Human organoid research identifies crucial 'traffic light' in gut cell differentiation

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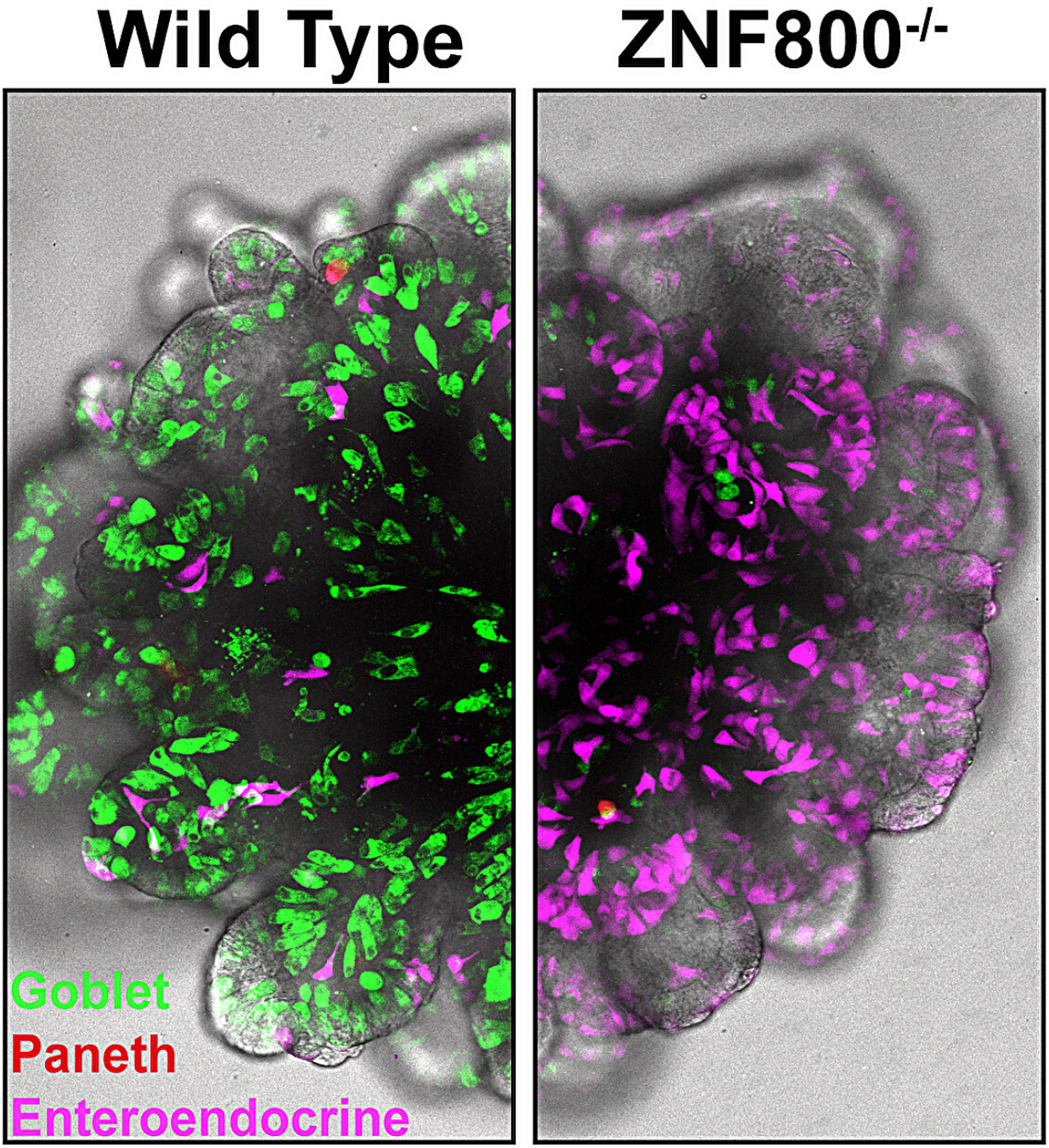

Human gut organoids with (left) and without (right) the transcription factor ZNF800. On the left, wild type (unaltered) gut organoids show the presence of goblet cells (green), enteroendocrine cells (EECs; magenta) and an occasional Paneth cell (red). On the right, the number of EECs is significantly increased after depletion of ZNF800, showing that ZNF800 represses the differentiation of

stem cells into EECs. Credit: Lin Lin. Hubrecht Institute.

The different cell types of the human gut develop from stem cells through a process of differentiation. Researchers from the Organoid group (Hubrecht Institute), together with researchers at the Princess Máxima Center and Maastricht University, used gut organoids to perform a systematic CRISPR screening of 1,800 human transcription factors and identified ZNF800 as a key regulator of the differentiation of a specific gut cell type, the enteroendocrine cells.

The results of the study were published in *Science* on 26 October 2023 and could have implications for our understanding of gastrointestinal diseases and endocrine disorders, as well as pancreatic development and diabetes.

The human gut contains various cell types, each with specific functions. These cell types all arise from the stem cells of the gut: cells that are not specialized yet, but have the potential to become functionally specialized cells.

Important cell types of the gut are enterocytes, responsible for the absorption of nutrients, goblet cells, which produce mucus, Paneth cells, which secrete antimicrobial peptides, and enteroendocrine cells (EECs), which produce various hormones. The hormones produced by the EECs regulate digestive processes, such as nutrient absorption, appetite and glucose metabolism.

In this study, researchers from the Organoid group investigated how stem cells become EECs through a process called differentiation. For this they used gut organoids: lab-grown miniature organs that mimic the structure and function of the actual gut.

Stem cell traffic lights

The differentiation of stem cells into specific cell types occurs through gene regulation: the 'on' and 'off' switching of genes in the DNA of the cells. Proteins called transcription factors play an important role in gene regulation, as it is their job to perform the on-off switching of genes.

First author Lin Lin explains, "You can compare it to a bustling intersection where different roads lead to various cell destinies, and the vehicles on the roads symbolize different cell types. The transcription factors act as traffic lights at the intersections, determining whether cells can follow a particular direction to become specialized cells."

"In our study, we used CRISPR technology, a gene-editing tool, to specifically target individual transcription factors. This is like switching the 'traffic lights' on or off. By doing this, we aimed to uncover the intricate signaling system that directs cells down their predetermined routes, in the same way that traffic lights govern the movement of vehicles in a busy city."

ZNF800 as a master repressor

The researchers used CRISPR to perform a systematic screening of the entire repertoire of human transcription factors, encompassing 1,800 genes, in order to find factors involved in stem cell differentiation into EECs.

"By screening all possible 'traffic lights,' we identified specific ones that play a crucial role in controlling cell fate decisions. Some of these factors acted as green lights, promoting the activation of genes that guide cells towards a particular destiny, while others acted as red lights, repressing certain gene expressions and diverting cells onto different

paths," says Lin.

The team identified ZNF800 as a critical transcription factor in determining the balance between EECs and other cell types in the gut.

"The presence of ZNF800 acts as a red light on the development of EECs: when we switched the ZNF800 'traffic light' off, we saw a significant 10-fold increase of EECs in the organoids. At the same time, other intestinal cell types like goblet cells and Paneth cells were suppressed," explains Lin.

ZNF800 was also shown to control the expression of other transcription factors involved in EEC differentiation, such as NEUROG3, INSM1, SOX4, and PAX4. Lin says, "The regulation of differentiation works in a hierarchical manner, where certain transcription factors act as master regulators and others as downstream effectors. We showed that ZNF800 acts as a master repressor, meaning that it influences other transcription factors and, in the end, inhibits EEC differentiation."

Clinical implications

The discoveries made by Lin and her colleagues could have implications for understanding gastrointestinal diseases and endocrine disorders.

"Our findings provide crucial insights into the molecular mechanisms that govern cell fate decisions in the human gut, which is essential for understanding these conditions and ultimately developing treatments," says Lin.

The fact that ZNF800 was shown to affect other transcription factors such as PAX4 and NEUROG3 suggests there could also be implications for diabetes research.

"These transcription factors are crucial for the regulation of insulin-producing beta cells in the pancreas, raising the possibility that ZNF800 may also play a role in pancreatic development and diabetes," concludes Lin.

More information: Lin Lin et al, Unbiased transcription factor CRISPR screen identifies ZNF800 as master repressor of enteroendocrine differentiation, *Science* (2023). DOI: 10.1126/science.adi2246

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