

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Timing of Metastasis in Breast Cancer**

Robert S. Schwartz, M.D., and John K. Erban, M.D.

In 2015, almost 232,000 women in the United States received a diagnosis of invasive breast cancer, and approximately 40,000 women died of metastatic breast cancer. The leading cause of these deaths was metastatic spread. The timing and distribution of breast-cancer metastases vary considerably. In approximately 5% of women with breast cancer, metastases are clinically evident at the time of diagnosis. In other women, metastases become apparent years or even decades after the initial diagnosis. Moreover, the number of metastases varies considerably.

The mechanisms that account for the wide variability in the propensity of breast cancer to metastasize are unknown, although this much is clear: metastatic spread from a primary breast tumor can occur at an early, presymptomatic stage, and disseminated cells often settle in the bone marrow where they can lie dormant for years before becoming clinically evident. In two recent studies, Hosseini et al.¹ and Harper et al.² provide insights into the metastatic process of breast-cancer cells that successfully establish themselves in the bone marrow (Fig. 1).

Mechanisms that influence the formation of metastases in breast cancer include the expression of the cell-surface protein human epidermal growth factor receptor 2 (HER2), a transmembrane tyrosine kinase receptor. HER2 augments the metastatic potential of breast-cancer cells; cells that express HER2 are more likely than HER2-negative cells to propagate metastases.³

There is a long-held view that the increase in malignant behavior during the course of a neoplasm is related to the stepwise acquisition by cancer cells of variants that predispose to the acquisition of additional variants that confer malignant and metastatic behavior.⁴ This “cascading” model may pertain to some tumors, but it has been challenged in the case of breast cancer.⁵ The model predicts that a subset of mutations in the primary tumor will also be found in meta-

static cells. Metastatic cells, however, can acquire mutations independent of mutations in the primary tumor — a phenomenon known as parallel progression. One may therefore ask whether samples from the primary tumor, blood, or metastases should be used for molecular analysis to guide therapy.

The reports by Hosseini et al. and Harper et al. together make a strong case for increased scrutiny of cells of the early lesion and of the establishment and proliferation of these cells at the site of metastasis. Hosseini et al. performed experiments using mice that were engineered to express high levels of the rat orthologue of HER2 in mammary epithelial cells. These mice are prone to breast cancer and are therefore used as a model of HER2-positive breast cancer. The authors observed that 80% of the mice had bone marrow metastases arising from cells that had disseminated from an early neoplastic lesion in breast epithelium soon after induction of HER2 expression. Moreover, Wnt4 and receptor activator of nuclear factor- κ B ligand (RANKL) signals secreted by early tumor cells that express both HER2 and the progesterone receptor were found to promote the dissemination of progesterone-receptor-negative cells from the lesion. Wnt4 supports cell proliferation, survival, and differentiation as well as carcinogenesis; RANKL supports the survival of metastatic cells. Hosseini et al. found that, although cells in early lesions are prone to metastasize, cells in late tumors are much less likely to disseminate from the tumor and are more prone to remain in situ and proliferate; these cells robustly express HER2 and lack expression of progesterone receptor. The authors also found that suppression of the progesterone receptor in late tumors is mediated by the high expression of HER2.

In the article by Hosseini et al., an analysis of disseminated cancer cells that were isolated from women who had received a diagnosis of

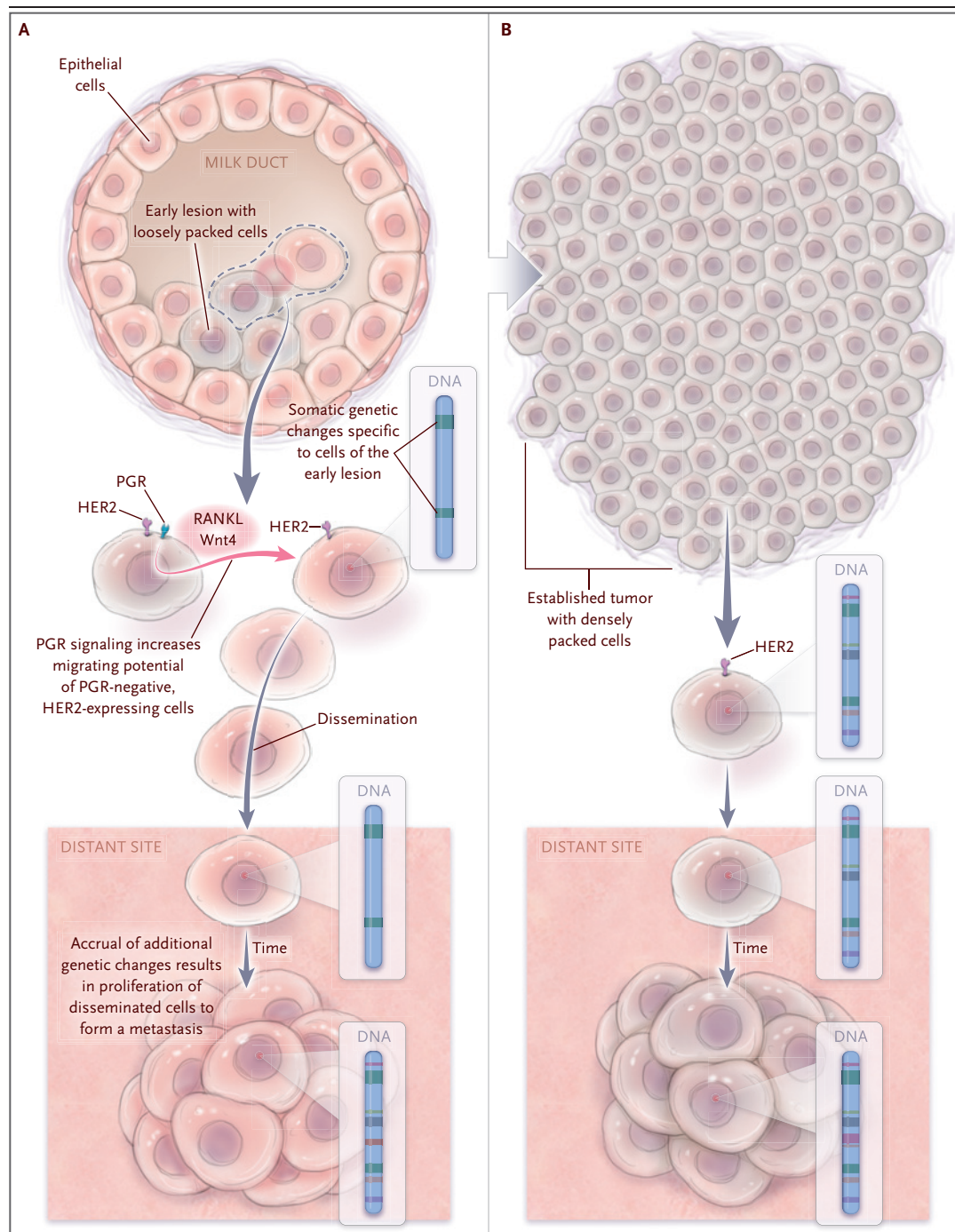


Figure 1. A Model of Parallel Progression after Early Dissemination.

Recent experiments with a mouse model of human epidermal growth factor receptor 2 (HER2)-positive breast cancer^{1,2} have uncovered events critical to the dissemination of cancer cells from the early lesion (Panel A) and cells of the established tumor (Panel B). The cells in the early lesion are loosely arranged; some of them express the progesterone receptor (PGR) in addition to HER2. These PGR-positive cells secrete soluble factors, such as receptor activator of nuclear factor- κ B ligand (RANKL) and Wnt4, that induce the migration of PGR-negative cells from the lesion. The cells in the established tumor (Panel B) are packed more tightly, typically do not express the PGR, and are less likely to metastasize. The cells that disseminate from established tumors to the bone marrow carry many of the genetic variants, such as loss of chromosome 8p, that are found in established tumors, whereas those that disseminate from early lesions do not and instead evolve in parallel with the primary tumor and other disseminated cells.

breast cancer (both women with and women without metastases) showed that disseminated cells isolated from women without metastases did not harbor chromosomal changes that were found in the primary tumor, such as loss of chromosome 8p. In contrast, an analysis of the disseminated cancer cells isolated from women with metastases showed that they had a genetic makeup similar to that of the primary tumor. This finding supports the hypothesis that cancerous cells that are disseminated from the early lesion follow an independent, or parallel, path of genetic evolution with respect to cells in the primary tumor.

Using a mouse model similar to the one used by Hosseini et al. (in which HER2 is expressed in the mammary ducts), Harper et al. found that HER2-positive cells examined early in the evolution of breast cancer in mice activate a program of Wnt-dependent dissemination. Both Harper et al. and Hosseini et al. concluded that progesterone-dependent signaling pathways may activate HER2, which activates processes (such as the weakening of cell adhesion) that favor metastasis. Hosseini et al. also found that the density of cells (the number of cells per unit volume, which was high in established tumors vs. low in early lesions) influences the expression of progesterone receptors and the propensity to metastasize.

What in the microenvironment of the bone marrow determines whether the metastatic cell remains dormant or becomes activated? Myeloid cells in the vascular niches of the marrow express RANK, the receptor of RANKL. Opportunity certainly exists for interactions between metastasized breast-cancer cells and the cells native to

the bone marrow. And what (if anything) in the bone marrow microenvironment influences the acquisition of mutations in cancer cells independently of mutations that accrue in the primary tumor? Mesenchymal cells also reside within the vascular niches of the bone marrow and regulate stem-cell renewal and proliferation, usually by means of secreted cytokines. These mesenchymal cells may regulate the dormancy and activation of metastatic cells, although there is much to be learned about the mechanisms that govern these states. Stromal cells in the bone marrow can also activate or suppress immune cells, including B cells, T cells, and macrophages — cell types that have an important role in the defense against cancer. Perhaps an improved understanding of the stromal-cell-immune-cell axis will yield therapeutic targets in the same way that the work by Hosseini et al. and Harper et al. has yielded insights into the process of metastasis.^{1,2,4}

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From Tufts University, Boston.

1. Hosseini H, Obradović MM, Hoffmann M, et al. Early dissemination seeds metastasis in breast cancer. *Nature* 2016 December 14 (Epub ahead of print).
2. Harper KL, Sosa MS, Entenberg D, et al. Mechanism of early dissemination and metastasis in Her2+ mammary cancer. *Nature* 2016 December 14 (Epub ahead of print).
3. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177-82.
4. Markowitz SD, Bertagnolli MM. Molecular basis of colorectal cancer. *N Engl J Med* 2009;361:2449-60.
5. Klein CA. Parallel progression of primary tumours and metastases. *Nat Rev Cancer* 2009;9:302-12.

DOI: 10.1056/NEJMcibr1701388

Copyright © 2017 Massachusetts Medical Society.

EARLY JOB ALERT SERVICE AVAILABLE AT THE NEJM CAREERCENTER

Register to receive weekly e-mail messages with the latest job openings that match your specialty, as well as preferred geographic region, practice setting, call schedule, and more. Visit the NEJM CareerCenter at NEJMjobs.org for more information.