Colon stem cells in Utrecht

A Comfortable Niche

The stem cells which continuously give rise to the renewal of gut tissue have remained elusive for a long time. By identifying a specific marker gene, Hans Clevers and his group have recently tracked them down.

Villi of the small intestine

Normality and abnormality are often only a few steps apart from each other. With regard to cancer, for instance, miniscule changes distinguish tumour cells from normal tissue cells. An inactive tumour suppressor, an overactive oncogene and an uncontrolled growth programme which should actually only be active during embryonic development, are examples.

"In the gut, the processes controlling the normal homeostasis of tissue renewal are the same as those causing cancer," says Hans Clevers, Director of the Hubrecht Institute for Developmental Biology and Stem Cell Research in Utrecht, The Netherlands. "Colon cancer cells activate very similar genes as normal cells do but the activation manner is different." This fact leads Clevers to a clear-cut opinion, "There is no way around this, if we want to understand cancer biology we must first understand normal processes." With his international research group of about 25 people, Hans Clevers has therefore set his focus on the molecular basics and the signal pathways crucial for normal gut tissue growth – which in consequence then also plays a role in tumour development.

Hans Clevers (insert, left), his team and a big friend

A new intestinal epithelium every 5 days

"The absorptive intestinal epithelium is the most rapidly proliferating and self-renewing tissue in adult mammals," Clevers points out. "Renewing the entire tissue every five days, the human being produces 200-300 g of epithelium every day." This massive proliferation getting out of control might be a reason for the high incidence of colon cancer.

In the small intestine the epithelium is ordered in crypts and villi. The one in the colon also contains crypts but has a flatter surface without villi. Intestinal cells are constantly produced from a stem cell reservoir in the crypt that cycle steadily to produce highly proliferating, so called transit-amplifying cells, capable of differentiating towards all cell lineages. The Wnt/Wingless-signalling pathway and especially the activated transcription factor TCF, represent the driving force, explains Clevers. "When we knock out the gut-specific TCF4, the entire proliferation in the epithelium is gone and the crypt disappears." Conversely, in colon cancer, TCF4 was seen to be always active. As the Wnt pathway determines the direction of cell growth, its role in normal proliferation in the crypt is closely related to that in cancer, says Clevers. The big difference in cancer is that there are mutations in the breaks of the pathway, for instance in APC, and thus the signal cannot be shut off. "The resulting constitutive activity of the Wnt signal apparently programmes the cell to be a cancer cell."

In mouse models the Dutch found about 200 genes to be under the direct control of TCF4, in tumour as well as in normal crypt cells. They also made quite a surprising discovery, "While most of these genes are always co-expressed in cancer cells, in crypts, they are expressed in different compartments," Clevers says. 90 percent of cells in the crypt are transit-amplifying cells, proliferating rapidly for a few days before they differentiate. "We found most of the identified TCF4 target genes to be expressed in the amplifying cells in the crypt," Clevers adds.

However, the stem cells, from which those highly proliferating cells emanate, were not identified for a long time. As those are the origin of the cell proliferation and tissue renewal, they are of special interest. "All the literature said, in the small intestine stem cells should be at position +4 counted from the bottom of the crypt," Clevers says. The popular idea is that the stem cell occasionally divides and one of its daughters very rapidly proliferates for a few days. As soon as the generated cells move up slowly out of the crypt onto the villus, they differentiate to enterocytes and goblet cells. Terminally differentiated Paneth cells reside at the bottom of the crypt.

If it's active, it's a stem cell

In search of the stem cells in the crypt, Hans Clevers and his team recently found one Wnt target gene, *Lgr5*, expressed in a unique manner (*Nature*, 2007, 449, p. 1003). "In contrast to most of the other genes, it is unexpressed in transit-amplifying cells but expressed in a limited number of cells that are located at the bottom of the crypts," describes the 50-year-old. The *Lgr5* gene marks six to eight slender cycling

cells interspersed between the Paneth cells, the so called cycling crypt base columnar (CBC) cells, which were already suggested as harbouring stem-cell activity more than 30 years ago.

Using an inducible knock-in with the recombinase Cre in the *Lgr5* locus in combination with activation of a Cre lacZ reporter, the scientists performed lineage-tracing experiments in adult mice. Cre-mediated excision of the blockade sequence in the lacZ reporter irreversibly marks $Lgr5^+$ cells. Thus, in potential progeny of these cells, the activated lacZ reporter acts as a genetic marker.

"Therefore, we saw that the *Lgr5*-positive CBC cell generated all epithelial lineages," says Clevers. "Thus, those cells – located at a very different place from where everybody thought, have turned out to be the real stem cells of the small intestine, as well as in the colon!"

Lgr5 can thus be taken as a good marker for stem cells. "Unlike other markers, the situation for *Lgr5* is very black and white, if you have the active gene, you are a stem cell," illustrates Clevers. This holds true not only for the gut: The researchers also identified Lgr5-positive cells in a number of other organs, including the stomach, mammary glands and hair follicles, which also seem to be stem cells.

Investigating whether stem cells from one organ can move to other related tissues, for example, if a stem cell from the small intestine can move to the colon, stomach, or pancreas, would be the next step.

Dividing like a clockwork

Clevers and Co. also found that controversially stem cells in the gut are not quiescent. "Following them for about one year in mice, we saw those stem cells constantly proliferating and dividing every day," he says. In a human life span this would be thousands of divisions. "This is very unusual and may again be the reason for the high frequency of colon cancer," Clevers remarks.

The function of *Lgr5* is not yet exactly known. "If we knock out *Lgr5*, we see no change in phenotype, so the gene is probably redundant and several genes do the same thing," he thinks. *Lgr5* belongs to a family of three much related genes that are all co-expressed in the same cells in the gut. By knocking out those other two genes *Lgr4* and *6* along with *Lgr5*, the researchers want to learn more about their role.

Hans Clevers' guess is that: "Lgr5 is a receptor for a small secreted protein, as it is closely related to the TSH or FSH receptors." He thinks there should be a ligand, which is probably secreted in all the stem cell niches, that activates the Lgr5 receptor and thus somehow controls the stem cell's behaviour. He is looking for this ligand now. Probably it is through Lgr5 that stem cells behave like stem cells, not dividing too rapidly, and staying at the bottom of the crypt, explains the scientist. "That is the most likely scenario but at the moment we have no evidence."

Regarding cancer, do stem cells also give rise to tumour cells? "It is not known whether cancer arises in the stem cell or in a slightly later, differentiated cell," says Clevers.

"There has been a lot of hype around the cancer stem cell hypothesis in the last 2-3 years," he laughs. Nevertheless, it is an attractive hypothesis but with too little good experimental data to support it at the moment.

Struggling back to the roots

"We know that the *Lgr5* gene is regionally expressed in very low numbers of cells in colon cancer that might serve as stem cells." Maybe these cells start re-expressing this gene, or they are the original stem cells that start the tumour. "So far, it has been very difficult to study this, because if you have cancer you can't go back!" Actually, with the Cre-system Clevers and Co. have the correct tools in their hands to address this in future.

TCF transcription factors and Hans Clevers have a long lasting scientific relationship. In 1991, in his time as a young professor at the Department of Immunology at the University of Utrecht, he cloned the T-cell specific family member TCF1. Coming from molecular immunology and especially T-lymphocyte biology the main subjects of Clevers' research have shifted over the years. As, since 1996, TCFs have been found to be crucial in Wnt signalling he has focused more and more on this pathway by studying it in diverse model organisms like frogs, flies and *C. elegans*. With the discovery, that the Wnt pathway is often mutated in colon cancer, Clevers then turned to gut research once again.

"In contrast to T-cell immunology, where thousands of highly competent labs work in a competitive fashion, gut biology, especially the normal processes in the epithelium has only been studied by a few good labs". Having been thus prompted to find his niche it seems that Clevers feels comfortable there.

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