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DNA Microarray Analysis of Gene Expression Regulated by Steroid Hormone in the Rat Uterus

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INTRODUCTION

The uterus during estrus cycle synthesizes a complex of signaling molecules with specific spatial and temporal modes of expression and which are critical for cell proliferation and differentiation. The mechanisms underlying the differential pattern of synthesis of these associated proteins is not understood very well. Previous studies have shown that the ovarian hormones modulate these cellular events through the gene expressions. The function of the endometrium is to support implantation of a fertilized embryo and the subsequent development and maintenance of the placenta. Cellular proliferation and differentiation are critical components of uterine remodeling prior to embryonic implantation. Implantation of the mammalian embryo into the wall of the uterus is regulated by a timely interplay of the ovarian hormones, estrogen and progesterone. The molecular and cellular mechanisms underlying this complex process, however, remain largely unknown. Therefore, our purpose is to identify the profile of the steroid hormone - regulated gene expressions in estrus cycle and implantation stage. In the present study, we used for a functional genomics approach applying cDNA expression array technology to identify novel candidate genes involved in the regulation of uterine cell proliferation and differentiation during estrus cycle and implantation. We found several steroid hormone responsible genes in the uterus.

MATERIALS & METHODS

Sample collection

Rat uterine tissues were obtained from the OVX, each stage of estrus cycles (pro-estrus, estrus, met-estrus, diestrus) and gestational day 1, 2, 3, 4. Uterine tissues were quickly dissected and frozen in liquid nitrogen, stored at -70℃.

RNA and probe preparation

Total RNA was extracted from the tissues using Tri-reagent (Sigma) following its recommended protocol. Genomic DNA contamination was removed by incubating the sample RNA with DNase I (0.1unit/μg of RNA) for 30 min at 37℃. Total RNAs were converted to cDNA using Atlas cDNA synthesis protocol (Atlas cDNA Kit; Clontech) in the presence of α-P^{32} dATP. The labelled cDNAs were purified from the free P^{32}-dATP using Nucleospin extraction kit (Clontech).

Hybridization and analysis

Identical cDNA array membranes (Atlas Rat 1.2 array II cDNA expression array, clontech), containing 1176 known genes were individually hybridized with their corresponding labelled cDNA probes. The cDNA array membranes were placed in separate glass bottles and prehybridized with 5 ㎖ of ExpressHyb buffer (Clontech) for 30 min at 68℃ in a rotating hybridization chamber. The labelled cDNA probes ($3-6\times10^6$cpm) were added to a prehybridized bottles and was hybridized at 68℃ overnight. The membranes were washed stringently (four washes of 2×SSC, 1% SDS;

one washes of 0.1×SSC, 0.5% SDS) and autoradiographed for 3 days at -70℃. The expression levels of the gene transcripts were quantified using a Adobe photoshop program and normalized as a percentage of the expression of several housekeeping genes that were also arrayed in the membranes. Consistent increased or decreased in gene expression levels were measured.

RT-PCR

Total RNAs were isolated from rat uterus obtained from each stage of estrus cycle and gestational day 1, 2, 3, 4. RT-PCR was performed using the GeneAmp Kit (Perkin-Elmer). For cDNA synthesis, 2-4 μg of total RNAs were reverse transcribed for 1 h at 42℃ in a volume of 20 $\mu\ell$ containing 1 mM each dNTP, 5 mM $MgCl_2$, 2.5 uM oligo(dT) primer, 20 U of RNase inhibitor, and 50 U of MuLV Reverse Transcriptase in 1 × reverse transcription (RT) buffer. PCRs were performed in 25 $\mu\ell$ containing 5 $\mu\ell$ of cDNA product, 200 uM dNTPs, 0.4 uM each oligo-nucleotide primer, 2 mM $MgCl_2$, and 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer). Oligonucleotide primers were designed using the oligomer program. For normalization a β -actin primer pair was also used under the same conditions. The PCR products were electrophoresed in a 2% agarose gel.

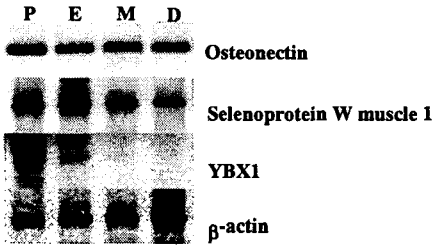

Fig. RT-PCR analyses on RNA isolated from estrus cycling uterus using a primer sets for estrogen responsible genes. 2ug of total RNA was reverse transcribed and amplified for 30 cycles using primers. β-actin primer set was also used for nomalization. P, proestrus; E, estrus; M, metestrus; D, diestrus.

CONCLUSION

We used the technique of hybridization of an Atlas rat 1.2 II cDNA expression array to identify the differences in the expression pattern of known genes between proestrus, estrus, pregnant, and OVX uterus. Differentially expressed genes were divided into two groups: increased or decreased one in each stages of uterus. The differentially expressed genes were studied by RT-PCR analyses. Using the cDNA expression array technique we identified several genes that were differentially expressed in estrus cycles and pregnant stages in the rat uterus. The identification of differentially expressed genes may provide important information in the regulation of uterine remodeling and is worthy of further investigation.

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