

## Cancer Stem Cells and Metastasis: Lethal Seeds

□□ Commentary on Balic et al., p. 5615

Max S. Wicha

In an article in the current issue of *Clinical Cancer Research*, Balic et al. (1) bring together the areas of tumor metastasis and cancer stem cells by showing that the majority of early disseminated cancer cells detected in the bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. The majority of cancer deaths are directly attributable to the development of metastatic disease. Over a century ago, Paget proposed the "seed and soil hypothesis," which stated that metastasis depends both on the properties of the tumor cells (the "seeds") as well as the environment at distant sites (the "soil"). A large body of experimental research by many investigators has elucidated the molecular mechanisms that underlie many of the events involved in tumor metastasis. However, many questions remain about the biological properties of both the seeds and the soil. Another old concept that has generated a great deal of interest recently is the "cancer stem cell hypothesis." This hypothesis posits that cancers arise from tissue stem or progenitor cells, and in turn are driven by a subset of tumor cells that retain stem cell properties (2). Previous studies by this group and others have shown that at the time of diagnosis, up to 30% of patients with apparently localized breast cancer have micrometastasis in their bone marrow as determined by immunochemistry of bone marrow aspirates (3). Although the presence of these cells in the bone marrow is associated with a poor prognosis, it is important to note that almost 50% of patients with micrometastasis in their bone marrow at the time of diagnosis have not developed clinically apparent macrometastasis at 10-year follow-up (3). However, even after 10 years, there is a significant rate of late relapse in these patients, highlighting the importance of understanding the phenomenon of metastasis and tumor dormancy at the molecular level. The study of Balic et al. represents an important first step in showing that micrometastases isolated from the bone marrow of early-stage breast cancer patients are highly enriched for cells that display the cell-surface phenotype CD44<sup>+</sup>CD24<sup>-</sup>. We have previously reported that cells with this phenotype isolated from either primary breast cancers or from clinically apparent metastatic lesions have stem cell properties (4). As few as 200 cells displaying this phenotype form tumors in nonobese diabetic-severe combined

immunodeficient mice, whereas 20,000 cells that do not display these markers fail to form tumors. Furthermore, consistent with a stem cell model, the CD44<sup>+</sup>CD24<sup>-</sup> cells generate tumors that recapitulate the phenotypic heterogeneity of the original tumor. Our study and a subsequent report by Ponti et al. (5) have shown that these cancer stem cells compose 1% to 10% of primary or metastatic lesions. The demonstration by Balic et al. showing significant enrichment of cells with this phenotype in micrometastasis suggests that these cells may display biological properties that facilitate their metastatic spread enabling them to colonize distant sites. The concept that tumor stem cells have such properties is supported by recent reports that cancer stem cells have increased angiogenic capacity and express receptors, such as CXCR4, which may facilitate their homing to metastatic sites (6). Indeed, CXCR4 has been reported to be suppressed by CD24, whereas CD44, a receptor for haluronic acid, has been reported to enhance tumor invasion and metastasis (7). Thus, these tumor stem cell markers actually may play a functional role in tumor metastasis. Furthermore, the demonstration that the molecular profile of metastatic tumors closely resembles that of the primary tumor (8) is consistent with the stem cell model as well as the data of Balic et al. because tumor stem cells in the primary tumor or at the metastatic site are able to generate the nontumorigenic cells comprising the bulk of the tumor.

Both the self-renewal and differentiation of normal stem cells are regulated by the stem cell microenvironment, which has been termed the "stem cell niche." Mutations in tumor stem cells may allow them to escape the constraints of the stem cell niche. However, the retention of components of niche signaling may constitute an important element of the tumor microenvironment at distance sites that may contribute to the propensity of particular tumors to metastasize to specific organ sites.

Although the study of Balic et al. represents an important advance in our understanding of the biology of micrometastasis, it raises a number of additional questions. Many previous investigators have used cytokeratin antibodies to detect disseminated tumor cells in lymph nodes or bone marrow aspirates. Some of these, such as cytokeratin 8 and cytokeratin 18, are selectively displayed on mature luminal breast epithelial cells. Cytokeratin 5 may be expressed on more primitive breast cells although it is unclear whether this includes the most primitive stem cell. This highlights the importance of using markers suitable for detecting primitive cancer cells in micrometastases. Immunohistochemical approaches are limited in the number of markers that can be simultaneously studied. To detect putative breast cancer stem cells, Balic et al. determined the percent of cells characterized as CD44<sup>+</sup>CD24<sup>-</sup>. Because of limitations of the number of antigens that can be simultaneously determined, they were unable to eliminate cells displaying lineage positive markers, which we previously used to enrich cancer stem cell

**Author's Affiliation:** University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan

Received 6/26/06; accepted 6/27/06.

**Note:** Dr. Wicha has financial holdings and is a scientific advisor for ONCOMED Pharmaceuticals.

**Requests for reprints:** Max S. Wicha, University of Michigan Comprehensive Cancer Center, 1500 East Medical Center Drive, 6302 CCGC, Ann Arbor, MI 48109-0942. Phone: 734-9367831; Fax: 734-647-9271; E-mail: mwicha@umich.edu.

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doi:10.1158/1078-0432.CCR-06-1537

populations (4). Furthermore, although cells displaying these markers are enriched for cancer stem cells, we have found that cells displaying cancer stem cell characteristics constitute only a subset of the CD44<sup>+</sup>CD24<sup>-</sup>lin<sup>-</sup> population.<sup>1</sup> It is also not known if different subsets of breast cancers contain stem cells with differing properties. Further studies will be needed to address these important questions.

<sup>1</sup>Manuscript in preparation.

In a recent review of the cancer stem cell hypothesis, Polyak and Hahn (9) described the “roots and stems of cancer.” Expanding on their analogy, we suggest that cancer stem cells might also be the “lethal seeds” postulated by Paget more than 100 years ago. Further study of the biology of these seeds should lead to improved methods for their detection. Furthermore, because these cells may be responsible for the development of clinically significant metastasis, development of strategies to target and eliminate them should lead to improved clinical outcomes.

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