Liver metastases: promising grounds for targeted therapies

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Metastases are the major cause of death from cancer. Liver is one of the top three organs (next to bone and lung) to be colonised by metastazing tumors. Studying hepatic metastases is thus an important component of developing new targeted therapies. Unfortunately, tumors harbour a heterogenous cell population, some of which are able to survive treatment. Based on available genetic data, the extent of tumor heterogeneity appears vast and unpredictable. But researchers from the University of Liège recently showed that cancer cells are not necessarily so heterogeneous at the protein level. They were also able to identify two potential targets for colorectal carcinoma (CRC) liver metastases.

Colon cancer or colorectal cancer is one of the most common cancers, afflicting both men and women. According to the World Health Organisation (WHO), more than 600,000 of the 7.6 million worldwide cancer deaths in 2008 were from colon cancer. One of the characteristics of cancer is the abnormally rapid and uncontrolled proliferation of cells that can spread to other bodily organs. Theses metastases are the main cause of cancer deaths.

At the time of diagnosis, people with colon cancer have metastases in 20-30% of cases. "When the tumour cells invade through the colon walls, the liver is often the first organ to develop metastases. Liver metastasis are also frequent in breast cancer, lung cancer, pancreas cancer and many others" explains Andrei Turtoi, FRS-FNRS Research Fellow with the GIGA-Cancer Metastasis Research Laboratory at ULg. The liver is thus a key element in the study of metastases.
When surgery is not enough

As long as no metastases develop, colon cancer can be cured. But when metastases reach the liver, only 30% of patients are operable. While this minority performs better (25% survive 5 years), 95% of non-operable patients die within next 5 years. "For 70% of patients with liver metastases, surgery isn’t an option," emphasises Andrei Turtoi. "This is why scientists are trying to find other kinds of treatment for metastases and particularly those in the liver." Today there are high hopes for targeted therapy. This term refers to medication that acts on a particular protein or a mechanism involved in tumour development. Theoretically, these therapies kill cancer cells without damaging healthy tissues. This is in contrast to chemo- or radiotherapy which often cause collateral damage, limiting the possibility to escalate the dose and kill the tumor. "The basic principle of targeted therapy is that it depends on the specific proteins within the tumour. In one kind of targeted therapy, these proteins must be easily accessible so that antibodies or antibody-drug conjugates can reach and bind them," the specialist explains. The chosen protein targets are thus generally proteins that are located on the surface of cancer cells.

From genes to proteins

"There is a strong need for new therapies other than surgery to treat patients with hepatic metastases. If we are able to discover an effective therapy for liver metastases, we could reduce the high mortality rates associated
with cancer," says Andrei Turtoi. "The targeted therapies that have been developed so far work at first, but then the tumour takes back over," indicates the researcher. It is in this context and following these observations that Andrei Turtoi and his colleagues at the Metastasis Research Laboratory became interested in CRC liver metastases. The tumour and the metastases are very heterogeneous in terms of cell populations. Yet a given targeted therapy targets only one population of cells within the tumour that share the same target. "We are able to kill these cells, but the other populations of cancer cells escape unharmed. Following Darwin’s theory of evolution, the fittest cancer cells surviving the selection pressure have an advantage and cause the tumour and metastases to regrow," explains Andrei Turtoi.

A number of studies have reported on the daunting genetic heterogeneity in cancer, but the Liège researchers wanted to know if this heterogeneity also existed at the protein level. "Cancer cells present many kinds of mutations, so it’s difficult to know what to target and how," continues the researcher. "But we weren’t targeting genes, we were targeting proteins," Andrei Turtoi reminds us.

**Organised proteomic heterogeneity**

In order to analyse the proteins in CRC liver metastases, the scientists worked in collaboration with the Department of Abdominal Surgery at Liège University Hospital and with GIGA’s Mass Spectrometry and Experimental Pathology Laboratories. "We had the opportunity to study human liver metastases from colorectal carcinoma and thanks to a specific mass spectrometry imaging technique we were able to screen first for different peptides in the metastases. The advantage of this approach is that we didn’t have to dissect and thus disturb the tumour," says Andrei Turtoi.

The research results that were published in the journal *Hepatology* (1) demonstrate that hepatic metastases do display proteomic heterogeneity, however this heterogeneity is actually organized. "The peptides are zonally delineated and these zones are very similar from one patient to another," the scientist reveals. The tumours are thus less phenotypically heterogeneous than expected given their genetic heterogeneity. "Although cancer cells carry different mutations, it seems that they can still behave in the same way," explains Andrei Turtoi. The zonal delineation can be explained by the fact that environmental factors in the tumor, such as oxygen and nutrient availability, favour certain phenotype. "This is very good news for targeted therapy because we can now envisage targeting the cells of the same zone together since they have the same phenotype," continues Andrei Turtoi.

**Two promising targets for new therapies**

After discovering these zones, the researchers then dissected the tumours to identify the proteins they contained. "We selectively isolated cancer cell surface proteins and matrix proteins. They are accessible and are the first point of contact between the tumour and its environment, and are thus good targets from a therapeutic point of view," explains Andrei Turtoi. The identification of these proteins led the scientists to several known proteins already used in clinics as targets for cancer therapy. These data can be used now to optimize existing treatment protocols. They also identified novel proteins, two of which are particularly interesting targets: LTBP2 and TGFBI. Indeed, these proteins could be very good markers given that they are expressed in the cells of most of the zones identified in CRC liver metastasis. And Andrei Turtoi and his colleagues have already found antibodies that can target these two proteins. The next step is to test the effect of these antibodies on a mouse model of colorectal cancer.
In addition to providing new prospects for targeted therapies for colorectal cancer, this research shows how important it is to study the proteomic aspect of tumours, and not just their genetics. "Proteins are finally determinant for the phenotype. Studying tumours by focusing on the phenotype may open the doors to new therapeutic treatments," Andrei Turtoi concludes.

(from left to right) Hepatic metastases are commonly encountered in advanced colorectal carcinoma patients. Following positive diagnosis through medical imaging (here PET-CT) and subsequent surgery the samples are used for pathological examinations and research. At MRL researchers are doing advanced protein analysis and sequencing of hepatic metastases in order to identify novel molecular targets useful for developing better anti-tumor therapies. They lay specific focus on the fact that tumors are heterogenous entities harboring cells with different genetic characteristics and abilities to escape therapeutic pressures. The most promising proteins with their functions to target are tested in animal tumor models (here mice) for their efficacy. This step is essential part of preclinical studies that should promote new drugs to help patients.
The human PET images used in this figure were generated by Department of Nuclear Imaging of CHU Liege, Prof. Roland Hustinx. Tomographic image of small rodent was performed with the support of Prof. Alain Vanderplasschen, Laboratory of Immunology - Vaccinology, Faculty of Veterinary Medicine, ULg.