발생과 생식 제 7권 제 1호, 41～48 (2003)
Dev. Reprod. Vol. 7, No. 1, 41～48 (2003)

Differential Expressions of Adhesion Receptor Genes in the Rat Uterus Associated with Ovarian Steroid Hormone

Han Seung Kang, Chae Kwan Lee[1], Deog Hwan Moon[1] and Sung Goo Kang†

School of Biotechnology and Biomedical Science, Institute of Basic Sciences, Inje University, Kimhae 621-749, Korea
[1]Institute of Industrial Medicine, Inje University, Busan 614-735 Korea

흰쥐 자궁에서 난소 스테로이드 호르몬에 의한 Adhesion 수용체 유전자 발현조절에 대한 연구

강한승 · 이채관[1] · 문덕환[1] · 강성구†

인제대학교 기초과학연구소 생명공학부, [1]인제대학교 산업의학연구소

ABSTRACT : This report aimed at investigating the differential gene expressions of the adhesion receptors between ovariectomized (OVX) and estrus stage rat uteri (OVX vs. estrus pair) using the cDNA expression array analysis. In addition, this report aimed at confirming of the differential gene expressions of the adhesion receptors between OVX and progesterone (P_4) injected OVX rat uteri (OVX vs. OVX+P_4 pair). RNA samples were extracted from the uterus and reverse-transcribed in the presence of [α^{32}P]-dATP. Membrane sets of Rat Atlas array 1.2 II (Clontech) were hybridized with cDNA probe sets. RT-PCR was employed to validate the relative gene expression patterns obtained by the cDNA array. The results were well consistent to cDNA array analysis data except the fold changes of gene expression.

Among a total of 1176 cDNAs, 5 genes of adhesion receotor including embigin protein, activated leukocyte cell adhesion molecule, afadin, neuroligin 2, semaphorin Z showed significant (more than 2-fold) changes in the OVX vs. late estrus pair. All of these genes were up regulated in estrus stage than OVX rat uterus. In the OVX vs. OVX+P_4 pair, 4 genes including osteonectin, afadin, neuroligin 2, semaphorin Z showed significant changes. All of these genes were also up regulated in OVX+P_4 injected rat uterus than OVX control. Three genes including afadin, neuroligin 2, semaphorin Z which were up regulated in estrus and OVX+ P_4 injected rat uteri of both experimental pairs than OVX rat uteri. These genes seem to be under the control of P_4.

Key words : Adhesion receptor gene, cDNA array, Estrus cycle, Progesterone, Uterus.

요 약 : 본 연구는 생식주기 중의 흰쥐 자궁에서 발현되는 유전자들 중 adhesion 수용체 유전자들의 발현이 프로게스테론(P_4)에 의하여 차별적으로 조절되는 실험을 하였다. 첫째 실험군은 난소절제 흰쥐와 배란기 흰쥐를 사용하였고(OVX/estrus), 둘째 실험군은 난소절제 흰쥐와 난소절제 후 P_4를 주사한 흰쥐를 사용하였다(OVX/OVX+P_4). 적출한 자궁조직에서 total RNA를 추출, [α^{32}P]-dATP로 probe를 제작한 후 Rat Atlas array 1.2 II(Clontech)을 이용하여 발현되는 유전자들을 선별하였으며, 그 중 adhesion 수용체 유전자들의 발현양상을 RT-PCR 방법으로 확인하였다.
OVX/estrus 자궁의 유전자 발현을 비교한 경우, 전체 1176개의 유전자들 중 P_4에 의해 발현이 증가되는 adhesion 수용체 유전자들은 embigin protein, activated leukocyte cell adhesion molecule, afadin, neuroligin 2, semaphorin Z, osteonectin 등 이었다. OVX/OVX+P_4 자궁의 유전자 발현을 비교한 경우, P_4에 의해 발현이 증가되는 adhesion 수용체 유전자들은 osteonectin, afadin, neuroligin 2, semaphorin Z 등 이었다. 그리고 afadin, neuroligin 2, semaphorin Z은 두 실험군에서 모두 유전자 발현이 증가되었다. 이러한 결과로 보아 이 유전자들은 P_4에 의하여 발현이 조절되어 배란 후 착상 준비에 관여할 것으로 추측된다.

This work was supported by grant No. 2000-1-20700-002-3 from the Basic Research Program and BPRC (2001) of the KOSEF and in part by Inje Research Foundation (2002).

† Correspondence: School of Biotechnology and Biomedical Science, Institute of Basic Sciences, 607 Obang-dong Kimhae-shi Kyongnam, 621-749 Korea. Tel: 82-55-320-3213, Fax: 82-55-336-7706, E-mail: biosgkan@inje. ac.kr

INTRODUCTION

The Rodent uterus is a classical target tissue for sexual steroid hormones. During menstrual cycle, uterus presents morphological changes including high proliferation of both luminal and glandular epithelia and important degeneration of the epithelium after ovulation (Bertalanffy and Lau, 1963; Marcus, 1974; Spornitz et al., 1994). Cell proliferation occurs in response to estrogen (E_2) and it is inhibited by P_4 (Martin et al., 1973; Kirkland et al., 1979). The action of steroids mediated by growth/differentiation factors that act in autocrine or paracrine fashion (Brigstok et al., 1989; Cullinan-Bove and Koos, 1993). For example, E_2 induces over expression of mRNA for platelet-derived growth factor, epidermal growth factor and their related receptors in both stromal and epidermal uterine cells (Di Augustine et al., 1988; Lingham et al., 1988; Huet-Hudson et al., 1990; Gray et al., 1995). Expression of insulin-like growth factor-I is also up regulated in human endometrium during the E_2-dominated proliferative phase (Zhou et al., 1994). Growth/differentiation factors are differently expressed in the E_2-dominated proliferative and P_4-dominated secretory phases. For example, vascular endothelial growth factor is expressed in E_2-responsive epithelium lining the uterus. Under the influence of P_4, when maximal development of secretory endometrium and development of new blood vessels take place, the site of expression of vascular endothelial growth factor shifts to the cells of the underlying stroma (Shweiki et al., 1993).

Cell death in tissues is highly regulated. It is involved in shaping tissues during development and in counter-balancing proliferation in adult tissues (Wyllie et al., 1980). Cell death by apoptosis has been demonstrated in the uterus of several mammals, including humans (Sandow et al., 1979; Pollard et al., 1987; Spornitz et al., 1994; Spencer et al., 1996). Apoptosis has been observed in rat uterus and vagina, where an inverse correlation was established between cell death and cell proliferation using a histomorphological approach (Spornitz et al., 1994). However, there is limited systematic information about the extent of proliferation, apoptosis and expression of genes involved in the processes during the menstrual cycle (Bourroughs et al., 2000). For these reason, this study performed to assess whether there are changes in adhesion receptor gene expressions in the rat uterus during menstrual cycle. We compared differential gene expressions between ovariectomized (OVX) and late estrus stage rat uteri using the cDNA expression array technique. And we also compared differential gene expressions between OVX and P_4 injected OVX rat uteri prepared according to the protocol described previously (Kennedy and Ross, 1997; Papay and Kennedy, 2000) Differential gene expression profiles were revealed. Relative expressions of genes were validated by RT-PCR.

MATERIALS AND METHODS

1. Preparation of Animals

Experiments were conducted on Sprague-Dawley rats purchased from Dae Han Bio-link (Seoul, Korea). Female virgin 2~3 month-old rats were kept 2~3 per cage in a light and climate controlled room with a 12 h light-dark cycle starting at 8 a.m. and received food and water ad libitum freely. Daily vaginal smears were obtained from each of the animals and uterine stages assessed according to criteria of Long and Evans (1922). Only animals exhibiting at least two consecutive 4~5 day cycles were used. Five animals per each in ovx and estrus stage were sacrificed. To assess the steroid hormone effects, rats were ovariectomized under ether anesthesia and allowed at least 10 days to withdraw the steroids. To obtain a state equivalent to pseudopregnancy, ovariectomized rats were given injections (s.c.) of E_2 and P_4 in sesame oil according to the protocol illustrated (Fig. 1), as described previously (Kennedy and Ross, 1997; Papay and Kennedy, 2000). Animals were sacrificed by decapitation on day 5 and referred as OVX+P_4. Uterus of each was dissected, immediately frozen with liquid nitrogen and stored at −70℃ until RNA isolation. Each group comprised more than 5 rat uteri.

Fig. 1. Schematic representation showing the sequence of hormone administration to ovariectomized rats to obtain uteri differentially sensitized. Black areas represent periods of darkness; numbers within light areas equivalent day of pseudopregnancy. Animals were sacrificed by decapitation on 5 and referred as OVX+P_4 (n=5). Dopp: day of pseudopregnancy.

2. cDNA Array Hybridization

RNA was extracted, reverse-transcribed and labeled using the Atlas Pure Total RNA Labeling System, as suggested by the manufacture (Clontech, Palp Alto, USA). Gene expression was analyzed using the Atlas Rat 1.2 array II cDNA expression array (Clontech), which consists of two sets, each with two identical membranes, on which are cDNA fragments specific for 1176 genes plus nine house-keeping genes. Briefly, total RNA was extracted and DNase treated. Total RNA was reverse-transcribed and labeled with [α^{32}P]-dATP (Amersham Pharmacia, Little Chalfont England). The resulting cDNA probes were purified on Nucleospin Extraction Kit (Clontech), used on the membranes at a final concentration of 5×10^7 cpm/ml. Hybridization was carried out overnight at 68°C according to the manufacturer's recommendation. After washing, the membranes were exposed to X-Omat X-ray film (Eastman Kodak, Rochester, NY) for 3 days. cDNA array hybridization was carried out by two pairs, OVX vs. estrus pair and OVX vs. OVX+ P_4 injected pair, for more than three times.

3. Data Analysis

The signals generated by cDNA expression arrays were analyzed as described previously (Life Science and Technology, Vol. 5, No. 3, Takara Biomedicals, Japan, 2000) with minor modifications. Briefly, autoradiographic signals were scanned with a flat-bed scanner (Epson, Model GT-9500) in a gray mode of Adobe Photoshop 5.5. The scanned autoradiograms were transformed to the RGB color mode with an 8-bit channel. A new canvas with an 8-bit RGB color mode was prepared, and the autoradiogram hybridized with the OVX cDNA probe was copied and pasted onto the prepared canvas with the green channel. The autoradiogram with the estrus or the OVX+ P_4 cDNA probe was copied and superimposed upon the same canvas using the red channel. Now the corresponding slots in the two pairs of autoradiograms (OVX vs. estrus pair and OVX vs. OVX+P_4 injected pair) combine at same slot and appear as either green or red or a combination according to their composition of hybridized probes. Using the information function of Adobe Photoshop, each corresponding slot was analyzed as to its color intensity, and the ratio of the resulting values were used to compare the two probe sets.

4. RT-PCR Analysis

To validate the expression patterns obtained by cDNA array hybridization, the same RNA samples from the same experimental groups were examined with relative RT-PCR analysis. Four genes that showed mutually a > 2-fold difference in both experimental pairs were selected. The PCR primer sets were designed based on the sequence deposited in NCBI GeneBank database using a PCR primer design program (MacVector software, Oxford Molecular, Oxford, UK) (Table 1). β-actin was used as an internal control. Briefly, total RNAs (1 mg) were reverse-transcribed using 2.5 U MMLV reverse transcriptase (Perkin Elmer, Norwalk, CT) and 0.5 mg of oligo d(T) primer (Perkin Elmer) in the presence of 1 mM dNTP (Perkin Elmer), and 1 U RNasin (Perkin Elmer) in a final volume of 20 ml. Following a hot start (5 min at 95°C) PCR continued with 60S at 95°C, 60S at each annealing temperature, 60S at 72°C for 30 cycles using 10 ml RT product, 0.2 mM primers and 2.5 U Taq polymerase (Perkin Elmer) in a final volume 50 ml containing 0.2 mM dNTP and 2 mM $MgCl_2$. The resulting PCR products were electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining. Gel photographs were scanned with a flat-bed scanner and analyzed using the NIH image program.

5. Statistics

The significance of differences in mean values between groups were analyzed by either an unpaired student's t-test for two groups or one-way analysis of variance (ANOVA) for more than two groups followed by Tukey's multiple comparison test. Prior to analysis, normal assumptions of the data and homogeneity of variance between the experimental groups were ascertained. The level of statistical significance was set at $p<0.05$. SPSS for Windows (version 10.0) was used to calculate probability values.

RESULTS AND DISCUSSION

cDNA expression array analysis between OVX and estrus stage rat uteri showed that 141 clones out of the 1176 cDNAs examined were expressed above background. Of these 141 cDNAs, 20 messages showed a significant (>2 fold) change in expression (data not shown). cDNA array data showed changes in gene expression of the following groups of proteins: (1) cell surface

Table 1. Primer pair sequences for RT-PCR and the lengths of the PCR products

Gene name	Primer sequences (5'-3') (upper:sense; lower: antisense)	PCR product (bp)	Accession number
Afadin	P : CTC AAG GGG ATG ACA GTG AG N : TCC TTA GCA CCT CTC TCA TC	387	U83230
Neuroligin 2	P : GGC GAG TGG TGG TGG TGA AGA N : ATG GCG AGA TGC GGC GTA AGA	537	U41662
Semaphorin Z	P : CCT GGG GAC TCA CAC TTC TAC N : TGA GAC ACT GGC ATT GGG CTT	516	AB000776
Osteonectin	P : TGG TGG AGG AGA CAG GGT TAC N : GCA AAG AAG TGG CAG GAA GAG	295	Y13714

antigen, (2) transcription factor, (3) adhesion receptor, (4) endocytosis protein, (5) energy metabolism, (6) ribosomal protein, (7) RNA processing, (8) calcium protein, (9) transducer & modulater, (10) filament protein, (11) unknown function protein. In the adhesion receptors group, expression of five genes coding embigin protein, CD 166 antigen, afadin, neuroligin 2 and semaphorin Z were up regulated in estrus stage than OVX rat uterus (Table 2). This may reflect the progress of cell-to-cell adhesion of differentiated endometrium cells that facilitate the dramatic events of reproduction.

cDNA expression array analysis between OVX and OVX+P_4 injected rat uteri showed that 132 clones out of the 1176 cDNAs examined were expressed above background (data not shown). In the adhesion receptors group, expression of four genes coding osteonectin, afadin, neuroligin 2 and semaphorin Z were up regulated in OVX+P_4 injected rat uterus (Table 3). And we confirmed six genes that is up-regulated in both experimental group by RT-PCR analysis (Fig. 2, 3). A linear relationship was observed as a function of increasing amplification cycles (data not shown). Moreover, the same tendency of difference between two group was observed at each point of amplification cycle. Increasing amounts of reverse transcribed RNA also resulted in a linear relationship between optical density and used amount of RNA. Similar tendency of difference was also found at each amount of used RNA samples (data not shown). These validation data support that our methodology for confirmation of cDNA array result with RT-PCR works well, though there are the discrepancy observed in expression folds regarding some genes.

The uterine endometrium undergoes cyclic growth and development with the sole purpose of successful establishment of pregnancy. Maternal endometrial cells are regulated directly by ovarian steroids and indirectly by various growth factors and cytokines. Establishment of uterine receptivity is still a great biological mystery that remains unsolved. In the rat, the level of progesterone increases markedly after ovulation, and remains high until the end of gestation (Wiest, 1970; Kalra and Kalra, 1974). The circulating level of estrogen is high in nonpregnant animals at estrus stage. After fertilization the level of estrogen

Table 2. List of adhesion receptor genes showing changed expression in the estrus stage rat uterus compared with the ovariectomized control

Genetype	Clones	Gene	Fold changes[a]	Accession number
Adhesion receptor	1	embigin protein	58.0	AJ009698
	2	activated leukocyte cell adhesion molecule (ALCAM); CD166 antigen	66.0	AB008538
	3	afadin	11.9	U83230
	4	neuroligin 2	8.8	U41662
	5	semaphorin Z (SEMAZ)	3.8	AB000776

[a] The fold change was obtained by comparing signal intensities of estrus stage with OVX rat uterus (estrus/ovx) in the cDNA expression array.

Table 3. List of adhesion receptor genes showing changed expression in the progesterone injected rat uterus compared with the ovariectomized control

Genetype	Clones	Gene	Fold changes[a]	Accession number
Adhesion receptors	1	osteonectin	2.5	Y13714
	2	afadin	9.0	U83230
	3	neuroligin 2	5.5	U41662
	4	semaphorin Z (SEMAZ)	2.48	AB000776

[a] The fold change was obtained by comparing signal intensities of OVX+P_4 with OVX rat uterus (OVX+P_4/OVX) in the cDNA expression array.

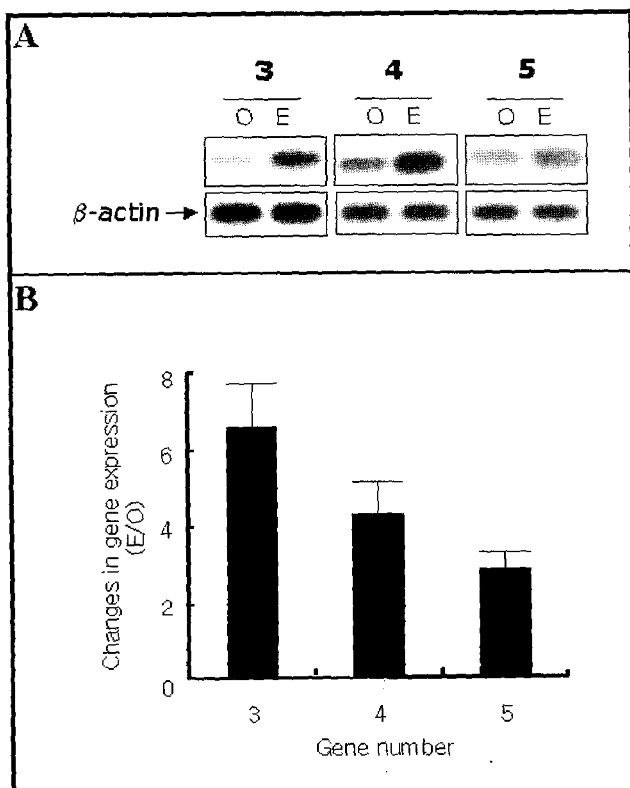

Fig. 2. RT-PCR analysis with PCR primer sets of cDNAs that showed difference between OVX and estrus stage rat uteri. (A) Gel scans of the RT-PCR analysis listed in Table 1. For each gene, the specific cDNA fragment (upper bands) was co-amplified with a specific fragment of the β-actin gene (lower bands) allowing determination of the relative expressions. (B) Optical densities were analyzed using NIH image software and represented as mean±S.D. (n=3). Numbers in (A) and (B) indicate the identification of genes in Table 1. O: ovariectomized rat uterus; E: estrus stage rat uterus.

declines and remains low throughout gestation, except for a transitory rise in estrogen level that occurs on day 4 of pregnancy (Yoshinaga et al., 1969).

The present data showed that the six genes, embigin protein, activated leukocyte cell adhesion molecule, afadin, neuroligin 2, semaphorin Z, osteonectin which are known as cell-to-cell or matrix adhesion receptor. Embigin is a transmembrane glycoprotein belonging to the immunoglobulin super family and enhances integrin-mediated cell adhesion (Tachikui et al., 1999). CD166 antigen facilitates cell-cell interactions (Van Kempen et al., 2001). Osteonectin is an extracellular Ca-binding glycoprotein that associates with cell populations undergoing migration, morphogenesis, and differentiation. Afadin is an actin filamentbinding protein that is associated with the cytoplasmic tail of nectin, a Ca^{2+}-independent immunoglobulin-like cell-cell adhesion molecular (Asada et al., 2002; Takai and Nakanishi, 2003). SEMAZ is one of the semaphorines/ collapsins protein family cloned

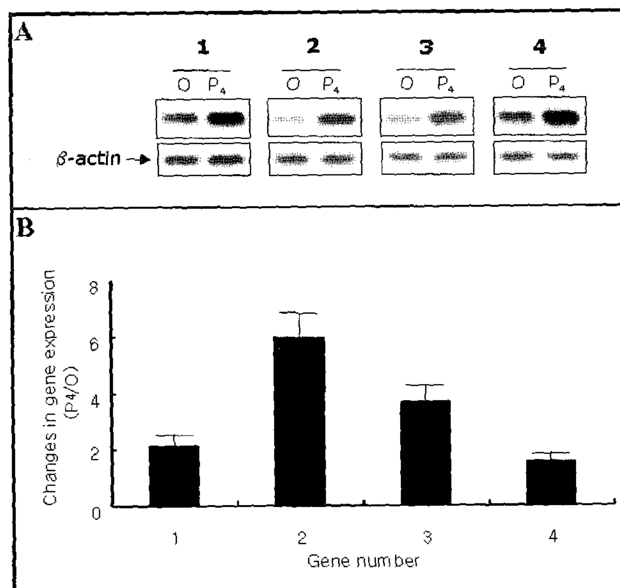

Fig. 3. RT-PCR analysis with PCR primer sets of cDNAs that showed difference between OVX and OVX+P_4 injected rat uteri. (A) Gel scans of the RT-PCR analysis listed in Table 2. For each gene, the specific cDNA fragment (upper bands) was co-amplified with a specific fragment of the β-actin gene (lower bands) allowing determination of the relative expressions. (B) Optical densities were analyzed using NIH image software and represented as mean±S.D. (n=3). Numbers in (A) and (B) indicate the identification of genes in Table 2. O: ovariectomized rat uterus; P_4:ovariectomized and progesterone injected rat uterus.

from a rat brain cDNA library. SEMAZ is thought to be an integral membrane glycoprotein (Kikuchi et al., 1997). Uterine endometrial epithelial cells are hormone-dependent (Harper, 1992) and accompanied by morphological and biochemical changes during the menstrual cycle (Pavelock et al., 2001; Mendosa-Rodrigues et al., 2002). And variety of molecules have implicated in the series of adhesive events in mammals' uterus (Domino et al., 2001; Julian et al., 1994; Spencer et al., 1999). On the line of this evidence, up regulation of these cell-to-cell adhesion related gene expressions in the estrus stage rat uterus is predictable. Even though uterus endometrial endothelial cells express estrogen and progesterone receptor genes (Iruela-Arispe et al., 1999), the regulatory mechanisms of the adhesion related genes response to the ovarian steroid hormones are not elucidated yet.

One of the most interesting data of present study is unexpected and significant increase in the expression of neuroligin 2 gene, originally described by Inchtchenko (1995). The rat neuroligin family comprises four distinct genes, neuroligin 1, 2, 3 (Inchtchenko et al., 1996), and 4 (Bolliger et al., 2001). In the rat, neuroligin 1, 2, 3 are expressed at high levels only in the central nerve system and constitute a family of brain-specific membrane proteins. Neuroligins bind to certain splice variants of b-neurexins (Inchtchenko et al., 1996) that are members of a polymorphic, brain specific family of cell-surface proteins (Ullrich et al., 1995; Ushkaryov et al., 1992). In addition, the intracellular C-terminal tail of neuroligins binds to postsynaptic scaffolding protein (PSD-95) (Irie et al., 1997), a PDZ-domain (PSD-95/Dlg/ZO-1) protein that is thought to be involved in the assembly and organization of signal-transduction complexes in postsynaptic densities (Kornau et al., 1997). Thus, neuroligins serve as synaptic cell-adhesion molecules that organize the postsynaptic assembly of protein complexes involved in signal transduction.

The Atlas Rat 1.2 array II cDNA expression array (Clontech), used in the present study, contains neuroligin 2, 3, PSD-95, PDZ domain 1,2,3 subunits cDNAs. However, neuroligin 2 gene was detected only in the rat uterus. And the expression of neurexin genes were not reported in the mammals' uterus yet. For these reason, it is difficult to hypothesis the precise role of neuroligin 2 gene in the rat uterus. However, it is presumed that neuroligin 2 gene expression in the rat uterus is under the control of P_4 cyclically secreted during the estrus cycle. It is the first time that reported the expression of neuroligen family gene in the mammals' uterus.

REFERENCES

Asada M, Irie K, Morimoto K, Yamada A, Ikeda W, Takeuchi M, Takai Y (2002) ADIP, a novel Afadin- and alpha-actinin-binding protein localized at cell-cell adherens junctions. J Biol Chem 278:4103-4111.

Bertalanffy FD, Lau C (1963) Mitotic rates, renewal times, and cytodynamics of the female genital tract epithelia in the rat. Acta Anat 54:28-37.

Bolliger MF, Frei K, Winterhalter KH, Gloor SM (2001) Identification of a novel neuroligin in humans which binds to PSD-95 and has a widespread expression. Biochem J 356: 581-588.

Bourroughs KD, Fuchs-Young R, Davis B, Walker CL (2000) Altered hormonal responsiveness of proliferation and apoptosis during myometrial maturation and the development of uterine leiomyomas in the rat. Biol Reprod 63:1322-1330.

Brigstock DR, Heap RB, Brown KD (1989) Polypeptide growth factors in uterine tissues and secretion. J Reprod Fertil 85: 747-758.

Cullinan-Bove K, Koos RD (1993) Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus:rapid stimulation by estrogen correlates with estrogen-induced increases in the uterine capillary permeability and growth. Endocrinology 133:829-837.

Di Augustine RP, Petrusz P, Bell GI, Brown CF (1988) Influence of estrogens on mouse uterine epidermal growth factor precursor protein and massenger ribonucleic acid. Endocrinology 122:2355-2363.

Dominio SE, Zhang L, Lpwe B (2001) Molecular cloning, genomic mapping, and expression of two secretor blood group a(1,2) Fucosyltransferase genes differentially regulated in mouse uterine epithelium and gastrointestinal tract. J Biol Chem 276:23748-23756.

Gray K, Eitzman B, Raszmann K, Steel T, Geboff A, McLachlan J, Bidwell M (1995) Coordinate regulation by diethylstilbestrol of the platelet-derived growth factor-A (PDGF-A) and B chain and the PDGF receptor alpha and beta subunits in the mouse uterus and vagina: potential mediators of est-

rogen action. Endocrinology 136:2325-2340.

Harper MJ (1992) The implantation window. Baillieres Clin Obstet Gynaecol 6:351-371.

Hute-Hudson YM, Chakraborty C, De SK, Suzuki Y, Andrews GK, Day SK (1990) Estrogen regulates the synthesis of epidermal growth factor in mouse uterine epithelial cells. Mol Endocrinol 4:510-523.

Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Sudhof TC. (1995) Neuroligin 1: a splice site-specific ligand for beta-neurexins. Cell 81:435-443.

Ichtchenko K, Naguyen T, Sudhof TC (1996) Structures, alternative splicing, and neurexin binding of multiple neuroligins. J Biol Chem 271:2676-2682.

Irie M, Hata Y, Takeuchi M, Ichtchenko K, Toyoda A, Hirao K, Takai Y, Rosahl TW, Sudhof TC (1997) Binding of neuroligins to PSD-95. Science 277:1511-1515.

Iruela-Arispe ML, Rodriguez-Manznaeque JC, Abu-Jawdeh G (1999) Endometrial endothelial cells express estrogen and progesterone receptors and exhibit a tissue specific response to angiogenic growth factors. Microcirculation 6:127-140.

Julian J, Chiquet-Ehrimann R, Erickson HP, Carson D (1994) Tenascin is induced at implantation sites in the mouse uterus and interferes with epithelial cell adhesion. Development 120:661-671.

Kalra SP, Kalra PS (1974) Temporal inter relationships among circulating levels of estradiol, progesterone and LH during the rat estrous cycle: Effects of exogenous progesterone. Endocrinology 95:1711-1718.

Kennedy TG, Ross HE (1997) Temporal- and hormone-dependent changes in uterine sensitization for the decidual cell reaction and decidualization *in vitro* of rat endometrial stromal cells. J Reprod Fertil 109:129-136.

Kikuchi K, Ishida H, Kimura T (1997) Molecular cloning of a novel member of semaphorin family genes, semaphorin Z. Brain Res Mol Brain Res 51:229-237.

Kirkland JL, LaPointe L, Justin E, Stancel GM (1979) Effects of estrogen on mitosis in individual cell types of the immature rat uterus. Biol Reprod 21:269-272.

Kornau HC, Seeburg PH, Kennedy MB (1997) Interaction of ion channels and receptors with PDZ domain proteins. Curr Opin Neurobiol 7:368-373.

Lingham RB, Stancel GM, Loose-Mitchell DS (1988) Estrogen regulation of epidermal growth factor receptor massenger ribonucleic acid. Mol Endocrinol 2:230-235.

Long JA, Evans HM (1922) The oestrus cycle in the rat and associated fenomena. Mem Univ Calif 6:1-148.

Marcus GJ (1974) Mitosis in the rat uterus during the estrous cycle, early preganacy, and early pseudopregnancy. Biol Reprod 10:447-452.

Martin L, Das RM, Finn CA (1973) The inhibition by progesterone of uterine epithelial proliferation in the mouse. J Endocrinolol 57:549-554.

Mendoza-Rodriduez CA, Merchant-Larios H, Segura-Valdez ML, Moreno-Mendoza N, Cruz ME, Arteaga0Lopez P, Camacho-Arroyo I, Dominguez R, Cerbon M (2002) Expression of *p53* in luminal and glandular epithelium during the growth and regression of rat uterus during the estrous cycle. Mol Reprod Dev 61:445-452.

Papay KD, Kennedy TG (2000) Characterization of temporal and cell-specific changes in transcripts for prostaglandin E(2) receptors in pseudopregnant rat endometrium. Biol Reprod 62:1515-1525.

Pavelock K, Braas KM, Ouafik L, Osol G, May V (2001) Differential expression and regulation of the vascular endometrial growth factor receptors neuropilin-1 and neuropilin-2 in rat uterus. Endocrinology 142:613-622.

Pollard BA, Pacey J, Cheng SVY, Jordan EG (1987) Estrogens and cell death in murine uterine luminal epithelium. Cell Tissue Res 249:533-540.

Sandow BA, West NB, Norman RL, Brenner RM (1979) Hormonal control of apoptosis in hamster uterine luminal epithelium. Am J Anat 156:15-36.

Shweik D, Itin A, Neufeld G, Gitay-Goren H, Keshet E (1993) Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. J Clin Invest 91:2235-2243.

Sounders JW Jr (1966) Death in embryonic systems. Science 154:604-612.

Spencer TE, Bartol FF, Bazer FW, Johnson GA, Joyce MM (1999) Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. Biol Reprod 60:241-250.

Spencer SJ, Cataldo NA, Jaffe RB (1996) Apoptosis in the hu-

man female reproductive tract. Obstet Gynecol Surv 51:314-323.

Spornitz UM, Rinderknecht BP, Edelmann A, Scheideggerm B, Cairili F (1994) Ultrastructure as a basis for dating of rat endometrium. Anat Rec 238:163-176.

Tachikui H, Kurosawa N, Kadomatsu K, Muramatsu T (1999) Genomic organization and promoter activity of embigin, a member of the immunoglobulin superfamily. Gene 240:325-332.

Takai Y, Nakanishi H (2003) Nectin and afadin: novel organizers of intercellular junctions. J Cell Sci 116:17-27.

Ullrich B, Ushkaryov YA, Sudhof TC (1995) Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron 14:497-507.

Ushkaryov YA, Petrenko AG, Geppert M, Sudhof TC (1992) Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin. Science 257:50-56.

Van Kempen LC, Nelissen JM, Degen WG, Torensma IE, Weidle UH, Bloemers HP, Figdor CG, Awart GW (2001) Molecular basis for the homophilic activated leukocyte cell adhesion molecule (ALCAM)-ALCAM interaction. J Biol Chem 276:25783-25790.

Wiest WG (1970) Progesterone and 20-alpha-hydroxypregn-4-en-3-one in plasma, ovaries and uteri during pregnancy in the rat. Endocrinology 87:43-48.

Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251-306.

Yoshinaga K, Hawkins RA, Stocker JF (1969) Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and Pregnancy. Endocrinology 85:103-112.

Zhou J, Dsupin BA, Giudice LC, Bondy CA (1994) Insulin-like growth factors system gene expression in human endometrium during the menstrual cycle. J Clin Endocrinol Metab 79:1723-1734.