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NEWS

From tumor dormancy to the vicious cycle of bone metastasis

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A recent *IBMS BoneKEy* cancer and bone webinar examined the role of VCAM-1 in awakening dormant breast cancer cells

Cancer and bone researchers accept the 'vicious cycle' of bone metastasis as a fundamental concept that helps explain why breast, prostate, lung and other cancers have a strong tendency to spread to bone. During the vicious cycle, tumor cells in the bone microenvironment produce molecules that stimulate osteoclastic bone resorption. The consequence of increased resorption is the release of growth factors from the bone matrix that feed back to the tumor cells, further stimulating their growth. At the same time, investigators are well aware of tumor dormancy, a phenomenon where tumor cells that have disseminated from a distant organ to the bone microenvironment remain dormant—even for a decade after a tumor is first detected. Before the vicious cycle can begin, these dormant cells must first be awakened.

The mechanisms that rouse disseminated tumor cells from their slumber were the focus of 'The Role of VCAM-1 in the Progression of Dormant Disseminated Breast Cancer to Bone Metastasis', a recent *IBMS BoneKEy* webinar (http://www.nature.com/bonekey/webinars/index.html?key=webinar13). Presenter Yibin Kang (Princeton University, USA) focused on the role of one particular molecule, vascular cell adhesion molecule 1 (VCAM-1), a protein expressed by endothelial cells and known to mediate the adhesion and migration of leukocytes during inflammation. Concentrating on research[1,2] published in 2003 and 2011, when he was a postdoctoral fellow and principal investigator, respectively, Professor Kang described the key experiments that have implicated VCAM-1 as a major player mediating the transition from dormancy to the vicious cycle in the breast cancer setting. Following his presentation, an esteemed panel of experts from the cancer and bone field pinpointed gaps in the current understanding of how VCAM-1 operates to stir dormant tumor cells to a more active and troublesome state, and also highlighted how to translate knowledge of VCAM-1 function into the clinic to help breast cancer patients.

An Experimental Path...

Professor Kang began his account of VCAM-1 in 2003 when, as a postdoc at the time in the lab of Joan Massagué at Memorial Sloan-Kettering Cancer Center in New York City, he and his colleagues were trying to understand the mechanisms of bone metastasis in breast cancer. To do so, they pursued an *in vivo* selection strategy in immunodeficient mice to isolate breast cancer cells with a strong tendency to spread to skeletal tissue. These investigators injected cells from a human breast cancer cell line into the mice, isolated cells from bone lesions that had formed in the animals, and then re-injected those cells into new mice. 'Even after just one round of selection, we selected tumor cells that were extremely efficient in metastasizing to bone,' Professor Kang said, also noting that microarray analyses identified a 'gene signature' characteristic of the metastatic cells.

In another set of experiments, the researchers isolated 46 single-cell-derived populations (SCPs) from the parental cell line and analyzed them for the expression of five bone metastasis genes they had identified in their previous experiments. While the SCPs varied in their expression of each of the five genes, most of the SCPs expressed none of them. Furthermore, unlike the other SCPs, these so-called 'Low-5' SCPs did not cause any bone metastases when injected into mice over an approximately 3-month period. However, X-rays would later reveal that, over ensuing months, about 10% of mice that had been injected with one of the Low-5 SCPs, called SCP6, did in fact exhibit bone metastases. Meanwhile, *in vivo* imaging studies of luciferase-labeled SCP6 tumor cells showed a repeated inability of the cells to form overt bone metastases in mice that had been injected with the cells; only in a small number of cases did the cells escape from dormancy to cause problems.

In short, somehow, in just a small percentage of the mice, the dormant SCP6 cells were emerging from dormancy to cause bone metastases—but how? Additional *in vivo* selection experiments enabled Professor Kang and colleagues to select for and then examine highly metastatic sublines derived from the parental SCP6 cells—and the results were unexpected. 'Surprisingly, the sublines did not express any of the bone metastasis genes published previously. This suggested that the cells somehow came up with a completely different way to escape from indolent growth and become able to form aggressive bone lesions,' Professor Kang said.

...leads to VCAM-1

Suspecting that a new gene must be involved, the team went to look for it. After narrowing the list of contenders to a handful of genes whose biological function suggested they might be involved in bone metastasis, Professor Kang discovered that knockdown of just one of those genes, *VCAM-1*, resulted in decreases in bone metastases in mice that had been injected with highly metastatic cells derived from SCP6. He noted that these findings were consistent with clinical data showing that breast cancer patients who relapse early after primary tumor removal express higher levels of VCAM-1 than patients who relapse later.

Further experiments solidified VCAM-1 as an important player in the escape from tumor dormancy, including studies using SCP6 sublines that had lost their bone metastatic ability; overexpressing VCAM-1 in these so-called revertant cells allowed them to regain it. Meanwhile, experiments in an immunocompetent mouse model that employed a different cell line derived from mouse mammary tumors also indicated that knockdown of VCAM-1 reduced bone metastasis. Antibody studies also supported a role for VCAM-1: inhibiting VCAM-1 function with an anti-VCAM-1 antibody, or directing an antibody against its receptor, the $\alpha4\beta1$ integrin, each decreased bone metastases formed by metastatic sublines of SCP6.

As the evidence built for an important role of VCAM-1, how the molecule was facilitating the escape from dormancy, at a cellular and molecular level, remained unclear. But experiments where tissue was stained for tartrate-resistant acid phosphatase, an osteoclast marker, revealed decreased levels of tartrate-resistant acid phosphatase-positive osteoclasts in bone lesions from animals treated with an anti-VCAM-1 antibody or an anti-$\alpha4\beta1$ antibody, compared with controls not receiving antibody treatment, indicative of decreased osteoclast activity. Additional studies showed that tumor cell-expressed VCAM-1 acted by stimulating osteoclast differentiation, rather than by working through an intermediate cell type. Furthermore, the effect of VCAM-1 on osteoclasts did not appear to be through effects on receptor activator of nuclear factor-κB ligand signaling, a pathway known to stimulate osteoclast differentiation. Rather, *in vitro*, VCAM-1 appeared to cause chemotaxis of osteoclast progenitors towards the tumor cells and the adhesion of those progenitors to each other. Experiments using light imaging of tumor cells and osteoclast progenitors also indicated that VCAM-1 was acting in a similar fashion *in vivo* in mice.

Finally, a picture of how dormant disseminated breast cancer cells awaken from dormancy had emerged. First, inflammation increases the expression of VCAM-1 by tumor cells in the bone microenvironment. Because VCAM-1 can be shed from the cell surface, soluble VCAM-1 then attracts osteoclast progenitors to the tumor cells. Then, by direct cell-to-cell contact with osteoclast progenitors—through the binding of VCAM-1 on tumor cells to $\alpha4\beta1$ on osteoclast progenitors—VCAM-1 facilitates the adhesion of the progenitors to each other, resulting in an increase in osteoclast activity, the end of dormancy, and the onset of the vicious cycle.

The Unknown

Many questions remain with regard to this conceptual framework, however. One concerns how VCAM-1 stimulates osteoclastogenesis—is inflammation-induced upregulation of VCAM-1, followed by effects on chemotaxis and adhesion of osteoclast progenitors, the sole or main mechanism? Evidence from the multiple myeloma setting, where VCAM-1 already has a known role, suggests that juxtacrine mechanisms in the bone microenvironment could be important. In multiple myeloma, stromal cells that express VCAM-1 bind to multiple myeloma cells that express $\alpha4\beta1$, which results in the release of factors like tumor necrosis factor-α and interleukin-6 from the stromal cells, with a consequent increase in osteoclastogenesis. Whether similar interactions also have a role in the breast cancer setting is unclear.

In fact, the failure of inflammatory triggers to stimulate VCAM-1 expression *in vivo* in mice suggests to Professor Kang that other factors, such as epigenetic mechanisms, may also have a role in upregulating the molecule. He emphasized that rather than relying on mouse models, obtaining tumor samples from patients—by examining primary tumors, disseminated tumor cells, or bone metastases—for VCAM-1 expression, and then using a technique like histone profiling could help to identify epigenetic phenomena that activate VCAM-1 in the first place.

Another issue to understand on the basic science side concerns the roles that VCAM-1 may have in processes other than bone metastasis. In fact, VCAM-1 appears to be important in lung metastasis. In addition, VCAM-1 is expressed in neoplastic cells at the site of primary tumors, where it could have a role in angiogenesis. Professor Kang noted that the multiple roles of VCAM-1 bode well for drug development, as an agent that targets VCAM-1 could have positive effects on all three processes.

While there is still much to understand about the cellular and molecular biology of VCAM-1 activity, researchers are excited about the potential clinical value of targeting it, both for prognostic and therapeutic purposes. Based on its biological function, VCAM-1 could potentially be used as a prognostic indicator of the escape from dormancy; because it is a transmembrane protein, possibly VCAM-1 could be detected on disseminated tumor cells and used as a biomarker to predict metastatic relapse. Detecting VCAM-1 on circulating tumor cells offers another prognostic possibility. When cancerous cells leave the site of a primary tumor, they must first circulate in the blood before they can disseminate to a distant organ. However, it is still unclear exactly what happens to circulating tumor cells once they leave the circulation and enter the bone marrow, and comparing the expression of VCAM-1 in circulating tumor cells to that in disseminated tumor cells may be instructive. Measuring VCAM-1 expression on circulating tumor cells could be quite useful; it is already known that circulating tumor cells are a predictor of metastasis. Finally, measuring circulating levels of soluble VCAM-1 could also be of value.

In addition to prognosis, VCAM-1 could potentially be targeted for treatment purposes. As VCAM-1 is known to have a role in inflammation, antibodies and small-molecule inhibitors of VCAM-1 already exist, some of which have been in clinical development for several years, and so using such agents in the context of bone metastasis could be an intriguing path to pursue. However, in order to target VCAM-1, it will be necessary to determine the proper time at which to block it. 'If you treat mice with a VCAM-1 antibody immediately after they are injected with the tumor cells or during the time window during which the tumors are growing in an indolent phase, there are clear and dramatic effects,' Professor Kang said. 'But if you wait until the cells have already broken out of dormancy and there is already

massive tumor growth, the effect is less significant,' he said. This finding suggests that blocking VCAM-1 could be useful as a preventive treatment. However, breast cancer patients can suffer relapse as many as 10 or more years after their diagnosis, so to treat those patients prophylactically for such a long time would be unrealistic, both in terms of potential side effects and cost. Perhaps a pulsatile treatment administered over a short time, and maybe combined with chemotherapy, could prove effective, Professor Kang suggested.

Conflict of Interest

The author declares no conflict of interest.

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Webinar Panelists: Theresa Guise, MD (Indiana University School of Medicine, USA); Klaus Pantel, MD, PhD (University Hospital Eppendorf, University of Hamburg, Germany); G David Roodman, MD, PhD (Indiana University School of Medicine, USA); Julie A Sterling, PhD (Vanderbilt Center for Bone Biology, USA). *Moderator:* Philippe Clézardin, PhD (INSERM, University of Lyon, France).

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