Review

Immortalization protocols used in cell culture models of human breast morphogenesis

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Abstract. Defining the key players in normal breast differentiation is instrumental to understanding how morphogenesis becomes defective during breast cancer progression. During the past 2 decades much effort has been devoted to the development of technologies for purification and expansion of primary human breast cells in culture and optimizing a relevant microenvironment, which may help to define the niche that regulates breast differentiation and morphogenesis. In contrast to the general property of cancer, normal human cells have a finite lifespan. After a defined number of population doublings, normal cells enter an irreversible proliferation-arrested state referred to as replicative senescence. To overcome this obstacle for continuous long-term studies, replicative senescence can be bypassed by treatment of cells with chemical agents such as benzopyrene, by radiation or by transfection with viral oncogenes or the gene for human telomerase (human telomerase reverse transcriptase, hTERT). A drawback of some of these protocols is a concurrent introduction of chromosomal changes, which sometimes leads to a transformed phenotype and selection of a subpopulation, which may not be representative of the tissue of origin. In recent years, we have sought to establish immortalized primary breast cells, which retain crucial characteristics of their original in situ tissue pattern. This review discusses various approaches to immortalization of breast-derived epithelial and stromal cells and the application of such cell lines for studies on human breast morphogenesis.

Key words. Breast morphogenesis; differentiation; immortalization; cell lines.

Introduction

The normal human breast comprises a branching ductal lobular system lined by an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells separated from the fibroblast-rich stroma by a basement membrane [1]. Disruption of the breast tissue architecture, including the balance between luminal epithelial and myoepithelial cells, is one of the earliest changes seen in breast cancer formation [2] and commonly used by pathologists to classify tumors into subtypes [3]. The luminal epithelial cells have received much attention in the past as the functionally active cell with tissue-specific milk secretion, and as the most likely target cell for carcinogens [4] (reviewed in [1]). This is supported by the fact that the majority of human breast carcinomas express luminal epithelial markers, and as such, most probably are derived from progenitor cells within the luminal epithelial compartment [5, 6]. Myoepithelial cells are present in normal and premalignant breast and in the major-
ity of carcinoma in situ lesions [7] but only in a minority of invasive breast cancers [8]. In general, myoepithelial tumors are of low malignancy, with the exception of malignant myoepithelioma, which is an aggressive, but rare tumor [8–10]. However, myoepithelial cells have recently attracted more attention along with the recognition of these cells as functionally active in branching morphogenesis and tumor suppression [11–13]. Taking into account the phenotypic and functional difference between luminal epithelial- and myoepithelial cells, it is of utmost importance to unravel the cellular relationship between these two lineages in the normal human breast and their roles in tissue morphogenesis, as this is a prerequisite for understanding breast cancer formation and progression. Furthermore, the breast epithelium is highly dependent on interactions with the surrounding stroma, including the fibroblasts [1, 14]. Understanding the subtleties of morphogenic signalling between cell-cell and cell-stroma will in our opinion, for the major part, rely on well-characterized immortalized breast cell lines and an appropriate cell culture assays that can capture critical aspects in breast structure and function. Studies on mouse mammary glands have provided important information regarding some aspects of mammary gland morphogenesis [15]. However, unlike human breast epithelial cells, mouse mammary cells exhibit a remarkable capacity for cell proliferation in culture and relatively often undergo spontaneous immortalization, a phenomenon almost never seen in primary human breast cells. The discussion of mouse mammary cells has been intensively covered by others [15–18] and is beyond the scope of this review.

A finite lifespan of normal breast cells

During the past 2 decades, the preferred sources of cells for studying human breast differentiation and morphogenesis have been reduction mammoplasties and milk from the lactating gland. This has provided information to an extent where cell-cell and cell-matrix interactions have proved to be important in patterns of the spatial organization of the normal and cancerous breast [13, 19–25]. However, like every other model system, there are advantages and disadvantages of employing primary epithelial cells for culture studies. The major advantage is that the cells do indeed represent the tissue of origin. Furthermore, the cells are cultured for a short period of time, and therefore have limited propensity to undergo transformation as sometimes seen in the long-term culture of immortalized cell lines [26]. The drawbacks, however, include limited access to biopsy material and a finite lifespan of the explanted cells, which may hamper long-term studies. Finally, primary cells may display inter-biopsy variations which may lessen the reproducibility of some of the morphogenic events in culture.

As first described by Hayflick [27], normal cells proliferate in culture until they reach a state where proliferation ceases. This state is named replicative senescence and is an indicator of aging [27, 28]. However, recent studies suggest that early senescence and premature cell death in culture may be attributed to inadequate culture conditions [29]. Indeed, routine tissue culture methods impose a state of oxidative stress on cells which can cause either premature senescence, cell death or adaptation [30]. Consequently, cells that do not adapt may enter growth arrest or die, and only those cells which are capable of adapting to particular culture conditions will survive for longer periods [30]. Forsyth et al. [31] compared the lifespan of fetal vs. adult human lung fibroblasts cultured at ambient (21%) and physiological oxygen concentrations (2–5%), respectively. Interestingly, the growth in low oxygen extended the lifespan of both fetal and adult strains. This is supported by the fact that when cells are grown at low O2, more cell doublings are possible [32]. The significance of O2 levels applies to breast epithelium as well. Thus, when breast epithelial cells are cultured at physiological O2 levels, the expression of the cdk (cyclin dependent kinase) inhibitor p16 is reduced, resulting in delayed senescence [33, 34].

Apparently, however, there is a distinct difference between primary breast epithelium and fibroblasts in terms of growth arrest and senescence in culture [35]. Whereas normal fibroblasts reach the plateau of senescence after approximately 50 population doublings, the isogenic breast epithelial cells can emerge from the first growth plateau [termed selection or mortality stage 0 (M0)] at a relatively high frequency (1 out of 104–10 5 cells). The postselection cells undergo further rounds of doublings before entering the second growth plateau and agonescence [previously termed senescence or mortality stage 1 (M1)] after ~75 population doublings (see fig. 1. in [35]). Cells that reach agonescence are phenotypically different from cells during selection. These cells have both a high proliferation rate and high death indexes, with no net increase in cell number [35, 36]. At the molecular level the postselection cells have low or undetectable expression of p16 [35]. Surprisingly, postselection cells exhibit chromosomal instability, which is reminiscent of the chromosomal changes observed in premalignant and malignant breast cancer [36, 37]. Recently, Holst et al. [36] have addressed the question whether the postselection epithelial cells originate as a result of proliferation arrest at the first growth plateau or whether these cells already preexist in the breast gland and are thus also present in preselection cell population. They showed that hypermethylation of the p16 promoter occurs in focal patches of the histologically normal breast [36], which indicates that cells with methylated p16 are already present in the preselection phase and that these cells could be more adapted to pass through selection than p16-expressing cells. Irrespective