continuing to smoke put them at high risk for lung cancer and other SRDs, and that many of them intended to quit smoking when they started the trial, most did not quit. The lung screening test did not appear to affect their risk perceptions and was not in and of itself a cue for changing their smoking behavior. (See infographic on page 1 for more key findings.)

In ongoing work, Dr. Park and Inga Lennes, MD, medical oncology director of Mass General's Lung Screening Clinic and director of quality at Mass General Cancer Center, are administering a modified questionnaire at the Lung Screening Clinic.

A NEED FOR INTERVENTION

"Lung screening may provide a teachable moment, but participants do not teach themselves," concludes Dr. Park. Recently, she and her team examined the effects of physicians' interventions with smokers following lung screening. The results were presented at the 2013 American Society of Clinical Oncology meeting.

If physicians simply ask these smokers about smoking and advise them to quit, they are not likely to do so. However, if physicians actually assist patients—by giving them a counseling referral, a stop-smoking medication prescription or by following up—this increases the likelihood that a patient will quit.

Drs. Park and Lennes plan to develop a computerized risk-based personalized intervention to guide clinicians. "Even a brief intervention," says Dr. Park, "whether by a physician, nurse or counselor, promotes smoking cessation."

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Circulating Tumor Cells Transition From State to State

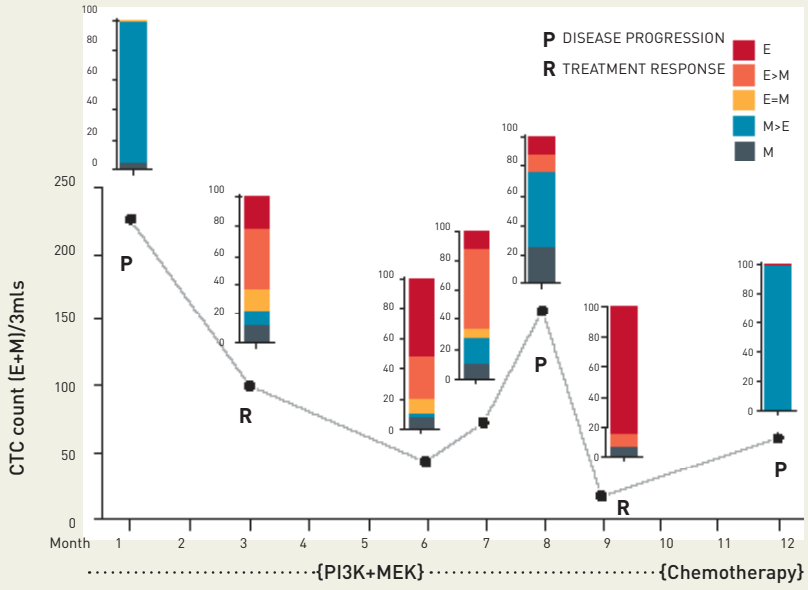
Can epithelial-mesenchymal transition status predict the aggressiveness of human cancers?

Cancer cells reinstate many processes observed mainly in embryonic cells and adult stem cells, including undergoing an epithelial-mesenchymal transition (EMT). In EMT, stationary epithelial cells become mesenchymal, gaining motility, invasiveness and resistance to cell death. In laboratory studies, cancer cells that undergo EMT are more resistant to drug treatment, more invasive and more likely to metastasize than cancers with an epithelial phenotype.

The correlation between EMT and cancer aggressiveness has been well studied in both ex vivo tumors and mice, particularly in breast cancer, which is an epithelial cancer. To investigate EMT in human cancers, Shyamala Maheswaran, PhD, scientific director of the Center for Cancer Risk Assessment at Massachusetts General Hospital Cancer Center, and Daniel A. Haber, MD, PhD, director of the Cancer Center, developed a new method to reliably identify the epithelial and mesenchymal phenotypes in breast cancers. They applied this method to the analysis of circulating tumor cells (CTCs), which may provide a window into the EMT process.[1] *(continued on page 4)*

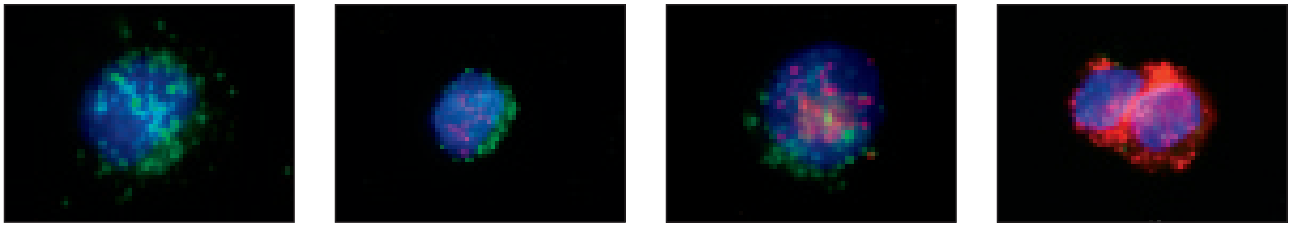
CTCs from a Breast Cancer Patient

This index patient underwent one round of treatment with P13K + MEK inhibition, relapsed, underwent a second round of treatment with chemotherapy, and relapsed again, over the course of 12 months. Shown below is the plot of the epithelial (E) and mesenchymal (M) circulating tumor cells (CTCs) isolated from the patient at different points in time. Predominantly epithelial states characterize periods of treatment response (R), while predominantly mesenchymal states characterize periods of disease progression (P).



A New View of Cells in Transition

A new technology, viewRNA, visualizes the RNA transcripts marking epithelial (in green) and mesenchymal (in red) phenotypes in breast cancer patients' circulating tumor cells (CTCs).



(continued from page 3) The team confirmed that EMT occurs in patients with breast cancer and correlates with cancer aggressiveness, and they discovered two unpredicted twists: EMT is reversible and dynamic, and mesenchymal CTCs can travel in clusters. The study appeared in the Feb. 1, 2013 issue of *Science*.[2]

MIXED PHENOTYPES

To differentiate the epithelial (E) and mesenchymal (M) states in primary breast tumors, the researchers used RNA transcripts to highlight multiple markers instead of the traditional one marker for each state. The tumors did not just have an either/or phenotype, but also exhibited both E+ and M+ markers to varying degrees. The more invasive breast cancer subtype, triple negative breast cancer, had more M+ markers than did hormone positive or HER2 positive breast cancers, but altogether, mesenchymal cells were very rare in primary breast tumors.

The team used a microfluidic device developed by Mehmet Toner, PhD, director of the Center for Bioengineering in Medicine and BioMEMS laboratory at Mass General, to capture CTCs in patients' bloodstreams. The CTCs had a much higher percentage of mixed and M+ phenotypes than cells in the primary tumors. These findings are consistent with the theory that CTCs carry the more motile cells that are the seeds for metastasis.

Surprisingly, some of the mesenchymal CTCs formed clusters ranging from 4 to more than 50 cells. The researchers had not predicted the cluster phenomenon because the prevailing theory holds that mesenchymal cells are solitary. The Haber Lab is investigating whether CTCs break off from the tumor as clusters or whether mesenchymal CTCs form clusters in the bloodstream, in interaction with platelets.

EMT DYNAMICS AND REVERSIBILITY

In other experiments, the researchers analyzed CTCs in 10 breast cancer patients before and after targeted therapy. The mesenchymal phenotype decreased in patients who responded to therapy but increased in patients whose disease progressed during therapy. Thus cancer cells that had acquired mesenchymal features disappeared as effective drugs were administered, but they tended to reappear along with emerging drug resistance.

In a follow-up, the researchers analyzed seven serial samples from one breast cancer patient. The M+ phenotype in CTCs decreased when the patient was responding to therapy, increased when the patient relapsed, decreased again under a new therapy and increased when the patient relapsed again.

BROAD IMPLICATIONS

The researchers are now analyzing RNA in CTCs to determine how gene activity changes with EMT dynamics. "The better our new drugs become, the more we need to know what they do to the cancer, and how cancers adapt and become resistant," Dr. Haber says. "This technique gives us a whole new window for drug testing."

The technology enabling this recent study still requires scale-up, standardization and commercialization. Still, the researchers predict that analyzing the EMT in CTCs will become an important tool for managing cancer.

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[2] Yu, Min, Aditya Bardia, Ben S Wittner, Shannon L Stott, Malgorzata E Smas, David T Ting, Steven J Isakoff, et al. "Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition." *Science* 339, no. 6119 (Feb. 1, 2013): 580–584.