Comparison of c-DNA microarray analysis of gene expression between eutopic endometrium and ectopic endometrium (endometriosis)

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Abstract *Problem:* As recent studies have suggested abnormalities in the regulation of specific genes in the development of endometriosis, we investigated differentially expressed genes in endometriosis compared to endometrium. *Method of study:* Gene expression profiles using the Atlas microarray were performed in endometriotic tissue and endometrium. Nine of the 13 genes of endometriotic tissue showed an up-regulation in relation to endometrium and four of the 13 genes a down-regulation. *Results:* Of the 1176 genes on the Atlas Human 1,2 array, only 13 differentially expressed identical genes were detected after repeating the gene analysis three times. *Conclusion:* According to our c-DNA analysis some differentially expressed genes may be involved in the pathogenesis of endometriosis. An imbalance in the genes responsible for the reproductive process may lead to a decrease in embryo implantation in patients with endometriosis.

Keywords Endometriosis · Endometrium · Microarray analysis

Introduction

The cause of endometriosis is unclear [1, 2]. It is believed to occur primarily as a result of retrograde menstruation; in

other words, back-flow of menstrual discharge through the fallopian tubes into the peritoneal cavity. Alternatively, it may occur in some women via lymphatic transport of menstrual debris. It is thought that retrograde menstruation occurs to some extent in most women. The question then arises, why do not all women get endometriosis? There must be unknown physiological differences that cause some women to develop endometriosis whereas others do not.

Recent studies have suggested that abnormalities in the regulation of specific genes are involved in the development of endometriosis [3–6] applied gene expression profiling using high-density oligonucleotide microarrays to investigate differentially regulated genes in endometrium from women with vs. without endometriosis during the window of implantation. A comparison of endometrial genes in the window of implantation from women with and without endometriosis identified three unique groups of target genes, which differ with respect to the implantation window and the presence of disease. The data support dysregulation of select genes leading to an inhospitable environment for implantation, including genes involved in embryonic attachment, embryo toxicity, immune dysfunction, and apoptotic responses, as well as genes likely contributing to the pathogenesis of endometriosis, including aromatase, progesterone receptor, angiogenic factors and others.

Some authors have studied the transcriptional characterizations of differences between eutopic and ectopic endometrium using laser capture microdissection and a cDNA microarray while others have used real-time reverse transcription-polymerase chain reaction [7–9]. Some of these genes are known to participate in oestrogen activities and antiapoptosis. They may play a role in the pathogenesis of endometriosis and may represent potential diagnostic markers or therapeutic targets for endometriosis.

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We consciously analysed the differences in gene expression between ES and EM groups in the proliferative phase of the cycle. We assume that particularly in this phase the balance between the expression of proliferation and apoptotic genes is important for reproductive function.

The current studies were undertaken to use DNA microarrays to search for new gene expression markers of endometriosis by identifying differentially expressed genes in endometriosis implants compared with normal endometrium, taken from other women as control tissue, in the proliferative cycle phase. Our long term goal is to identify gene expression markers of endometriosis that can be used for noninvasive diagnosis of endometriosis, for development of new treatment strategies or to gain an understanding of the pathophysiology and aetiology of this enigmatic disease.

We studied gene expression profiles using the Atlas microarray technique to investigate differentially regulated genes in endometriotic tissue compared to endometrium in the proliferative phase of non-endometriosis patients. In the present study we only tested endometrium from patients without endometriosis.

Material and methods

Tissue samples

Five patients scheduled for surgery of endometriosis participated in this study after their informed consent had been obtained. Tissue samples of endometrium were gained at operative laparoscopy and verified histologically before further assessment for RNA analysis. All five patients were in the proliferative phase of the menstrual cycle at the time of surgery. Eutopic endometrium was obtained from 5 non-endometriosis patients prior to hysteroscopy for infertility assessment by curettage. Endometriosis tissue was obtained at laparoscopy and histological diagnosis of the ectopic implant and endometrium was verified. The remainder of the sample was then placed in a RNA protective reagent, liquid nitrogen.

Samples of ectopic endometrium, taken exclusively from ovarian tissue, (endometriosis, $n = 5$) and eutopic endometrium ($n = 5$) were obtained from patients undergoing laparoscopy for the treatment of endometrioma (i.e. endometriotic ovarian cysts) and diagnostic hysteroscopy, respectively, during the proliferative phase of the cycle. Patients' mean age was $31 \pm SE$ (range 22–40 years) and none of them received hormonal treatment prior to their surgery. For the purpose of gene expression analysis, these samples were processed as follows:

- (1) Cryostat sections were prepared and stained with haematoxylin-eosin.

Table 1 Isolated RNA amount in ($\mu\text{g}/\mu\text{l}$) and total (μg) from ectopic (ES) and eutopic (EM) endometrium by Qiagen method

	ES	EM
RNA amount ($\mu\text{g}/\mu\text{l}$)	0,8	1,1
total RNA amount (μg)	58,0 μg	53,4 μg

- (2) For in vitro gene expression analysis, the tissue was immediately placed in an autoclaved cap, shock frozen in liquid nitrogen and stored at -80°C until further processing.
- (3) Eutopic and ectopic endometrium were subjected to histopathological assessment which confirmed their site of origin, i.e. proliferative endometrium and endometrioma cyst wall, respectively.

RNA preparation

For RNA-preparation the RNeasy Mini Kit (Qiagen) was used. Briefly: frozen tissues were disrupted and homogenized in lysis buffer RLT using a conventional rotor-stator homogenizer (Ultra Turrax^R, Ika). Tissue lysates were applied to RNeasy Mini columns and RNA purification was performed according to kit instructions. Table 1 shows the total isolated amount of RNA from ES and EM.

Figure 1 shows a formaldehyde agarosegelelectrophoresis (1.5%) of total isolated RNA from each 2 of 5 endometriosis and each 2 of 5 endometrium samples. The isolated RNA was in an excellent condition. The gel electrophoresis experiment showed a distinct band at 28 S respectively 18S rRNA in a model comparison of approximately 2:1.

cDNA probe synthesis

All reagents for probe synthesis (except [α -³²P]dATP), purification and array hybridisations were provided together with the AtlasTM cDNA expression arrays (Clontech).

Fig. 1 Formaldehyde agarosegelelectrophoresis with the total isolated RNA from each 2 of 5 endometriosis (line 1 and 2) and each 2 of 5 endometrium samples (line 3 and 4)

Five μg of total RNA was converted into [α - ^{32}P]dATP-labelled cDNA in a 20 μl reaction volume using 5 μl of [α - ^{32}P]dATP (10 $\mu\text{Ci}/\mu\text{l}$, 3,000 Ci/mmol, Amersham).

The labelled cDNA probe was purified from unincorporated labelled nucleotides by column chromatography using NucleoSpin Extraction Spin Columns (Clontech), followed by scintillation counting to check the radioactivity.

AtlasTM cDNA expression array hybridisation and exposure

The membrane of Atlas human 1,2 array (Clontech) used in this study contains 1176 human genes. The arrays were hybridized overnight at 68°C in 5 ml of ExpressHyb (Clontech) solution with 5×10^6 cpm for each cDNA probe. After hybridisation arrays were washed two times with 50 ml high salt wash buffer ($2 \times \text{SSC}$, 1% SDS), followed by a final wash with 50 ml low salt wash buffer ($0.1 \times \text{SSC}$, 0.5% SDS).

The damp membranes were immediately wrapped in plastic wrap and exposed to a phosphorimaging screen for 7 days.

Image analysis

For analysis of hybridisation results phosphorimage raw data were transferred to AtlasImageTM, Clontech's software for analyzing AtlasTM Arrays.

Statistical evaluation

The data analysis was performed with a special software provided by Clontech, especially adapted to the Atlas cDNA Expression Arrays.

The background was automatically subtracted and for normalization the intensity of all genes was used. The adjusted gene intensity from triplicate measurement of ectopic (ES) and eutopic endometrium (EM) were performed and variations were expressed as the mean $\pm$ SD.

Fold-change ratios between groups ES and EM were subsequently derived and a 2-fold difference, a generally adopted fold change difference for oligo nucleotide microarray profile analysis (7,8), was applied to select up-regulated (fold ≥ 2) and down-regulated (fold ≤ 2) genes. We also calculated the subtracted gene difference analysis between ectopic (ES) and eutopic endometrium (EM) (up regulated $\Delta (\text{ES-EM}) > 0$ and down regulated $\Delta (\text{ES-EM}) < 0$).

Results

Of the 1176 genes on the Atlas Human 1,2 Array, 940 (79.4%) were expressed in endometrial tissue (both eutopic and ectopic), however, this differed according to the tissue of origin; genes were expressed in 89.8% of endometrium but 79.4% only in endometriotic specimens. Further, the intensity of gene expression was different in 196/940 genes (20.8%) in ectopic endometrium tissues. Only 38/196 genes (19.4%) showed detectable signals between ES and EM. Differences between adjusted intensity of ES and EM are shown in Fig. 2.

Table 2 demonstrates these up and down regulated genes with gene bank accession, fold up of ES relation to EM, name of gene/protein and classification of gene/protein. Fold-change ratios between groups ES and EM were subsequently derived, and a difference of 2-fold, a generally adopted fold

Fig. 2 Gene difference analysis between ectopic (ES) and eutopic endometrium (EM), up regulated $\Delta (\text{ES-EM}) > 0$ and down regulated $\Delta (\text{ES-EM}) < 0$

Table 2 Gene expression array comparing endometriotic tissue (ES) with normal endometrium (EM) including genes/protein and gene classification

Gene Bank Accession	Fold up ES/EM	Gene/Protein Name	Gene/Protein Classification	Coordinate
AB003184	2,24	ISLR PRECURSOR.	Cell Surface Antigens Other Cell Adhesion Proteins	A02k
U20659	2,09	DNA + DIRECTED RNA POLYMERASE II 19 KD POLYPEPTIDE (EC 2.7.7.6) (RPB7).	RNA Polymerase	A03l
X53281	2,28	H.sapiens BTF3b mRNA	Basic Transcription Factor	A05a
AF017418	3,25	HOMEBOX PROTEIN MEIS2 (MEIS1+RELATED PROTEIN 1) (FRAGMENT).	Basic Transcription Factor	A08l
D31716	2,62	TRANSCRIPTION FACTOR BTEB1 (BASIC TRANSCRIPTION ELEMENT BINDING PROTEIN 1) (GC BOX BINDING PROTEIN 1).	Basic Transcription	A10e
U28686	0,49	PUTATIVE RNA+BINDING PROTEIN 3 (RNPL).	Other Transcription Proteins	A14j
L22009	0,39	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN H (HNRNP H).	Other Transcription Proteins. mRNA Processing, Turnover & Transport Proteins	B02j
M16342	4,24	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEINS C1/C2 (HNRNP C1 AND HNRNP C2).	Other Transcription Proteins. mRNA Processing, Turnover & Transport Proteins	B02m
U30825	0,35	SPLICING FACTOR, ARGININE/SERINE+RICH 9 (PRE+MRNA SPLICING FACTOR SRP30C)	Other Transcription Proteins. mRNA Processing, Turnover & Transport Proteins	B03e
L38486	3,18	MICROFIBRIL+ASSOCIATED GLYCOPROTEIN 4.	Other Cell Adhesion Proteins	B06i
AB021288	2,00	Homo sapiens mRNA for beta 2+microglobulin	Major Histocompatibility Complex Proteins	B07a
X00457	2,62	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DP ALPHA CHAIN PRECURSOR (HLA+SB ALPHA CHAIN) (MHC CLASS II DP3+ALPHA) (DP(W3)) (DP(W4))	Major Histocompatibility Complex Proteins	B07b
M14058	3,57	COMPLEMENT C1R COMPONENT PRECURSOR (EC 3.4.21.41).	Other Immune System Proteins. Serine Proteases	B09c
AF012106	3,13	DNAJ PROTEIN.	Protein Heat Shock Proteins. Chaperones & Heat Shock Proteins	B11l
AF018157	2,14	Homo sapiens glycine receptor alpha 3 subunit (GLRA3) mRNA	Ligand-Gated Ion Channels	B12m
U55054	0,50	K+CL COTRANSPORTER.	Symporters & Antiporters	B14c
M76559	3,12	DIHYDROPYRIDINE+SENSITIVE L+TYPE, CALCIUM CHANNEL ALPHA+2/DELTA SUBUNITS PRECURSOR	Other Membrane Channels & Transporters	B14g
D37991	0,36	Human SSR2 mRNA for beta+signal sequence receptor	Targeting Proteins Other Receptors (by Activities)	C05d
M11147	2,20	FERRITIN LIGHT CHAIN (FERRITIN L SUBUNIT).	Other Trafficking & Targeting Proteins	C06a
X13238	0,41	CYTOCHROME C OXIDASE POLYPEPTIDE VIC PRECURSOR (EC 1.9.3.1).	Energy Metabolism	C09m
AB006202	0,47	SUCCINATE DEHYDROGENASE [UBIQUINONE] CYTOCHROME B SMALL SUBUNIT PRECURSOR (CYBS) (SUCCINATE+UBIQUINONE REDUCTASE MEMBRANE ANCHOR SUBUNIT) (QPS2) (CII+4) (SUCCINATE DEHYDROGENASE COMPLEX SUBUNIT D).	Energy Metabolism	C11b
J03620	2,82	DIHYDROLIPOAMIDE DEHYDROGENASE, MITOCHONDRIAL PRECURSOR	Other Metabolism Enzymes	D01k

Table 2 Continued.

Gene Bank Accession	Fold up ES/EM	Gene/Protein Name	Gene/Protein Classification	Coordinate
AF102544	2,73	MOLYBDOPTERIN SYNTHASE SULFURYLASE.	Other Metabolism Enzymes	D03j
U12465	0,48	60S RIBOSOMAL PROTEIN L35.	Ribosomal Proteins	D07g
L38995	0,50	ELONGATION FACTOR TU, MITOCHONDRIAL PRECURSOR (P43)	Translation Factors tRNA Processing, Turnover & Transport Proteins	D10h
M88108	0,36	GAP+ASSOCIATED TYROSINE PHOSPHOPROTEIN P62.	mRNA Processing, Turnover & Transport Proteins	D13a
U54996	2,81	HZW10.	Centromeric Proteins	E03b
J02854	3,02	MYOSIN REGULATORY LIGHT CHAIN 2, SMOOTH MUSCLE ISOFORM (MYOSIN RLC) (LC20)	Calcium-Binding Proteins	E09k
AF015913	3,61	SHK1 KINASE+BINDING PROTEIN 1.	Microfilament Proteins	E10f
AB005047	2,90	SH3 BINDING PROTEIN.	Kinase Activators & Inhibitors	E10j
M64110	3,58	CALDESMON (CDM).	Other Intracellular Transducers, Effectors & Modulators	E11k
M96803	2,00	SPECTRIN BETA CHAIN, BRAIN (SPECTRIN, NON+ERYTHROID BETA CHAIN) (FODRIN BETA CHAIN) (SPTBN1)	Other Intracellular Transducers, Effectors & Modulators	E11l
X05607	3,48	CYSTATIN C PRECURSOR (NEUROENDOCRINE BASIC POLYPEPTIDE) (GAMMA+TRACE) (POST+GAMMA+GLOBULIN).	Other Cytoskeleton & Motility Proteins	F01b
U35398	2,68	PROBABLE G PROTEIN+COUPLED RECEPTOR OGR1.	Protease Inhibitors	F02d
D14678	2,11	KINESIN+RELATED PROTEIN (FRAGMENT).	G Protein-Coupled Receptors. Orphan Receptors	F03b
D49738	3,35	CYTOSKELETON+ASSOCIATED PROTEIN CKAPI (TUBULIN FOLDING COFACTOR B).	Motor Proteins	F05a
AF047439	2,35	HYPOTHETICAL 33.8 KD PROTEIN	Other Cytoskeleton & Motility Proteins	F10k
M11886	2,83	HLA class I histocompatibility antigen C-4 alpha subunit (HLAC)	Functionally Unclassified Proteins	G31
			Housekeeping Genes	

Note. Up regulated fold up (ES/EM) > 2 and down regulated fold up (ES/EM) < 0.5.

change difference for oligo nucleotide microarray profile analysis [10, 11], was applied to select up-regulated and down-regulated genes.

After repeating the gene analysis three times in all tissue samples, only 13 differentially expressed identical genes were detected (Fig. 3). Nine of 13 genes of ES showed an up-regulation in relation to EM and four of 13 genes a down-regulation.

The properties of the up-regulated nine genes distributed under their coordinate number are as follows:

- (1) AO2k pertains to the immunoglobulin super family containing LRR = Leucine-rich repeat [12].
- (2) A10e is a basic transcription element binding protein that may impair steroidogenesis.
- (3) B07 pertains to an abnormal HLA expression and could play a role in the pathogenesis of endometriosis [13].
- (4) B14g seems to act on the calcium channel alpha-2/delta subunits precursor.
- (5) C06a was found to be responsible for iron overload in the peritoneal cavity of women with pelvic endometriosis [14]. It is a differential gene encoding ferritin heavy chain (FHC).
- (6) E10j is an activin and as such implicated in a variety of biological effects, particularly in reproductive processes [15].
- (7) E11k caldesmon is described by Loddenkemper et al. 2003 in a study aimed to investigate oxytocin receptor expression in uterine smooth muscle tumours and endometrial stromal sarcomas [16]. CD10 has been recently reported to be expressed by normal and neoplastic endometrial stromal cells and has already been helpful in differentiating adenomyosis from endometrial carcinoma.

Fig. 3 Thirteen differentially expressed identical genes (after repeating the gene analysis three times). Nine of 13 genes of ectopic endometrium (ES) showed an up-regulation in relation to eutopic endometrium (EM) and four of 13 genes a down-regulation (select up factor was at least 2-fold)

- (8) F03b is a kinesin-related protein.
- (9) G31 is a HLA class 1 histocompatibility antigen and is responsible for the elastin distribution in the myometrial and vascular smooth muscle of the human uterus [17].

The properties of the down-regulated four genes distributed under their coordinate number are as follows:

- (1) B14c is a K-CL cotransporter.
- (2) C09m is described by Suske et al., 1987 as the gene interfering with cytochrome c oxidase subunits VIc and VIII [18].
- (3) D07g is a ribosomal protein.
- (4) D10h is an elongation factor and a mitochondrial precursor.

Discussion

In this preliminary report we compared a set of 940 genes expressed in endometrium and endometriosis and identified 38 genes (Table 2) which were differentially expressed in endometriotic implants compared to uterine endometrium. Only 13 of the 38 genes were found to be significantly expressed in both tissues after 3 repetitive genes analysis. A small group of up and down-regulated genes seem to be typical for endometriosis and endometrium. However, the observation of these genes in eutopic endometrial tissue in comparison to gene expression in endometriosis shows a specificity of endometriosis determined by DNA microarray which might be an effective tool for the development of new therapeutic strategies for the treatment of endometriosis. We anticipate that further studies of the genes identified herein will promote our understanding of

the pathophysiology and aetiology of endometriosis. All 13 genes may permit access to basis molecular mechanism in endometriosis.

Upregulated genes

Gene 1 with coordinate number (A02k)

In this study the immunoglobulin superfamily containing leucine-rich repeat (ISLR) classified as cell adhesion protein was significantly higher expressed in ES compared to genes in EM. According to Nagasawa et al. [12] these domains are known to be important for protein-protein interaction or cell adhesion, and therefore it is possible that the novel protein ISLR may also interact with other proteins or cells.

These authors showed by Northern blot analysis its presence in various human tissues including retina, heart, skeletal muscle, prostate, ovary, small intestine, thyroid, adrenal cortex, testis, stomach, and spinal cord as well as fetal lung and fetal kidney. We suggest that this protein may be responsible for adhesion formation, implantation and interaction of menstrual debris (host) (metaplasia or transplantation theory) in the internal and extragenital peritoneal surface (pouch of Douglas, ovaries, sacrouterine ligaments, broad ligaments, surface of the fallopian tubes, round ligaments, bladder, lungs, pleura and skin).

Gene 2 (A10e)

Basic transcription element binding protein (BTEB). A GC box-binding protein of Sp1 family transcription factors is known to regulate many cytochrome P450 genes [19].

Aromatase p450 (p450arom) is the key enzyme for biosynthesis of oestrogen [20], which is an essential hormone for the establishment and growth of endometriosis. There is no detectable aromatase enzyme activity in normal endometrium; therefore, oestrogen is not locally produced in endometrium. Endometriosis tissue, however, contains very high levels of aromatase enzyme which leads to production of significant quantities of oestrogen. Moreover, one of the best-known mediators of inflammation and pain, prostaglandin E (2), strikingly induces aromatase enzyme activity and formation of local oestrogen in this tissue. Additionally, oestrogen itself stimulates cyclo-oxygenase-2 and therefore increases the formation of prostaglandin E (2) in endometriosis. These authors connected the positive feedback to endometriosis, using aromatase inhibitors for treatment. In fact, pilot trials showed that aromatase inhibitors could decrease pelvic pain associated with endometriosis.

Gene 3 (B07) is classified under major histocompatibility complex proteins HLA class II. Gene expression analysis showed significantly higher secretion of HLA class II gene in ectopic endometrium than that in eutopic. In agreement with the findings of our study, Eyster et al. [21] reported over expression of histocompatibility antigen class I. They concluded that the immunological process is deeply involved in the formation of endometriosis.

Similarly, Xu et al. [13] demonstrated a significantly increased expression of HLA class molecules on ectopic endometrium compared to eutopic endometrium by immunohistochemical and flow cytometry methods. These authors suggest that the abnormal HLA expression could play a role in the pathogenesis of endometriosis.

Gene 4 (B14g) is classified under Transporters gene (dihydropyridine + sensitive L + type, calcium channel $\alpha + 2/\delta$ subunits precursor).

Burns et al. [22] and Riley et al. [23] described the role of calcium by cellular mechanisms by which oxytocin mediates uterine prostaglandin F2 α synthesis in bovine endometrium and suggests that both extracellular and intracellular Ca^{2+} may be required for oxytocin to stimulate PGF2 α secretion in bovine endometrial tissue.

The relationship between prostaglandin (PG) production and the mechanism of dysmenorrhea in endometriosis is poorly understood. Koike et al. [24] consequently investigated the role of PGs in dysmenorrhea of endometriosis.

These investigators reported significantly increased PG production by endometriosis and adenomyosis compared to other tissues as well as in women with severe dysmenorrhea. They concluded that increased PGI2 in the tissue of endometriosis seems to induce hyperalgesia during menstruation.

Gene 5 (C06a) was found to be responsible for iron overload in the peritoneal cavity of women with pelvic

endometriosis [14]. It is a differential gene encoding ferritin heavy chain (FHC).

In agreement with our finding of higher level of targeting proteins, ferritin light chain gene in ectopic endometrium tissue, Van Langendonck et al. reported significantly higher concentrations of iron and ferritin in the peritoneal fluid of patients with endometriosis compared with controls [14].

Further, these authors observed iron overloading in the cellular and peritoneal fluid compartments of the peritoneal cavity of women with endometriosis and found that iron deposits were related to the presence of endometriosis lesions, suggesting its role in the pathogenesis of endometriosis.

Gene 6 (E10j) classified under other intracellular transducers, effectors and modulators. SH3 binding protein is an activin and as such implicated in a variety of biological effects, particularly in reproductive processes such as embryonic development and folliculogenesis [15].

Vav is a signal transducing molecule containing SH2 and SH3 domains and a guanine nucleotide releasing factor-like domain. Its expression is thought to be highly specific for hematopoietic cells. Higuchi et al. [25] detected vav in human decidual, placental, and chorionic villous tissues, in a choriocarcinoma cell line BeWo, in the cytotrophoblast shell and columns and in the extravillous trophoblasts in the maternal decidua from the first through third trimesters. They suggest that vav plays an important role for successful implantation and placental development by regulating development of trophoblasts.

Gene 7 (E11k) caldesmon is described by Loddenkemper et al. [16] in a study aimed to investigate oxytocin receptor expression in uterine smooth muscle tumours and endometrial stromal sarcomas. CD10 has been recently reported to be expressed by normal and neoplastic endometrial stromal cells and differentiates adenomyosis from endometrial carcinoma [26]. Other correlations to adenomyosis are not known.

Gene 8 (F03b) is a kinesin-related protein, classified under “motor proteins”. Kinesin is one member of a large super family of microtubule-based motor proteins [27, 28]. This diverse group of motors drives a number of essential subcellular movements, including transport of membranous organelles and mitotic spindle functions. In our study, we observed a significantly higher level of kinesin and kinesin-related protein in ectopic endometrium compared to eutopic endometrium. However, further studies are required to explain this mysterious function in endometrium and endometriosis.

Gene 9 (G31) classified as housekeeping genes HLA class I histocompatibility antigen C-4 α subunit (HLAC). The major histocompatibility complex in humans contains at least 20 class I genes. Included within this family are three closely linked loci with 11–47 codominant alleles that encode the classical transplantation antigens HLA-A, -B, and -C [29].

In agreement with our results, Eyster et al. [21] reported over expression of histocompatibility antigen class I. These data suggest that an immunological process is deeply involved in the formation of endometriosis.

G31 is a HLA class I histocompatibility antigen and is responsible for the elastin distribution in the myometrial and vascular smooth muscle of the human uterus [17]. These data suggest that the external region of the myometrium is more elastic than the inner region and that elastin is found throughout the arteriolar tree of the human uterus.

Down regulated genes

Gene 1 (B14c), the so called “K-CL cotransporter”, responsible for an appropriate ionic environment within the uterus, is of central importance to reproductive function. For example, it has been suggested that sperm viability and fertilizing ability [30] as well as the cleavage rate of early embryos [31] are sensitive to the external K^+ concentration. However, little is known about the mechanisms involved in regulation of the intrauterine environment, particularly in the human.

In the rat, a transuterine potential difference is associated with active absorption of Na^+ and active secretion of K^+ , Cl^- and HCO_3^- [32, 33]. In our gene expression analysis we observed a significantly higher level of K-CL cotransporter gene in eutopic endometrium than in ectopic endometrium. This could explain the low pregnancy rate in patients with endometriosis.

Gene 2 (C09m) is described by Suske et al. [18] as the gene interfering with cytochrome c oxidase subunits VIc and VIII.

Cytochrome c Oxidase (COX) is the terminal component of the bacterial as well as the mitochondrial respiratory chain complex that catalyzes the conversion of redox energy to ATP. In eukaryotes, the oligomeric enzyme is bound to the mitochondrial inner membrane with subunits ranging from 7 to 13. Thus, its biosynthesis involves a coordinate interplay between nuclear and mitochondrial genomes [34]. The largest subunits, I, II, and III, which represent the catalytic core of the enzyme, are encoded by the mitochondrial DNA and are synthesized within the mitochondria. The rest of the smaller subunits implicated in the regulatory function are encoded on the nuclear DNA and imported into mitochondria following their synthesis in the cytosol.

Some of the nuclear coded subunits are expressed in tissue and developmental specific isoforms. The ubiquitous subunits IV, Va, Vb, VIb, VIc, VIIb, VIIc, and VIII (L) are detected in all the tissues, although the mRNA levels for the individual subunits vary in different tissues. The tissue specific isoforms VIa (H), VIIa (H), and VIII (H) are exclusive to heart and skeletal muscle. There are further studies required to explain these mystery function in endometrium and endometriosis.

Gene 3 (D07g) is classified under ribosomal proteins 60S, which contains over 40 different 60s ribosomal proteins, as ribosomal protein L35 (RPL 35) [35]. No specific information is available on the RPL 35. It is known that some of the ribosomal proteins have an effect on the biosynthesis of the ribosome. If the transcription of rRNA and ribosomal proteins in the cell is reduced, e.g. because of defects in secretion; repression is blocked by inactivation of RPL1B (a gene which contains the 60s ribosomal protein L1). This indicates an autoregulation in the biosynthesis of the ribosome [36].

Different ribosomal proteins are cited in connection with prostatic carcinoma (RPL10, RPL32, RPL16). They correlate with the progression of the androgen-independent growth of the hormone. Amazingly, the above-mentioned gene has an effect on gene expression and is connected with inflammatory processes and cell differentiation. These characteristics lead to the conjecture that the gene could be involved in the genesis of endometriosis.

Gene 4 (D10h) is classified under translation factors, tRNA processing, turnover and transport proteins elongation factor TU, mitochondrial precursor (P43).

Human p43 is associated with the macromolecular tRNA synthase complex and known as a precursor of endothelial monocyte-activating polypeptide II (EMAP II) [37]. Interestingly p43 is also secreted to induce proinflammatory genes. Although p43 itself seems to be a cytokine working at physiological conditions, most of the functional studies have been obtained with its C-terminal equivalent, EMAP II. Chang et al. [37] showed the potential interaction of p43/EMAP II with alpha-ATP synthase and its role in the proliferation of endothelial cells.

Wells et al. [38] found low amounts of P43 expressed in the spleen, skeletal muscle and pancreas. P43 is abundantly produced in the liver and in other tissues, such as the kidney, heart and brain which have levels of oxidative metabolism. In our results we found an over expression of elongation factor tu, mitochondrial precursor (p43) in human eutopic endometrium tissues compared to ectopic endometriotic samples.

In summary, our findings comparing c-DNA microarray analysis of gene expression in ectopic and eutopic endometrium revealed that some of the differently expressed genes may be involved in the pathogenesis of endometriosis. Other coexisting genes may be responsible for an immunological process leading to immune dysfunction and facilitating the implantation and interaction of menstrual debris in ectopic sites, such as internal and extragenital peritoneal surface. The genes responsible for the endocrinological process in endometriosis lead to its growth, proliferation and resistance in extragenital space. Furthermore, an imbalance in the genes responsible for the reproductive process may

lead to a decrease in embryo implantation and may cause infertility in patients with endometriosis.

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