Published in final edited form as:

Mol Cell Endocrinol. 2012 June 24; 357(1-2): 71–79. doi:10.1016/j.mce.2011.09.021.

Progesterone regulation of stem and progenitor cells in normal and malignant breast

Sunshine Daddario Axlund and Carol A. Sartorius*

Department of Pathology, University of Colorado Anschutz Medical Center, Denver, CO 80045, United States

Abstract

Progesterone plays an important, if not controversial, role in mammary epithelial cell proliferation and differentiation. Evidence supports that progesterone promotes rodent mammary carcinogenesis under some conditions, progesterone receptors (PR) are necessary for murine mammary gland tumorigenesis, and exogenous progestin use in post-menopausal women increases breast cancer risk. Thus, the progesterone/PR signaling axis can promote mammary tumorigenesis, albeit in a context dependent manner. A mechanistic basis for the tumor promoting actions of progesterone has thus far remained unknown. Recent studies, however, have identified a novel role for progesterone in controlling the number and function of stem and progenitor cell populations in the normal human and mouse mammary glands, and in human breast cancers. These discoveries promise to reshape our perception of progesterone function in the mammary gland, and have spawned new hypotheses for how progestins may increase the risk of breast cancer. Here we review studies on progesterone regulation of mammary stem cells in normal and malignant tissue, and their implications for breast cancer risk, tumorigenesis, and tumor behavior.

Keywords

progesterone; progesterone receptor; progenitor cells; stem cells; hormone therapy; mammary gland

1. Progesterone and progesterone receptors as promoters of mammary carcinogenesis

It was recognized in the late 19th century that ovarian hormones are critical for the survival of breast cancers, as removal of ovaries served as effective treatment for a subset of patients with advanced breast cancer (Beatson, 1896; Boyd, 1900). More than a century later, science has established that prolonged exposure to ovarian steroid hormones is a risk factor for breast cancer. This may occur as a result of a greater number of menstrual cycles, early menarche and late menopause, a transient risk tied to pregnancy, or estrogen plus progestin combination hormone therapies (HT) (Bernstein, 2002; Hankinson et al., 2004; Pike et al., 1993; Schedin, 2006). Dissecting the roles of the individual hormones estrogen (17 β -

© 2011 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author at: University of Colorado Anschutz Medical Center, 12801 E 17th Ave. MS8104, Aurora, CO 80045, United States. Tel: +1 303-724-3937; Fax: +1 303-724-3712. Carol.Sartorius@ucdenver.edu (C.A. Sartorius).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

estradiol unless otherwise noted) and progesterone, and later their receptors, in these processes became possible with the advent of molecular tools beginning in the late 1950's and 1960's. Since then, focusing on progesterone, an overwhelming body of evidence supports that progestins increase breast cancer risk. Here we summarize several milestones in this supposition.

In 1962, Huggins et al. tested the individual contribution of progesterone and estrogen to tumor incidence in a rat model of carcinogen-induced mammary tumors (Huggins et al., 1962). Female Sprague Dawley intact rats were fed a single dose of the mammary mutagen 17,12-dimethylbenz(a)anthracene (DMBA) followed by continuous treatment with estrogen, progesterone, or estrogen plus progesterone, and the incidence of mammary tumors assessed. Whereas mammary tumors formed with 100% penetrance in both control and progesterone treated animals, the time to tumor formation was greatly accelerated in the progesterone treated group. This was in contrast to estrogen alone, which delayed tumor formation, and estrogen plus progesterone, which decreased tumor incidence. Later studies also demonstrated that exogenous estrogen plus progesterone, given at the appropriate time, protects against carcinogen-induced mammary cancers in the rat model (Medina et al., 2001; Medina, 2004). The Huggins studies therefore implicate a role for progesterone alone in mammary tumor promotion. However, progesterone given in combination with estrogen does not have the same detrimental effect on tumor formation, implying that the context in which the hormone is given dictates whether it is tumor promotional. A critical role for progesterone receptors (PR) in murine mammary tumorigenesis was demonstrated in 1999 using a PR knockout mouse (PRKO) (Lydon et al., 1999). PRKO animals had significantly less DMBA-induced mammary tumors than wild-type animals. Overall, these studies support that progesterone and PR are necessary, but not sufficient, for carcinogen-induced mammary tumorigenesis in rodents.

The Women's Health Initiative and the Million Women Study established that there was a significant increase in breast cancer incidence in women taking combined estrogen plus progestin (medroxyprogesterone acetate (MPA) or norgestrel) HT compared to women taking estrogen therapy (ET) alone (Beral et al., 2011; Chlebowski et al., 2010). These results were somewhat unexpected, as the estrogen plus progestin arm of this study was projected to provide some protection against chronic estrogen exposure in the breast, as it does in the endometrium. The risk due to progestin exposure appears to be particular for post-menopausal women, as chronic exposure to progestins in birth control regimens has no significant effect on breast tumor incidence (Collaborative Group 1996). Exposure type (cyclic vs. continuous) and dose are likely to factor into these risks. Therefore, progestins play a pro-tumorigenic role in the breast in an age, and possibly dose and duration, dependent manner in women.

In recent years, studies have demonstrated that progesterone expands a transformation-sensitive stem cell population (Asselin-Labat et al., 2010; Graham et al., 2009a; Joshi et al., 2010). This led to hypotheses that progesterone may predispose the breast to carcinogenesis by activating the stem cell pool and accelerating tumor formation. This review describes the recent advances in progesterone regulation of mammary stem/progenitor cells, expansion of cells with stem-like features in breast cancers, and how this may explain progestin-driven tumorigenesis in rodent models and in women.

2. Progesterone regulation of mammary stem cells in the normal human breast and murine mammary gland

The increased risk of breast cancer due to long-term hormone exposure, and the speculation that stem cells are targets of transformation, prompted investigators to tie these two concepts

together by measuring hormonal control of stem cells in human and mouse models. To feasibly measure progesterone regulation of stem cells in the human breast, a model system that retains multiple cell types and intact PR signaling was needed. Graham et al. (Graham et al., 2009a; Graham et al., 2009b) established such a model using whole organoids isolated from reduction mamoplasties, placed into 3D culture. Their cultured organoids recapitulate the structure of normal breast lobules and retain functional PR and ER in a subset of cells, analogous to the punctate expression of these receptors in the intact human breast (Clarke et al., 1997). Progesterone treatment of human breast organoid cultures, compared to vehicle treated cultures, led to increased non-adherent mammosphere formation when cells were placed into suspension culture, and an increase in the number of cells with aldehyde dehydrogenase activity, two functions indicative of breast stem and/or progenitor cells (Dontu et al., 2003; Ginestier et al., 2007). Using triple marker immunostaining for cytokeratin 5 (CK5), mucin 1 (MUC1), and p63, they observed that organoids and mammospheres contained uncommitted progenitor cells (CK5⁺MUC1⁻p63⁻), as well as committed pre-luminal (CK5⁺MUC1⁺p63⁻), and committed pre-myoepithelial (CK5⁺MUC1⁻p63⁺) cells. Thus, the progesterone-dependent increase in mammosphere formation can likely be attributed to an increase in progenitor cell numbers in the treated organoids. Additionally, progesterone treatment of organoids led to a burst of proliferation, increasing the total number of cells in acini structures. Bromodeoxyuridine (BrdU) incorporation and dual immunostaining identified that the progesterone-induced proliferation occurred in PR-negative (PR⁻) cells (Graham et al., 2009a), a phenomenon described for the human breast where paracrine signaling from steroid receptor-positive cells triggers proliferation of neighboring steroid receptor-negative cells (Clarke et al., 1997). Expression profiling and gene ontology analyses identified that networks of genes involved in cell cycle progression and DNA replication licensing were significantly increased by progesterone treatment of organoids. These gene networks may play a pivotal role in the controlled proliferation of progenitor cells. These data thus demonstrate that progesterone treatment imparts increased proliferation to PR⁻ progenitor cells and increases measurable progenitor cell function in human breast organoid cultures. This is the only study using human breast tissue, to our knowledge, that implicates progesterone as a factor controlling human breast stem or progenitor cell numbers. One can therefore envision that progesterone contributes to controlling the abundance of transformation-sensitive stem/progenitor cells throughout a woman's life.

The murine mammary gland provides an excellent model to study the effects of hormones on stem cells. Murine mammary stem cells (MaSCs) are enriched for by fluorescent activated cell sorting (FACS) based on exclusion of lineage (Lin) positive (CD31, CD45) and erythrocyte cells (Ter-119), and expression of surface markers CD24, CD29, and CD49f. One type of gating uses the signature (Lin⁻)CD29^{hi}CD24⁺ to identify the epithelial population enriched in MaSCs (Shackleton et al., 2006). A single CD29^{hi}CD24⁺ MaSC can reconstitute an entire functional mammary gland in the cleared fat pads of recipient mice (Shackleton et al., 2006). It should be noted that the CD29^{hi}CD24⁺ fraction contains the whole of the basal/myoepithelial population *plus* the MaSCs. Conversely, the CD29^{low}CD24⁺ fraction collectively contains the luminal cell populations. A second type of gating utilizes (Lin⁻)CD24^{med}CD49f^{hi} to identify MaSCs, defined functionally as mammary repopulating units, separate from CD24^{low}CD49f^{low} myoepithelial cells, with virtually no repopulating capacity (Stingl et al., 2006). CD24^{hi}CD49f^{low} distinguishes the luminal cell fraction, which contains some progenitor cells capable of limited colony formation in adherent culture (Stingl et al., 2006). All cell fractionation scenarios will likely contain a mixture of cell populations to some degree and this should be taken into consideration when interpreting results of the discussed papers.

Two studies published in 2010 measured the number and function of murine MaSCs under different hormonal milieu in female mice (Asselin-Labat et al., 2010; Joshi et al., 2010). The investigators used surface marker signatures to measure the size of the MaSC pool during natural hormone fluctuations and in experimentally manipulated hormonal states of mice, and to isolate the MaSC-enriched and non-MaSC populations for functional analyses. The main functional measure of MaSCs used was mammary repopulating ability, measured by implanting isolated cell fractions into the cleared fat pads of recipient mice and determining the extent of formation of ductal outgrowth structures. It should be noted that this assay measures *active* stem cells, and may exclude stem cells that are in a quiescent state. Furthermore, progenitor cells have some capacity to produce outgrowths, although they tend to be smaller in size (Sleeman et al., 2007). Therefore, mammary repopulating ability should be interpreted to reflect the relative amount of activated stem/progenitor cells following hormonal manipulations.

Asselin-Labat et al. (Asselin-Labat et al., 2010) observed that although the relative number of MaSCs (using the signature CD29^{hi}CD24⁺) did not differ in mammary glands from ovariectomized (ovx) vs. wild-type mice, ovx MaSCs had significantly reduced repopulating capacity compared to wild-type MaSCs. They concluded that the original hormonal environment imparted a lasting influence on the MaSCs. By replacing individual hormones at physiological levels in ovx donor mice, they determined that neither estrogen nor progesterone alone was sufficient to influence repopulating activity, but that dual treatment with estrogen plus progesterone led to complete restoration of the repopulating function of MaSCs. The hormone combination estrogen plus progesterone therefore imparts a signal to the MaSC that influences its regenerative ability, and that signal is retained for some time. The progesterone antagonist RU486 itself did not reduce MaSC numbers, but could abrogate repopulating ability and ductal outgrowths. In aromatase deficient (ArKO) mice, which lack circulating estrogen, the number of MaSCs decreased. This either suggests a direct role for estrogen in maintaining MaSC numbers, or could be due to inefficient expression of PR in the absence of estrogen to transmit the progesterone signal. Therefore, blocking either estrogen or progesterone signaling affects the number (estrogen) or function (progesterone) of MaSCs, underscoring the importance of both hormones in maintaining a functional stem cell pool.

Joshi et al. (Joshi et al., 2010) observed that MaSC numbers (using the signature CD29^{hi}CD49^{hi}CD24⁺) peak during the diestrous phase of the murine estrous cycle at which progesterone levels are highest. This MaSC fraction is also increased 14-fold during pregnancy, when progesterone levels are high, compared to virgin mice. The authors therefore hypothesize that progesterone plays a critical role in regulating MaSC numbers in the murine mammary gland. Importantly, they propose that estrogen is merely necessary for induction of PR expression, and that progesterone is the predominant mediator of MaSC function. Studies on aged, non-cycling mice demonstrate that the MaSC pool becomes static upon cessation of the estrous cycle. In women, this more dormant population of MaSCs would likely be susceptible to a reawakening during post-menopausal hormone therapies, discussed below. In summary, these two studies prove that MaSCs are not stationary, but fluctuate with the hormone state, and importantly, that absolute MaSC numbers are not always indicative of function.

In mammary structures of humans and mice, ER⁺PR⁺ cells themselves do not necessarily proliferate upon ligand stimulation, but signal surrounding ER⁻PR⁻ cells to divide (Clarke et al., 1997; Clarke, 2004; Russo et al., 1999). Both of the above discussed papers observed that murine MaSCs, despite being void of ER and PR themselves, are subject to regulation in both number and repopulating ability by steroid hormones, particularly progesterone (Asselin-Labat et al., 2010; Joshi et al., 2010). Paracrine factors were hence hypothesized to

mediate the signal from the steroid receptor positive luminal cells to nearby basally located MaSCs. Receptor activator of nuclear factor kappa-B ligand (RANKL) was an attractive candidate since it is highly progesterone-regulated in mouse and rat mammary glands, has been shown to mediate progesterone signaling, and is critical for mammary gland development (Beleut et al., 2010; Brisken et al., 1998; Fernandez-Valdivia et al., 2009; Kariagina et al., 2010a; Mulac-Jericevic et al., 2003). Indeed, profiling studies on FACS-isolated fractions of luminal (measured as CD29^{low}CD24⁺) or myoepithelial/MaSCs (CD29^{hi}CD24⁺) showed that RANKL was increased in luminal cells at mid-pregnancy in a high estrogen/progesterone state and decreased upon ovx with concomitant sex hormone deficiency, compared to control mice (Asselin-Labat et al., 2010). Exogenous progesterone administration confirmed that P induces RANKL in the luminal cell fraction and concurrently RANK receptor expression in MaSC-enriched fraction. Treatment with RANK-Fc inhibitor or an anti-RANKL monoclonal antibody reduced clonogenicity specifically in the MaSC population. Likewise, RANKL expression increased 1000-fold in luminal cells (measured as CD24⁺CD49^{lo}) in ovx mice supplemented with estrogen plus progesterone, but not estrogen alone (Joshi et al., 2010). The latter report also found that the RANK receptor increased in estrogen plus progesterone treated mice specifically in the CD24⁺CD49^{hi} MaSC fraction. Together these data support that progesterone simultaneously drives expression of RANKL in the luminal compartment, and RANK in the MaSC compartment. Thus, RANKL is a major progesterone-mediated messenger to MaSCs. Other progesterone regulated paracrine signaling factors including amphiregulin (AREG) and Wnt4 were identified as being increased by estrogen and progesterone administration in luminal cells (Asselin-Labat et al., 2010; Joshi et al., 2010), and could potentially stimulate MaSC expansion. Table 1 lists progesterone regulated genes that may play a role in stem/progenitor cell function.

Two recent reports implicate RANKL as a key paracrine mediator in progestin-dependent mammary tumorigenesis (Gonzalez-Suarez et al., 2010; Schramek et al., 2010). These studies revisit the progestin (MPA) and DMBA mammary tumorigenesis model in mice with altered expression of the RANKL receptor (RANK) in the mammary gland, to define the role of RANKL signaling in this process. Genetic inactivation of RANK in the mammary epithelium of female RANK^{Δmam} mice prevented enrichment of MaSCs (CD24⁺CD49^{hi}) and sensitized this population to radiation-induced apoptosis following MPA treatment (Schramek et al., 2010). Furthermore, MPA/DMBA-induced mammary tumor invasiveness and overall incidence were decreased, and time to onset delayed, in female RANK^{Δmam} mice. Conversely, overexpression of RANK by a mouse mammary tumor virus (MMTV)-driven transgene accelerated pre-neoplastic mammary lesion and mammary tumor formation (Gonzalez-Suarez et al., 2010). Administration of the selective RANK-Fc inhibitor delayed the onset and decreased the incidence of MPA/DMBA-induced mammary tumors (Gonzalez-Suarez et al., 2010). Taken together, these findings provide compelling evidence of a central role for RANK/RANKL in progestin-mediated mammary tumorigenesis.

In contrast to these studies in murine models, RANKL has not been demonstrated as a paracrine factor mediating progesterone signaling in the human breast. Human breast cancer cells express RANK receptor and are responsive to RANKL when added exogenously (Jones et al., 2006). However, profiling studies of progesterone-regulated genes in normal human breast (Graham et al., 2009a), T47D breast cancer cells (Ghatge et al., 2005; Jacobsen et al., 2005; Richer et al., 2002), and PR-transfected MDA-MB-231 cells (Leo et al., 2005) have not identified RANKL as a progesterone target gene. Perhaps the loss of organized compartment signaling in breast cancers may contribute to the lack of observable progesterone-induced RANKL. Thus, while RANKL/RANK are clearly important for transmitting progesterone signals to surrounding cells in murine models, a similar role in humans has yet to be determined. Of note is that AREG appears to play a larger paracrine

role downstream of progesterone than RANKL in rat compared to mouse mammary glands (Kariagina et al., 2010a). Therefore, the human breast may utilize species specific paracrine mediators of progesterone signaling as well. Figure 1 outlines potential mechanisms of progesterone regulation of MaSCs in mouse, rat, and human.

3. Progestins reprogram breast cancer cells to a more stem-like phenotype

To study the actions of progestins in breast cancer, our laboratory utilizes a model in which xenograft tumors of ER⁺PR⁺ human breast cancer cells are grown in ovx athymic nude or NOD/SCID female mice, that are then supplemented with physiologic levels of continuous estrogen, without or with progestin (progesterone or MPA). Initially, we performed expression profiling studies on tumors derived from T47D human breast cancer cells and observed that cells grown with estrogen plus progestin had decreased mRNA expression levels of luminal CKs (CK8, CK18, and CK19) and increased mRNA expression levels of basal CKs (CK5, CK6, and CK17) (Sartorius et al., 2005). These results were confirmed by immunohistochemical staining, which demonstrated that the percent of CK5⁺ tumor cells increased from ~2% with estrogen treatment to 10–20% in estrogen plus progestin treated tumors. In 2002, Boecker and colleagues had described a population of CK5⁺ cells in the normal human breast as “common adult stem cells” (Boecker et al., 2002; Boecker and Buerger, 2003). These studies were based on immunohistochemical data demonstrating that CK5⁺ cells often transiently express the luminal marker CK18 (CK5⁺CK18⁺) or the myoepithelial marker smooth muscle actin (CK5⁺SMA⁺), but dual CK18⁺SMA⁺ cells were never observed. Therefore, they concluded that CK5⁺ cells could give rise to both luminal and myoepithelial cell lineages, and thus represent stem or bi-potent progenitor cells in the breast. Subsequent studies in the human breast have identified that CK5 is one of several markers (including SSEA-4, CK6A, CK15, and Bcl-2) signifying a candidate stem cell zone (Villadsen et al., 2007), and is expressed in both the luminal progenitor (CD49f⁺epithelial specific antigen(ESA)⁺) and MaSC (CD49f^{hi}ESA⁻) compartments, but not the differentiated luminal (CD49f⁻ESA⁺) compartment (Lim et al., 2009). We hypothesized that the progesterone-amplified CK5⁺ cells in tumors may have properties of tumor initiating, or *cancer stem cells* (CSCs). Such cells have been identified in primary human breast tumors as CD44⁺CD24^{-/low} ESA⁺ (Al-Hajj et al., 2003). Isolated CD44⁺CD24^{-/low}ESA⁺ breast cancer cells were able to form tumors when implanted in small numbers (100 cells) in NOD/SCID mice with significantly greater frequency than other cell fractions (CD44⁺CD24⁺ESA⁺, or CD44⁺CD24^{-/low}ESA⁻) collected from the same tumor. We isolated CK5⁺ and CK5⁻ cells from T47D xenograft tumors treated with estrogen or estrogen plus progestin (MPA), and compared their gene expression profiles. One of the genes with the highest CK5⁺/CK5⁻ expression ratio regardless of hormone treatment was CD44 (Horwitz et al., 2008). Several markers of the poor prognostic basal breast cancer molecular subtype, such as epidermal growth factor receptor (EGFR) and CK17 (Perou et al., 2000), also had high CK5⁺/CK5⁻ expression ratios in both estrogen and estrogen plus progestin treated tumors. Interestingly, a subset of genes including the multi-drug resistant pump ABCG2 (breast cancer resistance protein) and delta and notch-like epidermal growth factor-related receptor (DNER) had high CK5⁺/CK5⁻ expression ratios only in tumors grown with estrogen plus progestin, but not estrogen alone. These data suggest that progestins stimulate a gene expression program specifically within the more stem-like CK5⁺ tumor cells.

In xenograft human breast tumors, CK5⁺ cells are near ubiquitously CD44⁺ (>95% of CK5⁺ cells are also CD44⁺) (Horwitz et al., 2008). Thus, fractionating cells based on CD44 expression enriches for intracellular CK5 expressing cells. Using this method, the FACS-sorted Lin⁻CD44⁺ fraction of luminal tumors is 10-fold more efficient at both adherent 3D mammosphere formation *in vitro*, and tumor initiation *in vivo*, than the Lin⁻CD44⁻

population isolated from the same tumor (Horwitz et al., 2008). Colony outgrowth experiments demonstrated that while CD44⁺ cells expand proportionally with the colony (CD44⁺CK5⁻ cells), peripherally located CK5⁺ cells remain rare as colonies grow (CD44⁺CK5⁺ cells). Importantly, we observed that >95% of the rare CD44⁺CK5⁺ cells lack expression of ER and PR, while the majority of tumor cells are ER⁺PR⁺. This was in contrast to CD44⁺CK5⁻ cells, many of which are ER⁺PR⁺. One can speculate from these data that CK5⁺ tumor cells are progenitors of ER⁺PR⁺ tumor cells, and may be a more specific marker of breast CSCs than CD44, at least in some tumors.

The progesterone induction of CK5⁺ cells occurs in multiple human luminal breast cancer cell lines including T47D, MCF7, ZR-75-1, and BT-474 (Kabos et al., 2011)¹. The range of induction, starting from <1% positive cells in all cell lines, varies from 1–2% in MCF7 cells to up to 20% in T47D cells. In luminal breast cancer cell lines outside of T47D (which have constitutively high PR levels), estrogen is required for PR expression, and thus estrogen plus progesterone treatment is necessary to observe induction of CK5⁺ cells. Estrogen treatment alone has no effect on the CK5⁺ cell population of breast cancer cells (Horwitz et al., 2008; Sartorius et al., 2005). This parallels that observed for MaSC expansion in mice (Joshi et al., 2010); in this case, estrogen drives PR expression in tumor cells, and progesterone subsequently drives CK5⁺ cell expansion. It should be noted that not all ER⁺PR⁺ cell lines are susceptible to progesterone-induced expansion of CK5⁺ cells. Two cell lines isolated from pleural effusions of breast cancer patients, each of which have high endogenous ER and PR expression, have no detectable CK5⁺ cells upon progesterone or estrogen plus progesterone treatment^{2,3}. Therefore, not all PR⁺ tumor cells are capable of transducing a signal to expand the CK5⁺ cell population. Notably, dual staining of breast tumor tissue arrays demonstrates that ~50% of ER⁺ breast tumors have no detectable CK5⁺ cells within the tumor area (Kabos et al., 2011), while the other half contain various amounts of CK5⁺ cells and are considered “mixed” luminal/basal tumors with poorer prognosis than the pure luminal tumors (Abd El-Rehim et al., 2004; van de Rijn et al., 2002). This mixed luminal/basal phenotype (CK18⁺/CK5⁺) was also observed in carcinogen-induced rat mammary tumors when animals were treated with estrogen plus progesterone, but not estrogen alone (Kariagina et al., 2010b). Therefore, the two subsets of ER⁺PR⁺ tumors, one purely luminal and one with mixed luminal/basal features, may have different cells of origin - one that is susceptible to progesterone-mediated induction of CK5⁺ cells, and one that is not.

In subsequent studies, we found CK5⁺ tumor cells to be more quiescent compared to CK5⁻ cells, as measured by BrdU incorporation or Ki67 staining (Kabos et al., 2011). Consequently, CK5⁺ tumor cells were relatively resistant to apoptosis induced by docetaxel or 5-fluorouracil compared to CK5⁻ cells in the same tumors. *De novo* CK5⁺ cells or progesterone-induced CK5⁺ cells were equally resistant to chemotherapy. Interestingly, antiestrogen treatment with tamoxifen (Tam) or fulvestrant, or estrogen depletion, could mimic the effect of progestins and increase the percent of CK5⁺ cells in culture (Kabos et al., 2011). Importantly, this was also observed in tumors from patients undergoing neoadjuvant therapy with the aromatase inhibitor (AI) exemestane plus Tam. Post-therapy resected tumors had significantly increased CK5 mRNA expression compared to pre-therapy tumor biopsies from the same patients (Kabos et al., 2011). Larger clinical studies have demonstrated that the residual tumor cell population following endocrine (letrozole) or chemotherapy (docetaxel) treatment is enriched in cells with a CSC phenotype (measured as CD44⁺CD24^{-/low}) (Creighton et al., 2009). These data support the hypothesis that

¹C.A. Sartorius, unpublished data

²B.M. Jacobsen, personal communication

³C.A. Sartorius, unpublished data

exogenous progestin use could expand a population of therapeutic resistant CSCs, and thus affect breast cancer treatment outcomes.

Whereas paracrine signaling appears to be an important mechanism in progesterone-driven expansion and modulation of MaSCs in rodent mammary glands (Asselin-Labat et al., 2010; Gonzalez-Suarez et al., 2010; Joshi et al., 2010; Kariagina et al., 2010a; Schramek et al., 2010), and possibly the human breast (judging by the observed progesterone-induced proliferation of PR⁺ cells) (Graham et al., 2009a), this may not be the predominant mechanism underlying progesterone-mediated generation of breast CSCs (outlined in Figure 1). Our data suggest that progesterone acts directly on PR⁺ cells and converts them to a hormone receptor negative (ER⁺PR⁻), more stem-like (CK5⁺CD44⁺) state. Small outgrowths (<50 cells) derived from FACS isolated CD44⁺ cells contain mostly (>90%) PR⁺ cells and <10% CK5⁺ cells (1–5 per colony). When treated with progesterone for 24 hours, nearly 50% of cells become CK5⁺, with ~30% of these “trapped” in an intermediary CK5⁺PR⁺ state. At timepoints >48 hours, these intermediate cells are no longer visible and are fated to become CK5⁺PR⁻ or return to a CK5⁺PR⁺ state. Transient progesterone treatment is known to lead to a rapid proteasome-mediated downregulation of PR, which recovers in a short (1–2 day) time period (Shen et al., 2001). However, a small subset of cells appears to be primed for semi-permanent PR extinction and subsequent acquisition of stem cell characteristics. In these originally ER⁺PR⁺ cells, the receptors can remain “off” for months. Why it is that only a fraction of a cell culture or tumor population is susceptible to progesterone reprogramming remains unknown. This reprogramming can be abrogated by the antiprogesterin RU486, underscoring the importance of progesterone/PR signaling⁴. Collectively, these data strongly support that progesterone directly converts some PR⁺ cells to a more stem cell phenotype, likely through an autocrine mechanism. Of note are the recent seminal discoveries that non-CSCs can become CSCs either through spontaneous conversion or via external stimulation by factors such as interleukin 6 (Chaffer et al., 2011; Iliopoulos et al., 2011). This lends credence to progesterone being one factor that converts non-CSCs to CSCs in PR⁺ breast cancers.

Progestins increase the expression of a number of genes involved in the Notch and Wnt signaling pathways; both of these pathways help mediate the self-renewal of normal human stem cells and breast CSCs, a process by which stem cells divide asymmetrically to produce an identical multipotent stem cell and a progenitor cell, with limited replication capacity (Dontu et al., 2004; Farnie and Clarke, 2007; Lindvall et al., 2007; Roarty and Rosen, 2010). In the normal breast organoid model, progesterone treatment increased expression of delta-like 1 and 3 (DLL-1, DLL-3), the human homologues of the Notch-delta ligand, and the Notch receptor regulator presenilin 2 (PRSS2) (Graham et al., 2009a). In T47D breast cancer cells, progestins increase expression of Notch2, and Jagged 1 (JAG1), a Notch1 ligand (Ghatge et al., 2005; Horwitz et al., 2008). Interestingly, expression of Notch4, a basal-specific Notch receptor in the normal breast and mediator of breast stem cell activity (Dontu et al., 2004; Harrison et al., 2010; Leong et al., 2007), was elevated in CK5⁺, compared to CK5⁻ cells (Horwitz et al., 2008). Progesterone activates downstream Wnt4 signaling in the mouse mammary gland (Briskin et al., 2000), and increases expression of, and activates Wnt1 signaling in human breast cancer cells (Faivre and Lange, 2007). Other progesterone-regulated signaling molecules such as EGFR (Musgrove et al., 1991), are preferentially expressed in CK5⁺ compared to CK5⁻ breast cancer cells. However, blockade of EGFR in the mouse had no effect on hormone-mediated MaSC expansion (Asselin-Labat et al., 2010), and therefore its contribution to hormone regulation of stem cells, if any, remains unclear.

⁴S. D. Axlund and C.A. Sartorius, unpublished data

Whether the two naturally occurring PR isoforms, PRA and PRB, have the same propensity to stimulate stem cell expansion in the normal or malignant breast is unknown. PRA expression in murine mammary glands is restricted to luminal cells, while PRB is expressed in both luminal cells and a small population of basal and MaSCs (Joshi et al., 2010). This opens the possibility that PRB may help transmit the progesterone response in a minority population of basally located murine MaSCs. The fact that manipulating PRA:PRB ratios in the mammary gland alters cell fate decisions argues that MaSC regulation is differentially affected by PR isoforms (Shyamala et al., 2000). Utilizing breast cancer cell lines that express only PRA or PRB, we find that both PR isoforms are equally capable of reprogramming breast cancer cells to a PR⁻CK5⁺ state in tumors (Sartorius et al., 2005). Use of isoform-specific and conditional PR knockout mice (Fernandez-Valdivia et al., 2010; Mulac-Jericevic et al., 2003), as well as cells in which the PRA:PRB ratio is altered (Graham et al., 2005; Jacobsen et al., 2002), may help discern isoform-specific differences in MaSC regulation.

In conclusion, little is presently understood regarding the mechanism of regulation of CSCs by progesterone in human tumors. Progesterone appears to act directly on select PR⁺ cells, abolish PR expression, and activate a program of genes indicative of the basal breast cancer molecular subtype and CSCs. This occurs in some, but not all, PR⁺ breast cancers. Therefore, it remains to be seen how widespread this phenomenon is, and if this information could affect clinical behavior or response to treatment interventions. This mechanism appears to be distinct from the compartment-mediated MaSC expansion in the normal breast.

4. Luminal progenitor cells as the target population for progesterone expansion and transformation in HT related malignancies

Estrogen plus progestin HT as opposed to ET, or no hormone, confers a small but significant increased risk in breast cancer incidence (Beral et al., 2011; Chlebowski et al., 2010). There remains some debate over whether the natural hormone and synthetic counterparts impart the same risk (Fournier et al., 2008). Both progesterone and MPA regulate a similar program of genes in PR-rich T47D cells (Ghatge et al., 2005). Likewise, both hormones also expand CK5⁺ progenitor cells in xenograft breast tumors (Sartorius et al., 2005). A proliferative stimulus by progestins on the post-menopausal breast is suspected to facilitate the increased breast cancer risk. Based on recent advances highlighted here, it is plausible that the specific stimulation of stem or progenitor cells by progestins is at the root of the observed increase in breast tumorigenesis. In a murine model of progestin-induced mammary cancer, continuous depot MPA administration in female mice produces mammary tumors in 80% of animals at one year (Kordon et al., 1990; Lanari et al., 1986; Lanari et al., 2009). This implies that chronic stimulation of MaSCs over time leads to the appearance of tumors in the more aged gland. These MPA-induced tumors are ER⁺PR⁺, similar to the majority of tumors found in women taking HT, but uncommon for murine tumor models (Delgado and Lubian Lopez, 2001; Gajdos et al., 2000). It is therefore conceivable that the MPA-driven tumorigenic event occurred in the luminal progenitor population, resulting in the appearance of luminal ER⁺PR⁺ tumors. Of note is that the MPA stimulation is continuous, rather than intermittent, similar to many HT regimens used in women.

Evidence suggests that the post-menopausal human breast is uniquely prone to tumorigenesis instigated by progestins. Pre-menopausal women using oral contraceptives with a progestin component are not at significant risk for breast cancer (Collaborative Group 1996). While precise hormone combinations, dosing regimens, and age at HT are all likely to be contributing factors, signs still point to a fundamental change in the post-menopausal breast environment that causes susceptibility to HT tumorigenesis.

Extrapolating from the mouse model (Joshi et al., 2010), MaSC numbers likely fluctuate in the human breast in pre-menopausal women, but may become static post-menopause, when cycling hormones cease. This inactive MaSC population in post-menopausal women could be “awakened” by progestin-containing HTs (Horwitz and Sartorius, 2008). PR expression itself declines significantly post-menopause (Hargreaves et al., 1998). Therefore, the reintroduction of estrogen could restore PR expression levels, and consequently allow progestins to stimulate the dormant stem cells, thus making the estrogen/progestin HT combinations most damaging. One would speculate that these MaSCs were previously transformed, but remain dormant post-menopause, and thus require exogenous stimulation to materialize into a tumor. Progestin exposure would be one factor that supplies that stimulus. Since the vast majority of HT tumors are ER⁺PR⁺, this implies that the awakened stem cell, widely considered to be ER⁻PR⁻ (Asselin-Labat et al., 2006; Sleeman et al., 2007), is a luminal progenitor, programmed to produce ER⁺PR⁺ tumor cell progeny. A few reports have, however, identified putative ER⁺ progenitor cells (Clarke, 2005; Zeps et al., 1998), suggesting an alternative direct mechanism of progesterone-mediated tumorigenesis and expansion of ER⁺PR⁺ tumor cells from a steroid receptor-positive progenitor cell. Collectively, these data suggest that progesterone mobilization of the luminal progenitor cell population in dormant malignancies during HT mediates the increased breast cancer risk.

Several significant papers have identified the putative target cells of transformation in BRCA1 mutation carriers as luminal progenitor cells (Lim et al., 2009; Molyneux et al., 2010; Proia et al., 2011). In the human breast, these luminal progenitor cells are contained within the CD49f⁺ESA⁺ cell fraction and are CK5⁺ER⁻PR⁻ (Lim et al., 2009). Remarkably, the cell population expanded by progesterone in human breast cancer cells also displays this luminal progenitor phenotype (CD49f⁺ESA⁺CK5⁺ER⁻PR⁻) (Horwitz et al., 2008). BRCA1 tumors are typically triple negative, lacking ER, PR, or human epidermal growth factor 2, and have a basal breast cancer phenotype, signified by high CK5 and EGFR expression (Cheang et al., 2008; Perou et al., 2000). Therefore, luminal progenitor originators of BRCA1 breast cancers are considered “aberrant”, failing to produce ER⁺PR⁺ progeny (Lim et al., 2009). Pregnancy significantly elevates the risk of breast cancer in BRCA1 mutation carriers (Narod, 2001). Progesterone signaling is normally repressed by wild-type BRCA1, and PR expression is elevated in the luminal breast cells of BRCA1 mutation carriers (Katiyar et al., 2006; Ma et al., 2006). Thus, high progesterone levels during pregnancy could exacerbate breast cancer risk through excessive PR-mediated stimulation of BRCA1 mutant luminal progenitors.

While both human and mouse luminal progenitor cells are targets for breast tumor formation, it is unclear if the luminal progenitor population in the mouse correlates to the same population in humans. In fact, the human breast is unique in that it commonly contains luminal-located cells in the terminal duct lobular unit that are CK5⁺ (Boecker et al., 2002; Gusterson et al., 2005), whereas in the mouse CK5⁺ cells are considered to be markers of the myoepithelium. However, occasional staining of CK5⁺ cells is observed in the body cells and the luminal edge of mouse terminal end buds (Grimm et al., 2006). The use of CK5 as a basal breast epithelial lineage marker has been questioned (Lim et al., 2009), as has the nomenclature of “basal”, as it refers to human breast cancers that are prospectively derived from luminal-located progenitor cells (Gusterson, 2009). We put forth that these luminal-located progenitors, at least in humans, are targets of progesterone amplification, and the genesis of tumors in HT related malignancies.

Conclusions

We are now just beginning to understand the significance of progestins in the regulation of stem and progenitor cells in normal and malignant breast. The breast epithelial cell hierarchy

appears to be unique in its reaction to progesterone, as the hormone is protective in other reproductive tissues such as the endometrium. The traditional thought of progesterone as a differentiating hormone may be true, but in a context not previously considered. Progesterone serves as a master hormonal regulator of MaSCs, dictating their expansion and contraction (during low circulating hormone levels), repopulating ability, and thus affecting their differentiation into downstream progeny of different cell fates. Models to study progesterone and PR signaling in mammary stem and progenitor cells will be invaluable in pinpointing the cells of origin of hormone-dependent breast cancers. The finding that MaSC numbers and function both fluctuate with age and with circulating levels of female hormones may explain why the post-menopausal breast is particularly susceptible to progesterone-accelerated tumorigenesis. MaSCs may undergo their own unique transition at menopause that places them in more prolonged quiescence, sensitive to reintroduction of hormones, especially ones that are not cyclical. Post-menopausal hormone regimens that are deficient in MaSC amplification are conceivable; these would theoretically provide needed benefits, without increased risk of tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Unpublished studies on the role of progesterone and stem cells were funded by NIH R01 CA140985 (C.A.S.), and the Wendy Will Case Foundation (C.A.S.). The authors wish to thank Britta Jacobsen and Maggie Wierman for critical review of the manuscript. The authors wish to apologize to those whose work was not referenced due to space limitations.

Abbreviations

AI	aromatase inhibitor
AREG	amphiregulin
BrdU	bromodeoxyuridine
CK	cytokeratin
CK5	cytokeratin 5
CSC	cancer stem cell
3D	three dimensional
DMBA	7,12-dimethylbenz(a)anthracene
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ESA	epithelial specific antigen
ET	estrogen therapy
FACS	fluorescent activated cell sorting
HT	hormone therapy
Lineage	Lin
MaSC	mammary stem cells
MMTV	mouse mammary tumor virus

MPA	medroxyprogesterone acetate
MUC1	mucin 1
PR	progesterone receptor
PRKO	progesterone receptor knockout
RANK	receptor activator of nuclear factor kappa-B ligand
RANKL	receptor activator of nuclear factor kappa-B ligand
SMA	smooth muscle actin
Tam	tamoxifen

References

- Abd El-Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, Robertson JF, Nicholson RI, Ellis IO. Expression of luminal and basal cytokeratins in human breast carcinoma. *J Pathol.* 2004; 203:661–671. [PubMed: 15141381]
- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A.* 2003; 100:3983–3988. [PubMed: 12629218]
- Asselin-Labat ML, Shackleton M, Stingl J, Vaillant F, Forrest NC, Eaves CJ, Visvader JE, Lindeman GJ. Steroid hormone receptor status of mouse mammary stem cells. *J Natl Cancer Inst.* 2006; 98:1011–1014. [PubMed: 16849684]
- Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, Yasuda H, Smyth GK, Martin TJ, Lindeman GJ, Visvader JE. Control of mammary stem cell function by steroid hormone signalling. *Nature.* 2010; 465:798–802. [PubMed: 20383121]
- Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet.* 1896; 2:104–107.
- Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y, Schneider P, Briskin C. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. *Proc Natl Acad Sci U S A.* 2010; 107:2989–2994. [PubMed: 20133621]
- Beral V, Reeves G, Bull D, Green J. Breast cancer risk in relation to the interval between menopause and starting hormone therapy. *J Natl Cancer Inst.* 2011; 103:296–305. [PubMed: 21278356]
- Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia.* 2002; 7:3–15. [PubMed: 12160084]
- Boecker W, Moll R, Poremba C, Holland R, Van Diest PJ, Dervan P, Burger H, Wai D, Ina Diallo R, Brandt B, Herbst H, Schmidt A, Lerch MM, Buchwallow IB. Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept. *Lab Invest.* 2002; 82:737–746. [PubMed: 12065684]
- Boecker W, Buerger H. Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept. *Cell Prolif.* 2003; 36(Suppl 1):73–84. [PubMed: 14521517]
- Boyd S. On oophorectomy in cancer of the breast. *BMJ.* 1900; 2:1161–1167.
- 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. *Proc Natl Acad Sci U S A.* 1998; 95:5076–5081. [PubMed: 9560231]
- Briskin C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, McMahon JA, McMahon AP, Weinberg RA. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes Dev.* 2000; 14:650–654. [PubMed: 10733525]
- Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, Arendt LM, Kuperwasser C, Biehl B, Weinberg RA. Normal and neoplastic

- nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci U S A*. 2011; 108:7950–7955. [PubMed: 21498687]
- Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res*. 2008; 14:1368–1376. [PubMed: 18316557]
- Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH, Manson JE, Stefanick ML, Ockene J, Sarto GE, Johnson KC, Wactawski-Wende J, Ravdin PM, Schenken R, Hendrix SL, Rajkovic A, Rohan TE, Yasmeen S, Prentice RL. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *JAMA*. 2010; 304:1684–1692. [PubMed: 20959578]
- 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. [PubMed: 9371488]
- Clarke RB. Human breast cell proliferation and its relationship to steroid receptor expression. *Climacteric*. 2004; 7:129–137. [PubMed: 15497901]
- Clarke RB. Isolation and characterization of human mammary stem cells. *Cell Prolif*. 2005; 38:375–386. [PubMed: 16300651]
- Collaborative. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet*. 1996; 347:1713–1727. [PubMed: 8656904]
- Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, Rimm DL, Wong H, Rodriguez A, Herschkowitz JI, Fan C, Zhang X, He X, Pavlick A, Gutierrez MC, Renshaw L, Larionov AA, Faratian D, Hilsenbeck SG, Perou CM, Lewis MT, Rosen JM, Chang JC. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc Natl Acad Sci U S A*. 2009; 106:13820–13825. [PubMed: 19666588]
- Delgado RC, Lubian Lopez DM. Prognosis of breast cancers detected in women receiving hormone replacement therapy. *Maturitas*. 2001; 38:147–156. [PubMed: 11306203]
- Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev*. 2003; 17:1253–1270. [PubMed: 12756227]
- Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res*. 2004; 6:R605–615. [PubMed: 15535842]
- Faivre EJ, Lange CA. Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. *Mol Cell Biol*. 2007; 27:466–480. [PubMed: 17074804]
- Farnie G, Clarke RB. Mammary stem cells and breast cancer--role of Notch signalling. *Stem Cell Rev*. 2007; 3:169–175. [PubMed: 17873349]
- Fernandez-Valdivia R, Mukherjee A, Ying Y, Li J, Paquet M, DeMayo FJ, Lydon JP. The RANKL signaling axis is sufficient to elicit ductal side-branching and alveologenesis in the mammary gland of the virgin mouse. *Dev Biol*. 2009; 328:127–139. [PubMed: 19298785]
- Fernandez-Valdivia R, Jeong J, Mukherjee A, Soyak SM, Li J, Ying Y, Demayo FJ, Lydon JP. A mouse model to dissect progesterone signaling in the female reproductive tract and mammary gland. *Genesis*. 2010; 48:106–113. [PubMed: 20029965]
- Fournier A, Fabre A, Mesrine S, Boutron-Ruault MC, Berrino F, Clavel-Chapelon F. Use of different postmenopausal hormone therapies and risk of histology- and hormone receptor-defined invasive breast cancer. *J Clin Oncol*. 2008; 26:1260–1268. [PubMed: 18323549]
- Gajdos C, Tartter PI, Babinszki A. Breast cancer diagnosed during hormone replacement therapy. *Obstet Gynecol*. 2000; 95:513–518. [PubMed: 10725482]
- Ghatge RP, Jacobsen BM, Schittone SA, Horwitz KB. The progestational and androgenic properties of medroxyprogesterone acetate: gene regulatory overlap with dihydrotestosterone in breast cancer cells. *Breast Cancer Res*. 2005; 7:R1036–1050. [PubMed: 16457685]
- Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 Is a Marker of

- Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome. *Cell Stem Cell*. 2007; 1:555–567. [PubMed: 18371393]
- Giulianelli S, Herschkowitz JI, Patel V, Lamb CA, Gutkind JS, Molinolo A, Perou CM, Lanari C. MPA-induced gene expression and stromal and parenchymal gene expression profiles in luminal murine mammary carcinomas with different hormonal requirements. *Breast Cancer Res Treat*. 2010
- Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, Pinkas J, Branstetter D, Dougall WC. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. *Nature*. 2010; 468:103–107. [PubMed: 20881963]
- Graham JD, Yager ML, Hill HD, Byth K, O'Neill GM, Clarke CL. Altered progesterone receptor isoform expression remodels progestin responsiveness of breast cancer cells. *Mol Endocrinol*. 2005; 19:2713–2735. [PubMed: 15976005]
- Graham JD, Mote PA, Salagame U, van Dijk JH, Balleine RL, Hushchitscha LI, Reddel RR, Clarke CL. DNA replication licensing and progenitor numbers are increased by progesterone in normal human breast. *Endocrinology*. 2009a; 150:3318–3326. [PubMed: 19342456]
- Graham JD, Mote PA, Salagame U, Balleine RL, Hushchitscha LI, Clarke CL. Hormone-responsive model of primary human breast epithelium. *J Mammary Gland Biol Neoplasia*. 2009b; 14:367–379. [PubMed: 19936891]
- Grimm SL, Bu W, Longley MA, Roop DR, Li Y, Rosen JM. Keratin 6 is not essential for mammary gland development. *Breast Cancer Res*. 2006; 8:R29. [PubMed: 16790075]
- Gusterson B. Do 'basal-like' breast cancers really exist? *Nat Rev Cancer*. 2009; 9:128–134. [PubMed: 19132008]
- Gusterson BA, Ross DT, Heath VJ, Stein T. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. *Breast Cancer Res*. 2005; 7:143–148. [PubMed: 15987465]
- Hankinson SE, Colditz GA, Willett WC. Towards an integrated model for breast cancer etiology: the lifelong interplay of genes, lifestyle, and hormones. *Breast Cancer Res*. 2004; 6:213–218. [PubMed: 15318928]
- Hargreaves DF, Knox F, Swindell R, Potten CS, Bundred NJ. Epithelial proliferation and hormone receptor status in the normal post-menopausal breast and the effects of hormone replacement therapy. *Br J Cancer*. 1998; 78:945–949. [PubMed: 9764588]
- Harrison H, Farnie G, Howell SJ, Rock RE, Stylianou S, Brennan KR, Bundred NJ, Clarke RB. Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res*. 2010; 70:709–718. [PubMed: 20068161]
- Horwitz KB, Dye WW, Harrell JC, Kabos P, Sartorius CA. Rare steroid receptor-negative basal-like tumorigenic cells in luminal subtype human breast cancer xenografts. *Proc Natl Acad Sci U S A*. 2008; 105:5774–5779. [PubMed: 18391223]
- Horwitz KB, Sartorius CA. Progestins in hormone replacement therapies reactivate cancer stem cells in women with preexisting breast cancers: a hypothesis. *J Clin Endocrinol Metab*. 2008; 93:3295–3298. [PubMed: 18647813]
- Huggins C, Moon RC, Morii S. Extinction of experimental mammary cancer. I. Estradiol-17beta and progesterone. *Proc Natl Acad Sci U S A*. 1962; 48:379–386. [PubMed: 14449778]
- Iliopoulos D, Hirsch HA, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci U S A*. 2011; 108:1397–1402. [PubMed: 21220315]
- Jacobsen BM, Richer JK, Schittone SA, Horwitz KB. New human breast cancer cells to study progesterone receptor isoform ratio effects and ligand-independent gene regulation. *J Biol Chem*. 2002; 277:27793–27800. [PubMed: 12021276]
- Jacobsen BM, Schittone SA, Richer JK, Horwitz KB. Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology. *Mol Endocrinol*. 2005; 19:574–587. [PubMed: 15563544]
- Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, Morony S, Rubin E, Sarao R, Hojilla CV, Komnenovic V, Kong YY, Schreiber M, Dixon SJ, Sims SM, Khokha R,

- Wada T, Penninger JM. Regulation of cancer cell migration and bone metastasis by RANKL. *Nature*. 2006; 440:692–696. [PubMed: 16572175]
- Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, Stingl J, Waterhouse PD, Khokha R. Progesterone induces adult mammary stem cell expansion. *Nature*. 2010; 465:803–807. [PubMed: 20445538]
- Kabos P, Haughian JM, Wang X, Dye WW, Finlayson C, Elias A, Horwitz KB, Sartorius CA. Cytokeratin 5 positive cells represent a steroid receptor negative and therapy resistant subpopulation in luminal breast cancers. *Breast Cancer Res Treat*. 2011; 128:45–55. [PubMed: 20665103]
- Kariagina A, Xie J, Leipprandt JR, Haslam SZ. Amphiregulin Mediates Estrogen, Progesterone, and EGFR Signaling in the Normal Rat Mammary Gland and in Hormone-Dependent Rat Mammary Cancers. *Horm Cancer*. 2010a; 1:229–244. [PubMed: 21258428]
- Kariagina, A.; Kramer, AN.; Miller, KN.; Haslam, SZ. Progesterone alters tumor cell composition, increases tumor cell proliferation and survival, and promotes invasive behavior in carcinogen-induced mammary cancer in the rat. The 92nd Annual Meeting of the Endocrine Society; San Diego, CA. 2010b. p. OR12-2
- Katiyar P, Ma Y, Fan S, Pestell RG, Furth PA, Rosen EM. Regulation of progesterone receptor signaling by BRCA1 in mammary cancer. *Nucl Recept Signal*. 2006; 4:e006. [PubMed: 16741564]
- Kobayashi S, Stice JP, Kazmin D, Wittmann BM, Kimbrel EA, Edwards DP, Chang CY, McDonnell DP. Mechanisms of progesterone receptor inhibition of inflammatory responses in cellular models of breast cancer. *Mol Endocrinol*. 2010; 24:2292–2302. [PubMed: 20980435]
- Kordon E, Lanari C, Meiss R, Elizalde P, Charreau E, Dosne Pasqualini C. Hormone dependence of a mouse mammary tumor line induced in vivo by medroxyprogesterone acetate. *Breast Cancer Res Treat*. 1990; 17:33–43. [PubMed: 2151368]
- Lanari C, Molinolo AA, Pasqualini CD. Induction of mammary adenocarcinomas by medroxyprogesterone acetate in BALB/c female mice. *Cancer Lett*. 1986; 33:215–223. [PubMed: 2947675]
- Lanari C, Lamb CA, Fabris VT, Helguero LA, Soldati R, Bottino MC, Giulianelli S, Cerliani JP, Wargon V, Molinolo A. The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. *Endocr Relat Cancer*. 2009; 16:333–350. [PubMed: 19190078]
- Leo JC, Wang SM, Guo CH, Aw SE, Zhao Y, Li JM, Hui KM, Lin VC. Gene regulation profile reveals consistent anticancer properties of progesterone in hormone-independent breast cancer cells transfected with progesterone receptor. *Int J Cancer*. 2005; 117:561–568. [PubMed: 15945099]
- Leong KG, Niessen K, Kulic I, Raouf A, Eaves C, Pollet I, Karsan A. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. *J Exp Med*. 2007; 204:2935–2948. [PubMed: 17984306]
- Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, Feleppa F, Huschtscha LI, Thorne HJ, Fox SB, Yan M, French JD, Brown MA, Smyth GK, Visvader JE, Lindeman GJ. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med*. 2009; 15:907–913. [PubMed: 19648928]
- Lindvall C, Bu W, Williams BO, Li Y. Wnt signaling, stem cells, and the cellular origin of breast cancer. *Stem Cell Rev*. 2007; 3:157–168. [PubMed: 17873348]
- Lydon JP, Ge G, Kittrell FS, Medina D, O'Malley BW. Murine mammary gland carcinogenesis is critically dependent on progesterone receptor function. *Cancer Res*. 1999; 59:4276–4284. [PubMed: 10485472]
- Ma Y, Katiyar P, Jones LP, Fan S, Zhang Y, Furth PA, Rosen EM. The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. *Mol Endocrinol*. 2006; 20:14–34. [PubMed: 16109739]
- Medina D, Sivaraman L, Hilsenbeck SG, Conneely O, Ginger M, Rosen J, Omalle BW. Mechanisms of hormonal prevention of breast cancer. *Ann N Y Acad Sci*. 2001; 952:23–35. [PubMed: 11795441]

- Medina D. Breast cancer: the protective effect of pregnancy. *Clin Cancer Res.* 2004; 10:380S–384S. [PubMed: 14734495]
- Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, Mackay A, Grigoriadis A, Tutt A, Ashworth A, Reis-Filho JS, Smalley MJ. BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell.* 2010; 7:403–417. [PubMed: 20804975]
- Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc Natl Acad Sci U S A.* 2003; 100:9744–9749. [PubMed: 12897242]
- Musgrove EA, Lee CS, Sutherland RL. Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-fos, and c-myc genes. *Mol Cell Biol.* 1991; 11:5032–5043. [PubMed: 1922031]
- Narod SA. Hormonal prevention of hereditary breast cancer. *Ann N Y Acad Sci.* 2001; 952:36–43. [PubMed: 11795442]
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature.* 2000; 406:747–752. [PubMed: 10963602]
- Pike MC, Spicer DV, Dahmouch L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev.* 1993; 15:17–35. [PubMed: 8405201]
- Proia TA, Keller PJ, Gupta PB, Klebba I, Jones AD, Sedic M, Gilmore H, Tung N, Naber SP, Schnitt S, Lander ES, Kuperwasser C. Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. *Cell Stem Cell.* 2011; 8:149–163. [PubMed: 21295272]
- Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem.* 2002; 277:5209–5218. [PubMed: 11717311]
- Roarty K, Rosen JM. Wnt and mammary stem cells: hormones cannot fly wingless. *Curr Opin Pharmacol.* 2010; 10:643–649. [PubMed: 20810315]
- Russo J, Ao X, Grill C, Russo IH. Pattern of distribution of cells positive for estrogen receptor alpha and progesterone receptor in relation to proliferating cells in the mammary gland. *Breast Cancer Res Treat.* 1999; 53:217–227. [PubMed: 10369068]
- Santos SJ, Aupperlee MD, Xie J, Durairaj S, Miksicek R, Conrad SE, Leipprandt JR, Tan YS, Schwartz RC, Haslam SZ. Progesterone receptor A-regulated gene expression in mammary organoid cultures. *J Steroid Biochem Mol Biol.* 2009; 115:161–172. [PubMed: 19383543]
- Sartorius CA, Harvell DM, Shen T, Horwitz KB. Progestins initiate a luminal to myoepithelial switch in estrogen-dependent human breast tumors without altering growth. *Cancer Res.* 2005; 65:9779–9788. [PubMed: 16266999]
- Schedin P. Pregnancy-associated breast cancer and metastasis. *Nat Rev Cancer.* 2006; 6:281–291. [PubMed: 16557280]
- Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, Hanada R, Joshi PA, Aliprantis A, Glimcher L, Pasparakis M, Khokha R, Ormandy CJ, Widschwendter M, Schett G, Penninger JM. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature.* 2010; 468:98–102. [PubMed: 20881962]
- Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE. Generation of a functional mammary gland from a single stem cell. *Nature.* 2006; 439:84–88. [PubMed: 16397499]
- Shen T, Horwitz KB, Lange CA. Transcriptional hyperactivity of human progesterone receptors is coupled to their ligand-dependent down-regulation by mitogen-activated protein kinase-dependent phosphorylation of serine 294. *Mol Cell Biol.* 2001; 21:6122–6131. [PubMed: 11509655]
- Shyamala G, Yang X, Cardiff RD, Dale E. Impact of progesterone receptor on cell-fate decisions during mammary gland development. *Proc Natl Acad Sci U S A.* 2000; 97:3044–3049. [PubMed: 10737785]

- 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. *J Cell Biol.* 2007; 176:19–26. [PubMed: 17190790]
- Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ. Purification and unique properties of mammary epithelial stem cells. *Nature.* 2006; 439:993–997. [PubMed: 16395311]
- van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J, Torhorst J, Sauter G, Zuber M, Kochli OR, Mross F, Dieterich H, Seitz R, Ross D, Botstein D, Brown P. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. *Am J Pathol.* 2002; 161:1991–1996. [PubMed: 12466114]
- Villadsen R, Fridriksdottir AJ, Ronnov-Jessen L, Gudjonsson T, Rank F, LaBarge MA, Bissell MJ, Petersen OW. Evidence for a stem cell hierarchy in the adult human breast. *J Cell Biol.* 2007; 177:87–101. [PubMed: 17420292]
- 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]

Highlights

- The female hormone progesterone expands stem cells in the murine mammary gland.
- Progesterone increases progenitor cells in normal human breast organoids.
- Progesterone reprograms breast cancer cells to a more stem/progenitor like state.
- Progestins in hormone therapies may reactivate stem cells in postmenopausal women

Figure 1.

Model for paracrine and autocrine signaling by progesterone and PR in normal breast and breast cancer cells. In the normal murine mammary gland, progesterone (P) stimulates production of RANKL, which mediates paracrine signaling from PR⁺ luminal cells, to PR⁻ RANK expressing basal-located MaSCs. This leads to increased proliferation and repopulating function of MaSCs. In the rat, AREG, an EGFR ligand, mediates progesterone signaling and expands rat MaSCs. In humans, paracrine signaling from PR⁺ luminal cells may occur through unidentified factors to basal-located PR⁻ stem cells, as well as luminal-located PR⁻ progenitor cells. In human breast cancer models, progesterone produces an autocrine signal in PR⁺ cancer cells, and converts them to a PR⁻ state, with heightened expression of stem cell genes. These PR⁻ cells are more quiescent and drug resistant than surrounding PR⁺ cells, and produce PR⁺ outgrowths when isolated and placed in 3D cell culture models.

Table 1

Progesterone regulated genes in normal and malignant mammary epithelial cells potentially involved in stem cell regulation.

Gene symbol	Description	Function	Species ^a	Cell type	Reference
ABCG2	ATP-binding cassette, sub-family G, member 2	multi-drug resistant pump, side-population	H	tumor	(Horwitz et al., 2008; Leo et al., 2005)
AREG	Amphiregulin	paracrine signaling	m, r	normal	(Asselin-Labat et al., 2010; Kariagina et al., 2010a)
CD44	CD44 antigen	cell adhesion, cancer stem cell marker	H	tumor	(Ghatge et al., 2005; Horwitz et al., 2008)
DLL-1	delta-like 1	notch pathway; self-renewal	H	normal	(Graham et al., 2009a)
DLL-3	delta-like 3	notch pathway; self-renewal	H	normal	(Graham et al., 2009a)
DNER	delta and notch-like epidermal growth factor-related receptor	notch and EGF signaling	H	tumor	(Horwitz et al., 2008)
IL6	Interleukin 6	stem cell survival, expansion	H	tumor	(Horwitz et al., 2008)
IL8	Interleukin 8	stem cell survival, expansion	H	normal	(Kobayashi et al., 2010)
JAG1	jagged 1	notch pathway; self-renewal	H	tumor	(Ghatge et al., 2005)
NOTCH2	notch homolog 2	notch pathway; self-renewal	H	tumor	(Ghatge et al., 2005; Horwitz et al., 2008)
PRSS2	presenilin 2	notch pathway; self-renewal	h, m	normal, tumor	(Giulianelli et al., 2010; Graham et al., 2009a)
RANK ^b	Receptor activator of NF- κ B ligand receptor	mediates RANKL paracrine signaling, stem cell expansion	M	normal	(Asselin-Labat et al., 2010; Joshi et al., 2010)
RANKL ^c	Receptor activator of NF- κ B ligand	paracrine signaling, stem cell expansion	m, r	normal	(Asselin-Labat et al., 2010; Joshi et al., 2010; Kariagina et al., 2010a; Mulac-Jericevic et al., 2003; Santos et al., 2009)
WNT1	wingless-type MMTV integration site family, member 1	wnt signaling, self-renewal	H	tumor	(Faivre and Lange, 2007)
WNT4	wingless-type MMTV integration site family, member 4	wnt signaling, paracrine signaling	M	normal	(Briskin et al., 2000; Joshi et al., 2010; Santos et al., 2009)

^a m, mouse; r, rat; h, human

^b official gene symbol TNFSF11A, tumor necrosis factor receptor superfamily, member 11A, NFKB activator

^c official gene symbol TNFSF11, tumor necrosis factor (ligand) superfamily, member 11