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Review

Cellular foundations of mammary tubulogenesis

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ABSTRACT

The mammary gland is composed of a highly branched network of epithelial tubes, embedded within a complex stroma. The mammary epithelium originates during embryonic development from an epidermal placode. However, the majority of ductal elongation and bifurcation occurs postnatally, in response to steroid hormone and growth factor receptor signaling. The process of pubertal branching morphogenesis involves both elongation of the primary ducts across the length of the fat pad and a wave of secondary branching that elaborates the ductal network. Recent studies have revealed that mammary epithelial morphogenesis is accomplished by transitions between simple and stratified organization. During active morphogenesis, the epithelium is stratified, highly proliferative, has few intercellular junctions, and exhibits incomplete apico-basal polarity. In this review, we discuss recent advances in our understanding of the relationship between epithelial architecture, epithelial polarity, and ductal elongation.

Contents

1. Introduction

The mammary gland is composed of bilayered tubes embedded within an adipocyte rich stroma. The epithelial cells that constitute these tubes are connected to each other by extensive intercellular junctions and have a high degree of apico-basal polarity. This polarity is manifested both by morphological specializations, such as apically localized microvilli, and by segregated membrane domains containing distinct apical and basal polarity complexes [1–3]. These adhesive junctions must be remodeled for either individual or collective cell movement to occur. The strong apico-basal polarity also places constraints on tubulogenesis, as many of the molecular determinants of epithelial polarity serve distinct roles in migratory cells [1]. In this review, we will focus on the cellular mechanisms used by the mammary epithelium to build and elaborate the ductal network during puberty. Other authors have reviewed the genetic requirements for mammary gland formation [4] and the cellular steps in embryonic mammary development [5,6].

1.1. Embryonic origins of the mammary epithelium

Mammary ducts in the adult have a bilayered organization, with keratin-14+ (K14+) myoepithelial cells positioned basally relative

Abbreviations: ECM, extracellular matrix; TEB, terminal end bud.

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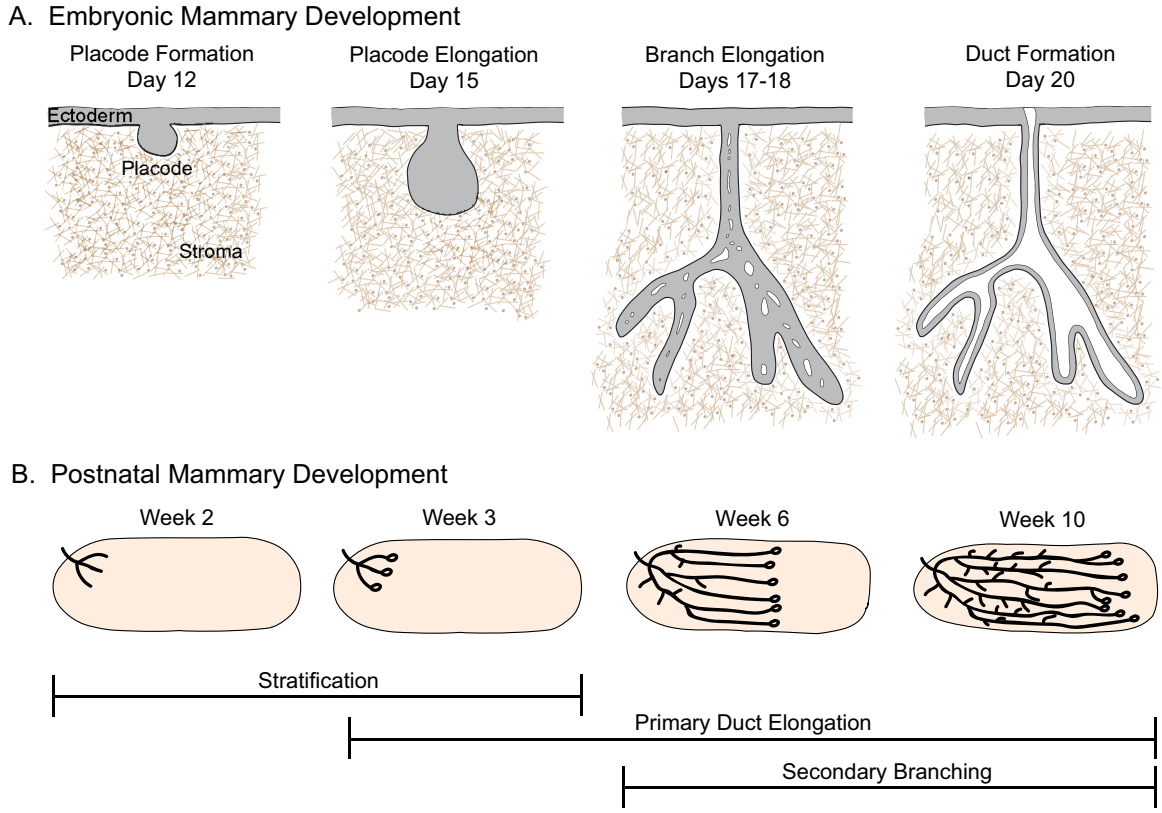

Fig. 1. (A) Mammary development in the mouse begins at embryonic day 12 when an epidermal placode invades the mammary stroma. The stratified epithelium then elongates and hollows to form a rudimentary bilayered duct that remains essentially quiescent until puberty. Modified from Hogg et al. [7]. (B) Mammary ducts are elaborated during puberty to form the functional ductal network. Pubertal development initiates during postnatal week 3 with the formation of terminal end buds (TEBs), which are a stratified epithelium at the ductal tips. Over the next 7 weeks, primary ducts elongate across the mammary fat pad, and secondary branching completes the ductal tree.

to the K8+ luminal epithelial cells. In contrast, the embryonic mammary epithelium originates as an epidermal placode and invades into the mesenchyme with a stratified organization and without a morphologically evident lumen [7] (Fig. 1A). Both the cell type specific gene expression and relative positioning of myoepithelial and luminal epithelial cells are late features that are acquired progressively during fetal and early postnatal development [8–10]. As the mammary bud initiates elongation, many of the epithelial cells are K8 + K14+ and K14+ cells are detected in both basal and interior positions [8,11]. It is only late in fetal development that a mature lumen forms, lined with K8+ luminal epithelial cells and surrounded by basally positioned K14+ cells [7,11]. Myoepithelial cells are named for their contractile properties, which are regulated by genes such as smooth muscle actin (SMA). This contractile gene expression program is activated postnatally and so the mature myoepithelial phenotype of K8-K14 + SMA+ is not established until the onset of puberty [9]. Taken together, the embryonic mammary epithelium during morphogenesis is stratified, lacks a contiguous lumen, and is composed of cells with combinations of "cell type specific" gene expression that are uncommon in adult ducts. While the result of embryonic mammary development is a small network of polarized simple ducts, the organization of the epithelium during morphogenesis is neither simple nor polarized [7] (Fig. 1A).

1.2. Postnatal branching morphogenesis of the mammary epithelium

The mammary epithelium finishes fetal development as fully polarized bilayered ducts [7]. These simple tubes are then essentially quiescent until the rise in steroid hormones that triggers the onset of puberty [12,13](Fig. 1B). Before puberty the mammary ducts are all simple (Fig. 2A and A′) and they then stratify to produce stratified terminal end buds [14–16] (TEBs) (Fig. 2B and B′). Terminal end buds have multiple layers of epithelial cells, referred to as body cells, surrounded by a basally positioned cap cell layer [15,16]. Most body cells stain positive for luminal epithelial markers, such as K8 and E-cadherin [17]. However, there are basal marker positive body cells as well [18,19]. Cap cells stain positive for basal cytokeratins and P-cadherin [17]. Consistent with the organization of the embryonic mammary epithelium, the body cells exhibit high levels of proliferation, have few intercellular junctions, and incomplete apico-basal polarity [19,20]. Many body cells lack contact with either the main lumen or the basement membrane and isolated microlumens are observed within the body cell region [19,20], similar to the microlumens observed in the early fetal mammary ducts [7] (Figs. 1A vs. 2C′). TEBs form in response to iterative signals exchanged between the epithelium and surrounding mesenchymal cells, mediated through both steroid hormone receptors and growth factor receptors [4,12,13].

TEBs begin elongating at the onset of puberty, 3 weeks of age in mice. These stratified TEBs build centimeters of ducts as they cross the mammary fat pad and there is an abrupt transition from the stratified organization of the TEB to the simple organization of the trailing duct (Fig. 2C and C′) [16,19]. A secondary wave of side branching initiates a couple of weeks later and fills in the complexity of the network (Fig. 1B). The details of the branching pattern are highly variable and can be quite distinct even when comparing different glands in the same mouse. Common themes emerge at a more global level: TEBs stop elongating and revert to simple epithelial architecture when they reach the edges of the mammary fat pad. The result is a similar openness in the branching pattern across mice of the same strain.

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