Estrogen regulation of mammary gland development and breast cancer: amphiregulin takes center stage
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Adipin = a disintegrin and metalloproteinase; AREG = amphiregulin; EGFR = epidermal growth factor receptor; ER = estrogen receptor; GFP = green fluorescent protein; HELU = hyperplastic enlarged lobular unit; TDLU = terminal duct lobular unit; TGF = transforming growth factor; WT = wild type.
Notably, previous studies have shown that EGFR in the stroma, but not the epithelium, is essential for ductal morphogenesis [5,6]. Studies by Werb and coworkers revealed a potential mechanism by which epithelial-derived AREG activates EGFR on neighboring stromal cells [7]. They demonstrated that in culture, ADAM17 (a disintegrin and metalloproteinase 17) activates AREG, which is normally secreted as an inactive, transmembrane precursor [7]. Similar to the phenotype observed in AREG-null mammary glands, ductal outgrowth was inhibited in ADAM17ΔΔ epithelium, and exogenous AREG could rescue ADAM17ΔΔ transplants [7]. These data suggest a key role for ADAM17 in the paracrine interaction elicited by estrogen signaling.

While intriguing, these results raise several critical questions. First, is AREG necessary and sufficient to rescue ductal morphogenesis in ERα-null mammary epithelium, or are other paracrine factors involved? These types of genetic rescue experiments are required to validate the models proposed by Brisken and Werb and colleagues. Second, how does AREG signal through the basement membrane and the myoepithelium to act on EGFR in the stroma? Likewise, what is the reciprocal paracrine signal that stimulates the epithelium? Stromal-derived fibroblast growth factor 7 has been suggested to activate epithelial fibroblast growth factor receptor 2b during mammary branching [3], but genetic evidence for this mechanism has yet to be provided.

Estrogen receptor-α and mammary stem cells
What insights do these studies provide with respect to the potential role of estrogen-mediated proliferation in mammary stem cells? Although considerable progress has been made in the characterization of mammary stem cells, the mechanisms that regulate their self-renewal remain poorly understood. Previous studies involving the role of steroid hormones in stem/progenitor cell activity have reported conflicting results. It has been proposed that slow-dividing, label-retaining ERα-positive cells comprise a stem/progenitor cell population that can directly respond to hormones [8,9]. In striking contrast, Brisken and colleagues’ studies suggest that non-proliferating ERα-positive cells exploit a paracrine mechanism to stimulate the proliferation of neighboring stem/progenitor cells [2,4]. Shackleton et al. [10] demonstrated that a single, self-renewing LinCD29CD24+ cell could repopulate a cleared mammary fat pad. Further analysis of this basal population revealed that these cells were exclusively ERα-negative, but did express proliferating cell nuclear antigen and EGFR [11]. Recent studies by Smalley and coworkers [12] have also demonstrated that a subpopulation of ERα-positive epithelial cells lacked in vivo stem cell activity. Limiting dilution transplantation revealed that CD24lo ERα-negative basal epithelial cells displayed the highest stem cell activity, while ERα-positive luminal cells exhibited very little stem cell activity, as defined by mammary repopulating units [12].

These studies raise the following critical question: how can a single ERα-negative stem cell give rise to a complete, functional ductal tree when transplanted into the cleared mammary fat pad? Wicha and colleagues [13] have proposed that ERα-negative stem cells give rise to undifferentiated steroid receptor-positive cells, which proliferate in response to estrogen and secrete paracrine factors that regulate the proliferation and differentiation of adjacent ERα-negative cells. In accordance with this model and the findings of Brisken and coworkers, we propose that an ERα-negative basal stem cell may divide asymmetrically once to give rise to a luminal ERα-positive progenitor cell. In response to estrogen stimulation, the resulting ERα-positive cell may secrete paracrine factors, such as AREG or growth hormone, that can feedback on ERα-negative stem cells (Figure 1). In addition, those paracrine factors may stimulate the proliferation and/or differentiation of adjacent ERα-negative and ERα-positive progenitor cells. Finally, epithelial-derived AREG, for example, may bind EGFR on more differentiated, neighboring stromal cells that induce other local growth factors, resulting in massive proliferation during ductal morphogenesis (see Figure 3 of [3]).

Amphiregulin and breast cancer
What implications do these studies of normal mammary ductal morphogenesis have for our understanding of human breast cancer? Specifically, how do quiescent ERα-positive epithelial cells become proliferative in hormone-dependent breast cancers? Some insights have come from recent studies by Allred and coworkers [14], who used DNA microarrays to determine differences in gene expression between terminal duct lobular units (TDLUs) and hyperplastic enlarged lobular units (HELUs). HELUs result from abnormal enlargement of TDLUs and are the earliest histologically identifiable lesions with premalignant potential observed during the progression from normal ductal epithelium to hormone-dependent breast cancer. Interestingly, HELUs,
which were previously shown to contain highly proliferative cells with elevated ERα expression, showed a ten-fold increase in AREG compared to normal TDLUs. This increase was accompanied by a 14-fold decrease in epidermal growth factor, while the expression of EGFR was unchanged. These results suggest an early switch in EGFR ligands that may be important for breast cancer initiation [14]. Whether this switch is mediated by estrogen remains to be determined.

AREG is overexpressed in most ERα-positive primary breast tumors, and EGFR expression in breast cancer has been associated with poor prognosis and resistance to hormone therapy [15]. Using a three-dimensional culture model of human breast cancer progression, Bissell and colleagues [16] recently described an ADAM17-dependent autocrine loop that induced a malignant phenotype. Importantly, this phenotype was reversed by inhibiting ADAM17, which is important for breast cancer initiation [14]. Whether this switch is mediated by estrogen remains to be determined.

Conclusions
Estrogen-mediated proliferation is fundamental to mammary gland development and breast tumorigenesis. Recent studies have established an important relationship between ERα, AREG, proliferation and breast cancer. AREG is a key paracrine factor that not only mediates ductal elongation, but may also play an important role in mammary stem cell self-renewal and differentiation. Elucidating the molecular mechanisms that regulate AREG signaling in the mammary gland, including the switch from estrogen-mediated paracrine events to an autocrine pathway, will be required for a complete understanding of the early events in breast cancer progression.

Competing interests
The authors declare that they have no competing interests.

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