SIBLINGs, cancer's multifunctional weapons

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Akeila Bellahcène and Vincent Castronovo of the Metastasis Research laboratory of the University of Liège are among the first researchers to have discovered the role played by certain bone and tooth proteins in the progression of certain cancers. These proteins, called SIBLINGs, are currently the object of numerous research studies worldwide.

In March 2008, Nature Reviews Cancer published a paper(1) whose first two authors are Akeila Bellahcène, a researcher at the Metastasis Research Laboratory of the University of Liège, and Vincent Castronovo, director of this same laboratory. The center has since received considerable recognition for its pioneering role in the field of SIBLINGs, a family of bone and tooth proteins involved in various stages of the progression of certain cancers: during malignant transformation, invasion and metastatic dissemination.

The paper summarizes current knowledge on SIBLINGs (Small integrin-binding ligand N-linked glycoproteins) and underlines their potential both as diagnostic and prognostic tools as well as preferred targets in what has come to be known as "intelligent cancer treatments", i.e. those which can destroy malignant cells while leaving healthy cells unharmed.
The family of SIBLINGs currently includes five members which, in addition to being associated with bones and teeth and implicated in the cancer process, are also linked by structural homologies in their respective genes. Osteopontin (OPN) is the protein most studied today; in 1994, Akeila Bellahcène and Vincent Castronovo discovered the significance of bone sialoprotein (BSP) in oncology, thereby becoming pioneers in the field; the other three proteins are dentin matrix protein 1 (DMP1), dentin sialolphosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE).

Osteomimicry

Professor Castronovo uses a metaphor to describe the multifunctional characteristic of SIBLINGs, comparing them to do-it-yourself tools which can be used to drill, screw, saw or sand depending on the situation. Likewise, cancer cells secreting these proteins use them to act on their own micro-environment at various stages of tumor invasion and the metastasis cascade. In the case of metastasis development, malignant cells must first leave the primary tumor, pass through vessel walls in order to be transported via blood or lymphatic circulation, re-cross the same vessel walls to migrate toward their destination site, and finally penetrate the organ where they will settle. Continuing with the same metaphor, these cancer cells need drills, grapples, and a number of other tools provided to them, at least in part, by the SIBLINGs they produce.

Detection of BSP (colored in brown) in a bone metastatic lesion in cancer mammary cells using an immunohistochemistry technique and a specific antibody. The marked cancer cells appear on both sides of a bone trabecula colored in blue. x400 enlargement.

Professor Castronovo explains that "... by attacking these proteins, we attack cancer on different fronts at the same time. We now know that through the cells that compose it, the tumor is constantly at different stages of its development: at any one time, certain malignant cells may be dividing while others, for example, may
be trying to pierce through basement membranes or attempting to induce vessel genesis. Ideally, a multiple therapeutic approach would be necessary to stop the process, using specific molecular tools for each stage, or acting on a group of molecules (in this case SIBLENg) involved in several stages."

The Metastasis Research Laboratory, thanks to the dedication of Akeila Bellahcène and Vincent Castronovo, has made a significant contribution to the validation of the role played by these molecules outside the context of the bone. The starting point of their research was Prof. Castronovo’s astonishing observation in the early 1990’s that microcalcifications composed essentially of hydroapatatite were often present in screening mammographies that led to cancer diagnosis. In attempting to find an explanation for this phenomenon, researchers at the MRL demonstrated both that mammary cancer cells were able to synthetize proteins necessary for bone mineralization and that there was a relation between the genesis of microcalcification in the breast and the development of bone metastases. Vincent Castronovo’s conclusion was that tumor cells present in the majority of mammary cancers "disguise themselves" as bone cells - the so-called osteomimicry phenomenon - in order to implant metastases in bones.

**Osteotropic Cancers**

Akeila Bellahcène and Vincent Castronovo focused their attention on the study of bone sialoprotein (BSP), a protein that ensures the adhesion of osteoclasts and osteoblasts to the bone matrix during bone remodelling, a "permanent work site" which results in the renewal of ten percent of the adult skeleton over a period of one year. During this process, BSP binds to integrin, a cell surface receptor. The biologists from Liège demonstrated that not only do mammary cancer cells overexpress BSP, but that the protein acts as a sort of harpoon by hooking on to the bone matrix in addition to having a pro-angiogenic action. Other laboratories later discovered that this protein is implicated in the degradation of basal membranes blocking malignant cell migration. It proceeds by activating metalloproteases in the bone matrix. In short, the multifunctional character of sialoprotein is undeniable, in particular in tumoral progression.
Clinical practice also elucidated its own but very similar truth. As underlined by Akeila Bellhcène, the Metastasis Research Laboratory has proven that in breast cancer, the tumoral cells which induce metastases in bone produce more BSP than their homologues, which spread towards other organs such as the liver. In addition, the greater the protein level at the time of the diagnosis of breast cancer, the greater probability there is of bone metastases developing. Professor Castronovo adds that "This observation has great clinical significance, as it should make it possible to determine which patients would most benefit from a preventive treatment with bisphosphonate. Bisphosphonates are molecules which are commonly administered in the treatment of osteoporosis, but are also very effective in blocking the development of bone metastases."

The MRL then became briefly interested in osteopontin, which is similar to BSP and would later become a member of the SIBLINGs family, as it came to be known, and osteonectin, a more unconventional protein. The Liège researchers were able to prove that these two extracellular matrix bone proteins expressed by osteoblasts were also present in mammary cancers. This discovery by Professor Castronovo’s team was reported in an article published in the American Journal of Pathology in 1995.

Generally speaking, the MRL was able to shed light on the abundant expression of bone proteins in all osteotropic cancers - i.e. breast, prostate, thyroid and lung - contrary to what may be observed in tumors whose metastases have little affinity for the skeleton.

Osteopontin’s Variable Action

The MRL findings were well received indeed. Many laboratories turned their attention to the relationship between bone proteins and cancer. Osteopontin, discovered in 1979, had already been the object of studies in cancerology, although not in its relation to metastases. Today, osteopontin has become the "star" protein of the SIBLINGs family. On one hand, it is overexpressed in all types of cancer; on the other, we now know that
it has a kaleidoscopic activity in the sphere of malignant tumors, since it is implicated in several of their stages such as inflammation, angiogenesis, invasion, the metastatic cascade, etc. 

The term *Small integrin-binding ligand N-linked glycoproteins* appeared in the scientific literature for the first time in 2001. Even if the five proteins concerned are all bone or tooth glycoproteins, they are no longer exclusively associated with osteotropism. Their presence has indeed been detected in diverse cancers where bone metastases rarely develop. Vincent Castronovo adds that "...we finally began to question whether these proteins used their multifunctionality as a weapon to invade most types of tissues". It is likely that they participate in the development of numerous types of metastases but until now this has only be proven for osteopontin; it is still unknown whether this is also the case for DMP1, DSPP, MEPE and BSP, with the latter remaining the MRL's main focus.

If it is generally accepted today that SIBLINGs proteins are overexpressed in several cancers, it is still largely unknown how and under what conditions some of them, i.e. DMP1, but especially DSPP and MEPE, contribute to the development of these malignancies. It appears more and more likely that, as is the case for OPN and BSP, they have a role to play in *angiogenesis*. Numerous research studies have been undertaken to determine the exact nature of the relationship between the SIBLINGs and the successive stages of tumoral progression. It has already been demonstrated that these proteins, by preferentially binding to integrins or CD44, their surface receptors, transmit a signal to the cancer cell which may modify its behavior and "strategy" (i.e. whether it divides, migrates, secretes an enzyme, etc.). We also know that OPN, BSP and DMP1 are implicated in the resistance to programmed malignant cell death (*apoptosis*). Professor Castronovo adds that "while protein P-53 contributes to the apoptosis of cells whose genomes are damaged, these three SIBLINGs oppose it". It has also been demonstrated that OPN, BSP and DMP1 contribute to cellular proliferation and tissue invasion by recruiting metalloproteases. Akeila Bellahcène explains that "...each of these SIBLINGS could take on a specific metalloprotease: BSP with MMP2, OPN MMP3, and DMP1 MMP9. However, we cannot exclude the possibility that other associations will be discovered in the future."

Metalloproteases are *proteolytic enzymes* capable of selecting and degrading proteins. By attacking extracellular matrix proteins - collagen for instance - they damage the physical barriers (basal membranes in particular) which prevent cancerous cells from invading tissues. Metalloproteases play a similar role in the metastatic cascade, as they make it possible for malignant cells to digest the surrounding matrix and reach the blood circulation by penetrating into the blood and lymphatic capillaries, and to be carried through the circulatory system until they reach their target, a distant organ which they will penetrate.

During their journey, metastatic cells are the target of attacks by the immune system. The expression and presentation of BSP, DMP1 and OPN at their surface apparently induces the sequestration and activation of *factor H*, which blocks the cell lysis activity of the *complement system*. consequently, cancer cells benefit from a protective effect, even if a majority will finally be destroyed.

When they reach their destination, the "survivors" stop at the distant organ's capillary bed by adhering either to the capillary endothelium or the sub-endothelial basal membrane (when it is accessible). They then extravasate by molecular mechanisms similar to those activated during invasion. Proliferation in the parenchyma of the colonized organ completes the metastatic process. The intervention of osteopontin and bone sialoprotein is once again observed.
New Horizons

Even if SIBLINGs are potential therapeutic targets in cancer treatment because of their multifunctional character, they are first and foremost a diagnostic and prognostic tool. These biomarkers could prove very useful, particularly at a time when much hope is placed in a personalized therapeutic approach to cancer. Akeila Bellahcène reports that "In prostate cancer for instance, we now know that the presence of BSP in the primary tumor is a negative prognostic indicator in terms of both metastatic invasion and dissemination."

The implication of SIBLINGs in the different stages of the cancerous process (2) opens the door to the possibility of different types of therapeutic intervention. Experiments carried out on animals have already shown that suppressing OPN expression slows tumor growth. In addition, administering anti-BSP antibodies hinders metastatic dissemination towards the bone. There exist other examples of similar phenomena, particularly in pancreatic cancer.

Osteopontin is currently considered the "star" of the SIBLINGs family and therefore tends to be the main focus of study. According to Prof. Castronovo, it would be an error, however, to neglect its "sisters", especially since BSP, the object of most research efforts at the Metastasis Research Laboratory of the University of Liège, seems to possess very similar properties. Knowledge of DMP1 and especially DSPP and MEPE remains very fragmentary. Professor Castronovo concludes that "there probably exist redundancies between these five glycoproteins. This is why a therapeutic strategy focusing on only one of them might not be successful."

(2) This only applies to tumors where the presence of SIBLINGs has been demonstrated.