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Review of: A putative human breast stem cell population is enriched for steroid receptor-positive cells

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Abstract of the original article
Breast epithelial stem cells are thought to be the primary targets in the aetiology of breast cancer. Since breast cancers mostly express oestrogen and progesterone receptor (ERalpha and PR), we examined the biology of these ERalpha/PR-positive cells and their relationship to stem cells in normal human breast epithelium. We employed several complementary approaches to identify putative stem cell markers, to characterise an isolated stem cell population and to relate these to cells expressing the steroid receptors ERalpha and PR. Using DNA radiolabelling in human tissue implanted into athymic nude mice, a population of label-retaining cells were shown to be enriched for the putative stem cell markers p21(CIP1) and Msi-1, the human homologue of Drosophila musashi. Steroid receptor-positive cells were found to co-express these stem cell markers together with cytokeratin 19, another putative stem cell marker in the breast. Human breast epithelial cells with Hoechst dye-effluxing 'side population' (SP) properties characteristic of mammary stem cells in mice were demonstrated to be undifferentiated 'intermediate' cells by lack of expression of myoepithelial and luminal apical membrane markers. These SP cells were 6-fold enriched for ERalpha-positive cells and expressed several fold higher levels of the ERalpha, p21(CIP1) and Msi-1 genes than non-SP cells. In contrast to non-SP cells, SP cells formed branching structures in matrigel which included cells of both luminal and myoepithelial lineages. The data suggest a model where scattered steroid receptor-positive cells are stem cells that self-renew through asymmetric cell division and generate patches of transit amplifying and differentiated cells.

Review

Mammary gland development, which largely occurs postnatally, involves ductal elongation and branching during puberty, followed by cycles of cellular proliferation, differentiation and apoptosis that accompany each round of pregnancy, lactation and involution. Steroid (oestrogen and progesterone) and peptide (prolactin) hormones and their cognate receptors are key drivers of these developmental processes. A requirement for tissue-specific stem cells (which by definition are pluripotent and self-renewing) would seem intuitive, given the remarkable proliferative activity that accompanies each successive round of pregnancy, as well as a need to maintain tissue homeostasis between

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pregnancies. Furthermore, the relevance of stem cells to breast cancer has been recently highlighted by the suggestion that tumours may arise from 'cancer stem cells' [1], and a report that human breast cancers contain a subset of 'tumour-initiating cells' [2].

Several studies on breast epithelial cells using human and mouse tissue support the existence of stem cells. For human breast, this evidence includes the presence of large contiguous fields of cells that exhibit identical X inactivation [3] and distinct phenotypes that arise during the culture or transplantation of primary mammary epithelial cells [4,5]. In mice, there is evidence for a subset of 'label-retaining cells' (LRCs) that retain DNA precursors (such as tritiated thymidine or bromodeoxyuridine) for several weeks following *in vivo* administration [6] and presumably correspond to stem cells that undergo asymmetric division. Electron microscopic studies have also identified 'small light' cells thought to represent putative stem cells resident in specialised niches [7]. In addition, serial transplantation of retrovirally tagged, clonally derived epithelial fragments into de-epithelialised mammary fat pads supports the notion that a single progenitor cell is capable of repopulating an entire mammary gland [8], analogous to studies on haemopoietic stem cells. Definitive proof of this model will rely on transplantation studies with single cells, and will likely require the identification of stem cell markers. In an alternative model, it has been argued that there may be an absolute requirement for more than one type of progenitor/stem cell to occupy a stem cell 'niche', to permit cellular cross-talk and reveal true stem cell behaviour. Evidence to support this model is, in part, based on the appearance of distinct limited outgrowths following serial transplantation of large numbers of cultured cells [9]. However, these findings are not incompatible with the single stem cell model, but suggest that under physiological conditions, stem/progenitor cells cooperate to govern expansion of the tissue.

New markers are clearly required to advance both the mammary stem cell and breast cancer stem cell field. There have been several recent publications that have shed light on stem/progenitor cells through the identification of putative mammary stem cell markers. Stem cell antigen-1 (Sca-1) has been reported to be enriched in mammary progenitors identified from short-term cultures of mammary epithelial cells [10]. Breast cancer resistance protein 1 (Bcrp1), also known as the ABCG2 transporter, is capable of excluding Hoechst 33342 dye [11]. Several (but not all) types of stem cells have been reported to have an increased ability to efflux Hoechst 333342 dye, identified as a 'side-population' (SP) by flow cytometry. There have been reports that SP cells in the mouse mammary gland are indeed enriched for progenitor activity [10,12]. Furthermore, nonadherent mammospheres can be preferentially derived from the SP population of human mammary epithelial cells. These mammospheres contain multilineage progenitor cells and exhibit substantial SP cell enrichment [13]. Human 'bi-potent' stem cells have been reported, based on the expression of epithelial specific antigen (ESA), mucin 1 (MUC1) and α6 integrin in normal breast tissue [14], or in an immortalised $ESA^{+ve}MUC1^{-ve}$ cell line that generates $MUC1/CK18^{+ve}$ luminal cells and $CALLA/CK14^{+ve}$ myoepithelial cells [5]. Nevertheless, the role of existing mammary stem cell markers as true markers of 'stemness' remains presumptive. Most current models could equally accommodate the markers as representing quiescent stem cells, activated stem cells or even lineage-committed progenitor cells.

In this paper, Clarke *et al.* use an athymic nude mouse model to explore putative stem cell markers in human breast tissue explants, with emphasis on their colocalisation with LRCs and steroid hormone receptor expression. Breast tissue was implanted subcutaneously into BALB/c nu/nu mice, followed by exposure to the breast mitogen oestradiol via a slow release implant [15] to permit the *in vivo* study of the human explants. LRCs were studied for up to 14 days after a ^{3}H-dT pulse and were also evaluated for proliferative capacity by BrdU (injected shortly prior to harvesting tissue for analysis) and Ki67 staining, as well as for $p27^{KIP1}$, $p21^{CIP1}$, cytokeratin 19, Msi-1 (the human homologue of *Drosophila* musachi) and steroid hormone receptor co-expression. Those studies suggested that LRCs cease to proliferate by 2 weeks following injection of radiolabel. Although LRCs were enriched for $p21^{CIP1}$ and Msi-1, the two putative stem cell markers were rarely co-expressed, suggesting that the LRCs comprise different subsets of progenitors. The $p21^{CIP1}$ positive cells were found to be located at an intermediate location between basal myoepithelial and luminal cell layers in the xenograft lobules, reminiscent of the location of the ultrastructurally defined 'small light' cell [7]. Msi-1 was expressed in cells in a luminal position, in addition to those in an intermediate position.

Dual labelling of breast epithelium for putative stem cell markers ($p21^{CIP1}$ or Msi-1) and steroid hormone receptors (either ERα or PR, since they are invariably co-expressed [16]), revealed a remarkably high proportion of cells co-expressing both sets of markers. Different subsets of steroid receptor positive-cells were found to be labelled, since p21 and Msi-1 are expressed on separate cellular subgroups. In addition, most steroid receptor-positive cells were CK19 positive, although CK19 staining was apparently widespread in some lobules.

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Msi-1 has been shown to be a positive regulator of Delta/Notch signalling and to promote asymmetric cell division. Since Notch was absent from Msi-1-positive cells, the authors inferred that Notch may be activated and cleaved from Msi1-positive cells, as has been described for neural stem cells [17]. It would be of interest to determine whether the two genes are co-expressed in breast cells at specific developmental stages, perhaps more easily shown at the RNA level. Both products were absent in primary breast tumours, possibly reflecting a 'switch' to symmetric division during tumourigenesis.

Clarke *et al.* also studied primary breast tissue for the presence of the SP cells, reported by others to contain stem/progenitor cells in both mouse and human mammary tissue [10,12]. In the current study, a substantial proportion of cells (at least 5%) were found to efflux Hoechst and were enriched for Msi-1, $p21^{CIP1}$ and ERα mRNA. In short-term 3D cultures using Matrigel and a lactogenic stimulus, SP cells gave rise to pleomorphic structures resembling alveoli, while main population (non-SP) generated small spherical colonies. These data suggested that the SP fraction contains progenitor and/or stem cells.

The mammary stem cell area is truly a field in transit [18], and this work provides important clues regarding breast stem/progenitor cells. Clarke *et al.* report new data regarding Msi-1, $p21^{CIP1}$ and ERα expression, revealing a substantial enrichment within the LRC population and SP cells and therefore progenitor populations. Studies using human tissue are complicated by the technical difficulties in establishing both the pluripotent and self-renewing properties of these cells, the hallmarks of a true stem cell. This work also raises the question as to which xenograft model provides the best milieu in which to study human breast tissue. Kuperwasser *et al.* have developed a 'humanised' fat-pad model, in which the cleared fat pad is humanised by the prior addition of human fibroblasts [19]. While the athymic nude xenograft model has considerable utility, the humanised fat pad model may facilitate the analysis of small numbers of breast epithelial cells.

There are several key questions that remain to be answered. For example, are the majority of mammary stem cells necessarily quiescent during normal tissue homeostasis? Certainly the concept of quiescent and activated stem cells is emerging in other systems, and such cells may have different phenotypes and 'niche' requirements. What proportion of stem cells undergo asymmetric vs. symmetric division and under which physiologic conditions? The observations reported by Clarke *et al.,* particularly with respect to steroid hormone receptor expression, provide important insights into the hierarchical nature of progenitor/stem cells within breast tissue.

References

1. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105–111.
2. Al-Hajj M, Wicha MS, Benito-Hernandez A, *et al*. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; **100**: 3983–3988.
3. Tsai YC, Lu Y, Nichols PW, *et al*. Contiguous patches of normal human mammary epithelium derived from a single stem cell: implications for breast carcinogenesis. *Cancer Res* 1996; **56**: 402–404.
4. Stingl J, Eaves CJ, Kuusk U, Emerman JT. Phenotypic and functional characterization in vitro of a multipotent epithelial cell present in the normal adult human breast. *Differentiation* 1998; **63**: 201–213.
5. Gudjonsson T, Villadsen R, Nielsen HL, *et al*. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. *Genes Dev* 2002; **16**: 693–706.
6. Zeps N, Dawkins HJ, Papadimitriou JM, *et al*. Detection of a population of long-lived cells in mammary epithelium of the mouse. *Cell Tissue Res* 1996; **286**: 525–536.
7. Chepko G, Smith GH. Three division-competent, structurally-distinct cell populations contribute to murine mammary epithelial renewal. *Tissue Cell* 1997; **29**: 239–253.
8. Kordon EC, Smith GH. An entire functional mammary gland may comprise the progeny from a single cell. *Development* 1998; **125**: 1921–1930.
9. Smith GH. Experimental mammary epithelial morphogenesis in an *in vivo* model: evidence for distinct cellular progenitors of the ductal and lobular phenotype. *Breast Cancer Res Treat* 1996; **39**: 21–31.
10. Welm BE, Tepera SB, Venezia T, *et al*. Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. *Dev Biol* 2002; **245**: 42–56.
11. Zhou S, Schuetz JD, Bunting KD, *et al*. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 2001; **7**: 1028–1034.
12. Alvi AJ, Clayton H, Joshi C, *et al*. Functional and molecular characterisation of mammary side population cells. *Breast Cancer Res* 2003; **5**: R1–R8.
13. Dontu G, Abdallah WM, Foley JM, *et al*. *In vitro* propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* 2003; **17**: 1253–1270.
14. Stingl J, Eaves CJ, Zandieh I, Emerman JT. Characterization of bipotent mammary epithelial progenitor cells in normal adult human breast tissue. *Breast Cancer Res Treat* 2001; **67**: 93–109.
15. Laidlaw IJ, Clarke RB, Howell A, Owen AW, Potten CS, Anderson E. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone. *Endocrinology* 1995; **136**: 164–171.
16. Clarke RB, Howell A, Potten CS, Anderson E. Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res* 1997; **57**: 4987–4991.
17. Okano H, Imai T, Okabe M. Musashi: a translational regulator of cell fate. *J Cell Sci* 2002; **115**: 1355–1359.
18. Smalley M, Ashworth A. Stem cells and breast cancer: a field in transit. *Nat Rev Cancer* 2003; **3**: 832–844.
19. Kuperwasser C, Chavarria T, Wu M, *et al*. Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc Natl Acad Sci USA* 2004; **101**: 4966–4971.