Novel detection method unmasks circulating breast cancer cells

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Circulating metastatic breast cancer cells can lose their epithelial receptors, a process that enables them to travel through the bloodstream undetected, according to research from The University of Texas M. D. Anderson Cancer Center.

The findings were presented today at the CTRC-AACR San Antonio Breast Cancer Symposium.

Levels of these circulating tumor cells (CTCs) - which are shed from a primary tumor or its metastases - have been used to monitor and tailor cancer therapy and to predict a patient's prognosis. CTCs that have undergone epithelial-mesenchymal transition (EMT), however, evade current detection methods and lose their traditional prognostic and therapeutic value. Those cancer cells also become more resistant to chemotherapy and radiation therapy. Finding a reliable method to detect these stealth breast cancer cells may reveal additional therapeutic targets that could help eradicate micrometastatic disease in patients with breast cancer or other epithelial tumors.

EMT and the Invasion-Metastasis Cascade

EMT is a process in which cancer cells undergo transdifferentiation (transformation into a different type of cell). "The carcinoma cells activate a transdifferentiation program in order to acquire the ability to execute the multiple steps necessary for the invasion-metastasis

cascade," said the study's first author Michal Mego, M.D., Ph.D., formerly a fellow at M. D. Anderson. "During EMT, epithelial cells acquire a mesenchymal appearance with increased motility and invasiveness."

The researchers hypothesized that these changes render the EMT-CTCs undetectable by current detection assays, such as CellSearch (Veridex). The cells' acquired resistance to chemotherapy and radiotherapy also suggested that EMT-CTCs are tumor-initiating cells and are responsible for tumor dissemination. Moreover, the researchers had found subgroups of high-risk patients with brain metastases, triple receptor-negative disease, or inflammatory breast cancer whose blood tests did not reveal elevated levels of CTCs, further supporting their hypothesis.

Detecting CTCs Through EMT Gene Expression

The researchers then set out to develop a detection method that could identify EMT-CTCs in the peripheral blood of breast cancer patients. They took approximately 5 mL of peripheral blood from 27 patients ranging in age from 34 - 72 years, with a median age of 54. Sixteen of the women had metastatic disease, 19 had inflammatory breast cancer, and 12 had primary, non-inflammatory breast cancer.

"Using magnetic beads coated with monoclonal antibodies capable of capturing the majority of hematopoietic cells in peripheral blood, we obtained a fraction of cells enriched for CTCs," said Mego, who is now a scientist at the National Cancer Institute in the Slovak Republic. "Next we isolated RNA from these cells to detect genes that are involved in epithelial-mesenchymal transition, using molecular biology technology, such as the polymerase chain reaction."

Five EMT genes were identified: TWIST1, SNAIL1, SLUG, ZEB1, and FOXC2. At least one of these genes was over-expressed in 21 percent of

the patients. Over-expression of EMT genes was more common among women with triple receptor-negative breast cancer than among those without this high-risk signature. The researchers found no correlation between EMT gene expression and CTC count as measured by CellSearch or the carcinoma-associated antigen known as Ep-CAM (epithelial cell adhesion molecule).

"We found that current CTC detection methods underestimate the most important subpopulation of CTCs involved in tumor dissemination-those with tumor-initiating properties," said James Reuben, Ph.D., professor in M. D. Anderson's Department of Hematopathology, the study's senior author. "A novel detection method such as ours that is capable of detecting CTCs after EMT could add important new prognostic information and could be useful for monitoring treatment efficacy in real time."

The M. D. Anderson and the Slovak National Cancer Institute teams have initiated a confirmatory study among patients with metastatic breast cancer, prostate cancer, or colon cancer. They also have initiated studies designed to identify therapeutic targets on EMT-CTCs. In addition to Mego and Reuben, other authors on the M. D. Anderson study include: Massimo Cristofanilli, M.D., Eleni Andreopoulou, M.D., and Summer Jackson, all of the Department of Breast Medical Oncology; Hui Gao, Ph.D. Changping Li, M.D., Sanda Tin, M.D. and Evan Cohen, all of the Department of Hematopathology; and Sendurai Mani, Ph.D., Department of Molecular Pathology.

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