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PROGESTERONE AND THE CONTROL OF UTERINE CELL PROLIFERATION AND DIFFERENTIATION

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1. ABSTRACT

Progesterone is the only steroid hormone that is essential for the establishment and maintenance of pregnancy in all mammalian species that have been studied. Mice lacking the progesterone receptor (PR) by targeted mutagenesis exhibit abnormalities in all aspects of reproduction including sexual behavior, mammary gland development, ovulation, and implantation. Implantation in PR null mice fails, in part, because the uterine stromal cells cannot undergo differentiation (the decidual cell reaction). Uterine stromal cells do not divide without progesterone and proliferation is blocked by progesterone antibodies and PR antagonism. In spite of the preeminence of progesterone in female reproduction, its molecular mechanisms of action on target cell proliferation and differentiation are not well understood. Recent studies suggest that progesterone plays a direct role in regulating cell cycle transit by increasing the expression and activation of cell cycle regulatory complexes. Furthermore, this progesterone-dependent regulation of cell cycle transit may provide a unique window of opportunity for uterine stromal cells to exit the proliferative cycle, and if exposed to appropriate agents, enter into the differentiation pathway.

2. INTRODUCTION

The growth and function of any tissue is dependent on regulated proliferation and differentiation of its cellular components. Within endocrine target tissues of

the reproductive tract, hormones exert specific temporal, spatial and interactive effects. Estrogens are associated generally with cell proliferation in uterine and breast tissues, while progesterone is considered more as the hormone promoting cellular differentiation in these organs. However, progesterone is a potent mitogen for stromal cells in the uterus and the lobuloalveolar cells in the mammary gland (1). The preeminence of progesterone in female reproduction has been highlighted by recent studies from mice lacking the progesterone receptor (PR) by targeted mutagenesis (2). The PR "knockout" mouse exhibits abnormalities in all aspects of reproduction including sexual behavior, mammary gland development, ovulation and implantation (2). Progesterone is the only steroid that is essential for the establishment of pregnancy in all mammalian species studied (3), but its mechanisms of action on target cells are poorly understood. The function of progesterone as a proliferative hormone has been difficult to assess because in most target tissues the levels of PR are regulated by estrogen (4). Many systems therefore require the addition of estrogen, progesterone and growth factors for cell proliferation, confounding elucidation of the role of progesterone action alone in the cell cycle. Moreover, progesterone serves dual functions in target cells stimulating both proliferation and differentiation. In spite of these complexities, it is imperative that the molecular mechanisms of progesterone action on target cells be elucidated since PR deficient mice unexpectedly revealed that progesterone regulates a wider range of reproductive functions than previously suspected (2).

3. CELL PROLIFERATION

The process by which cells replicate their DNA, segregate their chromosomes and divide forming two daughter cells is collectively referred to as cell proliferation. Most rapidly dividing mammalian cells have a cell cycle that lasts approximately 24 hours. By convention (5), the cell cycle has been subdivided into four distinct phases. DNA replication occurs during S phase while mitosis, lasting only about 30 minutes, is when actual segregation of the chromosomes takes place. Cell growth and mitosis are separated by a two gap interphases called gap 1 (G1) and gap 2 (G2). The regulatory points that control orderly progression through the cell cycle occur primarily during G1 and G2 phases.

3.1. Cell Cycle

Mammalian cells use a small family of related cyclin and cyclin dependent kinases (Cdks) to regulate progression through the cell cycle (6,7). Cell cycle transit involves the temporal and sequential activation of a number of genes, as well as the inactivation of a series of crucial checkpoints. Formation of active cyclin-Cdk complexes affects both basal and activated transcription of genes necessary for cell cycle progression (8). Three different mechanisms that control cell cycle progression have been well-characterized: (i) the timing of synthesis and proteolysis of cyclin subunits; (ii) the presence or absence of Cdk inhibitors; (iii) the modulation of the kinase activity of the Cdk partner through phosphorylation/dephosphorylation modifications. As cells progress through the cell cycle, phosphoproteins undergo ubiquitin mediated degradation (9,10). Degradation of specific proteins is essential for entry into S phase, separation of sister chromatids and exit from mitosis. The importance of this pathway as a regulatory mechanism is suggested from data base searches that revealed the F-Box motif, which is a tag for ubiquitylated proteolysis by the protein SKP1, is present in over 40 different proteins (10). Each phase of the cell cycle is dependent on events in the preceding phase and complex feedback control mechanisms exist to ensure orderly transit from one phase to the next. Regulation of feedback controls is still not well understood at the molecular level and even less is known about how cells can monitor processes like DNA replication and DNA damage. However, it is the integration of positive and negative regulators, and the means by which they control cell cycle progression, that determines the sequence of molecular events that link cell cycle exit with the selection of the differentiation pathway. It therefore seems essential to study cellular differentiation in the context of cell cycle regulation.

3.2. Cyclins

Cyclins are a related group of proteins that function during G1 phase of the cell cycle (cyclins D and E) or during the G2 to M transition such as cyclins A and B. Cyclin-Cdk complexes are heterodimeric proteins composed of two subunits. The first subunit is a cyclin protein that regulates substrate specificity. The concentration of each respective cyclin oscillates in phase with the cell cycle so that the peak expression of a given cyclin corresponds to the specific cell-cycle phase in which it functions (11-13). The current model for the action of growth factors during G1 phase of the cell cycle predicts that cyclin D and cyclin E levels increase resulting in the formation of different cyclin-Cdk complexes that drive cells through the restriction point of G1 into DNA replication. Once DNA replication is complete, all eukaryotic cells are stimulated to undergo cell division in response to maturation promoting factor (MPF), more broadly called mitosis promoting factor (12). MPF is a dimeric protein containing cyclin B (the regulatory subunit), and a catalytic subunit Cdk1, also referred to as Cdc2 from its homolog in yeast (12,13). MPF activity is controlled by the assembly of the cyclin B-Cdk 1 complex, activation by phosphorylation and dephosphorylation modifications and the translocation of the complex from the cytoplasm into the nucleus.

3.3. Cyclin Dependent Kinases

Binding of cyclin to the Cdk is necessary but not always sufficient for full activation of the complex (14). For full kinase activity, the Cdk is usually phosphorylated on the threonine residue adjacent to the kinase active site by a kinase called Cdk-activating kinase (CAK). The activity of CAK is usually present at high levels throughout the cell cycle but the activating phosphorylation occurs only after the binding of cyclin. In multicellular eukaryotes there are two primary Cdks that function in S phase (Cdk2), and M phase (Cdk1), respectively. Mammalian cells also contain Cdk4 and Cdk6 that function during G1 phase of the cell cycle. Interestingly, assembly of cyclin D and Cdk4/6 into high affinity binding complexes does not occur in the absence of other factors. It was surprising when the

other factors that promoted stable complex assembly turned out to be CKIs that associate with complex but do not destroy its catalytic activity.

3.4. Cyclin Dependent Kinase Inhibitors

Two types of CKIs are candidates for suppressing cyclin-Cdk activity during G1or at the G1-S boundary. First, are the cyclin D-Cdk4/6 specific inhibitors of which p16 ^{Ink4} is the prototype (15,16). Second, is the family of inhibitors containing the p21 and p27 prototypes that act on a broader range of substrates (17,18), and have been shown to increase in response to progesterone in breast cancer cell lines (19,20). Of the many CKIs that have been isolated, to date only p57 has been shown to be required for early development (21,22). The absence of functional p57 resulted in an embryonic lethal and the tissues in those mice failed to differentiate normally. Mice lacking other CKIs by targeted mutagenesis, such as p27, showed proliferative disorders in most organs of adult animals. However, the p27 null mice undergo apparently normal embryonic and fetal development.

4. SEX STEROIDS AND UTERINE CELL PROLIFERATION

One of the major functions of sex steroids in target organs is to stimulate cell proliferation and differentiation. In the endometrium of female mammals, sex steroids prepare the maternal endometrium for implantation of the embryo. The uterus does not normally constitute a receptive environment for the embryo until a precise sequence of changes takes place in response to the female sex steroids estrogen and progesterone. In rodents these changes involve proliferation of luminal and glandular epithelial cells under the influence of pro-estrus estrogen at days 2 and 3 *post coitum* (23). With the development of the corpus luteum following ovulation, and the resulting rise in circulating progesterone at day 4 of pregnancy, cell division switches from the luminal and glandular epithelium to the endometrial stroma (23-27). It is the action of ovarian hormones on the uterus followed by a stimulus from the implanting blastocyst that stimulates the proliferation and the subsequent differentiation of these cells to form the decidua (28). Decidualization occurs in many mammals, including the human female (29), and the differentiated stromal cells ultimately form the maternal part of the placenta. Unlike the process in rodents, decidualization of the stroma in the human female does not require the stimulus of an embryo and instead it occurs spontaneously in response to sex hormones.

Stromal cells in mice and rats do not divide without progesterone, and proliferation is blocked by progesterone antibodies and progesterone receptor (PR) antagonists (30,31). Progesterone is required to stimulate the proliferation of cultured human (32) and rat (33-36) endometrial stromal cells, while the PR antagonist RU 486 blocks the proliferative response in human (37,38) and rat (34) stromal cells *in vitro*. Progesterone alone is not sufficient to stimulate proliferation since cultured stromal cells also need growth factors (32-36). Sex steroids including progesterone and estrogen act on target tissues by stimulating or repressing the transcription of specific genes whose products alter cell function. Ligand bound steroid hormone receptors interact with defined hormone response elements along the DNA of target genes and change the rates of transcription (39,40). While it is clear that hormonal control of proliferation can be mediated, in part, by controlling the expression of a variety of growth factors and/or growth factor receptors (41), recent evidence from breast cancer (41,42) and uterine stromal cell (36) studies show direct regulation of cell cycle genes by progesterone.

In rat uterine stromal (36) and breast cancer (43) cell lines progesterone increases the expression of cyclin D1 which accelerates passage of cells through G1 and increases the proportion of cells engaged in cell cycle transit. Progesterone transiently stimulates transcription of c-myc in breast cancer and uterine cells and this stimulation is reversed by the progesterone receptor antagonist RU 486 (41). A progesterone response like element (PRE-like) is located in the 5'-flank of the human c-myc gene that confers progesterone responsiveness when coupled with a c-myc promoter-CAT reporter construct (44). Evidence thus exists for progesterone-regulated transcription of cell cycle genes although the molecular mechanisms are not known (36,42). Other progesterone controlled cell cycle regulators include the MPF complex that is utilized by mammalian cells to phosphorylate substrates that function in mitosis. Progesterone stimulates the activation of MPF in the immature frog egg through a complex signal transduction pathway (45,46) resulting in the resumption of the first meiotic division.

Since progesterone is known to also inhibit cell proliferation clues regarding the mechanisms invovled in its dual function have been obtained primarily from breast cancer cell studies. Progesterone mediated inhibition of breast cancer cell entry into S phase is preceded by decreased Cdk4 activity and cyclin E-Cdk2 activity (47), suggesting progesterone-dependent inhibition occurs from inhibitory proteins with broad substrate specificity. Inhibitory actions arise from the binding of distinct proteins including the specific inhibitors p21, p27 and p57 to cyclin-Cdk complexes (48). At low concentrations of p21, assembly of active cyclin D-Cdk4 complexes occurs. As the concentration of p21 increases, activation of the cyclin D-Cdk4 complex is inhibited. Other inhibitors such as p15, p16, p18 and p19 interact with the kinase subunit alone (48). The action of progesterone as a growth inhibitor in breast cancer cell lines correlates with increased expression of both p21and p27 (19,20,47). Progress in understanding progesterone action in target cells has been complicated by the fact that progesterone exerts dual functions in target cells stimulating both proliferation and growth inhibition within the same cell type. In order to understand the complexity of progesterone action, it therefore may be necessary to identify and characterize co-regulatory proteins that impart subtle changes in specificity to the function of the PR.

4.1. Uterine Epithelium

The epithelial cells of the glandular and luminal epithelium proliferate during the first few days of

pregnancy (25) prior to increased progesterone secretion from the newly formed corpus luteum (49). We have shown (24) that at day 3 of pregnancy in the rat, the expression of PR in the luminal epithelium is heterogenous while all of the glandular epithelial cells are positive. By day 4 of pregnancy, the time corresponding to the proliferative switch from epithelial to stromal compartments, PR was homogeneously expressed throughout the epithelium (24). Moreover, administration of the PR antagonist RU486 resulted in epithelial hypertrophy and continued proliferation in the absence of progesterone action (24). Consistent with our observations, a single injection of estrogen to ovariectomized mice will induce a wave of epithelial cell proliferation (50) while progesterone inhibits the estrogen-dependent response (51) indicating that estrogen alone stimulates epithelial cell proliferation while progesterone suppresses the estrogen-dependent response. More recently in a very elegant set of experiments, Tong & Pollard (52) showed that the stimulation of epithelial cell proliferation by estrogen is mediated by the estrogen-dependent translocation of cyclin D1 from the cytosol into the nucleus within 2 h of estrogen administration. Progesterone inhibited epithelial cell proliferation because it blocked the translocation of cyclin D1 from the cytosol into the nucleus. When mice were pre-treated with progesterone, the estrogen-dependent translocation of cyclin D1 was suppressed and the subsequent phosphorylation of pRB and p107 that is necessary for G1 transit did not take place.

4.2. Uterine Stroma

Cell cycle regulatory molecules are responsive to both estrogen and progesterone action in the uterine stroma. In ovariectomized rats there is a time dependent increase in the uterine expression of the D-type cyclins and cdk 4, 5 and 6 mRNA in response to estrogen (53). Localization studies of the D type cyclins and p27 showed a cell specific increase of p27 in the uterine stroma of ovariectomized rats in response to estrogen (54). The lack of stromal cell proliferation in response to estrogen was postulated to be the result of increased p27 expression in these cells. Expression of cyclins and Cdks correlated with ER and PR positive cells in the human uterine stroma and glandular epithelium (55). Progesterone increased p27 expression in normal human glandular epithelium and endometrial carcinoma cell lines (55). Previous studies in our laboratory showed rat stromal cells express cyclins D1 and D3 (56) *in vitro*, while others (57) have detected transcripts for cyclins D1 and D3 in the mouse uterus. We were unable to detect cyclin D2 in proliferating rat uterine stromal cells (56). Taken together, the data suggest that sex steroids regulate cell cycle progression in target cells, at least in part, by increasing the cell specific expression of cell cycle regulators. The future challenge is to not only identify the role for steroids in the formation and activation of cyclin-Cdk complexes, but also to discern the mechanisms by which sex steroid promote cell cycle exit and entry into the differentiation pathway.

Research in our laboratory is focused on the mechanisms of progesterone action on rat uterine stromal cell proliferation and differentiation (58-62). Specifically, we are characterizing the normal transit of stromal cells through the cell cycle and we are asking how cell cycle control is altered when cells select the differentiation pathway. A large number of growth factors have been implicated in the hormonal control of endometrial cell proliferation (reviewed in 63). We postulate that many growth factors, particularly those with tyrosine kinase activity can, in conjunction with appropriate hormonal stimulation, serve as co-regulators of uterine cell proliferation. Our initial studies focused on the spatial distribution of basic fibroblast growth factor (FGF) expression during the proliferative switch from epithelial to stromal cell proliferation in pregnant rats (24,58). We reasoned that if FGF is involved in stromal cell proliferation then inhibition of stromal cell division should alter FGF expression. Stromal cell proliferation was inhibited significantly ($p < 0.05$) in rats treated with PR antagonist (24). Importantly, that study showed that without active PR stromal cell FGF content is lost and cell division is suppressed. In contrast, expression of FGF in luminal and glandular epithelial cells was unaffected by PR antagonism indicating that repression of PR function in early pregnancy results in a cell-specific loss of FGF and inhibition of stromal but not epithelial proliferation.

Since FGF was evident in the uterine stromal cell extracellular matrix of pregnant rats, we measured the amount of FGF mRNA at days 2-4 of pregnancy using RNase protection (60). FGF transcripts were detected at each of the 3 days of pregnancy suggesting that the protein we identified in cells at these same times (58) could arise from newly synthesized FGF and not strictly from the uptake of extracellular stores. Steady-state FGF mRNA levels are controlled by sex steroids in the endometrium. Estrogen increased FGF mRNA 8.5-fold over that in control ovariectomized rats, while progesterone significantly ($p < 0.05$, approximately 27-fold) increased mRNA levels over the amount measured in ovariectomized controls (60).

In order for FGF to exert biological effects, FGF receptors (FGFRs) must be present in stromal cell membranes. We localized FGFR1 to stromal cell nuclei at days 4-6 of pregnancy (59). In contrast, immunoreactivity was limited to the cytoplasm in both glandular and luminal epithelial cells. Binding assays using ^{125}I-FGF and uterine membrane proteins from day 4 pregnant rats confirmed that uterine FGFR1 binds FGF with high affinity ($k_d \sim 1$ nM) and specificity (59). Taken together, these results implicate FGF and progesterone as co-regulators of uterine stromal cell proliferation in the rat.

While such *in vivo* analysis is important to establish key regulatory molecules in pregnant animals, in order to study the mechanisms involved in progesterone control of cell proliferation and differentiation it was important to develop an in vitro system where cells were regulated appropriately by progesterone and estrogen. We have now isolated and characterized three separate rat uterine stromal cell lines (34-36). The proliferative response of quiescent stromal cells to progesterone and

Figure 1.. Uterine stromal cell lines express transcripts for ER-alpha. Rat uterine stromal cells (UII-35) were cultured in growth medium (34) and grown to confluence. Total RNA was isolated and reverse transcribed in cDNAs. Nested polymerase chain amplification (PCR) was used to amplify ER-alpha and ER-beta using primers and methods described in detail elsewhere (82). The amplified products were separated on 2% (wt/vol) agarose gels stained with ethidium bromide. Lane 1, 100 bp size marker; Lane 2, uterine stromal cells (UII); Lane 3, HUVEC cells (positive control); Lane 4, uterine stromal cells; Lane 5, human T cells (positive control), Lane 6, no template, negative control.

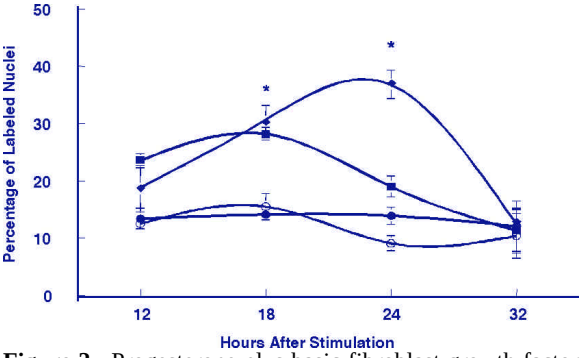

Figure 2. Progesterone plus basic fibroblast growth factor (FGF) increase the number of cells entering S phase and extend the time of stromal cell entry into DNA replication. Quiescent uterine stromal cells were stimulated to re-enter the cell cycle with serum-free medium containing the synthetic progestin medroxyprogesterone acetate (MPA, 1 ΦM), FGF (50 ng/ml), the two agents together (FGF plus MPA), or ethanol vehicle (Control). At the indicated times cells were pulsed for 30 min with bromodeoxyuridine (BrdU)-containing medium (10 Φmol/L). Samples were fixed and stained with anti-BrdU antibody as described in detail elsewhere (36). Data are the mean +/- SEM of three (18 h, 24 h) or two (12 h, 32 h) independent experiments. * ($p = 0.001$) compared to the control cells (ANOV). At 18 h, the percent of labeled nuclei increased in cells treated with FGF alone ($p = 0.01$) and MPA plus FGF ($p = 0.004$) compared to the control cells. At 24 h, the number of labeled nuclei in cells cultured with MPA plus FGF was significantly greater than for cells cultured in FGF alone ($p = 0.001$), MPA alone ($p = 0.001$) and the control medium ($p = 0.001$). The number of labeled nuclei in cells treated with FGF alone remained increased ($p = 0.023$) over that in the control cells at 24 h, Bonferroni post-hoc tests. **,** MPA plus FGF; #, FGF; !, MPA; ", serum-free medium (Control). Copied from reference 36 by permission of the Endocrine Society .

FGF were tested by the MTT assay to measure proliferation (34), flow cytometry to assess cell cycle progression (35), and entry into S phase using ^{3}H thymidine incorporation (35,36). The response among different cell isolates (UI-UIII) and cells at different passages (between 4 and 30) is similar. These cells fulfill 2 essential criteria for use as a model system to tease out the mechanisms of steroid hormone action on the stroma. First, as originally reported by Cohen et al (33), and now confirmed in our laboratory (34-36), isolated stromal cells retain an absolute requirement for progesterone to proliferate as do stromal cells *in vivo*. Second, the response of uterine stromal cells to progesterone and FGF is consistent and repeatable. While non-transformed lines of rat uterine cells exist (64), those cells contain a temperature sensitive mutant of the SV40 large T antigen. Uterine stromal cell lines (34-36) provide a system free of foreign DNA as an essential model to study endocrine-dependent cell cycle control and differentiation mechanisms.

Stromal cells proliferating in response to progesterone and FGF expressed the A and B forms of PR (34) and at least one of the receptors for FGF (FGFR1, 59). In addition, progesterone was the only hormone tested that stimulated proliferation significantly ($p < 0.01$). Addition of the PR antagonist RU 486 at 10-fold molar excess reduced significantly ($p < 0.01$) the proliferative response while at equimolar concentrations of hormone and RU 486, the PR antagonism was overcome suggesting that RU 486 exerts cytostatic rather than cytotoxic action in vitro. Suramin (500 ΦM), which blocks FGF binding to FGFR, inhibited stromal cell proliferation. We were surprised that estrogen alone did not stimulate proliferation. However, that earlier study (34) showed that estrogen potentiated the proliferative response in the presence of progesterone, but the differences were not statistically significant. We have subsequently determined that under normal growth conditions, uterine stromal cell lines express estrogen receptor (ER)-alpha but not ER-beta transcripts (Figure 1). To verify authenticity of ER-alpha, the PCR product was eluted form the gel and subcloned into the pGem T vector (Promega). Three independent clones were sequenced and all 3 had identical sequence to ER-alpha (data not shown).

Recently we have obtained some of the first clues regarding the mechanisms involved in progesterone-dependent control of uterine stromal cell proliferation (36). We investigated specific progesterone-dependent control of G1 phase after quiescent stromal cells were stimulated to re-enter the cell cycle by adding serum-free medium containing medroxyprogesterone acetate (MPA) and FGF. Cells stimulated with MPA plus FGF incorporated significantly more ($p=0.01$) [^{3}H] thymidine than cells treated with FGF alone. Flow cytometric analysis of stimulated cells showed FGF alone and MPA plus FGF increased significantly ($p=0.002$) the percentage of cells in S phase at 12 h (Figure 2, 36). Interestingly, while both FGF alone and MPA plus FGF increased bromodeoxyuridine (BrdU) incorporation into stromal cell nuclei at 18 and 24 h, MPA plus FGF increased ($p=0.001$) the number of cells entering S phase at 24 h compared with FGF alone (Figure 2). When we inhibited stromal cells from re-entering G1 by blocking mitosis with nocodazole,

MPA plus FGF accelerated entry into S phase compared with FGF alone (36).

Stromal cells cultured with MPA plus FGF showed increased ($p < 0.01$) cyclin D1 mRNA at 9, 12 and 15 h. Addition of RU 486 to cells stimulated with these agents for 9 h reduced cyclin D1 mRNA by 40%. Western blot analysis of cyclin D1 immunoprecipitates indicated complex formation with both Cdk4 and Cdk6. Cyclin D1-Cdk complexes, and kinase activity as measured by the phosphorylation of pRb, correlated temporally with increased cyclin D1 expression in cells cultured with both MPA plus FGF. The results from this study indicate that progesterone-FGF interactions increase cyclin D1 expression in uterine stromal cells, correlating with accelerated stromal cell entry into S phase compared with cells treated with FGF alone. Most importantly, progesterone plus FGF sustains the timing of stimulation for transit of uterine stromal cells through G1 into S phase compared with FGF alone (Figure 2).

5. PROGESTERONE AND STROMAL CELL DIFFERENTIATION (DECIDUALIZATION)

Labeling of stromal cells *in vivo* results in the appearance of ^{3}H thymidine in decidual cells without positional restriction (26) conclusively demonstrating that the stromal cells are the progenitors of decidual cells. The differentiation of uterine stromal cells involves a distinct spatial and temporal sequence in vivo since stromal cells in the anti-mesometrial region of the endometrium differentiate initially followed by those cells located in the periluminal stroma. As differentiation proceeds there are distinctions in gene expression between decidual cells from the mesometrial and anti-mesometrial regions (65). However, when uterine stromal cells are cultured, expression of those differential markers are lost suggesting that there are positional effects exerted on decidual (stromal) cells *in vivo* that are not maintained in culture (65). We suggest that continued proliferation of stromal cells in culture (34-36), accompanied by the loss of differentiated gene expression (65), is consistent with the idea that decidual cells dedifferentiate *in vitro*. These stromal cell lines therefore provide an excellent model system for dissecting the role of sex hormones not only on cell proliferation but also the mechanisms involved in cellular differentiation.

5.1. Cell Cycle Exit

Cellular differentiation involves exit from the cell cycle followed by changes in gene expression resulting in specialized cell function. Central to the differentiation cascade in uterine stromal cells is the process of endoduplication which involves the uncoupling of DNA replication from mitosis. Decidual nuclei are polyploid as the chromosomes fail to separate at metaphase and increased ploidy from 4 to 32 n is reported (66). While endomitosis is not common in mammalian cells, it occurs in trophoblast cells (67,68) and in bone marrow megakaryocytes during terminal differentiation (69,70). The available evidence indicates that control of the uncoupling process may be cell-type dependent and can involve cyclin E-Cdk (68,69), cyclin A-Cdk (69) and MPF (70). The available evidence thus suggests that changes in cell cycle regulators, particularly during G1 phase of the cell cycle, are necessary in order for cellular differentiation to occur.

5.2. The Differentiation Pathway

Of the several proteins that have been proposed as markers of decidualization, decidual/trophoblast prolactin related protein (d/t PRP) seems particularly suitable as a differentiation marker. While d/t PRP expression is not unique to decidual cells, the protein is not expressed in stromal cells (71). Furthermore, d/tPRP transcripts cannot be detected using RT-PCR in uterine stromal cell lines (71) including those isolated and characterized in our laboratory (34). We have obtained preliminary evidence, however, that d/t PRP expression can be stimulated in these same uterine stromal cell lines (72) by agents that are associated with decidualization *in vivo* (73-79). Prostaglandins have been implicated in the process of implantation because decidualization is considered to be analogous to a proinflammatory response (73-75). Cyclooxygenase (Cox) is the rate-limiting enzyme in the biosynthesis of prostaglandins. Mice lacking COX-2 by targeted mutagenesis cannot undergo a decidual cell reaction (74,75). Intraluminal instillation of cholera toxin partially restored the decidual response in COX-2 null mice (74). Subsequent experiments (75) suggested that the endogenous ligand for the decidual response is prostacyclin (PGI2) that stimulates early decidual events after binding to peroxisome proliferator-activator receptors (PPARs). While the endogenous prostaglandin that promotes decidualization in rats has not been identified, in hormonally primed rats, cholera toxin induces decidualization by stimulating COX-2 expression and perhaps by increasing the levels of cyclic AMP. Interleukin-11(IL-11) is a cytokine that has a spectrum of different functions in various cell types (76). Mice lacking the IL-11 receptor alpha (IL-11R∀) are infertile because the uterine stroma does not undergo decidualization (77,78). Temporal analysis of IL-11 expression revealed maximal expression at the time of decidualization in both mice and rats (79). IL-11 and IL-11R∀ transcripts were present in the developing decidual cells as shown by *in situ* hybridization. Together, these results provide strong evidence for cholera toxin (prostaglandins) and IL-11 as two candidate regulators of stromal cell differentiation (decidualization).

5.3. Molecular Mechanisms

To explore this possibility further, we reasoned that if cholera toxin and IL-11 are involved in stromal cell differentiation then we would expect increased expression of d/tPRP in cells treated with these two agents since d/tPRP is a cell-specific differentiation product. To test this proposal, the d/tPRP-luc construct was transiently transfected into uterine stromal cells using a liposome-mediated delivery system. Cells (1×10^{5}) were plated in 35 mm-culture dishes and transfected with 2 Φg d/tPRP-luc, RSV-luc (positive control) or pGL-2 basic vector (negative control). RSV-∃-GAL (0.5 Φg) was co-transfected with all constructs and used to correct for transfection efficiency. The results shown in Figure 3 indicate that the two

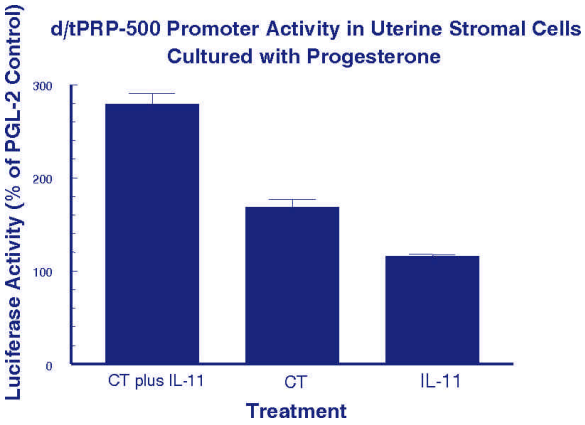

Figure 3. Cholera toxin (CT) and interleukin-11 (IL-11) activate the d/tPRP promoter. Rat uterine stromal cells were transfected with the d/tPRP promoter ligated to a luciferase reporter gene as described in detail elsewhere (72). Transfected cells were cultured in low-serum medium containing progesterone (1 micromolar). Agents (CT, 10 ng/ml) and IL-11 (10 ng/ml), reported to promote decidualization in vivo, activated the d/tPRP promoter, a marker of differentiated stromal cells. Data are mean differences +/- SEM from triplicate samples and are representative of three independent assays. $P < 0.05$ compared to the PGL-2 control, Mann Whitney U test.

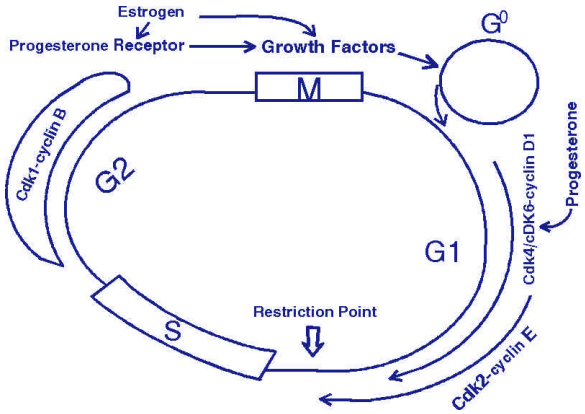

Figure 4. Progesterone controls the proliferation of rat uterine stromal cells by regulating cell cycle transit. Estrogen action *in vivo* is necessary to upregulate PR (34,80) and perhaps growth factors and/or their receptors (34). Progesterone plus FGF increase cyclin D1 expression which correlates temporally with cyclin D1-Cdk complex formation and activation. Stromal cell entry into S phase is accelerated by progesterone and the hormone sustains the timing of stimulation through G1 into S phase. We postulate that this sustainment provides the "window of opportunity" for cells to exit the cell cycle and if exposed to additional agents, such as interleukin-11 and cholera toxin (prostaglandins), enter into the differentiation pathway.

candidate agents (cholera toxin, IL-11), that have been shown to stimulate decidualization in vivo (73-79), activate the d/tPRP promoter in vitro. The regulation of key steps involved in the differentiation of uterine stromal cells *in vivo*, using cultured endocrine-dependent stromal cells as we show here, provides an important and unique opportunity to tease out the signal transduction pathways involved in cellular differentiation. Moreover, it may be possible using this system to delineate the process of differentiation of these cells in the context of cell cycle regulation and the unique and important role for sex steroids in this process.

6. PERSPECTIVES

The mammalian uterus is a complex tissue comprised of different cell types and an abundance of extracellular matrix proteins that undergo cyclical changes (61). Cell-cell interactions between heterologous cell types (80,81) cannot be studied in isolated stromal cells. However, signal transduction mechanisms cannot be readily studied *in vivo*. Therefore, we have used a multifaceted approach to understand the action of progesterone on cell proliferation and differentiation in the mammalian endometrium. While such an approach may not reflect all of the complex cell-cell, cell-extracellular matrix interactions, our data suggest that a well characterized stromal cell culture system does provide a valid model to study the molecular mechanisms invovled in steroid action that regulate uterine stromal cell proliferation and differentiation. Isolated rat uterine stromal cells express both ER and PR. Moreover, the control of proliferation by progesterone is consistent with the pattern of regulation of stromal cell proliferation *in vivo*. Teasing out the molecular mechanisms underlying progesterone action has lead to a novel hypothesis currently being investigated in our laboratory (Figure 4). We propose that progesterone, acting through the PR, helps to establish a G1 phase from which differentiation can occur. This hypothesis is consistent with our data showing a sustainment in the transit of stromal cells through G1 into S phase only in the presence of progesterone. We postulate that progesterone-dependent alterations in key cell cycle regulators permit cells to exit the proliferative cycle, and if exposed to the appropriate agents, enter into the differentiation pathway. Testing of this hypothesis will provide important new insight about the mechanism of progesterone action in target cells and increase our knowledge of the molecular mechanisms underlying mammalian reproduction and pregnancy.

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8. REFERENCES

1. Clarke C.L, & R.L. Sutherland: Progestin regulation of cellular proliferation. *Endocr Rev* 11, 266-301 (1990)

2. Lyndon J.P, F.J. DeMayo, C.R. Funk, S.K. Mani, A.R. Hughes, C.A. Montgomery Jr., G. Shyamala, O.M..Conneely & B.W. O'Malley: Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes & Dev* 9, 2266-2278 (1995)

3. D.W. Bullock, D.T. Lamb, V.C. Rider, & P.A. Kima: The rabbit progesterone receptor and uteroglobin gene expression. In: Cell Biology of the Uterus: Eds: W.W Levitt, K. Yoshinaga, Plenum Press, NY, 79-97 (1987)

4. Kraus W.L, & B.S. Katzenellenbogen: Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen action by progesterone and sex steroid hormone antagonists. *Endocrinology* 132, 2371-2379 (1993)

5. A Murray & T Hunt: The Cell Cycle: An Introduction. Oxford University Press, Oxford (1993)

6. Pardee A.B: G1 events and regulation of cell proliferation. *Science* 246, 603-608 (1989)

7. Murray A.W & M.W. Kirschner: Cyclin synthesis drives the early embryonic cell cycle. *Nature* 339, 275-280 (1989)

8. Dynlacht B.D: Regulation of transcription by proteins that control the cell cycle. *Nature* 389, 149-152 (1997)

9. Schwob E, T. Bohm, M.D. Mendenhall & K. Nasmyth: The B-type cyclin kinase inhibitor $p40^{SIC1}$ controls the G1 to S transition in S. cerevisiae. *Cell* 79, 233-244 (1994)

10. Bai C, P. Sen, K. Hofmann, L. Ma, M. Goebl, J.W. Harper & S. J. Elledge: SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 91, 263-274 (1996)

11. Masui Y, & C.L. Markert: Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *Exp Zool* 177, 129-145 (1971)

12. Dunphy W. G, L. Brizuela, D. Beach, & J. Newport: The Xenopus cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* 54, 423-431 (1988)

13. Evans T, E.T. Rosenthal, J.Youngblom, D. Distel & T. Hunt: Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* 389, 149-152 (1983)

14. Morgan D.O: Cyclin-dependent kinases: engines, clock, and microprocessor. *Annu Rev Cell Dev Biol* 13, 261-291 (1997)

15. Serrano M, G.J. Hannon, & D.Beach: A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366, 704-707 (1993)

16. Sherr, C.J, & J.M. Roberts: CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13, 1501-1512 (1999)

17. Harper J.W, N. Adami, N. Wei, K. Keyomarsi, S.J. Elledge: The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75, 805-816 (1993)

18. Toyoshima H & T. Hunter: p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell* 78, 67-74 (1994)

19. Musgrove E.A, C.S.L. Lee, A.L. Cornish, A. Swarbrick & R.L. Sutherland: Antiprogestin inhibition of cell cycle progression in T-47D breast cancer cells is accompanied by induction of the cyclin-dependent kinase inhibitor p21. *Mol Endocrinol* 11, 54-66 (1997)

20. Groshong S.D, G.I. Owen, B. Grimison, I.E. Schauer, M.A. Todd, T.A. Langan, R.A. Sclafani, C.A. Lange, & K.B. Horwitz: Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and $p27^{Kip1}$. *Mol Endocrinol* 11, 1593-1607 (1997)

21. Yan Y, J. Frisen, M.H. Lee, J. Massague, & M. Barbacid: Ablation of the CDK inhibitor $p57^{KIP2}$ results in increased apoptosis and delayed differentiation during mouse development. *Genes & Dev* 11, 973-983 (1997)

22. Zhang P, N.J. Liegeois, C.Wong, M. Finegold, H. Hou, J.C. Thompson, A. Silverman, J. W. Haper, R.A. Depinho, & S.J. Elledge: Altered cell differentiation and proliferation in mice lacking p57 ^{KIP2} indicates a role in Beckwith-Wiedemann syndrome. *Nature* 387, 151-158 (1997)

23. Finn C.A, & L. Martin: Patterns of cell division in the mouse uterus during early pregnancy. *J Endocrinol* 39, 593-597 (1967)

24. Rider V, & A. Psychoyos: Inhibition of progesterone receptor function results in loss of basic fibroblast growth factor expression and stromal cell proliferation during uterine remodelling in the pregnant rat. *J Endocrinol* 140, 39-249 (1994)

25. Martin L, & C.A. Finn: Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. *J Endocrinol* 41, 363-371 (1968)

26. Galassi L: Autoradiograph study of the decidual cell reaction in the rat. *Dev Biol* 17,75-84 (1968)

27. Finn C.A, & L. Martin: Hormone secretion during early pregnancy in the mouse *J Endocrinol* 45, 57-66 (1969)

28. C.A Finn: The biology of decidual cells. *Adv Reprod Physiol* 5, 1-26 (1971)

29. Noyes R.W, A.T. Hertig & J. Rock: Dating the endometrial biopsy. *Fertil Steril* 1, 3-25 (1950)

30. Rider V, M-Y Wang, C. Finn, R.B. Heap & A. Feinstein: Anti-fertility effect of passive immunization against progesterone is influenced by genotype. *J Endocrinol* 108, 117-121(1986)

31. Cullingford R.W, & J.W. Pollard: RU 486 completely inhibits the action of progesterone on cell proliferation in the mouse uterus. *J Reprod Fert* 83, 909-914 (1988)

32. Irwin J.C, W.H. Utian, & R.L. Eckert: Sex steroids and growth factors differentially regulate the growth and differentiation of cultured human endometrial stromal cells. *Endocrinology* 129, 2385-92 (1991)

33. Cohen H, HF Pageaux, C. Melinand, JM Fayard, & C. Laugier: Normal rat uterine stromal cells in continuous culture:characterization and progestin regulation of growth. *Eur J Cell Biol* 61, 116-125 (1993)

34. Piva M, O. Flieger & V. Rider: Growth factor control of cultured rat uterine stromal cell proliferation is progesterone dependent. *Biol Reprod* 55, 1333-1342 (1996)

35. Rider V, B.F. Kimler & W.M. Justice: Progesterone-growth factor interactions in uterine stromal cells. *Biol Reprod* 59, 464-469 (1998)

36. Jones S.R, B.F Kimler, W.M Justice & V. Rider: Transit of normal rat uterine stromal cells through G1 phase of the cell cycle requires progesterone-growth factor interactions. *Endocrinology* 141, 637-648 (2000)

37. Murphy A.A, M.H. Zhou, S. Malkapuram, N. Santanam, S. Parthasarathy & N. Sidell: RU486-induced growth inhibition of human endometrial cells. *Fertil Steril* 74, 1014-1019 (2000)

38. Prange-Kiel J, G.M. Rune, D. Wallwiener & L. Kiesel: Inhibition of proliferation and differentiation by RU 486 in human endometrial stromal and epithelial cells in vitro. *Exp Clin Endocrinol Diabetes* 108, 275-281 (2000)

39. Carson-Jurica M, W.T. Schrader & B.W. O=Malley: Steroid receptor family: structure and function. *Endocr Rev* 11, 201-220 (1990)

40. Dean D.M, & M.M. Sanders: Ten year after: reclassification of steroid-responsive genes. *Mol Endocrinol* 10, 1489-1495 (1996)

41. Musgrove E.A, C.S.L. Lee & R.L. Sutherland: Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of TGFalpha, epidermal growth factor receptor, c-fos and c-myc genes. *Mol Cell Biol* 11, 5032-5043 (1991)

42. Musgrove E.A, & R.L. Sutherland: Cell cycle control by steroid hormones. *Cancer Biol* 5, 381-389 (1994)

43. Musgrove E.A, C.S.L. Lee, M.F. Buckly & R.L. Sutherland: Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. *Proc Natl Acad Sci USA* 91, 8022-8026 (1994)

44. Moore M.R, J-L Zhou, K.A. Blanenship, J.S. Strobl, D.P. Edwards, R.N. Gentry: A sequence in the 5' flanking region confers progesterone responsiveness on the human c-myc gene. *J Steroid Biochem Mol Biol* 62, 243-252 (1997)

45. Frank-Vaillant M, O. Haccard, R. Ozon & C. Jessus: Interplay between Cdc2 kinase and the c-Mos/MAPK pathway between metaphase I and metaphase II in Xenopus oocytes. *Dev Biol* 231, 279-288 (2001)

46. Karaiskaou A, A. Dupre, O. Haccard & C. Jessus: From progesterone to active Cdc2 in Xenopus oocytes: a puzzling signalling pathway. *Biol Cell* 93, 35-46 (2001)

47. Musgrove E.A, A. Swarbrick, C.S.L. Lee, A.L. Cornish, & R.L. Sutherland: Mechanisms of cyclin-dependent kinase inactivation by progestins. *Mol Cell Biol* 18, 1812-1825 (1998)

48. La Baer J, M.D. Garrett, L.F. Stevenson, J.M. Slingerland, C. Sandhu, H.S. Chou, A. Fattaey, & E. Harlow: New functional activities for the p21 family of CDK inhibitors. *Genes & Dev* 11, 847-862 (1997)

49. Vu Hai M.T, F. Lagged & E. Milgrom: Progesterone receptors in the rat uterus: variations in cytosol and nuclei during the oestrous cycle and pregnancy. *J Endocrinol* 76, 43-48 (1978)

50. Finn C.A & L. Martin: The role of oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *J Endocrinol* 47, 431-438 (1970)

51. Martin L, R.M. Das & C.A. Finn: The inhibition by progesterone of uterine epithelial proliferation in the mouse. *J Endocrinol* 57, 549-554 (1973)

52. Tong W, & J.W. Pollard: Progesterone inhibits estrogen-induced cyclin D1 and cdk4 nuclear translocation, cyclin E- and cyclin A-cdk2 kinase activation, and cell proliferation in uterine epithelial cells in mice. *Mol Cell Biol* 19, 2251-2264 (1999)

53. Altucci L, R. Addeo, L. Cicatiello, D. Germano, C. Pacilio, T. Battista, M. Cancemi, V.B. Petrizzi, F. Bresciani, & A. Weisz: Estrogen induces early and timed activation of cyclin-dependent kinases 4, 5, and 6 and increases cyclin messenger ribonucleic acid expression in rat uterus. *Endocrinology* 138, 978-984 (1997)

54. Zhuang Y.H, D. Sarca, A. Weisz, L. Altucci, L. Cicatiello, E. Rollerova, P. Tuohimaa, & T. Ylikomi: Cell type-specific induction of cyclin D and cyclin-dependent kinase inhibitor p27 (kip1) expression by estrogen in rat endometrium. *J Steroid Biochem Mol Biol* 78, 93-199 (2001)

55. Shiozawa T, S.F. Li, K. Nakayama, T. Nikaido & S. Fujii: Relationship between the expression of cyclin/cyclin-dependent kinases and sex-steroid receptors/Ki67 in normal human endometrial glands and stroma during the menstrual cycle. *Mol Hum Reprod* 2, 745-752 (1996)

56. V Rider & SR Jones: Maternal preparation for implantation: molecular mechanisms of progesterone action. In: Cloned animal and placentation. Eds. R.M. Roberts, R Yanagimachi, T. Kariya and K. Hashizume, Yokena Ltd, Tokyo, Japan (2000)

57. Das S.K, H. Lim, B.C. Paria & S.K. Dey: Cyclin D3 in the mouse uterus is associated with the decidualization process during early pregnancy. *J Mol Endocrinol* 22:91-101 (1999)

58. Carlone D.L, & V. Rider: Embryonic modulation of basic fibroblast growth factor in the rat uterus. *Biol Reprod* 49, 653-665 (1993)

59. Rider V, M. Piva, M.E. Cohen & D.L. Carlone: Alternative splicing and differential targeting of fibroblast growth factor receptor 1 in the pregnant rat uterus. *Endocrinology* 136, 3137-3145 (1995)

60. Rider V, D.L. Carlone & R.T. Foster: Oestrogen and progesterone control basic fibroblast growth factor messenger RNA in the rat uterus. *J Endocrinol* 154, 75-84 (1997)

61. Rider V, D.L. Carlone, D. Witrock, C. Cai, & N. Oliver: Uterine fibronectin content and localization are modulated during implantation. *Dev Dynamics* 195, 1-14 (1992)

62. V Rider: Molecular mechanisms regulating uterine decidualization and implantation: cell-cell and cell-extracellular matrix interactions. In: Embryo implantation: molecular, cellular and clinical aspects. Ed. D Carson, Springer Verlag, NY, 271-286 (1999)

63. V Rider & M. Piva: Role of growth factors of uterine and fetal-placental origin during pregnancy. In: Endocrinology of pregnancy, chapter 4, Ed: F Bazer, The Humana Press, NJ, 83-124 (1998)

64. Arslan A, A. Guillermina & H.H. Zingg: Characterization and co-culture of novel nontransformed cell lines derived from rat endometrial epithelium and stroma. *In Vitro Cell Dev Biol* 31, 140-148 (1995)

65. Gu Y, & G. Gibori: Isolation, culture and characterization of the two cell subpopulations forming the rat decidua: differential gene expression for activin, follistatin and decidual-related prolactin protein. *Endocrinology* 136, 2451-2458 (1995)

66. McConnell K.N, R.G. Sillar, B.D. Young, & B.Green: Ploidy and progesterone-receptor distribution in flow-sorted deciduomal nuclei. *Mol Cell Endocrinol* 25, 99-104 (1982)

67. Ilgren E.B: Control of trophoblastic growth. *Placenta* 4, 307-328 (1982)

68. MacAuley A, J.C.Cross & Z.Werb: Reprogramming the cell cycle for endoreduplication in rodent trophoblast cells. *Mol Biol Cell* 9, 795-807 (1998)

69. Garcia P, & C. Cales: Endoreplication in megakaryoblastic cell lines is accompanied by sustained expression of G1/S cyclins and downregulation of cdc25C. *Oncogene* 13, 695-703 (1996)

70. Datta N.S, J.L. Williams, J. Caldwell, A.M. Curry, E.K. Ashcraft & M.W. Long: Novel alterations in CDK1/Cyclin B1 kinase complex formation occur during the acquisition of a polyploid DNA content. *Mol Biol Cell* 7:209-223 (1996)

71. Orwig K.E, G. Dai, C.A. Rasmussen & M.J.Soares: Decidual/trophoblast prolactin-related protein: characterization of gene structure and cell-specific expression. *Endocrinology* 138, 2491-2500 (1997)

72. Rider V,G. Dai & M.J. Soares: Regulation of rat uterine stromal cell differentiation. *Biol Reprod* 64, Suppl 1, 330 (2001)

73. Kennedy T.G: Evidences for the involvement of prostaglandins throughout the decidual cell reaction in the rat . *Biol Reprod* 33,140-146 (1985)

74. Lim H, B.C. Paria, S.K. Das, J.E. Dinchuk, R. Langenbach, J.M. Trzaskos & S.K. Dey: Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91, 197-208 (1997)

75. Lim H, R.A. Gupta, W-G Ma, B.C. Paria, D.E. Moller, J.D. Morrow, R.N. DuBois, J.M. Trzaskos & S.K. Dey: Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta. *Genes & Dev* 13, 1561-1574 (1999)

76. Du X, & D.A. Williams: Update on development of interleukin-11. *Curr Opin Hematol* 2, 182-188 (1995)

77. Robb L, R. Li, L. Hartley, H.H. Nandurkar, F. Koentgen & C.G. Begley: Infertility in female mice lacking the receptor for interleukin11 is due to a defective uterine response to implantation. *Nat Med* 4, 303-308 (1998)

78. Bilinski P, D. Roopenion & A. Gossler: Maternal IL-11R alpha function is required for normal decidua and fetoplacental development in mice. *Genes & Dev* 12, 2234-2243 (1998)

79. Li R, L. Hartley & L. Robb: Cloning of rat interleukin 11 and interleukin 11 receptor alpha chain and analysis of their expression in rat uterus in the peri-implantation period. *Reproduction* 122, 593-600 (2001)

80. Cooke P. S, D.L. Buchanan, P. Young, T. Setiawan, J. Brody, K.S. Korach, J. Taylor, D.B. Lubahn & G.R. Cunha: Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. *Proc Natl Acad Sci USA* 94, 6535-6540 (1997)

81. Kurita T, K. Lee, P. T. Saunders, P.S. Cooke, J.A. Taylor, D.B. Lubahn C. Zhao, S. Makela, J.A. Gustafsson, R. Dahiya & G.R. Cunha: Regulation of progesterone

receptors and decidualization in uterine stroma of the estrogen receptor-alpha knockout mouse. *Biol Reprod* 64, 272-283 (2001)

82. Rider V, S.R. Jones, M. Evans & N.I. Abdou: Molecular mechanisms invovled in the estrogen-dependent regulation of calcineurin in systemic lupus erythematosus. *Clin Immunol* 95, 126-134 (2000)

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