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Previews

Tumor Dissemination: An EMT Affair

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A recent paper reports that circulating tumor cells (CTCs) from metastatic breast cancer patients exhibit heterogeneous epithelial and mesenchymal phenotypes and that CTCs display higher frequencies of partial or full-blown mesenchymal phenotype than carcinoma cells within primary tumors. Mesenchymal-like CTCs are also elevated in patients who are refractory to therapy.

Cancer cell dissemination is a hallmark of tumor progression that can potentially lead to the establishment of clinically detectable metastases (Talmadge and Fidler, 2010). Cancer cells released from primary tumors intravasate through lymph or blood vessels. Much effort has been devoted to the detection and characterization of circulating tumor cells (CTCs) in the blood, tumor cells residing in the sentinel lymph nodes, or disseminated tumor cells (DTCs) in the bone marrow (Pantel et al., 2009). Most current studies focus on CTCs because a much less invasive clinical procedure is required to obtain them, which permits frequent monitoring. In addition, different enrichment methods have been successfully developed for blood but not for bone marrow samples. These methods are currently based on antibodies (Pantel et al., 2009), cell deformability (Tan et al., 2009), or cell size (Zheng et al., 2007). CTC phenotyping and its comparison with resident primary tumor cancer cells should help to enhance our understanding of the mechanism of escape.

Experimental models show evidence for collective or individual cell migration at the periphery of primary tumors (Thiery, 2009). Collective cell migration was postulated to be involved in lymph node metastasis; such a mechanism could also account for the dissemination of tumor cell clusters and the formation of microemboli within the primary tumor bed vessels or in vessels at distant sites. Epithelial-mesenchymal (EM) transition (EMT), a well-described mechanism driving major morphogenetic events in metazoans, was also postulated to be responsible for CTC formation (Thiery, 2002). A recent study by Yu et al. (2013) reported a detailed phenotypic analysis of CTCs from patients with metastatic breast cancer, revealing that a significant number of CTCs exhibited a partial or a full-blown EMT phenotype, supportive of an EMT-driven mechanism (Figure 1).

CTCs can be enriched by loading a blood sample through a high-throughput microfluidic herringbone-chip or ^{Hb}CTC-Chip (Stott et al., 2010). This chip comprises a herringbone-patterned surface that increases the interactions of CTCs with an antibody directed against EpCAM, an epithelial marker. However, this method has been limited by the use of a single antibody directed against one relatively specific epithelial marker; this could prevent the capture of CTCs exhibiting mesenchymal characteristics. To overcome this issue, the authors coated the surface of the ^{Hb}CTC-Chip with epithelial and breast molecular subtype-specific antibodies, comprising a cocktail of EpCAM, EGFR, and HER2. The captured CTCs were phenotyped using the ^{Hb}CTC-chip via a quantitative immunofluorescence-based RNA-in situ hybridization (ISH) technology. The chip was hybridized with a mixture comprising custom-designed RNA probes directed against epithelial (E) markers (CDH1, EpCAM, KRT5, KRT7, KRT8, KRT18, and KRT19) and mesenchymal (M) markers (FN1, CDH2, and SERPINE1). The captured cells were then classified into five categories: purely E, intermediate (E > M, E = M, and M > E), or purely M. The RNA-ISH detection allowed customized probes to be used against multiple genes, enabling dual staining and the precise spectral analysis of the EM states of the CTCs. Using a threshold of five CTCs in 3 ml of blood, 17 of 41 breast cancers patients were considered positive, suggesting that this method may not detect CTCs in all metastatic patients. All 17 patients showed evidence of phenotypic changes in the CTCs. Interestingly, a large fraction of the CTCs were either double E/M- or M-positive, particularly among the HER2-positive and triple negative subtypes. Analysis of a breast cancer tissue microarray revealed that all ductal carcinoma in situ showed an E phenotype, as expected, whereas invasive carcinomas often contained cells with dual phenotype (3.3% in estrogen/progesterone [ER/PR]-positive, 2.7% in HER2-positive, and ~12.1% in triple negative [TN] tumors). These data are consistent with a tissue microarray study of 479 samples that found that TN breast cancers were particularly enriched in carcinoma cells expressing a number of mesenchymal markers, but it also showed sporadic expression of some of these mesenchymal markers in ER/PR-positive tumors (Sarrió et al., 2008). Most interestingly, the longitudinal study of ten patients showed that, after targeted therapy, CTCs from responding patients were fewer in numbers and had a more epithelial phenotype. Conversely, CTCs from refractory patients were more numerous and retained or acquired an M phenotype. CTCs with a pronounced M phenotype were also found in clusters. The question then arises as to how these cells should have formed aggregates, as it would be expected that cells with a mesenchymal

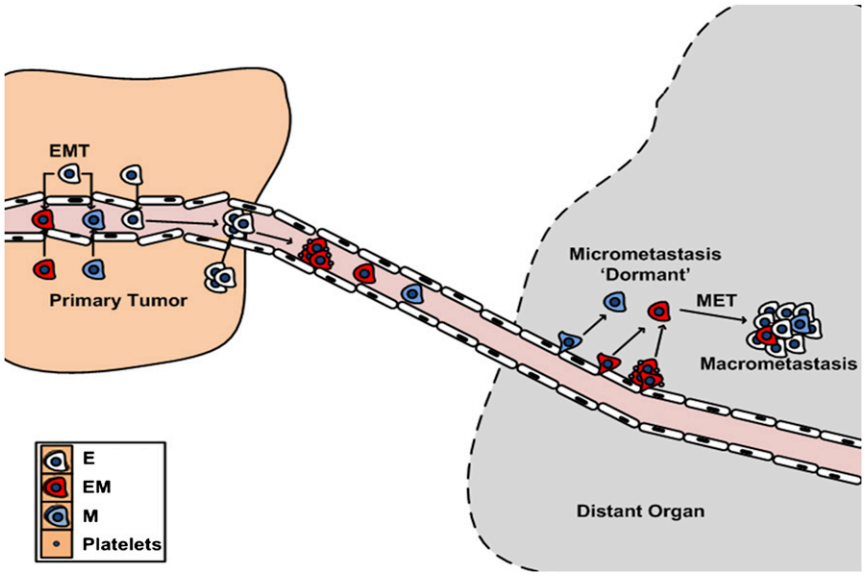

Figure 1. Hypothetical Schematic to Describe the Origin and Subsequent Fate of CTCs
Cells with different epithelial/mesenchymal (EM) phenotypes can exit the primary tumor. Epithelial (E) cells exit either through an epithelial-to-mesenchymal transition (EMT) or by other mechanisms that allow cells to be released as single cells or clusters. Microemboli can arise from EM phenotypes or from E cells. Subsequent EM phenotypes can be acquired through binding to platelets. At secondary sites, solitary cells or clusters may acquire dormancy before resuming growth through an MET (mesenchymal-to-epithelial transition) process. The metastatic tumor may express a similar proportion of EM phenotypes than the primary tumor.

phenotype should remain solitary (Figure 1). CTCs in blood can form microemboli, the presence of which indicates a worsened prognosis, but mechanisms driving microembolism formation remain unclear. Microemboli could result from the detachment of a cohesive group of cancer cells from the primary tumor, or they may be engulfed by invading neovessels. Microemboli could also form from isolated CTCs that undergo proliferation in a confined environment within the capillary bed. Several intercellular adhesion molecules, including N-cadherin, type II cadherins, or CAM-Ig family members, could be involved in cluster formation, even in E-cadherin-negative CTCs, thus promoting survival in the blood flow. Platelets also interact with CTCs and promote CTC aggregation to ensure immune escape. The present study provides evidence that CTC clusters are associated with platelets, which ensures cohesion and simultaneously induces a partial EMT phenotype through TGF-β secretion and documents the EMT phenotype by single-molecule RNA sequencing. An elegant study performed by Labelle et al. (2011) showed that platelet-secreted TGF-β and the direct interaction between platelets and cancer cells can induce an EMT phenotype through Smad and NF-κB pathways, respectively.

The study by Yu et al. (2013) prompts further investigations to better define the EMT spectrum of CTCs and ascertain their clinical relevance. Not all CTCs and DTCs have a prognostic value (Pantel et al., 2009). It will, therefore, be critical to assess their clonogenic potential in secondary sites and determine if the EMT intermediate phenotype is, in fact, most appropriate for the subsequent clonal expansion of CTCs following extravasation due to their ability to reacquire an epithelial-like phenotype (Thiery, 2002).

REFERENCES

Labelle, M., Begum, S., and Hynes, R.O. (2011). Cancer Cell *20*, 576–590.

Pantel, K., Alix-Panabières, C., and Riethdorf, S. (2009). Nat. Rev. Clin. Oncol. *6*, 339–351.

Sarrió, D., Rodriguez-Pinilla, S.M., Hardisson, D., Cano, A., Moreno-Bueno, G., and Palacios, J. (2008). Cancer Res. *68*, 989–997.

Stott, S.L., Hsu, C.H., Tsukrov, D.I., Yu, M., Miyamoto, D.T., Waltman, B.A., Rothenberg, S.M., Shah, A.M., Smas, M.E., Korir, G.K., et al. (2010). Proc. Natl. Acad. Sci. USA *107*, 18392–18397.

Talmadge, J.E., and Fidler, I.J. (2010). Cancer Res. *70*, 5649–5669.

Tan, S.J., Yobas, L., Lee, G.Y., Ong, C.N., and Lim, C.T. (2009). Biomed. Microdevices *11*, 883–892.

Thiery, J.P. (2002). Nat. Rev. Cancer *2*, 442–454.

Thiery, J.P. (2009). Curr. Biol. *19*, R1121–R1123.

Yu, M., Bardia, A., Wittner, B.S., Stott, S.L., Smas, M.E., Ting, D.T., Isakoff, S.J., Ciciliano, J.C., Wells, M.N., Shah, A.M., et al. (2013). Science *339*, 580–584.

Zheng, S., Lin, H., Liu, J.Q., Balic, M., Datar, R., Cote, R.J., and Tai, Y.C. (2007). J. Chromatogr. A *1162*, 154–161.