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Functional Characterization of Stem Cell Activity in the Mouse Mammary Gland

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Abstract

Any portion of the mouse mammary gland is capable of recapitulating a clonally derived complete and functional mammary tree upon transplantation into an epithelial divested mammary fat-pad of a recipient host. As such, it is an ideal model tissue for the study somatic stem cell function. This review will outline what is known regarding the function of stem/progenitor cells in the mouse mammary gland, including how progenitor populations can be functionally defined, the evidence for and potential role of selective DNA strand segregation, and the role of the niche in maintaining and controlling stem cell function.

Keywords

Stem cells; Mammary gland; Niche; Label-retaining cells; Asymmetric DNA segregation; Parity identified mammary epithelial cells; Lobule progenitor; Ductal progenitor

Introduction

The preponderance of current somatic stem cell research focuses on the characterization and identification of stem and progenitor cells in various tissues. This has led to structural and molecular definitions of these cells, based on their expression of various surface antigens and/or transcription factors. However, stem cells can really only be defined by their functional role in their tissue of origin—that is, their ability to give rise to all the other cells in the tissue. To achieve this, stem cells must actively traverse the cell cycle and undergo both symmetric and asymmetric cell divisions. Therefore, one must proceed with caution when using cellular somatic markers (i.e. epitope expression) to characterize cells ex-vivo, as the cells cannot be assumed to be static with respect to any one marker. With this proviso, we will review what is known about stem and progenitor cell function within the context of mouse mammary gland development, including how cellular hierarchies can be defined by function, the role of asymmetric DNA segregation in the maintenance and function of stem cells, and the influence of the tissue microenvironment in the regulation of these functions.

Evidence for Stem Cells in the Mouse Mammary Gland

The study of stem cell biology in the context of the mouse mammary gland really began with the pioneering studies of Charles DeOme and colleagues [1–3]. The model they

developed was the serial transplantation of mammary tissue into the cleared mammary fat pad of syngenic mice. Using this method, they demonstrated that the adult mammary gland contained cells that were capable of reconstituting a complete and functional mammary gland with a normal ductal tree and hormonal response in the epithelium-divested fat pad of the transplant recipient [3]. As transplantation of mammary fragments recapitulates mouse mammary development in its entirety, it therefore also recapitulates stem and progenitor cell activities in the intact gland with the exception that expansion of these populations is required during transplantation. For this reason, the transplantation model remains essential to the study of mammary stem cell function today. Serial transplantation of normal mammary tissue revealed that the normal gland was capable of generating multiple transplant generation (generally 5–8) before senescing. This was a direct contrast to hyperplastic glands, which could be transplanted indefinitely [4].

Subsequent studies demonstrated that cells capable of regenerating an entire mammary gland were present throughout the entire intact epithelial tree, and they persisted through various developmental states [5]. Age and reproductive history have no effect on the regenerative capacity of the mammary gland, as cells taken from 26-month old virgins have the same transplant potential as those taken from 3 week old mice, both capable of producing five serial transplant generations before reaching growth senescence. Importantly, transplantation of mammary fragments taken from mice infected with the mouse mammary tumor virus (MMTV) confirmed that outgrowths taken from any portion of the gland are clonal [6], and that the same MMTV proviral insertions could be detected through five transplant generations. This was the first direct evidence to suggest that a single pluripotent cell was capable of producing all the progeny necessary for a complete functional mammary gland, and that these functional, clonally derived “stem cells” were located throughout the entire epithelial tree of the gland.

Cellular Hierarchies as Defined by Function

In both mouse and rat, transplantation studies using limiting dilutions of mammary epithelial cells (MEC) have identified two distinct lineage limited progenitor cellular functions producing either lobule-limited or duct-limited outgrowths (Fig. 1) [6–8]. These lineage-limited outgrowths contain both luminal epithelial and basal myoepithelial cell populations. The apparent distinction between the two progenitor functions is that lobule-limited progenitors cannot produce cap cells at the tips of terminal end buds, and are therefore unable to penetrate the fat pad. Duct-limited progenitors, on the other hand, do not produce progeny capable of generating or sustaining alveolar growth and development during pregnancy (Fig. 2). Both lineage-limited activities could be identified through serial transplantations of MMTV marked clonal mammary populations, indicating that both lineage-limited progenitors were derived from a single pluripotent antecedent [6, 9]. Furthermore, serial transplantation of MMTV marked fragments revealed that ductal elongation and lobulogenesis are independently lost as the gland reaches senescence after multiple transplant generations [9].

Utilizing WAP-CRE and Rosa26-lox-stop-lox-*lacZ* (WC/R26-*lacZ*) double transgenic mice, Wagner et al. identified a cellular population in parous hosts that displayed the functional characteristics of a lobule-limited progenitor population [10]. The cells, termed parity identified mammary epithelial cells (PI-MEC), were capable of surviving involution and giving rise to all the cells comprising lobules in subsequent pregnancies (Fig. 3) [10, 11]. In this model, the transient expression of Cre recombinase (driven from the whey acidic promoter) during pregnancy permanently activates the ubiquitously expressing transgene, Rosa26-*lacZ*. PI-MEC were found to be multipotent, self-renewing, and capable of retaining their activity through serial transplantations [11, 12]. Through subsequent pregnancies, the

PI-MEC were found to proliferate to produce luminal progeny that were positive for estrogen receptor alpha or progesterone receptor, as well as luminal cells that did not express either steroid receptor. In addition, *lacZ*⁺ myoepithelial cells were seen in secretory acini, demonstrating that the PI-MEC were capable of producing both luminal and myoepithelial progeny. Originally, PI-MEC were thought to arise from de-differentiated secretory epithelial cells. However, they were subsequently found to exist in nulliparous glands by treating fragments with growth factors that induced CRE expression, but did not result in lactogenic differentiation [13]. These cells possessed all the same properties as PI-MEC including self-renewal and pluripotency. This result is consistent with the previous outlined findings of limiting dilution experiments from nulliparous donors, which produced both duct-limited and lobule-limited outgrowths in pregnant transplant hosts [7].

Multiple lines of evidence suggest PI-MEC are indispensable in mammary gland regeneration. First, when 5,000 dispersed cells from multiparous WC/R26-*lacZ* mice were inoculated into gland-free fat-pads, it was found that all resulting outgrowths contained *lacZ*⁺ cells at day 10 of pregnancy—which is prior to the de novo activation of WAP [11]. This result was somewhat surprising as *lacZ*⁺ cells represented only ~20% of the initial inoculated epithelial cell population. In the resulting outgrowths, *lacZ*⁺ cells were present in the body cells of growing terminal end buds, subtending ducts, as well as developing secretory acini. Furthermore, PI-MEC labeled with GFP following pregnancy using Wap-Cre/Chicken actin gene promoter (CAG)-flox-stop-flox-GFP double transgenic mice, were found to be exclusively in the CD49^{hi} fraction of fluorescent activated cell sorted (FACS) MEC [14]. The CD49^{hi} fraction was previously shown to contain essentially all of the mammary repopulating activity [15], and transplantation of the GFP⁺/CD49^{hi} (PI-MEC) and GFP⁻/CD49^{lo} mammary epithelial cell fractions into gland free fat pads confirmed that all of the repopulating activity was associated with the GFP⁺/CD49^{hi} fraction [14]. Importantly, outgrowths generated from transplants of tissue or cells from involuted WC/R26-*lacZ* mice never contained exclusively *lacZ*⁺ cells, suggesting PI-MEC are incapable of forming a complete gland per se. Therefore, PI-MEC, although indispensable for mammary gland regeneration, likely do not function as pluripotent mammary stem cells.

Several lines of evidence suggest that progenitor cells, particularly lobule-limited progenitors, are the targets of mammary tumorigenesis in mice. It has been shown that PI-MEC derived from WAP-TGFβ1:WC/R26-*lacZ* triple transgenic mice are incapable of self renewal upon transplantation [11]. Expression of TGF-β1 from the WAP promoter had previously been shown to reduce mammary cancer risk [12, 16]. Recently, direct evidence has shown that PI-MEC are the target of MMTV-Erb2 induced tumorigenesis [17, 18] and that lobule-progenitors are also the likely targets of tumorigenesis in ETV6-NTRK3 transgenic mice [19]. The susceptibility of functional progenitor populations to tumorigenesis may reflect their relatively active proliferative state as compared to that of stem cell function in the intact gland.

It should be noted that FACS analysis of MEC cells have identified cell populations that are capable of yielding mammospheres in-vitro that exclusively express basal or luminal markers [20, 21]. Such cell populations are often referred to as luminal and myoepithelial progenitors, respectively, and are often incorporated in mammary stem/ progenitor hierarchy models that differ from what is outlined in Fig. 2 [22]—which is based solely on in-vivo transplantation data. However, no functional evidence exists to demonstrate these cells are capable of self-renewal in-vivo, and transplantation of limiting dilutions of MEC never produce structures containing only luminal or basal cells. The latter point suggests that these cell populations are, at the very least, incapable of functioning per se, without the presence of a common antecedent (i.e. duct-limited or lobule-limited progenitors), and therefore may represent cells capable of only transient amplification in-vivo.

Is Asymmetric DNA Segregation a Function of Stem/ Progenitor Cells?

In 1975, John Cairns first postulated that somatic stem cells might protect their genome by selectively retaining their template DNA strand, sending the newly synthesized DNA to their daughter cell during asymmetric divisions [23]. Armed with DNA labels such as [3H]-thymidine (3HTdR) and 5-bromo-deoxyuridine (BrdU), researchers have long searched for evidence to support Cairns' hypothesis in various cells and tissues [24–28]. In the mouse mammary gland, epithelial cells labeled with 3HTdR during allometric growth retain label following extended chase periods (Fig. 4) [28–30]. Cells that retain their label over long periods of time must have either survived but not divided during the extended chase period, or divided but selectively retained their template DNA strands. To differentiate these two possibilities, mice were treated with BrdU following extensive chase of 3HTdR to label any newly synthesized strands of DNA [28]. Following a two-day BrdU pulse, approximately 82% of the 3HTdR-marked LREC were also positive for BrdU, suggesting at least 82% of the LREC were, in fact, actively synthesizing DNA (Fig. 4c). Following a 5 day chase period, the percent of LREC that were negative for BrdU rose to ~85%, consistent with the idea that the newly synthesized label had been passed to daughter cells during asymmetric divisions (Fig. 4d). As discussed earlier, cells capable of regenerating the entire gland are present throughout the intact mammary tree. Therefore, stem cells must divide symmetrically during allometric growth to account for their presence throughout the tree. The preceding results were interpreted to mean that 3HTdR is incorporated into stem/progenitor cells at their inception (during symmetric division) and is then retained through subsequent asymmetric cell divisions, in which newly synthesized DNA strands (marked with BrdU) are passed onto daughter cells.

Selective segregation of DNA label was observed in ER α -positive, PR-positive, and ER α /PR-negative mammary epithelium [31, 32], as well as in PI-MEC [28]. Furthermore, selective DNA segregation persists in LREC during pregnancy [32]. It is clear from the diversity of cells that retain and/or asymmetrically segregate DNA label, that asymmetric DNA segregation and label retention are not unique properties of stem cells. It would seem beneficial for any cell that must survive repeated replicative cycles to selectively retain its DNA template strand, as this would prevent the accumulation of mutations in long-lived dividing cells.

Protection against mutations may not be the only reason a cell might selectively retain its template strand. Klar has proposed the strand-specific imprinting and selective chromatid segregation (SSIS) model as a mechanism to drive asymmetric cell divisions [33–35]. In the model, differential epigenetic markings on complementary strands of DNA would allow for the generation of non-equivalent sister chromatids, which are then partitioned non-randomly to generate two cells with differing epigenetic states at specific genetic loci. This would “prime” one daughter cell for differentiation, and the other to retain stem/progenitor cell function. This concept was first described in fission yeast, where asymmetric DNA segregation controls expression of the *mat1* locus, conferring developmental asymmetry in the daughter cells [36]. In mammalian cells, mouse chromosome 7 has been shown to have a cell-type specific inheritance pattern, consistent with an SSIS model for differentiation [37]. It is important to note that Klar's SSIS model and Cairn's “immortal strand” hypothesis are not mutually exclusive, and may both account for the observation of cycling LREC in the mouse mammary gland. Future research designed to identify which chromosomes are retained in cycling LREC is needed to determine whether the “immortal strand,” SSIS, or a combination of both hypotheses accounts for the observed asymmetric DNA segregation in the mammary gland.

As discussed earlier, the mammary gland can be transplanted through 5–8 generations and the age and reproductive history of the donor mouse has no effect on the glands regenerative capacity [4, 5]. Therefore, the mouse mammary stem cell is a long-lived cell that is relatively quiescent, and likely segregates its DNA asymmetrically if and when it does divide in the intact gland. Upon transplantation, the stem cell must divide symmetrically to repopulate the gland. Therefore, the lifespan of the stem cell may only be limited by the amount of symmetric divisions it undergoes during gland regeneration. In other words, the stem cell may be essentially immortal so long as it interacts with the correct niche in the intact gland. The ability of the stem cell to out-survive the organism likely explains why label-retaining cells could be identified even after extended chase periods.

Defining Stem Cells by Their Niche

Schofield first postulated the concept of the stem cell niche as a specific location that supported and maintained hematopoietic stem cell function [38]. The theory was proposed to explain why cells isolated from aged mice were as capable of long-term engraftment in young recipients as hematopoietic stem cells isolated from young donors. Schofield postulated that the stem cells were essentially immortal so long as they resided in the niche. In this way, Schofield's model fits well with our understanding of mammary gland stem cell function.

One intriguing aspect of Schofield's theory was that cells other than the native stem cells (e.g. slightly more mature descendants of the stem cell) could function as normal stem cells when placed into the correct niche. This concept was put to the test in the mammary gland by combining stem/progenitor cells from non-mammary tissues with normal differentiated MEC during transplantation into a gland-free fat pad [39–41]. In the initial experiments, cells isolated from the seminiferous tubules of male WC/R26-*lacZ* mice were inoculated into the gland-free fat pads of Nu/Nu mice in a 1:1 ratio (50,000:50,000 cells) with normal mouse MEC (Fig. 5a–c) [39]. Following pregnancy and involution, *lacZ*⁺ cells were seen throughout the epithelial tree. By using the WC/R26-*lacZ* model, it can be determined that the male testicular cells had not only contributed to the formation of ducts and lobules, but had activated the WAP promoter during pregnancy and survived involution (i.e. had functioned as PI-MEC). Furthermore, the *lacZ*⁺ cells were shown by immunofluorescence to differentiate into luminal and basal cells, and produce milk proteins during lactation (Fig. 5b–c), and contributed to second-generation outgrowths upon transplantation.

In a follow-up experiment, isolated neuronal stem cells (NSC) from male WC/R26-*lacZ* mice were utilized in lieu of the cells isolated from the seminiferous tubules and were found also to contribute to mammary gland regeneration (Fig. 5d–f) [40]. Interestingly, it was shown that in second generation outgrowths generated from the NSC/MEC chimeric glands, that some *lacZ*⁺ cells retained expression of the NSC markers nestin and Sox2. In normal mammary outgrowths, Sox2 was not detected and nestin was expressed in a much smaller population of cells. Despite the presence of these markers in second-generation outgrowths, NSC displaying differentiation potential similar to that of the parental population could not be recovered. This demonstrates that the NSC, while functioning as mammary stem/progenitor cells, retained some of the properties of normal NSC. These results demonstrate that a strict epitope surface expression pattern is not required for stem/progenitor cell function, and underscores potential pit-falls in defining cells in this way. Further emphasizing this point, Streuli and colleagues demonstrated by genetic knockout that the purported mammary stem cell marker CD49f (α -6 integrin) is dispensable in mammary gland development [42]. Thus, the ability of a cell to function as a stem cell does not appear to be tied to surface integrin expression.

The remarkable ability of the stem cell niche to control cell fate is underscored by our recent papers that demonstrates that the normal mouse mammary niche is capable of reprogramming the human embryonal carcinoma cell line NTERA-2cl (NT2; Fig. 6) [43]. When inoculated into a clear fat-pad alone, NT2 cells either did not grow or formed tumors, but when mixed with normal MEC in a 1:10 and 1:50 ratio, they contributed progeny to the resulting normal mammary outgrowth. Like the testicular and neuronal stem cells in the previous outlined experiments, the NT2 cells were shown to express keratins (Fig. 6b1–2), produce human milk proteins during lactation (Fig. 6c), and persist in second-generation outgrowths. The latter point suggests that the NT2 cells remained viable and capable of self-renewal, and yet did not produce tumors when placed into the appropriate niche. These results obviously have important implications for stem cell and cancer research in general.

The factors that control stem cell function within the mammary niche are still poorly understood. However, it is known that signals from PR-positive MEC are essential to secretory alveolar development [44] and that ER α positive cells are necessary for ductal growth and expansion [45]. Recently, Taddei et al. demonstrated that β 1 integrin (β 1) may be an important factor in the stem cell niche [46]. Using Keratin 5-CRE/flox- β 1-flox (K5C/ β 1) double transgenic mice, the authors demonstrated that knockout of β 1 in the basal compartment resulted in a diminished transplant potential of mammary fragments. While primary transplants of mammary fragments taken from 3 week old K5C/ β 1 mice grew in 82% (79/92) of the cleared fat pads of wild-type recipient hosts, only 2/18 second-generation transplants grew out to form mammary structures. Furthermore, along with various biochemical and structural defects, the authors demonstrated that K5C/ β 1 mammary glands had an altered basal cell division axis as compared to wild-type controls [46]. Therefore it seems that β 1 is an important component of the mammary stem cell niche that may serves to sustain stem cells over time. As knockout of β 1 disrupts the division axis of epithelial cells, it is interesting to speculate that this may have an effect on stem/progenitor cells' ability to asymmetrically divide their chromosomes, and this in turn results in loss of long-term stem cell function. A similar alteration of the division axis was recently shown to disrupt asymmetric DNA segregation in the colon [47].

Studies with PI-MEC (outlined earlier) suggest that these cells (i.e. lobule-limited progenitors) are essential for mammary regeneration and therefore can be considered necessary components of the mammary stem cell niche. This paints a complex picture in which pluripotent stem cells, lineage-limited progenitors, fully differentiated cells, and the stromal environment all signal and contribute to the stem cell niche during growth and development of the mammary gland. Furthermore, the components of the mammary niche need not be necessarily static throughout development. These observations serve to blur the lines of the traditional mammary stem/progenitor hierarchy. If a cell from the seminiferous tubules can function as a mammary stem/progenitor cell when it interacts with the correct niche, perhaps cellular niches also control the differentiation potential of lineage-limited progenitors. In this way, the stem cells, lobule-limited and duct-limited progenitors may not have significant intrinsic differences, but rather display different functions through interactions with different mammary niches. This model is analogous to a recent finding in the drosophila midgut [48]. In this system, adult midgut progenitors are amplified through successive symmetric divisions leading to an island of identical progenitor cells which are maintained by a transient niche formed from a peripheral cell. As the gland develops, the peripheral cell is lost and one of the progenitors is recruited to a stem cell niche and becomes the intestinal stem cell, while the others become enteroblasts. Whether or not clonal populations of mammary stem/progenitor cells are partitioned into specific functional roles as the gland develops, or whether a primordial stem cell asymmetrically divides to create progenitor cells in the earliest stages of gland development is unknown. Future

experiments will look to elucidate the role of mammary niche(s) in the control of lineage-limited progenitor functions.

Conclusions

Mammary outgrowths are clonally derived, and cells capable of regenerating an entire functional mammary gland are present throughout the entire epithelial tree. In addition to the functional stem cells, repeated passage of tissue fragments or transplantation of limiting dilutions of dispersed cell populations has revealed two lineage-limited progenitor populations—lobule-limited and duct-limited. Lobule-limited progenitor cells appear to be represented by the PI-MEC cells in involuted WC/R26-*lacZ* mice. Populations of cells exist within the mammary gland that are capable of selectively retaining their template DNA strand. These cycling LREC are not a homogenous population and include cells with different hormone receptor expression and are present within the PI-MEC population. Therefore it is incorrect to define LREC as “stem cells.” The role of selective strand segregation in stem cell maintenance and function is not yet understood. Finally, evidence from reprogramming studies in the mouse mammary gland suggest that the niche is responsible for the control of stem/progenitor cell function and that cells act differently when they are allowed to interact with different niches.

The studies outlined in this review serve as a reminder for what is understood about stem cell function within the mammary gland. In the modern fast paced scientific community, it is important that these studies not be forgotten and replaced by misconceptions generated by defining stem cells solely based on their protein expression patterns. “Stemness” is best understood as a cellular function, and if we are to gain a greater understanding of the dynamics that control tissue growth and development, it is important not to lose sight of the dynamic interplay at work controlling the function of all cells. Future studies should focus on how all of the various cellular components of the mammary gland contribute to the niche to control and maintain stem/progenitor cell function. Elucidating these complex interactions is pivotal for understanding tissue development and tumorigenesis.

References

1. Deome K, Faulkin LJ, Bern H, Blair P. Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Research*. 1959; 19:515–520. [PubMed: 13663040]
2. Faulkin LJ Jr, Deome KB. Regulation of growth and spacing of gland elements in the mammary fat pad of the C3H mouse. *Journal of the National Cancer Institute*. 1960; 24:953–969. [PubMed: 13821714]
3. Daniel CW, Deome KB. Growth of mouse mammary glands in vivo after monolayer culture. *Science*. 1965; 149:634–636. [PubMed: 14331183]
4. Daniel CW, Aidells BD, Medina D, Faulkin LJ Jr. Unlimited division potential of precancerous mouse mammary cells after spontaneous or carcinogen-induced transformation. *Federation Proceedings*. 1975; 34:64–67. [PubMed: 162797]
5. Smith GH, Medina D. A morphologically distinct candidate for an epithelial stem cell in mouse mammary gland. *Journal of Cell Science*. 1988; 90(Pt 1):173–183. [PubMed: 3198708]
6. Kordon EC, Smith GH. An entire functional mammary gland may comprise the progeny from a single cell. *Development*. 1998; 125:1921–1930. [PubMed: 9550724]
7. Smith GH. Experimental mammary epithelial morphogenesis in an in vivo model: evidence for distinct cellular progenitors of the ductal and lobular phenotype. *Breast Cancer Research and Treatment*. 1996; 39:21–31. [PubMed: 8738603]
8. Kamiya K, Gould MN, Clifton KH. Quantitative studies of ductal versus alveolar differentiation from rat mammary clonogens. *Proceedings of the Society for Experimental Biology and Medicine*. 1998; 219:217–225. [PubMed: 9824544]

9. Smith GH, Boulanger CA. Mammary stem cell repertoire: new insights in aging epithelial populations. *Mechanisms of Ageing and Development*. 2002; 123:1505–1519. [PubMed: 12425957]
10. Wagner KU, Boulanger CA, Henry MD, Sgagias M, Hennighausen L, Smith GH. An adjunct mammary epithelial cell population in parous females: its role in functional adaptation and tissue renewal. *Development*. 2002; 129:1377–1386. [PubMed: 11880347]
11. Boulanger CA, Wagner KU, Smith GH. Parity-induced mouse mammary epithelial cells are pluripotent, self-renewing and sensitive to TGF-beta1 expression. *Oncogene*. 2005; 24:552–560. [PubMed: 15580303]
12. Boulanger CA, Smith GH. Reducing mammary cancer risk through premature stem cell senescence. *Oncogene*. 2001; 20:2264–2272. [PubMed: 11402321]
13. Booth BW, Boulanger CA, Smith GH. Alveolar progenitor cells develop in mouse mammary glands independent of pregnancy and lactation. *Journal of Cellular Physiology*. 2007; 212:729–736. [PubMed: 17443685]
14. Matulka LA, Triplett AA, Wagner KU. Parity-induced mammary epithelial cells are multipotent and express cell surface markers associated with stem cells. *Developmental Biology*. 2007; 303:29–44. [PubMed: 17222404]
15. Stingl J, Eirew P, Ricketson I, et al. Purification and unique properties of mammary epithelial stem cells. *Nature*. 2006; 439:993–997. [PubMed: 16395311]
16. Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G, Smith GH. Ectopic TGF beta 1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Developmental Biology*. 1995; 168:47–61. [PubMed: 7883078]
17. Jeselsohn R, Brown NE, Arendt L, et al. Cyclin D1 kinase activity is required for the self-renewal of mammary stem and progenitor cells that are targets of MMTV-ErbB2 tumorigenesis. *Cancer Cell*. 2010; 17:65–76. [PubMed: 20129248]
18. Henry MD, Triplett AA, Oh KB, Smith GH, Wagner KU. Parity-induced mammary epithelial cells facilitate tumorigenesis in MMTV-neu transgenic mice. *Oncogene*. 2004; 23:6980–6985. [PubMed: 15286714]
19. Li Z, Tognon CE, Godinho FJ, et al. ETV6-NTRK3 fusion oncogene initiates breast cancer from committed mammary progenitors via activation of AP1 complex. *Cancer Cell*. 2007; 12:542–558. [PubMed: 18068631]
20. Asselin-Labat ML, Sutherland KD, Barker H, et al. Gata-3 is an essential regulator of mammarygland morphogenesis and luminal-cell differentiation. *Nature Cell Biology*. 2007; 9:201–209.
21. Sleeman KE, Kendrick H, Robertson D, Isacke CM, Ashworth A, Smalley MJ. Dissociation of estrogen receptor expression and in vivo stem cell activity in the mammary gland. *The Journal of Cell Biology*. 2007; 176:19–26. [PubMed: 17190790]
22. Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes & Development*. 2009; 23:2563–2577. [PubMed: 19933147]
23. Cairns J. Mutation selection and the natural history of cancer. *Nature*. 1975; 255:197–200. [PubMed: 1143315]
24. Potten CS, Owen G, Booth D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. *Journal of Cell Science*. 2002; 115:2381–2388. [PubMed: 12006622]
25. Bickenbach JR. Identification and behavior of label-retaining cells in oral mucosa and skin. *Journal of Dental Research*. 1981; 60(SpecC):1611–1620. [PubMed: 6943171]
26. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell*. 1990; 61:1329–1337. [PubMed: 2364430]
27. Karpowicz P, Morshead C, Kam A, et al. Support for the immortal strand hypothesis: neural stem cells partition DNA asymmetrically in vitro. *The Journal of Cell Biology*. 2005; 170:721–732. [PubMed: 16115957]
28. Smith GH. Label-retaining epithelial cells in mouse mammary gland divide asymmetrically and retain their template DNA strands. *Development*. 2005; 132:681–687. [PubMed: 15647322]

29. Zeps N, Dawkins HJ, Papadimitriou JM, Redmond SL, Walters MI. Detection of a population of long-lived cells in mammary epithelium of the mouse. *Cell and Tissue Research*. 1996; 286:525–536. [PubMed: 8929355]
30. Zeps N, Bentel JM, Papadimitriou JM, D'Antuono MF, Dawkins HJ. Estrogen receptor-negative epithelial cells in mouse mammary gland development and growth. *Differentiation*. 1998; 62:221–226. [PubMed: 9566307]
31. Booth BW, Smith GH. Estrogen receptor-alpha and progesterone receptor are expressed in label-retaining mammary epithelial cells that divide asymmetrically and retain their template DNA strands. *Breast Cancer Research*. 2006; 8:R49. [PubMed: 16882347]
32. Booth BW, Boulanger CA, Smith GH. Selective segregation of DNA strands persists in long-label-retaining mammary cells during pregnancy. *Breast Cancer Research*. 2008; 10:R90. [PubMed: 18950502]
33. Klar AJ. A genetic mechanism implicates chromosome 11 in schizophrenia and bipolar diseases. *Genetics*. 2004; 167:1833–1840. [PubMed: 15342521]
34. Klar AJ. A model for specification of the left-right axis in vertebrates. *Trends in Genetics*. 1994; 10:392–396. [PubMed: 7809944]
35. Klar AJ. Support for the selective chromatid segregation hypothesis advanced for the mechanism of left-right body axis development in mice. *Breast Disease*. 2008; 29:47–56. [PubMed: 19029624]
36. Klar AJ. Differentiated parental DNA strands confer developmental asymmetry on daughter cells in fission yeast. *Nature*. 1987; 326:466–470. [PubMed: 3561486]
37. Armakolas A, Klar AJ. Cell type regulates selective segregation of mouse chromosome 7 DNA strands in mitosis. *Science*. 2006; 311:1146–1149. [PubMed: 16497932]
38. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978; 4:7–25. [PubMed: 747780]
39. Boulanger CA, Mack DL, Booth BW, Smith GH. Interaction with the mammary microenvironment redirects spermatogenic cell fate in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:3871–3876. [PubMed: 17360445]
40. Booth BW, Mack DL, Androutsellis-Theotokis A, McKay RD, Boulanger CA, Smith GH. The mammary microenvironment alters the differentiation repertoire of neural stem cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:14891–14896. [PubMed: 18809919]
41. Boulanger CA, Smith GH. Reprogramming cell fates in the mammary microenvironment. *Cell Cycle*. 2009; 8:1127–1132. [PubMed: 19282662]
42. Klinowska TC, Alexander CM, Georges-Labouesse E, et al. Epithelial development and differentiation in the mammary gland is not dependent on alpha 3 or alpha 6 integrin subunits. *Developmental Biology*. 2001; 233:449–467. [PubMed: 11336507]
43. Bussard KM, Boulanger CA, Booth BW, Bruno RD, Smith GH. Reprogramming human cancer cells in the mouse mammary gland. *Cancer Research*. 2010; 70:6336–6343. [PubMed: 20647316]
44. Briskin C, Park S, Vass T, Lydon JP, O'Malley BW, Weinberg RA. A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:5076–5081. [PubMed: 9560231]
45. Mallepell S, Krust A, Chambon P, Briskin C. Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:2196–2201. [PubMed: 16452162]
46. Taddei I, Deugnier MA, Faraldo MM, et al. Beta1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells. *Nature Cell Biology*. 2008; 10:716–722.
47. Quyn AJ, Appleton PL, Carey FA, et al. Spindle orientation bias in gut epithelial stem cell compartments is lost in precancerous tissue. *Cell Stem Cell*. 2010; 6:175–181. [PubMed: 20144789]
48. Mathur D, Bost A, Driver I, Ohlstein B. A transient niche regulates the specification of *Drosophila* intestinal stem cells. *Science*. 2010; 327:210–213. [PubMed: 20056890]

Fig. 1.

a and b Example of a lobule-limited outgrowth. **a** Whole mounted mammary fat pad of lobule-limited outgrowth in a full term pregnant host developed from a limiting dilution inoculum of mammary cells. **b** A cross-section of a lobule limited outgrowth reveals presence of luminal and myoepithelial cells (*arrows*). **c and d** Example of a duct-limited outgrowth. **c** A whole-mounted mammary fat pad of a duct limited outgrowth from a full term pregnant host with attendant terminal end buds (*arrows*) and no secretory lobule development. **d** Section through the duct-limited growth shown in (**c**) demonstrating the presence of both luminal and myoepithelial cells (*arrows*)

Fig. 2.

Model of stem/progenitor cellular hierarchy in the mouse mammary gland based on in-vivo transplantation. The pluripotent stem cell gives rise to duct-limited and lobule-limited progenitors. Both progenitor populations are multipotent. The lobule progenitor gives rise to ER+/- and PR+/- luminal epithelial cells and myoepithelial cells. Duct-limited progenitors also give rise to ER+/- and PR+/- luminal epithelial cells as well as the cap cells during ductal elongation. The cap cells ultimately differentiate into the myoepithelial cells of the ducts. Importantly, stem, lobule-limited, and duct-limited functions all seem to be required for complete mammary development

Fig. 3. Identification of PI-MEC in WC/R26-*lacZ* mice. **a** Tissue section of a nulliparous WC/R26-*lacZ* mouse is negative for lacZ expression. **b** Tissue sections from parous WC/R26-*lacZ* mouse following involution demonstrates presence of lacZ positive cells (i. e. PI-MEC) near the extremities of ducts. **c–e** Mammary whole mount (**c**) and tissue sections (**d** and **e**) of WC/R26-*lacZ* mice at day 8 of second gestation period reveal PI-MEC proliferate and function as alveolar progenitors (**c** and **d**) in subsequent pregnancies but remain largely absent and inactive in primary ducts (**e**). **f–h** Transplantation of PI-MEC results in second generation outgrowths that contain lacZ positive cells along primary ducts (**f**) and in the terminal end bud (**g**). **h** PI-MEC continue to function as alveolar progenitors in second generation outgrowths and contribute blue progeny to developing secretory acini

Fig. 4. Label retaining epithelial cells in the mouse mammary gland asymmetrically divide their DNA and retain their template strand. Mice were treated with $^3\text{HTdR}$ on 5 consecutive days 10 days following transplantation of mammary fragments into their epithelium divested #4 and #9 mammary glands. Following a 5 week chase period, mice were treated with BrdU on 2 consecutive days. **a** and **b** Cells that are single positive for either BrdU (**a**) or $^3\text{HTdR}$ (**b**). **c** A cell that is double positive for BrdU and $^3\text{HTdR}$. **d** A juxtaposed single positive BrdU cell and a $^3\text{HTdR}$ positive cell possibly resulting from a recent asymmetric division. Scale bar=10 μM

Fig. 5.

Testicular cells (**a–c**) and NSC (**d–f**) from WC/R26-*lacZ* mice are reprogrammed by the mammary microenvironment to adopt a mammary epithelial differentiation repertoire. Testicular cells or NSC from WC/R26-*lacZ* mice were mixed in a 1:1 ratio with wild-type FVB mammary epithelial cells. **a** and **d** Tissue sections reveal *lacZ*⁺ cells within mammary structures. **b** and **e** Co-localization of beta-gal (*green*) and caseins (*red*) demonstrates testicular (**b**) and NSC (**e**) produce secretory luminal epithelial cell progeny. **c** and **f** Co-localization of beta-gal (*green*) and smooth muscle actin (*red*) demonstrates testicular (**c**) and NSC (**f**) produce myoepithelial progeny

Fig. 6.

When mixed with normal mouse mammary epithelial cells (MEC), human embryonal carcinoma (NT2)-derived cells are incorporated into the mammary epithelium and differentiate. **a1** CD133-positive NT2 cells (*red*) are present within the confines of the fat pad containing regenerated mammary ducts. **a2** human NT2 cells are present within the mammary outgrowths as determined by human-specific FISH (green, nuclear; identified with *green arrows*) and human-specific immunocytochemical staining for CD133 (*red*). **a3** NT2 cells in regenerated mammary epithelium, as determined by expression of human CD133 (*red*), differentiate into luminal epithelial cells and express ER- α (*green*). **b1** and **b2** the location of basal cells (**b1**, *red*) expressing hK14 and mouse K14 (**b2**, *green*) in consecutive sections showing the same chimeric duct. **c** human and mouse proteins are secreted into the same lumen. Simultaneous staining of chimeric mammary outgrowth for human α lactalbumin (*green*) and mouse caseins (*red*) shows that production and secretion of both mouse and human milk proteins are present in the same chimeric duct. All fluorescent sections are counterstained with DAPI (*blue*). Scale bars, 20 μ m (**a1**), 15 μ m (**a2** and **a3**), 10 μ m (**b1** and **b2**), and 25 μ m (**c**)