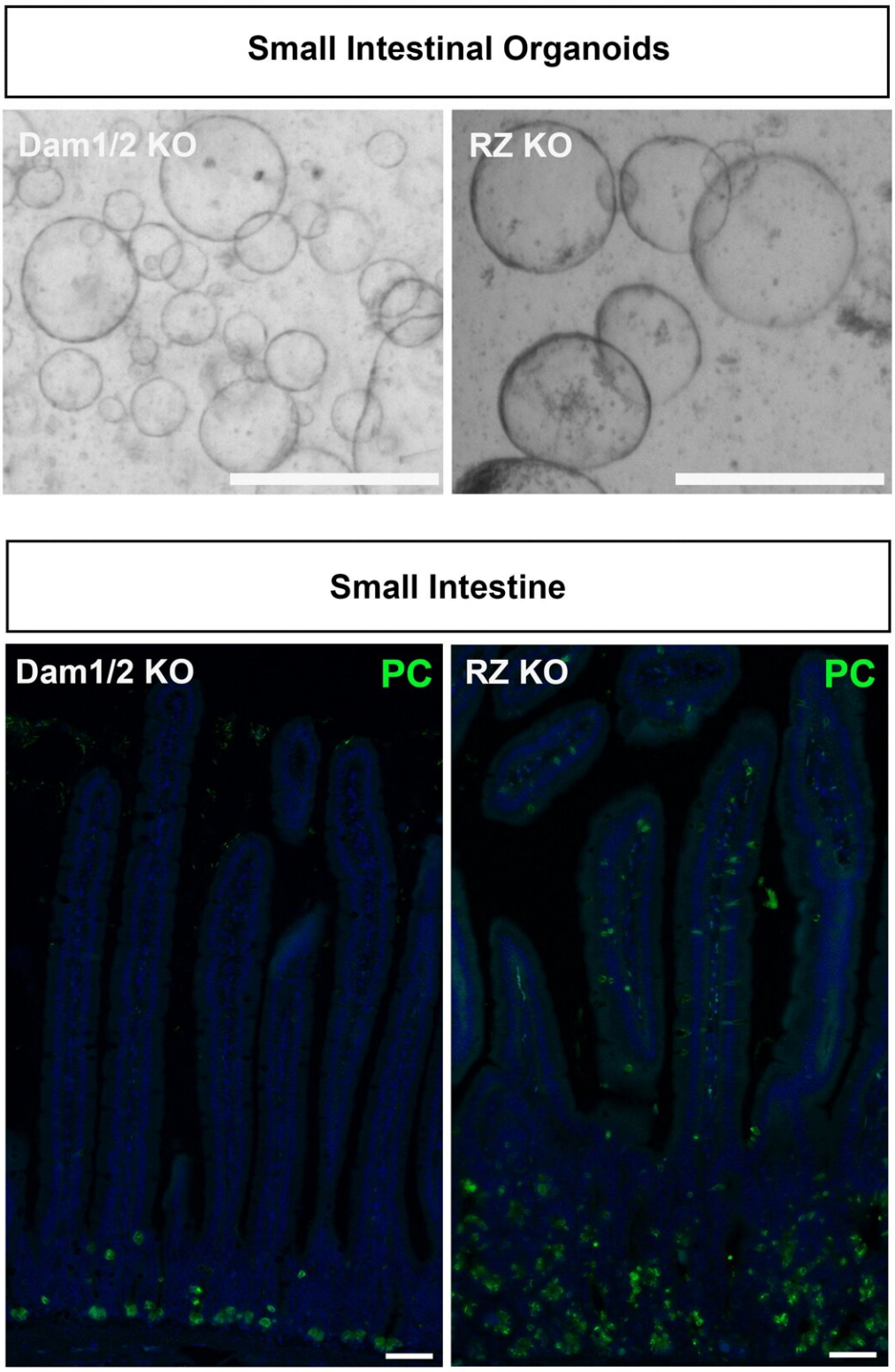
Decoding cell fate: Key mechanism in stem cell switch identified

November 24 2023


Small Intestinal Organoids
Dam1/2 KO
RZ KO
Small Intestine
Dam1/2 KO
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RZ KO
PC

Top: Intestinal stem cells lacking Daam1 (left) or Rnf43 (right) form tumor-like organoids. Credit: Gabriele Colozza/IMBA

Stem cells can differentiate to replace dead and damaged cells. But how do stem cells decide which type of cell to become in a given situation? Using intestinal organoids, the group of Bon-Kyoung Koo at IMBA and the Institute for Basic Science identified a new gene, Daam1, that plays an essential role, switching on the development of secretory cells in the intestine.

This finding, published on November 24 in *Science Advances*, opens new perspectives in cancer research.

Our bodies are, in some ways, like cars—to keep functioning, they need to be checked and repaired regularly. In the case of our bodies, any cells that are damaged or dead need to be replaced to keep organs functioning. This replacement occurs thanks to tissue-resident adult stem cells.

In contrast with embryonic stem cells, which can form any cell type in the body, adult stem cells will only form the cell types that are found in the tissue they belong to. But how do tissue-specific stem cells know which cell type to give rise to?

Gabriele Colozza, a postdoctoral researcher in the lab of Bon-Kyoung Koo at IMBA—now director at the Center for Genome Engineering, Institute for Basic Science in South Korea—decided to investigate this question using intestinal stem cells.

Intestines—a constant construction site

"In our intestines, cells are exposed to extreme conditions," Colozza explains. Mechanical wear and tear, but also digestive enzymes and varying pH values all affect intestinal cells. In turn, stem cells in the intestine's mucosa differentiate to form new intestinal cells.

"Damaged cells have to be replaced, but it is a delicate balance between stem cell renewal and differentiation into other cell types: uncontrolled stem cell proliferation may lead to tumor formation; on the other hand, if too many stem cells differentiate, the tissue will be depleted of stem cells and ultimately unable to self-renew."

This balance is delicately tuned by signaling pathways and feedback loops, which allow cells to communicate with each other. One important pathway is called Wnt. The Wnt pathway is known for its role in embryonic development, and if left unchecked, an overactive Wnt pathway can lead to excessive cell division and the formation of tumors.

Molecular partner identified

A well-known antagonist of Wnt signaling—keeping Wnt in check—is Rnf43, which was originally identified by Bon-Kyoung Koo. Prior to this study, Rnf43 was known to target the Wnt receptor Frizzled and mark it for degradation.

"We wanted to know how Rnf43 works, and also what, in turn, controls Rnf43 and helps it to regulate Wnt signaling." From earlier research, the scientists knew that Rnf43 on its own was not sufficient to break down the Wnt receptor Frizzled, which sits in the plasma membrane.

"In our project, we used biochemical assays to identify which proteins interact with Rnf43." A key partner of Rnf43 turned out to be the protein Daam1.

To understand how Daam1 regulates Rnf43 and affects the tissues it acts in, Colozza turned to intestinal organoids.

"We found that Daam1 is required for Rnf43 to be active, so for Rnf43 to regulate Wnt signaling at all. Further work in cells showed Rnf43 needs Daam1 to move the Wnt receptor Frizzled into vesicles called endosomes. From the endosomes, Frizzled is shuttled to the lysosomes where it is degraded, dampening Wnt signaling," Colozza adds.

Intestinal organoids are three-dimensional cell cultures grown from adult intestinal stem cells, allowing the researchers to mimic the intestinal mucosa.

For Colozza, organoids were an opportunity to understand how Rnf43 and Daam1 affect the delicate balance of stem cell renewal and differentiation in the intestine. "We found that when we knock-out Rnf43 or Daam1, the organoids grow into tumor-like structures. These tumor-like organoids keep on growing, even if we withdraw the growth factors they usually depend on, such as R-spondin."

Switching on Paneth cell formation

When Colozza followed up this result in mouse tissue, the researchers were in for a surprise.

"When Rnf43 was missing, the intestines grew tumors, as expected. But when Daam1 was missing, no tumors grew. We were puzzled by this striking difference: how can the loss of factors in the same pathway, that behave similarly in organoids, lead to such different outcomes?"

Looking closely at the intestines, Colozza saw that intestines lacking Rnf43 were full of a specific type of secretory cells, the Paneth cells. Intestines lacking Daam1, on the other hand, contained no extra Paneth

cells. Paneth cells secrete growth factors, such as Wnt, that stimulate cell division.

"Daam1 is required for the efficient formation of Paneth cells. When Daam1 is active, stem cells differentiate to form Paneth cells. When Daam1 is not active, the stem cells differentiate into another cell type."

Tumors modify their niche to grow

This link between the molecular results and Paneth cells explains the puzzling difference between intestines and organoids.

"In organoid culture, we scientists provide growth factors, so the knockout of both Rnf43 and Daam1 lead to tumor-like organoids. But in the intestine, there is no little scientist providing growth factors. Instead, Paneth cells provide growth factors, like Wnt, and create the right conditions for stem cells to survive and divide."

"When Paneth cells are lacking—such as when Daam1 is not active to drive cells into becoming Paneth cells—stem cells will not divide much. But when there are too many Paneth cells—such as in intestines lacking Rnf43—the excessive growth factors can contribute to the formation of tumors."

Colozza's and colleagues' study is the first genetic proof that Daam1, a member of the non-canonical Wnt pathway, is important for specifying Paneth cells, and directly involved in the development of this crucial secretory cell. The results also shed light on the importance of the stem cell niche. "We show that tumor cells modify their microenvironment, and influence their supporting environment so that they can grow better."

More information: Gabriele Colozza et al, Intestinal Paneth cell

differentiation relies on asymmetric regulation of Wnt signaling by Daam1/2, *Science Advances* (2023). DOI: 10.1126/sciadv.adh9673. www.science.org/doi/10.1126/sciadv.adh9673