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Review

Stromal regulation of embryonic and postnatal mammary epithelial development and differentiation

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ABSTRACT

The stroma, which is composed of supporting cells and connective tissue, comprises a large component of the local microenvironment of many epithelial cell types, and influences several fundamental aspects of cell behaviour through both tissue interactions and niche regulation. The significance of the stroma in development and disease has been increasingly recognised. Whereas normal stroma is essential for various developmental processes during vertebrate organogenesis, it can be deregulated and become abnormal, which in turn can initiate or promote a disease process, including cancer. The mouse mammary gland has emerged in recent years as an excellent model system for understanding stromal function in both developmental and cancer biology. Here, we take a systematic approach and focus on the dynamic interactions that the stroma engages with the epithelium during mammary specification, cell differentiation, and branching morphogenesis of both the embryonic and postnatal development of the mammary gland. Similar stromal–epithelial interactions underlie the aetiology of breast cancer, making targeting the cancer stroma an increasingly important and promising therapeutic strategy to pursue for breast cancer treatment.

Contents

Abbreviations: CAF, cancer associated fibroblast; ECM, extracellular matrix; EDA, Ectodysplasin; ERα, oestrogen receptor alpha; FGF, fibroblast growth factor; FPP, fat pad precursor; MPE, mammary primordial epithelium; MEC, mammary epithelial cell; MM, mammary mesenchyme; MP, mammary primordium; NRG3, Neuregulin3; PTH1R, parathyroid hormone 1 receptor; PTHRP, parathyroid hormone related protein.

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1. Introduction

Understanding the mechanisms whereby the stroma influences the fundamental aspects of cell and tissue behaviour is an area of intense research efforts in both developmental biology and cancer biology. The importance of mesenchymal influence on organ identity was demonstrated in a number of classic embryological studies that showed that the fate of the epithelium originating from another organ type is determined by the organ type of the embryonic mesenchyme, when the two tissues were surgically recombined [1–3]. While this concept of stromal influence on epithelial cells was recognised relatively more slowly in cancer biology, it has been increasingly accepted that normal stroma is essential for cell fate maintenance during organ homeostasis and function, whereas abnormal stroma promotes cancer initiation and progression [4].

As an ectodermal appendage, the mouse mammary gland is a non-vital organ, and embryological studies are amenable to a variety of experimental techniques such as, genetic and transgenic manipulation, transplantation surgery, and *ex vivo* culture. In recent years the mouse mammary gland emerged as a powerful model to understand the genetic and cellular basis of stromal function. For example, embryonic mammary mesenchyme and its postnatal derivative, often referred to as mammary stroma, are both essential for organ specification, cell differentiation, morphogenesis, and other important aspects of mammary gland development [3,5–8]. For the purpose of simplicity, throughout this review we use the term "stroma" in a broad sense that refers to both embryonic mesenchyme and postnatal stroma.

Decades of research of vertebrate organs have shown that epithelial development is a dynamic and complex process. Importantly, the stroma is intricate and constantly changing as it develops together with the epithelium. Their association is indispensable for differentiation and morphogenesis of the epithelium. Such developmental synchrony is accomplished by constant and persistent niche–epithelial crosstalk throughout different stages of mammary gland formation and tissue homeostasis. As is also observed with the epithelium, stromal composition increases in complexity during development as stromal cells mature and differentiate and cellular products, including paracrine factors and the extracellular matrices (ECM), start to accumulate and elaborate [9].

In light of its important influences on cell behaviour, the dynamic and complex nature of the stroma has posed a great challenge to understanding its function in mammary gland biology as taking cells out of their native microenvironment is routinely done for practical purposes in experimental studies. Here, we take a holistic approach to examine mammary gland development, emphasising the changes that the stroma undergoes throughout critical embryonic and postnatal stages. We highlight recent progress in understanding the mechanism whereby abnormal stroma, especially deregulated ECM biology, facilitates the formation of the tumour microenvironment and promotes breast cancer formation.

2. Development of the mammary gland stroma is a dynamic process

The emergence of the mammary gland is a late event during metazoan evolution [10–12]. Coinciding with its position along the evolutionary timeline is the relatively late development of the mammary gland during embryogenesis and postnatal life. The ectodermal appendages reviewed in this issue initiate relatively late in development. In mouse, which is the most widespread model organism used in mammary gland developmental studies, and the basis for most of the experimental studies reviewed here. The first morphological indication of mouse mammary gland development, does not occur until mid-gestation at around embryonic day (E) 11, when most other vertebrate organs have been patterned and are well in the process of cell differentiation [13]. Likewise, epithelial branching morphogenesis of the mammary gland does not start until late gestation and persists for weeks after birth, whereas in most other branched organs, such as the lungs, the process occurs only during embryogenesis [14].

The embryonic mammary organ is referred to as the mammary primordium (MP) (Fig. 1A–C). In the E11.0-stage mouse embryo, the MP is comprised of a multilayered lens-shaped epithelium with non-descript mesenchyme underlying it [13,15,16]. By E13.0-stage, several layers of dermal mesenchyme adjacent to the mammary bud epithelium have condensed and aligned into a concentric orientation. Mesenchymal condensation is critical for organogenesis, yet little is known about how this process is controlled. Coinciding with this morphological change is the expression of distinguishing molecular markers, including oestrogen receptor-alpha (ERα), that provide evidence that these cells have differentiated into the primary mammary mesenchyme (MM) (Fig. 1B).

Several important events occur starting at E15.5-stage when the MP forms a sprout and invades the underlying stroma. A morphologically distinct population of cells referred to as the secondary mammary mesenchyme or the fat pad precursor (FPP) tissue joins the primary MM and becomes a part of the embryonic stroma surrounding the invading mammary sprout which invades into it (Fig. 1C). At present, the early developmental events of the white adipose tissue, an essential cell population of the mammary gland stroma, remain largely unclear [17,18]. Within a few days after birth, however, the FPP appears to have differentiated into mature adipocytes [19]. By birth, a primitive epithelial tree can be found in the proximal area of the fat-pad (FP) [14]. The primitive ductal epithelial tree develops very slowly in the first a few weeks until puberty at three weeks of age. At puberty, the mammary epithelial cells respond to hormonal stimulation by a phase of rapid invasive growth into the stromal fat-pad with concurrent bifurcation of the terminal end bud (TEB) at the tip of each primary duct until the FP is completely filled with the epithelial tree within ten weeks after birth (Fig. 1D–I) [20].

The FP barely changes in size after birth and enlarges only in proportion with the overall growth of the whole organism, such that the ratio of the fat pad to body size is constant. The cellular composition of FP, however, increases in complexity during its maturation. For example, in addition to adipocytes, periductal fibroblasts, and endothelial and nerve cells, the mammary stroma is also composed of a variety of immune cells including macrophages and eosinophils, which both play an important role in postnatal branching [21–23]. Mouse mammary stroma is highly distinct from that found in the human breast. Mouse mammary epithelium is surrounded small number of fibroblasts, which are in turn, encompassed by a very large amount of fat cells. Human breast epithelium is closely associated with dense accumulations of fibroblasts, such that a distinct fibrous stromal component surrounding the mammary epithelium is found embedded within fatty tissue [24,25]. In both species, another essential component of the stroma is the extracellular matrix (ECM), which is composed of the basement membrane separating the epithelium and stroma, and the interstitial matrix filling the spaces among the non-adherent stromal cells. The ECM is a highly dynamic and functionally versatile structure that constantly undergoes a remodelling process owing to enzymes secreted by both the epithelium and stromal cells [9].

The epithelium continues to undergo further morphogenetic and differentiation events, including formation of alveoli and generation of milk producing cells during pregnancy [7]. During pregnancy, mammary stroma undergoes dramatic changes, especially in adipocyte morphology, which decrease in size, in order