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STEM CELLS

# R-spondin 3 is a critical regulator of gastric antral stem cell homeostasis

> "...growth of antral epithelial organoids derived from mice was impaired when R-spondin 3 was absent from the culture medium..."

A new study published in *Nature* identifies R-spondin 3, a protein released by gastric myofibroblasts, as an important regulator of gastric epithelial stem cells and antral gland homeostasis. These findings also have important implications for how *Helicobacter pylori* infection can lead to gastric cancer.

The gastric epithelium undergoes constant self-renewal from stem cells located within gastric glands. In a previous study, Michael Sigal and colleagues observed in a mouse model that *H. pylori* is able to penetrate deep into the glands, increasing stem cell turnover and providing a mechanism for *H. pylori*-induced gastric hyperplasia. Despite these findings, the precise identities of the gastric stem cells involved, or the signals controlling their turnover, were unclear. "We decided that it was necessary to clarify these relationships to understand whether the bacterium directly affects stem cell turnover," explains Sigal.

On the basis that Wnt signalling is a regulator of stem cell identity in other tissues, the researchers traced the lineage of cells expressing *AXIN2*, a Wnt target gene, to identify cells with active Wnt signalling in gastric gland stem cell niches. When combined with labelling of LGR5, which marks long-lived stem cells at the base of antral glands, two distinct stem cell populations were identified in the antrum: AXIN2$^+$LGR5$^+$ cells in the base of the glands, and AXIN2$^+$LGR5$^-$ cells in the lower isthmus. Whereas AXIN2$^+$LGR5$^+$ cells were unable to repopulate glands within 7 days, AXIN2$^+$LGR5$^-$ cells were more proliferative, entirely repopulating glands in the same timeframe. Importantly, AXIN2$^+$LGR5$^-$ cells were also capable of replenishing AXIN2$^+$LGR5$^+$ cells in the gland base when the LRG5$^+$ population was specifically depleted.

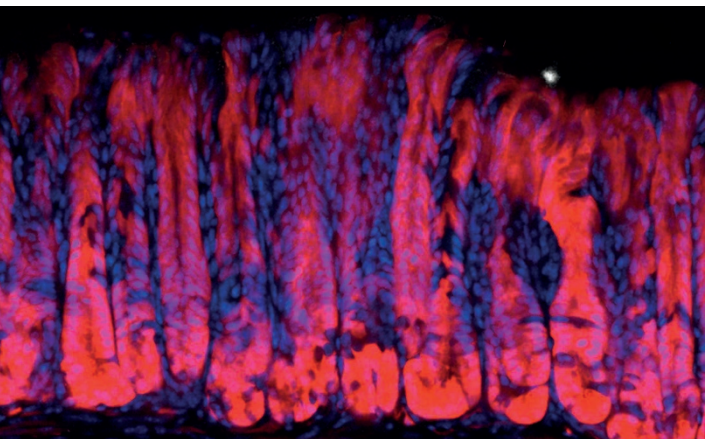
"We also used *in situ* hybridization in order to pinpoint which signalling molecules had a distribution pattern that was restricted to the stem cell niche at the bottom of the gland," comments author Thomas F. Meyer. Of the Wnt signalling proteins investigated, only the Wnt enhancer protein R-spondin 3 — expressed by stromal myofibroblasts located beneath the glands — showed this pattern.

Next, the researchers explored how R-spondin 3 regulates the turnover of gastric stem cells. The growth of antral epithelial organoids derived from mice was impaired when R-spondin 3 was absent from the culture medium, yet was rescued when organoids were co-cultured with stromal cells that produced the protein. In addition, whereas AXIN2$^+$ cells in mice given R-spondin 3 expanded in number and increased their proliferation, LGR5$^+$ cells did not. However, by day 14, glands had been fully repopulated by LGR5$^+$ cells, suggesting that the effect of R-spondin 3 is transient and dependent on the stem cell subtype. "This mechanism is likely to regulate the differential response of stem cell subpopulations when increased regeneration is required," Sigal says. "In this way, one population can be activated, the other silenced. Once the damage is repaired, the silenced population becomes active again — this may help to preserve genetic integrity."

Finally, the researchers characterized the role of R-spondin 3 in *H. pylori* infection. Mice infected with the pathogen for 2 months showed increased proliferation of AXIN2$^+$ cells in the antrum and gland hyperplasia, which was associated with greater numbers of gland-adjacent myofibroblasts and upregulated R-spondin 3 expression.

"We were particularly excited to observe that deletion of R-spondin 3 [in this mouse model] leads to increased colonization with *H. pylori*," reveals Meyer. "The finding that long-lived stem cells increase their turnover in response to infection and that this plays a role in controlling *H. pylori* colonization has wide-reaching implications for understanding the mechanism by which this chronic bacterial pathogen can cause cancer."

*Hugh Thomas*



Lineage tracing of AXIN2$^+$ cells (red) in gastric antral glands. Courtesy of M. Sigal.

**ORIGINAL ARTICLE** Sigal, M. *et al.* Stromal R-spondin orchestrates gastric epithelial stem cells and gland homeostasis. *Nature* **548**, 451–455 (2017)