VCAM-1 engine drives bone metastases

By Michael J. Haas, Senior Writer

A team lead by **Princeton University** researchers found that inhibiting signaling between vascular cell adhesion molecule-1 and its receptor, integrin α_4 , prevented bone metastases in mouse models of breast cancer.¹ Although further mechanistic studies are needed to determine whether the ligand or receptor will be the better cancer target, the findings represent a repurposing opportunity for the three integrin α_4 inhibitors in the clinic or on the market to treat autoimmune diseases.

In breast cancer patients, primary tumor cells can metastasize to the bone and remain dormant for years or decades before growing into metastatic tumors. The molecular mechanisms driving tumor cell dormancy and reactivation in the bone microenvironment are poorly understood, in part because of a dearth of animal models that mimic those processes.

To create an improved model, a team led by Yibin Kang started with a series of human breast cancer cell lines with varying bone metastatic potential that it had co-developed in 2003.² The team injected mice with cell lines from the series and measured how long it took for bone metastases to arise.

Some cell lines had short *in vivo* dormancy (aggressive cell lines), and others had long *in vivo* dormancy (indolent cell lines). Genomic analyses showed that the aggressive cell lines expressed *vascular cell adhesion molecule-1* (VCAM-1) more highly than indolent cell lines.

In mice injected intracardially with an aggressive cell line, antibodies to VCAM-1 or integrin α_4 (VLA-4; CD49D) reduced the formation of bone metastases, metastatic tumor growth and bone damage compared with an inactive control antibody. Cells directly injected into the heart enter the circulation and exclusively form metastatic tumors, which Kang said allowed the team to study bone metastatic tumor growth.

In mice injected with an indolent cell line, the team found high levels of VCAM-1 in the bone metastases that eventually developed. Those data indicate that, despite having initially low VCAM-1, indolent tumor cells can upregulate the protein after disseminating to the bone and thus initiate metastatic growth, the team wrote in its report in *Cancer Cell*.

Microarray studies showed that VCAM-1 expression in primary human breast tumors correlated with metastatic relapse. Further studies in mice uncovered the role of VCAM-1 in the growth of bone metastases. VCAM-1 expressed by metastatic tumor cells in the bone recruited osteoclasts expressing integrin $\alpha_4\beta_1$ (CD49D/CD29), thereby initiating an early step in the process of bone destruction that enables metastatic tumor growth,³ Kang said.

Collectively, Kang said the findings suggest inhibitors of VCAM-1 or integrin α_4 , given just before surgical resection of the primary tumor or in the months immediately after surgery, could prevent bone metastases in breast cancer patients whose tumors express high levels of VCAM-1.

Kang is an assistant professor of molecular biology at Princeton. The team included researchers from **Qilu Hospital of Shandong University**, the **Memorial Sloan-Kettering Cancer Center**, the **Howard Hughes Medical Institute**, the **Cancer Institute of New Jersey**, **University Medical Center Hamburg-Eppendorf** and the **University of Medicine and Dentistry of New Jersey**.

Integral targets?

Dean Sheppard, associate chair of biomedical research at the **University of California, San Francisco**, said the therapeutic relevance of the findings to human cancer remains murky. Sheppard is also director of UCSF's Lung Biology Center and chief of the division of pulmonary, critical care, allergy and sleep medicine.

For example, he said it was unclear from the study how many primary breast tumors actually express high levels of VCAM-1. "The frequency of VCAM-1 upregulation in primary tumors would be important for determining the fraction of patients in which the team's findings would be therapeutically useful" to prevent bone metastases, he said.

Sheppard said the ability of tumors to upregulate VCAM-1 at the metastatic site will further complicate efforts to determine which breast cancer patients could benefit from VCAM-1 or integrin α_4 inhibitors.

Kang noted that only 5%–10% of primary tumors expressed high levels of VCAM-1, but suggested detection of VCAM-1 expression in circulating tumor cells could help identify patients who would benefit from the inhibitors.

Sheppard also pointed out that the function of individual integrins is not exactly the same in mice and humans. "Another receptor for VCAM-1 in human cells is integrin $\alpha_5\beta_1$," he said. "It is theoretically possible human cells might use this integrin as a receptor for VCAM-1 expressed by metastatic tumor cells" instead of integrin $\alpha_4\beta_1$.

If that turns out to be the case, he said VCAM-1 might be a better target than integrin α_4 to prevent bone metastases.

Sheppard also was concerned about the risk of progressive multifocal leukoencephalopathy (PML) associated with integrin α_4 inhibitors such as Tysabri natalizumab.

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Elan Corp. plc and **Biogen Idec Inc.** market Tysabri to treat multiple sclerosis (MS) and Crohn's disease. The drug's label includes a black box warning of an increased risk of PML, a potentially fatal CNS disease that results from reactivation of latent human polyomavirus JC infection in immunocompromised individuals.

Sheppard acknowledged the risk of PML might be acceptable to prevent bone metastases in breast cancer patients. "But breast cancer can remain indolent for years, so a patient might have to take an integrin $\alpha_4\beta_1$ inhibitor for long time," he said. "You would have to think about the risk-benefit profile of that therapeutic option very carefully."

Kang agreed the risk of PML was a concern. "This might mean VCAM-1 is a safer target," he said.

Brake tests

Kang said his team's ongoing work includes examining the pathways up- and downstream of VCAM-1/integrin α_4 signaling. The goal is to better understand the processes behind the formation of bone metastases and potentially identify additional targets that interrupt the process.

"We are also collaborating with companies to test their anti-integrin α_4 compounds because we think those might work better in our mouse models" than the research antibodies his team used, Kang said. He declined to disclose the companies.

Integrin α_4 inhibitors in the clinic for MS include **GlaxoSmithKline plc's** Fingertegrast (SB-683699) and Elan's ELND002. The former is in Phase II testing and the latter is in Phase I/II trials.

Princeton has filed a patent application covering the findings reported in the paper, and the IP is available for licensing or partnering, Kang said.

Elan, Biogen Idec and GSK declined to comment on the team's findings.

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2. Kang, Y. *et al. Cancer Cell* 3, 537–549 (2003)
3. Haas, M. *SciBX* 3(18); doi:10.1038/scibx.2010.544

COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.

The Cancer Institute of New Jersey, New Brunswick, N.J.

Elan Corp. plc (NYSE:ELN), Dublin, Ireland

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.

Howard Hughes Medical Institute, Chevy Chase, Md.

Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Princeton University, Princeton, N.J.

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