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Stem Cells and the Mammary Microenvironment

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Abstract

An entire mammary epithelial outgrowth, capable of full secretory differentiation, may comprise the progeny of a single cellular antecedent. This conclusion is based upon the maintenance of retroviral insertion sites within the somatic DNA of successive transplant generations derived from a single mammary fragment. In addition, dissociation of these clonal dominant glands and implantation of dispersed cells at limiting dilution demonstrated that both duct-limited and lobule-limited outgrowths were developed as well as complete, fully differentiated glands. Thus, transplantation has revealed three distinct mammary epithelial progenitors in the mouse. Recently, using cre-lox conditional activation of reporter genes, the lobule-limited progenitor was lineally marked by lacZ expression. In situ, these cells were shown to regenerate secretory lobules upon successive pregnancies. In transplant studies, they demonstrated the capacity for self-renewal and contributed to the new generation of all of the epithelial cell types among mammary secretory lobules. Using this conditional activation model, cells isolated from other tissues of the WAP-Cre/Rosa26/lacZReporter mice, co-mingled with normal wild type mammary epithelial cells and transplanted into epithelium-divested mammary fat pads, were shown to be amenable to redirection of their cell fate by interaction with the mammary microenvironment in vivo. This suggests the ascendancy of the microenvironment over the intrinsic nature of somatic stem cells.

Keywords

Mammary; stem cell; epithelium; transplantation; senescence

INTRODUCTION

It was an interest in premalignant lesions of the breast that led K.B. DeOme and his colleagues [1] to develop a biologic system to recognize, characterize and study hyperplastic nodules (HAN) in the mammary glands of mouse mammary tumor virus (MMTV)-infected mice. In the quest for a means to demonstrate that these structures were precursors to frank mammary adenocarcinoma, these investigators developed a method for removing the endogenous mammary epithelium from a mammary fat pad. Subsequently, the “cleared” pad was used as a site of implantation where suspected premalignant lesions could be placed and their subsequent growth and development could be observed. Using this approach, they were able to show that both premalignant and normal mammary implants could grow and fill the empty fat pad within several weeks. During this growth period the premalignant implants recapitulated their hyperplastic phenotype, whereas normal implants produced normal branching mammary ducts. Serial transplantation of normal and premalignant outgrowths demonstrated that while normal gland invariably showed growth senescence after several

generations, hyperplastic outgrowths did not. It soon became apparent that any portion of the normal mammary parenchyma could regenerate a complete mammary tree over several transplant generations suggesting the existence of cells capable of reproducing new mammary epithelium through several rounds of self-renewal. However it was some time later before this property was recognized as representative of the presence of mammary epithelial stem cells [2,3].

It was discovered that all portions of the mouse mammary gland appeared competent to regenerate an entire new gland upon transplantation; this triggered a series of papers relating to the reproductive lifetime of mammary cells [4–7]. The results indicated that no difference existed in the regenerative ability of mammary tissue taken from very old mice versus that taken from very young mice during serial transplantation. In addition, neither reproductive history nor developmental state had a significant impact on the reproductive longevity of mammary tissue implants. That grafts from old donors could proliferate equivalently to those from young donors in young hosts suggested to these authors that the life span of mammary cells was primarily affected by the number of mitotic divisions rather than by the passage of chronological or metabolic time. The authors in a series of experiments tested this, where mammary implants were serially transplanted [5]. The authors concluded that growth senescence in transplanted mammary epithelium was related primarily to the number of cell divisions. Conversely, mouse mammary epithelium could be transformed to unlimited division potential either spontaneously, by MMTV infection, or by treatment with carcinogens [8–10]. At the time this observation was taken to signify that “immortalization”, i.e. attainment of unlimited division potential, was an important early step in malignant transformation. In recent reports, it was conclusively shown that accelerated senescence of mammary stem cells, in situ, resulted in increased refractoriness to mouse mammary tumor virus (MMTV)-induced neoplastic transformation [11,12].

In collaboration, with D. Medina [2] an earlier marker was identified that held promise for distinguishing mammary stem cells by their ultrastructural appearance. Undifferentiated (pale) cells were found which exhibited the expected behavior of stem cells in mammary explants induced in vitro, to differentiate toward secretory cell fates. In this study, it was discovered that mouse mammary explants, like mammary epithelium in situ, contained pale or light-staining cells, and that it was only these cells that entered mitosis when mammary explants were cultured.

Chepko and Smith [13] analyzed light cells in the electron microscope utilizing their ultrastructural features to distinguish them from other mammary epithelial cells. In a retrospective analysis of light and electron micrographs, a careful and detailed scrutiny of mammary tissue was performed to determine the range of morphological features among the cell types than had previously been reported. The samples evaluated included mouse mammary explants, pregnant and lactating mouse mammary glands, and rat mammary glands from 17 stages of development beginning with nulliparous through pregnancy, lactation and involution [13–15]. Both small light cells (SLC) and undifferentiated large light cells (ULLC) were observed with condensed mitotic chromosomes indicative of their replicative competence. Partially differentiated ULLC or differentiating large light cells (DLLC) were observed in rapidly proliferating mammary epithelium during pregnancy and probably represent transient-amplifying epithelial cells committed to a secretory fate. Using all of the above features we were able to develop a more detailed description of the epithelial subtypes that comprise the mammary epithelium (Fig. 1).

A total of 3552 cells through 17 stages of rat mammary gland development were counted and the percent of each morphotype was calculated. A similar analysis was made in the mouse. This evaluation showed that the population density (number of cells/mm²) of SLC

among mammary epithelium did not change from puberty through post-lactation involution. The proportion of SLC in the epithelial population remained unchanged. This means that although the number of mammary epithelial cells increased by 27 fold during pregnancy in the mouse [17,18] the percent of SLC in the population did not change. Therefore SLC increase and decrease in absolute number at the same relative rate as the expanding epithelial cell population, suggesting that they have a capacity for self-renewal. In contrast, ULLC numbers were much more variable, perhaps indicative of their transitional nature.

ABSENCE OF SLC AND ULLC IN GROWTH SENESCENT MAMMARY TISSUES

Cell and developmental biologists who have examined growing and regenerating tissue by transmission electron microscopy have postulated that the undifferentiated cells observed within these tissues represent tissue-specific stem or progenitor cells. However, no studies have addressed the issue of whether these undifferentiated, putative stem cells persist in growth senescent tissues. Serially transplanted mammary epithelium consistently displays growth senescence beginning at the third transplant generation. The rate of aging is not uniform throughout the transplanted population and complete growth quiescence for all portions of a given outgrowth is reached subsequent to the 6th transplant generation. Mammary epithelial cells bearing the morphological characteristics of undifferentiated stem cells (i.e., SLC and ULLC) likewise disappear from senescent populations simultaneous with growth cessation [19]. In premalignant mammary epithelial populations, which exhibit indefinitely prolonged growth potential, both of these cell types (SLC and ULLC) are maintained. This observation provides further support for the conclusion that these ultrastructurally distinct mammary cells represent the mammary stem/progenitor cell population.

A study [20] of human breast epithelium demonstrated the presence of mammary epithelial cells possessing the ability to regenerate elaborate branching structures resembling mammary terminal ductal lobular units both by morphology and marker expression, in vivo and in vitro. The authors based their experimental approach upon our ultrastructural studies in the mouse mammary gland [21], which described SLC and ULLC as putative epithelial stem cells. Since SLC and ULLC do not commonly contact the duct or lobule lumen, they predicted similar cells in the human breast would be negative for sialomucin (a surface marker for luminal epithelial cells) but positive for epithelial specific antigen (ESA). Indeed they found suprabasal breast epithelial cells with these properties and demonstrated that they possessed stem cell properties. This discovery lends strong experimental support for the conclusion that the undifferentiated SLC and ULLC described here represents a multipotent epithelial cell population in the mouse and that a similar epithelial subset exists in the human breast.

EVIDENCE FOR DUCT-LIMITED AND LOBULE-LIMITED MAMMARY EPITHELIAL PROGENITORS

Limiting dilution transplantation studies provide evidence of three distinct multipotent stem/epithelial cell activities within the mouse mammary gland [22]. These distinct stem/progenitor activities are characterized by their distinctive ability to produce secretory lobules, generate branching mammary ducts or recreate the entire functional (lactating) mammary epithelium upon transplantation into a breeding host. Each appears to have the capacity for self-renewal, but the lobule-limited and ductal-restricted progenitors appear to have a smaller reproductive capacity than the fully competent progenitor. Cap cells and terminal bud formation seem to be within the province of the ductal-restricted progenitor,

whereas lobule development and expansion is absent. The opposite is true for the lobule-limited progenitor (Fig. 2). Both duct-limited and lobule-limited outgrowths possessed luminal and myoepithelial mammary subtypes (Fig. 2) and contained ER α and PR-positive epithelial cells (Fig. 3). This was taken as indicative of the multipotent nature of the lineage-limited progenitors [22]. Similar experiments with dispersed mammary cells in the rat have also demonstrated lobule-limited and ductal-limited epithelial progenitors, among the mammary epithelial population confirming and extending our observations in the mouse [23,24].

CLONAL-DOMINANT MAMMARY OUTGROWTHS COMPRISED OF THE PROGENY OF A SINGLE ANTECEDENT

Our earlier studies indicate that individual, retrovirally-tagged, epithelial stem cells, positioned throughout the mature fully developed mammary gland, have the capacity to produce sufficient differentiated progeny to recapitulate an entire functional gland [17]. Second generation outgrowths from the original transplant generation produced mammary populations, which exhibited MMTV proviral insertions identical to the original. These second generation populations were shown by dissociation and limiting dilution transplantation studies to contain all three multipotent mammary cell types described above. We therefore proposed that all three types arise from a single pluripotent precursor and that this precursor was capable of self-renewal and existed in all epithelial portions of the mouse mammary gland.

Normal mammary transplants in nulliparous hosts show growth senescence during serial propagation. Some implants that showed senescent ductal growth were able to respond to pregnancy and produce secretory lobules and milk protein *in situ* [6]. From this observation, it seemed to us that both lobule-committed and ductal-committed progenitors might exist at any one time within an implant and possess different reproductive capacities. Alternatively, a primary antecedent responsible for the generation of both of these lineage-committed progenitors loses its capacity to produce one type independent of the other. Originally, we postulated that the entire clonal-dominant outgrowth was generated from the progeny of one or a few lineally related stem cells, whose predecessor had acquired MMTV proviral insertions. During ductal growth and extension these cells were self renewed and distributed throughout the ductal tree. Then, upon subsequent transplantation the process was repeated and the same proviral tagging pattern was maintained in the subsequent generation. However, because of the existence of multipotent lineage-limited progenitors within the outgrowths, the pattern of proviral tagging observed in the original outgrowth might result from the sum of several different provirally tagged cellular clones that were maintained at a similar relative fraction throughout the gland. Further, these sub-populations might possess similar or identical growth potentials such that they were maintained in the second outgrowth generation. In an attempt to distinguish between these possibilities, we serially transplanted apparent clonal mammary populations through several generations in breeding recipients (where both ductal and lobular development are supported) to growth senescence [19]. All subsequent generations were evaluated regarding their proviral content.

The proliferative lifespan of mammary stem cells was examined in serially transplanted clonal-dominant epithelial populations. Five successive transplant generations were done (Fig. 4). The epithelial cell number in each outgrowth expands ~ 500 fold in nulliparous hosts and ~ 10,000 fold in impregnated hosts. Despite this, all resulting mammary outgrowths showed lineal identity with the original. Growth senescence was observed in some implants beginning at the third generation in impregnated recipients. The ability of an individual implant to support ductal morphogenesis and also secretory lobule development decayed at independent rates. This supports the conclusion that duct and lobule-limited

epithelial progenitors share a common antecedent. Individual implants from a single clonal-dominant outgrowth occasionally gave rise to markedly different patterns of ductal development within the same host indicating that ductal patterning may be considered an epithelial cell autonomous mechanism. This observation suggests that the epithelial progeny of local progenitor/stem cells receive specific intrinsic signals at their inception that modify their response to the local environment during duct morphogenesis. Both premalignant and malignant populations appeared focally within the aging transplants. These populations were also lineally related to the original outgrowth supporting the conclusion that the primary growth was derived clonally from one or a few lineally related antecedents. The premalignant and malignant descendant populations no longer exhibit growth senescence suggesting that they are supported by a perpetually self-renewing progenitor. Importantly both luminal and myoepithelial mammary subtypes are represented in the premalignant lobular lesions (Fig. 5). Our evidence indicates that a single mammary cell may have the capacity to self-renew through 5 transplant generations. Even some sixth generation implants show vigorous growth [19].

A PARITY-IDENTIFIED, SELF-RENEWING, MULTIPOTENT EPITHELIAL MAMMARY SUBPOPULATION

With the development of the WAP-Cre model used in combination with the Rosa26LacZ reporter mice (R26R), evidence for a LacZ-marked lobule-limited progenitor observable in parous mouse mammary epithelium surfaced [25]. These LacZ-positive, parity-identified mammary cells (PI-MEC) were found to be pluripotent, self-renewing and capable of maintaining their lobule-limited progenitor activities following serial transplantation in epithelium-free mammary fat pads when the hosts were subsequently impregnated [26]. During pregnancy in these hosts, the PI-MEC proliferated and gave rise to LacZ⁺ luminal progeny that were PR or ER α -positive and luminal progeny that were bereft of these steroid receptors. Further in the developing secretory acini, they contributed not only secretory progeny but also, LacZ-positive myoepithelial cells. Originally, it was proposed that the LacZ⁺ PI-MEC arose from de-differentiated secretory epithelial cells that had survived involution and remodeling of the mammary tissue however, further study indicated that these cells were present in the mammary tissue of nulliparous females and that they could be detected in explant cultures after treatment of the fragments with growth factors that do not induce lactogenic differentiation [27]. These cells were shown to possess all the properties of PI-MEC including self-renewal and pluripotency. These observations support and confirm those reported earlier [22], which indicated the presence of lobule-limited progenitor activity in limiting dilution transplants of epithelium from nulliparous donors in pregnant transplant hosts. More recent evidence demonstrates that PI-MEC are marked by the expression of GFP in WAP-Cre/Chicken actin gene promoter (CAG)-flox-stop-flox-GFP parous females. In these studies GFP⁺ PI-MEC were fluorescent activated cell sorted (FACS) and found to be virtually 100% present in the CD49f^{hi} population [28]. This population was shown earlier to possess essentially all of the mammary repopulating activity [29]. Subsequent transplantation of GFP⁺/CD49f^{hi} positive PI-MEC and the GFP⁻/CD49f^{lo} epithelial cells into epithelium-divested mammary fat pads indicated that all the repopulating activity was associated with the GFP⁺ fraction [28]. The foregoing data and the observations reported earlier [26] suggest strongly that PI-MEC (i.e., lobule-limited progenitors) are indispensable to mammary gland ductal reconstitution in transplanted mammary fat pads.

With respect to tumorigenesis, both lobule-limited and duct-limited hyperplasia have been repeatedly isolated and propagated from mouse mammary glands [30]. These populations do not exhibit growth senescence upon serial passage and most exhibit an increased predilection for developing stochastic mammary tumors. Their existence strongly implicates the lobule-limited and duct-limited mammary stem/progenitor cells as targets for

tumorigenic transformation. These premalignant lesions have been variously induced in the mammary epithelium by hormonal treatment, mouse mammary tumor virus (MMTV), chemical carcinogens and combinations of these agents [30,31]. The MMTV-infected populations have been definitively demonstrated to be clonally derived indicating that the hyperplasia and the subsequent mammary tumors have arisen from a single transformed cell [32].

SELECTIVE SEGREGATION OF DNA DURING ASYMMETRIC CELL DIVISION

It has been postulated that the stem cells of somatic tissues protect themselves from mutation and cancer risk by selective segregation of their template DNA strands [33,34]. Self-renewing mammary epithelial stem cells that were originated during allometric growth of the mammary ducts in pubertal females were labeled using [³H]-thymidine (³HTdR) [35]. After a prolonged chase during which much of the branching duct morphogenesis was completed, ³HTdR-label retaining epithelial cells (LREC) were detected among the epithelium of the maturing glands. Labeling newly synthesized DNA in these glands with a different marker, 5-bromodeoxyuridine (5BrdU), resulted in the appearance of doubly labeled nuclei in a large percentage of the LREC. In contrast, label-retaining cells within the stroma did not incorporate 5BrdU during the pulse indicating that they were not traversing the cell cycle. Upon chase, the second label (5BrdU) was distributed from the double-labeled LREC to unlabeled mammary cells while ³HTdR was retained. These results demonstrate that mammary LREC selectively retain their ³HTdR-labeled template DNA strands and pass newly synthesized 5BrdU-labeled DNA to their progeny during asymmetric divisions (Fig. 6). Similar results were obtained in mammary transplants containing self-renewing, LacZ-positive, lobule-limited mammary stem/progenitor epithelial cells (PI-MEC) suggesting that cells capable of expansive self-renewal may repopulate new mammary stem cell niches during the allometric growth of new mammary ducts.

Immunohistochemistry was used on murine mammary glands that had been labeled with ³HTdR during allometric growth to investigate the co-expression of DNA label-retention and estrogen receptor- α (ER α) or progesterone receptor (PR). Using the same methods, we investigated [36] the co-localization of ³HTdR and ER α or PR in mammary tissue from mice that had received treatment of estrogen, progesterone and prolactin subsequent to a long chase period to identify label-retaining cells.

Label retaining epithelial cells (LREC) comprised approximately 2.0% of the entire mammary epithelium. ER α -positive (ER α ⁺) and PR-positive (PR⁺) cells represented roughly 30–40% of the LREC subpopulation. Administration of estrogen, progesterone and prolactin altered the percent of LREC expressing ER α .

The results support the premise that a subpopulation of LREC in the murine mammary gland is ER α and/or PR-positive. This suggests that certain mammary LREC (sic stem cells) remain stably positive for these receptors, raising the possibility, that LREC comprise a hierarchy of asymmetrically cycling mammary stem/progenitor cells distinguished one from another by the presence or absence of nuclear steroid receptor expression.

DOMINION OF THE MAMMARY MICROENVIRONMENT OVER CELLULAR FATES

Somatic stem cells are maintained and regulated by their surrounding microenvironment (niche). A tissue-specific niche is a restricted locale that supports self-renewing division of stem cells and prevents them from differentiating. A simple stem cell niche has three

components, localized signaling cells and extracellular matrix controlling stem cell behavior, a specified range of signaling and stem cell(s) [37]. Any portion of the mouse mammary gland can regenerate an entire functional gland upon transplantation into a cleared mammary fat pad [1,5,38]. This capacity remains undiminished regardless of age or reproductive history of the donor [7]. Therefore, mammary stem cells are stably maintained within specific microenvironments throughout the gland for life. Mammary regeneration also occurs when dispersed epithelial cells from the glands are transplanted, suggesting that complete mammary epithelial stem cell niches may be reconstituted de novo [22,39,40]. Stepwise (limiting) dilution of dispersed mammary cells results in a reduction of the percentage of inoculated fat pads positive for mammary epithelial growth, implying reduction in the number of mammary epithelial stem cells [17,22]. We have shown that the remaining cells comprised the epithelial signaling components and might support glandular regeneration if supplied with an extraneous source of stem/progenitor cells when injected into the mammary stroma. This was proven by isolating cells from an alternative adult tissue (testis) known to possess active stem/progenitor cells throughout lifetime and introduced them along with the diluted mammary epithelial cells into epithelium-divested mammary fat pads. These testicular cells were isolated from adult male Wap-Cre/Rosa26StopLacZ bitransgenic mice, which express lacZ from the Rosa 26 promoter with the activation of Wap, which allows Cre to remove the stop signal [41]. Wap is activated during pregnancy in the mammary gland; so all subsequent host mice were bred to activate the LacZ gene for the detection of the male cells. The presence of LacZ⁺ (blue) cells signals the occurrence of testicular cell progeny within the mammary outgrowth (Fig. 7).

These results have been repeated using bona fide stem cells derived from neural tissue and bone marrow. These alternative stem cells are able to provide progeny to the developing mammary gland that differentiate into many cell types including functional milk-producing epithelial cells, differentiated ER α ⁺ and PR⁺ cells, and myoepithelial cells. Our results provide evidence for the dominion of the stem cell microenvironment over the intrinsic nature of the alternative stem cells.

The deterministic power of the mammary niche is not limited to directing normal cells derived from other tissues. Tumorigenic mammary epithelial cells can also be directed by normal niche. Cells derived from MMTV-neu induced mammary tumors are also redirected and contribute to the development of a normal, functional mammary gland. When added alone the MMTV-neu cells are tumorigenic and form mammary tumors but when the MMTV-neu cells are mixed with normal mammary epithelial cells and transplanted the resulting mammary outgrowth is chimeric with tumor-derived progeny forming secretory epithelial cells and myoepithelial cells (Booth et al., manuscript submitted). The ratio of tumorigenic cells to normal cells plays a decisive role as to whether tumors form suggesting that a niche comprised of normal cells can influence the fate of tumorigenic cells but if the microenvironment is comprised of tumor-derived cells the outcome is tumorigenesis.

The study of human mammary stem cells has been hampered until recently by the lack of in vivo models. The generation of immuno-compromised animals that minimizes tissue rejection has advanced study immensely. Recently, Kuperwaser and colleagues have developed a new in vivo model in which they “humanize” a mouse mammary fat pad by transplanting human fibroblasts into the cleared murine fat pad [42]. The human fibroblasts are allowed to establish prior to the addition of human mammary epithelial cells allowing for the epithelial to engraft in a more natural environment. The results of successful implantations of normal human organoids indicate that independent ductal, lobular and acinar structures may be generated within humanized mouse mammary fat pads by human mammary epithelial cells. This result suggests similar stem/progenitor cell hierarchies exist in human breast epithelium and mouse mammary epithelium.

We have recently begun employing human cells within our transplantation model (Booth, unpublished data). NTERA-2 cells, a human embryonal carcinoma line from a male [43], can be directed by a niche comprised of normal mammary epithelial cells to form ducts and acinar structures. These human/mouse chimeric structures within the mammary outgrowth contain cells that maintain human markers such as CD133 and ESA (epithelial specific antigen) that are expressed by the N-TERRA2 cells prior to transplantation. We are hoping to expand the types of cells we can utilize in this model to encompass additional human cell lines, specifically those derived from human breast cancers.

Using alternative tissues as sources of stem cells is not a new concept. It is currently being investigated in numerous clinical aspects. Mesenchymal stem cells (MSC) derived from bone marrow directed towards a hepatocyte fate are being used for liver transplantation [44], mature liver cells and cord blood are being used as a source of cells for islet transplantation to treat diabetes [45,46], and MSC are also being used for cartilage and bone regeneration [47]. Not only is the mammary microenvironment dominant over foreign stem cells stem cell niches in many other tissues and organs display a similar dominion.

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Cell Renewal in Murine Mammary Epithelium

Fig. 1. Schematic illustration depicting self-renewal and differentiation of stem/progenitor cells in the murine mammary epithelium.

Fig. 2.

Whole mount examples of lobule-limited and duct-only mammary outgrowths with their corresponding cross sections. Note the absence of ductal tree in the lobule only outgrowth and the absence of developing acinar structures in duct only outgrowth. The white arrows in lobule only cross section and black arrows in duct only cross section denote myoepithelial cells. Arrows in duct only whole mount indicate cap cells.

Fig. 3. Both duct-limited and lobule-limited outgrowths possessed ER α and PR-positive epithelial cells. Arrows indicate cells expressing the nuclear steroid receptors in lobule-limited and ductal-limited mammary outgrowths.

Fig. 4.

Total DNA, from serially transplanted clonal dominant epithelial populations of five successive generations, was subjected to digestion with EcoR1 followed by Southern Blot analysis. The subsequent blot was probed with a P³² – labeled MMTV-LTR-specific probe. EcoR1 cuts within the genome of the MMTV producing 2 host-viral junction fragments of each provirus insertion. In panel A, five specific host-viral restriction fragments (arrows) were found in the original outgrowth, lane 6, and in all of the succeeding generations. Transplant generations 2 through 5 are represented by the middle 4 lanes, # 2 through 5. The DNA in lane 1 is from the mammary tumor that arose in a 4th generation outgrowth. In panel B, DNA from fully developed R12 outgrowth (lane 7) is compared to DNA from a lobule-limited outgrowth in the contra-lateral gland at parturition (lane 8). In both cases, all five MMTV-host restriction fragments are detected.

Fig. 5. Hyperplastic mammary outgrowths contain both luminal and myoepithelial cells. SEM of HOG demonstrating the presence of MMTV and the development of luminal epithelial cells around the lumen with the underlying myoepithelial cells. A small light cell (SLC) is also present in a suprabasal position.

Fig. 6.

Mouse mammary glands contain nuclear label retaining cells. Nuclei positive for $^3\text{HtdR}$ alone (A, E) or 5BrdU alone (B–D). Doubly labeled 5BrdU/ $^3\text{HtdR}$ nuclei, singly labeled $^3\text{HtdR}$ -positive nuclei and 5BrdU-labeled nuclei were often juxtaposed suggesting that their labeling resulted from a recent mitotic event (double arrows in K–M). Singly labeled $^3\text{HtdR}$ nuclei in 5BrdU-labeled mammary tissues (E, N). Scale bars = 10 μm .

WAP-Cre/Rosa26-fl-stop-fl-LacZ testicular cells combine with wild type mammary epithelial cells to produce mammary gland

Fig. 7.

The mammary gland microenvironment is dominant over foreign stem cells. Freshly isolated WAP-Cre/Rosa26-fl-stop-fl-LacZ testicular cells were mixed with equal numbers of wild type mammary epithelial cells and transplanted into the “cleared” mammary fat pads of 3-week old Nu/Nu host females. After pregnancy-induced recombination lacZ expressing progeny of the testicular cells are observed throughout the recapitulated mammary gland and these cells remain after involution and into second generation mammary transplants.