Targeting a protein in order to fight against intestinal cancer

As recently stated by The Foundation Against Cancer, "cancer of the large intestine is the second most common form of cancer in both males and females in Belgium and it is also the second most common cause of death by cancer in our country". More than 8,500 new cases were diagnosed in 2012. In a recent study (1), Alain Chariot and Pierre Close, of GIGA (University of Liege) identified the gene coding for the ELP3 protein as a key factor in the development of intestinal tumours.

The structure of the intestine is today well-known and understood. Its inner lining is composed of villi (folds of the intestinal mucosa and the underlying connective tissue) which broaden the surface of the intestine. It is therefore designed for an optimal exchange between the commensal bacteria and nutrients and for the absorption of the nutrients. At the bottom of these villi are intestinal crypts which are composed of different types of cells. "In a normally-functioning intestine, there is a large amount of cell regeneration: these cells can be considered as completely renewed, giving rise, in a manner of speaking, to a new intestine every week", explains Professor Alain Chariot, Director of research at the FNRS in the GIGA research centre of the University of Liege and principal investigator at WELBIO. "At the bottom of these intestinal crypts, there are stem cells surrounded by Paneth cells which protect them. Every day, the stem cells divide and proliferate to create the transit-amplifying compartment. These proliferating cells push older cells towards the top and can differentiate and specialise. They stop dividing in order to become either enteroendocrine cells, which produce various hormones, or enterocytes which absorb the nutrients or Goblet cells which synthesise a mucus the function of which is to protect against disease-causing infectious bacteria. Higher up in the crypt are Tuft cells which are rarer and whose exact function is as yet unknown. The cells that reach the top of the villi therefore die over time". This is what is happening during normal intestinal function. The molecular mechanisms which enable differentiation are beginning to reveal their secrets.
Double role of Wnt

To ensure that this intestinal regeneration and therefore the proliferation of stem cells and the differentiation of cells emerging from the transit-amplifying compartment occurs, there is a mechanism called the Wnt signalling pathway. When this is not activated, there is no proliferation. It must be activated in a transitory manner at the right time. When the Wnt signalling pathway is not active, a complex of proteins - axin, glycogen synthase kinase 3β (GSK3β) and APC (adenomatous polyposis coli) - degrade the β-catenin, another protein which plays a role in cellular signalling. The objective is to make sure that β-catenin is expressed in small quantities. If β-catenin is no longer degraded, it accumulates in the cytoplasm then moves into the nucleus of the cell, associates with LEF-1 /TCF transcription factors to induce the expression of several genes which will ultimately leads to cell proliferation. The transient stabilization of β-catenin takes place when ligands bind to a specific receptor and trigger the Wnt signalling pathway which ultimately prevents the complex of proteins required for the degradation of β-catenin from fulfilling its role. "In the case of cancer cells", explains Alain Chariot - and this applies to 80% of human intestinal cancers - "the activity of the Wnt signalling pathway is not controlled: it remains active and therefore the β-catenin is not sufficiently degraded and it accumulates: this leads to an uncontrolled proliferation". A defect in the degradation of β-catenin can occur when the APC protein is mutated, which interferes with the assembly of the degradative complex. Therefore, the Wnt pathway plays a double role here: on one hand it is essential for the proliferation of stem cells, an essential step for the generation of new intestinal crypts but only when it is transiently activated. On the other hand, when permanently active, it favours the production and proliferation of cancer cells.

A total inhibition of the Wnt signalling pathway is not appropriate, given its key role in homeostasis. "Ideally, a more targeted effect on proteins only involved in the constitutive activation of Wnt signaling is required. We think we have identified this protein: it is ELP3".

For several years now, Pierre Close, a research associate at the FRS-FNRS and head of the Laboratory of Cancer Signalling (GIGA-Research, "Molecular Biology of Diseases" Unit), who published this study with Alain Chariot, has been working on Elongator, a six subunits complex (ELP1-6). He has been working more specifically on ELP3, which is involved in other areas (Read : Neuron migration 'under the wing' of Elongator). Both collaborators have therefore sought to understand its involvement in the development of intestinal cancer cells.
APC and ELP3

When APC is lost, #-catenin stabilises and cells proliferate in an uncontrolled manner. "Mice lacking APC spontaneously develop tumours due to constitutive Wnt signaling. When the Wnt pathway was constitutively activated, ELP3 expression increased which led us to envision that its inactivation may impact on Wnt-driven tumour initiation. We then looked at mice in which we had inactivated the gene encoding ELP3 in intestinal stem cells. In healthy mice, the architecture of intestinal crypts was unchanged. The stem cells remained functional and we only detected a lower number of Tuft cells upon ELP3 deficiency. But as their exact role is still unclear, it is difficult to know what the implications of such defect. We can, however, conclude from this observation that ELP3 is involved in the production of Tuft cells."

On the other hand, in mice lacking APC in which intestinal tumours quickly develop, we observed a striking difference between mice expressing ELP3 or not: "Mice lacking APC and ELP3 had a much longer survival rate because they developed much less tumours. Tumour initiation in the intestine was blocked". In short, when APC is blocked, cancerous stem cells are produced; if APC and ELP3 are absent, the maintenance of cancer cells is limited."

Furthermore, when a healthy mouse has been irradiated, which causes massive cell death in intestinal crypts, we can nevertheless observe cell regeneration in a few crypts thanks to ELP3. This regeneration relies on the
Wnt pathway, and consequently on ELP3. Therefore, ELP3 expression is required in pathological situations in which the Wnt pathway is urgently needed (cancer development and intestinal regeneration after irradiation).

**A potential target**

The tumour has to synthesise several proteins, such as SOX9, for example, to develop and grow: the essential role of ELP3 can be explained by the fact that many proteins, including SOX9, require ELP3 in order to be synthesised. "We are trying to find a specific pharmacological *inhibitor* for ELP3 to see whether its inhibition leads to tumour regression in circumstances in which Wnt signaling is constitutively activated. ELP3 is an interesting target because it is a Wnt effector and is involved in the development of cancer cells while being dispensable to intestinal homeostasis..."

This inhibitor has yet to be found and some twenty researchers at ULg are actively working on it. However, several years will be needed to isolate some ELP3 inhibitors, to test and to validate them.

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