Countering chemo-induced metastasis

By Michael J. Haas, Senior Writer

Two independent research teams have elucidated mechanisms by which cisplatin and paclitaxel can promote lung metastases in mice, providing links between the chemotherapies and the known metastatic effects of upregulating VEGF receptor 1 and matrix metalloproteinase 9, which acts downstream of the receptor.[1,2]

The findings could create additional uses for inhibitors of VEGF receptor 1 (FLT1; VEGFR-1) and revive work on matrix metalloproteinase (MMP) inhibitors, a class of beleaguered cancer compounds that has all but disappeared from development.

Multiple preclinical studies have shown that chemotherapy can promote metastatic tumor growth,[3–5] and a handful of clinical trials have suggested chemotherapy can accelerate the growth of cancer cells.[6,7]

In the past five years, researchers have shown that chemotherapy can increase levels of circulating endothelial progenitor cells that contribute to tumor regrowth and angiogenesis,[8,9] and they have shown that these progenitor cells correlated with tumor progression and poor survival in patients.[10]

Despite the known link, the molecular mechanisms underlying chemotherapy's tumorigenic and prometastatic effects remained unclear. Part of the reason was that most studies had tracked the drugs' tumor-promoting effects in animals that already had tumors. This made it difficult to tease apart the specific mechanisms of drug-induced tumor growth from the drug's tumor-killing cytotoxicity.

To isolate the effects of chemotherapy on metastatic tumor growth, a team from **University Medical Center Utrecht** and, independently, an Israeli-Italian group both used an approach whereby they treated normal mice with a chemotherapeutic, allowed the drug to clear from circulation and then injected the mice with tumor cell lines. The stepwise process permitted the teams to model what happens in patients whose primary tumors have been decreased to undetectable levels by chemotherapy but who have aggressive or metastatic tumor cells in circulation.

The UMC Utrecht group pretreated mice with cisplatin and injected the animals with colon cancer or melanoma cell lines. Those animals developed more lung metastases than control mice pretreated with vehicle. The team also found higher expression of *Vegfr-1* on lung endothelial cells in cisplatin-pretreated mice than on endothelial cells in other organs or on cells from vehicle-pretreated controls.

In mice injected with the cancer cell lines, pretreatment with cisplatin and an antibody against Vegfr-1 resulted in fewer lung metastases than pretreatment with cisplatin, the antibody or vehicle monotherapy.

Collectively, the findings showed that chemotherapy can promote the growth of metastatic tumors by upregulating VEGFR-1 on endothelial cells, thus providing a rationale for adding VEGFR-1 inhibitors to chemotherapy regimens, the team wrote in its *Cancer Research* report.

The team leader was Emile Voest, head of the department of medical oncology at UMC Utrecht.

The two most advanced VEGFR-1 inhibitors are Votrient pazopanib and axitinib.

Votrient, a broad-spectrum inhibitor of VEGFR-1 and other tyrosine kinases from **GlaxoSmithKline plc**, is approved to treat renal cancer and in Phase III testing to treat ovarian and breast cancers and sarcoma.

Axitinib (AG-013736), an inhibitor of VEGFR-1, VEGFR-2 (KDR/Flk-1) and VEGFR-3 (FLT4) from **Pfizer Inc.**, is in registration to treat renal cancer and Phase II testing to treat breast, gastrointestinal, lung and thyroid cancers.

Enter the matrix

Concurrent to the Dutch team's work, researchers from **Technion–Israel Institute of Technology**, **Emek Medical Center** and the **European Institute of Oncology** began to connect the dots between chemotherapy, tumor metastasis and MMP9 expression on bone marrow–derived cells.

The Israeli-Italian team took note of other groups' preclinical studies showing that mouse bone marrow–derived cells expressing *Vegfr-1* contributed to tumor metastasis[11] and that MMP9 contributed to tumorigenesis.[12] Taken together with its own studies of the role of bone marrow–derived cells in tumor regrowth,[8,9] the team hypothesized that chemotherapy induced MMP9 expression on those cells to promote metastatic tumor growth.

To investigate this hypothesis, first the researchers collected plasma and bone marrow–derived cells from normal mice treated with paclitaxel or vehicle. Plasma from paclitaxel-treated mice increased the *in vitro* migration and invasive activity of a normal human endothelial cell line, two human breast cancer cell lines and two mouse cancer cell lines compared with plasma from vehicle-treated controls.

The team also found that plasma from paclitaxel-treated mice increased *MMP9* expression in a human breast cancer cell line. Moreover, bone marrow–derived cells from those mice expressed higher levels of *Mmp9* than cells from vehicle-treated mice.

In mice pretreated with paclitaxel, compared with vehicle-pretreated controls, a lung carcinoma cell line increased the number of lung metastases and decreased survival. Pretreatment with paclitaxel and an MMP9 inhibitor reduced lung metastases and improved survival compared with pretreatment using paclitaxel monotherapy.

Data were published in *Cancer Research*.

Collectively, the findings suggest chemotherapy may have protumorigenic side effects that upregulate host factors such as

MMP9 to decrease its effectiveness in some patients, team leader Yuval Shaked, a senior lecturer in molecular pharmacology at Technion, told *SciBX*.

Such protumorigenic effects could explain why some clinical studies have shown that chemotherapy increased progression-free survival but not overall survival, he said. "Our results suggest that we may be able to enhance the efficacy of chemotherapy by identifying and blocking the targets that have protumorigenic effects."

However, he cautioned that the results do not mean chemotherapy was ineffective or always promoted metastatic tumor growth. For instance, the team also showed that in a mouse model of spontaneous lung metastasis, surgical resection of mammary tumors followed by three rounds of paclitaxel increased survival compared with resection followed by vehicle. Thus, "Protumorigenic effects of chemotherapy on metastases might also be blocked by multiple rounds of therapy, as is done in the clinic," he said.

Nevertheless, "Clearly these two *Cancer Research* papers are complementary in that they both suggest that chemotherapy can promote metastatic tumor growth under some circumstances," Shaked said.

"I hope these papers will spur further studies that address the mechanisms of chemotherapy-induced metastasis and consider combination therapies in light of those mechanisms, because clinicians aren't going to stop using paclitaxel and other chemotherapies," said Sara Courtneidge, professor and director of the Tumor Microenvironment Program and director of academic affairs at **Sanford-Burnham Medical Research Institute**.

John Ebos, assistant professor of oncology in the genitourinary section of **Roswell Park Cancer Institute**'s Department of Medicine, agreed. "The findings in these two very important papers may suggest explanations for why the efficacy of chemotherapy is sometimes limited in patients and, more importantly, what can be done to improve that efficacy."

Ebos was coauthor of an April paper that included a review of chemotherapy's known prometastatic effects.[13]

"The bottom line is that the majority of current cancer treatments were developed to target some aspect of primary tumor growth, not metastasis—which is what kills the vast majority of cancer patients," said Irwin Gelman, professor of oncology in Roswell Park's Department of Cancer Genetics. "The holy grail is development of metastasis-targeting drugs based on sound molecular data."

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> ***—Yuval Shaked, Technion–Israel Institute of Technology***

Convergence and separation

The development of compounds that specifically counter chemotherapy's prometastatic effects will depend on how many additional mechanisms are involved and whether those mechanisms share targets or pathways.

Courtneidge said MMP9 could be one such shared target. "MMP9 upregulation was the focus of one team's paper and cited in the other as a known downstream effect of *VEGFR-1* expression," she said.

She added that *MMP* expression is also a feature of invadopodia—foot-like cell membrane protrusions that enable tumor cells to become invasive. "The ability of invadopodia to degrade the extracellular matrix is largely due to MMPs," she said. In July, a team co-led by Courtneidge reported that paclitaxel promoted the formation of invadopodia in a panel of human cancer cell lines.[14,15]

Thus, Courtneidge suggested MMP inhibitors could be reexamined for use with chemotherapies to treat cancer.

In the late 1990s and early 2000s, several companies—including **Bayer AG**, **Bristol-Myers Squibb Co.**, British Biotech plc (now **Vernalis plc**) and Warner-Lambert Co. (now part of Pfizer)—terminated development of MMP inhibitors to treat cancer when Phase II to Phase III trials showed dose-limiting toxicities and a lack of efficacy.

"Additional preclinical studies might indicate which cancers could be treated by a combination of chemotherapy and MMP inhibitors," Courtneidge said.

She said that to tease out the full range of mechanisms by which chemotherapies can promote metastatic tumor growth, "the way forward is for researchers to study cell lines and primary tumor models that are resistant to those drugs" and thus separate their prometastatic effects from their cytotoxicity.

She also wanted to know what plasma components from the chemotherapy-treated mice were involved in promoting *MMP9* expression and metastatic tumor growth.

Shaked said his team is already studying several plasma components that might account for those effects.

He added, "We are also in the process of establishing a consortium to further study the protumorigenic effects of cytotoxic drugs and to identify new targets for therapy. I would definitely welcome collaborators on this project." He said the consortium so far includes undisclosed academic groups and companies from Sweden, Greece, France, Germany, Ireland, Italy and Israel.

The findings in the paper are unpatented, "but we are thinking of patenting the new factors once we have identified them," he said.

The Dutch team's findings also are unpatented. Voest declined to disclose his team's ongoing work.

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