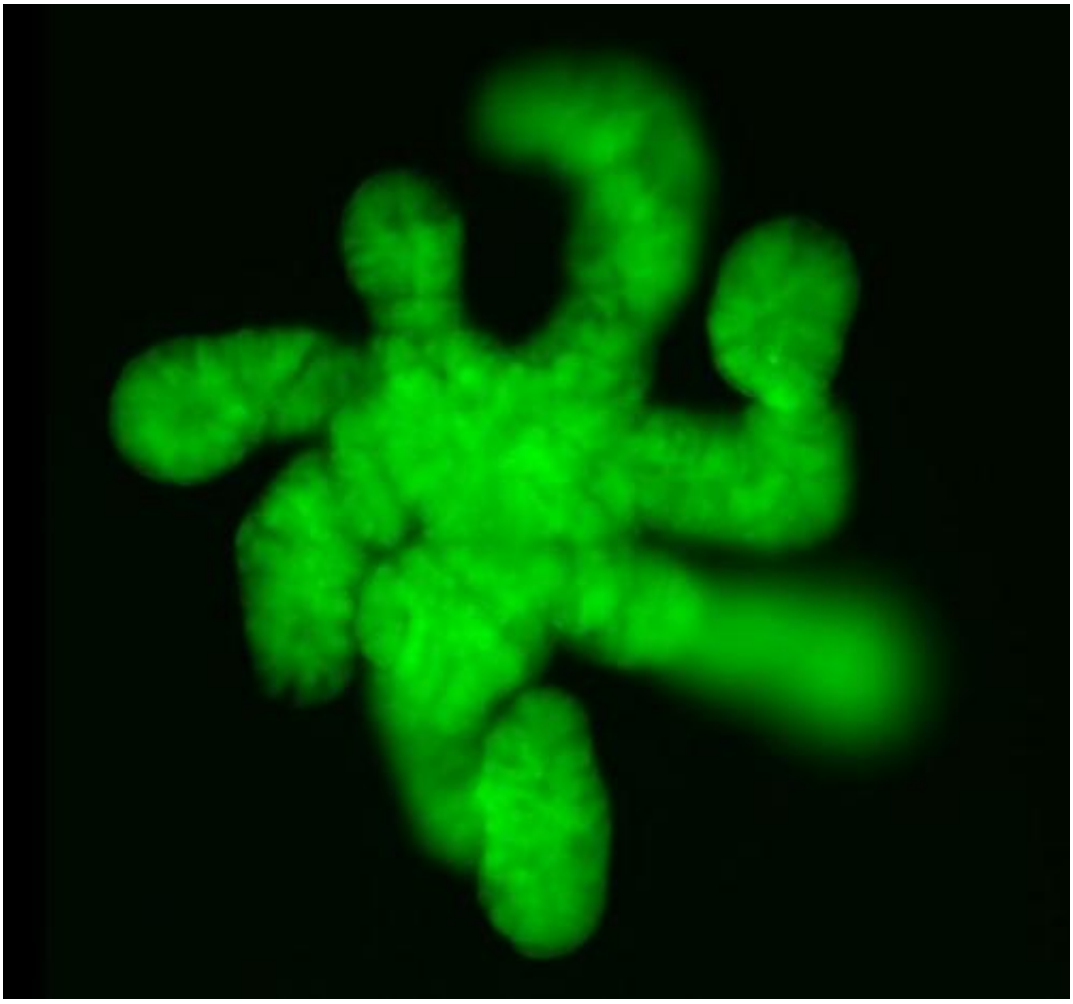
New means of growing intestinal stem cells

December 2 2013, by Anne Trafton



Intestinal stem cells. GFP (stem cell) expression of cells cultured in our condition.

The small intestine, like most other body tissues, has a small store of immature adult stem cells that can differentiate into more mature,

specialized cell types. Until now, there has been no good way to grow large numbers of these stem cells, because they only remain immature while in contact with a type of supportive cells called Paneth cells.

In a new study appearing in the Dec. 1 online edition of *Nature Methods*, the researchers found a way to replace Paneth cells with two small molecules that maintain stem cells and promote their proliferation. Stem cells grown in a lab dish containing these molecules can stay immature indefinitely; by adding other molecules, including inhibitors and activators, the researchers can control what types of cells they eventually become.

"This opens the door to doing all kinds of things, ranging from someday engineering a new gut for patients with intestinal diseases to doing drug screening for safety and efficacy. It's really the first time this has been done," says Robert Langer, the David H. Koch Institute Professor, a member of MIT's Koch Institute for Integrative Cancer Research, and one of the paper's senior authors.

Jeffrey Karp, an associate professor of medicine at Harvard Medical School and Brigham and Women's Hospital, is also a senior author of the paper. The paper's lead author is Xiaolei Yin, a postdoc at the Koch Institute and Brigham and Women's Hospital.

From one cell, many

The inner layer of the intestines has several critical functions. Some cells are specialized to absorb nutrients from digested food, while others form a barrier that secretes mucus and prevents viruses and bacteria from entering cells. Still others alert the immune system when a foreign pathogen is present.

This layer, known as the intestinal epithelium, is coated with many small

indentations known as crypts. At the bottom of each crypt is a small pool of epithelial stem cells, which constantly replenish the specialized cells of the intestinal epithelium, which only live for about five days. These stem cells can become any type of intestinal epithelial cell, but don't have the pluripotency of embryonic stem cells, which can become any cell type in the body.

If scientists could obtain large quantities of intestinal epithelial stem cells, they could be used to help treat gastrointestinal disorders that damage the epithelial layer. Recent studies in animals have shown that intestinal stem cells delivered to the gut can attach to ulcers and help regenerate healthy tissue, offering a potential new way to treat ulcerative colitis.

Using those stem cells to produce large populations of specialized cells would also be useful for drug development and testing, the researchers say. With large quantities of goblet cells, which help control the immune response to proteins found in food, scientists could study food allergies; with enteroendocrine cells, which release hunger hormones, they could test new treatments for obesity.

"If we had ways of performing high-throughput screens on large numbers of these very specific cell types, we could potentially identify new targets and develop completely new drugs for diseases ranging from inflammatory bowel disease to diabetes," Karp says.

Controlling cell fate

In 2007, Hans Clevers, a professor at the Hubrecht Institute in the Netherlands, identified a marker for intestinal epithelial stem cells—a protein called Lgr5. Clevers, who is an author of the new *Nature Methods* paper, also identified growth factors that enable these stem cells to reproduce in small quantities in a lab dish and spontaneously

differentiate into mature cells, forming small structures called organoids that mimic the natural architecture of the intestinal lining.

In the new study, the researchers wanted to figure out how to keep stem cells proliferating but stop them from differentiating, creating a nearly pure population of stem cells. This has been difficult to do because stem cells start to differentiate as soon as they lose contact with a Paneth cell.

Paneth cells control two signaling pathways, known as Notch and Wnt, which coordinate cell proliferation, especially during embryonic development. The researchers identified two small molecules, valproic acid and CHIR-99021, that work together to induce stem cells to proliferate and prevent them from differentiating into mature cells.

When the researchers grew mouse intestinal stem cells in a dish containing these two small molecules, they obtained large clusters made of 70 to 90 percent stem cells.

Once the researchers had nearly pure populations of stem cells, they showed that they could drive them to develop into particular types of intestinal cells by adding other factors that influence the Wnt and Notch pathways. "We used different combinations of inhibitors and activators to drive stem cells to differentiate into specific populations of mature cells," Yin says.

This approach also works in mouse stomach and colon cells, the researchers found. They also showed that the small molecules improved the proliferation of human intestinal stem cells. They are now working on engineering intestinal tissues for patient transplant and developing new ways to rapidly test the effects of drugs on intestinal cells.

Another potential use for these cells is studying the biology that underlies stem cells' special ability to self-renew and to develop into

other cell types, says Ramesh Shivdasani, an associate professor of medicine at Harvard Medical School and Dana-Farber Cancer Institute.

"There are a lot of things we don't know about stem cells," says Shivdasani, who was not part of the research team. "Without access to large quantities of these cells, it's very difficult to do any experiments. This opens the door to a systematic, incisive, reliable way of interrogating intestinal stem cell biology."

More information: Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny, DOI: 10.1038/nmeth.2737

Provided by Massachusetts Institute of Technology