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Mammary epithelial stem and progenitor cells and the prolactin pathway

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1. ABSTRACT

Prolactin is a pleiotropic peptide hormone and cytokine that is secreted from the pituitary gland and locally within various tissues of the body for autocrine and paracrine signal transduction. It controls proliferation and differentiation in a number of body tissues and increasing evidence indicates that it controls these functions in undifferentiated stem and progenitor cells of adult tissues, such as mesenchymal stem cells, hematopoietic progenitors, neural stem cells, oligodendrocyte precursor cells and possibly in mammary gland stem/progenitor cells. These roles in these undifferentiated cell types also implicate prolactin in the stem cell theory of cancer, supporting its known roles in cancer formation and progression.

2. INTRODUCTION

Prolactin is a peptide hormone and cytokine that has multiple roles in the adult body and increasing evidence demonstrates its importance in the development of different tissues. It is well known that the prolactin-janus kinase-2-(Jak2)-signal transducer and activator of transcription-5 (Stat5) pathway is essential for functional development of the adult mammary gland; responsible for the proliferation of alveolar precursors and the differentiation of alveolar cells (1-8). However, it is not known at what level prolactin functions with respect to the most undifferentiated cells that contribute to mammary gland development, the stem or progenitor cells. Prolactin does function in a number of different vertebrate stem cell systems, such as mesenchymal stem cells (9), neural stem cells (10),

hematopoietic progenitor cells (for review see (11)) and oligodendrocyte progenitor cells (12) for the development and maturation of those tissues, the evidence for which is discussed in this review. The question of whether or not these functions are conserved within the mammary gland and whether the effect is direct, or not, will also be addressed.

A number of genetically engineered mouse models provide evidence to support the essential role for the prolactin-Jak-Stat5 pathway in mammary gland development (1-8). The mammary phenotypes of these mice support the idea that prolactin communicates to undifferentiated cells that are capable of expanding into a large population of cells that eventually differentiate into the functional alveolar cells that secrete milk proteins. The identity of that undifferentiated cell population is an area of current investigation, which will be covered in this review.

The undifferentiated cells of a stem cell system include stem and progenitor cells. Stem cells will be defined here, with respect to the mammary gland, as undifferentiated cells capable of self-renewing as well as capable of forming all the cell lineages of a given tissue and are therefore mulitpotent. Progenitor cells are more restricted than stem cells and can only give rise to one or two lineages of a given tissue. Multi-potent progenitors are thought to be also capable of extended self-renewal and a progenitor cell is capable of limited self-renewal. Progenitor cells are thought to have a high capacity for proliferation, but whether they themselves differentiate or produce a cell capable of functional differentiation, is unknown. Is it possible to identify distinct cell types based upon these functional definitions? Current research is attempting to define these cell types at the molecular level.

The functions of prolactin in the mammary gland include both proliferation and differentiation, which are essentially two opposing cellular responses. These two different functions likely require either different signal transduction pathways downstream from the prolactin receptor in one cell type and/or the prolactin responses of different cell types, and the distinction cannot be discerned until the undifferentiated cell types in the mammary gland have been fully characterized. The cellular identification and functional definition of the potential cell types that respond to prolactin are currently being established through numerous *in vitro* and *in vivo* assays designed to identify and characterize the undifferentiated cell types in the mammary gland. While the mammary gland field has yet to truly define the nature of the undifferentiated cells of the mammary gland and the role of prolactin in these cells, a number of studies have provided evidence to support a role for prolactin signal transduction in undifferentiated mammary epithelial cells. It is possible that this prolactin function is conserved, though perhaps deregulated, during cancer development. Cancer has been proposed to arise from undifferentiated cells (stem cell theory) or in mature cells that dedifferentiate (clonal evolution). These models are not necessarily mutually exclusive (for review see (13)).

The model of clonal evolution takes into account that any cell can acquire transforming mutations, and that genetic drift, providing selective growth advantages to different subpopulations within the tumor, accounts for tumor heterogeneity. It is plausible that prolactin-responsive cells in the tumor are contributing to tumor growth, therapy resistance and/or the metastatic nature depending upon the mutations accumulated in those cells and additionally the contributing role of prolactin. The target genes of prolactin in breast cancer cells have not been fully elucidated, and this is required in order to understand the full effect of prolactin on breast cancer cells.

Applying the stem cell theory of carcinogenesis, malignant transformation occurs in undifferentiated cells, which are either stem cells or possibly progenitor cells that acquire stem cell functions. The cells with stem cell functions of self-renewal and differentiation and are thought to be the only cells in the tumor capable of generating a second tumor in an animal model (14) and are referred to as breast cancer stem cells or tumor-initiating cells. Uncontrolled self-renewal is thought to produce a large stem cell population capable of producing large numbers of proliferating cells that undergo an altered differentiation program. Prolactin contributes to mammary cancer initiation in the mouse (15, 16) and to increased breast cancer risk and cancerous progression in the human (for review see (17)). The question of whether tumour-initiating cells are responsive to prolactin is currently an open one, but based upon the evidence that supports a role for prolactin in progenitor's cells and possibly stem cells, it is likely that prolactin affects the tumor-initiating cell population.

While few details regarding the target genes of prolactin in breast cancer are known, it has been demonstrated that prolactin contributes to proliferation and survival of breast cancer cells. Neutralizing antibodies (18) and receptor antagonists (19-21) both interfere with the ability of prolactin to induce proliferation in breast cancer cells. Antisense oligonucleotides against cyclin D1 inhibit the proliferative response to prolactin (22). The prolactin receptor antagonist, G129R-human- prolactin, also induced apoptosis, indicating a role for prolactin in survival in breast cancer cells.

3. THE PROLACTIN RECEPTOR AND PROLACTIN SIGNAL TRANSDUCTION PATHWAYS

There are a number of major downstream signaling pathways from the activated prolactin receptor, a type 1 cytokine receptor, including the Jak2-Stat pathway (23, 24), Fyn (25, 26), Src (27), PI3K (25, 28), Tec-Vav (29, 30), Nek3-Vav2 (31), ZAP-70 tyrosine kinase (32), and MAPK (33-36) pathways. The form of the receptor that is expressed in the cell is one factor that restricts the availability of these downstream pathways. The prolactin receptors differ by the length of the cytoplasmic domain and for the most part are derived from alternate splicing. Three short forms differing by a few amino acids and one long form have been identified in the mouse (37-39). Long, short, intermediate and soluble forms of the receptor of the

receptor have been described in rat (40, 41) and human (42) (for review see (17)).

One piece of evidence that implicates prolactin in the non-mammary cell systems described below is the expression of the prolactin receptor, which interestingly is predominantly present as either the intermediate or the short form, versus the long form. Box 1, which is present in all prolactin receptors, is thought to mediate Jak, Fyn and MAPK signaling (for review see (43, 44)), although it may not activate Fyn via the human intermediate form (45). The long form is also capable of binding to Vav2, a guanine nucleotide exchange factor, and the serine/threonine NIMA (never in mitosis A)-related family kinase p56Nek3 (31). Additionally the long form of the receptor is capable of binding to Vav1 and the tyrosine kinase p70Tec, and there is a possibility that the intermediate form of the receptor could also bind to the Vav1 (30). The short form of the receptor (PR1) transduces signals despite its small size and has been reported to be involved in proliferation (46, 47). It is capable of activating Jak2, although transduction of the signal to the Stats may not be possible due to a lack of traditional Stat docking sites. This short form of the receptor is able to partially compensate for a heterozygous loss of the long form of the receptor *in vivo* and restore mammary gland function (46). Although the mechanism was not elucidated, it may be through some interaction with the functional long form of the receptor. This is in contrast to the observed function of the short F3 form of the rat prolactin receptor (48), which acted as a dominant negative *in vivo* with respect to prolactin signaling (49). Differential expression of these different prolactin receptor isoforms may provide an additional level of signal transduction modulation responsible for different cellular responses.

4. EVIDENCE FOR A PROLACTIN ROLE IN NON-MAMMARY STEM CELL SYSTEMS

There are several lines of evidence that indicate a role for prolactin in stem and progenitor cells is conserved in different tissues of the vertebrate including evidence from human mesenchymal stem cells, hematopoietic progenitors, mouse neural stem cells and mouse oligodendrocyte precursors.

4.1. Mesenchymal stem cells

Pluripotent mesenchymal stem cells from the bone marrow are capable of forming cartilage, bone, and even neurons and astrocytes (for review see (50)). The three main cell types produced from the marrow that constitute bone are chondrocytes and osteoblasts from mesenchymal stem cells, and osteoclasts that are of hematopoietic origin. Osteoclasts, derived from monocyte and macrophage precursors from bone marrow, do not express the prolactin receptor, although osteoblasts express two short forms and the long form of the receptor, as identified by reverse-transcriptase PCR (51).

Human mesenchymal stem cells isolated from the bone marrow express an intermediate form of the prolactin receptor, as identified by reverse-transcriptase PCR (9). The addition of prolactin increases proliferation of human mesenchymal stem cells (9), such that they resemble proliferative chondrocytes that are not terminally differentiated (52). Prolactin was also demonstrated to induce chondrocyte differentiation of human mesenchymal stem cells. During *in vitro* chondrogenic differentiation, when mesenchymal stem cells are encouraged to form cell aggregates in defined medium as a first step in the differentiation regime, expression switches to the long form of the prolactin receptor with a concomitant increase in expression of extrapituitary prolactin. Prolactin treatment increases the production of type II collagen transcripts, proteoglycan extracellular matrix and induction of cell-cell contacts, all indicative of chondrogenic differentiation (9). Prolactin and dexamethasone together also induce chondrogenic differentiation of mesenchymal stem cells (52). Synovial tissue is another source of mesenchymal stem cells (53) and prolactin was found in synovial fluid (9). PCR analysis of synovial and cartilage tissue from adult human donors identified an intermediate form of the prolactin receptor in synovial tissue and the long form of the prolactin receptor in cartilage (9), supporting a role for prolactin in cartilage formation. These studies indicate that mesenchymal stem cells express the intermediate form of the prolactin receptor and respond to prolactin by proliferating, and upon differentiation into the osteochondral fate express the long form of the prolactin receptor, which may help promote differentiation. Together these observations indicate an autocrine/paracrine role for prolactin in the acquisition of the chondrocyte phenotype from mesenchymal stem cells and in the regulation of adult cartilage formation.

Consistent with these observations, bone formation is diminished in mice with germline null mutations of the prolactin receptor. In the fetus, ossification is reduced and in the adult animals the rate of bone formation is reduced by 60% (51). Together this indicates that prolactin has an important role in the formation and maintenance of bone mass, acting upon the undifferentiated cells of the bone.

4.2. Hematopoietic progenitor cells

Defining the *in vivo* role of prolactin in hematopoiesis is complicated, given that the majority of cells derived from hematopoietic stem cells, including lineage-precursors, B and T lymphocytes, differentiated natural killer cells, monocytes, granulocytes and also the stromal microenvironment are prolactin responsive (11). Evidence suggests that prolactin plays a role in normalizing hematopoietic homeostasis in times of stress, rather than modulating basal level homeostasis. Prolactin null mice show no defects in the steady-state hematopoietic system (1) nor do prolactin receptor null mice exhibit defects in the developing immune system (54), indicating that prolactin either has no role in basal level homeostasis or is compensated for by another pathway or molecule. Prolactin is, however, capable of expanding the hematopoietic progenitor cell population after bone marrow transplantation (55).

One of the short prolactin receptors (43kDa) is expressed within the diverse human CD34 positive (+)

population, which consists of hematopoietic stem cells, progenitors and stromal cell precursors (56). The addition of prolactin to $CD34^+$ cells, in colony forming assays, stimulated the proliferation of granulocytic and erythrocytic progenitors in combination with hematopoietic growth factors. Limiting dilution experiments indicated that the effect of prolactin was directly on the progenitor cells. Erythroid differentiation was also enhanced by prolactin, by the upregulation of receptors for the lineage-specific factor erythropoietin. Although only the short form of the prolactin receptor was reported (56), prolactin is capable of activating Stat5 in $CD34^+$ cells (57). The specific and important roles of Stat5 activated by cytokines in normal and cancerous hematopoietic stem and progenitor cells will not be discussed in this review as it was recently reviewed elsewhere (58). Certainly, given the expression of the prolactin receptor on developing blood cell progenitors, prolactin does have a role in modulating the lineages of the blood. Detection of the prolactin receptor on a pure population of hematopoietic stem cells and its effects in single cell assays are required to determine if prolactin plays a direct role in early hematopoietic development.

4.3. Neural stem cells

Olfactory memory is essential for good maternal behavior, and prolactin contributes to this phenomenon. Neural stem cells from the subventricular zone produce neuroblasts that migrate to the olfactory bulb and form interneurons. Prolactin stimulates neurogenesis and differentiation that results in the production, proliferation and migration of functional cells to the olfactory bulb (10). Prolactin was able to stimulate neurogenesis during pregnancy and lactation, the two phases when prolactin is naturally secreted at high levels in the mouse, and when provided directly to the brain or systemically. In mice that were heterozygous for the prolactin receptor, the increase in neurogenesis was half of the wild type. When cultured neural stem cells were treated with prolactin, in the presence of epidermal growth factor, it resulted in increased self-renewal, proliferation, as well as differentiation. Therefore, prolactin stimulates neural progenitor production and proliferation *in vivo* and helps to stimulate self-renewal of cultured neural stem cells and their differentiation *in vitro*.

The mechanism by which prolactin increases neurogenesis is unknown, but the authors provided a few indications that it could involve an indirect and/or a direct response. The choroid plexus is positive for the prolactin receptor by immunofluorescence and the dorsolateral corner of the subventricular zone and cultured neural stem cells both express the short form of the prolactin receptor, a size similar to the short form found in the spleen. The choroid plexus is known to secrete growth factors that regulate neural stem cells, and this may indicate that prolactin also has an indirect effect on neural stem cells (10). The expression of the short form of the receptor on the dorsolateral corner of the subventricular zone, where cells begin their migration, and on cultured neural stem cells themselves, supports a direct effect of prolactin in neurogenesis and differentiation.

4.4. Oligodendrocyte precursor cells

More recently, prolactin was discovered to regulate oligodendrocyte precursors and the production of oligodendrocytes that are responsible for central nervous system myelination. Pregnancy related hormones resulted in an increase in the number of platelet derived growth factor receptor-$alpha^+$ oligodendrocyte precursor cells in mice (12). The pregnancy-associated (high serum prolactin levels) increase in oligodendrocytes was associated with an increase in myelination postpartum, and this held true when substituting prolactin for pregnancy. Mice heterozygous for the prolactin receptor that were pregnant did not show a significant increase in oligodendrocyte precursor cells above virgin controls, whereas the pregnant wild-type mice showed a 90% increase in the number of dividing oligodendrocyte precursors in the corpus callosum. Prolactin treatment increased the number of dividing oligodendrocyte precursors and new oligodendrocytes in the corpus callosum and the spinal cord. In this study, it was found that a large proportion of oligodendrocyte precursors (42% in the corpus callosum and 47% in the spinal cord) are prolactin $receptor^+$. PCR and western blot analysis showed expression and production of the long form of the prolactin receptor and none of the short forms of the receptor in both locations. Oligodendrocyte precursors that were isolated from the corpus callosum and grown as neurospheres, responded to prolactin treatment with increased self-renewal as well as increased oligodendrocyte cell numbers upon differentiation compared to cells treated with platelet derived growth factor alone (12). These results identify prolactin as a therapeutic tool for white matter damage that occurs in multiple sclerosis.

5. PROLACTIN SIGNALING IN THE MAMMARY GLAND

5.1. Animal models indicating mammary cell targets for prolactin signaling

Mouse models have been engineered to specifically address the role of prolactin-Jak2-Stat5 pathway members, using null copies of the genes for the ligand prolactin (1), the prolactin receptor (4), the associated tyrosine kinase Jak2 (5, 8), or the transcription factors Stat5a or Stat5b (3, 6, 7). Each of these models indicates that this pathway is essential for the final functional development of the mammary gland during pregnancy and lactation. Disruption of any of those genes disrupts the massive proliferation of alveolar precursor cells and their final differentiation into milk-secreting alveolar cells. Whether this production of alveolar cells involves prolactin-mediated specification of an alveolar progenitor from a stem cell or bipotent progenitor or alternatively involves prolactin-mediated activation of a previously determined alveolar progenitor is unknown. Given the lack of ductal defects in these animal models, it is unlikely that prolactin transduces an early signal to stem cells and more likely that it communicates to an alveolar progenitor.

Prolactin also acts earlier, though indirectly, in mammary gland development, by supporting the

production of progesterone from the ovary. The loss of the prolactin receptor in the germline results in a loss of ductal branching as well as a loss of alveolargenesis, however, it was determined using mammary gland transplants that the effect of prolactin on ductal branching was not due to its loss in the mammary gland. Prolactin receptor null tissue transplanted into wild-type mouse fat pads did not result in abnormal ductal branching. Prolactin acts indirectly in order to contribute to ductal branching, through progesterone (59, 60). Progesterone receptors contribute to ductal branching as well as alveolargenesis, as indicated by progesterone null mice (61, 62), and progesterone levels are lower in the prolactin receptor null mice. It is possible that prolactin induces progesterone-mediated specification of alveolar progenitors; although it is also possible that alveolar progenitor production occurs early in gestation in response to prolactin. The hormonal stimulus that controls the origin of alveolar progenitor cells is unknown.

The lack of alveoli in mice with targeted disruption of genes in the prolactin-Jak2-Stat5 pathway, indicates that the alveolar progenitors are either not specified or that they are specified but cannot respond to prolactin. The recent identification of a luminal progenitor marker, CD61 (beta-3 integrin) (63), would be a useful marker to test whether the epithelium in these genetically engineered mice possess appropriate numbers of luminal progenitor cells versus the wild-type. An alveolar progenitor-specific marker would also be useful to determine if the prolactin-Jak2-Stat5 pathway is required for alveolar progenitor specification.

6. THE STEM CELL HIERARCHY IN THE MAMMARY GLAND

Some of the questions in the mammary gland field include whether prolactin communicates with a stem cell, and/or a progenitor cell and whether prolactin induces proliferation and differentiation in different or in the same cell type. It appears that it is more likely that prolactin controls the specification of alveolar progenitors and/or controls the activation of existing alveolar progenitors; however, there is also evidence to suggest that prolactin may affect the stem/progenitor cell population indirectly in the stem cell niche. Emerging evidence that alveolar progenitors may exhibit multipotent capacity upon *in vivo* transplantation (64), making them stem cells by functional definition, indicates that we have a lot to learn about the mammary stem/progenitor cell system.

Evidence supports that prolactin is responsible for the proliferation and differentiation of alveolar cells that arise from alveolar progenitors and may possibly be responsible for alveolar progenitor specification (1, 3, 4, 7, 8, 65). An important issue is the proper identification of stem versus progenitor cells, which have not been well-defined biochemically due to nonspecific mammary stem cell markers and different enrichment protocols. Multiple pools of stem cells and/or bipotent progenitor cells have been identified, although the relationship between the cells identified in these various studies is yet to be determined. The specific protein markers used to enrich for mouse mammary stem and progenitor cells has been reviewed elsewhere (66-68). This review will focus on the characteristics of the possible hierarchal pools in the mammary gland and the origins for breast tumours. In general, the relationship between stem and progenitor cells identified using various cell markers, to the cell populations identified using other techniques described below, such as label retaining cells, has not yet been demonstrated. Essentially, there have been multiple pools of multipotent cells identified by different marker expression patterns by different groups and a consensus on the definitive stem or progenitor cells has not yet been established. One concern is whether each of these pools is indeed multipotent stem cells or bipotent progenitor cells that give rise to both luminal and myoepithelial lineages. An *in vitro* stem cell assay is also lacking although mammary gland transplantation and regeneration is used as a definitive *in vivo* stem cell assay. We have adapted the original surgical procedure to make it more efficient (69).

The stem and progenitor cell markers also appear to be different from the mouse mammary gland to the human breast, complicating the unification of studies from different systems. The relationship of normal mammary epithelial stem or progenitor cells to cancer stem cells has also yet to be determined. These observations underlie the importance of identifying additional stem or progenitor cell markers and establishing the precise characteristics of each functional pool of cells. Given the recent exciting steps forward in the mammary gland field, we are on the verge of uniting these observations.

6.1. Label retaining cells

Based on the idea that stem and progenitor cells would protect their DNA templates throughout each cell division, it was postulated they would retain the original DNA strands while passing on the new strands to differentiating cells (70). Cells that retain a labeled nucleotide incorporated during cell division, called label-retaining cells, are thought to be long-lived cells. It was proposed that label-retaining cells the mammary gland, created during allometric ductal growth, are labeled mammary epithelial stem cells that are in a process of expansion (71-73). A portion of label-retaining mouse mammary cells was found to be estrogen receptor-a$^+$ or progesterone receptor$^+$, and these cells were capable of self-renewal and differentiation as well as mammary gland regeneration, supporting their identification as stem cells (71, 73). Although it was not investigated in those experiments whether the markers were coexpressed in the same label-retaining cell, the estrogen receptor-a and progesterone receptor are known to be co-expressed in mammary epithelial cells (74, 75). The identification of estrogen receptor-a$^+$ or progesterone receptor$^+$ cells as stem cells is consistent for what has been reported in human mammary epithelial cells, where the estrogen receptor-a/progesterone receptor$^+$ cells (of the side population) were capable of morphologically differentiating in Matrigel (72). The difficulty in testing human stem cells by transplantation assay makes it problematic to confirm the stem cell nature of this population. The estrogen receptor is not always found on stem cells in all studies, again

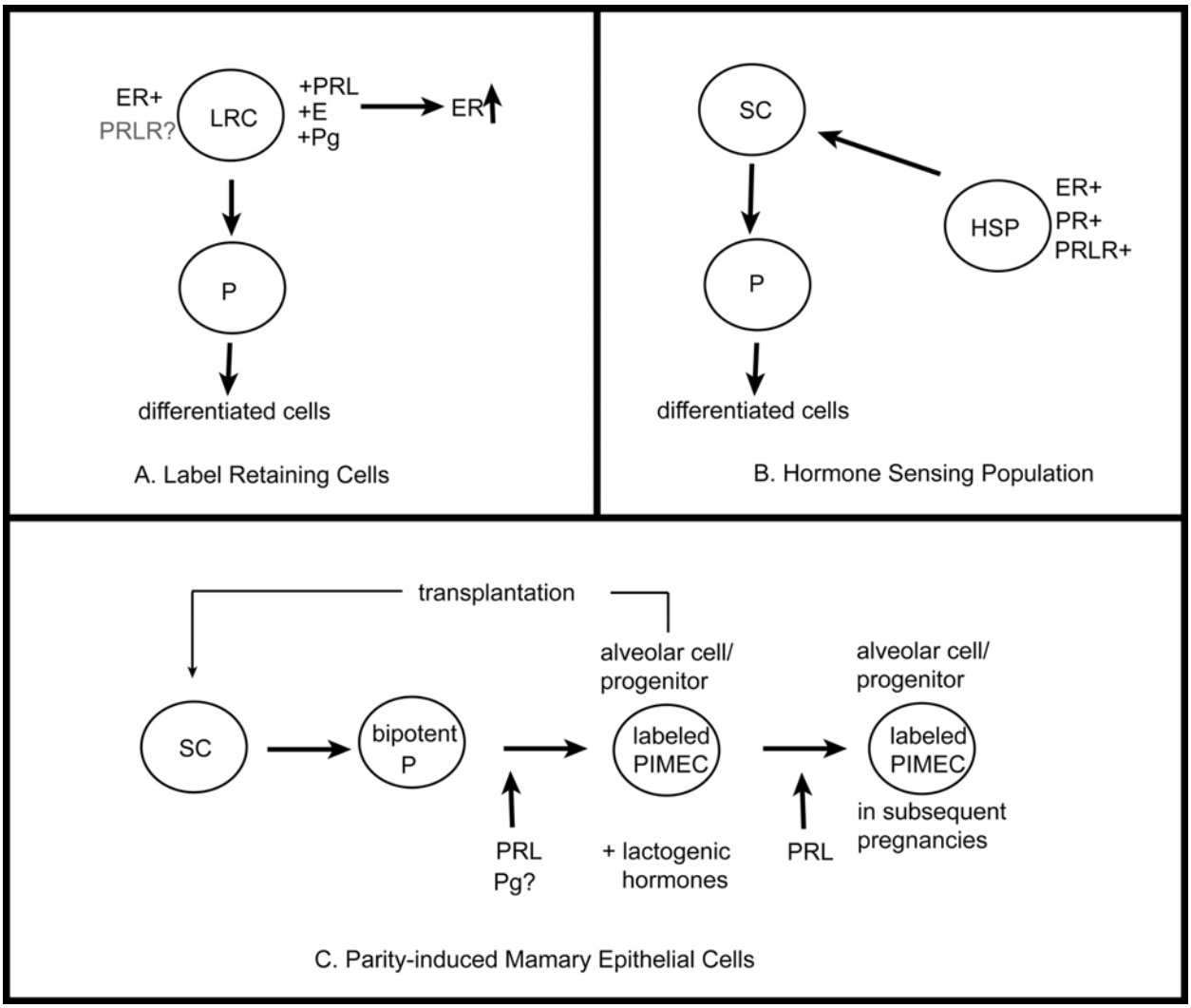

Figure 1. The possible roles of prolactin in stem or progenitor cells. A. Prolactin (PRL), in conjunction with progesterone (Pg) and estrogen (E), increases expression of the estrogen receptor (ER) in label retaining cells (LRC). B. The prolactin receptor (PRLR) mRNA is expressed in a hormone sensing population (HSP) that may modulate the stem cell (SC)/progenitor cell (P) population. C. A subset of alveolar cells that are labeled based upon expression of a prolactin responsive trans-gene, parity-induced mammary epithelial cells (PI-MECs), are capable of multipotency upon transplantation into a cleared mammary fat pad, and possess a $CD24^{+}/CD49f^{high}$ phenotype. Prolactin and lactogenic hormones, but also progesterone at an earlier stage, may be involved in formation of these alveolar progenitors. Labeled PIMECs are indicated as alveolar/progenitor cells, indicating the differentiated phenotype as well as the progenitor function contributing to the alveolar population of subsequent pregnancies.

illustrating the different multipotent cell populations that have been identified in the mammary gland, and that the estrogen receptor may not be expressed on the most primitive cells.

The prolactin receptor is also known to be coexpressed with the estrogen and progesterone receptor in breast cancer cells (76) and normal mammary epithelial cells (77), although it was not tested directly if the prolactin receptor is coexpressed within this estrogen receptor-a/ progesterone $receptor^{+}$ label retaining cell population. What also needs to be determined is if these label-retaining cells are prolactin $receptor^{+}$ at their time of labeling. A combination of prolactin, estradiol and progesterone did increase the percentage of label retaining cells expressing the estrogen receptor (73) (Figure 1a). It has been previously reported that prolactin is capable of increasing expression of the estrogen receptor as well as the progesterone receptor (78). So therefore, prolactin is capable of affecting this steroid $receptor^{+}$ label retaining population in this manner, and given its known pattern of coexpression with the steroid receptors, is likely to have a direct effect.

6.2. A hormone sensing population

In contrast, Sleeman *et al* (77) presents evidence to suggest that luminal cells ($CD24^{high}$/$prominin^{+}$) expressing the estrogen receptor (and genes for

progesterone and prolactin receptors) form a hormone-sensing population (Figure 1b) that possesses some colony forming capacity *in vitro* and only possesses a small amount of stem cell activity, as determined by mammary gland transplantation assays (77). It is possible that pre-selection using antibodies to CD24 and prominin will have resulted in recovery of a slightly different cell population than previously studied in the above-mentioned reports where estrogen receptor$^+$ cells have greater stem cell capacity. The label-retention capacity of the hormone-sensing population has also yet to be determined. The results of this study support an indirect role for prolactin as part of a hormone sensing mechanism that would direct the stem cell pool, perhaps in a stem cell niche.

Therefore, prolactin receptor expressing cells may actually be part of the stem/progenitor pool, as it appears in the label-retaining cell studies, or alternatively, may regulate the stem/progenitor pool indirectly, as supported by its presence in the hormone-sensing cell population. In support of the latter, a role for secreted factors, such as insulin-like growth factor-2, in an indirect mechanism of prolactin signaling has been identified (79), and it is likely that additional paracrine factors will be identified.

6.3. Parity induced / identified mammary epithelial cells

One stem/progenitor cell candidate known to be prolactin-sensitive is the parity-induced mammary epithelial cell (80). Parity-induced mammary epithelial cells were identified by induction of a whey acidic protein gene promoter activated Cre recombinase that removes a STOP codon and resulted in permanent activation of a Rosa promoter-beta-galactosidase transgene in prolactin treated cells (64, 80). The gene for whey acidic protein is a prolactin regulated target gene that is activated in the second half of pregnancy, mid-gestation. These parity-induced cells are identified by expression of the prolactin regulated target gene, and expand and differentiate during pregnancy and lactation. It was also discovered that EGF and IGF-2, as well as a combination of lactogenic hormones (prolactin, hydrocortisone, insulin), could lead to identification of this population *in vitro*. With this method, it has been shown that a portion of alveolar cells survives involution and is capable of forming alveolar cells upon subsequent pregnancies. Additionally, upon transplantation, a portion of the parity-induced mammary epithelial cells behaves as multipotent cells capable of contributing to the alveolar and ductal cells populations (64, 81), which indicates stem cell capacity. Their stem cell nature is also supported by the expression of mouse mammary epithelial stem cell markers. Overall, this provides support for the role of prolactin in the identification, activation and expansion of alveolar cells that act as stem/progenitor cells in future rounds of pregnancy and in transplantation assays (Figure 1c). Identification of the specific form of the prolactin receptor on these parity-induced cells, as well as the timing of when and how these alveolar progenitors are specified, remains to be clarified.

Interestingly, parity-induced mammary epithelial cells were observed to develop from undifferentiated testicular cells when directed by the mammary microenvironment (82). Co-injection of epithelial cells from the seminiferous tubules of the whey acidic protein-CRE-Rosa26 R mice (80) with mammary epithelial cells into a mammary fat pad cleared of endogenous epithelium resulted in the production of labeled cells in the regenerated mammary gland. These beta-galactosidase-labeled cells were present within normally functioning mammary epithelial ductal trees and alveoli and were able to produce the milk protein beta-casein. These experiments demonstrate that the microenvironment plays a more significant role than cell fate, and emphasizes the important role of the stem cell niche. The microenvironment also plays a significant role in cancer, with contributions from cells in the stroma.

Parity-induced mammary epithelial cells are also thought to be one of the cellular targets of mouse mammary tumor virus-based transformation with the Neu oncogene (83). A reduction in parity induced mammary epithelial cells led to a reduction in tumor incidence, supporting a role for prolactin-sensitive alveolar progenitors in tumor formation (83).

7. BREAST CANCER AND BREAST CANCER STEM CELLS / TUMOUR INITIATING CELLS

It is theorized that breast cancer arises from a transformed stem cell or a transformed progenitor cell that has gained stem cell attributes. While transformation of a stem cell would be the shortest route to a cancer stem cell, the possibility that a progenitor cell is the target of transformation is also high, given their greater number. Increasing evidence also suggests that progenitors may be the target of transformation. For example, the predominance of estrogen-receptor$^+$ breast cancers, if stem cells are estrogen-receptor negative (77, 84), suggests that progenitors are the target of transformation (68). The alternate possibility is that the cancerous stem cells acquire estrogen-receptor expression, or that the stem cell is indeed estrogen-receptor$^+$ (71, 73, 85).

The observation that different phenotypes of tumors formed in a mouse model highlighting the role of local prolactin production indicates that prolactin may communicate with early progenitors cells in tumourigenesis (15). The evidence that prolactin transduces a signal directly and/or indirectly to a mammary progenitor is mounting. While the roles of prolactin and progenitors in cancer formation or progression still needs to be tested directly, it presents important implications for prolactin in modulating progenitor cell function, as well as modulating cancer formation and/or progression.

The concepts of prolactin responsiveness and the progenitor cell identity are important. Most human breast cancers arise in hormone responsive terminal ductal lobular units (86). Parity-induced mammary epithelial cells were observed to be located at similar structures in the mouse (80), and these prolactin responsive cells are known progenitor cells that are also targets of transformation. While most human breast cancers arise in the terminal

ductual lobular units, it is unknown if they arise specifically from progenitor cells. Most breast cancer cell lines and breast tumors are prolactin-receptor$^+$ (42, 87, 88).

Recently mesenchymal stem cells have been highlighted with respect to their role in breast cancer. Mesenchymal stem cells are capable of homing to human breast xenografts in mice. Tumors with mixtures of mesenchymal stem cells and breast cancer cell lines were associated with increased metastasis to the lungs of mice. The mesenchymal stem cell-produced cytokine, CCL5, was shown to contribute to the metastatic properties of the breast cancer cells (89). Given the local production of prolactin in breast tumors, and the prolactin responsiveness of mesenchymal stem cells, it would be interesting to determine if there is an important effect of prolactin on the mesenchymal stem cell-mediated metastases of breast cancer cells.

8. PERSPECTIVES

Increasing amounts of evidence in mesenchymal stem cells, hematopoietic progenitors, oligodendrocyte progenitors and neural stem cells, point to a conserved role for prolactin in stem and progenitor cell function, with an emphasis on proliferation and differentiation of progenitor cells in a variety of tissues. Part of what remains to be examined is the role of each of the different phosphorylated, glycosylated and proteolytically cleaved forms of prolactin (90). In time, more direct evidence will better define the function of local and systemic prolactin in undifferentiated mammary cells and/or in the mammary stem cell niche (91, 92), as well as in the undifferentiated tumorigenic cells of the breast.

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Abbreviations: PRL: Prolactin, Pg: progesterone, E: estrogen, ER: estrogen receptor, LRC: label retaining cells, PRLR: prolactin receptor, HSP: hormone sensing population, SC: stem cell, P: progenitor cell, PIMEC: parity induced mammary epithelial cell

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