Cancer's long-distance plan

By Tim Fulmer, Senior Writer

Researchers at the **Massachusetts Institute of Technology** have uncovered a new role for the endocrine factor osteopontin as a 'long-distance' modulator of tumor growth. Researchers polled by *SciBX* felt that the findings not only opened new avenues for the design of tumor-targeting strategies but also had important implications for current surgical and therapeutic procedures aimed at tumor shrinkage.

Cancer researchers have long appreciated that cells in the immediate vicinity of tumors—the tumor microenvironment—support tumor growth and are therefore potential therapeutic targets.^{1,2} However, the paper published in *Cell* by Robert Weinberg, professor of biology at MIT, and colleagues now suggests a potentially equally important tumor-supportive role for cells further away from a tumor.

Osteopontin (secreted phosphoprotein 1), the systemic endocrine factor that potentially mediates such long-distance interactions, could be a new cancer target. Osteopontin had previously been shown to be overexpressed in tumors and had been suggested to be involved in metastasis, but a causal connection had not yet been established.³

Weinberg and colleagues developed a mouse model for identifying systemic mechanisms that could be targeted to prevent or slow progression of solid tumors.⁴ They implanted two different human mammary epithelial tumor cell lines on opposite sides of the same nude mouse. One of these cell lines, the 'instigator', resembled aggressively invasive adenocarcinomas often found in breast cancer. The other cell line, the 'responder', mimicked much slower growing indolent tumors.

Initial experiments revealed that responder cells implanted opposite instigator cells had higher growth kinetics and tumor mass than responder cells implanted opposite Matrigel control matrix.

Moreover, conditioned media from instigator tumor cells did not alter the growth of responding cells *in vitro*, suggesting that growth factors derived from the instigator tumor were not directly responsible for increasing the growth of responder tumors.

The authors speculated that "the instigating tumors might mobilize stromal cell precursors from the bone marrow into circulation, thereby making them available for recruitment by responding tumors."

A second set of experiments in the same models revealed that responder tumors opposite instigator tumors incorporated sig-

nificantly more host bone marrow cells into their stroma than did responders opposite Matrigel control matrix ($p=0.039$).

A plasma analysis of mice bearing only instigator tumors revealed that circulating levels of the glycoprotein osteopontin were threefold higher than those seen in mice with only responder tumors. Circulating levels of 80 other cytokines were not significantly altered between the two groups.

The researchers thus suggested that osteopontin released by instigator tumors might be responsible for recruiting bone marrow cells to responder tumor cells. In the same mouse models, they found that osteopontin-deficient instigator tumors induced less bone marrow cell recruitment and tumor growth in responder tumors than was seen in responder tumors opposite osteopontin-expressing instigator tumors.

In conclusion, the authors proposed a "Model of Systemic Instigation," whereby primary tumors secrete soluble osteopontin, which stepwise leads to activation of bone marrow cells, release of stromal precursor cells into systemic circulation and instigation of outgrowth at previously indolent distal tumors.

Weinberg told *SciBX* that his lab now plans to use the same mouse models to study the effects of antiosteopontin antibodies on tumor instigation, as well as to identify additional systemic factors that potentially cooperate with osteopontin to promote outgrowth of distal, secondary tumors.

"The *Cell* paper illustrates the critical importance of *in vivo* context to our understanding of cancer. In particular, the researchers have shown that primary tumors can co-opt host systemic factors to promote the growth of tumors that might otherwise remain latent. Thus, at least in this case, host context provides the necessary means to cancer progression," said Murray Robinson, SVP of oncology for **Aveo Pharmaceuticals Inc.**

New role for old player

Researchers told *SciBX* that the *Cell* paper had important implications for tumor-targeting strategies. Moreover, they said the findings throw some doubt on surgical and therapeutic procedures aimed at tumor shrinkage.

In healthy individuals, the glycoprotein osteopontin is produced by multiple cell types, including epithelial cells, bone cells, macrophages and lymphocytes. The protein interacts with a variety of cell-surface receptors, such as integrins and CD44, to stimulate cell adhesion, migration and intracellular signaling.⁵

At the same time, elevated osteopontin has correlated with the metastatic potential of multiple cancer cell lines.^{6,7} Moreover, abnormally elevated plasma levels of osteopontin have been observed in some patients with metastatic renal cell carcinoma and breast cancer.^{8,9}

"The *Cell* paper is an impressive piece of work because it does more than suggest a correlative relation between osteopontin levels and cancer," said Dennis France, VP of molecular oncology at **ArQule Inc.** "Indeed, Weinberg and colleagues have perhaps uncov-

ered a causal role for osteopontin in modulating tumor-stroma interactions.”

ArQule's lead compound is ARQ 197, a small molecule inhibitor of c-Met receptor tyrosine kinase in Phase II trials to treat Microphthalmia Transcription Factor (MiT) tumors and Tarceva erlotinib-resistant non-small cell lung cancer (NSCLC). Tarceva, a small molecule inhibitor of epidermal growth factor receptor, is marketed by **Genentech Inc.**, **OSI Pharmaceuticals Inc.** and **Roche**.

The *Cell* paper shows for the first time that osteopontin promotes tumor outgrowth by influencing the contribution of bone marrow-derived cells to the tumor stroma, noted Natalie Direkze, a researcher in the histopathology unit of the **Cancer Research UK**, London Research Institute. Direkze and colleagues have shown that bone marrow cells contribute to fibroblast populations in the tumor stroma of a mouse model of pancreatic cancer.¹⁰

According to David Young, president and CEO of **Arius Research Inc.**, “the paper's model, whereby primary tumors enhance the growth of secondary tumors, stands in contrast to much of our current understanding of how metastasis works. Multiple studies have established that resecting primary tumors can lead to metastasis because intact primary tumors release factors that suppress tumor outgrowth at secondary sites. The *Cell* paper now suggests that primary tumors can also release factors that drive growth of secondary tumors.”

Young added: “Another somewhat counterintuitive implication of the paper is that a comparatively small instigating tumor can perhaps drive the growth of a larger, previously indolent secondary tumor. Thus, surgically removing the larger tumor may, at least in this case, have little curative effect—which is certainly surprising.”

Arius plans to start a Phase I cancer trial of its AR001 anti-CD44 antibody at year end.

Inhibiting a generalist

Andrew Mazar, CSO of **Attenuon LLC**, said a straightforward approach to antagonizing osteopontin would be an mAb.

“I would try to come up with a panel of antibodies that targeted the various domains and interactions of osteopontin and then use these to figure out which interaction was most important to the anti-tumor activity of osteopontin,” he said. “The high degree of homology between mouse and human osteopontin should make this doable in mice.”

Nevertheless, Mazar and other researchers polled by *SciBX* suggested that inhibiting osteopontin in cancer could bump into the usual challenges associated with targeting a molecule that interacts with a variety of receptors and cell types.

Key issues include determining which of these interactions are important for tumor-promoting activity and whether blocking a single interaction or multiple interactions is necessary to show clinically meaningful activity, Mazar said.

Attenuon's ATN-224, a small molecule inhibitor of superoxide dismutase 1 (SOD1) that disrupts multiple kinase signaling pathways, is in separate Phase II trials to treat multiple myeloma (MM), advanced melanoma, prostate cancer and breast cancer.

Young noted that any therapeutic strategy must take into account that tumors have alternative means of replenishing their stroma.

“An antiosteopontin antibody or a soluble osteopontin decoy

receptor could both be effective at blocking recruitment of bone marrow-derived cells to tumor stroma,” he said. “However, these strategies may have little or no effect on stromal precursors that are in circulation or on stromal cells that are already proliferating within the tumor microenvironment.”

Moreover, Aveo's Robinson noted that “tumors have considerable genetic and epigenetic heterogeneity. This, in turn, makes it unlikely that a single therapeutic agent will be equally efficacious against all tumors, even of the same tissue type. Therefore, in the case of osteopontin antagonists, we ought to look for antitumor efficacy across a whole population of, say, breast cancer tumors rather than just the two types used in the paper.”

Robinson added: “Such a population would better represent the heterogeneity that characterizes breast cancer in patients and also help differentiate breast cancers where osteopontin is perhaps worth targeting from those types that may be more resistant to osteopontin [targeting].”

Aveo's AV-951, a small molecule inhibitor of VEGF receptor 1, VEGF receptor 2 and VEGF receptor 3, is in Phase II trials to treat metastatic renal cell carcinoma (RCC).

Modifying the model

While the therapeutic issues involved in inhibiting osteopontin are sorted out, the models in the *Cell* paper also could be modified to investigate osteopontin–bone marrow and bone marrow–tumor stroma interactions.

France pointed out that the models in the paper were variations on standard human tumor xenograft models and therefore were immunocompromised. “Such models likely don't have a fully responsive bone marrow that supports production of all hematopoietic cells—which means these models may not be ideal for studying osteopontin–bone marrow interactions,” he said. “Consequently, I would like to see some of this work repeated in a nonimmunocompromised syngeneic rodent model.”

Young, however, told *SciBX* that engineering knockouts into the same immunocompromised models could provide insights into osteopontin–bone marrow interactions.

“First of all, knockout of osteopontin or the osteopontin receptor in bone marrow could reveal whether other unidentified factors also mobilize stromal precursors to secondary tumors,” he said. “Secondly, knockout of hematopoietic precursor populations within the bone marrow could help elucidate the relative importance of these subpopulations for recruitment to distal tumor stroma.”

A third option, according to Peter Lassota, divisional VP of imaging biology and oncology at **Caliper Life Sciences Inc.**, would be to engineer the models to express tumor-specific or bone marrow-specific bioluminescent markers. This would allow for noninvasive monitoring of tumor growth in response to osteopontin knockout or osteopontin overexpression.

Caliper markets a range of transgenic mouse models that can be engineered to express the *luciferase* gene in a tissue-specific manner.

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