Metastases: preparing for the crime

11/17/09

In May 2009, ULg GIGA-Cancer researchers under the supervision of Agnès Noël, co-director of the Tumour Biology and Development Laboratory, published an article in the *Journal of Cellular and Molecular Medicine*, focusing on the roles of a matrix metalloproteinase (MT4-MMP) in the formation of pulmonary metastases stemming from breast cancer. And on 2nd September, Agnès Noël and Françoise Bruyère were responsible for another article, on lymphangiogenesis, in *The FASEB Journal*. These studies are in perfect symbiosis with the vast European Microenvimet programme, co-ordinated by Agnès Noël, whose ultimate goal is to find the means to eradicate the formation of metastases by acting on the tumoral microenvironment.

The development of metastases, the major cause of death in cancer patients, bears witness to the aggressive nature of the primary tumour. In addition, the question of the "metastatic cascade" has attracted the attention of numerous research groups. One of the essential aspects of the problem certainly relates to the way in which the tumoral microenvironment favours the spreading of the cancerous cells which leave the primitive lesion, through the blood or lymph system, to spread through the body and form one or more secondary foci.

Under the aegis of the 7th European Union Framework Programme, the Microenvimet (1) network, whose work began in March 2008, includes nine European research centres - Belgium, Finland, Germany (2 laboratories), Italy, Spain, France, Denmark and Slovenia. Financed by the European Commission for the sum of EUR 2 999 689 for four years, its overall objective is to contribute to understanding and fighting metastases by modulating the tumoral microenvironment.

In order to succeed in its research programme, it is relying on the specific expertise of each of its nine members and on innovative technological platforms - genomic platform for the analysis of messenger RNA and micro-RNA, phage bank for the development of antibodies that block identified targets, computer-assisted image analysis platform, transgenic platform.

As the co-ordinator, Professor Agnès Noël, co-director of the Tumour Biology and Development Laboratory (TBDL) within the University of Liège's GIGA-Cancer explains, a tumour is not only made up of tumoral cells, but also of cells from the host it has recruited for itself through molecular messages and which infiltrate it. Among other things, they can be endothelial cells required for the neoformation of blood vessels (angiogenesis) and lymph vessels (lymphangiogenesis), and fibroblasts, elements that enter into the constitution of the extracellular matrix, favourable to the migration of tumoral cells, and which, in addition, will produce growth factors and proangiogenic factors, or even inflammatory cells. "While some immune or inflammatory cells defend the host against the tumour, others will work to its advantage", Agnès Noël points out. "Thus, some types of macrophages turn out to be antitumoral while others stimulate angiogenesis. A whole range of work to detect the sub-populations of cells that infiltrate the tumour has been underway for the past few years, but there’s still a long way to go."

(1) Microenvimet for Understanding and fighting metastasis by modulating the tumour microenvironnement through interference with the protease network.

Strange niches

In fact, many beliefs are currently being re-explored. For instance, we were sure that the fibroblasts that infiltrated the primary tumour came from its direct environment. Everything now seems to indicate that they also result from mesenchymal stem cells present in bone marrow. In addition, some twenty years ago, we
believed that the **proteases** expressed in cancer cells were only there to ensure the perforation of the **basal membranes**, in order to allow the migration of the tumoral cells. Nothing of the sort. They can also activate growth factors as well as **cytokines**, which recruit inflammatory cells among other things.

As for the cancerous cells, the remote colonisation of tissues (metastases) is a veritable assault course. In the course of their journey, they inevitably break through several basal membranes, since they have to leave the primitive tumour, cross vessel walls in order to be conveyed by the blood or lymph system, cross it to migrate towards their final destination and, finally, penetrate the organ where they are going to settle. But that is not all. To accomplish this journey, they will have had to induce angiogenesis, resister **apoptosis** and the blood flow, which is an inhospitable environment for them. "*Because of the mutations its has undergone, the malignant cell has acquired a certain number of particular abilities as regards proliferation, resistance to the dead cell, and migration, but this is not enough to allow it to invade the neighbouring tissue or generate metastases; it is essential its environment is remodelled*,", emphasises Agnès Noël.

However, is the primary tumour’s microenvironment the only one to be taken into consideration? No. Recent data (2) suggests that the implantation site of secondary tumoral foci is not random, but predefined by the
establishment of a microenvironment favourable to this implantation. This had led to the concept of the "premetastatic niche", which is situated at the heart of the Microenvimet network’s research. What is original about this work is that it does not only focus on the primary tumour's microenvironment, but also on that of the potential target organs and established metastases.

Professor Noël points out that the goal is to elucidate and understand the early mechanisms of metastatic dissemination by studying the tumoral microenvironment’s contribution throughout the different stages of the evolution of the epithelial tumours (carcinomas): the primary tumour, the premetastatic phase preceding the dissemination of cancerous cells and the metastatic phase during which the secondary foci develop. She also adds that "Our programme aims to identify molecular targets that intervene very early in the progress of the tumour." As a result, the main point of the project is to reveal mechanisms underlying the elaboration of an area favourable to the implantation of metastases, a reality translated by the concept of the premetastatic niche.


**Intelligent and lazy…**

Signals are therefore sent from the primary tumour to the target organ even before the spreading of the cancerous cells, so that, as Agnès Noël metaphorically describes, they can be "welcomed" into a "cosy nest" when they arrive. "This process may well possibly involve intermediary stages", adds the TBDL co-director. "For instance, experimental data suggests that messages from the primary tumour end up in the bone marrow, which sends precursors of the hematopoietic cells to the target organ, which will create a swelling there and modify the environment."

In any case, the conditioning of the target organ for the future colonisation by tumoral cells emanating from a primary foci gives rise to the idea of programming and finality, so to speak. This is something we can relate to. Agnès Noël even goes so far as to define the metastatic cancer cell as "intelligent" and "lazy", insofar as it prepares its future implantation but can only succeed with "outside help".

**The importance of proteases**

One of the key areas of the Microenvimet research programme consists of clarifying the role played by certain proteases, which are known to be important regulators in the interactions that are established between the tumoral cells and their cellular and molecular microenvironment. The article published last May(3) by GIGA-Cancer researchers on the involvement of the MT4-MMP metalloprotease in the formation of pulmonary metastases stemming from breast cancer, responds perfectly to this concern.

In the more global perspective of the Microenvimet project, researchers use knock-out mice, among others, for the coding gene for such or such protease. "By comparing these mice and wild mice, we are trying to determine how the absence of the studied protease influences the respective microenvironments of the primary tumour, the premetastatic niches and the metastases", explains Agnès Noël.

It is well known that certain proteases produced by malignant cells or cells that infiltrate the tumour encourage angiogenesis, the invasion of the surrounding tissues or migration. The European research group has already been able to pinpoint a membrane protease that encourages the formation of metastases and other proteases that, contrary to all expectations, fights off tumoral invasion: it is a metalloprotease, MMP-8, and ADAMTS (A Disintegrin And Metalloprotease with Thrombospondin domain), which are members of a family of enzymes related to metalloproteases.
The discovery of the existence of proteases capable of preventing metastatic dissemination is of great importance. In fact, metalloprotease inhibitors were tested clinically. They turned out to be relatively ineffective and the cause of major side effects, such as the return of the treated tumour. This was surprising, considering the results of the experiments carried out on animals, and they were abandoned in humans. "Now, we are starting to understand why these drugs did not meet expectations", states the head of the Microenvimet network. "It's certainly a question of inhibitors with too great a spectrum. They should undoubtedly be replaced with more targeted inhibitors."


**Therapeutic targets**

Another innovative aspect of the project resides in using microarray technology to determine the gene expression profile in the form of messenger RNA both in the primary tumour and in the premetastatic niche and secondary foci. The analysis also relates to micro-RNA, small fragments of non-coding RNA from some twenty or thirty nucleotides, discovered only several years ago and whose role remains uncertain even though we know that they participate in controlling the translation of messenger RNA into proteins. Literature seems to increasingly underline their importance in the development of cancers. "One of the project's partners, the Institut de Pharmacologie Cellulaire et Moléculaire (IPMC - CNRS) led by Professor Pascal Barbry and Dr. Bernard Mari at the University of Sophia Antipolis, Nice, has developed microarrays for the micro-RNA", Agnès Noël informs us. "We are using this technique."
The identification of therapeutic targets and the elaboration of antibodies directed against them in an effort to curb the metastatic cascade are also two closely related objectives of the Miroenvimet programme. Antibodies are produced using the so-called "phage display" technique, which allows humanised antibodies to be obtained. "This technique is perfectly controlled by our Finnish partner, the Molecular Cancer Biology Research Program group from the University of Helsinki, led by Professor Kari Alitalo and Dr. Pirjo Laakanen”, specifies Professor Noël. "As for the development of antibodies in mice, this will be entrusted to Professor Gunilla Høyer-Hansen's Finsen Laboratory in Denmark."

Cancerous stem cells

In the 1970s, Isaiah Fidler, from the MD Anderson Centre at the University of Texas, considered that only a few tumoral cells were able to form metastases; to be specific, those that had accumulated the most mutations - overexpression of proliferation genes, extinction of tumour suppressor genes, etc. Now, another similar yet original view has taken form: cancer-inducing cells, capable of subsequently migrating to generate metastases, are in fact "cancerous stem cells", i.e. stem cells that, after genetic mutation, have become dissident. This new view is at the heart of numerous pieces of research and animated scientific debates. Attention is particularly
focused on the identification of markers that would allow us to detect these cells in the blood and lymph systems and to thus predict possible metastatic dissemination.

The subject is primarily of interest to research centres in the Microenvimet network, especially since the cancerous stem cells may be highly resistant to chemotherapy and radiotherapy and, consequently, may be at the source of recurring episodes. For the time being, there are few markers that allow us to identify them. At the University of Turin, the group of professors Paolo Comoglio and Carla Boccaccio, who specialises in this theme, has established a protocol to isolate these human tumour cells in the colon. Subsequently, it will be extended to other types of cancer, while similar work is currently being carried out on mice in parallel. "Here too, we remain faithful to our initial "ideology", Agnès Noël stresses. "Indeed, the originality of our approach is not to apprehend the cancerous stem cells in isolation, but in their environment."

The whole body

On a methodological level, participants in the Microenviment project are trying to develop culture models that are increasingly close to in vivo reality. This would, incidentally, have the advantage of limiting the use of laboratory animals.

For instance, for the lymphangiogenesis study, the researchers have recourse to a model of lymph vessel formation that relies on a system of three-dimensional culture. This model developed by the TBDL (Françoise Bruyère, Agnès Noël) at GIGA-Cancer, in collaboration with the laboratories of professors Claude Libert (Ghent), Peter Carmiliët (Leuven), Jonathan P. Sleemæn (Germany) and Kari Alitalo (Finland), has been the subject of publications in *Nature Methods* (4), 20th May 2008, and online in *The FASEB Journal* (5), 2nd September 2009.

"Up until now, researchers only had two-dimensional cellular proliferation models when they wanted to study lymphangiogenesis", points out Françoise Bruyère. "Our three-dimensional model should allow this process to be better understood and to test molecules likely to inhibit the formation of lymph vessels."

After a year and a half in existence, the Microenvimet programme has already been able to establish the existence of premetastatic phases (specific modifications to the environment of the target organ) in several in vivo models, thus validating the concept of premetastatic niches, at least in these models. A fact is now starting to be revealed in this work in progress: cancer must be studied as a phenomenon that affects the body in its entirety.
