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行政院國家科學委員會專題研究計畫 成果報告

以微矩陣排列晶片偵測性腺釋放激素在人類胎盤細胞上的基因調節功能

計畫類別： 個別型計畫

計畫編號： NSC 94－2314－B－038－036

執行期間： 94 年 08 月 01 日 至 95 年 07 月 31 日

執行單位： 臺北醫學大學婦產科

計畫主持人： 周遵善

共同主持人： 曾啟瑞

報告類型： 精簡報告

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計畫主持人：«主持人» 周遵善

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成果報告類型(依經費核定清單規定繳交)：□期中報告　■期末報告

Elucidation of the functional roles of GnRH I and GnRH II in human placenta under the microarray basis

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一、中文摘要

以微矩陣排列晶片偵測性腺釋放激素在人類胎盤細胞上的基因調節功能

近年來微矩陣排列晶片已經普遍應用在研究大量基因調控上，我們希望運用這個平台去篩選 GnRH Ⅰ， GnRH II 在人類胎盤細胞上的基因調節功能，同時我們在縱向運用微矩陣排列晶片來分析不同時間中 GnRH Ⅰ， GnRH II 對不同基因的調節，之後，再運用生物資訊分析不同時間中受調節的基因之質量改變，便可順利分析出 GnRH Ⅰ， GnRH II 在人類胎盤細胞上的功能，並將其訊息傳遞路徑闡明。

我們過去五年的研究中發現 GnRH Ⅰ， GnRH II 不但在下視丘有表達， 而且在胎盤也有 GnRH Ⅰ及 GnRH II 蛋白質的分泌， 我們也發現 GnRH Ⅰ， GnRH II 自胎盤分泌出來之後可活化蛋白酶，進而利於胎盤進入子宮內膜。GnRH Ⅰ， GnRH II 於胎盤及子宮內膜內表達，它們在細胞內的訊息傳遞路徑為何至今尚未明瞭。如果能夠瞭解它們在細胞內的訊息傳遞方法，不但可以知道這兩種荷爾蒙如何作用，也可藉此增加或降低它們在細胞內訊息傳遞的強度，以控制這兩種荷爾蒙的功能。

本計畫運用了微矩陣排列晶片及生物資訊分析這兩種荷爾蒙在胎盤細胞內訊息傳遞的路徑並闡明其在胎盤細胞上的功能。

GnRH Ⅰ， GnRH II 於胎盤細胞上的功能及訊息傳遞路徑至今尚未明瞭，證據顯示 GnRH 對早期胚胎發育與著床以及胎盤的形成扮演著關鍵的角色，除此之外，GnRH Ⅰ，GnRH II 似乎對細胞外間質的重塑也有某種程度之影響。 細胞外間質的重塑過程如果有任何錯誤將會導致許多臨床上的疾病，然而控制這一群蛋白酶系統的因素至今尚未完全被了解。但是在臨床上發現 GnRH 的使用可以增加胚胎的著床及試管嬰兒的成功率， 我們在一系列的研究中發現 GnRH 似乎與 uPA 及 MMP 和它們的抑制劑 PAI 及 TIMP 等蛋白酶系統間有著相當密切的關聯。

在此研究結果中， 我們發現受 GnRH Ⅰ調節兩倍以上的基因約有 61 個，向上調節的基因有 50 個，向下調節的基因有 11 個，另一方面受 GnRH II 調節兩倍以上的基因約有 36 個，向上調節的基因有 14 個，向下調節的基因有 22 個。以基因分類看來受 GnRH 調節的基因可分為訊息傳遞、細胞生長、分裂、蛋白胨、核酸等等。

由微矩陣排列晶片偵測性腺釋放激素在人類胎盤細胞上的基因調節功能的結果中分析看來，GnRH 似乎在人類胎盤細胞上掌管生長、分裂以及分化等重要功能。

關鍵詞：性腺釋放激素、微矩陣排列晶片 。

Abstract

Elucidation of the functional roles of GnRH I and GnRH II in human placenta under the microarray basis

The spatiotemporal expression of GnRH I and GnRH II in human placenta is believed to exert their functions, at least in part, the process of placenta invasion and embryo development. In particular, MMP-2 and MMP-9 expression has been associated with the GnRH I and GnRH II in human trophoblastic cells. The composition of the decidual ECM is also modulated by MMPs secreted by the trophoblastic cells of the implanting embryo. To date,the functional roles and signaling transduction pathways of GnRH I and GnRH II in human placenta remain poorly characterized.

Microarray techniques have emerged as important approaches for the simultaneous analysis of multiple gene transcripts. These methods have proven valuable in providing qualitative assessment of the global gene programs that accompany cell division, development, and the responses to specific stimuli. It has been shown that GnRH I and GnRH II play regulatory roles in human implantation and placentation. To date, the regulatory effects of down stream genes by these two hormones in trophoblasts remains poorly characterized. We validate the microarrays data from trophoblastic cells treated by GnRH I and GnRH II in different time periods and analyze all the gene changes at different time scales by bioinformatics. The data analyses resulting from DNA microarray may help verify the downstream genes and the signaling transduction pathways of GnRH I and GnRH II in human placenta.

Owing to their capacity to induce strong, sequence-specific, gene silencing in cells, short interfering RNAs (siRNAs) represent new tools to elucidate the signaling transduction pathways and functions of GnRH I and GnRH II in human placenta. In the present study, siRNA techniques are applied for these purposes.

In the past five years, we found GnRH I and GnRH II are expressed not only in human hypothalamus but also in human placenta and endometrial tissues. We also demonstrated that both GnRH I and GnRH II were capable of regulating the expression of uPA and MMP proteolytic systems at the maternal-fetal interface to facilitate placenta invasion and placentation.

In the present studies, we analyzed 13420 genes on a DNA chip to identify the regulatory net work of GnRH I and GnRH II. The microarray data showed that there are 61 genes regulated above two times by GnRH I . Among those genes, 50 genes were upregulated. In contrast, 11 genes were down regulated. On the other side, there are

36 genes regulated above two times by GnRH II. Of which 14 genes were upregulated and 22 genes were down regulated. Taken together, genes regulated by either GnRH I or GnRH II could be categorized as signaling transduction, cell growth, mitosis, differentiation, proteas, and nucleic acid etc.

Key words: GnRH I, GnRH II, Placenta, **microarray**, Signaling transduction, siRNA

Introduction

The establishment of a successful pregnancy is dependent on the coordinated development of the implanting embryo and the maternal endometrium (Tabibzadeh and Babaknia, 1995; Paria et al., 2000; 2002). In particular, the endometrium must have undergone a series of hormonally-regulated morphogenetic events in preparation for pregnancy. The blastocyst, in turn, must have attained the ability to interact with the diverse cell types that constitute the endometrium and subsequently form a functional placenta. As the endometrium is only receptive to the implanting embryo during a defined period of the menstrual cycle, known as the "window of implantation" (Hertig and Rock, 1956; Nikas, 1999; Wilcox et al., 1999), dyschrony in the spatial or molecular development of the maternal and/or the fetal compartment often results in spontaneous abortion and, in less severe cases, defects in the formation and organization of the placenta that often compromise continuation of pregnancy to term (Paria et al., 2000, 2002; Kao et al., 2002).

Microarray techniques have emerged as important approaches for the simultaneous analysis of multiple gene transcripts. These methods have proven valuable in providing qualitative assessment of the global gene programs that accompany cell division, development, and the responses to specific stimuli. It has been shown that GnRH I and GnRH II play regulatory roles in human implantation and placentation. To date, the regulatory effects of down stream genes by these two hormones in trophoblasts remains poorly characterized. We analyze the microarrays data from trophoblastic cells treated by GnRH I and GnRH II in different time periods and validate all the gene changes by bioinformatics. The data analyses resulting from DNA microarray may help verify the downstream genes and the signaling transduction pathways of GnRH I and GnRH II in human placenta. In these studies, we have examined the gene responses of GnRH I and GnRH II receptor-couple organization in the human trophoblasts.

Owing to their capacity to induce strong, sequence-specific, gene silencing in cells, short interfering RNAs (siRNAs) represent new tools to elucidate the signaling transduction pathways and functions of GnRH I and GnRH II in human placenta. In the present study, siRNA techniques are applied for these purposes.

In the present studies, a model for analysis of GnRH gene network is

established. We have determined the genes regulated by GnRHs and tried to identify the effects of SiRNA on GnRH functions.

Materials and Methods

Tissue and cell isolation

Samples of first trimester placental tissues were obtained from women undergoing elective termination of pregnancy (gestational ages ranging from 6–12 wk). The use of these tissues was approved by the Committee for Ethical Review of Research on the use of human subjects. All women provided informed written consent.

Trophoblast

Extravillous cytotrophoblasts (EVTs) were propagated from first trimester placental explants as described by Graham *et al.* Briefly, chorionic villi were washed thoroughly in DMEM (Life Technologies, Inc., Burlington, Ontario, Canada) containing antibiotics. The villi were minced finely and plated in 25-cm^2 tissue culture flasks containing DMEM supplemented with antibiotics and 10% heated-inactivated fetal bovine serum (FBS). The fragments of chorionic villi were allowed to adhere for 2–3 d, after which the nonadherent material was removed. The villous explants were cultured for a further 10–14 d with the culture medium being replaced every 48 h. The EVTs were separated from the villous explants by a brief (2–3 min) trypsin digestion [0.125% (vol/vol) trypsin-EDTA/Ca^{2+}-, Mg^{2+}-free PBS] at 37 C and plated in 60-mm culture dishes (Falcon, Becton Dickinson and Co. Labware, Franklin Lakes, NJ) penicillin/streptomycin (100 IU/ml, 100 µg/ml, respectively) and supplemented with 10% fetal bovine serum (Life Technologies, Inc.). The purity of the EVT cultures was determined by immunostaining with a monoclonal antibody directed against cytokeratin 8 and 18 (Becton Dickinson and Co.) according to the methods of MacCalman *et al.*. Only cell cultures that exhibited 100% immunostaining for cytokertain were included in these studies.

Microarray Development and Quality Control The cells treated by GnRH I or GnRH II were harvested for RNA purified using the mRNA extraction kit. The product was dried, dissolved in either 18 µl of H_2O, 50% Me_2SO, or 3× SSC, and spotted (3 hits/feature) with a GMS 417 Arrayer (Affymetrix) on CMT-GAPS-coated glass slides (Corning). CDNA was fixed either by incubating the slide for 3.5 h at 40 °C followed by 10 min at 100 °C or for 2 h at 85 °C or by UV cross-linking with 90 mJ (Stratalinker, Stratagene). The suspension solutions and fixing protocols were compared in a 3×3 design because feature morphology may be influenced by surface tension effects arising from surface and spotting solution chemistry and by attachment efficiency. The optimum morphology and intensity were reproducibly observed, independently of insert sequence or size, with the PCR product dissolved in 50% Me_2SO and fixed for 2 h at 85 °C to CMT-GAPS-coated slides, which was used for subsequent array production.

Real time Polymerase Chain Reaction (PCR) confirmation

We used a previously described protocol. Briefly, 5 µg of total RNA was converted into cDNA, and 1:400 (~250 pg) was utilized for 40-cycle three-step PCR in an ABI Prism 7700 in 20 mM Tris, pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 200 µM dNTPs, 0.5× SYBR green (Molecular Probes), 200 nM each primer, and 0.25 units of Platinum *Taq* (Life Technologies). Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis. The number of target copies in each sample was interpolated from its detection threshold (C_T) value using a plasmid or purified PCR product standard curve included on each plate. The sequence of the 60 primer sets utilized can be found in the supplementary material. Each transcript in each sample was assayed five times, and the median C_T values were used for analysis.

Plasmid

AP1-TA-Luc vector (Clontech, Polo Alto, CA, USA) is designed to monitor the induction of AP1 signal transduction pathway and contains the firefly luciferase (*luc*) gene from *Photinus pyralis*.AP1-TA-Luc contains a AP-1 response element, located upstream of the minimal TA promoter, the TATA box from the herpes simplex virus thymidine kinase promoter (P_{TA}). Located downstream of P_{TA} is the firefly luciferase reporter gene (*luc*). After transcription factors bind to the AP1-response elements, transcription is induced and the reporter gene is activated. pTAL-Luc (Clontech) was used as a negative control to determine the background signals associated with the cell lysates. The enhancerless pTAL-Luc contains HSV-TK upstream of the Luciferase coding sedquence.

Transient transfections

Transient transfections were performed using Lipofetamine^{TM} 2000 transfection reagent (Invitrogen life technologies Carlsbad, CA), following the manufacturer's protocol. To correct for different transfction efficiencies of various luciferase constructs, the Rous sarcoma virus (RSV)-*lacz* plasmid was cotransfected into the cells with 1µg vectors that contained a specific cis-acting DNA sequence (enhancer element) and a sensitive luciferase reporter gene. Briefly, 4 x 10^5 of cells were seeded into six-well tissue culture plates before the day of transfection. One microgram of the various luciferase reporter constructs and 1 µg of RSV-*lacZ* vector were cotransfected into the cells under serum-free conditions. After 6 h of transfection, 1 ml of medium containing 20% FBS was added, and the cells were further incubated for 24 h. After incubation, the old medium was removed and the cells were cultured for another 24 h with normal fresh medium containing 10% FBS. The treatments were carried out 40 hours after transfection and the cells were exposed in the presence of 100nM GnRH I or II for 6 hr.

Luciferase Reporter Assays

Cellular lysates were collected with 150µl reporter lysis buffer (Promega Corp., Nepean, Canada) and immediately assayed for luciferase activity with the Luciferase Assay System (Promega Corp. Nepean,

Canada) at room temperature. The transfected cells were rinsed twice with phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 M KH_2PO_4) and incubated for 15 min after addition of 200 µl of cell lysis buffer. Cells were then collected by centrifugation at 14000 rpm for 10 s at 4°C. Fifty µl of cell lysates was mixed with 100 µl each of Substrate A and Substrate B, and luminescence was immediately measured by Lumat LB9507 luminometer (EG&G Berthold, Bad Wildbad, Germany). Beta-galactosidase (ß -Gal) activity was also measured and used to normalize for varying transfection efficiencies. Promoter activity was calculated as luciferase activity/ß-galactosidase activity. A promoterless pGL2-Basic vector was included as a control in the transfection experiments.

Preparation of nuclear extracts

Nuclear extracts were prepared from human EVTs according to the methods described previously (Lassar et al. 1991). Briefly, cells at 70% confluency will be lysed in 2ml of Lysis Buffer, containing 20mM HEPES pH 7.6, 20% Glycerol, 10mM NaCl, 1.5mM $MgCl_2$, 0.2mM EDTA, 0.1% Triton X-100, 1mM DTT, 1mM PMSF, 10 µg/ml leupeptin, 10 µg/ml pepstatin, and 100 µg/ml aproteinin. Cells were dislodged by scrapping and collected by centrifugation for 5 min at 2000 rpm at 4°C. Nuclei were resuspended at 2.5 x 10^7 nucleus per ml in Nuclear Extraction Buffer (Lysis Buffer plus 500mM NaCl). Nuclei were gently rocked for 1h at 4°C and centrifuged at 10,000 rpm for 10 min, and supernatant was aliquoted, quick frozen in liquid nitrogen, and stored at -80°C.

Data analysis

All data were shown as the means +/- SD of triplicate assays in at least three independent experiments. For Western blot analysis, data are obtained from three independent experiments. All data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test or *t*-test using the computer software PRISM GraphPad Version 2 (GraphPad Software, Inc., San Diego, USA). Data were considered significantly different from each other when $P < 0.05$.

Results

Fig1A. Exploratory data analysis and quality control: results of exploratory analysis of one cDNA microarray experiment (GnRH I vs. control). GnRH I was labeled with Cy5, and the control with Cy3. The hybridization data was examined using the following scatter plots exploratory data analysis tool. **Fig1B.** Similar Results of the exploratory data analysis for one cDNA microarray experiment (GnRH II vs. control).

In the present studies, we analyzed 13420 genes on a DNA chip to identify the regulatory net work of GnRH I and GnRH II. The microarray data showed that there are 61 genes regulated above two times by GnRH I . Among those genes, 50 genes were upregulated(Table 1A). In contrast, 11 genes were down regulated(Table 1B). On the other side, there are 36 genes regulated above

two times by GnRH II. Of which 14 genes were upregulated(Table 2A) and 22 genes were down regulated(Table 2B).

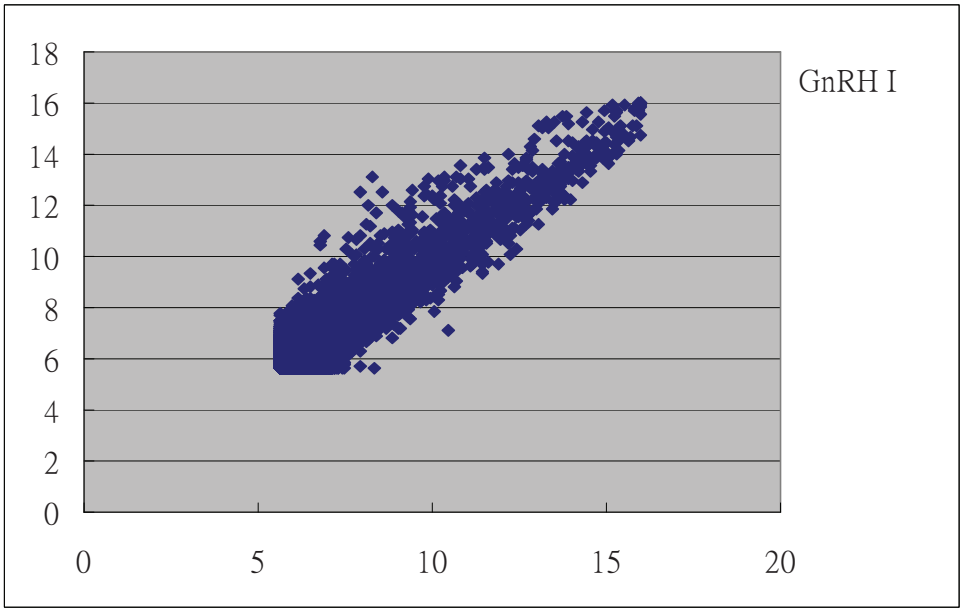

Fig 1 A

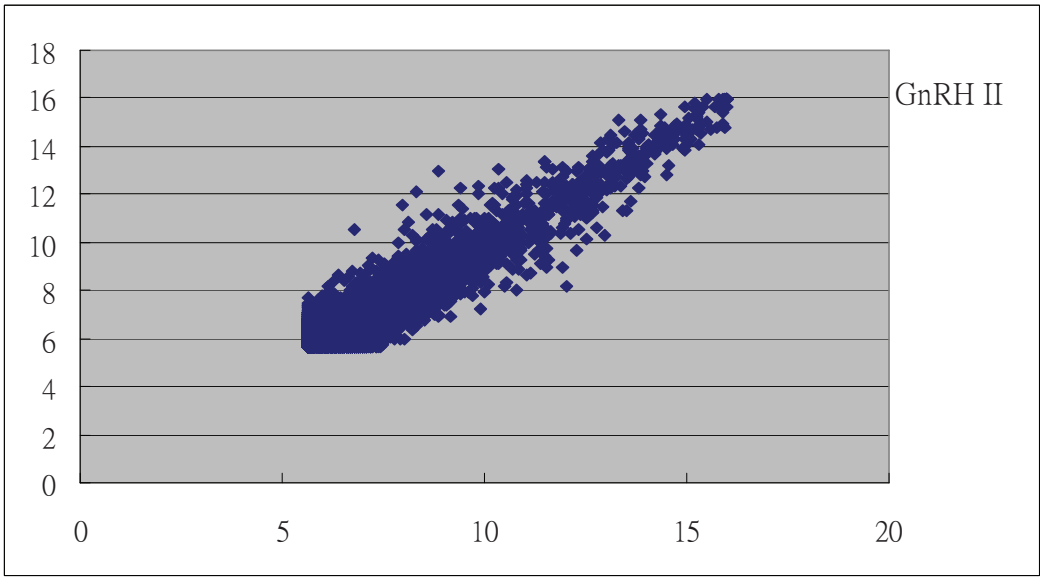

Fig 1 B

Table 1A

AB016898^Homo sapiens HGC6.4 mRNA_ complete cds
AB007970^Homo sapiens mRNA_ chromosome 1 specific
U89942^lysyl oxidase-like 2
M22333^LINE retrotransposable element 1
X16869^eukaryotic translation elongation factor 1 alpha 1
AL137163^Novel human gene mapping to chomosome X
U65410^MAD2 (mitotic arrest deficient_ yeast_ homolog)-like 1
U32576^apolipoprotein C-IV
AB007941^KIAA0472 protein
X56932^ribosomal protein L13a
AB004064^tomoregulin
X13839^actin_ alpha 2_ smooth muscle_ aorta
AK000702^hypothetical protein FLJ20695
S48220^deiodinase_ iodothyronine_ type I
NM_014270^solute carrier family 7 (cationic amino acid_ transporter_ y+ system)_ member 9
AK001798^hypothetical protein FLJ10936
AF151037^hypothetical protein
AF070529^hypothetical protein
AB033025^Homo sapiens mRNA; cDNA DKFZp586O0118 (from clone DKFZp586O0118)
AK001968^hypothetical protein FLJ11106
U58090^cullin 4A
AL078612^reticulocalbin 1_ EF-hand calcium binding domain
AK001536^Homo sapiens cDNA FLJ10674 fis_ clone NT2RP2006436
NM_005490^novel SH2-containing protein 1
AL137304^hypothetical protein DKFZp434H247
D10202^platelet-activating factor receptor
AJ224741^matrilin 3
AF023456^protein phosphatase_ EF hand calcium-binding domain 2
NM_016343^PRO1779 protein
X14420^collagen_ type III_ alpha 1 (Ehlers-Danlos syndrome type IV_ autosomal dominant)
X04802^Homo sapiens UBBP2 pseudogene for ubiquitin UBB
AB010994^Homo sapiens hedgehog gene
AK001937^Homo sapiens cDNA FLJ11075 fis_ clone PLACE1005046
AL022146^Human DNA sequence from clone 780M13 on chromosome 1q24-25. Contains the alternatively sp SELP
AJ243797^three prime repair exonuclease 1
AK000939^Homo sapiens cDNA FLJ10077 fis_ clone HEMBA1001864
L27560^Human insulin-like growth factor binding protein 5 (IGFBP5) mRNA
AC006539^Homo sapiens chromosome 19_ BAC 39498 (CIT-B-26L23)
AK000463^hypothetical protein FLJ20456
AF053004^class I cytokine receptor

AK001700^amino acid transporter 2
AB037785^Homo sapiens mRNA for KIAA1364 protein_ partial cds
AB037799^Homo sapiens mRNA for KIAA1378 protein_ partial cds
X04106^calpain_ small polypeptide
AF109161^Cbp/p300-interacting transactivator_ with Glu/Asp-rich carboxy-terminal domain_ 2
U46691^suppressor of Ty (S.cerevisiae) 6 homolog
AJ006692^Homo sapiens UHS KerB gene
AL161951^Homo sapiens mRNA; cDNA DKFZp564G163 (from clone DKFZp564G163)
NM_001219^calumenin
X83543^apical protein_ Xenopus laevis-like
AK001106^hypothetical protein FLJ10244
L06499^ribosomal protein L37a
AK001037^Homo sapiens cDNA FLJ10175 fis_ clone HEMBA1003989
AK000707^hypothetical protein FLJ20700
AL117485^hypothetical protein
AK001948^Homo sapiens cDNA FLJ11086 fis_ clone PLACE1005266
D38524^purine 5 nucleotidase
L11566^ribosomal protein L18
AF000989^thymosin_ beta 4_ Y chromosome
AK000930^Homo sapiens cDNA FLJ10068 fis_ clone HEMBA1001533
NM_003926^methyl-CpG binding domain protein 3

Table 1B

GI down-regulation	GI vs Control
D10522^myristoylated alanine-rich protein kinase C substrate (MARCKS_ 80K-L)	-2.0058
U17997^GFP marker	-2.0320
AL137681^eukaryotic translation initiation factor 4A_ isoform 2	-2.0637
D14696^KIAA0108 gene product	-2.0858
AK000887^Homo sapiens cDNA FLJ10025 fis_ clone HEMBA1000682	-2.1530
NM_012445^spondin 2_ extracellular matrix protein	-2.1940
AC003973^zinc finger protein 208	-2.209
AF070617^Homo sapiens clone 24812 mRNA sequence	-2.2139
AB012911^frizzled (Drosophila) homolog 6	-2.7019
AK001588^hypothetical protein FLJ10726	-3.3847

Table 2A

NM_014270^solute carrier family 7 (cationic amino acid_ transporter_ y+ system)_ member 9
U65410^MAD2 (mitotic arrest deficient_ yeast_ homolog)-like 1
U32576^apolipoprotein C-IV
AJ243797^three prime repair exonuclease 1
AK001798^hypothetical protein FLJ10936
X04802^Homo sapiens UBBP2 pseudogene for ubiquitin UBB
AB007941^KIAA0472 protein
AF151037^hypothetical protein
AL137304^hypothetical protein DKFZp434H247
AB010994^Homo sapiens hedgehog gene
AB033025^Homo sapiens mRNA; cDNA DKFZp586O0118 (from clone DKFZp586O0118)
AB023165^KIAA0948 protein
AC006539^Homo sapiens chromosome 19_ BAC 39498 (CIT-B-26L23)
AB032990^hypothetical protein KIAA1164

Table 2B

AB037769^Homo sapiens mRNA for KIAA1348 protein_ partial cds
AF073308^deafness_ autosomal dominant 5
M57729^complement component 5
AL050179^tropomyosin 1 (alpha)
AL022240^Human DNA sequence from clone 328E19 on chromosome 1q12-21.2 Contains a cyclophilin-like
AB037822^hypothetical protein FLJ10534
X80910^protein phosphatase 1_ catalytic subunit_ beta isoform
AB018266^matrin 3
M14328^enolase 1_ (alpha)
AK001588^hypothetical protein FLJ10726
D10522^myristoylated alanine-rich protein kinase C substrate (MARCKS_ 80K-L)
M97815^cellular retinoic acid-binding protein 2
AK000011^hypothetical protein FLJ20004
AB037761^Homo sapiens mRNA for KIAA1340 protein_ partial cds
AF208844^hypothetical protein
X52104^DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase_ 68kD)
AJ001189^oligophrenin 1
X75042^v-rel avian reticuloendotheliosis viral oncogene homolog
U86136^telomerase-associated protein 1
AL137509^hypothetical protein DKFZp761A052
AL031313^Human DNA sequence from clone 581F12 on chromosome Xq21. Contains Eukaryotic Translation
X60489^eukaryotic translation elongation factor 1 beta 2

Discussion

Gonadotropin-releasing hormone (GnRH) is a neuropeptide that plays a pivotal role in reproductive processes. In recent years, it has become clear that it is also an anti-proliferative agent. GnRH analogs are now used clinically in the treatment of prostate cancer as well as endometriosis and precocious puberty. The target cells of GnRH include the gonadotropes of the anterior pituitary gland and the cells of various hormone-dependent tumors. Only a few target genes have been identified in these cells, however, and little is known concerning their regulation by GnRHs. Therefore, we used a quantitative microarray assay to identify the genes that are regulated by GnRHs in trophoblasts. Treatment of trophoblasts with GnRH I or II for 1 h resulted in alterations in the levels of expression of genes that ranged in magnitude from2- to 6-fold, with a total of 61 genes exhibiting a twofold or greater alteration in expression compared to vehicle treated cells for GnRH I. Of these 61 genes, 50 were up-regulated and, 11 were down-regulated by GnRH I treatment. After 24 h of treatment, the expression of most of the genes that had exhibited altered expression after 1 h of treatment had returned to baseline levels. Moreover, a different profile was observed after 24 h of treatment by GnRH II with 36 genes exhibiting a twofold or greater alteration. Of these, 14 were up-regulated and 22 down-regulated. Most of the affected genes were not known to be responsive to GnRH prior to this study. Treatment with GnRH I or II was found to affect the expression of a diverse range of genes, including oncogenes and those that encode transcription factors, ion channel proteins, and cytoskeletal proteins as well as other proteins that are involved in signal transduction, the cell cycle, cell proliferation and apoptosis. The altered expression of six of the genes that were found by microarray analysis to be regulated by GnRH I was confirmed by semiquantitative reverse transcriptase–polymerase chain reaction. This is first application of the microarray technique in the study of the global profile of genes regulated by GnRH I or II, and should prove to be a powerful tool for future analysis of the mechanisms by which GnRH regulates the expression of gonadotropins and the growth of tumor cells.

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