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Macrophages stimulate mammary stem cells

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The adult mammary gland is a bilayered branching epithelial structure consisting of an outer layer of basal cells and an inner layer of luminal cells. The neonatal structure expands rapidly during puberty and then undergoes cyclic growth in response to the changing hormonal stimuli (progesterone and estrogen) in each menstrual cycle (1). These dynamic responses of the mammary gland involve important interactions with various surrounding nonepithelial cells that constitute its “niche.” Circulating macrophages are important constituents of this niche although the mechanism through which they influence mammary cell proliferation has remained unclear. On page 1421 of this issue, Chakrabarti *et al.* (2) show that mouse mammary stem cells [cells with an ability to regenerate an entire mammary gland (3, 4)] are enriched within a subset of cells with a phenotype of basal layer cells and express the delta-like 1 (DLL1) NOTCH ligand, which allows them to interact with nearby NOTCH-expressing macrophages. This interaction triggers the production of multiple Wnt (Wingless-related integration site) ligands that, in turn, induce an expansion of the mammary stem cell population. This finding is important because it has implications for understanding how breast cancer may develop.

The demonstration that mammary stem cells lack steroid hormone receptors [progesterone receptor (PR) and estrogen receptor (ER)] (5) predicted that their ability to respond to changing amounts of progesterone and estrogen depends on a multistep mechanism involving the production of intermediate signals by hormone-responsive cells. Such a process would then explain how oscillations in blood concentrations of these key ovarian hormones during the estrous cycle cause changes in the size of the entire gland (see the figure). Further support for this concept came from the subsequent demonstration of a significant expansion and contraction of this functionally defined mouse mammary stem cell population during the estrous cycle and pregnancy (6, 7), mediated indirectly by PR-expressing luminal cells stimulated to produce receptor activator of nuclear factor κB ligand (RANKL) and WNT4 (8, 9).

A potential involvement of macrophages in this regulatory activity was first suggested by the finding that a bone marrow transplant from immunologically matched (syngeneic) mice could reverse the blunted development of the mammary gland that occurs when mice are exposed to ionizing radiation before puberty (10). Further evidence of such a functional role of macrophages was provided by the demonstration of a similarly constrained development of the mammary gland in mice with a genetic depletion of macrophages (11). Together,

these studies established the dependence of postnatal development of the mammary gland on an associated population of tissue-resident macrophages. The compelling temporal coincidence of cyclic changes in mammary stem cell numbers with corresponding oscillations in Wnt-expressing macrophages reported by Chakrabarti *et al.* now provides an attractive mechanistic explanation of how macrophages execute a regulatory role in the mammary gland in response to cyclic changes in the concentration of circulating progesterone. The implication is that even the peak concentrations of progesterone achieved in the blood during the diestrous phase of the menstrual cycle (see the figure) and late pregnancy do not induce either the responsive luminal cells or the resident macrophages to produce sufficient Wnt to stimulate an expansion of the mammary stem cell population. But together, these two sources can cooperatively increase the amount of Wnt above a rate-limiting concentration.

As exemplified in the study of Chakrabarti *et al.*, mammary stem cells and their role in situ have been most frequently investigated in mice, although there is persisting controversy as to the extent of postnatal maintenance of the two cell layers of the mammary gland by unipotent versus bipotent cells (12, 13). An understanding of the roles of WNT4 and RANKL in mediating the effects of progesterone on mouse mammary stem cells is also incomplete (8, 9). In addition, despite evidence of functionally and phenotypically analogous subsets of luminal and basal cell populations in the mammary glands of mice and humans, and the accompanying presence of stromal cells and resident macrophages, considerable differences exist between the breast tissue of both species. These include differences in the detailed structure of the mammary gland, differences in the types and distribution of stromal elements that surround the gland, and differences in the growth requirements of the mammary cells ex vivo (1). Thus, further investigation to determine the relevance of these findings to the hormonal control of human mammary cell dynamics will be of interest.

The discovery by Chakrabarti *et al.* of a role of tissue-resident macrophages in regulating mammary stem cell expansion raises additional interesting questions for future study. Because the emergence of embryonic mammary cells and their early rapid growth seem to be independent of ovarian function, are these cells also Wnt dependent? If so, might embryonic macrophages, which are early migrants from the yolk sac or fetal liver, be involved in stimulating their growth? Expansion of the population of mammary stem cells during and after puberty appears to be under the joint control of ovarian hormones and macrophages. What then regulates the observed changes in macrophage numbers? And will macrophages affect mammary stem cell proliferative responses in vitro to enable further dissection of their effects? Although mammary-macrophage signaling via a DLL1-Wnt pathway may create a positive-feedback loop that is important for the rapid expansion of mammary stem cells in vivo, are there separate mechanisms that counterbalance and fine tune the restoration of baseline mammary stem cell numbers?

Notably, these findings are also relevant to understanding the potential role of macrophages in the genesis and dissemination of breast cancer. Fluctuations in macrophage numbers may not be limited to playing a key role in the normal physiology of the mammary gland. Chronically increased numbers of macrophagic “crown-like” structures, particularly associated with inflammation in benign mammary adipose tis-sue, appear to correlate with

an increased risk of breast cancer (14). In mouse models of metastatic breast cancer, the presence of macrophages, or progesterone-associated RANKL and WNT4 signals in mammary tumor lesions, were also found to be sufficient for early dissemination of malignant cells before a primary tumor was evident (15). The mammary gland and its surrounding tissue environment is clearly more complex than historically anticipated, with emerging evidence that macrophages play different roles throughout mammary gland development, aging, and tumorigenesis.

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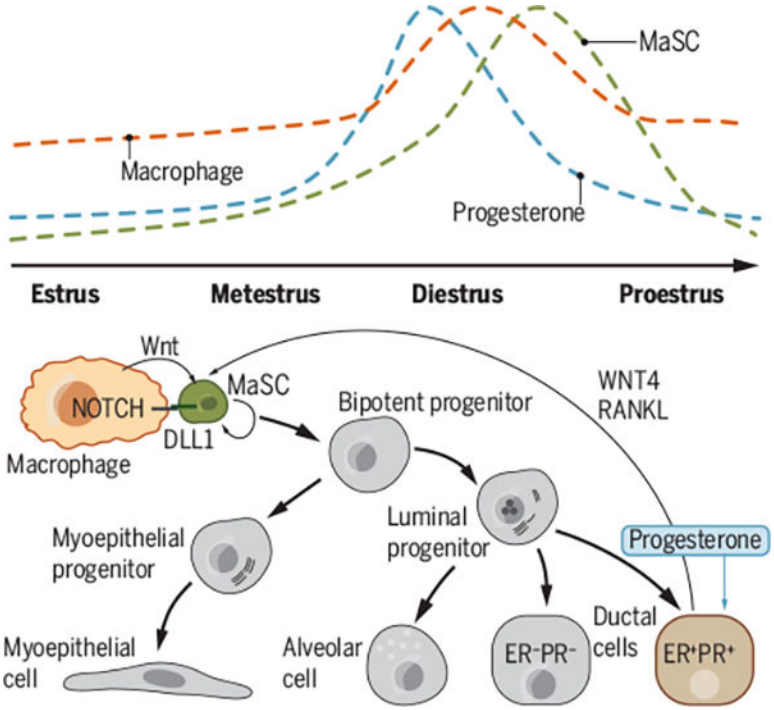

Proposed macrophage control of MaSCs

Mammary stem cell (MaSC) numbers oscillate during the estrous cycle, mirroring similar oscillations of serum progesterone and macrophage numbers in the breast. NOTCH macrophages interact with DLL1 expressed by a subpopulation of MaSCs with basal features. This triggers macrophages to secrete Wnt ligands. In response to progesterone, the ER+PR+ ductal cells secrete WNT4 and RANKL. Together with macrophages, this brings Wnt ligands above a threshold that stimulates MaSC proliferation.