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Review

Interplay between progesterone and prolactin in mammary development and implications for breast cancer

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ABSTRACT

Progesterone and prolactin remodel mammary morphology during pregnancy by acting on the mammary epithelial cell hierarchy. The roles of each hormone in mammary development have been well studied, but evidence of signalling cross-talk between progesterone and prolactin is still emerging. Factors such as receptor activator of NFkB ligand (RANKL) may integrate signals from both hormones to orchestrate their joint actions on the epithelial cell hierarchy. Common targets of progesterone and prolactin signalling are also likely to integrate their pro-proliferative actions in breast cancer. Therefore, a thorough understanding of the interplay between progesterone and prolactin in mammary development may reveal therapeutic targets for breast cancer. This review summarises our understanding of Pg and PRL action in mammary gland development before focusing on molecular mechanisms of signalling cross-talk and the implications for breast cancer.

Contents

1. Introduction

The mammary gland undergoes dramatic tissue remodelling events in response to hormonal stimuli during puberty and pregnancy (Richert et al., 2000; Hovey et al., 2002). Oestrogen and growth hormone drive the elongation of the mammary ductal network during puberty, while progesterone (Pg) and prolactin (PRL) co-operate during pregnancy to stimulate the formation of alveolar structures that produce milk post-partum. Underlying these tissue-remodelling events is a mammary cell hierarchy composed of multipotent stem and lineage restricted progenitor cells (Shackleton et al., 2006; Stingl et al., 2006; Asselin-Labat et al., 2007; Visvader, 2009). Hormones elicit morphological changes in the mammary gland by acting on a complex regulatory network of paracrine signals and transcription factors to modulate the activity of mammary stem cells (Asselin-Labat et al., 2010; Joshi et al., 2010; Schramek et al., 2010).

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Like normal mammary gland development, breast carcinogenesis is commonly a hormonally dependent process. Since the original observation that bilateral ovariectomy was effective in treating breast cancer, more than a century of research has been devoted to understanding how hormones control this disease (Medina, 2005). Therefore, a thorough understanding of how hormones co-operate to promote mammary development will have applications not only to normal physiology but also to carcinogenesis.

This review focuses on Pg and prolactin PRL, the two key drivers of mammary development during pregnancy. Since early endocrine ablation and replacement studies first established progesterone Pg and PRL as master regulators of mammary gland development (Lyons, 1958), genetically modified animal models and tissue recombination techniques have been used to clarify the role of each hormone. The marked similarity between the phenotypes of PRL receptor (PRLR) and Pg receptor (PR) deficient mammary glands, and the significant overlap in transcriptional targets of Pg and PRL (Fernandez-Valdivia et al., 2008), indicate that these hormones act synergistically to drive mammary development during pregnancy. Our current understanding of the role of each hormone in mammary development is summarised below before potential interactions are investigated. The implications of these interactions for breast carcinogenesis are also discussed.

2. Progesterone action in the mammary gland

In mammary glands deficient for PR, ductal elongation proceeds as normal, demonstrating that Pg is not essential for pubertal mammary development (Lydon et al., 1995; Humphreys et al., 1997). Rather, Pg plays a critical role in inducing ductal side-branching of the mammary gland (Atwood et al., 2000), which is essential for lobuloalveolar development during pregnancy (Brisken et al., 1998). PR null mammary glands fail to undergo alveolar morphogenesis during pregnancy, and this effect is epithelial cell autonomous, as demonstrated by transplants into cleared mammary fat pads of wild-type (WT) mice (Brisken et al., 1998). Conversely, forced over-expression of PR resulted in increased ductal side-branching in adult virgin animals (Shyamala et al., 1998).

Animals with selective knockout of PR-A and PR-B have been used to study the effects of each PR isoform. Ablation of PR-A had no effect on mammary gland development in animals treated with oestrogen and Pg (Mulac-Jericevic et al., 2000, 2003), whereas there was reduced ductal side-branching in PR-B null mammary glands (Mulac-Jericevic et al., 2003). Similarly, mammary glands from PR-B null mice exhibited limited ductal side-branching and lobuloalveologenesis during pregnancy (Mulac-Jericevic et al., 2003). These results suggest that PR-A is not essential for mammary gland development, and that PR-B is the primary mediator of progesterone's proliferative effects during pregnancy.

3. Prolactin action in the mammary gland

Disruption of the PRL gene in mice did not effect mammary gland development during puberty but prevented the formation of alveolar buds upon reaching adulthood (Vomachka et al., 2000). Similarly, deletion of the PRLR had no influence on pubertal mammary development but resulted in failure to form secondary branches and alveolar buds in adults (Ormandy et al., 1997a,b; Brisken et al., 1999). Since PRLR null mice have reduced serum Pg levels, it was unclear whether these defects were direct effects or due to the endocrine disturbance in these animals. Pg replacement was able to rescue the failed side-branching of PRLR null mammary glands, but could not compensate for the defect in alveolar bud formation (Ormandy et al., 2003). This result indicates that PRL has a direct effect on alveolar bud formation, but not on ductal side-branching in adult mammary glands. Animals carrying only one allele of the PRLR were fertile but failed lactation following their first pregnancy (Ormandy et al., 1997a,b; Brisken et al., 1999). This phenotype was characterized by incomplete lobuloalveolar development during pregnancy, with alveoli failing to expand and engorge with milk at parturition (Brisken et al., 1999). Since PRLR null mice are infertile, epithelial transplants into cleared mammary fat pads were performed to study development in pregnant hosts. These mammary glands underwent normal ductal side-branching but failed alveologenesis, indicating that epithelial PRLR is required for mammary development during pregnancy. Conversely, recombined glands with PRLR null stroma and WT epithelium developed normally in pregnant hosts, indicating that stromal PRLR is not required for mammary development (Ormandy et al., 2003).

4. Signalling interactions between Pg and PRL in the mammary gland

4.1. Progesterone and prolactin interactions at the level of their receptors

PR is a ligand-activated transcription factor that binds to DNA as a protein dimer, and activates transcription of a suite of target genes (Fernandez-Valdivia et al., 2008). PR is restricted to the luminal cell lineage with no expression in the myoepethelial and stromal compartments of the mammary gland (Shyamala et al., 2002). In adult virgin mice, PR is expressed in approximately 55% of luminal epithelial cells (Seagroves et al., 2000), but during pregnancy this proportion decreases dramatically to around 5% (Shyamala et al., 2002). Importantly, PR and oestrogen receptor (ER) are usually co-expressed in the mammary gland and define a steroid receptor positive subset of epithelial cells (Mukherjee et al., 2010).

The PRLR is a membrane-bound protein of class I of the cytokine receptor superfamily (Bole-Feysot et al., 1998), that is closely related to the growth hormone receptor (GHR). Lack of a reliable antibody has precluded attempts to analyse PRLR expression during mouse mammary gland development in detail, however PRLR has been detected in both the stroma and epithelium of the rat mammary gland (Camarillo et al., 2001). Interestingly, microarray analysis of sorted epithelial populations has indicated that PRLR is expressed predominantly in the steroid receptor positive luminal cells in virgin mice (Kendrick et al., 2008). Whether or not PRLR expression is restricted to this population throughout development remains to be seen.

Interactions between PRL and Pg have been described at the level of cross-regulation of their receptors. Progestin treatment causes increased PRLR expression in MCF-7 cells, and conversely PRL treatment upregulates PR (Ormandy et al., 1997c). Similarly, ectopic expression of PR in a normal mammary epithelial cell line led to increased PRLR expression (Goldhar et al., 2011). The mechanism by which PRL induces PR expression is unknown; however, it has been shown that Pg induction of PRLR expression involves co-operative activation of Sp1 and C/EBP signalling (Goldhar et al., 2011). Studies *in vivo* have also demonstrated that acute Pg exposure can upregulate PRLR expression in virgin mice (Fernandez-Valdivia et al., 2008), however there is also evidence indicating that Pg can suppress PRLR expression in late pregnant mammary glands (Nishikawa et al., 1994). These results suggest that PR and PRLR interactions may depend upon the cellular and physiological context.

4.2. Convergence of progesterone and prolactin signals on STAT5

The PRLR lacks intrinsic kinase activity but associates constitutively with Janus kinase (JAK2) (Campbell et al., 1994; DaSilva

et al., 1994; Lebrun et al., 1994; Rui et al., 1994). Upon PRL stimulation, JAK2 is rapidly activated by auto-phosphorylation (Ali et al., 2003), and then phosphorylates tyrosine residues in the cytoplasmic domain of the PRLR (Lebrun et al., 1994, 1995). Signal transducer and activator of transcription (STAT) proteins bind to phosphotyrosine residues on the PRLR and are themselves phosphorylated by JAK2 (Gouilleux et al., 1994; Lebrun et al., 1995; DaSilva et al., 1996; Pezet et al., 1997; Freeman et al., 2000). Once activated, STAT proteins dimerise and migrate to the nucleus, where they act as transcription factors by binding to response elements in the promoter regions of target genes.

Consistent with an important role in PRL signal transduction, animals deficient for STAT5a display failed alveologenesis and lactation (Liu et al., 1997). Conditional deletion of both STAT5a and 5b under the MMTV promoter also resulted in reduced tertiary branching and alveolar bud formation in mature females that was associated with reduced cellular proliferation (Cui et al., 2004). Further experiments using conditional knockout of STAT5 under the whey acidic protein (WAP) promoter demonstrated an essential role in the survival and maintenance as well as the differentiation of alveolar cells (Cui et al., 2004).

While STAT5 is well established as a mediator of PRL action, there is also evidence to suggest that Pg can influence this signalling pathway. Combined treatment of ovariectomised virgin mice with oestrogen and Pg leads to increased STAT5 expression in the mammary epithelium (Santos et al., 2008). PRL treatment, alone or in combination with Pg, had no effect on the level of STAT5 expression, but inhibition of pituitary PRL secretion prevented nuclear translocation of STAT5. These results indicate that oestrogen and Pg co-operatively maintain STAT5 expression in the mammary gland, while PRL induces STAT5 transcriptional activity. In T47D breast cancer cells, progestin treatment induces STAT5 expression, phosphorylation, and nuclear translocation, and this led to an increased sensitivity to PRL treatment (Richer et al., 1998). Co-immunoprecipitation experiments have also demonstrated that STAT5 and PR can directly interact in HeLa cells, and progestin treatment of T47D cells causes co-recruitment of STAT5 and PR to the progestin responsive MYC promoter (Cerliani et al., 2011). In addition, STAT5 and PR were found to be co-localized in the nuclei of ER and PR positive human breast cancer samples. Further work is required to clarify the importance of PR and STAT5 interactions in various physiological contexts.

4.3. RANKL as a potential integrator of progesterone and prolactin signals

The osteoclast differentiation factor, receptor activator of NFkB ligand (RANKL) and its receptor (RANK) are essential for lobuloalveolar development during pregnancy (Fata et al., 2000). In transplants of RANKL null mammary epithelium ductal outgrowth proceeded as normal during puberty, but side-branching and alveolar development were impaired during pregnancy (Beleut et al., 2010). Conversely, forced expression of RANKL resulted in increased ductal side-branching and alveolar bud formation during puberty and increased epithelial cell proliferation in adult virgin animals (Fernandez-Valdivia et al., 2009).

Upon co-treatment with oestrogen and Pg, RANKL is induced in the mammary glands of WT but not PR null animals, indicating that Pg regulates RANKL expression (Mulac-Jericevic et al., 2003). Furthermore, Pg alone but not oestrogen alone, induced RANKL expression in OVX females (Fata et al., 2000). RANKL is expressed in the same cells as PR, and induces proliferation of neighbouring cells via a paracrine mechanism. (Mulac-Jericevic et al., 2003; Beleut et al., 2010). The importance of RANKL in mediating progesterone's paracrine action was decisively established by Belut and colleagues (2010) who ectopically expressed RANKL in PR null epithelial cells to demonstrate that RANKL can rescue the failed development of PR null mammary transplants during pregnancy. Further work has also demonstrated that ectopic RANKL expression in the ER positive cells of PR null mammary glands is able to induce ordered branching morphogenesis and alveologenesis (Mukherjee et al., 2010). Together, these results demonstrate that Pg induction of RANKL expression in the steroid receptor positive luminal epithelial cells can stimulate proliferation of mammary epithelial cells.

Interestingly, it has also been suggested that PRL promotes STAT5 binding to the RANKL promoter to induce its expression (Srivastava et al., 2003), consistent with the observations that RANKL is decreased in PRLR null mammary glands in early pregnancy (Ormandy et al., 2003), and in STAT5a null mammary glands treated with oestrogen and Pg (Santos et al., 2010). In addition, progestin induction of RANKL expression in the mammary epithelium depends, at least in part, on PRLR signalling (Schramek et al., 2010). Together, these results identify RANKL as a potential point of convergence between Pg and PRL action in the mammary gland.

4.4. Elf5 as a potential integrator of progesterone and prolactin signals

A functional role for E74-like factor (Elf5) in the mammary gland was first identified in $Elf5^{+/-}$ mice, which failed to lactate (Zhou et al., 2005). The role of Elf5 has since been studied in $Elf5^{+/-}$ mice (Zhou et al., 2005), $Elf5^{-/-}$ mammary transplants (generated by tetraploid embryonic stem cell rescue to avoid the placentation defect) (Oakes et al., 2008), and a mammary specific Elf5 knock-out mouse (Choi et al., 2009). These studies have all demonstrated that loss of Elf5 has no effect on ductal elongation or branching morphogenesis during pubertal mammary development, but causes a severe impairment of mammary gland development during pregnancy. The effects of forced Elf5 expression on mammary gland development have also been studied in an inducible mammary-specific transgenic mouse (Oakes et al., 2008). Forced Elf5 expression in virgin mice resulted in the formation of alveolar structures and milk production, while induction of Elf5 caused increased milk protein production in mid-pregnant mice.

Several lines of evidence have established Elf5 as a PRL regulated gene in mammary cells. Firstly, Elf5 expression is reduced in PRLR deficient mammary glands, whilst PRLR expression is not reduced in Elf5 deficient glands, suggesting that Elf5 acts downstream of the PRLR (Zhou et al., 2005; Harris et al., 2006; Choi et al., 2009). Secondly, in a positive model of PRL action, Elf5 is induced upon PRL treatment of differentiating Scp2 mammary epithelial cells (Harris et al., 2006). Finally, Elf5 was confirmed to be the primary mediator of PRL action by re-expressing Elf5 in PRLR null mammary epithelial cells. Outgrowths of PRLR null cells expressing Elf5 were able to develop normally during pregnancy, demonstrating that Elf5 can rescue the PRLR null phenotype (Harris et al., 2006). The position of Elf5 within the PRL signalling cascade has recently been further defined by Choi et al. (2009), who demonstrated that Elf5 null mammary glands have reduced STAT5 expression and phosphorylation. This result implied that Elf5 acts upstream of STAT5 signalling, and accordingly Elf5 was shown to bind to the STAT5 promoter (Choi et al., 2009). To test the possibility that STAT5 and Elf5 form a positive feedback loop, it will be interesting to investigate whether STAT5 can directly induce Elf5 expression.

Although Elf5 is a well-established mediator of PRL action during mammary development, recent reports have indicated that other hormonal factors may also regulate Elf5 expression (Menzies et al., 2009, 2010). The marked similarity between mammary glands null for PRLR, PR and Elf5 suggested that PRL, Pg and Elf5 comprise a regulatory network pivotal to mammary development. Accordingly, Pg has been shown to induce Elf5 expression in both

T47D cells and mouse mammary glands (Fernandez-Valdivia et al., 2008; Hilton et al., 2010). In T47D cells, progestin induction of Elf5 expression was associated with recruitment of PR to a site within the 4th intron of the Elf5 gene. This result suggests that PR directly activates Elf5 transcription in T47D cells. An inducible shRNA knock-down model was also used to prevent the Pg induced rise in Elf5 expression levels. In these cells, the anti-proliferative effects of Pg were enhanced, suggesting that Elf5 opposes Pg action in T47D cells. Whether or not Elf5 has the same effect on Pg signalling in normal mammary development remains to be seen.

5. Consequences of Pg and PRL signalling interactions for mammary biology

5.1. Progesterone and prolactin interactions and regulation of β-casein expression

In addition to promoting mammary development during pregnancy, PRL is required for maintenance of post-partum lactation in most species (Neville et al., 2002). Suckling stimulates PRL secretion from the pituitary, and plasma concentrations fluctuate with the feeding cycle. Reduced proliferation in PRL null mammary transplants during lactation also indicates that PRL has an autocrine or paracrine role in maintaining cell proliferation during lactation (Naylor et al., 2003). On the contrary, Pg is considered to be an inhibitor of active milk production, with Pg withdrawal at parturition being required for the onset of active lactation (Neville and Morton, 2001). Ovariectomy of mice during pregnancy results in increased β-casein expression in the mammary gland, and concurrent administration of Pg can prevent this increase (Nishikawa et al., 1994). Early explant studies have demonstrated that Pg antagonism of PRL induced milk expression occurs within the mammary tissue, potentially by preventing PRL mediated PRLR up-regulation (Nishikawa et al., 1994; Buser et al., 2007). More recently, an *in vitro* model of lactogenesis has been used to examine whether Pg can directly inhibit milk protein expression.

The HC11 cell line was derived from the mammary glands of a healthy mid-pregnant mouse and selected for PRL responsiveness (Ball et al., 1988). When treated with lactogenic hormones these cells can be differentiated to express milk proteins such as β-casein. STAT5 binds to the β-casein promoter in HC11 cells, and this binding is essential for PRL activation of transcription (Gouilleux et al., 1994). The β-casein promoter also contains multiple C/EBPb binding sites, and several gluccocorticoid response element (GRE) half sites. In HC11 cells, transfected PR binds to a GRE half site in the β-casein promoter and inhibits PRL activated transcription (Buser et al., 2007). This inhibition of PRL signalling was not associated with a change in STAT5 phosphorylation, but with reduced STAT5 DNA binding (Buser et al., 2007), and disruption of long-range interactions between regulatory elements in the β-casein promoter (Kabotyanski et al., 2009). PR and STAT5 are co-expressed in virgin mammary glands (Santos et al., 2008), but this relationship is unlikely to be maintained during pregnancy when PR expression is restricted to a small subset of epithelial cells (Shyamala et al., 2002). Since β-casein expression is seen in the vast majority of epithelial cells during lactation, the mechanism of PR inhibition of milk expression requires further *in vivo* investigation.

5.2. Pg and PRL interactions and the mammary epithelial cell hierarchy

The mammary epithelial hierarchy is hypothesized to consist of multipotent stem cells, lineage committed progenitor cells and mature terminally differentiated cells. Regulation of this hierarchy by hormones underpins the morphological changes seen during post-natal mammary development with mammary stem cell activity being regulated through both the oestrous cycle and pregnancy (Asselin-Labat et al., 2010; Joshi et al., 2010; Lydon, 2010). The steroid hormone regulation of mammary epithelial subpopulations has been studied using ovariectomised (OVX) mice. While ovariectomy did not effect the proportion of cells in the basal (stem cell enriched) fraction, these cells had reduced repopulating capacity in transplantation assays (Asselin-Labat et al., 2010). Similarly, treatment of OVX mice with exogenous Pg and oestradiol resulted in expansion of the stem cell containing fraction and increased their repopulating efficiency (Asselin-Labat et al., 2010; Joshi et al., 2010).

These results were somewhat surprising since the stem-cell containing sub-population does not express steroid receptors (Asselin-Labat et al., 2006). Rather, approximately 50% of the luminal cells express ERa and PR, the majority of which are the "mature" or non-proliferating luminal cells (Asselin-Labat et al., 2007). To determine how oestrogen and Pg exert their effects on ER and PR negative stem cells, RT-PCR was performed on sub-populations isolated at different stages of mammary development or from hormone treated animals. The paracrine factors RANKL and Wnt4 were increased in the luminal population following oestradiol and Pg treatment (Joshi et al., 2010), and luminal RANKL expression was also increased during pregnancy (Asselin-Labat et al., 2010). Conversely, expression of RANK and LRP5 (a Wnt4 receptor) were increased in the basal population of hormone treated and pregnant mammary glands. Further, mammary stem cells isolated from mice treated with an anti-RANKL antibody had reduced clonogenic activity when grown in culture (Asselin-Labat et al., 2010), and mammary glands deficient for RANK had reduced numbers of stem cells (Schramek et al., 2010). Together, these results suggest that Pg and oestrogen stimulate the activity of basally located stem cells via paracrine signals such as RANKL and Wnt4.

PRL regulation of the mammary epithelial cell hierarchy has not been directly investigated, however several mediators of PRL action have been shown to influence mammary stem and progenitor cells. STAT5 deficient mammary glands display reduced proportions of luminal progenitor cells in virgin animals (Yamaji et al., 2009). This suggests that STAT5a is important for the generation of alveolar progenitor cells from stem cells. Consistent with this idea STAT5a is expressed in the mammary epithelial stem cell population. Elf5 is not expressed in the stem cell enriched population of normal mammary glands, and expression of Elf5 in the luminal progenitor population was dependant upon STAT5 (Yamaji et al., 2009). In virgin Elf5 null mammary glands, there was no difference in the proportion of luminal progenitor cells (Oakes et al., 2008). During pregnancy, however, luminal progenitor cells accumulated in Elf5 null transplants and in $Elf5^{+/-}$ mammary glands, suggesting that Elf5 is required for the differentiation of these cells. Consistent with this hypothesis, forced expression of Elf5 in virgin mice resulted in erosion of the luminal progenitor population accompanied by decreased colony forming ability of these cells (Oakes et al., 2008). These results suggest that PRL signalling may be important for maintenance and differentiation of the luminal progenitor population.

While these studies suggest that Pg and PRL may act on different cell populations within the epithelial cell hierarchy, it is important to remember that cross-regulation exists between Pg and PRL signalling pathways. RANKL can be activated by PRL as well as Pg (Srivastava et al., 2003), so PRL may also contribute to increased stem cell activity via paracrine signalling during pregnancy. Since Pg is required for maintenance of STAT5 signalling (Santos et al., 2008), and can also stimulate Elf5 expression (Hilton et al., 2010), this hormone may also be able to act on the luminal progenitor population.

6. Pg and PRL interactions in breast cancer

Evidence from epidemiological studies and mouse models demonstrates that both PRL and Pg can promote mammary tumourigenesis. Women with high levels of circulating PRL carry a 1.6-fold increase in risk of developing ER positive breast cancer, an effect that is independent of serum oestrogen and testosterone levels (Tworoger and Hankinson, 2008). Retardation of mammary tumour formation in PRL and PRLR deficient mice also indicates that PRL can accelerate this disease (Vomachka et al., 2000; Oakes et al., 2007), and mammary epithelial transplants have demonstrated that PRL achieves this effect by acting directly on the epithelium (Oakes et al., 2007).

Epidemiological evidence of progesterone's role in breast cancer is less clear, but several well-publicized studies have demonstrated that synthetic progestins used in hormone replacement therapy confer an increased risk of developing the disease (Rossouw et al., 2002; Beral, 2003). These results are consistent with the reduced frequency of mammary tumours in carcinogen treated PR knock-out mice (Lydon et al., 1999). Several recent reports have used animal models to establish RANKL as a key player in progestin-driven breast cancer. In the absence of RANK, mammary tumours had increased latency to formation (Schramek et al., 2010), while forced RANK expression accelerated tumour formation (Gonzalez-Suarez et al., 2010). Importantly, blockade of RANKL using a neutralising antibody was able to prevent mammary tumourigenesis in the majority of WT mice (Gonzalez-Suarez et al., 2010), providing justification for clinical trials in breast cancer. Anti-RANKL therapies may also be able to delay breast cancer metastasis, as tumour infiltrating T cells have been shown to encourage breast cancer metastasis to the lungs by producing RANKL (Tan et al., 2011).

PRLR and PR are frequently co-expressed in breast cancer cells (Ormandy et al., 1997c), but very little is known about how these hormones may co-operate during disease progression. Common targets of Pg and PRL signalling, such as Elf5 and RANKL, are likely to integrate their pro-proliferative actions in breast cancer as well as in normal mammary development.

7. Conclusion

Emerging evidence is casting light upon the molecular mechanisms by which Pg and PRL co-operate to promote mammary development. Pg and PRL interplay occurs at the levels of their receptors and downstream signalling pathways. Interactions between Pg and PRL are likely to be cell and physiological context dependent (Fig. 1). In ductal luminal cells PR and PRLR may promote each other's expression and induce RANKL production, while in alveolar luminal cells activated PRLR drives milk expression. Pg suppression of milk production is likely to occur via an indirect mechanism since mammary PR is scarce during lactation. For instance, paracrine signalling from PR positive luminal cells may prevent STAT5 activation of milk protein expression. Both Pg and PRL may influence the mammary epithelial hierarchy by inducing RANKL feedback to stem cells. Further, both Pg and PRL may be required for STAT5 dependent maintenance of the luminal progenitor population. Pg and PRL signalling may also converge on the luminal progenitor population to induce Elf5 expression and drive the expansion of the alveolar lineage.

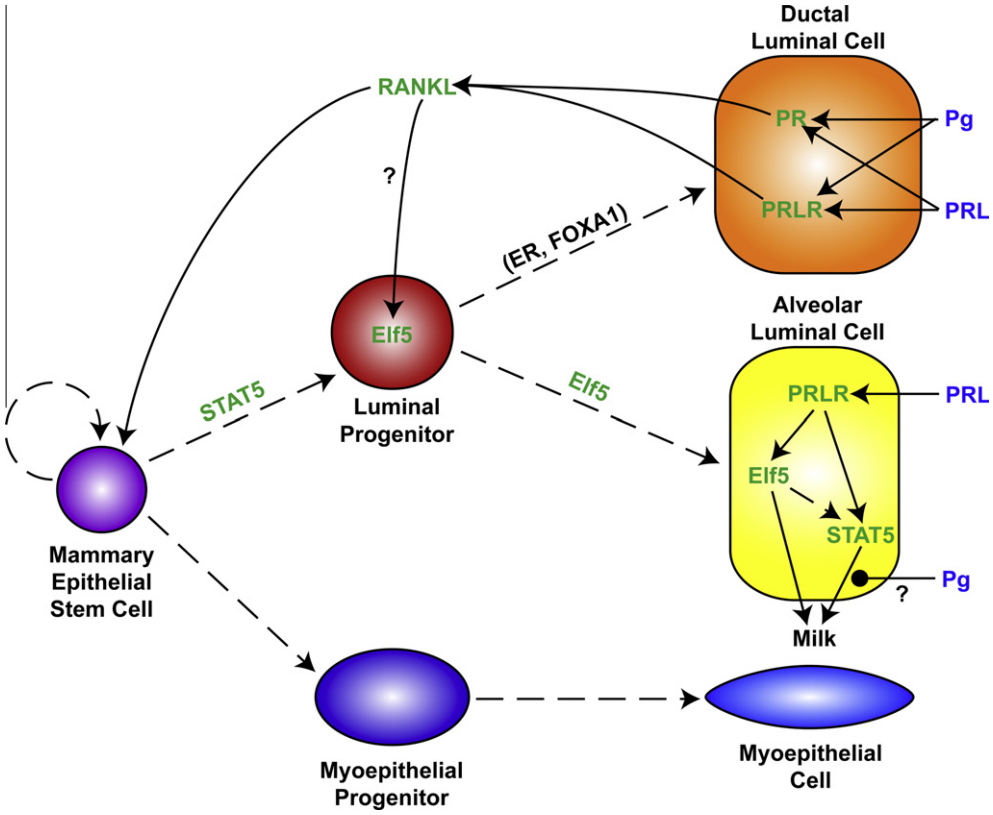

Fig. 1. Interplay between Pg and PRL in mammary development. Pg and PRL synergise to promote mammary development during pregnancy by acting on the mammary epithelial hierarchy. In ductal luminal cells PR and PRLR may promote each other's expression and induce RANKL production, while in alveolar luminal cells activated PRLR drives milk expression. Pg suppression of milk production is likely to occur via indirect mechanism such as paracrine signalling from PR positive cells. Both Pg and PRL influence STAT5 and Elf5, which are required, respectively, for the maintenance and differentiation of luminal progenitor cells.

Given the evidence of PRL and Pg interactions in the developing mammary gland, future research should focus on shared signalling pathways in breast cancer. By therapeutically targeting points of convergent signalling, such as Elf5 and RANKL, it may be possible to simultaneously suppress pro-proliferative signals from both Pg and PRL.

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