The Origin of Estrogen Receptor $\alpha$-Positive and $\alpha$-Negative Breast Cancer

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Introduction

Recent advances in global gene expression analyses have led to a classification of breast tumours based on their intrinsic molecular signature rather than histological appearance, or presence/absence of one particular molecular marker. In a series of seminal papers, Perou and Sorlie working in Botstein’s laboratory in Stanford delineated four basic molecular sub-types of breast cancer (BC) that have been generally confirmed and corroborated by subsequent studies (1–5). The four major sub-types, termed basal, HER2 and luminal types A and B, have been demonstrated to engender different prognostic outcomes (6). This is partly explained by their heterogeneous expression of the ER$\alpha$, with absent or low levels in the first two sub-types and moderate or strong expression in the latter two sub-types. In this chapter, we review what is known about normal breast epithelial stem and progenitor cells, expression of ER$\alpha$ and how this informs us about the likely cellular origins of these cancer sub-types and their ER$\alpha$ status.

Breast Epithelial Stem Cells

Adult tissue stem cells are long-lived, generally quiescent cells defined by their ability both to self renew and to produce progeny that can differentiate into all the functional cell types of a particular tissue (7, 8). This may occur by symmetric or asymmetric cell division giving rise to either two new stem cells or a stem cell and an undifferentiated progenitor cell. The progenitor cells will then divide by transit amplification and generate the lineage-restricted progenitors that subsequently undergo terminal differentiation to form the functional cells of a tissue (9–11). Experimental and clinical data suggest that tissue-specific stem cells, because of their longevity, may represent the major target for mutations leading to cancer (7).

The adult mammary gland has a lobulo-alveolar structure, composed of two basic cell lineages: myoepithelial cells that form the basal layer of ducts and luminal epithelial cells that synthesize milk proteins (12). As is the case in other tissues,
the cellular repertoire of the human mammary gland is quite likely to be generated by a stem cell component. Evidence for the existence of mammary stem cells is suggested by the cyclic development, involution, and subsequent redevelopment of the mammary gland with each successive pregnancy and lactation. Seminal transplantation experiments in mice first demonstrated nearly half a century ago that isolated segments from any portion of the mammary gland are capable of regenerating a complete mammary ductal and alveolar network (13, 14). More recently, this transplantable, reconstitutive capacity was shown in the progeny of a single retrovirally marked mammary epithelial cell (15). In the past year, parallel experiments in two laboratories have confirmed that an entire mouse mammary gland can be regenerated by transplanting single cells with defined cell surface markers into cleared mammary fat pads (16, 17).

To study the functional properties of stem cells, one needs to identify and prospectively purify them, a task that has proved technically difficult because of the scarcity of stem cells in the tissue of origin, and the lack of universal morphologic traits for stem cells. Using cell surface markers, as in the studies described earlier, the purity of stem cells achieved by selection was never higher than approximately 5% of sorted cells. In the normal breast, by definition, stem cells should rarely divide and persist throughout reproductive life. They can be identified on the basis that they will retain label after the administration of labelled DNA precursors, such as [3H]-thymidine or bromodeoxyuridine (BdUr). This approach has been used by a number of groups including our own demonstration using human mammary tissue implanted into athymic nude mice (18–21). In our study, 7–9% of label-retaining cells (LRCs) after two weeks chase expressed the putative stem cell markers p21CIP1/WAF1 (cyclin-dependent kinase inhibitor) and Musashi-1 (Msi1; RNA-binding protein) (18). A characteristic also shared by a number of stem cells is their ability to exclude dyes like Hoechst or rhodamine as a result of increased expression of membrane transporter proteins, such as P-glycoproteins or BCRP (breast cancer resistance proteins) (22). This increased dye exclusion has been used to identify a sub-population of mouse mammary epithelial cells termed the “side population” (SP) similar to the SP containing haematopoietic stem cells in bone marrow (23). The multipotency of breast SP cells was demonstrated by their ability to regenerate the mouse mammary gland upon transplantation (24). A similar SP to that observed in the mouse mammary gland has also been identified by several groups in normal human breast tissue obtained from reduction mammoplasty and other non-cancer breast surgery (18, 24, 25). In the three groups who have performed human breast tissue SP analyses, the proportion of breast SP cells varied from ∼0.2% to ∼5%. Their stem cell nature has been analysed and compared with the non-SP cells using various in vitro cell culture methods. In support of their putative stem cell nature, only the cells within the SP possessed the ability to produce colonies comprising both myoepithelial and luminal epithelial cell types. In our own study, we identified a SP fraction of undifferentiated cells isolated from normal human mammary tissue, which lacked both the myoepithelial and luminal markers CALLA and MUC-1. In contrast to non-SP cells, a small fraction of SP cells, ∼1/50, formed branching structures in matrigel, reminiscent of lobular structures in vivo, and which included