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NEWS

RANKL: A Soil Factor for Cancer Metastasis to Bone?

Evidence builds for a key role for RANKL in the spread of cancer to bone, but further experiments are necessary to cinch the case

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A collaborative effort by an international team of researchers has produced suggestive evidence that RANKL, a protein that controls the differentiation of several cell types, including bone-resorbing osteoclasts, also regulates the migration of cancer cells and perhaps their preferential spread to bone (1). While definitive experiments that could prove a role for RANKL in cancer metastasis have yet to be performed, the current findings confirm similar observations made in the past and make RANKL an intriguing candidate for the so-called "soil" factor that makes bone a particularly fertile environment for metastasizing cancer cells.

The notion that bone provides a favorable environment or soil for the spread of cancer was first postulated by the English surgeon Stephen Paget in the late nineteenth century. "Paget wanted to know why breast cancer patients suffered so much with bone metastases while other cancers didn't seem to have that same attraction to bone," says Holly Jones, lead author of the current study and an assistant professor at the University of Ontario Institute of Technology. "So he hypothesized that the bone microenvironment must make some kind of protein or other molecule that attracted cancer, especially breast cancer, so readily to bone."

The particular molecule that has caught the attention of Jones and her fellow investigators, as well as that of other researchers interested in the metastasis of breast and other cancers to bone, is receptor activator of NF-κB ligand, also known by its acronym, RANKL. That RANKL might play a role in cancer metastasis makes sense, because of its distribution and activity both in the bone microenvironment and in other tissues where tumors arise and then spread to bone. For instance, RANKL acts through its receptor, RANK, to stimulate the differentiation, activation and survival of osteoclasts, the cells that dissolve bone, and it also stimulates breast epithelial cells to differentiate during pregnancy. Since RANKL is able to stimulate cells in both breast and bone tissue, the investigators reasoned that perhaps breast cancer cells are also able to respond to RANKL signaling.

To test this hypothesis, the researchers first examined tumor samples from the breasts, and from the lymph nodes to which cancer cells had spread, of breast cancer patients and found the presence of RANK in both tissues. RANK was also present in human prostate and breast cancer cell lines examined during the study. In addition, experiments revealed that stimulating human breast cancer cells that express RANK with a genetically engineered form of RANKL activated a signaling pathway known to mediate the movement of cancer cells in response to chemical signals.

These initial findings led to a detailed examination of whether RANKL, acting through its receptor, could directly influence the migration of cancer cells expressing the receptor. Jones and her colleagues found that, *in vitro*, RANKL induced the migration of human breast cancer cell lines and that osteoprotegerin (OPG), a decoy receptor for RANKL that blocks the binding of RANKL to RANK, inhibited this migration. They also found that RANKL induced the migration of human prostate cancer cell lines and a mouse melanoma cell line, and in both cases the migration was inhibited by OPG. Furthermore, RANKL stimulated the migration of normal cells, including mouse

mammary epithelial cells and a human mammary epithelial cell line. Finally, RANKL also induced the migration of mature osteoclasts.

"These results suggest a new function for RANKL," says Dr. Jones of the migration experiments. "It's not just a signaling molecule for the differentiation of cells, but it may also be a signaling molecule for epithelial cell movement." Experts familiar with the study concur. "The new component of this study is the *in vitro* data demonstrating that RANKL activity, through RANK on the target cells, does actually enhance the migration of these cells," says Evan Keller, a professor of urology and pathology at the University of Michigan and senior author of the first study, published in 2001 in *The Journal of Clinical Investigation*, to link RANKL with cancer. "The migration data are new and interesting," agrees Gordon Strewler, a professor of medicine at Harvard Medical School and Editor-in-Chief of BoneKEy. "They show a functional effect of RANKL on tumor cells for the first time and raise the question of whether such effects promote targeting of tumor cells to bone or their survival in bone."

The results of the *in vitro* migration studies described above led to an effort that aimed to answer that very question – to test whether the regulation of cancer cell migration by RANKL also plays a significant part in the spread of tumors to bone *in vivo*. To test this idea, the researchers aimed to determine whether OPG, by blocking the interaction between RANKL and RANK, could prevent the metastasis of melanoma cells to bone. They chose to examine the behavior of melanoma cells because these cells, unlike breast and prostate cancer cells, do not activate osteoclasts and thus do not induce bone remodeling. Using a model where cancer cells do induce remodeling would make it difficult to determine exactly what impact OPG, itself an inhibitor of bone remodeling, might be having on the metastasis of cancer cells to bone. Indeed, OPG might block metastasis because it actually blocked the migration of the tumor cells themselves, but it also might block metastasis because it blocked the cancer cell-induced bone remodeling that enables cancer cells to spread successfully to bone. "Now, when we give the inhibitor OPG," Dr. Jones says of the choice to use a melanoma model, "we can really look at whether we inhibited cancer migration, and separate it from the fact that cancer cells can activate bone independently."

Consequently, in the initial melanoma cell experiments, Jones and her colleagues injected mouse melanoma cells into the left cardiac ventricles of mice and observed that the cells spread into the animals' long bones, vertebrae, ovaries, and adrenal glands, as well as into the brain. However, when they performed the same experiments with animals treated with OPG, the mice exhibited noticeably fewer tumors in the bones, though they exhibited similar numbers of tumors in all the other organs examined. Furthermore, while control mice, many of whom died before the experiments concluded, developed paralysis of their hind legs after receiving the melanoma cell injections, none of the mice treated with OPG exhibited paralysis or died during the course of the experiments. From these findings, the investigators suggest that RANKL, through its induction of tumor cell migration, is a prominent factor in the bone microenvironment that mediates the metastasis of cancer cells to bone.

While experts conversant with the study find the results of the *in vitro* migration studies novel and compelling, they find the results from the *in vivo* metastasis studies less so, primarily because the particular experiments used to define a role for RANKL are open to more than one interpretation. For instance, while the investigators suggest that OPG inhibited cancer cell metastasis to bone by blocking RANKL-induced migration, it is also possible that OPG instead merely inhibited basal bone remodeling that makes the bone microenvironment a hospitable one for metastasizing cancer cells. So argues Dr. Keller, whose 2001 study mentioned above found that OPG prevented the growth of prostate cancer cells that had been injected directly into the tibial bones of mice. Keller

suggests a similar experiment using a model system that doesn't induce bone remodeling, like the melanoma model used by the investigators from the current study; this kind of experiment would address whether RANKL impacts metastasis to bone specifically through an effect on cell migration. "With direct injection of tumor cells into the bone, if the study authors' hypothesis is correct, when OPG is administered, the tumor cells should grow in the bone now, because you have circumvented the inhibition of the migration," Keller says. "It would have been nice to see that, now that you have circumvented the migration component, the tumor cells can grow in the bone in the face of OPG."

Experiments with OPG, however, are less than ideal in resolving the question of whether RANKL activity plays a key role in metastasis to bone, according to Dr. Strewler. "Previous results such as Evan Keller's were interpreted to mean that OPG affects the environment–it reduces bone resorption and thereby makes the soil for tumor cells less rich," he says. "The present interpretation is that OPG affects the seed – the tumor cell – by blocking RANKL. Meanwhile, more recent studies show that OPG, when expressed in cancer cells themselves, has the opposite effect and enhances bone metastasis, so OPG experiments are hard to interpret."

Consequently, Dr. Strewler suggests a different kind of experiment with the potential to generate more compelling evidence. "I would regard a 'gain of function' experiment as more decisive," he argues. "If you express RANK in tumor cells that do not ordinarily metastasize to bone and thereby induce bone metastasis, that, together with what is shown in the current paper, would make a strong case that RANK expression is important. In that experiment, the effect of RANK is cell-autonomous." Dr. Strewler also notes that more solid evidence that tumors that spread to bone produce greater levels of RANK than those that don't spread to bone would also strengthen the argument. "The authors look at a couple of cell lines, but they are highly selected. What is needed is a rigorous look at tumors themselves," he says.

More definitive experiments capable of pinning down a role for RANKL in metastases to bone are desirable because RANKL makes a very practical target for pharmacological intervention. In fact, RANKL inhibitors are already being tested in clinical trials for the treatment of osteoporosis, as well as for patients who already suffer from bone metastases, and the hope is that these inhibitors could also be used to prevent primary metastases to bone. If so, these agents might offer distinct advantages over bisphosphonates, which are currently used to treat bone metastases, according to Theresa Guise, a professor of internal medicine at the University of Virginia. "RANKL inhibitors could have distinct mechanisms on the cancer cell," Dr. Guise says. "If cancer cells express RANK, and signaling of RANK through RANKL in cancer cells is important, it could be a different mechanism by which anti-RANKL therapy targets bone metastasis."

Developing additional therapeutic options that might work differently than current treatments is paramount, considering the scope of the problem of cancer metastasis to bone. Indeed, it is estimated that 70% of patients with progressive breast cancer develop metastases, as do 84% of those with prostate cancer, and these metastases cause a lot of pain. Bisphosphonates, which inhibit bone resorption, treat bone metastases effectively, but are unable to cure them. Clearly, the problem of cancer metastasis to bone is acute, and the desirability for new treatment options is immense. For now, though, the field awaits the definitive experiments and evidence that will turn the idea that RANKL, through its impact on cell migration, regulates cancer metastasis to bone from an intriguing hypothesis into an established tenet.

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