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PROGESTERONE RECEPTORS: VARIOUS FORMS AND FUNCTIONS IN REPRODUCTIVE TISSUES

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1. ABSTRACT

The unequivocal role of progesterone in a variety of events like ovulation, mammary gland development, establishment and maintenance of pregnancy etc are well established. Also the data are accumulating on its role in male reproductive events. In vertebrates and humans, the biological activity of progesterone is mediated by two progesterone receptor proteins PR-A and PR-B, that arise from the same gene and are the members of nuclear receptor superfamily of transcriptional factors. Several studies have demonstrated that the blockage of progesterone receptor using antiprogestins impairs folliculogenesis, ovulation, implantation and pregnancy. Progesterone receptor (PR), have also been detected in human spermatozoa. However, unlike the conventional PR, sperm PR was localized on the membrane and showed distinct characteristics in terms of its size. There are data to demonstrate the inhibition of progesterone driven functions such as hyperactive motility, acrosome reaction on neutralization of sperm membrane PR with specific antibodies against PR. Further significant decrease in the % of PR positive spermatozoa was observed in infertile cases as compared to the fertile men. This indicated that PR can serve as the marker to define the fertilizing potential of the spermatozoa. Recently we have also shown that the PR is expressed in human testis. This reinforced that this PR protein is an inherent testicular protein and not a secretion of accessory reproductive organs. This review compiles the major observations on the forms of the progesterone receptor in various reproductive tissues.

2. INTRODUCTION

Progesterone, an ovarian steroid plays a major role in regulating female reproductive events. These events include ovulation, luteinization, the maintenance of luteal structure and function, development of uterine and

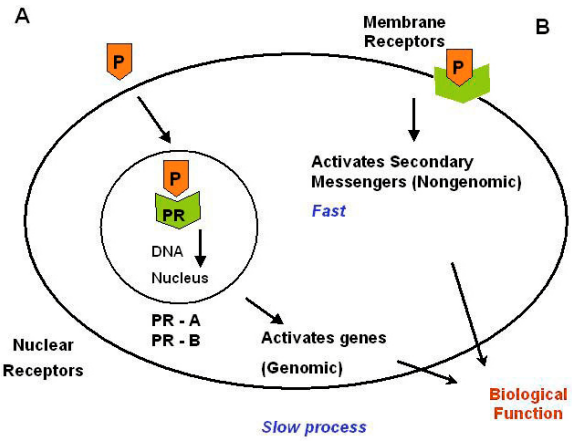

Figure 1. Mode of progesterone action by genomic (A) and nongenomic (B) pathways. P- progesterone, PR- progesterone receptor.

mammary gland, implantation, sustenance of pregnancy and neurobehavioural expression associated with sexual responsiveness (1,2).

The biological actions of progesterone are mediated via progesterone receptor (PR), a member of the nuclear/intracellular receptor superfamily of ligand dependent transcription factors (3,4). Following the binding of progesterone to its receptor, a conformational change occurs in PR, the receptor ligand complex is translocated to the nucleus where it interacts with DNA binding elements in the genome (Figure 1) and alters the transcription of progesterone responsive genes (5). In addition, rapid actions of PR that occur independent of transcription, have also been observed in several tissues like brain, liver, mammary gland and spermatozoa (6). PRs have been detected in variety of tissues but not in spleen (7).

2.1. Genomic Mode of Progesterone Action

The classical mode of progesterone action is mediated via intracellular or nuclear PR. The genomic organizations of these PR are fairly well characterized. Nuclear/genomic PR contains three functional domains, the N-terminus, the DNA-binding domain (DBD) and the C-terminal ligand-binding domain (LBD). Activation domains present within these functional domains are required for the DNA bound receptor to transmit a transcription activation response and these sites also serve as specific binding sites for co-activators. The N-terminal domain, the least conserved domain with respect to length and amino acid sequence, is required for the full transcriptional activity of the receptor and for other cell and target gene specific responses (3). This domain contains the transcription activation domains AF-1 and AF-3 that recruit co-activator proteins to the receptor and thereby regulate promoter specificity and also the transcriptional activity (8).

The DBD is highly conserved centrally located domain consisting of approximately 66-68 amino acids (8). Two type II zinc finger structures in this domain facilitate the binding of the receptor to specific cis-acting DNA structures (9). DBD also contains a nuclear localization sequence (NLS), dimerization domain (DI) and a highly conserved AF-2 domain.

The LBD is involved in binding to progesterone and heat shock proteins. It contains another determinant of dimerization, which functions in absence of DNA binding and a second NLS. Progesterone binding induces a conformational change in the receptor that creates a hydrophobic binding pocket of the p160 family of steroid receptor co-factors, which then activates AF-2 (3). Many of the nuclear receptor co-activators such as TRBP, CBP, PNRC-2, SRC-1, SRA, L7/SPA, SRC2, E6-AP and RPF-1 are known to act via the ligand-binding domain (10-12**).** However, recently a tissue specific nuclear receptor co-activator GT198 has been identified which interacts with several nuclear receptors including PR through their DNA-binding domain. GT198 is a 217 amino-acid nuclear protein that contains a leucine zipper required for its dimerization. Further, protein kinase A, protein kinase C, and mitogen-activated protein kinase can phosphorylate GT198 *in vitro*, and are found to regulate the transcriptional activity of GT198 (10). DBDs have also been known to interact with other transcriptional factors such as NF-κB and AP-1, with resultant regulation of transcriptional repression (13).

The binding of progesterone to the LBD domain induces a conformational change in the receptor, leading to its phosphorylation and dissociation from heat shock proteins and dimerization. The ligand receptor complex enters the nucleus and then binds and activates specific response elements in the promoter region of the target genes. The activation of these genes requires the interaction of steroid receptor complexes (SRCs) with the AF-2 region, resulting in the recruitment of other SRC-associated histone acetyltransferases (CBP and pCAF) and the methyltransferases, CARM1 (14-16).

Three isoforms of nuclear progesterone receptors are known, namely PR-A (94 kDA), PR-B (116 kDa) and PR-C (60 kDa). PR-A and PR-B are translated from the same gene; however, their transcription is initiated at different promoters (17). PR-B contains additional 164 amino acids at the N-terminal domain called the B-upstream sequence (18). Loss of two regions in the B-upstream sequence i.e. amino acids 55-90 (R1) and 120-150 (R2) reduces AF3 activity by 90%. These sequences (R1 and R2) contain two LXX and LL motifs and a conserved tryptophan residue in the R2 sequence (19). PR-A and PR-B demonstrate both overlapping and distinct activities in vitro. To delineate the individual roles of PR-A and PR-B *in vivo*, mouse models have been developed in which expression of a single PR isoform has been ablated. Progesterone receptor A knockout (PRAKO) mice with ablation of the PR-A isoform shows normal mammary gland response to progesterone but abnormal uterine and ovarian function. On the other hand, ablation of the PR B isoform did not affect biological responses of the ovary or uterus to progesterone but led to altered mammary gland morphogenesis during pregnancy. It has been proposed that the individual PR isoforms regulate distinct subsets of progesterone dependent target genes in distinct tissues (20).

The ratios of PR-A and PR-B have been found to vary in reproductive tissues as a consequence of developmental and hormonal status. Both receptors exhibit similar DNA and ligand binding affinities although their activation properties differ (17). PR-A is transcriptionally less active than PR-B and is a dominant inhibitor of PR-B (22). An inhibitory domain (ID/IF) is present in the N-terminal domain of PR-A, which is responsible for recruitment of transcriptional inhibitory corepressor proteins (23). Removal of IF/ID converts PR-A into a strong transcriptional activator. ID represses AF-1 and AF-2 but not AF-3 (15). PR-A and PR-B share AF-1 and AF-2 but PR-A lacks AF-3. The presence of AF3 in PR-B allows binding of a subset of co- activators to PR-B, making it more transcriptionally active. The lower transactivation potential of PR-A may be due to its higher affinity for the co-repressor SMRT (Silencing Mediator of Retinoid and Thyroid hormone receptor) and its less efficient recruitment of the co-activator SRC-1 (24).

In cell contexts where PR-A remains transcriptionally inactive, it acts as a progesterone dependent inhibitor of estrogen receptor function (25). Antiprogestins like mifepristone and onapristone have been shown to inhibitestrogen receptor mediated gene transcription. This has been proposed as a basis of tissue specific antiestrogenic effects of antiprogestins. Interestingly, hPR-A but not hPR-B was found to be capable of glucocorticoid, androgen and mineralocorticoid receptor mediated gene transcription. Further it has been suggested that the inhibitory function of hPR-A does not require DNA binding (26).

PR-C is smaller than the other two isoforms, truncated at the N-terminal domain and known to have unique transcriptional potentiating properties although its progestin and antiprogestin autoregulation parallels PR-A and PR-B. PR-C has dissociation constant (kda) 5 times higher than PR-A and PR-B and is found to enhance the transcriptional activity of the larger PR proteins (27). It has been demonstrated that PRs can be activated in a ligand independent manner (28-29). Progesterone can stimulate cellular response without the involvement of the transcription regulatory function of PRs, either through PR mediated activation of intracellular phosphorylation cascades (30-31) or through interaction with specific membrane receptors for progesterone. Human PR, that localizes to the cytoplasm or associated with the plasma membrane has been shown to mediate rapid progesterone activation of the Src/Ras/Raf/mitogen activated protein kinase signaling pathway in mammalian cells by a direct interaction with the Src homology 3 domain of Src tyrosine kinase through a motif located in the N terminal domain of the receptor. This extranuclear action of PR is distinct from its transcriptional activity (32). A novel membrane protein that shows characteristics of a G protein coupled receptor for progesterone, has been identified and shown to be involved in mediating the extranuclear signaling actions of progesterone to promote oocyte maturation in fish. The role of this membrane PR (mPR) in mammalian cells is not very clear (33).

In the proliferative phase human endometrium, PR was found to be distributed evenly and both PR isoforms were colocated. In the secretary phase human endometrium, there was a marked increase in the proportion of nuclei containing PR distributed into discrete foci and PR-B was found to be predominant isoform in nuclear foci. An inverse relationship was observed between even and focal PR distribution in the menstrual cycle, suggesting the hormonal regulation of PR movement into focal nuclear locations (21).

2.2. Non-genomic Mode of Progesterone Action

Several investigations in different cell systems support either the existence of the novel membrane receptors encoded by the genes different than the genomic ones or the relocation of the classical receptors to the inner side of the membrane. Membrane bound progesterone receptors are also being localized on human aortic endothelial cells (34), hepatocytes (35), brain cells (36), mammary glands (37), ovary (38), granulosa cells (39) and spermatozoa (40-41). Progesterone is responsible for the resumption of meiosis in amphibian oocyte leading to an increase in intracellular calcium (42, 43). In mammals, nongenomic progesterone actions are shown to localize to regulate relaxation of intestinal (44) and uterine muscle (45). Intracellular PR have been demonstrated to the inner side of the plasma membrane by interaction with Src Kinase in breast cancer cells and upon binding to SH2 domain, PR directly activates the Src/ERK signal transduction pathway leading to proliferation in breast cancer cells (46). However, till date, clear characterization and sequencing of the putative membrane receptors mediating the rapid nongenomic effects of progesterone are still unclear.

Spermatozoa represent a very good model for studying rapid effects of progesterone. The sperm DNA is highly packed and inaccessible for transcription and these cells lack ribosomes and translational machinery (47). Several rapid nongenomic effects of progesterone have been shown in human spermatozoa. For example, progesterone has been shown to stimulate calcium influx, tyrosine phosphorylation of proteins, including extracellular signaling regulated kinases, chloride efflux and increase in cAMP (Figure 1), finally leading to activation of pivotal processes such as capacitation, hyperactivated motility and acrosome reaction (6). Conversely, estradiol, acting rapidly on calcium influx and on protein tyrosine phosphorylation, seems to negatively modulate sperm responsiveness to progesterone by inhibiting the plateau phase of calcium influx and acrosome reaction induced by progesterone (48, 49).

3. PROGESTERONE RECEPTOR IN FEMALE REPRODUCTIVE TRACT

PRs have been detected in the hypothalamic neurons and LH secreting cells of the pituitary (50-52). These PR in the hypothalamus, however are not regulated by progesterone (53). Progesterone is known to exert its inhibitory effect on GnRH secretion and control of

gonadotropin release through PR (54). The gonodotrophin secreting cells of the human pituitary are known to express PR. Progesterone regulation of gonadotropin release in the pituitary is mediated via GnRH1 (the classical form of GnRH) via PR-B (55). A short exposure to physiological levels of progesterone stimulated LH secretion by the pituitary in women with functional hypothalamic amenorrhoea (56). PR status can also reliably predict the recurrence of meningiomas and benign brain tumors (57).

The protein 25-DX, the rat homolog of the membrane associated progesterone binding protein has been identified in the ventromedial hypothalamus and its expression has been shown to be repressed by progesterone. 25-DX expression was found to be higher in the hypothalamus of female PRKO mice than in their wild type littermates. There findings suggest a mechanism in which the action of nuclear progesterone receptor suppresses expression of membrane progesterone receptor 25-DX during lordosis facilitation (58).

3.1. Mammary Gland

Progesterone receptors play a critical role in mammary gland development during and after the attainment of puberty. PRs are exclusively expressed by the epithelium throughout postpubertal mammary gland development (8, 59). The expression pattern of PR in mammary gland changes from a uniform to a non-uniform pattern during development from juvenile to adult state, becoming localized to a subset of epithelial cells scattered throughout the adult ductal epithelium. The change in PR pattern is regulated by the bZIP transcription factor CEBP and is required for elaboration of proliferative responses to progesterone. However, PR-positive cells are segregated from proliferating cells in both developmental stages. The PR-positive non-proliferating cells are assumed to induce the expression of proliferative signal that promotes proliferation of neighboring PR-negative cells in a paracrine manner (8).

Both estrogen and progesterone are critical to maintain postnatal development plasticity of the mammary gland. In the mammary gland of virgin mouse, PR-A as well as PR-B are expressed (60) and during pregnancy there is increase in the expression of PR-A as compared to the PR-B protein by at least 2:1 ratio (61). Most of the information on the role of PR in breast development and changes during pregnancy has been obtained from studies in PR-A Knockout (PRAKO) and PR-B Knockout (PRBKO) Mice.

The impaired development of the pregnancy associated side branching of the ductual epithelium, alveolar differentiation has been observed in PRKO mice. This indicates that the PRs are specifically required for pregnancy associated ductual proliferation and lobuloalveolar differentiation of the mammary epithelium. It has been investigated that when PRKO cells are placed in proximity with PR positive cells or wild type epithelial cells, branching and differentiation defects can be overcome. This is suggestive of the paracrine signaling of progesterone mediator (62). Recently it had been shown that a wnt-4, a glycoprotein is regulated by progesterone and essential for the ductual branching via paracrine regulation of proliferation (63).

In PRKO mice, ablation of PR-A did not affect the ability of PR-B to elicit normal progesterone responsiveness in the mammary gland. Mammary epithelium proliferation, ductual branching and lobular alveolar development in these PRAKO mice were similar to that of wild type mice (64). This indicates that progesterone acts via PRB isoform to elicit the normal proliferation and differentiation of the mammary epithelium. Further, pregnancy associated ductual side branching and lobulo-alveolar development was found to be reduced in PRBKO mice.

PRs are considered to be good prognostic markers of breast cancers irrespective of the patient's progestational status (65). PR-A and PR-B are found in equimolar amounts in normal breast but their ratios are altered in advanced disease. Patients having PR-A rich tumors have much faster disease recurrence than those with PR-B specific tumors and have poor disease free survival rates (65, 66). In breast cancer, PR-A and PR-B are also regulated by estradiol (67). Ablation of PR expression in these mice also resulted in a significantly reduced incidence of mammary tumor growth in response to carcinogen challenge (68). This demonstrates a crucial role of PRs as obligate mediators of the intracellular signaling pathways for the initiation of mammary tumors induced by the carcinogen.

Various truncated forms of PR are known to be associated with breast cancer. A significant proportion of breast cancers contain a truncated progesterone receptor (78 kDa). This smaller protein lacks the N-terminal domain. Further, this protein has ligand binding properties. However its role in breast cancer needs to be deciphered (69). Another truncated form of PR of approximately 38 kDa (PR-M) has also been reported in human aortic endothelial cells and T47D breast cancer cells, which contains the signal peptide but lacks the DBD. However, its function is still unknown (70).

3.2. Fallopian Tube

The fallopian tube responds to progesterone produced by the corpus luteum and cumulus oophorus of the fertilized oocyte after ovulation. This responsiveness is effected by PR present on the stromal and epithelial cells of human fallopian tube (71). These PRs undergo cyclic changes during various phases of the menstrual cycle (72, 73). The expressions of PR-A and PR-B in the human fallopian tube were found to be higher in follicular and early luteal phases of the menstrual cycle and almost undetectable in menopausal women (74). PR expression completely declined in the late luteal phase (72). Epithelial PR staining in various regions of the fallopian tube did not differ, however, stromal PR expression was found significantly less in the ampullar than in the isthmic region (71). In women with ectopic pregnancy, PR expression could not be detected in the glandular epithelium or the stroma of the fallopian tube. This absence of PR expression was postulated to be correlated with poor decidualization

and also with the failure of treatment of ectopic pregnancy with antiprogestin RU486 (75). Cytosolic PR expression was increased in fallopian tubes of females with proximal endometriotic lesions as compared to normal individuals (76) and decreased significantly in women with hydrosalpinx (77). Thus, PR appears to play an important role in the various fallopian tube related infertilities.

3.3 Ovary

Human ovary synthesizes all the three isoforms of PR- PR-A, PR-B and PR-C. In macaque luteal tissue, PR-A levels decreased over the course of the luteal phase while PR-B levels were unchanged from early to very late luteal phase (7). PRs have been detected in the granulosa and thecal cells of the preantral and antral follicles of human ovaries (78). Primordial and preantral follicles in human ovary lack PR. Prior to the LH surge, the granulosa cells of the antral follicles lack PR but start expressing PR with the onset of LH surge. Granulosa cells of the non-dominant cells, however lack PR. The theca interna and the surrounding stromal cells express PR throughout the menstrual cycle (79). PR were found to be absent in pre-ovulatory follicles and they appear in peri-ovulatory follicles after exposure to gonadotropin surge in rhesus monkeys (80, 81) indicating that PR expression is induced by LH (82). Stimulation of immature PRAKO mice with gonadotropins indicated that superovulation is severely impaired in these mice as compaired to their wild type (WT) counterparts. However in PRBKO mice, superovulation was unaffected. These studies indicate that ovulation is induced by progesterone through PR-A.

Corpora lutea, the main site for progesterone synthesis, in monkeys and humans are also known to express PR (83, 84). PRs are expressed by the steroidogenic cells, the granulosa cells, luteal cells and the stromal fibroblast in the corpus luteum of the human ovary (84). PR expression has been detected in luteinized granulosa and thecal cells of the corpus luteum from ovulation to the mid-secretory phase of the menstrual cycle in humans (85). Treatment of luteinizing Mice granulosa cells with progesterone antagonists led to decreased secretion of progesterone (82), highlighting the importance of PR in modulating progesterone synthesis.

Rapid action of progesterone mediated via Ca^{+2}, IP_3 and PLC-γ have also been observed in pig granulosa cells. A membrane bound PR (mPR) has been isolated from pig granulosa cells, which shows non-genomic mode of action. Two putative human homologues have been identified. The human mPR contains three exons separated by two introns. The 5' region lacks a typical TATA box but has high homology to a transcription initiator consensus sequence while the proximal region is GC rich with CpG island spanning the putative transcription start site. Further several upstream regulatory DNA motifs have been identified such as AF-2, NF-AT, C/EBF and Ahr/Amt (90).

Disruption in the expression of PRs has been found to lead to various ovarian dysfunctions. Ovarian PR expression was found to decrease in polycystic ovarian syndrome (91). The expression of PR was increased in preneoplastic and early neoplastic lesions of ovarian cancers (92). Polymorphisms in the hormone-binding domain of PR (PROGINS) i.e. an Alu insertion in intron G and two additional mutations in exon 4 and 5 have been linked with increased risk of ovarian cancer (93, 94); although reports to contradict such observations do exist (95). PR was found to be favorable prognosis marker for serous ovarian cancer and is associated with high degree of tumour differentiation (96). Further PR-positive tumor status can act as an independent prognostic variable for improved progression-free survival among patients showing advanced epithelial ovarian cancer (97).

3.4. Uterus

The role of progesterone in endometrial receptivity during implantation and in maintenance of pregnancy has been well understood. Progesterone, down regulates its own receptors while estradiol upregulates PR expression in the endometrium (98-100). It has been demonstrated that endometrial cancer cells that express PR-B are more invasive *in vitro* than cells that express only PR-A (101). Another study also supported this inference by demonstrating macroscopically detectable abdominal tumor outgrowth on administration of cells that express exclusively PR-B, in ovariectomized nude mice (8).

The support for the relevance of PR in regulating endometrium receptivity and pregnancy has come from the studies in PR knockout mice. Progesterone dependent regulation of calcitonin, histidine decarboxylase and amphiregulin (102-104) and lactoferrin (105) genes was found abolished in PRKO mice, leading to defective uterine implantation/ a lack of decidualization of uterine stromal cells in response to progesterone. (2). The WT embryos failed to implant when transferred into uteri of psuedopregnant PRKO females, and the mating attempts between the superovulated PRAKO females and WT males also failed to induce successful pregnancies.

The expression of PR decreases significantly in the glandular epithelium from the proliferative to the secretory phases of menstrual cycle in humans and monkeys (106,107). In the glandular epithelium of the functionalis in the human endometrium, although both isoforms are expressed in comparable amounts in the proliferative phase, PR-B is persistent in the mid-secretory phase suggesting its role in glandular secretion (108). PR-B has been implicated in the regulation of proliferation of the uterine endometrium (109). In the endometrial stroma, PR-A showed a predominant expression throughout the menstrual cycle (110).

PR-B has been shown to act as the supporter/ enhancer for the abnormal progesterone-dependent induction of epithelial cell mass proliferation in PRAKO mice. This is in contrast to its ability to inhibit estrogen-induced proliferation in the WT uterus (64). This is suggestive of the fact that the proliferative activity of progesterone is PR-B dependent and uterine expression of PR-A isoform is required to inhibit potentially adverse proliferation (21, 111).

Several reports have supported the role of PR in mediating the effects of progesterone on differentiation of endometrial stroma i.e. decidualization. During decidualization stromal cells are converted into decidualized cells in the late luteal phase of the menstrual cycle and pregnancy in humans. PR-A is the dominant form expressed in the decidualizing stroma while PR-B expression was down regulated during decidualization (reviewed in Gellerson, 2003). However the abundance of PR-A expression gradually declines with the decidualization process (112). IGFBP-1, a marker of decidualization was found to be more strongly induced by PR-A than PR-B (113).

The stroma from first trimester pregnant decidua showed predominant nuclear expression of PR, with varying intensities (114, 115). This expression remained fairly constant in the stroma and spiral arterial wall in the endometrium during pregnancy, indicating a role in decidualization and modulation of blood flow. Increased decidualization of the stroma and angiogenesis are very critical for pregnancy to provide nutrition to the developing embryo. However, the glandular epithelial cells lacked PR expression during the first trimester (116).

Alteration in PR-A/PR-B expression pattern has been found to be associated with certain endometrial dysfunctions. The presence of PR-A isoform and the absence of PR-B isoform are found to be associated with the resistance of progesterone action in endometriotic lesions (117). Cytosolic stromal PR expression was found to be down regulated in endometrium of patients with early pregnancy loss as compared to control women (118). Low expression of PR may be the cause of early pregnancy loss of unknown etiology.

In women with endometriosis, the expression of stromal PR was shown to be lesser in the ectopic endometrium as compared to eutopic endometrium while in the epithelial cells, the expression of PR was increased in the ectopic endometrium but only during the late secretory phase (119). PR-B expression was also found to be a useful prognostic marker for endometrial adenocarcinoma (109). PR expression was found higher in hyperplastic tissue as compared to normal endometrium while it decreased in hyperplastic tissue showing atypia (120).

3.5. Cervix

Functional withdrawal of progesterone is required to activate the myometrium and initiate labor in females. Progesterone receptors are thought to be involved in this process via induction of Polypyrimidine tract binding Protein-associated Splicing Factor (PSF), a co-repressor of PR (121). In fact, in women, the onset of term labor is associated with a significant increase in the myometrial PR-A/PR-B expression ratio. PR-A blocks progesterone action by inhibiting the transcriptional activity of PR-B and its expression is increased during labor (122, 123). The expressions of both these isoforms were decreased after parturition (124). A decline in the expressions of PR co-activators like cAMP-response element-binding (CREB)-binding protein and steroid receptor co activators 2 and 3 has also been observed in the fundal uterine tissue of women in labor, causing histone acetylation at term which contributes towards progesterone withdrawal induced labor (125).

Another phenomenon critical for the onset of labor is cervical softening or ripening i.e. remodeling of the dominating cervical extra cellular matrix (ECM), which is found to be under hormonal control. PRs were found to be actively associated with this process. PRs were found down regulated in the human cervix at term and during cervical ripening (126, 127). Further, PR in postmenopausal women was higher than in premenopausal women while it decreased significantly in women taking oral contraceptives (128). An association of PR expression and cervical cancer has also been observed. Increased PR expression was associated with the proliferation of cervical squamous epithelium in neoplastic cervical carcinoma (128). The predominant expression of PR-B form was found to be associated with malignant cervical cancer.

4. PROGESTERONE RECEPTOR IN MALE REPRODUCTIVE TRACT

4.1. Testis

Progesterone-binding sites have been detected in the testes of several lower species including immature rats (129), shark (130), sea trout (131) and octopus (132). However, the testicular PR in these species is membrane bound and has a different mode of action as compared to the intracellular PR (6). Although, the reproductive phenotype of male PRKO mice has not been reported in detail, mice null for steroidal receptor coactivator-1 (SRC-1; an intracellular PR co-activator) show reduced testicular growth and fertility as compared to the wild type littermates (133). Our group was the first to report existence of both intracellular and membrane-bound PR in the adult human testis (134). In this study, along with the 120 kDa and 94 kDa bands corresponding to PR-B and PR-A isoforms respectively, an additional band of 55 kDa was detected in the testis and spermatogenic cell lysates. These results suggest that three forms of PR protein are expressed in human testis, two are similar to the conventional PR isoforms and one is of the smaller size.

Based on the sequence analysis of testicular PR clones demonstrating some novel exons, PR protein of smaller size (~29 kDa) has been proposed to be present in the testis (135). However according to another report, the N terminal regions of the PR-A and PR-B isoforms are spliced out and a part of DBD and full HBD are conserved resulting in a protein of lesser molecular weight (136). Our studies demonstrating the reactivity of a 55 kDa protein with PR-B specific monoclonal antibody, it is likely that this protein retains a part of the amino terminus of the conventional PR along with the DBD.

The pattern of PR expression in human testicular sections was found to be stage specific. Immunoreactive PR was detected maximally during stages IV-VI of spermatogenesis. Although, at present the precise role of progesterone in the testis remains speculative, an increase

in PR expression after stage III hints at the possible involvement of progesterone and progesterone regulated gene products in the regulation of spermatogenesis post spermeation. Indeed, administration of supraphysiological levels of progestins to males has been shown to result in the disruption of spermiation (reviewed in 137) that is independent of gonadotropin suppression. It will be of potential interest to investigate the genomic and non-genomic mode of progesterone action in the testis.

4.2. Spermatozoa

A conjugate of fluorescein isothiocynate (FITC)-labelled bovine serum albumin (BSA) and progesterone has been used to localize PR in the sperm plasma membrane. The results of these studies show that a small percentage of spermatozoa (10-30%) binds to the hormone conjugate and the binding is marked at whole or equatorially acrosomal region of human spermatozoa (138). Immunocytolocalization studies demonstrated the expression of PR in 50% of spermatozoa at the equatorial region of sperm head (139). In contrast to these observations, the recent studies carried out in our laboratory, where the localization pattern of the PR on digitonin treated spermatozoa was investigated by three different techniques viz. immunocytochemistry, direct immunofluroscence and flow cytometry, more than 90% of spermatozoa showed the presence of PR on the acrosomal or equatorial region (140). Our data support a report where image analysis technique was performed to evaluate the number of spermatozoa responding to progesterone (141). The significance of this membrane bound PR in sperm function is evident from our studies that showed reduced number of PR positive spermatozoa from infertile men (140).

We also detected the presence of PR transcript in human spermatozoa (142). Cloning and sequencing of the amplified transcript demonstrated the presence of the DBD and a part of HBD along with the hinge region (143). As compared to conventional PR, no deletions, insertions or mutations were identified in this transcript indicating complete homology of the sperm PR transcript to the conventional PR transcripts. However, recently a study has reported a novel exon in the DBD of the testicular PR transcript (137) but our studies have failed to detect this exon by RT-PCR in the sperm or testicular PR. Interestingly, using a strategy similar to ours, Luconi et al (143) also detected the PR transcript containing the DBD in the sperm cDNA population, however as compared to placental PR, the size of the DBD differed in sperm PR (144). At present it is unclear if the transcript detected by this group contains the additional exon reported by Hirata et al (135), but other regions of the sperm PR showed homology to the conventional PR.The transcripts detected in spermatozoa may be remnants of the transcription activity during spermatogenesis (145-147). Interestingly, sperm specific transcripts have been demonstrated in fertilized zygote but not in unfertilized eggs. Therefore, it has been hypothesized that the sperm mRNA may serve as a source of paternal genome for the embryo post fertilization (148).

Our previous studies have demonstrated that these progesterone receptors are masked antigens and when treated with mild detergent like digitonin (0.1%), binding sites are exposed (149). These results were further substantiated by the flow cytometric analysis of digitonin treated and untreated spermatozoa showing binding of progesterone to more than 90% of cells after the treatment and whereas only 20% spermatozoa showed binding to progesterone, if not treated.

Several proteins of apparently different molecular masses have been implicated as sperm PR. Using an antibody directed against the C terminal of the conventional PR (C 262), a 52-kDa antigen has been found on the sperm head (150). The same antibody detected four bands of molecular masses of 28, 54, 57 and 66 kDa in the human sperm preparations (40). Interestingly, in the same study ligand blot assays detected only two bands of 54 and 57 kDa that could be displaced by unlabelled progesterone or the C 262 antibody indicating that the 54 and 57 kDa sperm membrane proteins are involved in progesterone binding, the others could be nonspecific bands or isoforms of the 54 and 57 kDa proteins. Two proteins of 70 kDa and 58.5 kDa were immunoprecipitated by antisperm antibodies that block the progesterone initiated acrosome reaction (151). Other putative human sperm PR was reported to be of 220 kDa that blocks progesterone induced Ca^{++} influx (152). Another protein is observed to be of size 94 kDa in human sperm preparations, which displayed progesterone mediated tyrosine phosphorylation (15).

Recently, a novel PR isoform (termed as S) has been reported and this isoform consists of a previously unidentified 5` sequence called exon S and the C-terminal of the classical PR.This form lacks the DNA binding domain and N terminal regions. The predicted molecular mass of this gene sequence is of 29 kDa (135). More recently, immunoprecipitation of sperm membrane proteins using an antibody against the C terminus of the conventional PR followed by 2D electrophoresis identified 2 protein bands of approximately 59 and 29 kDa. Database search of the molecular masses and the sequence of the peptides obtained after tryptic digestion of the spots did not find a significant match with known protein sequences (143).

At present, the reasons for the discrepancies in the molecular sizes reported for sperm PR is unclear. It is apparent that the sperm PR has a molecular weight of 50-60 kDa, unlike the uterine PRs (PR-B of 120 kDa and PR-A of 90 kDa). According to Sabeur et al, (1996), the lower molecular weight sperm PR is probably a proteolytically cleaved form of the larger protein that occurs during processing without protease inhibitors. It would be of interest to note that most of the sperm antigens of various molecular masses are detected by monoclonal antibodies against the C-terminal and N terminal regions of the conventional progesterone receptor suggesting that the sperm PR has some homology to the conventional PR. In this context, it is of interest to note that a monoclonal antibody (MA 410) against the C terminal of the conventional PR inhibits follicular fluid derived

progesterone mediated acrosome reaction (41). However it is not clear whether one or more of these proteins are involved in mediating progesterone actions.

5. PERSPECTIVES

Our knowledge on the endocrine regulation of various reproductive events in male and female has increased substantially because of our understanding of PR structure, regulation and function. Studies to determine the identity of PR regulated target genes during various events have been undertaken but these studies are not exhaustive. Many answers especially to the queries related to the events following binding of progestin to these receptors are still awaited. These answers have the potential to redefine our understanding of various reproductive processes.

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