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Review

Stroma in breast development and disease

Lisa M. Arendt[a,b], Jenny A. Rudnick[a,b], Patricia J. Keller[a,b], Charlotte Kuperwasser[a,b,*]

[a] Department of Anatomy & Cellular Biology, Sackler School, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111, USA
[b] Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA 02111, USA

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ABSTRACT

It is increasingly apparent that normal and malignant breast tissues require complex local and systemic stromal interactions for development and progression. During development, mammary cell fate specification and differentiation require highly regulated contextual signals derived from the stroma. Likewise, during breast carcinoma development, the tissue stroma can provide tumor suppressing and tumor-promoting environments that serve to regulate neoplastic growth of the epithelium. This review focuses on the role of the stroma as a mediator of normal mammary development, as well as a critical regulator of malignant conversion and progression in breast cancer. Recognition of the important role of the stroma during the progression of breast cancers leads to the possibility of new targets for treatment of the initial breast cancer lesion as well as prevention of recurrence.

Contents

1. Introduction

The mammary gland is a complex tissue comprised of an epithelial parenchyma embedded in an array of stromal cells that regulate its proliferation, differentiation and survival. The mammary gland undergoes dynamic changes over the lifetime of a woman, from the expanded development at puberty, to hormonally controlled proliferation and apoptosis during the menstrual cycle, to full lobuloalveolar development for lactation. Pioneering mouse mammary epithelial cell transplant work by DeOme et al. demonstrated the regenerative plasticity of the mammary epithelium and the dependence on the stroma for its development [1,2]. Moreover, through similar epithelial transplant experiments, non-mammary

Abbreviations: E, embryonic day; ECM, extracellular matrix; FGF, fibroblast growth factor; PTHrP, parathyroid hormone related peptide; TEB, terminal end bud; ER, estrogen receptor; EGFR, epidermal growth factor receptor; GH, growth hormone; IGF, insulin-like growth factor; TGFβ, transforming growth factor beta; MMTV, mouse mammary tumor virus; αSMA, alpha smooth muscle actin; FAP, fibroblast activated protein; HIM, human-in-mouse; RMFs, reduction mammary fibroblasts; HGF, hepatocyte growth factor; DCIS, ductal carcinoma in situ; CAF, cancer-associated fibroblast; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; MSC, mesenchymal stem cell; EMT, epithelial–mesenchymal transition; MD, mammographic density.

* Corresponding author at: Tufts University School of Medicine, 800 Washington Street, Box 5609, Boston, MA 02111, USA. Tel.: +1 617 636 2364; fax: +1 617 636 6127.
E-mail address: Charlotte.Kuperwasser@tufts.edu (C. Kuperwasser).

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cells were reprogrammed to perform mammary epithelial cell functions due, in part, to the contribution of paracrine interactions with the host mammary stroma [3,4].

Breast cancers are also highly complex tissues with carcinoma cells constituting only one of many distinct cell types. Indeed, within many breast tumor masses, the cancer cells may represent only a small proportion (<20%) of the total cell number. The remaining cell types are often grouped together under the collective term of "tumor-associated stroma", which includes fibroblasts, myofibroblasts, macrophages, other immune cells, adipocytes and endothelial cells, among others. The role of this stroma in breast cancer pathogenesis has become an area of intense investigation due to the mounting evidence demonstrating its ability to promote tumorigenesis [5,6]. It has been repeatedly demonstrated that breast cancer development and progression is highly dependent on specialized stroma, as tumors rarely develop in the absence of this microenvironment [7,8].

This narrative focuses on reviewing the parallels between the role of stroma during normal mammary gland development with that of stroma during breast tumor development and progression. The critical function of the stroma during malignant transformation and progression, suggests that targeting it in conjunction with the carcinoma cells may be a synergistic strategy for therapeutic intervention.

2. Normal mammary development

2.1. Stromal influence on mammary fate

Mammary gland development in rodents occurs with the thickening of the ectoderm, forming an epidermal "mammary crest." Between embryonic day 11 (E11) and E12, mammary placodes develop, which give rise to the mammary nipple and the underlying ductal tree [9]. The placode is surrounded by a primary mesenchyme that is indistinguishable from the rest of the dermis, but by E14, the concentric layers of fibroblasts surrounding the placodes exhibit specialized differences in gene expression such as upregulation of steroid receptors and components of the extracellular matrix (ECM) [10,11]. As development proceeds, the placodes elongate and penetrate the secondary mesenchyme, a cluster of preadipocytes in the deeper dermis that will become the mammary fat pad.

During this developmental stage, the mesenchyme is the critical determinant of mammary fate. In elegant tissue recombination studies, non-mammalian chick and duck epidermis recombined with rabbit mammary mesenchyme was able to develop branched glandular tissue [12]. To explore the effect of the mesenchyme on functional mammary differentiation of non-mammary epithelium, dorsal skin epithelium from mouse embryos was combined with syngeneic mammary mesenchyme and grafted under the renal capsule of syngeneic hosts. When grown in hosts implanted with prolactin secreting pituitary isografts, the epithelial cells of the resulting ductal structures expressed the milk proteins casein and alpha-lactalbumin [13]. Similarly, when embryonic mammary epithelium was recombined with salivary mesenchyme and grafted under the renal capsule, the resulting outgrowths were morphologically similar to salivary glands. However, in response to hormonal stimulation, the grafted epithelium was capable of synthesizing milk proteins [14]. These studies suggest that epithelial cell contact with the mesenchyme determines the architecture of the epithelial outgrowth, however, regulation of its biosynthetic function is less clear.

While the primary fibroblastic mammary mesenchyme defines the cellular fate of the mammary gland, the secondary preadipocyte mesenchyme is critical for the characteristic shaping of ductal branching structures. Recombination of embryonic or adult mammary epithelial cells with the fibroblastic mesenchyme led to atypical ductal branching and hyperplasia, whereas grafting with preadipocytes led to normal ductal elongation [15], possibly due to differences in the composition of the basement membrane [16]. It is not clear if the preadipocytes play a similar role in human mammary development. While the mature murine mammary fat pad consists primarily of adipocytes, the developing mammary epithelium in humans remains encased in fibroblastic stroma, eventually resulting in the development of specialized interlobular and intralobular stroma in the mature tissue; further, it is thought that adipose rich tissue inhibits the growth of the human mammary epithelium [17].

Complex signaling through multiple families of ligands and their cognate receptors appear to function through temporally restricted and highly localized expression in the epidermis and mesenchyme to control development during the embryonic period. The most characterized of these families include Wnt, fibroblast growth factor (FGF), parathyroid hormone related peptide (PTHrP), and hedgehog; their signaling patterns at specific times during embryonic development have been recently reviewed [9,18–20]. Gene knockout studies in mice have demonstrated non-redundant roles for specific genes. For example, failure to express FGF10 or its receptor FGFR2b during placode development results in the inability to form mammary buds 1, 2, 3, and 5, and maintain bud 4 [21]. Although expressed during similar points in embryonic development, FGF family members appear to act in parallel with the Wnt family, as inhibition of Wnt pathways do not alter expression of FGF10 or FGFR1 [11,22]. However, these families appear to influence each other indirectly through induced transcription factors [23], such as Tbx3 [22]. While these interactions are starting to be elucidated in the mouse, little is known about the roles these families play during development in the human gland.

2.2. Stroma and growth of the ductal tree

Unlike the embryonic phase of growth, full development and differentiation of the mouse mammary gland relies on coordinated communication between circulating hormones and localized growth factors. Terminal end buds (TEBs) form at the tips of the ducts and begin to grow allometrically into the mammary fat pad [24]. At puberty, elevated circulating estrogen acts through its receptors, estrogen receptor alpha (ERα) and beta (ERβ). Transplants of ERα$^{-/-}$ epithelium into wild type glands developed only a rudimentary ductal structure limited to the nipple region [25,26], demonstrating that this receptor is critical for estrogen-induced growth of the ductal tree. Early studies suggested that ERα expression in the stroma was critical during puberty for ductal elongation, and expression within both the epithelial cells and stroma were necessary for function in the adult [27]. However, these studies were confounded by incomplete removal of ERα activity, and further investigation with a complete functional knockout revealed that epithelial ERα expression was critical at both points [26]. Epithelial cells expressing ERα do not proliferate [28–30], suggesting a paracrine interaction for growth. Interestingly, ERβ$^{-/-}$ mice do not show any overt mammary abnormalities and lactate normally [31].

Although expression of ERα in the epithelium is critical for development, stromal ERα expression appears to have a role in modulating the expression of the growth hormone receptors and their ligands that are necessary for development. Through transplant studies, roles for stromal epidermal growth factor receptor (EGFR) and growth hormone (GH) receptor in ductal elongation have been uncovered. Although embryonic lethal, EGFR$^{-/-}$ females showed normal mammary ductal development before birth, however, transplant studies demonstrated impaired ductal outgrowth