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The need for complex 3D culture models to unravel novel pathways and identify accurate biomarkers in breast cancer☆

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ABSTRACT

The recent cataloging of the genomic aberrations in breast cancer has revealed the diversity and complexity of the disease at the genetic level. To unravel the functional consequences of specific repertoires of mutations and copy number changes on signaling pathways in breast cancer, it is crucial to develop model systems that truly recapitulate the disease. Here we discuss the three-dimensional culture models currently being used or recently developed for the study of normal mammary epithelial cells and breast cancer, including primary tumors and dormancy. We discuss the insights gained from these models in regards to cell signaling and potential therapeutic strategies, and the challenges that need to be met for the generation of heterotypic breast cancer model systems that are amenable for high-throughput approaches.

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1. Introduction

Breast cancer is a heterogeneous disease, encompassing multiple entities with distinct biological and clinical features [1]. The massively parallel sequencing endeavors performed by The Cancer Genome Atlas (TCGA; www.cancergenome.nih.gov), the International Cancer Genome Consortium (ICGC [2]) and individual investigators have provided a comprehensive characterization of breast cancer mainly at the genomic, but also the transcriptomic and epigenomic level. The use of this technology has demonstrated that breast cancers harbor heterogeneous constellations of somatic mutations and only few highly recurrently mutated driver genes [3–6]. In fact, at base-pair resolution each breast cancer appears to be unique in its repertoire of genetic aberrations [6]. Despite this genetic heterogeneity seen between breast cancers, it is important to note that the number of specific signaling pathways activated in each molecular subtype of the disease seems to be limited [5]. In addition, massively parallel sequencing analyses of breast cancers have revealed intra-tumor genetic heterogeneity in a substantial proportion of cases [4,7,8]. In fact, it is currently accepted that at least a subset of breast cancers are composed of mosaics of tumor cell clones, which in addition to the founder genetic events present in all cells, also display additional genomic alterations.

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It has been posited that the microenvironment exerts selective pressures on cancer cells, such as during the metastatic process or when the environment is changed due to an external selective pressure, such as drug treatment. Indeed, there is burgeoning evidence stemming from sequencing endeavors to demonstrate that primary breast cancers and their matched distant metastases are distinct in their mutational landscapes, and enrichment of populations of cancer cells harboring specific genetic alterations in the primary versus the metastatic site and vice versa has been observed [9,10]. Also drug treatment has been reported to result in the selection of subclones, present in varying frequencies in the primary tumors, harboring mutations conferring resistance to the therapeutic agent [11–14].

The impact of environmental cues on cancer is not restricted to biological or exogenous bottlenecks as exemplified above. In fact, it is plausible that throughout tumorigenesis and tumor progression, the microenvironment plays a pivotal role, as cancer cells are exposed to local selective pressures stemming from the structural and cellular microenvironment. In fact, a tumor cell is not an island [15]; instead, breast cancer cells interact with each other and with their surrounding non-malignant cells, hormones, secreted factors and the extracellular matrix (ECM). These complex microenvironmental interactions and forces contribute profoundly to the behavior, phenotype and evolution of cancer cells. For example, in estrogen receptor-negative breast cancer, increased expression levels of immune response pathway genes or increased presence of lymphocytic infiltration have been shown by independent investigators and studies to be the strongest predictor of outcome and, potentially, of chemotherapy benefit [16–19].

Given the genomic complexity of breast cancer, understanding the epistatic interactions between mutations, as well as their effects on tissue function and endocrine, paracrine and autocrine signaling is germane for the development and validation of prognostic and predictive strategies. Most studies investigating the effect of genetic/epigenetic aberrations in vitro on specific aspects of cellular processes such as transformation, proliferation or signaling have been performed in oversimplified model systems, not taking alterations in tissue architecture, cell–cell interactions, or cell–microenvironment interactions into account. The understanding of the functional consequences of specific repertoires of genomic aberrations on signaling and pathway dependencies within and between the cancers cells but also with their surrounding microenvironment require model systems that truly recapitulate the disease. To date, the vast majority of functional studies using cancer cell lines are performed in traditional monolayer cultures, however, and culture systems that fully mirror human breast cancer, primary and metastatic, and its diverse cellular microenvironment have yet to be developed further.

Here, we provide an overview of the three-dimensional (3D) cell culture models currently being employed for the study of breast cancer, including co-culture systems. In addition, we discuss how these models can be used for the dissection of cell–cell and cell–stroma interactions and of the role of specific genetic aberrations or signaling pathways in normal and malignant mammary epithelial cells.

2. Three-dimensional cell culture models

The acini (also called alveoli in breast) and ducts of the normal mammary gland are highly organized structures, with a central lumen lined by polarized luminal epithelial cells and surrounded by an outer layer of myoepithelial cells. The epithelium is separated from the surrounding stromal ECM and stromal cells by a basement membrane (BM) (reviewed in [20]). In contrast, in invasive breast cancer, the neoplastic epithelial cells are in direct contact with the stroma [20] comprised of stromal ECM, adipose tissue, blood vessels, lymphatics as well as lymphocytes, macrophages, and fibroblasts, amongst other cell types. It has been observed that in invasive breast cancers the myoepithelial cells are generally lost, whereas there is an increase in myofibroblasts and immune cells in the stroma and enhanced vascularization [20–24].

In the 1970s it was shown that collagen gels, once floated in the cell culture medium, could allow epithelial cells of different tissues and origins to maintain much of their tissue structure and some of their differentiated functions [25–27]. The mechanisms by which the collagen gel could allow partial functional tissue-specific differentiation was not at all clear, in particular because on similar floating gels, mammary cells would produce milk proteins whereas liver cells would produce albumin [28]. Using patterns of C14-labeled glucose metabolites, we showed initially that patterns of functional differentiation indeed were cell- and tissue-specific (for review see [15]). We also showed that the milk protein (beta-casein) detected was synthesized de novo [29,30]. Subsequently, our studies have also demonstrated that there is a temporal and direct relation between the endogenous production of BM by the cells in culture and the subsequent expression of tissue-specific genes [31,32]. Thus we suggested then, and proved later in our acini model, that what allows tissue specific functions to be expressed on floating collagen gels is not the collagen gel itself, but the ability to lay down an endogenously made BM upon floatation to which the cells respond (Fig. 1).

Based on these observations we proposed that the ECM in general [33] and BM in particular, which is composed of laminins, collagens, tenascin, and proteoglycans [34], are more than simple scaffolds. Instead, we postulated a “dynamic reciprocity” between the ECM and the nucleus of a cell where the ECM provides signaling cues via transmembrane receptors and the cytoskeleton to the nuclear matrix and chromatin to maintain tissue integrity [35]. This would explain why normal epithelial cells when grown as monolayers on tissue culture plastic lose morphological organization and almost all of their tissue-specific functions and resemble cancer cells [15]. To address these problems, we developed 3D culture systems using a BM gel derived from Engelbreth–Holm–Swarm (EHS) tumors [36] (see below) to study tissue-specific functions of normal mouse mammary cells [37–39], and later normal and malignant human breast cells [40]. The basement membrane-like gels, which we call laminin-rich ECM (lrECM) gels, restore more of functional and morphological differentiation than do floating collagen gels. In fact, these 3D lrECM models allow cells to organize into structures that mimic their in vivo architecture (i.e. acini) and

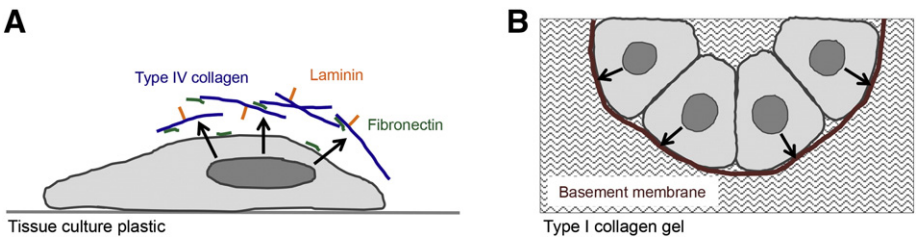

Fig. 1. De novo synthesis of extracellular matrix components of cultured normal mammary epithelial cells is dependent on the substratum. (A) Normal mammary epithelial cells cultured on tissue culture plastic secrete laminin and other ECM proteins such as fibronectin and type IV collagen but fail to organize, whereas (B) normal mammary epithelial cells cultured on type I collagen gels deposit an endogenously synthesized basement membrane and recapitulate their in vivo phenotype [31,32].