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EXPRESSION OF PROGESTERONE RECEPTOR mRNA IN THE FIRST TRIMESTER HUMAN PLACENTA

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SUMMARY

The human placenta produces large quantities of progesterone, the function and target tissues of action of which during pregnancy are not completely understood. Although it has been suggested that placenta itself could be a potential target tissue for progesterone action, there is no conclusive evidence for the presence of progesterone receptors in the human placenta. We provide evidence for the expression of progesterone receptor mRNA in the human placenta by reverse transcription coupled to polymerase chain reaction (RT-PCR). This was further confirmed by Southern hybridization, restriction analysis and sequencing of the PCR amplified fragment.

Keywords : Progesterone receptor , Human Placenta, Progesterone, RT-PCR.

INTRODUCTION

The human placenta produces as much as 250-600 mg of progesterone (P_4) daily during late pregnancy. Although the role of such large quantities of P_4 remains as yet unclear, it is well established that P_4 is indispensible for the maintenance of pregnancy in all mammals studied so far. One of the target tissues suggested for the action of P_4 during human pregnancy is the placenta itself.

Most of the actions of P_4 are mediated through the interaction of P_4 with specific progesterone receptors (PR). Over the years, several reports have appeared claiming the presence or absence of receptors for P_4 in the human placenta. Using ^3H- P_4 binding assays, Younes *et al* [1] and Rivera *et al* [2] have reported the presence of PR in the human

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ABBREVIATIONS: P4: progesterone; PR: progesterone receptor; RT-PCR: reverse transcription coupled to polymerase chain reaction; FTHP: first trimester human placenta.

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placenta. However, using the same method as well as immunoassays or immunoblots, Padayachi *et al* [3], McCormick *et al* [4] and Karalis *et al* [5] were unable to detect PR in the human placenta. There is also a wide variation in the concentration of PR reported in literature. Furthermore, most of these studies have used binding assays, which are equivocal due to interference by the high levels of endogenous P_4 in the human placenta. In the present study, we demonstrate the expression of PR mRNA in the human placenta by RT-PCR.

MATERIALS AND METHODS

1. *Reverse Transcription-Polymerase Chain reaction (RT-PCR)*

First trimester human placenta (FTHP) was collected from cases of medical termination of pregnancy and processed as described earlier [6], taking additional care to avoid uterine tissue contamination. Total RNA was isolated by the method of Chomczynski and Sacchi [7]. First strand cDNA was synthesised using the SuperScript preamplification system (Gibco-BRL) according to the manufacturer's instructions. PCR was performed for 40 cycles of denaturation (94°C - 1 min), annealing (48°C - 1 min) and extension (72°C - 2 min) in a buffer containing 25 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT, 0.2 mM dNTPs and 2.5 units of *Taq* DNA polymerase. Primers specific for the human PR were designed based on the published sequence of the human PR cDNA [8] in order to amplify the region corresponding to amino acids 730-840. The forward primer used was 5'GGCGGATCCGTCAAGTGGTCTAAATCATTG 3' and the reverse primer used was 5' GGCGAATTCCTGGGTTTGACTTCGTAGCCC 3'. The forward primer has a *Bam* HI site and the reverse primer, an *Eco* RI site, to facilitate directional cloning. The expected size of the amplified product with these primers is 351 bp. Primers were designed to amplify PP14 (based on the published sequence [9]) which has been used as a negative control. The sequences of the primers for PP14 are 5' GACCAACAACATCTCCCTCAT 3' (forward primer) and 5'AAACGGCACGGCTCTTCCATC 3' (reverse primer). PCR for PP14 was performed for 40 cycles of denaturation (94°C - 1 min), annealing (57°C - 1 min) and extension (72°C - 2 min). The products of PCR were resolved by electrophoresis on a 1.5% agarose gel. Southern hybridization was done according to the procedures of Sambrook *et al* [10] using ^{32}-P labeled full length human PR cDNA as a probe.

2. *Cloning and sequencing of the amplified fragment*

The PR fragment amplified upon RT-PCR was digested with *Bam* HI and *Eco* RI, and was purified by gel-elution. The cloning vector, pRSET A (Invitrogen corp.), was also digested with *Bam* HI and *Eco* RI and gel-purified to remove the stuffer fragment. Ligation was carried out as described by Sambrook *et al* [10] with an 8-fold molar excess of the PCR amplified PR DNA. Ten percent of the ligation reaction was used to transform *E.coli* DH 5α and plasmid DNA was prepared from several transformants and digested with *Bam* HI and *Eco* RI to identify the clones. Twelve clones were identified of which two, viz. pGS 6 and pGS 12 were sequenced by cycle-sequencing on an automated DNA-sequencer (ABI Prism, Model No 377).

RESULTS

The results presented in Fig.1 indicate that a fragment of 351 bp is amplified upon RT-PCR from first trimester human placental RNA and this is the size expected of genuine amplification from PR mRNA with the given set of primers. The absence of any amplification in the control lane (lane 3) where reverse transcriptase was omitted and its presence in the lane (lane 5) where the RNA was treated with DNase I prior to reverse transcription confirms the specificity of amplification of the 351 bp fragment. We have been able to amplify a fragment of this size from several batches of FTHP. Supportive evidence for the amplification of a product of expected size is obtained from results of Southern hybridisation with the full length human PR cDNA as the probe, as presented in Fig.2 . It can be seen that the 351 bp fragment hybridises with the full length human PR cDNA and there is no hybridization in the "no RT" control. Furthermore, restriction analysis (Fig.3) of the PCR product confirms that the 351 bp product is a part of the PR cDNA. The region amplified has restriction sites for 3 enzymes viz., *Hin*d III, *Alu* I and *Hin*f I. *Alu* I digestion should result in three fragments of sizes 160 bp, 99 bp and 92 bp ; *Hin*d III digestion should result in two fragments of sizes 250 bp and 101 bp and *Hin*f I digestion should result in two fragments of sizes 193 bp and 158 bp. Restriction analysis with all the three enzymes conformed to the expected pattern.

In order to rule out the possibilty that the amplification is due to contamination from uterine tissue, primers were designed for PP14, a gene that is expressed only in the uterus but not in the placenta [9]. It is evident from results presented in fig. 4 that the cDNA used

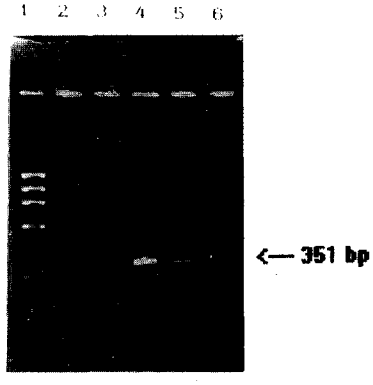

Fig.1: RT-PCR of PR in FTHP. 1: ϕx174/*Hae* III markers, 2: No template, 3: No RT, 4: RT-PCR of FTHP RNA, 5: RT-PCR of DNase I treated FTHP RNA, 6: PCR of human genomic DNA.

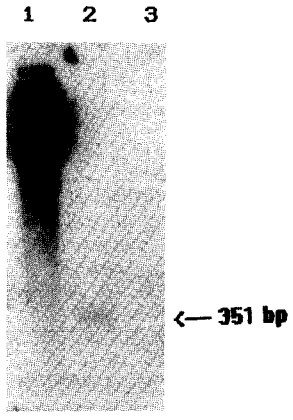

Fig.2: Southern analysis. 1: Human PR cDNA, 2: RT-PCR product from FTHP, 3: No RT control.

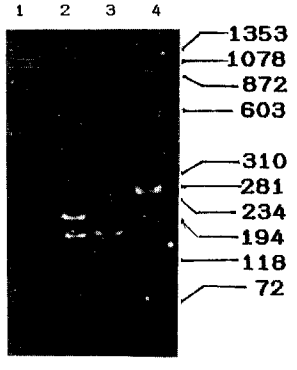

Fig.3: Restriction Analysis. 1: Undigested PCR fragment, 2: *Hin*f I digest, 3: *Alu* I digest, 4: *Hin*d III digest.

for amplification of PR does not amplify PP14 mRNA, establishing that the PR amplification is due to PR mRNA expressed in the human placenta.

Additional evidence for the above conclusion was obtained by sequencing the PCR amplified fragment. The PR fragment amplified from human placenta was cloned into pRSET A using the *Bam* HI and *Eco* RI sites. We were able to pick up twelve clones of which we sequenced two, viz. pGS6 and pGS12, by cycle sequencing. The sequence of both these clones showed complete identity with the published sequence [7] except at one nucleotide in pGS 6 (Fig.5) and two nucleotides in pGS 12 (data not shown), which can be attributed to errors during PCR or sequencing, as the changes are at different positions in pGS 6 and pGS 12.

DISCUSSION

The search for progesterone receptors in the human placenta has been on for over a decade. The reports available in literature are ambiguous and do not provide any definite molecular evidence for the expression of PR [1-5]. This is primarily due the heavy reliance of these studies on binding assays which are equivocal due to the high levels of endogenous P_4. In contrast, we have made use of RT-PCR , a highly sensitive technique, to detect the presence of PR mRNA and our results provide conclusive evidence for the presence of PR mRNA in the human placenta.

The human placenta produces large quantities of P_4 during pregnancy, the function of which is still unclear. One of the major potential roles of P_4 could be in the regulation of gene expression in the placenta itself. In fact, the role of P_4 in the regulation of the hCG α and ß subunit gene expression has been demonstrated in our laboratory [11]. Considering this, it is reasonable to expect that PR must be expressed in the placenta. Our attempts to detect the mRNA for PR by northern analyses have not been successful. The amplification of a fragment of expected size (351 bp), it's ability to hybridise to the human PR cDNA, the correct restriction fragment pattern, and the sequence identity with the cloned human PR provide sufficient evidence to prove that the human placenta expresses PR, albeit at low levels (below the sensitivity limit of northern analysis). Also, the possibility that the

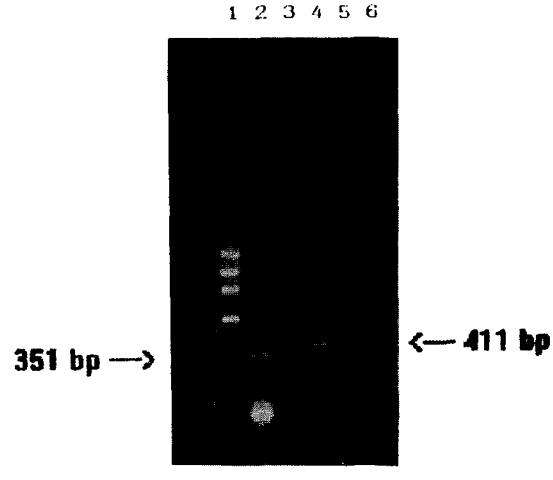

Fig.4: PP14 PCR. 1: ϕx174/*Hae* III markers, RT-PCR with: 2: PR primers in FTHP, 3: PP14 primers in FTHP, 4: PP14 primers in decidua, 5: No RT, 6: No template

gtcaagtggtctaaatcattgccaggttttcgaaacttacatattgatgaccagataactctcattcagtattcttggatgagcttaat ggtgtttggtctaggatggagatcctacaaacacgtcagtgggcgatgctgtattttgcacctgatctaatactaaatgaacagcg gatgaaagaatcatcattctattcattatgccttaccatgtggcagatcccacaggagtttgtcaagcttcaagttagccaagaaga gttcctctgtatgaaagtattgttacttcttaatacaattccttttggaagggctacgaagtcaaacccag.

Fig.5: Nucleotide Sequence of the PR insert of pGS6. The nucleotide "t" at the boxed position is an "a" in the published PR sequence.

detection of PR in placenta is due to the contamination of placental tissue by uterine tissue, which is known to express PR, was ruled out by the fact that we were unable to detect a product following the RT-PCR of placental RNA using primers designed to amplify PP14, which is a gene specifically expressed in the uterus. In summary, to the best of our knowledge this is the first conclusive evidence for the presence of progesterone receptor mRNA in the human placenta.

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