

## EMT Research Surges

# Epithelial-to-Mesenchymal Transition Is Important to Metastasis, But Questions Remain

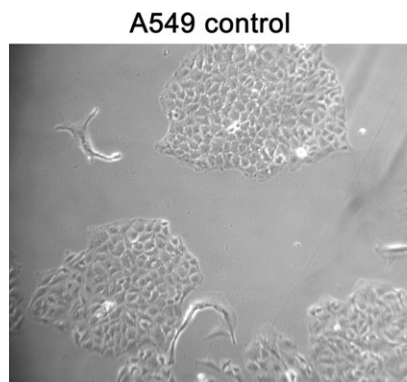
By Ken Garber

**H**ow tumors spread and kill their hosts remains a mystery. To metastasize, cancer cells must break many fundamental rules of normal cellular behavior: They detach from neighboring cells, move freely on their own, enter the bloodstream and survive there, and finally exit into new tissue and

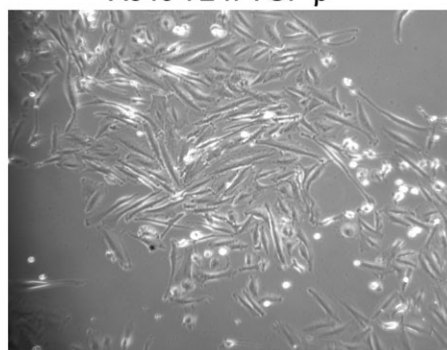
colonize it. The whole area of metastasis has been “one of unfathomable complexity,” MIT cancer researcher Robert Weinberg, Ph.D., said at the 2006 American Association for Cancer Research annual meeting.

It’s especially hard to conceive how epithelial tumors—about 90% of all cancers—could ever metastasize. Epithelial cells, which line body surfaces and cavities, are tightly zipped together and largely immobile. But now a potential mechanism for metastatic spread is gaining traction: the epithelial-to-mesenchymal transition (EMT). EMT is a normal process in embryonic development in which epithelial cells transform into mesenchymal cells, the highly mobile cells that give rise to bone, muscle, connective tissue, and blood vessels. Now researchers, including Weinberg, are proposing that cancer cells can hijack EMT to spread. If so, then metastasis is “much simpler than one had imagined,” Weinberg said.

Interest in the EMT idea “has grown exponentially in the last several years,” said Erik Thompson, Ph.D., of the University of Melbourne in Australia. The number of published reports on



A549 control



A549 72 h TGF-β

Spread formation: Following growth factor treatment, these tightly-bound epithelial lung cancer cells separated and transformed into spindle-shaped mesenchymal-like cells.

Venkateshwar Keshamouni, University of Michigan

EMT and cancer grew 12-fold between 2002 and 2007. EMT “is one of the things that everybody’s doing nowadays,” said Robert Cardiff, M.D., Ph.D., a cancer researcher at the University of California, Davis. “I can’t tell you how many papers I’m getting to review about EMT.”

The EMT idea has intuitive appeal and is increasingly popular, but it’s

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also controversial. How EMT occurs in cancer has not been well worked out. It is not even proven to happen at all in human tumors. Some clinical pathologists are skeptical of the idea. “I’m totally unconvinced,” said David Tarin, M.D., Ph.D., a pathologist and cancer researcher at the University of

California, San Diego. The EMT field now must capitalize on its growing popularity to answer fundamental questions about metastasis—and to silence the remaining skeptics.

## An Exploding Field

EMT was first recognized as such in 1982, when Gary Greenburg, Ph.D.,

then a student in the lab of Harvard cell biologist Elizabeth Hay, M.D., put epithelial tissue from chick embryos into collagen gel as a control for an experiment and watched in amazement as mesenchymal cells poured into the gel. The first cancer link came in the late 1980s, when Jean-Paul Thiery, Ph.D., of the Centre National de la Recherche Scientifique in France found that rat bladder carcinoma cells in culture could transform into invasive mesenchymal tumor cells and back. But no one knew how this amazing change took place.

Clues to explain EMT in cancer have gradually emerged. In 1991, “scatter factor,” a protein known to promote cancer cell movement and invasion, was found to be identical to hepatocyte growth factor, and since then other cellular regulators of EMT have been found. These are probably secreted by the surrounding connective tissue to trigger EMT. EMT results in loss of e-cadherin, the adhesion protein that helps form the zipperlike junctions between epithelial cells, and researchers have also identified other markers of EMT.

Two advances led directly to the current EMT boom in cancer research. First, researchers found links between EMT and fibrosis, a form of scarring that follows injury. Fibrosis can lead to kidney failure, and in 2002 Eric Neilson, M.D., and his group at Vanderbilt University in Nashville showed that many of the fibrosis-causing cells in a mouse model of kidney fibrosis arise from epithelial cells through EMT. EMT links to fibrosis of the liver and heart have since been reported. Fibrosis and cancer are related in the sense that a tumor mimics a wound that doesn't heal, so some of the same mechanisms, including EMT, probably contribute to both disorders.

The other major advance has been the identification of transcription factors—proteins that regulate the expression of genes—triggering EMT in tumors. In 2002, Angela Nieto, Ph.D., then at the Cajal Institute in Madrid showed that expression of Snail, a transcription factor crucial for EMT in embryonic development, correlated closely with invasiveness in primary human tumor samples. Two years later, Weinberg's group showed that Twist, another transcription factor important in development, is highly expressed in tumor cell lines and invasive human tumors and promotes EMT as well. At least three other EMT-implicated transcription factors have been linked to cancer. "There are multiple ways that cells can trigger, or carry out, an EMT," Thompson said.

### Persuading the Pathologists

But EMT's very legitimacy was challenged in 2005 with the publication in *Cancer Research* of a negative review

by Tarin. He dismissed EMT as a "fallacy" and called it "an unfortunate misconception resulting from erroneous interpretation of pathologic data." Tarin's views haven't changed. Invited to an upcoming EMT meeting, he has declined, saying that he doesn't want to spend 3 days "listening to people talking about a topic that I think doesn't exist."

Tarin makes four basic arguments against EMT: Pathologists haven't seen EMT in tumor samples, and it's unlikely that they'd miss it if it were happening often. There is no convincing evidence for EMT in live animals with cancer. Markers used in EMT studies aren't specific for mesenchymal cells. Such markers are unreliable anyway because tumors are genetically unstable.

"When cells become malignant, they can express almost any gene you would like to name," Tarin said. "That does not indicate that the thing has changed its lineage and its capabilities to do the kind of job that mesenchyme is supposed to do: make cartilage, bone, blood vessels, tendon." The kind of job that mesenchyme is supposed to do: make cartilage, bone, blood vessels, tendon ... I defy [EMT proponents] to show that cells which have undergone EMT can then make tendon or bone."

EMT researchers haven't done that, but they argue that cancer cells probably undergo only a partial EMT, retaining some epithelial qualities. "It's more of an intermediate thing," Thompson said. "And now that people are looking for that, they're finding it." And EMT proponents agree that better markers are needed. "The problem here is that there is no specific marker for a mesenchymal cell that derives from a

cancer cell," said Raghu Kalluri, Ph.D., a fibrosis and cancer researcher at Harvard Medical School. "I think the animal studies are really going to clear this up [and] maybe come up with new markers."

The most cited animal study to date is from Neilson's group, published in 2003. Neilson extracted cells from transgenic mouse tumors expressing a fluorescent protein linked to the EMT marker fibroblast-specific protein 1 (FSP1). Tracking tumor spread by fluorescence, Neilson showed that mice injected with these cells developed metastases more often than non-FSP1-injected mice, and the metastases disappeared when FSP1 was blocked. As perhaps the first study to demonstrate cancer EMT in vivo, it was "a revolutionary paper," Thompson said.



David Tarin, M.D., Ph.D.

But Tarin dismissed the results, arguing that FSP1 is not a reliable marker for EMT or for fibroblasts, the mesenchymal wound-healing cells that Neilson's EMT tumor cells resembled. "We don't have evidence from comprehensive studies that [FSP1] is absolutely characteristic of fibroblasts," he said. Neilson, in an e-mail, responded that he's "pretty comfortable" that FSP1 is a reliable marker for fibroblasts, cells undergoing EMT to become fibroblasts, or invasive cancer cells.

As for why pathologists haven't seen EMT in their human tumor biopsies, Kalluri points out that it's hard to distinguish cancer cells that have undergone EMT from mesenchymal cells in the surrounding connective tissue, and pathologists may be mistaking one for the other. He also pointed out that mesenchymal-like tumor cells undergo a reverse conversion, a mesenchymal-to-epithelial transition (MET). "By the time they reach the metastatic site, they're probably converting back [to epithelial

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EMT, continued from page 233

cells] again,” Kalluri said. However, direct evidence for MET is limited.

But tumor cells don't need to convert from one cell type to another to explain metastasis, Tarin argued—genetic instability can account for it. EMT proponents counter that it's far more likely that tumors co-opt an existing “hard wired” mechanism than create one themselves through random mutation.

“To a certain extent, both parties are correct,” Kalluri said. “I think there is EMT going on, and I think the molecular biologists have to find ways to convince pathologists that it actually happens.”

Not all pathologists dismiss EMT. One believer is Cardiff, who works on both transgenic mouse and human tumors. For example, he cites work by Lewis Chodosh, M.D., Ph.D., and his group at the University of Pennsylvania, showing increased Snail activity in recurrent transgenic mouse mammary tumors, along with expression of EMT markers. Adding Snail to primary human tumor cells also caused EMT. (High Snail expression predicted lower relapse-free survival for breast cancer.) Chodosh's report was “direct experimental proof that Snail promotes recurrence and promotes EMT,” Cardiff said.

Then why haven't clinical pathologists reported EMT? “We don't quite know what to look for,” Cardiff said, adding that human EMT tumors are going to be hard to spot until researchers develop specific identifiers. “Rather than making it a closed case, I think the pathologists need to look [for EMT], and they need to look with informed eyes.”

### Exploiting EMT

Academic laboratories and companies are already developing ways to exploit EMT to treat cancer. OSI Pharmaceuticals of Melville, N.Y., hopes to eventually combine its lung and pancreatic cancer drug erlotinib (Tarceva) with a drug that will target epithelial tumors that may have undergone EMT. (OSI studies have shown that tumors expressing markers of EMT are less sensitive to erlotinib treatment.) EMT is a general phenomenon of aggressive tumors, said OSI senior vice president for oncology David Epstein, Ph.D., and not just an element of metastasis. “EMT is really a part of the normal development of cancer,” he said. OSI is now refining a marker set to identify tumors that have undergone EMT to possibly stratify patients for treatment with different drug combinations. And it has launched a collaboration with Aveo Pharmaceuticals in Cambridge, Mass., to develop new therapies to block EMT initiation or to kill cancer cells that have undergone EMT.

The EMT idea is expanding in other ways. For example, researchers are probing the reverse transition, MET. In 2006, Thompson and Elizabeth Williams, Ph.D., of the Monash Institute of Medical Research in Melbourne reported a mesenchymal-like bladder cancer cell line that spontaneously reverted to an epithelial form, suggesting MET. By switching the cells between the two forms, they showed in mice that the mesenchymal-type cells were better at escaping from the primary tumor, and the epithelial-like cells were better at colonizing distant sites, consistent with the EMT–MET concept. Another emerging concept is EndoMT, or the endothelial-to-mesenchymal transition.

Kalluri's group has demonstrated EndoMT in heart fibrosis and last year showed that many activated fibroblasts surrounding tumors originate as endothelial cells, probably from sprouting blood vessels.

Another theme is cancer stem cells, which have signaling pathways in common with mesenchymal-like cancer cells. Whether that means stem cells arise through EMT, or vice versa, nobody knows. “Maybe all tumor cells have the ability to undergo EMT, but only tumor stem cells can undergo EMT and have the ability to grow in distant sites,” speculated EMT researcher Venkateshwar Keshamouni, Ph.D., of the University of Michigan. “These are all open questions.”

Weinberg is enthusiastic about EMT, he wrote in an e-mail, although many key questions remain unanswered.



Raghu Kalluri, Ph.D.

For example, no one knows how widespread EMT and MET are in human cancer or what internal and external signals trigger EMT in the cancer cell.

The EMT field is trying to cope with its sudden popularity while searching for legitimacy. “Part of the field is built on speculation,” Cardiff said. “People...have a penchant for over-interpreting results, because it's fashionable. So you've got to be careful.” So far, EMT has been demonstrated in only a few cell and animal models of cancer. “There's an ever-growing amount of supportive evidence [for clinical EMT],” Thompson said, “although the definitive milestone paper has yet to be published.”

But the EMT–cancer connection is now a given to many researchers. “The phenomenon is quite well understood and accepted,” Kalluri said. “But now we need to ... nail down some of the mechanisms that would lead these cells to behave that way.”

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