FINAL SUMMARY REPORT

In the murine small intestine, the epithelium renews every five days. This vigorous self- renewing process is controlled by several secreted factors at the base of the crypt-villus axis. In the intestinal epithelium, Lgr5 is expressed selectively in a subset of cells called Crypt Base Columnar cells within the proliferative crypt compartment and, has been recently identified as a marker of adult stem cells in small intestine and colon.

It is firmly established that inappropriate activation of the Wnt signaling pathway via mutations in core pathway components such as APC is one of the initiating events in colon cancer. The cancer stem cell hypothesis postulates that a small reservoir of self-sustaining cells is exclusively able to self-renew and generates the cell population that constitutes the bulk of the tumour. Importantly, Cancer Stem Cells (CSC) not only fuel tumor growth but also tumor metastases. In 2002, Lgr5 was identified as a gene expressed in colon cancer. By using mouse genetic engineering we deleted APC exclusively in the Lgr5 intestinal stem cells. We observed transformation of the epithelia within days. Transformed stem cells remain located at the crypt bottoms, while fueling a growing microadenoma. We also observed that cell hierarchy between stem cells, progenitors and differentiated cells was still maintained in early stem cell derived adenomas, lending support to the “cancer stem cell”-concept. These results were published in the journal Nature 29th of January 2009 (Barker, Nature 2009).

Lgr5 expression is not only restricted to a subset of cells in the small intestine but also, we find Lgr5 expression in other adult tissues, suggesting that it may be a more general marker of adult stem cells. Therefore, we next set out to investigate whether Lgr5 could indeed be a bona fide marker for adult stem cells and cancer stem cell populations in other tissues. We knew that the

stomach shares a number of features with the intestine, including a common endodermal origin and a constantly renewing epithelium. In both organs, cell renewal is fuelled from stem cell populations located in pockets within the epithelium. Using mouse genetic manipulation and in vivo lineage tracing we found that indeed the intestinal stem cell marker Lgr5 also marks an active adult stem cell population at the base of the stomach glands. We isolated single Lgr5 cells from normal stomach epithelia and analyzed their genetic profiles using microarray gene expression analysis. We found robust canonical Wnt signaling activity on the Lgr5 stem cell population whereas several lineage-specific genes (enteroendocrine-pit cell) were absent in the stem cells but rapidly upregulated in their daughter cells.

Since it had been previously described that spontaneous inactivation of Apc not only results in colon cancer but also promotes the formation of gastric adenomas, we next sought to determine the tumorigenic potential of the Lgr5 stomach cells. We deleted specifically APC in the Lgr5 stem cells and \observed a clear expansion of the stem cell compartment and formation of adenomas in the stomach epithelia. Similarly as in the small intestine, the Lgr5-derived adenomas fulfilled the criteria of the Cancer stem cell hypothesis, since they were mainly formed by stem cells as well as differentiated cells (see Fig 1). These results were published in the journal Cell Stem Cell 8th January 2010.

Since no long-term culture system had been established that allow studying the gastric physiology *in vitro*, we next wondered whether these Lgr5 stomach cells could be maintained in culture, and whether they would form the basic structure of the gastric gland units. We attempted to design such a culture system by combining previously defined insights in the growth requirements of gastric epithelium. When isolated Lgr5 stomach cells were cultured under the right growth media and growth factors, they formed gastric organoid structures (mini-stomachs) that developed gland-like units and could be maintained for months in culture (see figure 2). This gastric culture system not only self-renews in vitro, but also contains the differentiated adult cells present in the normal adult stomach epithelia. This was the first time that an adult stomach culture that self-renews and differentiates in vitro can be maintain in

culture for many months without losing its properties. These results were published in the journal Cell Stem Cell 8th January 2010.

This work will have an important impact on the development of new therapies for gastric pathologies such as gastric epithelial infection, inflammation and stomach cancer. It also represents a major step forward for the development of new strategies for regenerative medicine.
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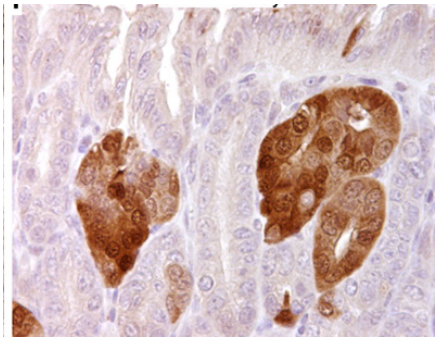

Figure 1: Image showing a Lgr5-derived gastric adenoma from an Lgr5-Cre APC mouse. Published by by Huch et al. at the journal Cell Stem Cell 2010

A **B**

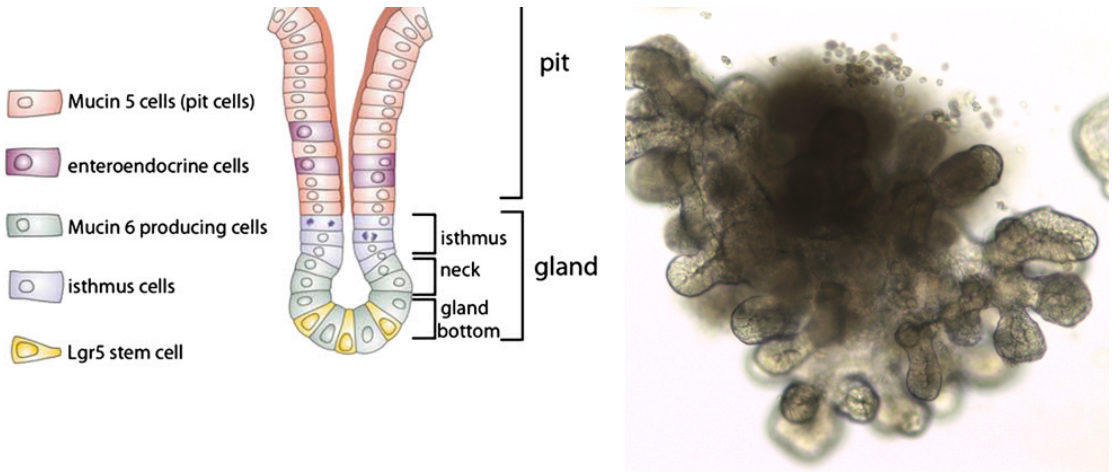

Figure 2. **A:** schematic representation of the stomach epithelia showing the position of the Lgr5 stem cells and the differentiated cells. Published by Huch et al. at the Journal Molecular oncology 2010. **B**: image of a growing stomach organoid culture.