Review

Paracrine signaling by progesterone

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ABSTRACT

Steroid hormones coordinate and control the development and function of many organs and are implicated in many pathological processes. Progesterone signaling, in particular, is essential for several important female reproductive functions. Physiological effects of progesterone are mediated by its cognate receptor, expressed in a subset of cells in target tissues. Experimental evidence has accumulated that progesterone acts through both cell intrinsic as well as paracrine signaling mechanisms. By relegating the hormonal stimulus to paracrine signaling cascades the systemic signal gets amplified locally and signaling reaches different cell types that are devoid of hormone receptors. Interestingly, distinct biological responses to progesterone in different target tissues rely on several tissue-specific and some common paracrine factors that coordinate biological responses in different cell types. Evidence is forthcoming that the intercellular signaling pathways that control development and physiological functions are important in tumorigenesis.

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1. Introduction

The steroid hormone progesterone plays a prominent role in female reproductive tissues; its levels rise cyclically during menstrual cycles and are high throughout pregnancy. The hormone exerts its effects by binding to its cognate receptor, the progesterone receptor (PR) that acts as a transcription factor and is expressed only in a subset of cells in the target organs. The major functions of PR signaling are to trigger the release of the mature oocytes from the ovaries, to prepare the uterus for implantation of the blastocysts, and to increase the complexity of the milk duct system in the mammary gland essential for the expansion of secretory surface for milk production. Progesterone signaling also affects non-reproductive organs like thymus, bone and blood vessels, and impinges on the central nervous system. It exerts distinct biological functions in different target tissues that can be quite opposed. For instance, in the adult mammary gland progesterone signaling is mitogenic whereas it is anti-proliferative in the uterine epithelium. How can a single hormone elicit such

strong and diversified biological effects? Extensive studies addressing how progesterone affects reproductive tissues using *in vitro* and *in vivo* approaches, which include genetically engineered-mouse models such as mice that lack PR and mice deficient for either PR-A or PR-B isoforms have provided evidence that progesterone acts by paracrine signaling as reviewed below.

2. Mammary gland

The mammary gland is a branching organ that undergoes most of its development postnatally under control of female reproductive hormones. Progesterone is a key player in the adult mammary gland where it triggers the formation of side branches from the preexisting mammary ducts during estrus cycles and early pregnancy (Brisken and O'Malley, 2010). Analysis of mutant mice that lack either PR-A or PR-B isoform revealed that PR-B function is essential for mammary gland development (Mulac-Jericevic et al., 2003, 2000). The mammary ducts are composed of an inner layer
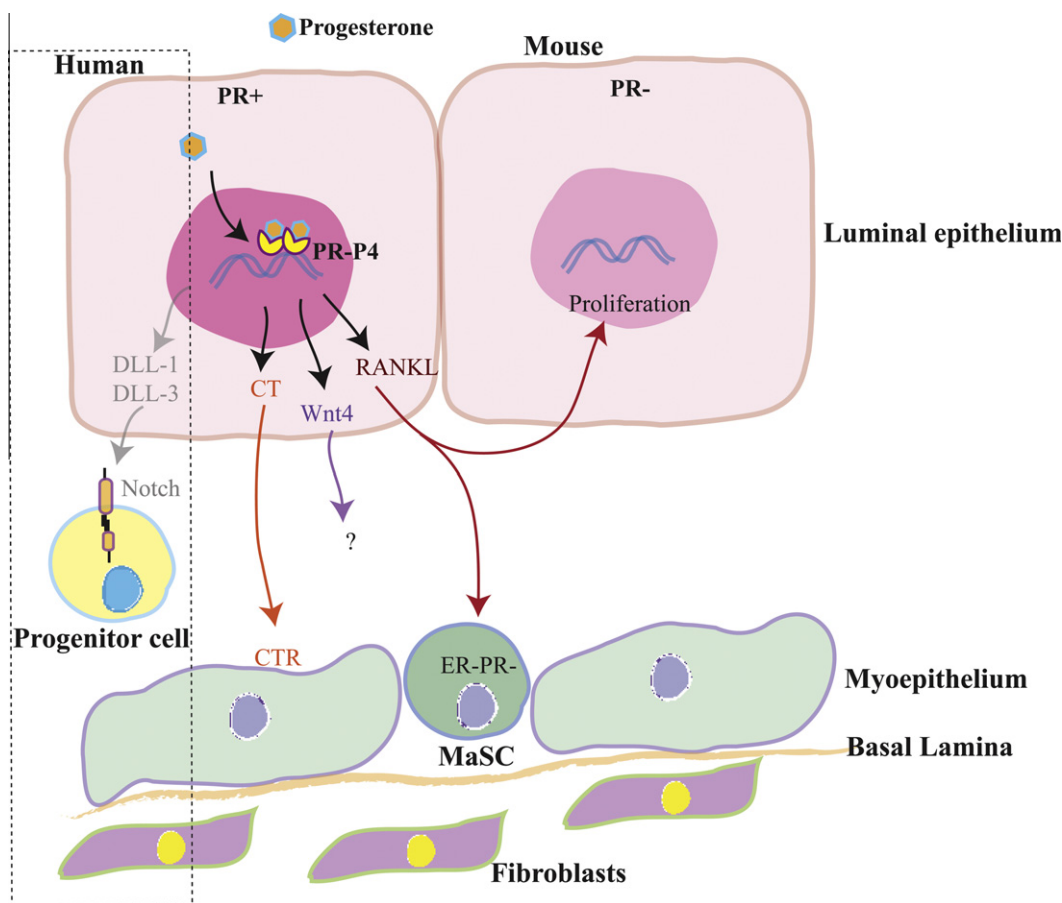
Fig. 1. Paracrine signaling induced by progesterone in mouse mammary gland and human breast. Progesterone binds to PR expressed in a subset of luminal mammary epithelial cells, and induces the expression of paracrine factors. In mouse mammary gland, RANKL, CT and Wnt-4 are induced which in turn act on their respective target cells to induce stem cell activation, cell proliferation, and tissue remodeling thereby increasing the complexity of the mammary gland. In human breast model that responds to progesterone, the hormone induces genes involved in Notch signaling pathway (shown in dashed frame) and was suggested to communicate with bipotent progenitors to increase the complexity of the breast (for details see text).

of luminal epithelium and an outer layer of myoepithelial cells that are surrounded by a basal lamina and stromal fibroblasts and are embedded within the fatty stroma (Fig. 1). In the adult mouse mammary gland, PR is expressed in 25% of luminal epithelial cells and distributed non-uniformly throughout the mammary ducts (Ismail et al., 2002; Seagroves et al., 2000). This expression pattern raises the question of how the PR positive (PR+) cells that are dispersed between PR negative (PR−) cells can elicit progesterone-mediated functions such as cell proliferation, activation of stem/progenitor cells and tissue remodeling all of which are required for the formation of side branches.

In the normal human breast epithelium, 98% of the proliferating cells are ER/PR− (Clarke et al., 1997). A similar segregation of PR− expressing and proliferating cells was observed in mice, rats and cows (Capuco et al., 2002; Russo et al., 1999; Seagroves et al., 2000). Two scenarios can account for this observation. Either nuclear hormone receptor expression is down regulated when cells proliferate or the effects of progesterone are mediated by paracrine mechanisms. The former hypothesis was supported by the finding that the estrogen receptor α (ERα) protein is rapidly degraded by proteasomes after activation in MCF-7 cells (Reid et al., 2003) and the observation that ERα expression was down modulated in the cells that entered the cell cycle in the mammary epithelium (Cheng et al., 2004). On the other hand, PR-deficient mammary epithelial cells (MECs) mixed with wild-type (WT) MECs and used to reconstitute fat pads cleared of endogenous epithelium were able to contribute to side branch formation in the resulting chimeric epithelium if they were in close proximity to WT cells (Brisken et al., 1998). This demonstrated that progesterone can act by paracrine mechanisms. Subsequently, several important paracrine mediators downstream of progesterone signaling have been identified.

2.1. Role of Wnt signaling in mammary gland development

Wnt-4, member of Wnt family was the initial paracrine mediator of progesterone identified in mammary gland. The first Wnt gene to be identified was cloned as a frequent integration site, int-1, for mouse mammary tumor virus (MMTV), a retrovirus responsible for mammary carcinomas. When it became apparent that int-1 was related to the Drosophila segment polarity gene wingless (Wg) it was renamed as Wnt-1 (Rijsewijk et al., 1987). The observation that Wnt-1 (Tsukamoto et al., 1988) and Wnt-3 (Roelink et al., 1990) were activated by an MMTV provirus in virus-induced mammary carcinomas suggested that Wnt signaling is an important oncogenic pathway in mouse mammary epithelial cells. Since then, studies using genetically modified mouse models have revealed the importance of Wnt signaling in several aspects of mammary gland development and carcinogenesis (Lindvall et al., 2007). Ectopic expression of Wnt-1 in PR deficient epithelium rescued the side-branching defect characteristic of this mutant and suggested that Wnt signaling acts downstream of the PR signaling. In addition, when MECs from MMTV-Wnt-1 transgenic mice and WT MECs were mixed and used to reconstitute cleared mammary fat pads, the WT MECs, also showed increased side-branching characteristic of MMTV-Wnt-1 mammary ducts, indicating that secreted Wnt-1 is sufficient to cause side branching (Brisken et al., 2000). As Wnt-1 is not expressed in the mouse mammary gland, it has been surmised that it mimics Wnt-4, which is expressed during early pregnancy and which acts likewise when ectopically expressed in the mammary epithelium (Bradbury et al., 1995; Gavin and McMahon, 1992; Weber-Hall et al., 1994). In line with Wnt-4 being the physiologically relevant Wnt, Wnt-4 mRNA is induced by progesterone treatment, its expression during pregnancy requires PR, and Wnt-4 deficient mammary epithelium fails to sidebranch during early pregnancy (Brisken et al., 2000). Consistent with Wnt-4 being a direct target of PR signaling, PR and Wnt-4 mRNAs show a similar expression pattern in the luminal epithelial cells, as assessed by *in situ* hybridization (Brisken et al., 2000). Recently, evidence was provided that in the PR(+) breast cancer cell line T47D, PR-B is recruited to a progesterone response element in the Wnt-4 promoter by ChIP assay (Ramamoorthy et al., 2010). Wnt-4 mRNA levels are not changed in progesterone receptor isoform-A knock out (PR-AKO) and progesterone receptor isoform-B knock out (PR-BKO) mutant mammary glands but significantly less in PRKO suggesting that both forms of the receptor can compensate for loss of each other in the regulation of Wnt-4 transcription (Mulac-Jericevic et al., 2003).

Whether the secreted Wnt-4 acts on neighboring PR− luminal epithelial and/or myoepithelial cells and/or stromal cells and to what extent the effects of Wnt-4 are mediated through canonical versus noncanonical Wnt signaling both of which have been implicated downstream of this ligand, remains to be determined (Fig. 1).

2.2. RANKL/RANK in mammary gland development

Recent studies have identified another paracrine mediator of PR, Activator of NF-κB Ligand (RANKL) member of the tumor necrosis factors (TNF) family. RANKL is required for osteoclast differentiation and lymph node organogenesis (Kong et al., 1999). In the absence of RANKL signaling, mice fail to lactate (Fata et al., 2000) and overexpression of RANKL in the mammary epithelium was sufficient to trigger side branching in virgin mammary glands (Fernandez-Valdivia et al., 2009). RANKL mRNA was induced in ovariectomized mice stimulated with progesterone (Brisken et al., 2002) and its expression was reduced in PR-B deficient mammary glands indicating that RANKL is a PR-B specific target (Mulac-Jericevic et al., 2003). The findings that ectopic expression of RANKL using retroviral vectors (Beleut et al., 2010) as well as expression of doxycycline-inducible RANKL in PR-deficient mammary epithelium was sufficient to trigger sidebranching (Mukherjee et al., 2010) have identified RANKL as an important downstream mediator of PR signaling. Immunohistochemistry on PR− deficient MECs infected with a retrovirus coexpressing ectopic RANKL and GFP revealed that proliferating cells were frequently found next to RANKL expressing cells. Similarly, in mammary glands of pregnant WT mice proliferating cells are often neighbors of RANKL expressing cells indicating that RANKL elicits proliferation by a paracrine mechanism (Beleut et al., 2010) (Fig. 1). Whether RANKL acts directly mitogenic or relies yet on other factors to elicit cell proliferation remains to be addressed.

The TNF family member, RANKL was also implicated in paracrine control of mammary gland stems cells (Asselin-Labat et al., 2010; Joshi et al., 2010). As they are located in basal epithelial compartment and are hormone receptor negative, endocrine stimulation of stem cell activation requires a paracrine mechanism (Asselin-Labat et al., 2006; Brisken and Duss, 2007; Tanos and Brisken, 2008). Consistent with RANKL being important for this, expression of mRNA of its cognate receptor, RANK was enriched in mammary stem cell (MaSC) population as opposed to the more differentiated cell populations isolated from mammary glands based on the expression of distinct cell surface markers by Fluorescent activated cell sorting (FACS) (Asselin-Labat et al., 2010; Joshi et al., 2010). Furthermore, *in vitro* assays demonstrated that RANK-Fc inhibited the clonogenic activity of MaSCs enriched population but not of the similarly treated luminal epithelial cells. Likewise, treatment of virgin or pregnant mice with anti-RANKL monoclonal antibody and subsequent *in vitro* assays showed impairment in clonogenicity of MaSCs enriched CD29^{hi} cells compared to their untreated counterpart (Asselin-Labat et al., 2010) (Fig. 1). Therefore, it is likely that progesterone controls mammary stem cells via RANKL mediated paracrine signaling.

2.3. Calcitonin involvement in mammary gland development

Calcitonin (CT), a 32-amino acid polypeptide hormone produced by thyroid involved in calcium homeostasis has been implicated as downstream target of progesterone signaling. The observation that the hormone is detected in the early human milk samples (Bucht et al., 1983) at levels that are independent of thyroid function first suggested that it might be produced locally (Bucht et al., 1986). Studies using rats and mice have shown that indeed CT and calcitonin receptor (CTR) are expressed in the mammary gland during pregnancy (Ismail et al., 2004; Tverberg et al., 2000). Based on the observation that progesterone induces CT in the uterus (Ding et al., 1994), it was put forward that the hormone may regulate CT expression in the mammary gland (Tverberg et al., 2000). In line with such hypothesis, CT mRNA expression is induced by progesterone treatment in adult WT mice but not in similarly treated PR−/− mouse mammary glands indicating that CT mRNA induction requires intact PR signaling. Immunohistochemistry revealed the expression of CTR in the myoepithelial cell layer (Ismail et al., 2004) (Fig. 1). Thus, CT mRNA is induced by progesterone and the spatial separation of CT expression in the luminal epithelium and CTR expression in the myoepithelium imply a paracrine mode of action for CT in mediating the luminal-myoepithelial cross-talk to elucidate yet unknown progesterone's function (Ismail et al., 2004).

3. Mammary tumorigenesis

Excitingly, in the mammary gland recent evidence supports the notion that deregulation of paracrine signaling pathways contributes to tumorigenesis. Deletion of the receptor for RANKL, RANK in MECs was shown to impair dimethylbenz(a)anthracene (DMBA) and medroxyprogesterone (MPA, synthetic progestin) induced tumorigenesis (Schramek et al., 2010). Similarly, MMTV-RANK transgenic mice showed accelerated tumor formation in response to MPA and DMBA. Pharmacological inhibition of RANK signaling reduced tumor formation in DMBA-MPA treated MMTV-RANK transgenic mice as well as in the hormone independent mammary tumor model, MMTV-neu/ErbB2 (Gonzalez-Suarez et al., 2010). Furthermore, RANK signaling was implicated in pulmonary metastasis in MMTV-neu transgenic mice (Guy et al., 1992). Metastatic spread of Erbb2-transformed carcinoma cells required the presence of CD4+CD25+ T cells, implying that these cells produce RANKL (Tan et al., 2011). These findings point to a more complex role of RANKL mediated paracrine signaling involving immune cells (Tan et al., 2011).

4. Human breast

4.1. Proliferation and stem/progenitor cell activation

Are the paracrine mediators identified in mouse mammary gland conserved in the human breast? The observation that most of the proliferating cells in human breast like in mouse, rat and cow mammary epithelium are PR− (Capuco et al., 2002; Clarke et al., 1997; Russo et al., 1999; Seagroves et al., 2000) suggests that paracrine mechanism(s) for progesterone action on mammary epithelial cells proliferation are evolutionarily conserved. Despite considerable progress in understanding mechanisms of progesterone action in the mouse mammary epithelium, little is known in the human breast due to the lack of suitable models that retain responsiveness to progesterone.

Only recently, 3-dimensional cultures of human mammary epithelial cells (HMECs) grown in matrigel were developed that retain estrogen receptor and PR expression and respond to progesterone

stimulation. In this system, progesterone induces cell proliferation and the majority of the proliferating cells are PR− negative (Graham et al., 2009). Furthermore, progesterone treatment of such HMEC cultures, increased the number of progenitor cells as assessed by number of mammosphere initiating cells (Dontu et al., 2003) and aldefluor positivity (Ginestier et al., 2007) suggesting that in human cells, at least in this model, progesterone activates similar processes as in the mouse mammary epithelium (Graham et al., 2009). However, important paracrine mediators of progesterone signaling identified in the mouse mammary epithelium, Wnt-4 and RANKL were not induced by progesterone in this study. Instead, the Notch signaling pathway was found to be positively regulated by progesterone, with induction of the Notch ligands, delta-like 1 and 3, as well as the notch signaling regulator presenilin2 (Graham et al., 2009) (Fig. 1). Whether this indicates that the paracrine circuitry induced by progesterone varies between mouse and human or whether the dissimilarities can be attributed to differences between in vitro and in vivo systems is unclear. It is conceivable that the in vitro system lacks factors required for progesterone regulation of these paracrine factors; this could be attributed to differences in the microenvironment with important cell types lacking such as fibroblasts, infiltrating immune cells, variations in the biochemical composition of extra cellular matrix components, ratio of luminal and basal cells, changes in physical properties such as tissue tension and/or fluctuations in hormone levels that fail to be reproduced.

5. Breast cancer

Epidemiologic studies have shown that a woman's risk of getting breast cancer is affected by her lifetime hormone exposure (Kelsey et al., 1993). Early pregnancies provide a protective effect (MacMahon et al., 1970) that, as established recently, applies specifically to progesterone receptor (PR) positive breast carcinomas (Colditz et al., 2004). Independent of hormone receptor status, breast cancer risk increases with early menarche and late menopause both of which result in an increased number of menstrual cycles during lifetime (Colditz et al., 2004). Mitotic activity in the breast epithelium and changes in tissue structure are observed during the luteal phase of menstrual cycles when progesterone levels are high (Ramakrishnan et al., 2002) suggesting that in particular exposure to progesterone relates to breast cancer risk. A further indication that PR signaling is related to the disease comes from studies on postmenopausal women on combined hormone replacement therapy (Pike et al., 1997; Pike and Ross, 2000). Women taking estrogen monotherapy had a relative risk of 1.3 whereas women using a combination of estrogens and progestin had a relative risk of 2.0 to acquire breast cancer (Beral, 2003).

Because of common developmental and hormonal characteristics, the mouse mammary gland has provided an important in vivo model for the normal human breast development and tumorigenesis (Lydon and Edwards, 2009). A question rises as to what extent the paracrine signaling pathways identified in mouse models are relevant in breast tumors that continue to express estrogen and progesterone receptors and are responsive to hormones?

5.1. Role of Wnts in breast cancer

Deregulation of the Wnt signaling pathway is linked to many different human tumor types with mutations in Wnt signaling pathway components reported (Lindvall et al., 2007). Importantly, despite substantial efforts, no alterations in intracellular Wnt pathway have been reported in breast cancer (Lindvall et al., 2007). Yet, down regulation of secreted frizzled-related protein-1 (sFRP-1), an

extracellular inhibitor of Wnt signaling is found in 80% of breast carcinomas (Ugolini et al., 1999, 2001).Similarly, secreted inhibitors of the Wnt signaling pathway, the Wnt inhibitory factor-1 (WIF1) and Dickkopf-3 (DKK-3) are targets of epigenetic silencing in 67% and 61% of primary breast tumors, respectively (Ai et al., 2006; Veeck et al., 2008). Upregulation of several Wnt ligands in breast cancer cell lines and tumor samples have been reported (Ayyanan et al., 2006; Benhaj et al., 2006; Milovanovic et al., 2004). The expression of frizzled (Fzd) 1, 2 and 7 were found to be upregulated in breast cancer (Milovanovic et al., 2004; Yang et al., 2011a). Together, these findings indicate Wnt activation is enhanced at the stage of Wnt ligand-Fzds interaction, via upregulation of Wnts ligands, Fzds as well as through the down regulation or epigenetic inactivation of secreted inhibitors.

It was not clear whether Wnts acts through paracrine mechanism(s) in tumors like in the normal mammary epithelium. Analysis of a panel of human breast cancer cell lines showed dishevelled (dvl) phosphorylation and presence of transcriptionally active form of β-catenin, as well as increased expression of several Wnt ligands; however no mutations in the Wnt pathway components were identified implying an alternative mechanism. In line with this, addition of FRP1 and DKK1, inhibitors of Wnt signaling at the level of Wnt-receptor interactions, caused down regulation of unphosphorylated β-catenin suggesting an autocrine mechanism for Wnt signaling activation in breast tumor cell lines and imply a switch from paracrine to autocrine mechanism for Wnt mediated functions in breast carcinogenesis (Bafico et al., 2004; Schlange et al., 2007).

5.2. RANKL/RANK in breast cancer

RANKL protein is expressed in 11% of human breast carcinomas and associated stromal cells such as infiltrating mononuclear cells or helper T cells (Gonzalez-Suarez et al., 2010; Tan et al., 2011). Because of the existence of an inhibitor of RANK signaling, a humanized antibody (Denosumab), a potential role of this pathway in breast tumorigenesis is an area of intense investigation (Gonzalez-Suarez, 2011; Tanos and Brisken, 2011).

5.3. Calcitonin involvement in breast cancer

The receptor for calcitonin, is expressed both in human breast cancer cell lines (Findlay et al., 1980) and primary breast cancers (Gillespie et al., 1997). As most breast cancers are held to be of luminal origin and expression of the CTR, a characteristic of myo-epithelial cells in the normal mammary gland epithelium may be connected to the neoplastic transformation. It is conceivable that this acquired expression of CTR in the tumor cells reflects a switch from paracrine to autocrine CT signaling (Ismail et al., 2004).
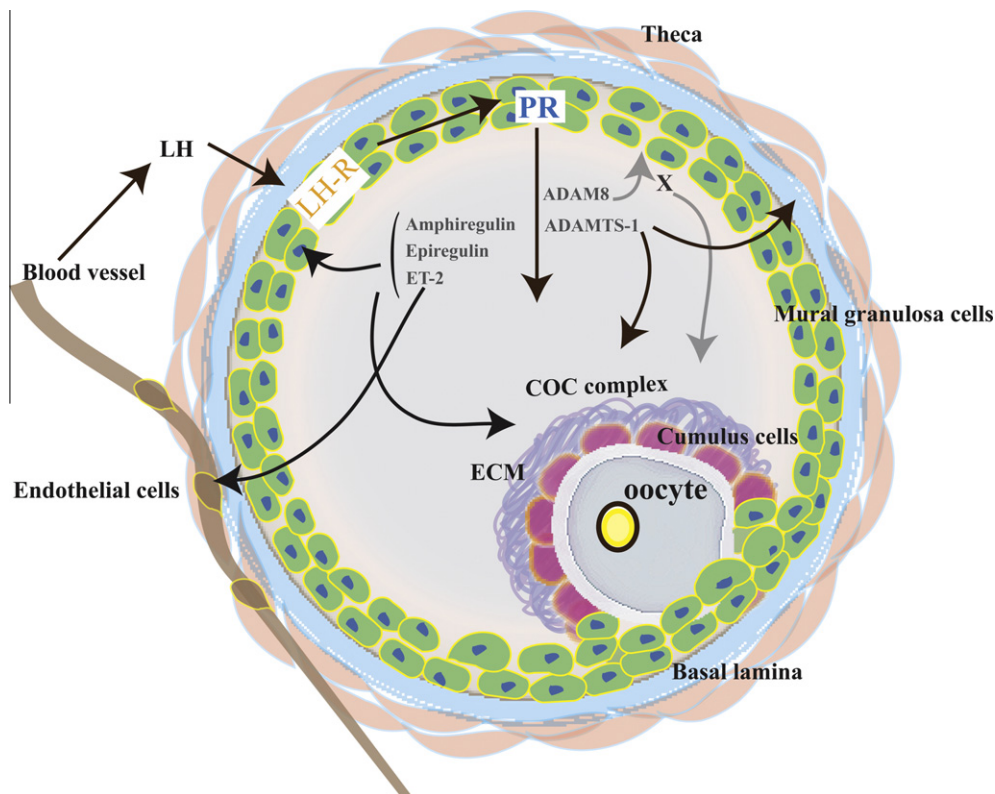
6. Ovaries

The ovaries support oogenesis and ovulation. These processes are tightly coordinated by hormones of the hypothalamic–pituitary–ovarian axis, in particular FSH (Follicle stimulating hormone) and LH (luteinizing hormone) released from the pituitary. The primary follicles that stem from primordial follicle pool develop in response to pituitary gonadotropins and mature into pre-ovulatory follicles. The pre-ovulatory follicle is composed of oocyte, surrounding cumulus cells, mural granulosa cells (MGCs), theca cells and endothelial cells of the blood vessels (Conneely, 2010; Richards and Pangas, 2010; Russell and Robker, 2007) (Fig. 2). In response to elevated levels of estrogens produced by the mature pre-ovulatory follicles, LH hormone is released by the pituitary gland. This pituitary LH surge ceases the follicular phase associated

events while at the same time induces the expression of genes required for the cumulus expansion, release of oocyte and luteinization (Conneely, 2010; Kim et al., 2009). LH, the key inducer of the ovulation, exerts its effects by binding to LH receptors, which are predominantly expressed in mural granulosa cells (Peng et al., 1991). Cumulus cells and the oocytes themselves are devoid of LH receptors, therefore LH-mediated cumulus expansion and oocyte release depend on paracrine signaling (Conneely, 2010; Kim et al., 2009; Park et al., 2004).

PR signaling is essential for ovulation, a complex process during which a pore is generated in the apical surface of the follicle wall to release the mature oocyte (Conneely, 2010; Russell and Robker, 2007). This is illustrated by the finding that treatment of mice with progesterone antagonist mifepristone (RU486) inhibits ovulation (Loutradis et al., 1991) and that PR− deficient mice are infertile because they fail to ovulate in response to exogenous gonadotropins treatment (Lydon et al., 1995). Analysis of mutants that lack either PR-A or PR-B isoform revealed that the PR-A isoform is specifically required to mediate ovulation (Mulac-Jericevic et al., 2003, 2000). Histological analysis of the ovaries of PR-deficient females showed that the pre-ovulatory follicles sustained normal cumulus cell expansion in response to pituitary LH surge; however, ovulation was severely impaired due to the lack of pore formation in the apical follicle wall, resulting in the entrapment of the oocytes inside the follicles (Robker et al., 2000). An elegant PR-lacZ transcriptional reporter mouse model and immunohistochemistry approaches revealed that LH surge induces transient PR mRNA and protein expression in mural granulosa cells (MGCs) of the pre-ovulatory follicles whereas cumulus, thecal cells and the oocytes do not express the receptor (Ismail et al., 2002; Robker et al., 2000). Such restricted expression of PR in MGCs suggests that effects of progesterone on the other cell types of the follicle essential for oocyte rupture rely on paracrine signaling.

Endothelin-2 (ET-2), potent vasoactive molecule was identified by global gene expression analysis as down regulated gene in the ovaries upon treatment with CDB-2914, a novel synthetic steroidal anti-progestin. Expression of ET-2 mRNA was undetectable in PR−/− mice treated with gonadotropins to induce superovulation, indicating that intact PR signaling is essential for induction of ET-2 expression (Palanisamy et al., 2006). The observation that ET-2 mRNA expression, like PR expression, is restricted to MGCs is compatible with ET-2 being a direct target of PR signaling. ET-2 binds to endothelin receptor (ETR-B) expressed in mural and cumulus granulosa cells of pre-ovulatory follicles as well as in endothelial cells of the capillaries present in theca interna (Palanisamy et al., 2006). Hence, it was proposed that ET-2 produced by MGCs acts in an autocrine manner on MGCs and in a paracrine fashion on cumulus oocyte complex (COC) and capillary endothelial cells to mediate vasodilatation and increase vascular permeability, thereby promoting ovulation (Fig. 2) (Palanisamy et al., 2006).

The LH surge induces expression of epidermal growth factor family (EGF) members, such as amphiregulin, epiregulin, and betacellulin in MGCs of pre-ovulatory follicles (Park et al., 2004). In a follicle culture model, amphiregulin and epiregulin induce meiotic maturation, as measured by germinal vesicle breakdown, to an extent comparable to that of LH suggesting that they are the central mediators of this effect of LH (Park et al., 2004). These two factors similarly regulate cumulus cell expansion through binding to epidermal growth factor family receptor (EGFR) (Park et al., 2004). Interestingly, both amphiregulin and epiregulin mRNA expression are markedly reduced in granulosa cells isolated from pre-ovulatory follicles of PR-deficient females suggesting that their expression downstream of LH is coregulated by PR signaling (Shimada et al., 2006). Reiterative as such, they present paracrine mediators of LH/progesterone action (Park et al., 2004) (Fig. 2).

Fig. 2. Schematic representation of pre-ovulatory follicle and the paracrine signaling involved in the control of PR and LH signaling. The LH surge induces PR expression in mural granulosa cells of pre-ovulatory follicles in the ovaries that are otherwise devoid of PR expression. Subsequent progesterone signaling induces expression of secreted factors such as amphiregulin, epiregulin and ET-2 that act as paracrine factors on cumulus cells that surround the oocyte to form the cumulus oocyte complex (COC) and vascular endothelial cells. In addition, progesterone induces the expression of the extracellular proteases ADAM-8 that may affect release of growth factors from the MGCs thereby further modulating intercellular cross talk and ADAMTS-1 initiates remodeling of cumulus oocyte complex associated extra cellular matrix as well as the follicular basement membrane.

In addition to the growth factors mentioned above, expression of two secreted proteases, a disintegrin and metalloproteinase 8 (ADAM-8) and disintegrin and metalloproteinase with thrombospondin motif-1 (ADAMTS-1) is regulated by PR signaling in MGCs (Robker et al., 2000; Sriraman et al., 2008). ADAM-8 sheds extracellular domains of transmembrane proteins by proteolytic cleavage and may release yet unidentified signaling molecules from MGCs that in turn act in a paracrine manner in other cell types of the follicles (Sriraman et al., 2008; Kim et al., 2009). ADAMTS-1 expression is required for ovulation as indicated by the observation that ADAMTS-1 deficient mice have reduced ovulation rates (Mittaz et al., 2004; Shozu et al., 2005). An inactive precursor of ADAMTS-1 is synthesized in MGCs; the secreted mature form is concentrated in extra cellular matrix (ECM) of the cumulus oocyte complex during matrix expansion and cleaves versican and important in remodeling of thecal/vascular invaginations (Brown et al., 2010; Russell et al., 2003) (Fig. 2).

7. Ovarian cancer

Ovarian cancer ranks fifth in cancer associated death in women and occurs mostly in menopausal women. The growing incidence of ovarian cancer has been linked to increased use of assisted reproduction techniques and fertility drugs (Ahmad and Kumar, 2011). Full term pregnancy, in particular twin pregnancies, has protective effects. This may be linked to the high levels of progesterone in maternal circulation during pregnancy, in particular, twin pregnancy (Adami et al., 1994; Ji et al., 2007; Lambe et al., 1999; Salazar-Martinez et al., 1999). Progesterone treatment induced

apoptosis in normal and malignant human ovarian surface epithelial cells *in vitro* (Syed and Ho, 2003) and in Macaque ovarian epithelium *in vivo* (Rodriguez et al., 1998). These findings suggest that progesterone signaling may be involved in ovarian carcinogenesis. The underlying mechanisms including the nature of potential paracrine mediators of progesterone tumor suppressive functions in tumor context have yet to be characterized. The ongoing debate that some of the ovarian cancer might originate from fallopian tubes which is also known to express PR further complicates our understanding how progesterone signaling is protective against ovarian cancer (Kurman and Shih Ie, 2010; Tone et al., 2011; Tuma, 2010).

8. Uterus

The endometrium, inner lining of the uterine cavity is composed of luminal epithelium, the glands attached to it, and the underlying stroma, which reciprocally cooperate to harmonize the functions of the uterus (Matsumoto et al., 2002). Progesterone signaling in the uterus is necessary to establish and maintain pregnancy (Lydon et al., 1995). In interaction with ovarian estrogens, progesterone prepares the uterine epithelium for blastocysts implantation and induces differentiation of endometrial stromal cells (Conneely et al., 2003). Implantation of the embryo occurs on day 4.5 in mice; the embryo attaches and invades the uterine epithelium while the stromal cells undergoes decidualization (Franco et al., 2008).

PR is expressed in epithelial as well as stromal cells of the endometrium (Ismail et al., 2002; Mote et al., 1999; Tibbetts et al.,
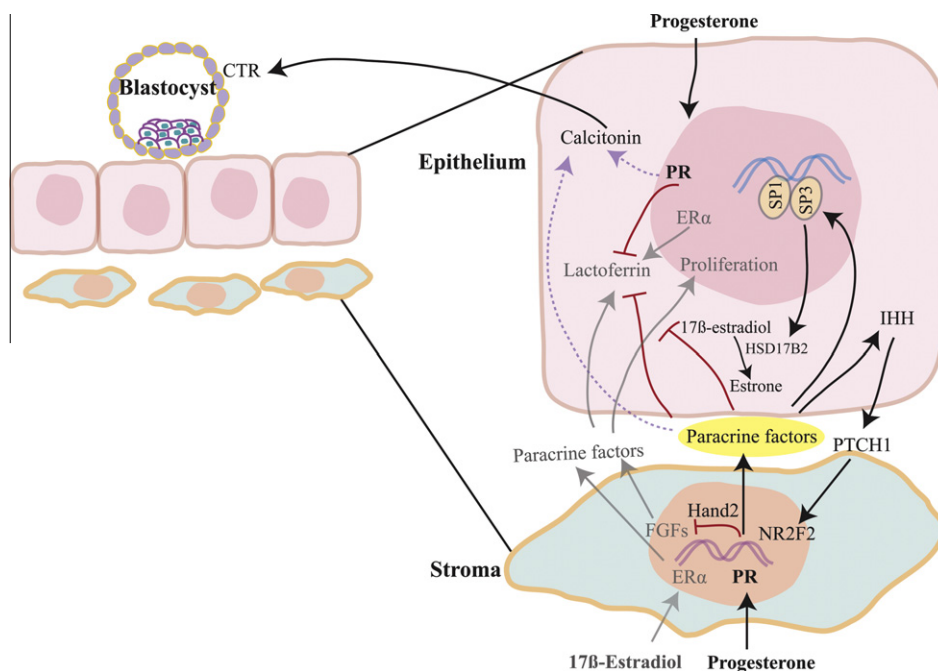
Fig. 3. Schematic representation of direct and paracrine signaling of progesterone mediated by epithelial and stromal PR respectively in the uterine endometrium (for details see text).

1998). In the periimplantation uterus, PR mRNA is undetectable by *in situ* hybridization on day 1 of pregnancy; but detected in the epithelium on day 2 of gestation (Tan et al., 1999). PR expression is further upregulated in both epithelium and stroma on days 3 and 4 of pregnancy. Uterine epithelial cells show decreases in PR expression before implantation while the stromal PR expression is strongly induced (Bazer and Slayden, 2008; Tan et al., 1999). It was suggested that progesterone signals either through stromal PR via paracrine signaling and/or through PR expressed in the epithelium which is under the current detection limit but sufficient to mediate progesterone function (Bazer and Slayden, 2008). Yet, evidence has accumulated that speaks to many of progesterone's function on the uterus being mediated by stromal PR.

Progesterone opposes estrogen-induced proliferation in the uterine epithelium (Das and Martin, 1973; Martin et al., 1973; Martin and Finn, 1968). Consistently, PR deletion results in epithelial hyperplasia (Lydon et al., 1995). The PR-A isoform was shown to be sufficient to mediate the anti-proliferative functions of progesterone (Mulac-Jericevic et al., 2003). Elegant tissue recombination experiments combining neonatal uterine stroma and epithelium from WT and PR-deficient mice under the kidney capsule demonstrated that the inhibitory effects of progesterone on uterine epithelial cell proliferation are mediated by stromal PR (Kurita et al., 1998). Estrogens similarly exert its proliferative effects in the epithelium through stromal ERα (Cooke et al., 1997). It was suggested that progesterone might exert its anti-mitogenic function by counteracting ERα-mediated signals directly in the stromal cells and/or indirectly acting on the epithelium via paracrine mechanisms (Kurita et al., 1998). Indeed, *in vitro*, treatment of endometrial epithelial cells with conditioned medium harvested from progestin-treated endometrial stromal cells induced the *mRNA* expression of 17-β-hydroxysteroid dehydrogenase type 2 (HSD17B2) that catalyzes the conversion of biologically potent estradiol to weakly estrogenic estrone whereas progestin failed to do so, implicating paracrine mechanism mediated through stromal PR (Yang et al., 2001). Yet unidentified factors secreted from endometrial stromal cells impinge on the downstream

transcription factors SP1 and SP3 in the epithelium to regulate HSD17B2 transcription (Cheng et al., 2006) (Fig. 3).

Indian hedgehog (IHH) was identified as essential mediator of progesterone involved in the epithelial–stromal cross-talk. Progesterone stimulates expression of IHH in the uterine epithelium which in turn induces patched homolog1 (patch1) and nuclear receptor super family 2 (NR2F2) in uterine stroma (Lee et al., 2006; Matsumoto et al., 2002; Takamoto et al., 2002). The functional significance of IHH in the uterus was demonstrated by conditional deletion using PR-Cre knock in mouse model that resulted in infertility due to the defect in implantation (Lee et al., 2006). Tissue recombination experiments demonstrated that stromal PR expression is required and sufficient for stimulation of IHH in the epithelium (Simon et al., 2009). Yet the nature of the paracrine factors released by the stroma that induce expression of IHH in the epithelium is unclear (Fig. 3).

Although the majority of progesterone functions in the uterus are mediated by stromal PR, evidence suggests that some of them require epithelial PR or both. Lactoferrin, for instance, is a protein secreted by epithelial cells whose expression is inhibited by progesterone (McMaster et al., 1992; Buchanan et al., 1999). Tissue recombination experiments demonstrated that progesterone can partially inhibit E2-induced lactoferrin expression via stromal PR, but both epithelial and stromal PR were required for complete abrogation of lactoferrin expression (Kurita et al., 2000) (Fig. 3). This illustrates that paracrine signaling can cooperate with cell intrinsic mechanisms of action of progesterone signaling to regulate uterine function. Progesterone-induced expression of the basic helix–loop–helix transcription factor Hand2 in the uterine stroma suppresses the production of several fibroblast growth factors (FGFs) induced by estrogens that can have mitogenic effects of estrogens on the epithelium (Li et al., 2011) (Fig. 3).

CT is upregulated by progesterone in uterine glandular epithelial cells, prior to implantation; its expression decreases as pregnancy progress (Ding et al., 1994; Wang et al., 1998). Such induction of CT mRNA was inhibited upon treatment with progesterone antagonist RU486 (Ding et al., 1994). In utero administration of CT

antisense oligodeoxynucleotides abolished implantation revealing a role for CT in this process (Zhu et al., 1998). Interestingly, CTR mRNA was detected in blastocyst (Wang et al., 1998) suggesting that CT induced by progesterone acts in a paracrine fashion to mediate maternal-embryonic cross talk (Wang et al., 1998). Whether stromal or epithelial PR mediates CT expression in the uterine epithelium is not known (Fig. 3).

As it emerges that stromal PR is important for uterine functions the identity of the paracrine signals released by the stroma downstream of progesterone that communicate with uterine epithelium comes into focus. *In situ* hybridization on uterine sections prepared from pregnant ewes revealed that hepatocyte growth factor (HGF) and fibroblast growth factor (FGF-10) mRNA were expressed in the stroma and their respective receptors c-met and FGFR2IIIb in the epithelium. Expression of HGF, FGF-10 and c-met was high in early pregnancy. Therefore, it was put forward that progesterone may play a role in regulation of HGF and FGF-10 expression in uterine stromal cells that express PR and c-met in uterine epithelial cells (Bazer and Slayden, 2008; Chen et al., 2000a,b). However, to what extent the stromal–epithelial crosstalk relies on these two factors is not clear. It is likely that additional paracrine signaling molecules downstream of PR will have essential roles in stromal–epithelial crosstalk in mediating progesterone's function in the uterus.

9. Endometrial cancer

Endometrial cancer is the fourth most common malignancy in women with most tumors originating from the glandular epithelium (Yang et al., 2011b). The inhibitory effect of progesterone on uterine epithelial proliferation has been the basis for treating endometrial hyperplasia and adenocarcinomas with progestins (Gambrell, 1986; Yang et al., 2011b). To what extent this therapeutic approach affects paracrine signaling identified in rodent uterus remains to be determined. Exposure to progesterone in menopausal combined hormone replacement therapy is known to increase the overall risk factor for breast cancer. Therefore, it is essential to identify downstream mediators of progesterone that are unique to different target tissues.

10. Progesterone signaling in non-reproductive tissues

PR is also expressed in non-reproductive tissues including thymus, bone, blood vessels and the central nervous system. In the thymus, PR is expressed by stromal cells. PR signaling is required for thymic involution during pregnancy and plays an essential role in blocking T-cell development early on which is important to maintain pregnancy. As PR is expressed in non-lymphocyte population but ultimately acts on T-cells, it was proposed that progesterone receptor blocks T-cell development by paracrine mechanisms (Tibbetts et al., 1999). Identification of the paracrine mediators will give new insights into how progesterone regulates pregnancy induced immunotolerance.

In the bone, PR is detected in osteoblasts and osteoclasts (MacNamara et al., 1995; Pensler et al., 1990; Yao et al., 2010). Histomorphometric and microcomputed tomographic studies on PR-deficient mice showed no gross abnormalities in bone growth suggesting that PR signaling is not absolutely required for bone growth and turnover. However, loss of PR signaling resulted in increased accumulation of cortical and cancellous bone mass (Rickard et al., 2008; Yao et al., 2010). Similarly, pharmacological inhibition of PR with RU486 in wt mice moderately increased bone mass, a finding of potential relevance for patients with osteoporosis (Yao et al., 2010).

PR expression has also been detected in the smooth muscle cells of the uterine arteries in rabbits and humans (Perrot-Applanat et al., 1988) and in 25–30% of endothelial cells in human arteries (Vazquez et al., 1999). Progesterone treatment inhibits endothelial cells proliferation *in vitro* and reduces aorta re-endotheliazation in response to experimentally induced injury pointing to potential biological functions in these cells (Vazquez et al., 1999). A carotid artery injury model in wt and PR-deficient animals established an important role for PR (Karas et al., 2001). For review see (Simoncini et al., 2003).

PR is also expressed by variety cell types in different regions of the central nervous system. Growing evidence suggest that the hormone controls reproductive behavior and several other non-reproductive functions in the central nervous system as reviewed in (Brinton et al., 2008; Mani, 2008).

Understanding the paracrine signaling elicited by progesterone to mediate non-reproductive functions has substantial clinical implications in light of the importance of hormone replacement therapy (HRT).

11. Conclusions

Progesterone induces a multitude of biological effects in different organs by acting on a subset of cells in distinct target tissues. Most of PR's functions are mediated indirectly via secreted paracrine factors. In this way the hormonal stimulus is amplified and the signal communicated to multiple cell types, an important aspect in order to coordinate the function of different cells. To date, several important paracrine factors downstream of progesterone signaling have been identified in mammary gland, ovaries and uterus. Evidence suggests that these mediators relegate paracrine signaling between similar cell types, for example RANKL synthesized in the PR(+) luminal epithelium acts on PR(−) luminal epithelial cells and/or paracrine communication involving different cell types such as epithelial–stromal cross talk, engaging endothelial cells and immune cells, thus progesterone orchestrates several cell types to execute its function. The diversity of progesterone's function in these tissues can be attributed partly to distinct paracrine mediators, which is also of relevance in understanding carcinogenesis and treatment. For example, the combined HRT replacement therapy with progestins seems to protect women against uterine cancer while increases the breast cancer risk. This may be explained by the fact that progesterone receptor signaling in the uterus is anti-proliferative whereas it is mitogenic in the mammary gland. This in turn may be attributed to distinct paracrine mediators that are induced by progesterone in specific tissues. Therefore, understanding the cross-talk between the PR expressing cells and target cells will shed new insights about the effects of progesterone and may help to identify novel downstream pathways for therapeutic applications that could specifically target the tissue type than having a global impact on all the progesterone target tissues and thereby avoiding adverse consequences.

Acknowledgements

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