Published in final edited form as:

Mol Cell Endocrinol. 2012 June 24; 357(1-2): 4–17. doi:10.1016/j.mce.2011.10.030.

The Biology of Progesterone Receptor in the Normal Mammary gland and in Breast Cancer

Alison Obr¹ and Dean P. Edwards¹

¹Departments of Molecular & Cellular Biology and Pathology and Immunology, Baylor College of Medicine, Houston, Texas, 77030, USA

Abstract

This paper reviews work on progesterone and the progesterone receptor (PR) in the mouse mammary gland that has been used extensively as an experimental model. Studies have led to the concept that progesterone controls proliferation and morphogenesis of the luminal epithelium in a tightly orchestrated manner at distinct stages of development by paracrine signaling pathways, including receptor of activated nuclear factor κ ligand (RANKL) as a major paracrine factor. Progesterone also drives expansion of stem cells by paracrine signals to generate progenitors required for alveologenesis. During mid-to-late pregnancy, progesterone has another role to suppress secretory activation until parturition mediated in part by crosstalk between PR and prolactin/Stat5 signaling to inhibit induction of milk protein gene expression, and by inhibiting tight junction closure. In models of hormone-dependent mouse mammary tumors, the progesterone/PR signaling axis enhances pre-neoplastic progression by a switch from a paracrine to an autocrine mode of proliferation and dysregulation of the RANKL signaling pathway. Limited experiments with normal human breast show that progesterone/PR signaling also stimulates epithelial cell proliferation by a paracrine mechanism; however, the signaling pathways and whether RANKL is a major mediator remains unknown. Work with human breast cancer cell lines, patient tumor samples and clinical studies indicates that progesterone is a risk factor for breast cancer and that alteration in progesterone/PR signaling pathways contributes to early stage human breast cancer progression. However, loss of PR expression in primary tumors is associated with a less differentiated more invasive phenotype and worse prognosis, suggesting that PR may limit later stages of tumor progression.

Keywords

Progesterone receptor; Breast cancer; Mammary gland; Prolactin; STAT5; RANKL

1. Introduction

The mammary gland is a hormonally responsive target tissue that develops predominantly after birth, and is capable of undergoing sequential cycles of development through pregnancy, lactation and involution. Because of common developmental and hormonal response features, the mouse mammary gland has served as an experimental system for

© 2011 Elsevier Ireland Ltd. All rights reserved.

Corresponding Author: Dean P. Edwards, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, deane@bcm.edu, Phone: 001-713-798-2326, FAX: 001-713-798-8711.

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 copy editing, 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.

modeling normal human breast and breast cancer (Conneely et al., 2003). Progesterone (P4) is a key cycling ovarian steroid hormone that is highest in the luteal phase and has a major role to promote glandular differentiation of the endometrium. P4 is also sustained at high levels during pregnancy and is required for maintenance of pregnancy (Anderson and Clarke, 2004; Graham and Clarke, 1997; Howard and Gusterson, 2000; Silberstein et al., 1996). Experimental manipulation of mouse ovarian function has demonstrated the importance of P4 for proliferation and ductal side branching of the mammary gland during puberty and for alveologenesis during pregnancy (Graham and Clarke, 1997). The essential nature of the P4 signaling axis for mammary gland development has also been defined through studies of the progesterone receptor (PR) knockout mouse (PRKO) (Lydon et al., 1995). PR is a member of the nuclear hormone receptor family of ligand-dependent transcription factors that functions by binding to specific target genes either through *cis*-acting progesterone response elements (PREs) or by tethering to other DNA bound transcription factors. DNA bound receptor recruits co-regulatory proteins that affect chromatin structure and rates of gene transcription co-activation (Bulyanko and O'Malley, 2010; Kastner et al., 1990; Li and O'Malley, 2003; Mangelsdorf et al., 1995). In addition to its action as a transcription factor, a subpopulation of PR functions outside of the nucleus to mediate rapid (minutes) P4 induced activation of protein phosphorylation signaling cascades (Ballare et al., 2003; Boonyaratanakornkit et al., 2001; Edwards, 2005). In both humans and mice, PR is expressed as two isoforms, PR-A and PR-B that have identical ligand-binding (LBD) and DNA-binding domains (DBD) and differ only in truncation of the amino-terminal domain (NTD) in PR-A (Aupperlee et al., 2005; Aupperlee and Haslam, 2007; Kastner et al., 1990; Li and O'Malley, 2003; Mangelsdorf et al., 1995; Mulac-Jericevic et al., 2000; Shyamala et al., 1998). In human breast cancer cell lines PR-B is a stronger transcriptional activator than PR-A, and the two isoforms have been characterized to regulate different but overlapping subsets of target genes (Graham et al., 2005; Jacobsen et al., 2003). Also, the proliferative effect of P4 in breast cancer cells is mediated primarily by PR-B (Boonyaratanakornkit et al., 2007; Daniel et al., 2009; Faivre et al., 2008; Jacobsen et al., 2003; Skildum et al., 2005). Based on phenotypes of PR isoform selective knockout mice, PR-B is more important for the proliferative responses to P4 in the mammary epithelium, while ovarian and uterine development and function rely primarily on PR-A (Mulac-Jericevic et al., 2000; Mulac-Jericevic et al., 2003). Distinct functions in the mouse *in vivo* are due in part to differential expression of PR-A and PR-B during development of the mammary gland and in cell compartments of the uterus (Aupperlee et al., 2005; Aupperlee and Haslam, 2007; Kariagina et al., 2007; Mote et al., 2006). This paper reviews the role of P4 and PR in normal mammary gland development, focusing primarily on the mouse model, and in breast cancer based on studies of mouse and human models of breast cancer.

2. Epithelial cell autonomous actions of PR in the mammary gland

During post-natal development and puberty the mouse mammary gland ductal cap cells proliferate leading to elongation of the ductal tree into the fat pad (Anderson and Clarke, 2004; Graham and Clarke, 1997; Scarpin et al., 2009). In the PRKO mouse, ductal elongation is similar to that of the wild type mouse, establishing that P4/PR signaling is not required for ductal elongation (Lydon et al., 1995). The phenotype of estrogen receptoralpha (ERα) knockout mice has established the requirement of estradiol-17β and ER for ductal proliferation and elongation (Mueller et al., 2002). Transplantation of mammary epithelial cells (MECs) derived from PRKO mice into the cleared mammary fat pad of wild type female recipients, and examination of the phenotype of tissue outgrowths, has been used as an experimental approach to distinguish systemic from cell autonomous effects of P4/PR signaling on mammary gland development. Since PRKO mice are infertile, this approach was also essential to determine the role of P4/PR signaling in the mammary gland

development during pregnancy. With the adult virgin mouse as the host, mammary gland lateral side-branching was impaired in tissue transplants derived from PRKO as compared to wild type MECs, establishing P4 as the primary ovarian hormone for proliferation and branching morphogenesis in the cycling adult animal (Briskin et al., 1998; Lydon et al., 1995). Transplant experiments have also confirmed that PR is only required functionally in the epithelial compartment. Ductal outgrowths were not observed from PRKO MECs transplanted into stromal fat pads of wild type mice, whereas extensive tissue outgrowths were obtained with wild type MECs transplanted with the stroma of PRKO mice (Briskin et al., 1998). Extensive epithelial cell expansion and alveolar morphogenesis occur during pregnancy to prepare the mammary gland for lactation (Oakes et al., 2006). Transplanted MECs from PRKO mice into wt hosts that were then mated, failed to undergo expansion and lobuloalveolar morphogenesis during pregnancy also providing evidence of the essential role of PR signaling in the epithelial compartment for growth and development of the mammary gland during pregnancy (Conneely et al., 2003; Ismail et al., 2003; Soyak et al., 2002).

3. Progesterone regulation of cell proliferation and morphogenesis by paracrine pathways

Based on immunohistochemistry PR is exclusively expressed in the epithelial cell compartment of mammary gland ducts with no evidence of expression in myoepithelial cells or stroma (Grimm et al., 2002; Ismail et al., 2003; Shyamala et al., 1997; Shyamala et al., 2002; Silberstein et al., 1996). PR is uniformly expressed in epithelial cells in juvenile mammary gland ducts but switches to a heterogeneous pattern during puberty and in the adult. In the adult, PR is expressed in ~40% of cells and PR positive cells are largely non-proliferative and reside nearby proliferative PR negative cells suggesting a paracrine mechanism for P4 induced proliferation (Ismail et al., 2002; Shyamala et al., 2002; Silberstein et al., 1996). As a more direct demonstration of a paracrine mechanism, transplantation of a mixture of wild type and PRKO MECs into a wt recipient, resulted in rescue of proliferation and morphogenesis of those PRKO MEC outgrowths that were in close proximity to wild type PR positive MECs (Briskin et al., 1998). Generation of the PR^{lacZ} reporter mouse model, where LacZ was knocked into one of the PR alleles leaving PR functional through the other allele, enabled an alternate approach to examine PR patterning during stages of mammary gland development. Consistent with immunohistochemistry of endogenous PR, β -galactosidase was expressed uniformly in the juvenile body cells of the terminal end buds (TEBs), and heterogeneously in adult luminal epithelial cells. Cells stained for LacZ and BrdU were largely segregated at the onset of pregnancy (Ismail et al., 2002; Ismail et al., 2003). These studies taken together establish a paracrine mechanism whereby PR negative cells undergo proliferation in response to P4 while PR positive cells are largely quiescent. The majority of epithelial cells that express PR also express ER α in the adult mouse mammary gland. PR is an upregulated target gene of ER and estrogen is required to maintain high expression of PR. Thus, the spatial segregation of PR from proliferating cells also applies to ER and estrogen induced proliferation by a paracrine mechanism (Mallepell et al., 2006; Mukherjee et al., 2010; Mulac-Jericevic et al., 2003).

The CCAAT/enhancer transcription factor C/EBP β has been implicated in regulating the segregation of PR positive non-dividing epithelial cells from PR-negative proliferating cells. Genetic deletion of C/EBP β resulted in a lack of lobuloalveolar development similar to that of the PRKO mouse (Grimm et al., 2005; Seagroves et al., 2000) and increased the fraction of dividing cells expressing PR as compared with wt mice. Deletion of C/EBP β was also associated with reduced proliferation of the mammary epithelium indicating that segregation of PR from non-proliferating cells is required for the proliferative effects of P4 (Grimm et

al., 2005). Studies have shown that transforming growth factor β (TGF β) plays a role in impeding proliferation of ER/PR positive cells. TGF β is a potent inhibitor of epithelial cell proliferation (Barcellos-Hoff and Ewan, 2000; Grimm and Rosen, 2006) and mammary epithelial cells that are positive for ER/PR tend to co-localize with active TGF β and phosphorylated nuclear Smad2/3 (Ewan et al., 2005; Grimm and Rosen, 2006). Reduced proliferation that resulted from deletion of C/EBP β was also noted to be associated with markedly increased TGF β 1 expression in ER/PR positive cells (Grimm et al., 2005). As more direct evidence for a role TGF β 1, *Tgfb1* heterozygous mice that have ~10% the level of wt mice, exhibited a dramatic increase in proliferation of ER⁺/PR⁺ cells at estrus and during pregnancy. Conversely, over-expression of active TGF β 1 further reduced the population of ER⁺/PR⁺ proliferating cells (Barcellos-Hoff and Ewan, 2000; Ewan et al., 2005; Nguyen et al., 2011). However, manipulation of active TGF β 1 did not affect the proportion of proliferating ER⁺/PR⁺ cells during puberty (Ewan et al., 2005) suggesting that other factors are involved in suppressing proliferation of ER⁺/PR⁺ cells at this stage of mammary gland development.

4. Paracrine and other mediators of P4/PR signaling in the mouse mammary gland

Several downstream targets have been implicated as effectors of P4 induced cellular proliferation and morphogenesis including Wnt-4, amphiregulin, calcitonin, inhibitor of DNA binding 4 (Id4) and receptor of activated nuclear factor κ ligand (RANKL). These genes are known to be involved in proliferation and/or morphogenesis and are induced by P4 in the mammary gland in a manner dependent on PR (Beleut et al., 2010; Briskin et al., 2000; Fernandez-Valdivia et al., 2008; Gavin and McMahon, 1992; Haslam et al., 2008; Ismail et al., 2004; Kariagina et al., 2010; Mulac-Jericevic et al., 2000; Mulac-Jericevic et al., 2003; Weber-Hall et al., 1994).

The Wnt protein family and respective downstream signaling pathways are responsible for regulation of differentiation and cell fate (Moon et al., 1997). Wnt activity is mediated by binding to frizzled (Fz), a transmembrane receptor expressed by target cells. Upon Wnt binding, downstream components are activated, subsequently leading to stabilization of β -catenin by inactivation of GSK-3 (glycogen synthase kinase-3). Once stabilized, β -catenin forms a complex with TCF, a transcription factor responsible for activating proliferative target genes (Cadigan and Nusse, 1997; Dale, 1998; Nusse and Varmus, 1992). Wnt4 is expressed in the mammary epithelium and is increased during pregnancy to activate ductal proliferation and morphogenesis (Gavin and McMahon, 1992; Robinson et al., 2000; Weber-Hall et al., 1994). Wnt4 regulates proliferation by activating the cell cycle targets, cyclin D1 and p21 (Nusse, 1999). Overexpression of Wnt4 in transplanted primary MECs resulted in extensive side-branching similar to pregnancy (Bradbury et al., 1995). Furthermore, transplanted MECs from Wnt4 knockout mice failed to undergo ductal side-branching during pregnancy (Briskin et al., 2000). Aberrant side -branching in Wnt4 overexpressing and loss of side -branching in knockout transplants demonstrated that Wnt4 is an important regulator of mammary gland proliferation and morphogenesis. Colocalization in mammary epithelium by *in situ* hybridization suggests a direct association between PR and Wnt4. Induction of Wnt4 in the mammary gland by P4 in adult ovariectomized mice, and loss of Wnt4 expression in PR^{-/-} cells, further demonstrates that Wnt4 expression is PR dependent; however, it is unknown whether Wnt4 is a direct nuclear target of PR (Fernandez-Valdivia et al., 2008; Mulac-Jericevic et al., 2003).

Another potential effector of P4 in the mammary gland is Id4, a member of the basic helix-loop-helix (HLH) family of Id proteins. Id proteins lack the basic DNA binding domain and act as a dominant-negative inhibitor of other bHLH transcription factors during

differentiation and development (Norton, 2000). In general, Id proteins act as inhibitors of differentiation and have been implicated in mammary gland development and various human cancers including breast cancer (Beger et al., 2001; Shan et al., 2003). Id4 mRNA expression was acutely upregulated by P4 in the mammary gland of ovariectomized adult mice. In intact mice, the Id4 protein was also observed to increase dramatically and transiently during early pregnancy and this increase was restricted to ER⁺/PR⁺ luminal epithelial cells, suggesting that Id4 could be a mediator of the early proliferative expansion phase of P4/PR dependent morphogenesis (Fernandez -Valdivia et al., 2008). Experiments with differentiated mouse mammary epithelial HC-11 cells in culture demonstrated that Id 4 is a direct PR target. P4 induced Id4 mRNA expression in HC-11 cells in a manner dependent on PR, and by chromatin immunoprecipitation (ChIP) assay, PR bound in response to progestin to the 5' regulatory region of the Id4 gene that contains PRE sequences (Fernandez-Valdivia et al., 2008). Evidence that Id4 is an effector of progesterone-induced proliferation of mammary epithelium has been further demonstrated with Id4 knockout mice (Dong et al., 2011). In the adult virgin animal, Id4 deletion resulted in reduced ductal side -branching morphogenesis and a reduction in alveolar expansion during pregnancy suggesting defects in P4 signaling. In intact and ovariectomized Id4-null mice, proliferation of the mammary epithelium stimulated by P4, or P4+ estradiol-17 β (E2), was reduced as compared with wt mice (Dong et al., 2011).

RANKL is a secreted cytokine member of the tumor necrosis factor (TNF α) superfamily that mediates its biological effects through binding to the RANK transmembrane receptor. Depending on the cell type, the RANK receptor, in response to binding RANKL, engages and activates a variety of down stream signaling cascades including transcription factor NF- κ B and upregulation of the NF- κ B target gene cyclin D1 (Gonzalez-Suarez, 2011). RANKL was initially defined as an essential factor for osteoclast differentiation and survival. However, it is expressed in a wide variety of other tissues and was subsequently shown in RANKL deficient mice to be required for cellular proliferation, survival and alveologenesis of mammary gland during pregnancy (Dougall et al., 1999; Fata et al., 2000; Kong et al., 1999). RANKL mRNA is induced acutely by treatment with E2+P4, or P4 alone, in the mammary gland of ovariectomized adult mice, but not in PRKO mice (Fernandez -Valdivia et al., 2008; Mulac-Jericevic et al., 2003). Furthermore, RANKL induced by P4 is expressed exclusively in a subset of PR positive luminal epithelial cells (Mulac-Jericevic et al., 2003) and both proteins are co-expressed in luminal epithelial cells during early to mid pregnancy. Cyclin D1 expression is also induced by P4 in a manner dependent on PR (Mulac -Jericevic et al., 2003; Said et al., 1997); however, cyclin D1 is largely segregated from PR positive cells suggesting that P4 regulation of cyclin D1 is indirect through a RANKL-RANK activated signaling pathway (Fernandez-Valdivia et al., 2009; Mulac-Jericevic et al., 2003). Progesterone-induced proliferation of the mammary epithelium in the adult mouse was shown to involve an early (24hr) wave of a small percentage of PR positive cells, and a subsequent larger sustained phase of proliferation of PR negative cells, indicating the existence of both autocrine and paracrine pathways (Beleut et al., 2010). Tissue reconstitution experiments with MECs derived from cyclin D1^{-/-}, RANKL^{-/-} or wt mice, revealed that the early phase of P4 induced autocrine proliferation was dependent on cyclin D1, where as the second paracrine phase was RANKL dependent. RANKL expressed ectopically in PRKO MECs from retroviral vectors and transplanted into cleared fat pads rescued ductal side branching and alveologenesis, indicating that RANKL is a major paracrine mediator of P4 (Beleut et al., 2010). To more definitively determine whether RANKL is a critical effector of P4/PR signaling in the intact mammary gland, a conditional system was developed that conditionally targets RANKL in the PRKO epithelium to ER positive epithelial cells that are equivalent to ER/PR/RANKL positive cells of wt epithelium (Mukherjee et al., 2010). In this system, ectopic RANKL was expressed in the same spatial pattern as P4 induced endogenous RANKL, and rescued the PRKO phenotype in the

absence of PR. Interestingly, constitutive overexpression of RANKL in the mammary gland as an MMTV transgene resulted in precocious ductal side branching morphogenesis and alveologenesis in adult virgin mice accompanied by increased expression of NF- κ B and cyclin D1 (Fernandez -Valdivia et al., 2009). However, the RANKL transgene was expressed indiscriminately in ER⁺/PR⁺ and ER⁻/PR⁻ cells, and prolonged exposure to the transgene led to epithelial hyperplasia indicating that correct spatial regulation of RANKL is required for ordered and tight regulation of proliferation and morphogenesis during mammary gland development (Fernandez -Valdivia et al., 2009). The molecular mechanisms by which P4 regulates expression of RANKL and the downstream paracrine signaling pathways responsible for mediating proliferation of ER⁻/PR⁻ epithelial cells has not been well defined. RANKL appears to be a direct PR target gene. ChIP assays have demonstrated that PR binds to enhancer regions of the RANKL gene in a progesterone dependent manner in HC-11 mouse mammary epithelial cells indicating that RANKL is a direct PR target (Obr et al., 2011). Furthermore, RANKL-luciferase reporter genes in cell culture experiments were induced by P4 in a PR-dependent manner by RANKL enhancers that also bound PR by ChIP assay (Obr et al., 2011).

Progesterone was recently shown to stimulate expansion of mouse mammary stem cells (MaSCs) during the reproductive cycle and pregnancy (Asselin-Labat et al., 2010; Joshi et al., 2010). In the mouse subpopulations of cells have been isolated, based on expression of a combination of cell surface markers (CD29, CD49f, CD24), that exhibit both the self-renewal and multi-differentiation properties of mammary stem cells. Limited numbers of cells enriched for these markers have the ability in serial transplantations to repopulate a complete mammary gland containing both luminal and myoepithelial cells as well as lobuloalveolar structures during pregnancy (Shackleton et al., 2006; Stingl et al., 2006; Vaillant et al., 2007). Mouse MaSCs are ER/PR negative and are localized in the basal compartment of mouse mammary epithelium nearby ER/PR positive mature luminal epithelial cells (Asselin-Labat et al., 2006; Vaillant et al., 2007). An expansion of the pool of MaSCs was observed during diestrus in cycling adult mice and pregnancy when P4 is highest, suggesting that P4 stimulates proliferation of MaSCs (Joshi et al., 2010). As more direct evidence that P4 can regulate MaSCs, administration of P4 in intact adult mice increased the population of MaSCs and ovariectomy reduced the total number of repopulating cells in the mammary gland and diminished ductal outgrowths in transplantation experiments (Asselin-Labat et al., 2010; Joshi et al., 2010). Furthermore, in ovariectomized mice, E2+P4, but not either hormone alone, led to an increase in MaSC numbers and repopulation activity. Since E2 in the adult gland is needed primarily to maintain PR expression, this suggests that MaSC expansion is stimulated largely by P4 (Asselin-Labat et al., 2010; Joshi et al., 2010). Transplantation assays have also revealed a marked increase in repopulating activity of MaSCs from mid-pregnancy vs. virgin mice, suggesting that P4 during pregnancy expands a MaSC pool that drives lobuloalveolar development (Asselin-Labat et al., 2010; Joshi et al., 2010). RANKL induced by P4 in mature luminal cells was not observed with enriched MaSC populations suggesting P4/PR affects MaSCs by a RANKL mediated paracrine signaling pathway (Asselin -Labat et al., 2010). As direct evidence for this, inhibition of RANKL with an anti-RANKL antibody decreased the clonogenicity of MaSCs implicating RANKL as a mediator of P4 induced MaSC proliferation (Joshi et al., 2010).

5. P4/PR suppression of differentiation during pregnancy

In mid-to-late pregnancy, progesterone inhibits milk protein production and closure of tight junctions until parturition (Neville et al., 2002). Tight junctions of the mammary epithelium remain open during pregnancy and their closure during lactation is important to prevent reflux of accumulated milk into the ductal lumen and interstitial spaces (Neville et al., 2002;

Nguyen et al., 2001). In the mouse, a precipitous decline in circulating P4 at parturition is associated with increased milk protein production and tight junction closure (Loizzi, 1985; Virgo and Bellward, 1974). The progressive reduction of PR expression that occurs during late pregnancy has also been suggested to contribute to the onset of lactation and terminal differentiation (Barcellos-Hoff and Ewan, 2000; Ismail et al., 2002). In humans, progesterone levels remain high during the onset of labor; however, lactation is delayed for 2–3 days when a post-partum drop in circulating progesterone also occurs (Neville et al., 2002). Thus, release from inhibition by progesterone appears to be a general mechanism required for secretory activation of the mammary gland at the transition from pregnancy to lactation. In the mouse, ovariectomy (day 17) at late pregnancy triggers premature milk secretion and closure of tight junctions enabling accumulation of milk proteins in the ductal lumen (Neville et al., 2002; Nguyen et al., 2011). Administration of P4, but not E2, blocked ovariectomy-induced tight junction closure while injection of the PR antagonist RU486 in intact mice induced tight junction closure (Nguyen et al., 2001). These data collectively demonstrate that tight junctions in the mammary gland are under the control of P4/PR to keep them open until parturition. The molecular mechanisms by which P4/PR regulates tight junctions in the mammary gland remains unknown.

In the mouse, induction of milk protein gene expression such as β -casein is primarily under the regulation of two lactogenic hormones, prolactin (PRL) and glucocorticoids (Doppler et al., 1990). The PRL receptor/Jak-2/Stat5 signaling pathway mediates PRL induction. Stat5 (signal transducer and activator of transcription) is a transcription factor that resides in the cytoplasm in an inactive form and becomes activated by Jak-2 mediated tyrosine phosphorylation in response to signaling by the PRL receptor. Activated Stat5 translocates to the nucleus and interacts with specific response elements in the promoter and enhancer of the β -casein gene (Groner et al., 1994; Rosen et al., 1999). Glucocorticoids potentiate PRL induced expression of β -casein through recruitment of glucocorticoid receptor (GR) to function as a Stat5 coactivator (Doppler et al., 1990; Lechner et al., 1997; Lechner et al., 1997; Stocklin et al., 1996; Wyszomierski et al., 1999). C/EBP β also potentiates Stat5a mediated transactivation of β -casein. Thus Stat5a, GR, and C/EBP β act as a complex to recruit coactivators such as p300 required for chromatin remodeling and transcriptional activation of the β -casein gene (Winklehner-Jennewein et al., 1998; Wyszomierski and Rosen, 2001). In addition to positive interacting factors the repressors, YY-1 (Yin and Yang) and HDAC3 interact constitutively with the β -casein promoter and their dissociation induced by PRL and glucocorticoids is also required for activation of β -casein (Meier and Groner, 1994).

In studies with HC -11 cells and primary MECs, PR, in a progestin-agonist dependent manner, inhibited PRL- and glucocorticoid-induced expression of β -casein by interfering with the PRLR/Jak-2/Stat5a signaling pathway (Buser et al., 2007). By ChIP assay, PR was recruited to the β -casein promoter and enhancer in a progesterone-dependent manner, and inhibited both the assembly of the Stat5/GR/CEBP β transcription complex and dissociation of the YY1/HDAC3 corepressors induced by PRL and glucocorticoids (Buser et al., 2011). Additionally, PR interaction with the β -casein gene resulted in sustained repressive histone modifications at the promoter and enhancer suggesting that epigenetic changes contribute to repressive effects of progesterone on milk protein genes in the mammary gland (Buser et al., 2011). Functional cooperativity between PRL and glucocorticoids was reported to involve a physical interaction between the promoter and enhancer of the β -casein gene (Kabotyanski et al., 2009). This long range looping interaction between the promoter and enhancer was inhibited by progestin-induced recruitment of PR (Kabotyanski et al., 2009). These studies define a novel mechanism for hormone agonist induced repression of gene transcription of a developmentally important target gene in the mammary gland.

6. Crosstalk between prolactin/Stat5 and progesterone/PR signaling pathways

Both P4 and prolactin (PRL) are required for epithelial cell expansion and alveologenesis. Endocrine ablation and gene knockout studies have shown that neither hormone alone is sufficient, and PRKO and PRL receptor knockout (PRLRKO) mice exhibit a similar phenotype suggesting crosstalk between P4 and PRL signaling pathways (Barash, 2006; Bole-Feysot et al., 1998; Briskin and Rajaram, 2006; Fendrick et al., 1998; Goff in and Kelly, 1997; Hennighausen and Robinson, 2008). Gene microarray analysis of mammary glands of ovariectomized adult mice identified a subset of genes unregulated by P4 (Fernandez-Valdivia et al., 2008) that were also down regulated in a microarray analysis of PRL receptor knock out mice, and thus were considered candidate PRL unregulated targets (Gass et al., 2003; Harris et al., 2006). These findings suggest a common set of gene targets down stream of the P4/PR and PRL/JAK2/Stat5 signaling pathways, most notably the Ets family transcription factor Elf5, the cell fate factor Gata 3, RANKL, WNT4, amphiregulin and Id4 (Fernandez-Valdivia et al., 2008). Most of these genes have yet to be confirmed as direct targets of PR or Stat5. With the exception of Id4 and RANKL, it is unclear whether P4 and PRL act independently or cooperatively to regulate expression of these genes. PRL was observed to stimulate RANKL expression in a mouse model with a pituitary graft, while P4 further enhanced induction by PRL (Santos et al., 2010; Srivastava et al., 2003). Knockout of Stat5a in the mouse mammary gland has a similar phenotype as PRKO or PRLRKO mice, and E2+P4 failed to induce RANKL expression in the mammary gland of Stat5a knockout mice indicating that P4 induction of RANKL is dependent on Stat5 (Santos et al., 2010). In HC-11 mouse mammary epithelial cells, PRL or P4 each substantially induced expression of Id4 (4-fold and 8-fold respectively), while the two hormones together gave an additive 32-fold induction (Fernandez-Valdivia et al., 2008). By ChIP assay, PR and Stat5 each bound to separate sites on the Id4 gene in response to P4 or PRL, whereas a PR-Stat5 complex was assembled in response to treatment with P4+PRL. These data suggest that interactions between PR and Stat5, at the target gene level, contribute to the additive functional effect of P4 and PRL on expression of Id4 (Fernandez-Valdivia et al., 2008).

Similar to segregation of a subset of ER/PR positive cells from dividing cells, Stat5a and PRLR are expressed heterogeneously in mammary epithelial cells, and cells that proliferate tend to be PRLR- and Stat5 -negative (Briskin and Rajaram, 2006; Hovey et al., 2001; Wagner and Rui, 2008). Furthermore, PR and Stat5a are co-expressed in predominantly the same population of mammary epithelial cells, thus providing the opportunity for direct interactions between PR and Stat5 in the mammary gland (Hovey et al., 2001). Indeed physical and functional interactions between all steroid hormone receptors and Stat5a have been reported (Doppler et al., 2001; Lechner et al., 1997; Stocklin et al., 1996; Stoecklin et al., 1997; Winklehner-Jennwein et al., 1998; Wyszomierski et al., 1999). (Bjornstrom et al., 2001; Bjornstrom and Sjoberg, 2002; Boerner et al., 2005; Cao et al., 2004; Engblom et al., 2007; Faulds et al., 2001; Tan et al., 2008; Wang and Cheng, 2004). PR interaction with Stat5 has been detected by co-immunoprecipitation assay in breast cancer cells and progesterone was observed to induce nuclear translocation of Stat5 (Richer et al., 1998). In addition to interactions between PR and Stat5 on the Id4 and β -casein gene described above, P4 induction of 11 β HSD in breast cancer cells was reported to involve the ring of PR to DNA bound Stat5 (Subtil-Rodriguez et al., 2008). Much work still needs to be done to define mechanisms and the functional role for these interactions between P4/PR and PRL/Stat5 signaling pathways in the mammary gland.

7. P4/PR signaling in mouse mammary gland vs. normal human breast

Segregation of ER/PR positive from steroid hormone induced proliferative cells appears to be conserved in the human breast. As determined by immunohistochemistry, the patterning of ER/PR expression in human breast tissue is similar to that of the mouse mammary gland. ER and PR expression is non-uniform and the two steroid receptors colocalize largely in non-proliferating luminal epithelial cells that are nearby ER/PR negative proliferating cells (Anderson et al., 1998; Anderson, 2002; Anderson and Clarke, 2004; Clarke et al., 1997; Rosen, 2003; Russo et al., 1999). It also appears that progesterone is a proliferative hormone in the human breast. The proliferative index of the epithelial compartment of normal human breast is highest during the progesterone-dominant luteal phase of the menstrual cycle (Anderson et al., 1998; Anderson, 2002; Anderson and Clarke, 2004; Anderson et al., 1982). Postmenopausal hormone replacement therapies (HRT) that include estrogens plus progestins, have been observed to increase human mammary proliferation above that of estrogen alone (Hofseth et al., 1999). More direct mechanistic studies to confirm proliferative responses to P4 and to define the paracrine signaling pathways have been hampered due to the lack of a suitable experimental system for the normal human breast. To solve this problem, a three-dimensional (3D) culture system of primary normal human breast cells was developed that retains the non-uniform patterning of ER/PR co-expression in non - proliferating luminal epithelial cells similar to that of intact tissue (Graham et al., 2009). Growth in 3D by embedding in Matrigel was essential to maintain PR expression and builds on previous work of Bissel, Brugge and colleagues (Debnath and Brugge, 2005; Novaro et al., 2003) establishing that cellular interactions with the basement membrane are essential to maintain proper polarity and differentiated functions of breast epithelial cells *in vitro*. Treatment of human breast epithelial cells in 3D culture with P4 resulted in increased proliferation and the majority of cells that responded were ER/PR negative (Graham et al., 2009). Gene microarray experiments to identify acute P4responsive target genes revealed a novel set of genes involved in proliferation including DNA replication and cell cycle (Graham et al., 2009). P4 also stimulated the expansion of a bipotent progenitor cell into luminal and myoepithelial cell descendants in this 3D culture system. Genes in the Notch signaling pathways were also upregulated by P4 suggesting that Notch maybe a paracrine mediator of P4 effects on progenitor cell fate. However, P4/PR targets such as RANKL, Wnt4, and amphiregul in that have been characterized as paracrine mediators in the rodent mammary gland were not observed to be upregulated by P4 in the 3D human breast culture system(Graham et al., 2009).

This result raises the question of whether the mouse and human mammary glandutilize different paracrine factors and pathways for P4 induced proliferation, or whether this reflects differences between *in vitro* systems and the mammary gland *in vivo*. In the mouse mammary gland *in vivo*, induction of RANKL required at least 24hr of P4 treatment with maximal induction occurring at 76hr (Fernandez-Valdivia et al., 2008). Human breast epithelial cells in 3D culture were treated for only 6hr (Graham et al., 2009); therefore, it is possible that RANKL and other paracrine mediators of P4 action require longer times for induction and were simply missed. Microarray analysis has also been performed with primary mouse mammary MEC 3D organoid culture system embedded in a collagen I gel matrix. P4 treatment for 24hr induced expression of RANKL, Wnt-4 and calcitonin as well as novel genes not previously reported (Haslam et al., 2008). Similarly, a robust induction of RANKL by P4 in 3D cultures of primary mouse MECs in Matrigel was observed over the same time frame required for induction by P4 (24–76hr) in the mouse mammary gland *in vivo* (Fernandez -Valdivia et al., 2008; Obr et al., 2011). These data suggest that, at least for RANKL and some other gene targets in mice, *in vitro* 3D cultures domimic P4 responses in the mammary gland *in vivo*. Novel P4 regulated genes detected in organoid cultures of mouse MECs were suggested to be attributable to enrichment of epithelial cells in culture

and enhanced sensitivity as compared with microarray analysis of intact mammary glands that have a large proportion of stromal cells (Haslam et al., 2008). Transcriptome analysis of isolated mouse and human mammary epithelial subpopulations revealed expression of the paracrine factors RANKL, Wnt4 and amphiregulin in both species. However, whether these factors are P4 regulated in human breast was not examined (Lim et al., 2010). Because of the importance of the RANK-RANKL signaling axis as a mediator of P4 induced proliferation in the mouse, additional experiments with normal human breast models are important to determine whether RANKL or other paracrine factors are the mediators of progesterone in human breast epithelium (Lydon and Edwards, 2009). A model for progesterone regulation of epithelial cell proliferation by a paracrine mechanism based on work with the mouse mammary glands depicted in Figure 1.

8. Progesterone regulated RANKL signaling in mammary tumorigenesis

A hallmark of pre-neoplastic lesions and mammary tumors is an increase in the proportion of proliferating ER/PR positive cells, suggesting a switch from a paracrine to an autocrine mode of regulation by steroid hormones (Anderson, 2002; Briskin, 2002; Briskin and Rajaram, 2006; Rosen, 2003). In mouse models of hormone-dependent mammary tumors, an increase in the percentage of proliferating ER/PR positive cells is associated with hyperplasias and development of ductal carcinoma in situ (DCIS) (Frech et al., 2005; Medina et al., 2003; Sivaraman et al., 2001). Breast cancer cell lines grown in 3D cultures under the same conditions as normal human breast cells, also exhibit an increase in the fraction of ER/PR proliferating cells (Graham et al., 2009). In human breast tissues, the frequency of ER/PR positive proliferating cells increases with age and is associated with breast cancer risk. In clinical samples, an increase in percentage of proliferating ER/PR positive cells has been observed in areas of normal tissues of breast cancer patients and in hyperplasias and DCIS, which correlates with risk of developing breast cancer (Khan et al., 1994; Lawson et al., 1999; Lawson et al., 2002; Shoker et al., 1999; Shoker et al., 2000). These data collectively suggest that a switch from a paracrine to an autocrine mechanism of regulation of cellular proliferation by steroid hormones is conserved in humans and contributes to pre-neoplastic progression in human breast cancer.

In mouse models, dysregulation of the RANKL-RANK signaling pathway has been implicated in the initiation and progression of P4-dependent mammary tumors. MMTV-RANK transgenic mice develop spontaneous pre-neoplastic lesions and adenocarcinomas after multiple pregnancies, and chemical carcinogen induced tumors that are dependent on the progestin MPA (medroxyprogesterone acetate) occur with increased frequency and shorter latency in MMTV-RANK transgenic mice as compared with wt animals (Gonzalez-Suarez et al., 2007; Gonzalez-Suarez et al., 2010). Pharmacological inhibitors of RANK or RANKL, and genetic deletion of RANK in the mammary gland, reduced the incidence and delayed the onset of progestin-induced mammary tumors (Gonzalez-Suarez et al., 2010; Schramek et al., 2010). In virgin mice, over expression of RANKL in the mammary gland as an MMTV transgene led to up regulation of the NF- κ B/cyclin D1 signaling pathway and aberrant alveolar budding and hyperplasias; however, these hyperplasias did not progress to adenocarcinomas (Fernandez-Valdivia et al., 2009). Failure to develop mammary tumors was suggested to be the result of down regulation of RANK in the RANKL-MMTV mouse (Fernandez-Valdivia et al., 2009). Whether RANK-RANKL signaling is involved in hormone-dependent breast cancer in women is not known. RANKL is over expressed in a fraction of breast cancers and its expression was reported to be inversely correlated with tumor progression (Gonzalez-Suarez, 2011). Future studies with models of normal human breast and human breast cancer progression will be important to determine whether RANK-RANKL signaling as a paracrine mediator of P4 action is a driver of human cancer as it is in the rodent mammary gland.

9) Progesterone/PR signaling in human breast cancer

The estrogen/ER signaling axis is a major player in stimulating growth and progression of the luminal subtype human breast cancers and indeed targeted anti-estrogen therapies have been successful in treatment of ER positive tumors (Bast et al., 2001). Early on, progesterone was assumed to be of little consequence in the etiology or progression of breast cancer, in part due to its well established role to inhibit proliferation of the uterine endometrium and to promote differentiation of uterine glands (Fernandez-Valdivia et al., 2005). However observational studies, clinical studies, and newer experimental models, have revealed that progesterone is a proliferative hormone in the normal human breast independent of estrogen, and is a risk factor for breast cancer (Anderson, 2002; Beral, 2003; Clarke et al., 2006; Hofseth et al., 1999; Lee et al., 2006; Ross et al., 2000; Rossouw et al., 2002). Two clinical studies, the Women's Health Initiative (WHI) and the Million Women study, have demonstrated that the progestin MPA is a risk factor for breast cancer in post-menopausal women receiving hormone replacement therapy (HRT) (Beral, 2003; Lee et al., 2006; Ross et al., 2000; Rossouw et al., 2002). Women taking progestin in combination with estrogen had a greater risk of breast cancer than those taking estrogen alone, and breast cancer mortality increased with combined HRT (Chlebowski et al., 2010). Mouse models have also illustrated the role of P4 and PR in promoting mammary tumorigenesis. Carcinogen-induced (DMBA) mammary tumors in mice are highly dependent on progestins. A marked reduction in the incidence of DMBA induced mammary tumors was observed in PRKO mice as compared to wt animals (Chatterton et al., 2002; Lydon et al., 1999). The progestin MP A induces ER/PR positive mammary adenocarcinomas in female Balb/c mice that require continuous progestin for growth (Carnevale et al., 2007; Labriola et al., 2003). BRCA1 deletion in the mouse mammary gland leads to overexpression of PR and a hyper-proliferative response to P4. The PR antagonist RU486 prevents development of these tumors, indicating that mammary tumor formation in BRCA1-null mice is dependent on PR (Ma et al., 2006; Poole et al., 2006). A p53 null mouse model of spontaneous hormone-dependent mammary tumors that progresses from hormone-dependent ductal hyperplasia to DCIS and invasive ductal cancer, is an excellent model to study P4/PR dependent tumor progression. To target deletion of p53 specifically to the mammary gland, this model employs transplantation of p53 null MECs into the cleared fat pads of wt host female mice. Mammary tissue outgrowths form hyperplasias that are ER/PR positive and are sensitive to growth stimulation by P4 by a mechanism that appears to involve a switch from a paracrine to an autocrine mode of P4 induced proliferation (Medina et al., 2002; Medina et al., 2003). Progesterone, but not estrogen, also increases aneuploidy and chromosome instability in this model (Goepfert et al., 2000). A fraction of DCIS and invasive tumors in this model retain expression of ER/PR and either E2+P4, or P4 alone, can enhance tumorigenesis while deletion of PR in the p53 null mouse model markedly reduced tumor incidence (Medina et al., 2002; Medina et al., 2003). These clinical data and mouse model experiments taken together indicate that cell proliferative signaling pathways regulated by P4/PR contributes to the initiation and development of breast tumors.

The mechanism(s) underlying progesterone as a breast cancer risk factor is not well defined, but hypotheses have been developed. The life-time cyclical proliferative effect of P4 on the breast epithelium is thought to be pre-disposing for breast cancer initiated by specific genetic changes. Indeed exposure of the human breast epithelium to ovarian sex steroids during the reproductive years is established to be a risk factor for breast cancer (Apter et al., 1989; Bernstein, 2002; Clemons and Goss, 2001; Hankinson et al., 2004; Pike et al., 1993; Spicer et al., 1995). Early menarche and late menopause, which results in a longer life time exposure to ovarian steroids, is associated with increased breast cancer incidence. Also, a transient increased incidence of breast cancer is associated with pregnancy implicating the proliferative actions of progesterone specifically (Lambe et al., 1994). As another

mechanism, the ability of progesterone to activate mammary stem cell pools has also been suggested to lead to expansion of MaSCs with mutations that could drive cell transformation. Over time, P4 expanded MaSC pools could accumulate mutations sufficient for MaSCs to acquire the properties of tumor initiating cells (Asselin-Labat et al., 2010; Joshi et al., 2010). Alternatively, P4 has been shown in luminal subtype ER⁺/PR⁺/CK5⁻ human breast tumor xenografts to increase a rare subpopulation of ER⁻/PR⁻/CK5⁺ cells with basal stem cell-like properties. Based on these studies, the progestin in HRT was proposed to be a risk factor due to the presence of occult pre-invasive breast cancer in post-menopausal women that contains ER⁻/PR⁻/CK5⁺ stem cells capable of being reactivated by progestins and mobilized as tumor initiating cells (Horwitz et al., 2008; Horwitz and Sartorius, 2008). Interestingly, this subpopulation of basal stem-like cells in the luminal breast cancer ER⁺/PR⁺ subtype was shown to be relatively resistant to chemotherapies and endocrine therapies *in vitro*, and in patient tumors in a neo-adjuvant setting, suggesting a novel therapeutic cell target and mechanism of resistance (Kabos et al., 2011).

The extent to which P4/PR signaling contributes to progression of breast cancer and the mechanisms involved are not clear, due in part to the lack of good experimental models of human breast cancer progression. In most breast cancer cell lines, progesterone induces a transient proliferation followed by cell cycle arrest due to induction of cell cycle inhibitors (Groshong et al., 1997; Skildum et al., 2005; Sutherland et al., 1998). Thus, similar to normal breast, the proliferative effect of P4 in breast cancer cells is transient with the long-term effect being to inhibit proliferation. However, several studies have shown that P4/PR signaling is altered during tumorigenesis, suggesting that dysregulation of PR pathways may contribute to pathogenesis. Gene expression microarray analysis of primary normal human breast epithelial cells in 3D culture, compared with a transformed human breast cancer cell line, revealed very little overlap of genes regulated acutely by P4 (Graham et al., 2009), suggesting that the P4/PR signaling axis is substantially altered during the cancer transformation process. However, it is not clear from these studies the extent to which the difference in the progesterone transcriptome is a reflection of a cell line that accumulates gene changes over time vs. primary cultures.

The relative level of expression of the two PR isoforms has been demonstrated to change during the tumorigenic process and this change has been suggested to have a direct impact on the pathogenesis of breast cancer. Equal amounts of PR-A and PR-B are expressed in normal human breast and in benign breast lesions, while PR-A/PR-B ratios are frequently altered in breast cancer (Bamberger et al., 2000; Graham et al., 1995; Mote et al., 2002; Mote et al., 2007). A high ratio of PR-A:PR-B was observed to be associated with a more aggressive tumor phenotype and with resistance to endocrine therapies (Hopp et al., 2004). Experimental manipulation of PR-A to PR-B ratios has also been shown to alter responses to P4 in breast cancer cells including gene expression network affected by P4 (Graham et al., 2005; Jacobsen et al., 2002; McGowan and Clarke, 1999; McGowan et al., 2004). Transgenic mice overexpressing PR-A develop abnormally and exhibit hyperplasias and disorganization of epithelial-basement membrane interactions (Shyamala et al., 1998). Mammary tumors in transgenic mice overexpressing the MAT1 oncogene exhibit an increased relative abundance of PR-A to PR-B as do mammary tumors that arise in BRCA1 mutated mice (Bagheri-Yarmand et al., 2004; Poole et al., 2006). Resistance of mammary tumors to antiprogestins can be modulated by altering the ratios of PR-A:PR-B (Wargon et al., 2009; Wargon et al., 2011).

A subpopulation of PR outside the nucleus is capable of mediating rapid P4 induced activation of different protein phosphorylation signaling cascades including p21/Ras/MAPK, Jak-1/-2/Stat3 and PI3K/Akt/NF- κ B. Rapid activation of these signaling pathways has been shown to be required for maximal proliferative effects of P4 in breast cancer cells

(Boonyaratanakornkit et al., 2007; Carnevale et al., 2007; Castoria et al., 1999; Migliaccio et al., 2007; Proietti et al., 2005; Saitoh et al., 2005; Skildum et al., 2005). Rapid activation of the PI3K/Akt/Rho/ROCK signaling cascade in breast cancer cells has also been reported to be involved in mediating the effects of P4 on cell motility and focal adhesion (Fu et al., 2010). The non-receptor tyrosine kinase c-Src is a central player in mediating rapid activation of signaling cascades by all steroid hormone receptors. Src is proximal in the signaling pathways affected, and it can bind steroid receptors either directly or indirectly (Ballare et al., 2003; Boonyaratanakornkit et al., 2001). PR interaction with c-Src is mediated by a polypro line domain in the NTD of receptor that binds to the SH3 domain of c-Src and activates the kinase via conformational changes (Boonyaratanakornkit et al., 2001). Rapid P4 activation of c-Src and kinase signaling cascades has also been shown to be integrated with the transcriptional activity of PR. In a feed-forward loop, kinase signaling activated by P4, in a manner dependent on PR interaction with the SH3 domain of Src, was shown to directly phosphorylate PR on a site (serine 345) required for P4 induction of selected non-canonical target genes (p21 and EGFR) that require PR tethering to SP1 (Daniel et al., 2009; Faivre et al., 2008). Extra-nuclear signaling actions of PR are mediated predominantly by PR-B. PR-A, which appears to traffic differently than PR-B, does not efficiently mediate rapid activation of protein kinase signaling pathways (Boonyaratanakornkit et al., 2007; Faivre et al., 2008; Skildum et al., 2005). An unknown question is whether the extra-nuclear signaling actions of PR occur in normal mammary gland or represents a mechanism by which P4/PR in breast cancer cells usurps signaling pathways normally used by growth factor or other cell surface receptors. Extra-nuclear signaling mediated by PR has been demonstrated only in breast cancer and other transformed cell lines. Its role in the normal mammary gland has not been explored.

Human PR in breast cancer cells is phosphorylated on as many as 10 sites. This post-translational modification has been demonstrated to affect multiple PR functions including receptor turnover, nuclear trafficking and transcriptional activity. Phosphorylation of PR is mediated by multiple kinases including MAPK, CDK2/cyclin A and casein kinase II, and specific sites that affect PR function can be phosphorylated in response to P4 or kinases activated by growth factors such as EGF (Daniel et al., 2007; Daniel et al., 2009; Hagan et al., 2011; Moore et al., 2007). EGF potentiates progesterone induction of selected PR gene targets, and enhances progestin-dependent growth of breast cancer cells, indicating that crosstalk between EGF and P4 produces a hyper activated PR. A MAPK consensus site at Ser294 of PR is phosphorylated either by EGF or P4 in breast cancer cells and appears to be required for this functional synergy. Mutation of Ser294 to an alanine or MEK inhibitors, blocked the synergy between P4 and EGF. Over-expression of MAPK in breast cancer cells also resulted in a strong enhancement of P4 dependent transcriptional activity of PR that was abrogated by MEK inhibitors or mutation of ser294 (Shen et al., 2001). Interestingly, phosphorylation of Ser294 promotes a down regulation of the PR protein and this event is required for the transcriptional activity of PR. These results, coupled with the fact that breast cancers frequently have elevated MAPK, have led to the proposal that cross talk between growth factor receptors and PR contributes to deregulated proliferation and progression of breast cancer (Daniel et al 2007 and 2009). Loss of detectable PR protein, or low PR, in breast cancers with elevated MAPK or growth factor receptors may be due to a hyper-activated persistently down regulated PR that contributes to tumor progression during transition to more aggressive PR negative tumors. Again, an important piece of information that is lacking is the extent to which cross talk between growth factor receptor/MAPK signaling and PR occurs in normal human breast epithelium or is a process usurped by breast cancer cells.

Despite the fact that progesterone is a proliferative hormone and a risk factor for breast cancer, the presence of PR in primary breast cancers is an independent marker of a favorable

prognosis and it is associated with a more differentiated, less invasive phenotype than PR negative tumors. ER⁺/PR⁺ tumors respond to endocrine therapies better than ER⁺/PR⁻ tumors in both pre- and postmenopausal women (Bardou et al., 2003; Baum et al., 2003; Ravdin et al., 1992). The presence of PR is also predictive of better overall survival (Bardou et al., 2003). Loss of PR in ER⁺ tumors is associated with a more aggressive tumor phenotype, reduced responsiveness to endocrine therapies, and a shorter overall survival (Bardou et al., 2003; Baum et al., 2003; Liu et al., 2010). Although PR is an upregulated ER target gene and originally thought to be a marker for a functional ER, the unfavorable outcome of ER⁺/PR⁻ breast cancers does not appear to be due to a non-functional ER. Molecular profiling indicates that ER is functional suggesting that other mechanisms must be responsible for the phenotype of ER⁺/PR⁻ tumors (Cui et al., 2003). Loss of PR in ER⁺ tumors is associated with overexpression of EGFR and HER2 (Bamberger et al., 1996; Dixon et al., 2004; Rimawi et al., 2010; Taucher et al., 2003), and upregulation of the PI3K/Akt/mTOR signaling pathway (Creighton et al., 2009; Tokunaga et al., 2006; Tokunaga et al., 2006). Mammary tumors in transgenic mice overexpressing the coactivator AIB1 have elevated ER, loss of PR, and increased IGF-I activation of the PI3K/Akt/mTOR pathway (Torres -Arzayus et al., 2004) indicating a conservation of hyperactive growth factor signaling associated with PR loss. Therefore, in addition to ER that is well established as a marker of favorable prognosis in breast cancer, PR appears to have a positive impact on the biology and progression of breast tumors.

These data taken together suggest that utilization of up regulated growth factor and PI3K signaling pathways contributes to the more aggressive phenotype of ER⁺/PR⁻ breast tumors. What has not been considered is whether PR itself may have tumor suppressor activity and limit progression of breast cancer, such that loss of a beneficial action of PR contributes to the tumor phenotype. Since the absence of PR is associated with a less differentiated tumor phenotype and worse prognosis, raises the possibility that PR may impede epithelial-to-mesenchymal transition (EMT). EMT is a process whereby epithelial cells lose polarity and interactions with the basement membrane, and acquire migratory properties and other characteristics of mesenchymal cells. Activation of an EMT process has been proposed to be a critical intermediate for metastasis of epithelial cell cancers including breast cancer (Blick et al., 2008; Chaffer and Weinberg, 2011; Kalluri and Weinberg, 2009). Other steroid receptors have been demonstrated to have tumor suppressor activity and to impair EMT. Interestingly, this appears to occur independent of hormonal ligand. For example, androgen receptor (AR) was shown to function in basal epithelial cells to suppress prostate cancer invasion and metastasis in an animal model (Niu et al., 2008). ER α suppressed expression of the EMT transcription factor Slug and the downstream lipocalin 2 gene that appears to be a major signaling axis driving EMT and invasion/metastasis of breast cancer cells (Berger et al., 2010; Niu et al., 2008; Yang et al., 2009). ER β was reported to repress EMT in prostate cancer cells induced by TGF β or hypoxia (Mak et al., 2010) and ER β in HC -11 cells was shown to play a role in maintaining a differentiated phenotype in the absence of hormone (Helguero et al., 2008). Loss of ER β in HC -11 cells resulted in mislocalization of E-cadherin, activation of β -catenin, and disruption of cell polarity of acini in 3D culture, suggesting that ER β suppresses changes associated with EMT. Gene microarray experiments with breast cancer cell lines engineered to induce PR, demonstrated that PR independent of ligand regulates the expression of a range of genes some of which are involved in cell adhesion, migration, and epithelial cell differentiation (Jacobsen et al., 2005). PR, in the absence of hormone, was also observed to inhibit growth of breast cancer cells *in vitro* and as xenografts *in vivo* (Jacobsen et al., 2005; Sartorius et al., 2003), and to inhibit inflammatory responses in breast cancer cells (Hardy et al., 2008). These data collectively suggest that PR, independent of ligand, exhibits some degree of tumor suppressor activity. Since the majority of breast cancer occurs in postmenopausal women when P4 levels are very low, it is reasonable to speculate that the potential beneficial

functions of PR to limit tumor progression may be independent of its hormonal ligand. We propose that PR in breast cancer, independent of hormone, promotes maintenance of epithelial cell differentiation and inhibits EMT through gene expression programming, and perhaps through PR interaction with a network of cell signaling pathways.

Steroid hormones, including P4, actively repress expression of many genes, and this action could also contribute to tumor suppressor activity of PR. For example, reactivation of PR expression in highly metastatic ER/PR negative MDA-231 breast cancer cells resulted in P4 mediating a reduction of genes required for cell proliferation and metastasis, and increased expression of other genes associated with tumor suppression (Leo et al., 2005). Additionally, P4 in PR reactivated MDA-231 exhibited growth inhibitory and other anti-cancer effects (Leo and Lin, 2008). Interestingly, reintroduction of ER had little impact on P4 effects in MDA -231 cells (Leo and Lin, 2008). Progesterone has also been reported to repress inflammatory response pathways in breast cancer cells induced by IL-1 β or TNF α . Repression is mediated by PR inhibition of NF- κ B dependent transcription of pro-inflammatory gene targets either through increased expression of the nuclear factor- κ B inhibitor I κ B α (Hardy et al., 2008), or physical tethering of PR with the p65 subunit of NF- κ B (Kobayashi et al., 2010). A model for the role of P4/PR at different stages of breast cancer progression is depicted in Figure 2. As a proliferative hormone, P4 is a risk factor and promotes pre-neoplastic progression. In more advanced stage breast cancer, PR, either independent of hormone or in response to P4, is proposed to impede invasion and metastasis by maintaining epithelial cell differentiation and inhibiting EMT.

Conclusion

Much of our understanding of the biology of PR in the normal mammary gland and the role of progesterone/PR signaling in tumor progression comes from studies with mouse experimental models. In part due to lack of suitable experimental models, uncertainties remain concerning the mechanism of action of progesterone and PR in the normal human breast and in the etiology and progression of human breast cancer. Available data indicates that progesterone as a proliferative hormone is a risk factor for human breast cancer and that it stimulates normal human breast epithelium through a paracrine mechanism. However, whether RANKL in humans is the major paracrine mediator or whether dysregulation of the PR/RANKL paracrine signaling axis contributes to tumor progression as it does in the mouse, is not known. Future development and experimentation with models of normal human breast and human breast cancer progression will be important to broaden our mechanistic understanding of the effects of progesterone/PR in human breast cancer. Such mechanistic information may be useful for improved diagnostic and therapeutic interventions in breast cancer.

Acknowledgments

Some of the work described in this review was supported in part by NIH grants DK049030 and HD038129 (DPE).

References

- Anderson E, Clarke RB, Howell A. Estrogen responsiveness and control of normal human breast proliferation. *J Mammary Gland Biol Neoplasia*. 1998; 3:23–35. [PubMed: 10819502]
- Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. *Breast Cancer Res*. 2002; 4:197–201. [PubMed: 12223124]
- Anderson E, Clarke RB. Steroid receptors and cell cycle in normal mammary epithelium. *J Mammary Gland Biol Neoplasia*. 2004; 9:3–13. [PubMed: 15082914]

- Anderson TJ, Ferguson DJ, Raab GM. Cell turnover in the “resting” human breast: influence of parity, contraceptive pill, age and laterality. *Br J Cancer*. 1982; 46:376–382. [PubMed: 7126427]
- Apter D, Reinila M, Vihko R. Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. *Int J Cancer*. 1989; 44:783–787. [PubMed: 2511157]
- 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]
- Aupperlee MD, Smith KT, Kariagina A, Haslam SZ. Progesterone receptor isoforms A and B: temporal and spatial differences in expression during murine mammary gland development. *Endocrinology*. 2005; 146:3577–3588. [PubMed: 15878961]
- Aupperlee MD, Haslam SZ. Differential hormonal regulation and function of progesterone receptor isoforms in normal adult mouse mammary gland. *Endocrinology*. 2007; 148:2290–2300. [PubMed: 17317767]
- Bagheri-Yarmand R, Talukder AH, Wang RA, Vadlamudi RK, Kumar R. Metastasis-associated protein 1 deregulation causes inappropriate mammary gland development and tumorigenesis. *Development*. 2004; 131:3469–3479. [PubMed: 15226262]
- Ballare C, Uhrig M, Bechtold T, Sancho E, Di Domenico M, Migliaccio A, Auricchio F, Beato M. Two domains of the progesterone receptor interact with the estrogen receptor and are required for progesterone activation of the c-Src/Erk pathway in mammalian cells. *Mol Cell Biol*. 2003; 23:1994–2008. [PubMed: 12612073]
- Bamberger AM, Bamberger CM, Gellersen B, Schulte HM. Modulation of AP-1 activity by the human progesterone receptor in endometrial adenocarcinoma cells. *Proc Natl Acad Sci U S A*. 1996; 93:6169–6174. [PubMed: 8650238]
- Bamberger AM, Milde-Langosch K, Schulte HM, Loning T. Progesterone receptor isoforms, PR-B and PR-A, in breast cancer: correlations with clinicopathologic tumor parameters and expression of AP-1 factors. *Horm Res*. 2000; 54:32–37. [PubMed: 11182633]
- Barash I. Stat5 in the mammary gland: controlling normal development and cancer. *J Cell Physiol*. 2006; 209:305–313. [PubMed: 16883580]
- Barcellos-Hoff MH, Ewan KB. Transforming growth factor-beta and breast cancer: Mammary gland development. *Breast Cancer Res*. 2000; 2:92–99. [PubMed: 11250698]
- Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol*. 2003; 21:1973–1979. [PubMed: 12743151]
- Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG, Somerfield MR. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol*. 2001; 19:1865–1878. [PubMed: 11251019]
- Baum M, Buzdar A, Cuzick J, Forbes J, Houghton J, Howell A, Sahmoud T. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. *Cancer*. 2003; 98:1802–1810. [PubMed: 14584060]
- Beger C, Pierce LN, Kruger M, Marcusson EG, Robbins JM, Welch P, Welch PJ, Welte K, King MC, Barber JR, Wong-Staal F. Identification of Id4 as a regulator of BRCA1 expression by using a ribozyme-library-based inverse genomics approach. *Proc Natl Acad Sci U S A*. 2001; 98:130–135. [PubMed: 11136250]
- 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. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*. 2003; 362:419–427. [PubMed: 12927427]

- Berger T, Cheung CC, Elia AJ, Mak TW. Disruption of the *Lcn2* gene in mice suppresses primary mammary tumor formation but does not decrease lung metastasis. *Proc Natl Acad Sci U S A*. 2010; 107:2995–3000. [PubMed: 20133630]
- Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia*. 2002; 7:3–15. [PubMed: 12160084]
- Bjornstrom L, Kilic E, Norman M, Parker MG, Sjoberg M. Cross-talk between Stat5b and estrogen receptor- α and - β in mammary epithelial cells. *J Mol Endocrinol*. 2001; 27:93–106. [PubMed: 11463580]
- Bjornstrom L, Sjoberg M. Signal transducers and activators of transcription as downstream targets of nongenomic estrogen receptor actions. *Mol Endocrinol*. 2002; 16:2202–2214. [PubMed: 12351686]
- Blick T, Widodo E, Hugo H, Waltham M, Lenburg ME, Neve RM, Thompson EW. Epithelial mesenchymal transition traits in human breast cancer cell lines. *Clin Exp Metastasis*. 2008; 25:629–642. [PubMed: 18461285]
- Boerner JL, Gibson MA, Fox EM, Posner ED, Parsons SJ, Silva CM, Shupnik MA. Estrogen negatively regulates epidermal growth factor (EGF)-mediated signal transducer and activator of transcription 5 signaling in human EGF family receptor-overexpressing breast cancer cells. *Mol Endocrinol*. 2005; 19:2660–2670. [PubMed: 15976008]
- Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*. 1998; 19:225–268. [PubMed: 9626554]
- Boonyaratankornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT, Edwards DP. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. *Mol Cell*. 2001; 8:269–280. [PubMed: 11545730]
- Boonyaratankornkit V, McGowan E, Sherman L, Mancini MA, Cheskis BJ, Edwards DP. The role of extranuclear signaling actions of progesterone receptor in mediating progesterone regulation of gene expression and the cell cycle. *Mol Endocrinol*. 2007; 21:359–375. [PubMed: 17138644]
- Bradbury JM, Edwards PA, Niemeyer CC, Dale TC. Wnt-4 expression induces a pregnancy-like growth pattern in reconstituted mammary glands in virgin mice. *Dev Biol*. 1995; 170:553–563. [PubMed: 7649383]
- 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]
- Briskin C. Hormonal control of alveolar development and its implications for breast carcinogenesis. *J Mammary Gland Biol Neoplasia*. 2002; 7:39–48. [PubMed: 12160085]
- Briskin C, Rajaram RD. Alveolar and lactogenic differentiation. *J Mammary Gland Biol Neoplasia*. 2006; 11:239–248. [PubMed: 17111223]
- Bulyanko YA, O'Malley BW. Nuclear Receptor Coactivators: Structural and Functional Biochemistry. *Biochemistry*. 2010
- Buser AC, Gass-Handel EK, Wyszomierski SL, Doppler W, Leonhardt SA, Schaack J, Rosen JM, Watkin H, Anderson SM, Edwards DP. Progesterone receptor repression of prolactin/signal transducer and activator of transcription 5-mediated transcription of the beta-casein gene in mammary epithelial cells. *Mol Endocrinol*. 2007; 21:106–125. [PubMed: 16973758]
- Buser AC, Obr AE, Kabotyanski EB, Grimm SL, Rosen JM, Edwards DP. Progesterone receptor directly inhibits beta-casein gene transcription in mammary epithelial cells through promoting promoter and enhancer repressive chromatin modifications. *Mol Endocrinol*. 2011; 25:955–968. [PubMed: 21527503]
- Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev*. 1997; 11:3286–3305. [PubMed: 9407023]

- Cao J, Wood M, Liu Y, Hoffman T, Hyde J, Park-Sarge OK, Vore M. Estradiol represses prolactin-induced expression of Na⁺/taurocholate cotransporting polypeptide in liver cells through estrogen receptor- α and signal transducers and activators of transcription 5a. *Endocrinology*. 2004; 145:1739–1749. [PubMed: 14684617]
- Carnevale RP, Proietti CJ, Salatino M, Urtreger A, Peluffo G, Edwards DP, Boonyaratanakornkit V, Charreau EH, Bal de KierJoffe E, Schillaci R, Elizalde PV. Progesterone effects on breast cancer cell proliferation, proteases activation, and in vivo development of metastatic phenotype all depend on progesterone receptor capacity to activate cytoplasmic signaling pathways. *Mol Endocrinol*. 2007; 21:1335–1358. [PubMed: 17440047]
- Castoria G, Barone MV, Di Domenico M, Bilancio A, Ametrano D, Migliaccio A, Auricchio F. Non-transcriptional action of oestradiol and progesterone triggers DNA synthesis. *EMBO J*. 1999; 18:2500–2510. [PubMed: 10228164]
- Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science*. 2011; 331:1559–1564. [PubMed: 21436443]
- Chatterton RT Jr, Lydon JP, Mehta RG, Mateo ET, Pletz A, Jordan VC. Role of the progesterone receptor (PR) in susceptibility of mouse mammary gland to 7, 12-dimethylbenz[a]anthracene-induced hormone-independent preneoplastic lesions in vitro. *Cancer Lett*. 2002; 188:47–52. [PubMed: 12406547]
- 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 progesterone and breast cancer incidence and mortality in postmenopausal women. *JAMA*. 2010; 304:1684–1692. [PubMed: 20959578]
- Clarke CA, Glaser SL, Uratsu CS, Selby JV, Kushi LH, Herrinton LJ. Recent declines in hormone therapy utilization and breast cancer incidence: clinical and population-based evidence. *J Clin Oncol*. 2006; 24:e49–50. [PubMed: 17114650]
- 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]
- Clemons M, Goss P. Estrogen and the risk of breast cancer. *N Engl J Med*. 2001; 344:276–285. [PubMed: 11172156]
- Conneely OM, Jericevic BM, Lydon JP. Progesterone receptors in mammary gland development and tumorigenesis. *J Mammary Gland Biol Neoplasia*. 2003; 8:205–214. [PubMed: 14635795]
- Creighton CJ, Kent Osborne C, van de Vijver MJ, Foekens JA, Klijn JG, Horlings HM, Nuyten D, Wang Y, Zhang Y, Chamness GC, Hilsenbeck SG, Lee AV, Schiff R. Molecular profiles of progesterone receptor loss in human breast tumors. *Breast Cancer Res Treat*. 2009; 114:287–299. [PubMed: 18425577]
- Cui X, Zhang P, Deng W, Oesterreich S, Lu Y, Mills GB, Lee AV. Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. *Mol Endocrinol*. 2003; 17:575–588. [PubMed: 12554765]
- Dale TC. Signal transduction by the Wnt family of ligands. *Biochem J*. 1998; 329(Pt 2):209–223. [PubMed: 9425102]
- Daniel AR, Qiu M, Faivre EJ, Ostrander JH, Skildum A, Lange CA. Linkage of progesterone and epidermal growth factor signaling: phosphorylation of progesterone receptors mediates transcriptional hypersensitivity and increased ligand-independent breast cancer cell growth. *Steroids*. 2007; 72:188–201. [PubMed: 17173941]
- Daniel AR, Knutson TP, Lange CA. Signaling inputs to progesterone receptor gene regulation and promoter selectivity. *Mol Cell Endocrinol*. 2009; 308:47–52. [PubMed: 19549591]
- Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. *Nat Rev Cancer*. 2005; 5:675–688. [PubMed: 16148884]
- Dixon JM, Jackson J, Hills M, Renshaw L, Cameron DA, Anderson TJ, Miller WR, Dowsett M. Anastrozole demonstrates clinical and biological effectiveness in oestrogen receptor-positive breast cancers, irrespective of the erbB2 status. *Eur J Cancer*. 2004; 40:2742–2747. [PubMed: 15571956]

- Dong J, Huang S, Cailovski M, Ji S, McGrath A, Custorio MG, Creighton CJ, Maliakkal P, Bogoslovskaja E, Du Z, Zhang X, Lewis MT, Sablitzky F, Briskin C, Li Y. ID4 regulates mammary gland development and tumorigenesis by suppressing p38MAPK activity. *Development*. 2011
- Doppler W, Hock W, Hofer P, Groner B, Ball RK. Prolactin and glucocorticoid hormones control transcription of the beta-casein gene by kinetically distinct mechanisms. *Mol Endocrinol*. 1990; 4:912–919. [PubMed: 2172795]
- Doppler W, Windegger M, Soratroi C, Tomasi J, Lechner J, Rusconi S, Cato AC, Almlöf T, Liden J, Ökret S, Gustafsson JA, Richard-Foy H, Starr DB, Klocker H, Edwards D, Geymayer S. Expression level-dependent contribution of glucocorticoid receptor domains for functional interaction with STAT5. *Mol Cell Biol*. 2001; 21:3266–3279. [PubMed: 11287629]
- Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev*. 1999; 13:2412–2424. [PubMed: 10500098]
- Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol*. 2005; 67:335–376. [PubMed: 15709962]
- Engblom D, Kornfeld JW, Schwake L, Tronche F, Reimann A, Beug H, Hennighausen L, Moriggl R, Schutz G. Direct glucocorticoid receptor-Stat5 interaction in hepatocytes controls body size and maturation-related gene expression. *Genes Dev*. 2007; 21:1157–1162. [PubMed: 17504935]
- Ewan KB, Oketch-Rabah HA, Ravani SA, Shyamala G, Moses HL, Barcellos-Hoff MH. Proliferation of estrogen receptor-alpha-positive mammary epithelial cells is restrained by transforming growth factor-beta1 in adult mice. *Am J Pathol*. 2005; 167:409–417. [PubMed: 16049327]
- Faivre EJ, Daniel AR, Hillard CJ, Lange CA. Progesterone receptor rapid signaling mediates serine 345 phosphorylation and tethering to specificity protein 1 transcription factors. *Mol Endocrinol*. 2008; 22:823–837. [PubMed: 18202149]
- Fata JE, Kong YY, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, Elliott R, Scully S, Voura EB, Lacey DL, Boyle WJ, Khokha R, Penninger JM. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. *Cell*. 2000; 103:41–50. [PubMed: 11051546]
- Faulds MH, Pettersson K, Gustafsson JA, Haldosen LA. Cross-talk between ERs and signal transducer and activator of transcription 5 is E2 dependent and involves two functionally separate mechanisms. *Mol Endocrinol*. 2001; 15:1929–1940. [PubMed: 11682624]
- Fendrick JL, Raafat AM, Haslam SZ. Mammary gland growth and development from the postnatal period to postmenopause: ovarian steroid receptor ontogeny and regulation in the mouse. *J Mammary Gland Biol Neoplasia*. 1998; 3:7–22. [PubMed: 10819501]
- Fernandez-Valdivia R, Mukherjee A, Mulac-Jericevic B, Conneely OM, DeMayo FJ, Amato P, Lydon JP. Revealing progesterone's role in uterine and mammary gland biology: insights from the mouse. *Semin Reprod Med*. 2005; 23:22–37. [PubMed: 15714387]
- Fernandez-Valdivia R, Mukherjee A, Creighton CJ, Buser AC, DeMayo FJ, Edwards DP, Lydon JP. Transcriptional response of the murine mammary gland to acute progesterone exposure. *Endocrinology*. 2008; 149:6236–6250. [PubMed: 18687774]
- 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]
- Frech MS, Halama ED, Tilli MT, Singh B, Gunther EJ, Chodosh LA, Flaws JA, Furth PA. Deregulated estrogen receptor alpha expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ. *Cancer Res*. 2005; 65:681–685. [PubMed: 15705859]
- Fu XD, Goglia L, Sanchez AM, Flamini M, Giretti MS, Tosi V, Genazzani AR, Simoncini T. Progesterone receptor enhances breast cancer cell motility and invasion via extranuclear activation of focal adhesion kinase. *Endocr Relat Cancer*. 2010; 17:431–443. [PubMed: 20233709]
- Gass S, Harris J, Ormandy C, Briskin C. Using gene expression arrays to elucidate transcriptional profiles underlying prolactin function. *J Mammary Gland Biol Neoplasia*. 2003; 8:269–285. [PubMed: 14973373]

- Gavin BJ, McMahon AP. Differential regulation of the Wnt gene family during pregnancy and lactation suggests a role in postnatal development of the mammary gland. *Mol Cell Biol.* 1992; 12:2418–2423. [PubMed: 1373817]
- Goepfert TM, McCarthy M, Kittrell FS, Stephens C, Ullrich RL, Brinkley BR, Medina D. Progesterone facilitates chromosome instability (aneuploidy) in p53 null normal mammary epithelial cells. *FASEB J.* 2000; 14:2221–2229. [PubMed: 11053243]
- Goffin V, Kelly PA. The prolactin/growth hormone receptor family: structure/function relationships. *J Mammary Gland Biol Neoplasia.* 1997; 2:7–17. [PubMed: 10887515]
- Gonzalez-Suarez E, Branstetter D, Armstrong A, Dinh H, Blumberg H, Dougall WC. RANK overexpression in transgenic mice with mouse mammary tumor virus promoter-controlled RANK increases proliferation and impairs alveolar differentiation in the mammary epithelia and disrupts lumen formation in cultured epithelial acini. *Mol Cell Biol.* 2007; 27:1442–1454. [PubMed: 17145767]
- 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]
- Gonzalez-Suarez E. RANKL inhibition: a promising novel strategy for breast cancer treatment. *Clin Transl Oncol.* 2011; 13:222–228. [PubMed: 21493182]
- Graham JD, Yeates C, Balleine RL, Harvey SS, Milliken JS, Bilous AM, Clarke CL. Characterization of progesterone receptor A and B expression in human breast cancer. *Cancer Res.* 1995; 55:5063–5068. [PubMed: 7585552]
- Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev.* 1997; 18:502–519. [PubMed: 9267762]
- 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, Huschtscha LI, Reddel RR, Clarke CL. DNA replication licensing and progenitor numbers are increased by progesterone in normal human breast. *Endocrinology.* 2009; 150:3318–3326. [PubMed: 19342456]
- Grimm SL, Seagroves TN, Kabotyanski EB, Hovey RC, Vonderhaar BK, Lydon JP, Miyoshi K, Hennighausen L, Ormandy CJ, Lee AV, Stull MA, Wood TL, Rosen JM. Disruption of steroid and prolactin receptor patterning in the mammary gland correlates with a block in lobuloalveolar development. *Mol Endocrinol.* 2002; 16:2675–2691. [PubMed: 12456789]
- Grimm SL, Contreras A, Barcellos-Hoff MH, Rosen JM. Cell cycle defects contribute to a block in hormone-induced mammary gland proliferation in CCAAT/enhancer-binding protein (C/EBP β)-null mice. *J Biol Chem.* 2005; 280:36301–36309. [PubMed: 16120603]
- Grimm SL, Rosen JM. Stop! In the name of transforming growth factor- β : keeping estrogen receptor- α -positive mammary epithelial cells from proliferating. *Breast Cancer Res.* 2006; 8:106. [PubMed: 16834786]
- Groner B, Altioek S, Meier V. Hormonal regulation of transcription factor activity in mammary epithelial cells. *Mol Cell Endocrinol.* 1994; 100:109–114. [PubMed: 8056143]
- Groshong SD, Owen GI, Grimison B, Schauer IE, Todd MC, Langan TA, Sclafani RA, Lange CA, Horwitz KB. Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and p27 (Kip1). *Mol Endocrinol.* 1997; 11:1593–1607. [PubMed: 9328342]
- Hagan CR, Regan TM, Dressing GE, Lange CA. ck2 -dependent phosphorylation of progesterone receptors (PR) on Ser81 regulates PR-B isoform-specific target gene expression in breast cancer cells. *Mol Cell Biol.* 2011; 31:2439–2452. [PubMed: 21518957]
- 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]
- Hardy DB, Janowski BA, Chen CC, Mendelson CR. Progesterone receptor inhibits aromatase and inflammatory response pathways in breast cancer cells via ligand-dependent and ligand-independent mechanisms. *Mol Endocrinol.* 2008; 22:1812–1824. [PubMed: 18483177]

- Harris J, Stanford PM, Sutherland K, Oakes SR, Naylor MJ, Robertson FG, Blazek KD, Kazlauskas M, Hilton HN, Wittlin S, Alexander WS, Lindeman GJ, Visvader JE, Ormandy CJ. Socs2 and elf5 mediate prolactin-induced mammary gland development. *Mol Endocrinol*. 2006; 20:1177–1187. [PubMed: 16469767]
- Haslam SZ, Drolet A, Smith K, Tan M, Aupperlee M. Progesterin-regulated luminal cell and myoepithelial cell-specific responses in mammary organoid culture. *Endocrinology*. 2008; 149:2098–2107. [PubMed: 18218689]
- Helguero LA, Lindberg K, Gardmo C, Schwend T, Gustafsson JA, Haldosen LA. Different roles of estrogen receptors alpha and beta in the regulation of E-cadherin protein levels in a mouse mammary epithelial cell line. *Cancer Res*. 2008; 68:8695–8704. [PubMed: 18974111]
- Hennighausen L, Robinson GW. Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. *Genes Dev*. 2008; 22:711–721. [PubMed: 18347089]
- Hofseth LJ, Raafat AM, Osuch JR, Pathak DR, Slomski CA, Haslam SZ. Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. *J Clin Endocrinol Metab*. 1999; 84:4559–4565. [PubMed: 10599719]
- Hopp TA, Weiss HL, Hilsenbeck SG, Cui Y, Allred DC, Horwitz KB, Fuqua SA. Breast cancer patients with progesterone receptor PR-A-rich tumors have poorer disease-free survival rates. *Clin Cancer Res*. 2004; 10:2751–2760. [PubMed: 15102680]
- 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]
- Hovey RC, Trott JF, Ginsburg E, Goldhar A, Sasaki MM, Fountain SJ, Sundararajan K, Vonderhaar BK. Transcriptional and spatiotemporal regulation of prolactin receptor mRNA and cooperativity with progesterone receptor function during ductal branch growth in the mammary gland. *Dev Dyn*. 2001; 222:192–205. [PubMed: 11668597]
- Howard BA, Gusterson BA. Human breast development. *J Mammary Gland Biol Neoplasia*. 2000; 5:119–137. [PubMed: 11149569]
- Ismail PM, Li J, DeMayo FJ, O'Malley BW, Lydon JP. A novel LacZ reporter mouse reveals complex regulation of the progesterone receptor promoter during mammary gland development. *Mol Endocrinol*. 2002; 16:2475–2489. [PubMed: 12403837]
- Ismail PM, Amato P, Soyal SM, DeMayo FJ, Conneely OM, O'Malley BW, Lydon JP. Progesterone involvement in breast development and tumorigenesis--as revealed by progesterone receptor "knockout" and "knockin" mouse models. *Steroids*. 2003; 68:779–787. [PubMed: 14667968]
- Ismail PM, DeMayo FJ, Amato P, Lydon JP. Progesterone induction of calcitonin expression in the murine mammary gland. *J Endocrinol*. 2004; 180:287–295. [PubMed: 14765981]
- 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, Richer JK, Sartorius CA, Horwitz KB. Expression profiling of human breast cancers and gene regulation by progesterone receptors. *J Mammary Gland Biol Neoplasia*. 2003; 8:257–268. [PubMed: 14973372]
- 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]
- 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]
- Kabotyanski EB, Rijnkels M, Freeman-Zadrowski C, Buser AC, Edwards DP, Rosen JM. Lactogenic hormonal induction of long distance interactions between beta-casein gene regulatory elements. *J Biol Chem.* 2009; 284:22815–22824. [PubMed: 19542223]
- Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest.* 2009; 119:1420–1428. [PubMed: 19487818]
- Kariagina A, Aupperlee MD, Haslam SZ. Progesterone receptor isoforms and proliferation in the rat mammary gland during development. *Endocrinology.* 2007; 148:2723–2736. [PubMed: 17332059]
- 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.* 2010; 1:229–244. [PubMed: 21258428]
- Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J.* 1990; 9:1603–1614. [PubMed: 2328727]
- Khan SA, Rogers MA, Obando JA, Tamsen A. Estrogen receptor expression of benign breast epithelium and its association with breast cancer. *Cancer Res.* 1994; 54:993–997. [PubMed: 8313390]
- 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]
- Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature.* 1999; 397:315–323. [PubMed: 9950424]
- Labriola L, Salatino M, Proietti CJ, Pecci A, Coso OA, Kornblihtt AR, Charreau EH, Elizalde PV. Heregulin induces transcriptional activation of the progesterone receptor by a mechanism that requires functional ErbB-2 and mitogen-activated protein kinase activation in breast cancer cells. *Mol Cell Biol.* 2003; 23:1095–1111. [PubMed: 12529413]
- Lambe M, Hsieh C, Trichopoulos D, Ekblom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. *N Engl J Med.* 1994; 331:5–9. [PubMed: 8202106]
- Lawson JS, Field AS, Champion S, Tran D, Ishikura H, Trichopoulos D. Low oestrogen receptor alpha expression in normal breast tissue underlies low breast cancer incidence in Japan. *Lancet.* 1999; 354:1787–1788. [PubMed: 10577642]
- Lawson JS, Field AS, Tran DD, Killeen J, Maskarenic G, Ishikura H, Trichopoulos D. Breast cancer incidence and estrogen receptor alpha in normal mammary tissue--an epidemiologic study among Japanese women in Japan and Hawaii. *Int J Cancer.* 2002; 97:685–687. [PubMed: 11807798]
- Lechner J, Welte T, Doppler W. Mechanism of interaction between the glucocorticoid receptor and Stat5: role of DNA-binding. *Immunobiology.* 1997; 198:112–123. [PubMed: 9442383]
- Lechner J, Welte T, Tomasi JK, Bruno P, Cairns C, Gustafsson J, Doppler W. Promoter-dependent synergy between glucocorticoid receptor and Stat5 in the activation of beta-casein gene transcription. *J Biol Chem.* 1997; 272:20954–20960. [PubMed: 9252424]
- Lee S, Kolonel L, Wilkens L, Wan P, Henderson B, Pike M. Postmenopausal hormone therapy and breast cancer risk: the Multiethnic Cohort. *Int J Cancer.* 2006; 118:1285–1291. [PubMed: 16170777]
- 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]
- Leo JC, Lin VC. The activities of progesterone receptor isoform A and B are differentially modulated by their ligands in a gene-selective manner. *Int J Cancer.* 2008; 122:230–243. [PubMed: 17893877]

- Li X, O'Malley BW. Unfolding the action of progesterone receptors. *J Biol Chem.* 2003; 278:39261–39264. [PubMed: 12893816]
- Lim E, Wu D, Pal B, Bouras T, Asselin-Labat ML, Vaillant F, Yagita H, Lindeman GJ, Smyth GK, Visvader JE. Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. *Breast Cancer Res.* 2010; 12:R21. [PubMed: 20346151]
- Liu S, Chia SK, Mehl E, Leung S, Rajput A, Cheang MC, Nielsen TO. Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. *Breast Cancer Res Treat.* 2010; 119:53–61. [PubMed: 19205877]
- Loizzi RF. Progesterone withdrawal stimulates mammary gland tubulin polymerization in pregnant rats. *Endocrinology.* 1985; 116:2543–2547. [PubMed: 2986954]
- Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 1995; 9:2266–2278. [PubMed: 7557380]
- 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]
- Lydon JP, Edwards DP. Finally! A model for progesterone receptor action in normal human breast. *Endocrinology.* 2009; 150:2988–2990. [PubMed: 19549883]
- 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]
- Mak P, Leav I, Pursell B, Bae D, Yang X, Taglienti CA, Gouvin LM, Sharma VM, Mercurio AM. ERbeta impedes prostate cancer EMT by destabilizing HIF-1alpha and inhibiting VEGF-mediated snail nuclear localization: implications for Gleason grading. *Cancer Cell.* 2010; 17:319–332. [PubMed: 20385358]
- Mallepell S, Krust A, Chambon P, Briskin C. Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proc Natl Acad Sci U S A.* 2006; 103:2196–2201. [PubMed: 16452162]
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell.* 1995; 83:835–839. [PubMed: 8521507]
- McGowan EM, Clarke CL. Effect of overexpression of progesterone receptor A on endogenous progestin-sensitive endpoints in breast cancer cells. *Mol Endocrinol.* 1999; 13:1657–1671. [PubMed: 10517668]
- McGowan EM, Saad S, Bendall LJ, Bradstock KF, Clarke CL. Effect of progesterone receptor a predominance on breast cancer cell migration into bone marrow fibroblasts. *Breast Cancer Res Treat.* 2004; 83:211–220. [PubMed: 14758091]
- Medina D, Kittrell FS, Shepard A, Stephens LC, Jiang C, Lu J, Allred DC, McCarthy M, Ullrich RL. Biological and genetic properties of the p53 null preneoplastic mammary epithelium. *FASEB J.* 2002; 16:881–883. [PubMed: 11967232]
- Medina D, Kittrell FS, Shepard A, Contreras A, Rosen JM, Lydon J. Hormone dependence in premalignant mammary progression. *Cancer Res.* 2003; 63:1067–1072. [PubMed: 12615724]
- Meier VS, Groner B. The nuclear factor YY1 participates in repression of the beta-casein gene promoter in mammary epithelial cells and is counteracted by mammary gland factor during lactogenic hormone induction. *Mol Cell Biol.* 1994; 14:128–137. [PubMed: 8264581]
- Migliaccio A, Castoria G, Auricchio F. Src-dependent signalling pathway regulation by sex-steroid hormones: therapeutic implications. *Int J Biochem Cell Biol.* 2007; 39:1343–1348. [PubMed: 17329144]
- Moon RT, Brown JD, Torres M. WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet.* 1997; 13:157–162. [PubMed: 9097727]
- Moore NL, Narayanan R, Weigel NL. Cyclin dependent kinase 2 and the regulation of human progesterone receptor activity. *Steroids.* 2007; 72:202–209. [PubMed: 17207508]

- Mote PA, Bartow S, Tran N, Clarke CL. Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. *Breast Cancer Res Treat.* 2002; 72:163–172. [PubMed: 12038707]
- Mote PA, Arnett-Mansfield RL, Gava N, de Fazio A, Mulac-Jericevic B, Conneely OM, Clarke CL. Overlapping and distinct expression of progesterone receptors A and B in mouse uterus and mammary gland during the estrous cycle. *Endocrinology.* 2006; 147:5503–5512. [PubMed: 16980438]
- Mote PA, Graham JD, Clarke CL. Progesterone receptor isoforms in normal and malignant breast. *Ernst Schering Found Symp Proc.* 2007:77–107. [PubMed: 18540569]
- Mueller SO, Clark JA, Myers PH, Korach KS. Mammary gland development in adult mice requires epithelial and stromal estrogen receptor alpha. *Endocrinology.* 2002; 143:2357–2365. [PubMed: 12021201]
- Mukherjee A, Soyal SM, Li J, Ying Y, He B, DeMayo FJ, Lydon JP. Targeting RANKL to a specific subset of murine mammary epithelial cells induces ordered branching morphogenesis and alveologenesis in the absence of progesterone receptor expression. *FASEB J.* 2010; 24:4408–4419. [PubMed: 20605949]
- Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science.* 2000; 289:1751–1754. [PubMed: 10976068]
- 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]
- Neville MC, McFadden TB, Forsyth I. Hormonal regulation of mammary differentiation and milk secretion. *J Mammary Gland Biol Neoplasia.* 2002; 7:49–66. [PubMed: 12160086]
- Nguyen DA, Parlow AF, Neville MC. Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation. *J Endocrinol.* 2001; 170:347–356. [PubMed: 11479131]
- Nguyen DH, Martinez-Ruiz H, Barcellos-Hoff MH. Consequences of epithelial or stromal TGFbeta1 depletion in the mammary gland. *J Mammary Gland Biol Neoplasia.* 2011; 16:147–155. [PubMed: 21590374]
- Niu Y, Altuwaijri S, Lai KP, Wu CT, Ricke WA, Messing EM, Yao J, Yeh S, Chang C. Androgen receptor is a tumor suppressor and proliferator in prostate cancer. *Proc Natl Acad Sci U S A.* 2008; 105:12182–12187. [PubMed: 18723679]
- Norton JD. ID helix-loop-helix proteins in cell growth, differentiation and tumorigenesis. *J Cell Sci.* 2000; 113(Pt 22):3897–3905. [PubMed: 11058077]
- Novaro V, Roskelley CD, Bissell MJ. Collagen-IV and laminin-1 regulate estrogen receptor alpha expression and function in mouse mammary epithelial cells. *J Cell Sci.* 2003; 116:2975–2986. [PubMed: 12808020]
- Nusse R, Varmus HE. Wnt genes. *Cell.* 1992; 69:1073–1087. [PubMed: 1617723]
- Nusse R. WNT targets. Repression and activation. *Trends Genet.* 1999; 15:1–3. [PubMed: 10087922]
- Oakes SR, Hilton HN, Ormandy CJ. The alveolar switch: coordinating the proliferative cues and cell fate decisions that drive the formation of lobuloalveoli from ductal epithelium. *Breast Cancer Res.* 2006; 8:207. [PubMed: 16677418]
- Obr, AE.; Grimm, SL.; Lydon, JP.; Bishop, KA.; Pike, JW.; Edwards, DP. Mechanism of gene regulation by cooperative actions of progesterone and prolactin analyzed in a 3D primary mammary epithelial cell culture system. *Annual Endocrine Society Meetings; Boston, MA.* 2011.
- 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]
- Poole AJ, Li Y, Kim Y, Lin SC, Lee WH, Lee EY. Prevention of Brca1-mediated mammary tumorigenesis in mice by a progesterone antagonist. *Science.* 2006; 314:1467–1470. [PubMed: 17138902]
- Proietti C, Salatino M, Rosembli C, Carnevale R, Pecci A, Kornblihtt AR, Molinolo AA, Frahm I, Charreau EH, Schillaci R, Elizalde PV. Progestins induce transcriptional activation of signal

- transducer and activator of transcription 3 (Stat3) via a Jak-and Src -dependent mechanism in breast cancer cells. *Mol Cell Biol.* 2005; 25:4826–4840. [PubMed: 15923602]
- Ravdin PM, Green S, Dorr TM, McGuire WL, Fabian C, Pugh RP, Carter RD, Rivkin SE, Borst JR, Belt RJ, et al. Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study. *J Clin Oncol.* 1992; 10:1284–1291. [PubMed: 1634918]
- Richer JK, Lange CA, Manning NG, Owen G, Powell R, Horwitz KB. Convergence of progesterone with growth factor and cytokine signaling in breast cancer. Progesterone receptors regulate signal transducers and activators of transcription expression and activity. *J Biol Chem.* 1998; 273:31317–31326. [PubMed: 9813040]
- Rimawi MF, Shetty PB, Weiss HL, Schiff R, Osborne CK, Chamness GC, Elledge RM. Epidermal growth factor receptor expression in breast cancer association with biologic phenotype and clinical outcomes. *Cancer.* 2010; 116:1234–1242. [PubMed: 20082448]
- Robinson GW, Hennighausen L, Johnson PF. Side-branching in the mammary gland: the progesterone-Wnt connection. *Genes Dev.* 2000; 14:889–894. [PubMed: 10783160]
- Rosen JM, Wyszomierski SL, Hadsell D. Regulation of milk protein gene expression. *Annu Rev Nutr.* 1999; 19:407–436. [PubMed: 10448531]
- Rosen JM. Hormone receptor patterning plays a critical role in normal lobuloalveolar development and breast cancer progression. *Breast Dis.* 2003; 18:3–9. [PubMed: 15687684]
- Ross RK, Paganini-Hill A, Wan PC, Pike MC. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. *J Natl Cancer Inst.* 2000; 92:328–332. [PubMed: 10675382]
- Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA.* 2002; 288:321–333. [PubMed: 12117397]
- 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]
- Said TK, Conneely OM, Medina D, O'Malley BW, Lydon JP. Progesterone, in addition to estrogen, induces cyclin D1 expression in the murine mammary epithelial cell, in vivo. *Endocrinology.* 1997; 138:3933–3939. [PubMed: 9275084]
- Saitoh M, Ohmichi M, Takahashi K, Kawagoe J, Ohta T, Doshida M, Takahashi T, Igarashi H, Mori-Abe A, Du B, Tsutsumi S, Kurachi H. Medroxyprogesterone acetate induces cell proliferation through up-regulation of cyclin D1 expression via phosphatidylinositol 3-kinase/Akt/nuclear factor-kappaB cascade in human breast cancer cells. *Endocrinology.* 2005; 146:4917–4925. [PubMed: 16123159]
- Santos SJ, Haslam SZ, Conrad SE. Signal transducer and activator of transcription 5a mediates mammary ductal branching and proliferation in the nulliparous mouse. *Endocrinology.* 2010; 151:2876–2885. [PubMed: 20392833]
- Sartorius CA, Shen T, Horwitz KB. Progesterone receptors A and B differentially affect the growth of estrogen-dependent human breast tumor xenografts. *Breast Cancer Res Treat.* 2003; 79:287–299. [PubMed: 12846413]
- Scarpin KM, Graham JD, Mote PA, Clarke CL. Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. *Nucl Recept Signal.* 2009; 7:e009. [PubMed: 20087430]
- 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]
- Seagroves TN, Lydon JP, Hovey RC, Vonderhaar BK, Rosen JM. C/EBPbeta (CCAAT/enhancer binding protein) controls cell fate determination during mammary gland development. *Mol Endocrinol.* 2000; 14:359–368. [PubMed: 10707954]

- 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]
- Shan L, Yu M, Qiu C, Snyderwine EG. Id4 regulates mammary epithelial cell growth and differentiation and is overexpressed in rat mammary gland carcinomas. *Am J Pathol*. 2003; 163:2495–2502. [PubMed: 14633621]
- 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]
- Shoker BS, Jarvis C, Clarke RB, Anderson E, Hewlett J, Davies MP, Sibson DR, Sloane JP. Estrogen receptor-positive proliferating cells in the normal and precancerous breast. *Am J Pathol*. 1999; 155:1811–1815. [PubMed: 10595909]
- Shoker BS, Jarvis C, Clarke RB, Anderson E, Munro C, Davies MP, Sibson DR, Sloane JP. Abnormal regulation of the oestrogen receptor in benign breast lesions. *J Clin Pathol*. 2000; 53:778–783. [PubMed: 11064673]
- Shyamala G, Barcellos-Hoff MH, Toft D, Yang X. In situ localization of progesterone receptors in normal mouse mammary glands: absence of receptors in the connective and adipose stroma and a heterogeneous distribution in the epithelium. *J Steroid Biochem Mol Biol*. 1997; 63:251–259. [PubMed: 9459191]
- Shyamala G, Yang X, Silberstein G, Barcellos-Hoff MH, Dale E. Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. *Proc Natl Acad Sci U S A*. 1998; 95:696–701. [PubMed: 9435255]
- Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, Nandi S. Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging. *J Steroid Biochem Mol Biol*. 2002; 80:137–148. [PubMed: 11897499]
- Silberstein GB, Van Horn K, Shyamala G, Daniel CW. Progesterone receptors in the mouse mammary duct: distribution and developmental regulation. *Cell Growth Differ*. 1996; 7:945–952. [PubMed: 8809412]
- Sivaraman L, Hilsenbeck SG, Zhong L, Gay J, Conneely OM, Medina D, O'Malley BW. Early exposure of the rat mammary gland to estrogen and progesterone blocks co-localization of estrogen receptor expression and proliferation. *J Endocrinol*. 2001; 171:75–83. [PubMed: 11572792]
- Skildum A, Faivre E, Lange CA. Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases. *Mol Endocrinol*. 2005; 19:327–339. [PubMed: 15486045]
- Soyal S, Ismail PM, Li J, Mulac-Jericevic B, Conneely OM, Lydon JP. Progesterone's role in mammary gland development and tumorigenesis as disclosed by experimental mouse genetics. *Breast Cancer Res*. 2002; 4:191–196. [PubMed: 12223123]
- Spicer DV, Kreckler EA, Pike MC. The endocrine prevention of breast cancer. *Cancer Invest*. 1995; 13:495–504. [PubMed: 7552817]
- Srivastava S, Matsuda M, Hou Z, Bailey JP, Kitazawa R, Herbst MP, Horseman ND. Receptor activator of NF-kappaB ligand induction via Jak2 and Stat5a in mammary epithelial cells. *J Biol Chem*. 2003; 278:46171–46178. [PubMed: 12952963]
- 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]
- Stocklin E, Wissler M, Gouilleux F, Groner B. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature*. 1996; 383:726–728. [PubMed: 8878484]
- Stoecklin E, Wissler M, Moriggl R, Groner B. Specific DNA binding of Stat5, but not of glucocorticoid receptor, is required for their functional cooperation in the regulation of gene transcription. *Mol Cell Biol*. 1997; 17:6708–6716. [PubMed: 9343435]
- Subtil-Rodriguez A, Millan-Arino L, Quiles I, Ballare C, Beato M, Jordan A. Progesterone induction of the 11beta-hydroxysteroid dehydrogenase type 2 promoter in breast cancer cells involves

- coordinated recruitment of STAT5A and progesterone receptor to a distal enhancer and polymerase tracking. *Mol Cell Biol.* 2008; 28:3830–3849. [PubMed: 18378698]
- Sutherland RL, Prall OW, Watts CK, Musgrove EA. Estrogen and progestin regulation of cell cycle progression. *J Mammary Gland Biol Neoplasia.* 1998; 3:63–72. [PubMed: 10819505]
- Tan SH, Dagvadorj A, Shen F, Gu L, Liao Z, Abdulghani J, Zhang Y, Gelmann EP, Zellweger T, Culig Z, Visakorpi T, Bubendorf L, Kirken RA, Karras J, Nevalainen MT. Transcription factor Stat5 synergizes with androgen receptor in prostate cancer cells. *Cancer Res.* 2008; 68:236–248. [PubMed: 18172316]
- Taucher S, Rudas M, Mader RM, Gnant M, Dubsy P, Bachleitner T, Roka S, Fitzal F, Kandoler D, Sporn E, Friedl J, Mittlbock M, Jakesz R. Do we need HER-2/neu testing for all patients with primary breast carcinoma? *Cancer.* 2003; 98:2547–2553. [PubMed: 14669272]
- Tokunaga E, Kataoka A, Kimura Y, Oki E, Mashino K, Nishida K, Koga T, Morita M, Kakeji Y, Baba H, Ohno S, Maehara Y. The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. *Eur J Cancer.* 2006; 42:629–635. [PubMed: 16464571]
- Tokunaga E, Kimura Y, Oki E, Ueda N, Futatsugi M, Mashino K, Yamamoto M, Ikebe M, Kakeji Y, Baba H, Maehara Y. Akt is frequently activated in HER2/neu-positive breast cancers and associated with poor prognosis among hormone-treated patients. *Int J Cancer.* 2006; 118:284–289. [PubMed: 16049961]
- Torres-Arzayus MI, Font de Mora J, Yuan J, Vazquez F, Bronson R, Rue M, Sellers WR, Brown M. High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. *Cancer Cell.* 2004; 6:263–274. [PubMed: 15380517]
- Vaillant F, Asselin-Labat ML, Shackleton M, Lindeman GJ, Visvader JE. The emerging picture of the mouse mammary stem cell. *Stem Cell Rev.* 2007; 3:114–123. [PubMed: 17873344]
- Virgo BB, Bellward GD. Serum progesterone levels in the pregnant and postpartum laboratory mouse. *Endocrinology.* 1974; 95:1486–1490. [PubMed: 4473330]
- Wagner KU, Rui H. Jak2/Stat5 signaling in mammaryogenesis, breast cancer initiation and progression. *J Mammary Gland Biol Neoplasia.* 2008; 13:93–103. [PubMed: 18228120]
- Wang Y, Cheng CH. ERalpha and STAT5a cross-talk: interaction through C-terminal portions of the proteins decreases STAT5a phosphorylation, nuclear translocation and DNA-binding. *FEBS Lett.* 2004; 572:238–244. [PubMed: 15304355]
- Wargon V, Helguero LA, Bolado J, Rojas P, Novaro V, Molinolo A, Lanari C. Reversal of antiprogesterin resistance and progesterone receptor isoform ratio in acquired resistant mammary carcinomas. *Breast Cancer Res Treat.* 2009; 116:449–460. [PubMed: 18677559]
- Wargon V, Fernandez SV, Goin M, Giulianelli S, Russo J, Lanari C. Hypermethylation of the progesterone receptor A in constitutive antiprogesterin-resistant mouse mammary carcinomas. *Breast Cancer Res Treat.* 2011; 126:319–332. [PubMed: 20440553]
- Weber-Hall SJ, Phippard DJ, Niemeyer CC, Dale TC. Developmental and hormonal regulation of Wnt gene expression in the mouse mammary gland. *Differentiation.* 1994; 57:205–214. [PubMed: 7988795]
- Winklehner-Jennewein P, Geymayer S, Lechner J, Welte T, Hansson L, Geley S, Doppler W. A distal enhancer region in the human beta-casein gene mediates the response to prolactin and glucocorticoid hormones. *Gene.* 1998; 217:127–139. [PubMed: 9795185]
- Wyszomierski SL, Yeh J, Rosen JM. Glucocorticoid receptor/signal transducer and activator of transcription 5 (STAT5) interactions enhance STAT5 activation by prolonging STAT5 DNA binding and tyrosine phosphorylation. *Mol Endocrinol.* 1999; 13:330–343. [PubMed: 9973262]
- Wyszomierski SL, Rosen JM. Cooperative effects of STAT5 (signal transducer and activator of transcription 5) and C/EBPbeta (CCAAT/enhancer-binding protein-beta) on beta-casein gene transcription are mediated by the glucocorticoid receptor. *Mol Endocrinol.* 2001; 15:228–240. [PubMed: 11158330]
- Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL, Strong RK, Zurakowski D, Moses MA. Lipocalin 2 promotes breast cancer progression. *Proc Natl Acad Sci U S A.* 2009; 106:3913–3918. [PubMed: 19237579]

Highlights

This paper reviews the role of progesterone and progesterone receptor (PR) in the mouse mammary gland used extensively as an experimental model of human breast cancer. Progesterone stimulates proliferation of mouse mammary epithelium by a paracrine mechanism and enhances pre-neoplastic progression through dysregulation of RANKL paracrine signals. In humans, progesterone is a risk factor for breast cancer with similar cell proliferative activities except for uncertainties on the paracrine signaling pathways.

Figure 1. Model for progesterone regulation of cell proliferation in the mammary epithelium mediated by the RANK-RANKL paracrine signaling pathway

The mechanism depicted is based on work with the mouse mammary gland as an experimental model. RANKL is a direct target of PR induced by progesterone (P4) and is then released to interact with the RANK receptor on either PR⁺ or nearby PR⁻ cells. In PR⁻ cells, RANKL activates the downstream IKK/IκB/NFκB/cyclin D1 signaling pathway to stimulate proliferation. RANKL does not generate a sustained activation of the NFκB/cyclin D1 proliferative pathway in PR⁺ cells due to the suppressive actions of TGFβ and/or other unknown mechanisms.

Figure 2. Model for progesterone and PR action at stages of breast cancer progression

Progesterone is a risk factor for breast cancer and promotes pre-neoplastic progression by stimulating cyclical proliferation of the mature breast epithelium and by activating mammary stem cell pools or occult tumor initiating cells. Alterations in the progesterone/PR signaling axis, including a switch from a paracrine to an autocrine regulation of proliferation, contribute to progression. In more advanced stage breast cancer PR, either in dependent of P4 or in response to P4, suppresses tumor invasion and metastasis through maintaining epithelial cell phenotype and impeding the epithelial-mesenchymal transition (EMT).