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Early effects of high concentrations of progesterone and Mifepristone A gene expression study of endometrial cancer cells (Ishikawa)

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ABSTRACT

Patients with endometrial hyperplasia representing preliminary stages of endometrial cancer have shown to respond to therapy in 100% of the cases when treated with levonorgestrel-impregnated intrauterine device. Anti-proliferative effect has also been reported after application of an anti-progestin impregnated intrauterine device which showed to induce endometrial atrophy. The intention of the present study was to obtain more information of novel therapeutic targets for hormonal treatment in endometrial hyperplasia and endometrial cancers. Gene expression of signaling pathways after stimulation of Ishikawa cells with high doses of progesterone (32 μM) or Mifepristone (32 μM) was performed. After using an oligo microarrays representing 24,650 human genes and 37,580 gene transcripts, 6154 genes remained after pre-processing and filtering. This resulted in a total of 993 up-regulated genes with 189 genes for progesterone and 255 genes for Mifepristone. The 550 down-regulated genes were distributed with 256 genes for progesterone, 127 genes for RU 486. The results showed that genes presenting the epidermal growth factor (EGF)/MAP-kinase pathway were significantly over-represented by progesterone treatment, whereas, by Mifepristone treatment genes involved in the p53 pathway were also up-regulated (data not shown). These genes may be interesting as potential new therapeutic targets in endometrial hyperplasia and endometrial cancer, as candidate genes for therapy response or as candidate markers for tumor progression.

1. Introduction

The sex steroid hormone progesterone and the synthetic mimics progestins have a strong anti-proliferative impact on the human female endometrial mucosa. The anti-proliferative effect of progestins has been utilized in therapy of pathological proliferative conditions like endometrial hyperplasia as well as in highly differentiated endometrial carcinoma [1–7]. This growth inhibitory effect has been assumed to be mediated through sex steroid receptors (PRs) acting as ligand-activated transcriptional factors to regulate the expression of specific sets of target genes [8]. Previous studies have shown that more than 60% of patients with endometrial hyperplasia respond to or intramuscular progestin therapy, however, treatment failure has been commonly observed after regular doses of per oral progestins [1,4,5]. High dose treatment with the levonorgestrel-impregnated intrauterine device has proved to have complete and continuous effect in endometrial hyperplasias. The actual patients showed no sign of recurring hyperplasia as long as the hormone impregnated intrauterine device remained in situ [7,9]. In the former study progesterone receptors were demonstrated to be completely down-regulated when investigated by immunohistochemistry [10]. Thus, there is strong evidence indicating that progestins may activate other transcriptional factors to communicate the anti-proliferative effect. Several extra-nuclear pathways utilizing alternative signaling routs for growth limitary effects of steroid hormones have been suggested. A recently published RNA microarray study of Ishikawa cells stimulated with a high dose of progesterone showed that the MAP kinase pathway as well as the p53 signaling pathways were activated [11].

The progesterone receptor antagonist, Mifepristone, was the first progesterone antagonist that exhibited anti-progestin activity in humans [12–14]. In a recent in vitro study Mifepristone was shown to induce potent growth inhibition in endometrial cancer cells (Ishikawa and Hec-1-A) [15]. Some authors have indicated that growth inhibition initiated by anti-progestins may occur through modulation of apoptosis related genes mediated by a variety of

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transcriptional factors like p53 [16–18]. Activation of mitogen-activated protein kinase has also been investigated [19,20]. In a therapeutic setting a new anti-progestin impregnated intrauterine device, showing to superior compared to the levonorgestrel-impregnated IUD in reducing breakthrough bleedings, also induced mucosal atrophy [21]. Mifepristone and other steroid receptor antagonists are assumed to be more potent than predicted by simple competition for progesterone binding and Mifepristone effectively antagonizes progesterone activation of the receptor at concentrations much less than sub-stoichiometric with the agonist [12]. However, the mechanism by which Mifepristone inactivates PR, however, is complex and incompletely understood [12]. The antagonist is also known to have increased affinity to the PR receptors compared to progesterone [22]. A growth—inhibitory effect of Mifepristone has been described and the antagonist has been evaluated in clinical trials as first-line as well as second-line agents in treatment of metastatic breast cancer [23].

For endometrial cancers and endometrial hyperplasia more exact knowledge concerning modulation of transcriptional mechanisms is important to obtain new therapeutic targets and new markers of therapy response. On this background the present gene expression study was undertaken to compare the effects of high concentrations of progesterone and Mifepristone on growth regulation in endometrial cancer cells to mimic possible choices of hormone impregnated intrauterine devices (Ishikawa).

2. Material and methods

2.1. Cell culture

Ishikawa cells, originally derived from a well-differentiated endometrial adenocarcinoma [24], was obtained from the European Collection of Cell Cultures (ECACC #99040201) (Sigma, UK). Monolayer cultures were established in RPMI 1640 medium (Sigma, St. Louis) with 10% dialyzed fetal calf serum (PAA-laboratories, Austria) without phenol red to avoid potential steroid-mimicking effects [25] and without antibiotics. Cells were seeded at a density of 1×10^5 cells/ml in 25 cm^2 plastic flasks in humidified atmosphere (5% CO_2) at 37 °C with daily change of supplemented growth medium.

The cells were grown for 5 days (showing approximate density of 1×10^6 cells/ml) with daily medium change before they were exposed to progesterone and RU 486.

2.2. Experimental design

Twelve independent hormone- and/or glucocorticoid antagonist-treatment experiments were carried out such that all cells were harvested simultaneously at the end of an experiment. Progestin (Sigma, St. Louis) and/or glucocorticoid antagonist Mifepristone (Sigma, St. Louis) were added from an ethanol stock to the cell culture medium to obtain a final concentration of 32 μM. The final ethanol concentration in the culture medium was 0.2%. The cells were incubated for 4 h before harvest. Four parallel controls (no treatment) were run in medium containing only 0.2% ethanol. The medium was always changed at the start of the experiment. All experiments were carried out using cells in the exponential phase of growth.

2.3. RNA extraction

After exposure, the cells were washed twice with PBS prior RNA extraction. The cells were homogenized directly in TRIzol (phenol–guanidinium–thiocyanate) (GIBCO BRL Life Technologies, NY) and RNA was prepared according to the manufacturer's protocol. RNA samples were examined for degradation by microfluidic analysis using the Agilent bioanalyzer with evaluation of 18S and 28S ribosomal RNA bands. The RNA integrity, represented by the RNA integrity number (RIN) was always >9.0 for all samples measured, indicating no degradation of any of the samples used for microarray analysis (data not shown).

2.4. Microarray procedures

35 K human oligo microarrays were obtained form the Norwegian Microarray Consortium (http://www.mikromatrise.no/). Briefly, the arrays contain spotted 70-mer oligonucleotides from the AROS Human oligo v3.0 set from OPERON (operon.com). The set contains 34,580 probes representing 24,650 human genes and 37,123 gene transcripts. The probe design is fully based on the Ensembl Human 13.31 Database (http://www.ensembl.org) and Human Genome Sequencing Project and allows detection of alternative splicing variants using common, partial common or individual transcript oligos. For more information see: (http://omad.operon.com/download/storage/human_V3.0.2_datasheet.pdf). As external control system, the SpotReport oligo validation system (Cat# 252170-7) from Stratgene was used.

Total RNA was reverse transcribed and labelled with Cy3- and Cy5-attached dendrimer, respectively, using the Genisphere 3DNA 350HS kit (Genisphere, Montvale, NJ) as described in the manufacturer's protocol. Hybridizations of transcribed probes were carried out in a TECAN HS4800 instrument using the formamide-based hybridization buffer from Genisphere containing 5% dextrane sulfate and 5.5 ng/μl COT1 DNA (GIBCO BRL Life Technologies) at 37 °C for 23 h. 3DNA dendrimer hybridizations were carried out in formamide-based hybridization buffer alone. Post-hybridization washes were carried out at room temperature with 2× SSC for 1 min, 0.2% SDS/2× SSC for 1 min and finally with 0.2× SSC for 30 s. The arrays were scanned with the GenePix 4000B scanner (Axon Instruments Inc.).

2.5. Data analysis––filtering and normalization

The features were extracted from the arrays using Genepix Pro 6.0 [Axon instruments Inc. (2004) Gene Pix 6.0 Pro, http://www.axon.com, documentation]. The background estimates were calculated using the morphological opening method [26]. Spots that displayed a signal-to-noise ratio of less than 2, or that were significantly saturated (more than 20% saturation among foreground pixels) were filtered out. The median was used as the averaging measure of the foreground pixels. After quality control, genes that were present in less than 50% of the arrays were filtered out. The arrays were normalized using the lowest method [27].

2.6. Statistical analysis

Statistical significance was assigned to the genes using the SAM methodology [28]. The bioconductor package was used in actual analysis. Missing expression levels were imputed using 10 nearest neighbors imputation [29]. The FDR threshold was set to 15%.

2.7. Database submission of microarray data

The microarray data were prepared according to minimum information about a microarray experiment (MIAME) recommendations [30] and deposited in the GEO database where it can be accessed at http://www.ncbi.nlm.nih.gov/geo/. The GEO accession number for the platform is GPL4790.

Table 1A
Genes up-regulated by progestin (10 μM) alone (receptor antagonist independent), clustered to the group of biological processes and subdivided into the indicated subgroups (apoptosis, cell adhesion, cell cycle, cell proliferation and differentiation, cell structure and motility, developmental process, immunity and defence, lipid, fatty acid and steroid metabolism, transport, other biological processes and unclassified biological processes) with $p < 0.01$ and a fold-change threshold set to ≥1.6.

Gene symbol	Gene name	Fold-change
Apoptosis		
TNFRSF10A	Tumor necrosis factor receptor subfamily member 10	2.1
Cell adhesion		
CDH9	Cadherin 9, type 2 (T1-CDH9 cadherin)	6.0
GARNL3	GTPase activating Ras/RanGAP domain-like 3	6.7
GARNL4	GTPase activating Ras/RanGAP domain-like 4	9.5
NELL1	NEL-like 1 (chicken)	3.1
Cell cycle		
CDC2	Cell division cycle 2, G1 to S and G2 to M	1.6
MPHOSPH6	M-phase phosphoprotein 6	1.7
SEPT2	Septin 2	1.7
Cell proliferation and differentiation		
DUSP6	Dual specificity phosphatase 6	1.8
ZNF43	Zinc finger protein 43	2.1
Cell structure and motility		
KLHL1	Kelch-like 1 (Drosophila)	7.2
PLS3	Plastin 3 (T isoform)	3.1
RAC1	ras related protein RAC	3.2
Developmental process		
MAML1	Mastermind-like 1 (Drosophila)	3.4
TNNI3	Troponin I type 3 (cardiac)	2.9
Immunity and defence		
CFB	Component factor B	3.9
HS3ST2	Heparin sulfate (glucosamine) 3-O-sulfotransferase 2	12.0
LIFR	Leukemia inhibitory factor receptor alpha	11.9
Lipid, fatty acid and steroid metabolism		
CYP21A2	Cytochrome p450, family 21, subfamily A, polypeptide 2	1.8
DGKB	Diacylglycerol kinase, beta 90 kDa	1.7
HSD17B12	Hydroxysteroid (17-beta) dehydrogenase 12	1.8
PLD1	Phospholipase D1, phosphatidylcholine-specific	1.8
Transport		
G3BP1	GTPase activating protein 1	1.9
KCNK13	Potassium channel, subfamily K, member 13	2.2
SLC5A5	Solute carrier family 5 (sodium iodide symporter), member 5	4.1
SLC26A2	Solute carrier family 26 (sulfate transporter), member 2	1.6
TM9SF2	Transmembrane 9 superfamily member 2	1.6
Other biological processes		
AK3	Adenylate kinase 3	1.7
ALDH9A1	Aldehyde dehydrogenase 9 family, member A1	1.7
ARHGEF4	Rho guanine nuleotide exchange factor (GEF) 4	5.4
ARF4L	ADP-ribosylation factor-like 4D	1.6
DNAJC9	DnaJ (Hsp40) homolog, subfamily C, member 9	1.6
ENTPD5	Ectonucleoside triphosphate diphosphohydrolase 5	2.1
GLS2	Glutaminase 2 (liver, mitochondrial)	3.8
GPR18	G protein-coupled receptor 18	9.5
GTPBP1	GTP binding protein 1	2.7
MAL2	T-cell differentiation protein 2	2.0
MRPL13	Mitochondrial ribosomal protein L1 3	1.7
P4HA1	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	1.6
RAB5A	RAB5A, member RAS oncogene family	1.7
RASA1	RAS p21 protein activator (GTPase activating protein) 1	4.6
RFK	Riboflavin kinase	1.6
RFX2	Regulatory factor X, 2 (influences HLA class II expression)	6.1
SAT1	Spermidine/spermine N1-acetyltransferase 1	1.6
SMN1	Survival of motor neuron 1, telomeric	1.6
SUZ12	Supressor of zeste 12 homolog (Drosophila)	2.2
THOP1	Thimet oligopeptidase 1	1.8
TFB2M	Transcription factor B2, mitochondrial	1.6
UBE2J1	Ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	5.5
UPF3B	UPF3 regulator of nonsense transcripts homolog B (yeast)	3.2
VSX1	Visual system homeobox 1 homolog, CHX10-like (zebrafish)	1.6
ZNF396	Zinc finger protein 396	1.6
ZRANB2	Zinc finger, RAN-binding domain containing 2	1.9
Unclassified biological processes		
BHLHB9	Basic helix-loop-helix domain containing, class B9	1.6
CEP55	Centrosomal protein 55 kDa	1.7
LENG4	Leukocyte receptor cluster (LRC) member 4	2.7
MRPL33	Mitochondrial ribosomal protein L33	1.6

Table 1A (*Continued*).

Gene symbol	Gene name	Fold-change
MRPL45	Mitochondrial ribosomal protein L45	49.4
ODF4	Outer dense fiber of sperm tails 4	2.7
OR52H1	Olfactory receptor, family 52, subfamily H, member 1	3.5
PJA2	praja 2, RING-H2 motif containing	1.6
RASAL2	RAS protein activator like 2	2.8
RNF6	Ring finger protein (C3H2C3 type) 6	1.8
SMEK1	SMEK homolog 1, suppressor of mek1 (Dictyostelium)	2.8
STK36	Serine/threonine kinase 36, fused homolog (Drosophila)	3.9
UBXD2	UBX domain containing 2	1.8
ZNF407	Zinc finger protein 407	2.0

2.8. Pathway search

The differentially expressed genes of the treatment groups were used in the pathway search in the PANTHER classification system. The human AB 1700 gene list was used as reference gene list and Bonferroni correction for multiple testing was applied (http://www.pantherdb.org/).

2.9. Quantitative real-time RT-PCR

A pool of total RNA of four samples used for microarray analysis was reverse transcribed using Transcriptor First Strand cDNA synthesis kit (Roche) and as described in the manufacturers protocol. TaqMan real-time PCR amplification was performed with an ABI HT7900 Instrument (Applied Biosystems) using

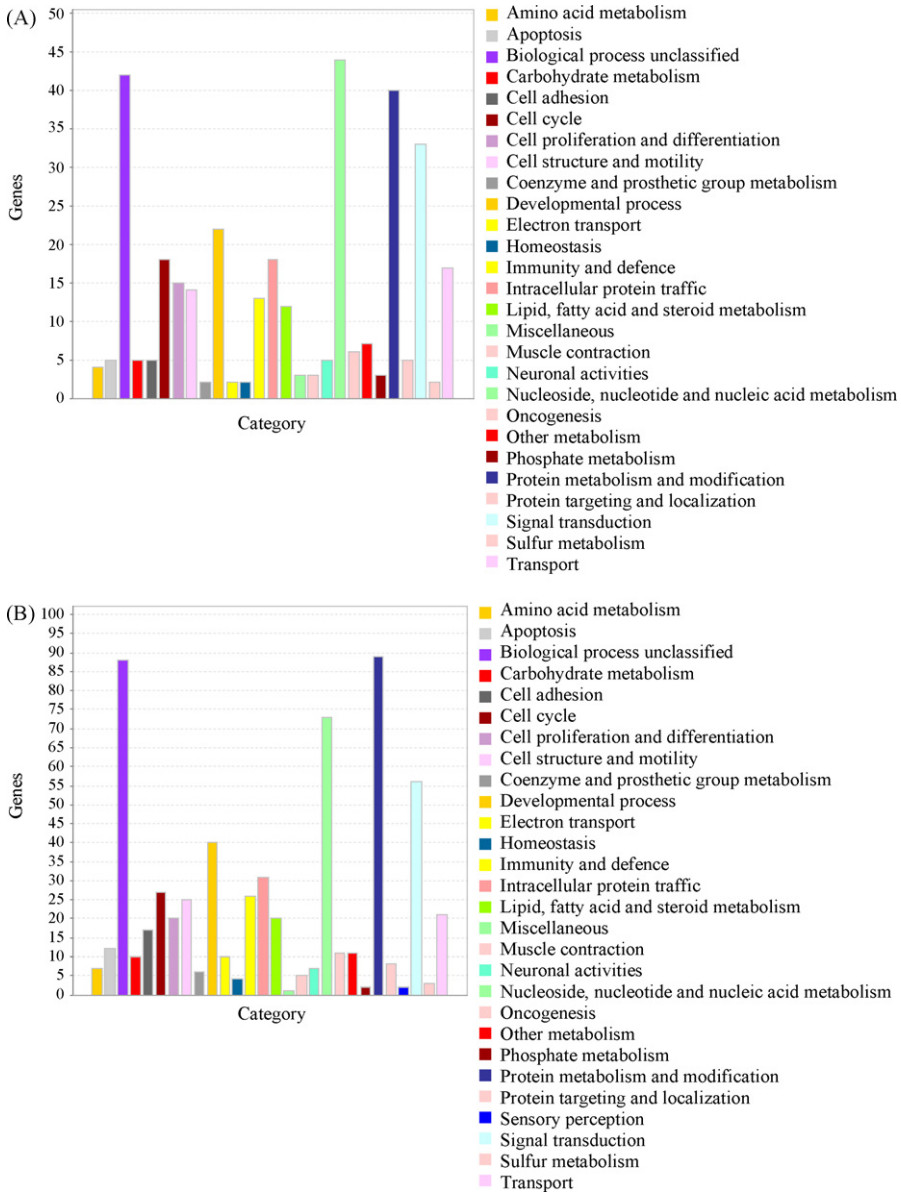

Fig. 1. Classification of up (A)- and down (B)-regulated genes with $p < 0.01$ in Ishikawa cells treated for 4 h with progestin (32 μM) classified for biological processes with the PANTHER classification system using the NCBI database. The figure depicts the number of genes distributed within the different categories, as indicated.

Table 1B
Genes down-regulated by progestin (10 μM) alone (receptor antagonist independent), clustered to the group of biological processes and subdivided into the indicated subgroups (apoptosis, cell adhesion, cell cycle, cell proliferation and differentiation, cell structure and motility, developmental processes, immunity and defence, lipid, fatty acid and steroid metabolism, transport, other biological processes and unclassified biological processes) with $p < 0.01$, and fold-threshold set to ≥1.6.

Gene symbol	Gene name	Fold-change
Apoptosis		
CLUL1	Clusterin-like 1 (retinal)	−1.7
HTRA	Htra serine peptidase 3	−2.2
Cell adhesion		
BCAR1	Breast cancer anti-estrogen resistance 1	−1.6
Cell structure and motility		
MYOM1	Myomesin 1 (skelemin) 185 kDa	−3.1
PARVB	Parvin, beta	−1.6
Developmental processes		
ARID3A	AT rich interactive domain 3A (BRIGHT-like)	−2.1
DCN	Decorin	−16.8
ELTD1	EGF latrophilin and seven transmembrane domain containing 1	−2.5
MBD3	Methyl-CpG binding domain protein 3	−1.6
NCDN	Neurochondrin	−1.7
RNF166	Ring finger protein 166	−1.8
TNC	Tenascin C (hexabrachion)	−1.6
TRIM 15	Tripartite motif-containing 15	−1.7
Immunity and defense		
CCR2	Chemokine (C-C motif) receptor 2	−4.5
HSPB1	Heat shock 27 kDa protein 1	−1.7
Lipid, fatty acid and steroid metabolism		
AMACR	Alpha-methylacyl-CoA racemase	−1.7
Transport		
ATP7B	ATPase, Cu^{2+} transporting, beta polypeptide	−2.9
HCN1	Hyperpolarization activated cyclic nucleotide-gated potassium channel 1	−3.5
TNPO1	Transportin 1	−13.8
Other biological processes		
APBA2	Amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)	−1.7
ALLC	Allantoicase	−3.5
COX15	COX15 homolog, cytochrome c oxidase assembly protein (yeast)	−1.7
DDX55	DEAD (Asp-Glu-Ala-Asp) box polypeptide 55	−2.2
DMRTA2	DMRT-like family A2	−31.9
GPR25	G protein-coupled receptor 25	−14.7
GOPC	Golgi-associated PDZ and coiled-coil motif containing	−3.9
H2AFY2	H2A histone family, member Y2	−2.5
HCCS	Holocytochrome c synthase (cytochrome c heme-lyase)	−1.8
HEPH	Hephaestin	−20.3
IL4I1	Interleukin 4 induced 1	−2.1
KYNU	Kynureninase (L-kynurenine hydrolase)	−3.1
NBEA	Neurobeachin	−29.6
OGDH	Oxoglutarate (alpha-ketoglutarate) dehydrogenase	−1.9
OR10Q1	Olfactory receptor, family 10, subfamily Q, member 1	−9.9
P4HA2	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	−1.8
RKHD1	Ring finger and KH domain containing 1	−1.8
SH3PXD2A	SH3 and PX domains 2A	−2.7
SMARCA5	Subfamily a, member 5	−4.2
ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	−2.3
SNX17	Sorting nexin 17	−1.6
TCEB3	Transcription elongation factor B (SIII), polypeptide 3 (110 kDa, elongin A)	−1.7
TRIM 15	Tripartite motif-containing 15	−1.7
Unclassified biological processes		
C9orf72	Chromosome 9 open reading frame 72	−3.5
C19orf29	Chromosome 19 open reading frame 29	−8.7
COG7	Component of oligomeric golgi complex 7	−3.6
COMMD1	Copper metabolism (Murr) domain containing 1	−2.0
GRIP AP1GRI	GRIP1 associated protein 1	−1.6
MY ADM	Myeloid-associated differentiation marker	−2.6
NOLC1	Nucleolar and coiled-body phosphoprotein 1	−2.5
PIGN	Phosphatidylinositol glycan anchor biosynthesis, class N	−29.6
RBM15B	RNA binding motif protein 15B	−1.7
REXO1	REX1, RNA exonuclease 1 homolog (S. cervisiae)	−1.9
RRP12	Ribosomal RNA processing 12 homolog (*S. cerevisiae*)	−1.7
SH3RF2	SH3 domain containing ring finger 2	−10.9
SLC38A5	Solute carrier family 38, member 5	−12.7
YPEL3	Yippee-like 3 (Drosophila)	−3.0

Table 2A
Genes up-regulated in Ishikawa cells by progesterone receptor antagonist RU-486 (10 μM) independent of progestin, clustered to the group of biological processes and subdivided into the indicated subgroups (apoptosis, cell adhesion, cell cycle, cell proliferation and differentiation, cell structure and motility, developmental processes, lipid, fatty acid and steroid metabolism, transport, other biological processes and unclassified biological processes) with $p < 0.01$, and fold-threshold set to ≥ 1.6.

Gene symbol	Gene name	Fold-increase
Apoptosis		
FGFRL1	Fibroblast growth factor receptor-like 1	1.7
GADD45A	Growth arrest and DNA-damage-inducible, alpha	1.8
Cell adhesion		
GPC1	Glypican 1	
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	1.7
NINJ1	Ninjurin 1	1.7
NLGN2	Neuroligin 2	1.6
PTPRK	Protein tyrosine phosphatase, receptor type, K	1.6
Cell cycle		
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.7
PEO1	Progressive external ophthalmoplegia 1	1.6
S100A11	S100 calcium binding protein A11 1	1.8
SESN2	Sestrin 2	2.7
Cell structure and motility		
ACTB	Actin, beta	
ARTN	Artemin	1.7
CFL1	Cofilin 1 (non-muscle)	1.8
MYL9	Myosin, light chain 9, regulatory	1.7
PLEC1	Plectin 1, intermediate filament binding protein 500 kDa	2.0
Developmental processes		
ARTN	Artemin	
ETV4	ets variant gene 4 (E1 A enhancer binding protein, E1 AF)	1.7
MFGE8	Milk fat globule-EGF factor 8 protein	1.6
MKNK2	MAP kinase interacting serine/threonine kinase 2	1.8
PILRB	Paired immunoglobin-like type 2 receptor beta	1.7
PRSS8	Prostasin	2.1
SALL2	Sal-like (Drosophila)	1.6
SPRY4	Sprouty homolog 4 (Drosophila)	2.0
TIMP1	TIMP metallopeptidase inhibitor 1	1.9
TLE3	Transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	1.6
Lipid, fatty acid and steroid metabolism		
AGPAT1	1-Acylglycerol-3-phosphate O-acyltransferase 1	1.7
RNPEPL1	Arginyl aminopeptidase (aminopeptidase B)-like 1	2.0
Transport		
NXF1	Nuclear RNA export factor 1	1.9
PKD1	Polycystic kidney disease 1 (autosomal dominant)	2.2
SLC4A11	Solute carrier family 4, sodium bicarbonate transporter-like, member 111	1.6
SLC7A5	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	1.7
SLCO2A1	Solute carrier organic anion transporter family, member 2A1	1.8
TM9SF4	Transmembrane 9 superfamily protein member 4	
Other biological processes		
ALDOA	Aldolase A, fructose-bisphosphate	1.9
ALG3	Asparagine-linked glycosylation 3 homolog (*S. cerevisiae*)	1.8
CAMTA2	Calmodulin binding transcription activator 2	1.9
CHGA	Chromogranin A (parathyroid secretory protein 1)	1.9
C8orf20	Receptor accessory protein 4	1.7
ENO2	Enolase 2 (gamma, neuronal)	1.7
IBRDC2	IBR domain containing 2	1.8
MGAT4B	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N acetylglucosaminyltransferase, isozyme B	1.9
MGRN1	Mahogunin, ring finger 1	1.6
NAPSA	Napsin A aspartic peptidase	
NCOR2	Nuclear receptor co-repressor 2	2.2
PHGDH	Phosphogluconate dehydrogenase	1.6
PLXNB2	Plexin B2	1.7
POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A, 220 kDa	2.2
RAD23A	RAD23 homolog A (*S. cerevisiae*)	1.9
RIN1	Ras and Rab interactor 1	1.9
RPLP2	Ribosomal protein, large P2	1.6
SCRIB	Scribbled homolog (Drosophila)	1.7
SNRPA	Small nuclear ribonucleoprotein polypeptide A	2.1
SPINT1	Serine peptidase inhibitor, Kunitz type 1	1.8
SRM	Spermidine synthase	1.8
STC2	Stanniocalcin 2	1.8
STK24	Serine/threonine kinase 24 (STE20 homolog, yeast)	2.0
TACC2	Transforming, acidic coiled-coil containing protein 2	1.7
TRIB3	Tribbles homolog 3 (Drosophila)	3.0
USP22	Ubiquitin specific peptidase 22	1.7
WFDC2	WAP four-disulfide core domain 2	1.8

Table 2A (*Continued*).

Gene symbol	Gene name	Fold-increase
Unclassified biological processes		
C16orf35	chromosome 16 open reading frame 35	
C22orf5	chromosome 22 open reading frame 5	1.9
COL4A1	collagen, type IV, alpha 1	1.6
DDIT3	DNA-damage inducible transcript 3	3.6
EVL	Enah/Vasp-like	1.8
KCTD13	Potassium channel tetramerisation domain containing 13	1.7
KIAA0284	KIAA0284	1.7
MT1B	Metallothionein 1B (functional)	2.2
MT1E	Metallothionein 1E (functional)	1.8
PFN1	Profilin 1	1.7
PHLDA2	Pleckstrin homology-like domain, family A, member 2	2.1
SHB	Src homology 2 domain containing adaptor protein B	1.7
SRRM2	Serine/arginine repetitive matrix 2	1.8
TNFAIP1	Tumor necrosis factor, alpha-induced protein 1(endothelial)	1.6
ZNF580	Zinc finger protein 580	

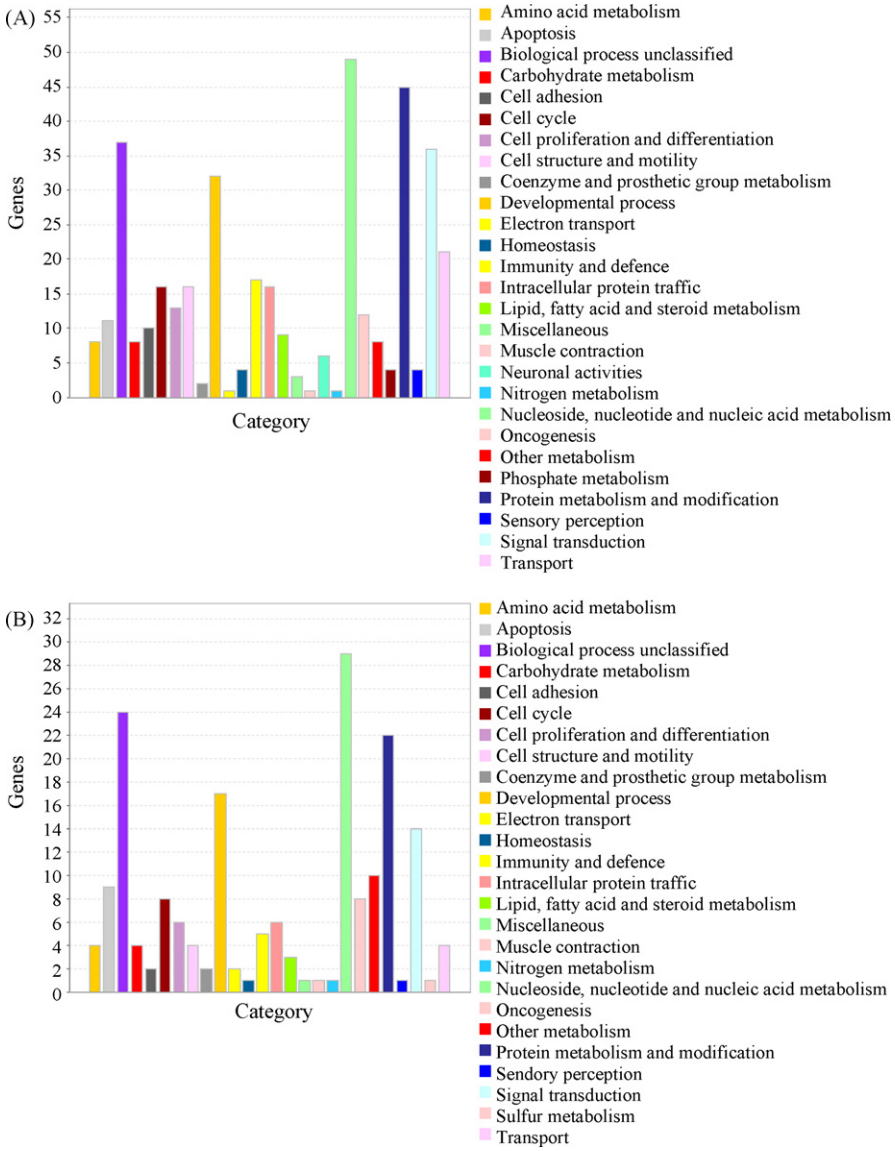

Fig. 2. Classification of up (A)- and down (B)-regulated genes with $p < 0.01$ in Ishikawa cells treated for 4 h with Mifepristone (32 μM) classified for biological processes with the PANTHER classification system using the NCBI database. The figure depicts the number of genes distributed within the different categories, as indicated.

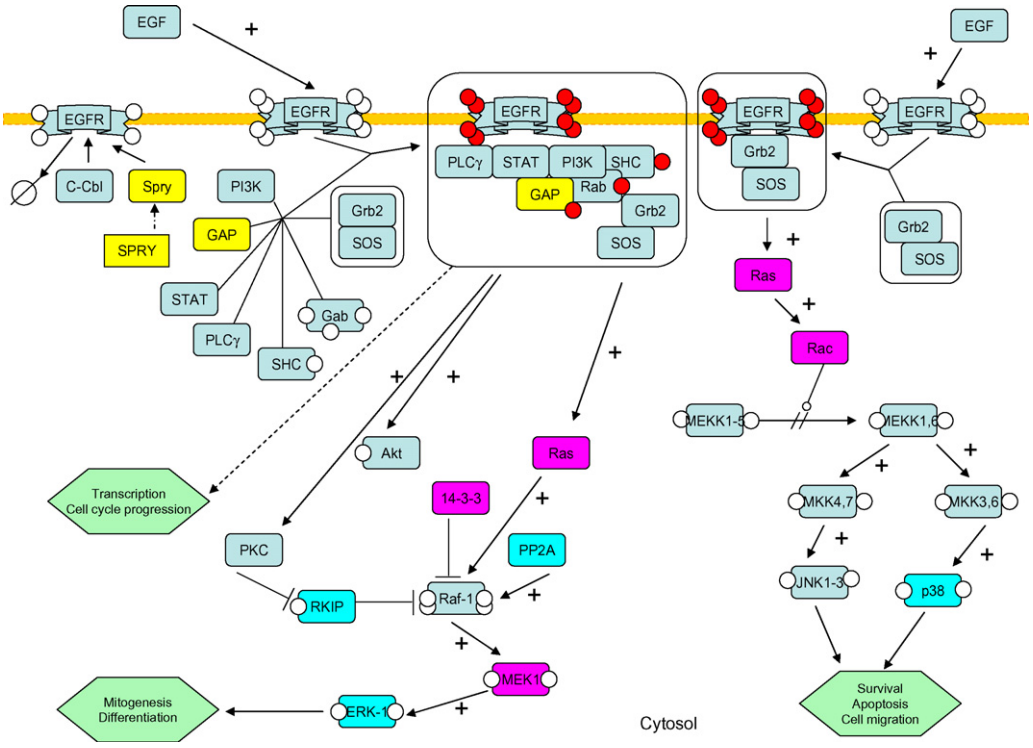

Fig. 3. EGF pathway in Ishikawa cells after treatment with progestin (32 μM) for 4 h. The coloured boxes represent the genes present in the gene list for differentially expressed genes, as indicated. Cyan represents all down- and up-regulated genes with $p < 0.01$, respectively. Yellow indicate genes found in both gene lists, gray represents members of the pathway, green biological processes and red indicates phosphorylation of proteins. The Human AB 1700 gene list, representing all genes of the Human Survey v.2.0 microarray was used as a reference list and Bonferroni for multiple testing was applied for statistical significance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

TaqMan Inventory PCR assays as described by the manufacturer.

The eight assays used for QPCR validation were YWHAQ (Hs00863277_g1), RASA1 (Hs002243115-m1), MAP2K1 (Hs00605615_m1), SFN (Hs00356613_m1), RAC1 (Hs00251654_m1), MAPK12 (Hs00268060_m1), MAPK3 (Hs00385075_m1) and RASAL1 (Hs00183013_m1). Samples for each experiment were run in triplicate and averaged for final quantification. Fold inductions were calculated as described previously [31].

3. Results

In the present study, the molecular responses in the endometrial cancer cell line Ishikawa receiving progestin or glucocorticoid antagonist Mifepristone for 4 h were studied by measuring genome-wide transcript-level changes using oligo microarrays representing 24,650 human genes and 37,580 gene transcripts. 6154 genes remained after pre-processing and filtering (see Supplemental Table S1). Genes that appeared to be regulated in all independent cell experiments were used for further analysis. The fold change expression level threshold was set to 1.5 with $p < 0.01$. This resulted in a total of 993 differentially expressed genes of which 550 were up-regulated and 443 were down-regulated. The 993 up-regulated genes were found to be distributed within the different treatment groups with189 genes for progesterone and 255 genes for Mifepristone (Tables 1A and 2A). The 550 down-regulated genes were distributed with 256 genes for progesterone- and 127 genes for Mifepristone (Tables 1B and 2B). To facilitate interpretation of the results, genes were grouped according to biological processes and molecular functions in which they are likely to be involved. Several genes were classified for more than one of the biological processes. Therefore, classifications of genes differentially expressed in the different treatment groups (progesterone and Mifepristone) were carried out by using the PANTHER database and the results of up- and down-regulated genes are depicted (Figs. 1A, 1B, 2A and 2B). In addition, the PANTHER pathway search algorithm was used to identify signaling pathways that were over-represented in the gene lists using genes expressed with a $p < 0.01$ and the Bonferroni correction for multiple testing. The results show that genes presenting the epidermal growth factor (EGF) were significantly over-represented by progesterone treatment (Fig. 3), whereas genes involved in the p53 pathway were significantly over-represented by Mifepristone treatment (data not shown).

The gene groups obtained for progesterone and Mifepristone treatment were further filtered to define the solely effects of the treatment components by excluding genes with p-values > 0.1 in the corresponding treatment group (e.g. progesterone vs. Mifepristone and Mifepristone vs. progesterone) and with a fold-change threshold set to ≥1.6. This resulted in 123 differential expressed genes solely influenced by progesterone of which 67 were up-regulated and 56 were down-regulated (Tables 1A and 1B), and 88 differentially expressed genes solely influenced by RU-486 of which 69 were up-regulated and 14 were down-regulated (Tables 2A and 2B).

Genes that were differentially expressed by progestin and are represented in the EGF/MAP kinase pathway, were validated by quantitative real-time PCR as described in Section 2. Prior to RT-PCR analysis RNA preparations of all experiments were verified for possible genomic DNA contamination by running a minus-RT-PCR directly on RNA samples and human genomic DNA as a positive control testing the housekeeping gene cyclophilin A. Genomic DNA was not detected in any of the RNA preparations (data not shown). The RT-PCR results obtained for 10 single genes are summarized in Fig. 3. All of the probes tested by RT-PCR gave detectable results. Similar expression changes for microarray and RT-PCR have been

Table 2B
Genes down-regulated in Ishikawa cells by progesterone receptor antagonist RU-486 (10 μM) independent of progestin, clustered to the group of biological processes and subdivided into the indicated subgroups (cell cycle, cell proliferation and differentiation, cell structure and motility, developmental processes, transport, other biological processes and unclassified biological processes) with $p < 0.01$, fold-change threshold set to ≥1.6.

Gene symbol	Gene name	Fold-decrease
Cell cycle		
RFC5	Replication factor C (activator 1) 5, 36.5 kDa	−1.6
Cell proliferation and differentiation		
RACGAP1	Rac GTPase activating protein 1	−1.7
Developmental processes		
EIF3S3	Eukaryotic translation initiation factor 3, subunit 3 gamma, 40 kDa	−1.6
RUVBL1	RuvB-like 1 (*E. coli*)	−1.6
Transport		
ATP1B3	ATPase, Na^+/K^+ transporting, beta 3 polypeptide	−1.6
Other biological processes		
IGFBP3	Insulin-like growth factor binding protein 3	−1.8
IL1R2	Interleukin 1 receptor, type II	−1.8
LDHB	Lactate dehydrogenase B	−1.9
MARS	Methionine-tRNA synthetase	−1.6
NDUFA9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9, 39 kDa	−1.8
PSMB7	Proteosome (prosome, macropain) subunit, beta type, 7	−1.6
Unclassified biological processes		
AIG1	Androgen-induced 1	−1.8
CKAP4	Cytoskeleton-associated protein 4	−2.0
TMEM59	Transmembrane protein 59	−1.8

observed for all tested genes. The differential expression of all 10 genes tested was confirmed by RT-PCR [32].

4. Discussion

A wide array of genes was significantly influenced in this short time high dose study of progesterone as well as by Mifepristone in endometrial cancer cell model. Interestingly, Mifepristone seemed to modulate the p53 regulating pathway, however, another conspicuous finding was the modulation of numerous members of the EGF/MAP kinase pathway mostly induced by progestins but also to some degree by Mifepristone (Tables 1A, 1B, 2A and 2B, Figs. 1A–3).

4.1. Expression of genes regulating p53, cell cycle, and apoptosis

In a recently published study, we demonstrated, the p53 related growth arrest and DNA-damage-inducible gene, GADD45A, to be up-regulated in Ishikawa cells by high-dose progesterone stimulation [11]. Comparable induction was also demonstrated in the current results for Mifepristone showing a 1.8-fold increase in GADD45A expression (Table 2A). In another study Kovalsky et al. showed that the GADD45A gene, known to be induced by genotoxic substances and other cellular stresses, was also significantly induced after high-dose progesterone [33].

Another interesting finding of the present study was the induction of death receptor 4, encoded by the tumor necrosis factor receptor subfamily member 10 gene (TNFRSF10A) which was induced by progesterone (Table 1A). This gene has been considered as an important mediator of apoptosis and its dysfunction may be related to cancer development and distant tumor spread. The role of the TNFRSF10A polymorphism in metastatic progression of prostate cancer after radiation therapy concluding that this gene might be a novel independent risk factor for prostate cancer metastases after radiation therapy [34]. Also tumor necrosis factor alpha induced protein 1 (endothelial); (TNFA1P1) is significantly up-regulated by Mifepristone stimulation in our current study. On the other hand, demonstrating the complexity of the interaction, the tumor necrosis factor receptor super family, member 12A (TNF RSF12A) was down-regulated when Mifepristone was added (Table 2B). The present results also revealed that the cyclin-dependent kinase inhibitor 1A (p21 cip1) named CDKN1A, was up-regulated by Mifepristone (Table 2A). Differently, Heikinheimo et al. reported that levels of cyclin-B, but not of p21 mRNA were markedly increased following RU486. This was presumed to be associated with a cell-cycle block at the G2-M interphase [35]. Whether mechanisms similar to these are associated with the beneficial clinical effects of RU486 observed in the treatment of various hormone dependent diseases remains to be determined [36]. RU486 also showed to up-regulate the sestrin 2 (SESN2), which was a significant finding after progestin stimulation in our former study [11]. Correspondingly, Donadelli et al. report that increased expression of p53 and the p53-regulated antioxidant SESN2 was correlated to decreased tumor volume in pancreatic carcinoma cells [37]. Also CDK 2 (cyclin-dependent kinase 2) (not shown in tables) were up-regulated by combination of antagonist and agonist, however, this biological effect is hard to explain. In a study by Meng et al. of ovarian cancer cell, it was shown that proliferation associated with the induction of p27 (KIP1) inhibiting cell cycle-associated proteins including cyclin D1 and CDK2 was reduced [38]. Estrogen-like effects of RU 486 have been described in a MCF-7 cell line showing increased proliferation after low doses of RU486 expressing reduced levels of TGFβ2 and TGFβ3 mRNA but not of TGF TGFβ1 mRNA. Similar mechanisms may explain some of the differential gene expression observed for progesterone and Mifepristone in the present study [39].

4.2. Expression of genes regulating EGF/MAP-kinase pathway

The complexity of the EGF/MAP kinase activated signaling pathways is focusing interest on the understanding of cellular growth regulation and the identification of additional modifiers, and particularly the RAS–Raf–MEK–ERK pathways have been studied [40]. In the present study the ras related protein RAC1, and the RAS protein activator like 2 (RAS-AL 2) showed to be significantly up-regulated by progesterone (Table 1A). These finding are consistent with the results of Sarkisian et al. showing that regulation of RAS activates tumor suppressor pathways and triggers an irreversible senescent growth arrest in vivo [41]. They suggest a three-stage model for RAS-induced tumor genesis consisting of an initial activating RAS mutation, over-expression of the activated RAS allele and, finally, evasion of p53-Ink4a-Arf-senescence checkpoints [41]. Also other related genes like RAS p21 protein activator (GTPase activating protein)1, (RASA1), and RAB5A, member RAS oncogene family (RAB5A) were significantly up-regulated by progesterone alone (Table 1A). Specificity of regulation is achieved for multiple members of the MAP kinase family by organization of MAP modules by use of scaffolding and anchoring proteins bringing together specific kinases for selective activation, sequestration and localization of signaling complexes [40]. Additionally, the Ras and Rab interactor1 (RIN1) and the Map kinase interacting serine/threonine kinase 2 (MKNK2) were induced by Mifepristone (Table 2A). The latter gene was also shown to be up-regulated by progesterone with a concentration of 30 μg/ml in our recently published study [11]. Thus, the MAP-kinase induced genes showed to be regulated by progesterone as well as by Mifepristone in the present study. In addition, the MAP kinase family related gene, dual specificity phosphatase 6 (DUSP6), was up-regulated by progesterone in the present study (Table 1A). The importance of the DUSP6 gene in human cancer progression has also been described by Furukawa

et al., suggesting that the abrogation of DUSP6 is associated with progression from pancreatic intraepithelial neoplasia to the invasive ductal carcinoma, these results were also associated with mutated KRAS2 [42].

4.3. Expression of genes regulating zinc finger proteins and metallothioneins

Several genes coding for zinc finger molecules and related proteins crucial for steroid receptor function were significantly up-regulated in the present study by progesterone as well as by Mifepristone. Thus zinc finger binding protein 396 (ZNF 396) and zinc finger RAN-binding domain containing 2 (ZRANB2) were up-regulated by progesterone (Table 1A) and zinc finger binding protein ZNF 580 was induced by Mifepristone (Table 2A). Although, little is known about high-dose progesterone or Mifepristone influence of these complex structures, however, such effects may be considered as another contribution to steroid receptor modulation. Reversible zinc exchange has been described between metallothioneins and estrogen receptor zinc finger [43]. Thus, metallothioneins may also show a strong affinity to the zinc rich progesterone receptor binding zinc fingers, representing still another potential control factor for the down-regulation of the steroid receptor. Metallothioneins being cystein rich, zinc and copper binding, low molecular weight proteins, have shown to be hormone dependent and expression of metallothioneins in the secretory transformed human endometrium and in other species under influence of progesterone, have been described [43–45]. Progestins, known to induce apoptosis in the endometrial mucosa, are important as regulatory mechanism in physiological as well as pathological conditions [7]. According to different authors metallothioneins may be considered as an attempt to rescue cells trying to minimize apoptotic events in the rat ovary during peri-ovulatory period under the influence of HCG hormone [46–48]. In endometrial proliferative conditions metallothioneins have been shown to be highly expressed in benign hyperplastic endometrial lesions but elevated metallothionein expression was associated with higher grade and stage of endometrial carcinoma [49] (Table 2A).

4.4. Expression of genes regulating other biological processes

In the present study the heparin sulfate (glucosamine) 3-O-sulfotransferase 2 (HS3ST2) gene being related to immunity and cell defense was significantly induced with a fold-change of 12.0 when stimulated by progesterone (Table 1A). When this gene was studied in cervical cytology specimens with high-grade squamous intraepithelial lesions, hypermethylation of HS3ST2 in 93% of cancer tissues and 70% of cytology specimens interpreted as CIN3 was reported, indicating HS3ST2 as potential markers of cervical cancer and its precursor lesions [43,50]. Some genes related to other biological categories showed more that 20-fold reduction after progesterone treatment like the DMRT-like family A2 (DMRTA2) and the hepaestin (HEPH) described with 31.9- and 20.3-fold reduction, respectively. However, no other studies can be detected relating these genes growth regulation (Table 1B).

New therapeutic targets for intrauterine treatment may be of importance to control endometrial cancer and its preliminary stages, but also as candidate markers for therapy response or as markers for tumor progression. Thus, studies of material from patients with different endometrial proliferative disorders receiving different treatment regimens have to be studied with similar techniques.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2008.12.003.

References

[1] A. Ferenczy, M. Gelfand, The biologic significance of cytologic atypia in progestogen-treated endometrial hyperplasia, Am. J. Obstet. Gynecol. 160 (1989) 126–131.
[2] D. Gal, Hormonal therapy for lesions of the endometrium, Semin. Oncol. 13 (1986) 33–36.
[3] B. Lindahl, P. Alm, M. Ferno, A. Norgren, Endometrial hyperplasia: a prospective randomized study of histopathology, tissue steroid receptors and plasma steroids after abrasio, with or without high dose gestagen treatment, Anticancer Res. 10 (1990) 725–730.
[4] B. Lindahl, R. Willen, Endometrial hyperplasia. Clinico-pathological considerations of a prospective randomised study after abrasio only or high-dose gestagen treatment. Results of 2 years follow-up of 292 patients, Anticancer Res. 11 (1991) 403–406.
[5] T.C. Randall, R.J. Kurman, Progestin treatment of atypical hyperplasia and well-differentiated carcinoma of the endometrium in women under age 40, Obstet. Gynecol. 90 (1997) 434–440.
[6] J.T. Thigpen, M.F. Brady, R.D. Alvarez, M.D. Adelson, H.D. Homesley, A. Manetta, J.T. Soper, F.T. Given, Oral medroxyprogesterone acetate in the treatment of advanced or recurrent endometrial carcinoma: a dose-response study by the Gynecologic Oncology Group, J. Clin. Oncol. 17 (1999) 1736–1744.
[7] A.B. Vereide, M. Arnes, B. Straume, J.M. Maltau, A. Orbo, Nuclear morphometric changes and therapy monitoring in patients with endometrial hyperplasia: a study comparing effects of intrauterine levonorgestrel and systemic medroxyprogesterone, Gynecol. Oncol. 91 (2003) 526–533.
[8] N.S. Kumar, J. Richer, G. Owen, E. Litman, K.B. Horwitz, K.K. Leslie, Selective down-regulation of progesterone receptor isoform B in poorly differentiated human endometrial cancer cells: implications for unopposed estrogen action, Cancer Res. 58 (1998) 1860–1865.
[9] A. Orbo, M. Arnes, C. Hancke, A.B. Vereide, I. Pettersen, K. Larsen, Treatment results of endometrial hyperplasia after prospective D-score classification A follow-up study comparing effect of LNG-IUD and oral progestins versus observation only, Gynecol. Oncol. 111 (1) (2008 Oct) 68–73, Epub 2008 Aug 6.
[10] A.B. Vereide, T. Kaino, G. Sager, M. Arnes, A. Orbo, Effect of levonorgestrel IUD and oral medroxyprogesterone acetate on glandular and stromal progesterone receptors (PRA and PRB), and estrogen receptors (ER-alpha and ER-beta) in human endometrial hyperplasia, Gynecol. Oncol. 101 (2006) 214–223.
[11] R.H. Paulssen, B. Moe, H. Gronaas, A. Orbo, Gene expression in endometrial cancer cells (Ishikawa) after short time high dose exposure to progesterone, Steroids 73 (2008) 116–128.
[12] S.A. Leonhardt, D.P. Edwards, Mechanism of action of progesterone antagonists, Exp. Biol. Med. (Maywood) 227 (2002) 969–980.
[13] E.A. Musgrove, R.L. Sutherland, Effects of the progestin antagonist RU 486 on T-47D breast cancer cell cycle kinetics and cell cycle regulatory genes, Biochem. Biophys. Res. Commun. 195 (1993) 1184–1190.
[14] I.M. Spitz, Progesterone antagonists and progesterone receptor modulators: an overview, Steroids 68 (2003) 981–993.
[15] M.A. Navo, J.A. Smith, A. Gaikwad, T. Burke, J. Brown, L.M. Ramondetta, In vitro evaluation of the growth inhibition and apoptosis effect of mifepristone (RU486) in human Ishikawa and HEC1A endometrial cancer cell lines, Cancer Chemother. Pharmacol. 62 (2008) 483–489.
[16] C.C. Schneider, R.K. Gibb, D.D. Taylor, T. Wan, C. Gercel-Taylor, Inhibition of endometrial cancer cell lines by mifepristone (RU 486), J. Soc. Gynecol. Investig. 5 (1998) 334–338.
[17] Y. Sun, p53 and its downstream proteins as molecular targets of cancer, Mol. Carcinog. 45 (2006) 409–415.
[18] S. Han, N. Sidell, RU486-induced growth inhibition of human endometrial cells involves the nuclear factor-kappa B signaling pathway, J. Clin. Endocrinol. Metab. 88 (2003) 713–719.
[19] A. Li, J.C. Felix, P. Minoo, C.A. Amezcua, J.K. Jain, Effect of mifepristone on proliferation and apoptosis of Ishikawa endometrial adenocarcinoma cells, Fertil. Steril. 84 (2005) 202–211.
[20] A. Skildum, E. Faivre, C.A. Lange, Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases, Mol. Endocrinol. 19 (2005) 327–339.
[21] N.R. Nayak, O.D. Slayden, K. Mah, K. Chwalisz, R.M. Brenner, Antiprogestin-releasing intrauterine devices: a novel approach to endometrial contraception, Contraception 75 (2007) S104–S111.
[22] R.N. Brogden, K.L. Goa, D. Faulds, Mifepristone, A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential, Drugs 45 (1993) 384–409.

[23] F. Cadepond, A. Ulmann, E.E. Baulieu, RU486 (mifepristone): mechanisms of action and clinical uses, Annu. Rev. Med. 48 (1997) 129–156.
[24] M. Nishida, The Ishikawa cells from birth to the present, Hum. Cell. 15 (2002) 104–117.
[25] Y. Berthois, J.A. Katzenellenbogen, B.S. Katzenellenbogen, Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 2496–2500.
[26] P. Soille, Morphological image analysis: principles and applications (1999), pp. 71–73.
[27] W. Cleveland, Robust locally weighted regression and smoothing scatterplots, J. Am. Stat. Assoc. 74 (1979) 829–836.
[28] V. Tusher, J. Tibshirani, G. Chu, Significance analysis of microarrays applied to ionizing radiation response, Proc. Natl. Acad. Sci. PNAS 98 (2001) 5116–5124.
[29] O. Troyanskaya, M. Cantor, G. Sherlock, P. Brown, T. Hastie, R. Tibshirani, D. Botstein, R.B. Altman, Missing value estimation methods for DNA microarrays, Bioinformatics 17 (2001) 520–525.
[30] A. Brazma, P. Hingkamp, J. Quackenbush, G. Sherlock, P. Spellmann, C. Stoeckert, J. Aach, W. Ansorge, C.A. Ball, H.C. Causton, T. Gaasterland, P. Glennison, F.C. Hofstege, I.F. Kim, V. Markowitz, J.C. Matese, H. Parkinson, A. Robinson, U. Sarkans, S. Schulze-Kremer, J. Stewart, R. Taylor, J. Vilo, M. Vingron, Minimum information about a microarray experiment (MIAME)-toward standards for microarray data, Nat. Genet. 29 (2001) 365–371.
[31] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[32] H. Chaib, E.K. Cockrell, M.A. Rubin, J.A. Macoska, Profiling and verification of gene expression patterns in normal and malignant human prostate tissues by cDNA microarray analysis, Neoplasia 3 (2001) 43–52.
[33] O. Kovalsky, F.D. Lung, P.P. Roller, A.J. Fornace Jr., Oligomerization of human Gadd45a protein, J. Biol. Chem. 276 (2001) 39330–39339.
[34] T. Langsenlehner, K.S. Kapp, U. Langsenlehner, TGFB1 single-nucleotide polymorphisms are associated with adverse quality of life in prostate cancer patients treated with radiotherapy. In regard to peters et al. (Int j radiat oncol biol phys 2008; 70:752–759), Int. J. Radiat. Oncol. Biol. Phys. 71 (2008) 960.
[35] O. Heikinheimo, J.G. Hsiu, K. Gordon, S. Kim, R.F. Williams, W.E. Gibbons, G.D. Hodgen, Endometrial effects of RU486 in primates—antiproliferative action despite signs of estrogen action and increased cyclin-B expression, J. Steroid Biochem. Mol. Biol. 59 (1996) 179–190.
[36] M.E. Taplin, J. Manola, W.K. Oh, P.W. Kantoff, G.J. Bubley, M. Smith, D. Barb, C. Mantzoros, E.P. Gelmann, S.P. Balk, A phase II study of mifepristone (RU-486) in castration-resistant prostate cancer, with a correlative assessment of androgen-related hormones, BJU Int. 101 (2008) 1084–1089.
[37] M. Donadelli, P.E. Dalla, C. Costanzo, M.T. Scupoli, P. Piacentini, A. Scarpa, M. Palmieri, Increased stability of P21(WAF1/CIP1) mRNA is required for ROS/ERK-dependent pancreatic adenocarcinoma cell growth inhibition by pyrrolidine dithiocarbamate, Biochim. Biophys. Acta 1763 (2006) 917–926.
[38] Q. Meng, C. Xia, J. Fang, Y. Rojanasakul, B.H. Jiang, Role of PI3K and AKT specific isoforms in ovarian cancer cell migration, invasion and proliferation through the p70S6K1 pathway, Cell Signal. 18 (2006) 2262–2271.
[39] M.H. Jeng, S.M. Langan-Fahey, V.C. Jordan, Estrogenic actions of RU486 in hormone-responsive MCF-7 human breast cancer cells, Endocrinology 132 (1993) 2622–2630.
[40] T.P. Garrington, G.L. Johnson, Organization and regulation of mitogen-activated protein kinase signaling pathways, Curr. Opin. Cell Biol. 11 (1999) 211–218.
[41] C.J. Sarkisian, B.A. Keister, D.B. Stairs, R.B. Boxer, S.E. Moody, L.A. Chodosh, Dose-dependent oncogene-induced senescence in vivo and its evasion during mammary tumorigenesis, Nat. Cell Biol. 9 (2007) 493–505.
[42] T. Furukawa, R. Fujisaki, Y. Yoshida, N. Kanai, M. Sunamura, T. Abe, K. Takeda, S. Matsuno, A. Horii, Distinct progression pathways involving the dysfunction of DUSP6/MKP-3 in pancreatic intraepithelial neoplasia and intraductal papillary-mucinous neoplasms of the pancreas, Mod. Pathol. 18 (2005) 1034–1042.
[43] D.F. Cano-Gauci, B. Sarkar, Reversible zinc exchange between metallothionein and the estrogen receptor zinc finger, FEBS Lett. 386 (1996) 1–4.
[44] C.I. Ace, W.C. Okulicz, Microarray profiling of progesterone-regulated endometrial genes during the rhesus monkey secretory phase, Reprod. Biol. Endocrinol. 2 (2004) 54.
[45] L.C. Giudice, Application of functional genomics to primate endometrium: insights into biological processes, Reprod. Biol. Endocrinol. 4 (Suppl. 1) (2006) S4.
[46] L.L. Espey, T. Ujioka, H. Okamura, J.S. Richards, Metallothionein-1 messenger RNA transcription in steroid-secreting cells of the rat ovary during the periovulatory period, Biol. Reprod. 68 (2003) 1895–1902.
[47] M. Penkowa, J. Hidalgo, Metallothionein treatment reduces proinflammatory cytokines IL-6 and TNF-alpha and apoptotic cell death during experimental autoimmune encephalomyelitis (EAE), Exp. Neurol. 170 (2001) 1–14.
[48] G.T. Tsangaris, F. Tzortzatou-Stathopoulou, Metallothionein expression prevents apoptosis: a study with antisense phosphorothioate oligodeoxynucleotides in a human T cell line, Anticancer Res. 18 (1998) 2423–2433.
[49] W.G. McCluggage, P. Maxwell, P.W. Hamilton, B. Jasani, High metallothionein expression is associated with features predictive of aggressive behaviour in endometrial carcinoma, Histopathology 34 (1999) 51–55.
[50] N. Shivapurkar, M.E. Sherman, V. Stastny, C. Echebiri, J.S. Rader, R. Nayar, T.A. Bonfiglio, A.F. Gazdar, S.S. Wang, Evaluation of candidate methylation markers to detect cervical neoplasia, Gynecol. Oncol. 107 (2007) 549–553.