基因晶片技術於生殖醫學之應用

The Applications of cDNA Microarray in Reproductive Medicine:

中文摘要

第一部分：分析研究子宮內膜異位症之病理及標記分子

子宮內膜異位症 (Endometriosis)，是一種常見的婦科疾病，約 40％ 的不孕婦女患有子宮內膜異位症，Gonadotropin-releasing hormone analog (GnRHa) 是目前臨床上普遍使用於治療子宮內膜異位症的一種藥物。但對子宮內膜異位症致病機轉與診斷的標誌分子，至今仍不清楚。在本篇研究論文中，我們利用 cDNA 基因晶片 (cDNA microarray) 的技術分析比較 GnRHa 治療前後的異位子宮內膜組織(Chocolate cyst) 之基因表現差異圖譜。

在經過台北醫學大學附設醫院人體試驗委員會核准，及病人同意下，取得實驗所需之檢體。子宮內膜異位囊腫組織檢體分別取自於接受GnRHa治療及未經GnRHa治療之子宮內膜異位症病人(n=4)。並萃取其mRNA 進行 cDNA 基因晶片分析。我們利用人類 cDNA 基因晶片系統 (包含 9600 個基因及 EST) 並結合酵素呈色系統分析基因表現之差異。再利用即時定量 PCR (Real time Q RT-PCR)、免疫組織染色 (Immunohistochemistry) 和西方點墨法 (Western blotting) 確認這些基因的差異性表現。

根據 cDNA 基因晶片的分析結果，75 個基因的表現在 GnRHa 治療後會被抑制；216 個基因的表現在 GnRHa 治療後則會被提升 (up regulated)。與細胞生長 (如：PCNA、topoisomerase II alpha 和 CDC2 delta T)、細胞轉化 (pituitary tumor transforming) 及細胞侵入 (Enolase 1 alpha) 的相關基因，皆高度表現於未經 GnRHa 治療之異位子宮內膜組織。我們進一步以免疫組織染色方法確認經過 GnRHa 治療之異位子宮內膜組織其細胞增殖標誌 PCNA 的表現降低。

根據 cDNA 基因晶片、即時定量 PCR 及西方點墨法的分析結果，我們確認 Enolase 1 alpha, Keratin 19, pituitary tumor-transforming gene (PTTG) 及 H-cadherin 在 GnRHa 治療前後的子宮內膜異位症病人之異位子宮內膜組織、腹腔液及血清檢體，皆具有差異性表現。

利用 cDNA 基因晶片確認這些表現差異的基因，將有助於我們對子宮內膜異位症導致不孕之致病機轉的瞭解，且也許能夠提供尋找診斷或治療標誌分子的相關資訊。

第二部分：研究胚胎孵化過程之基因調控

早期胚胎發育過程中，囊胚孵化 (blastocysts hatching) 對胚胎的著床而言很重要。在這時期許多因子會被高度調控，且與母體的子宮內膜相互作用。先前研究都著重於對子宮內膜的探討，但對囊胚的瞭解相對地較少。

在本篇研究論文中，我們整合 T7-based RNA 放大技術 (體外轉錄) 及 cDNA 基因晶片 (cDNA microarray) 技術，分析比較囊胚孵化前後之基因表現差異圖譜。

我們收集 ICR 小鼠胚胎 (未孵化及已孵化囊胚) 萃取其 RNA (實驗重複 3 次，每組使用 25 個囊胚)。利用體外轉錄技術放大 RNA，以便進行 cDNA 基因晶片分析。我們使用小鼠 cDNA 基因晶片系統 (包含 6,144 個基因及 EST) 結合酵素呈色系統進行分析。再利用即時定量 PCR

(Realtime Q RT-PCR) 確認這些基因的差異性表現。
根據 cDNA 基因晶片的分析結果，我們偵測到 2087 個基因在囊胚期會表現；其中 13 個基因在孵化前的囊胚表現較高；85 個基因則在孵化期的囊胚表現較高。我們繼續將這些表現差異的基因依其可能的特性分群，分爲：細胞黏附分子 (cell adhesion molecules)、賀爾蒙/細胞激素 (hormones/cytokines)、免疫調控因子 (immuno-regulators)、細胞外基質及相關酵素 (extracellular matrix and related enzymes)，還有 ESTs (expressed sequence tags)。我們選取其中表現差異比值 (ratio) 大於 2.5 倍的基因 (包括：copine III, neurotrophin-3, INF-g receptor-2, IL-4 receptor, IL-7 receptor, type II keratin 及 ESTs)，利用即時定量 PCR 確認這些基因自囊胚前期 (early blastocyst stage) 到孵化期囊胚 (hatched blastocyst stage) 的 mRNA 表現。我們發現 copine III 的表現在孵化期會被調昇 (up-regulated)，且已有研究指出 copine III 能夠與 MEK1 及 CDC42 regulated kinase 相互作用，顯示 copine III 可能參與孵化期囊胚的細胞分裂 (cell division) 與生長。利用專一性抗體進行免疫染色，孵化期囊胚高度表現 IL-4 receptor，可能 IL-4 receptor 會調控著床時的免疫反應 (immunoresponse) 及囊胚的生長。
本篇研究藉由建立囊胚孵化過程的基因表現差異圖譜，使我們更加了解囊胚孵化的機制，以及提供我們研究囊胚與子宮內膜間相互作用的資訊

英文摘要

Part I: To Study the Pathological Mechanisms and Markers for Endometriosis
Endometriosis is one of the most common gynecological diseases and about 40 % of infertile women have endometriosis. The gonadotropin releasing hormone analog (GnRHa) has been widely used in the treatment of endometriosis for many years. However, the genetic mechanisms and diagnostic marker of endometriosis are still unclear. In this study, the global gene expression profiles in endometric tissues (chocolate cysts) which have been treated with or without GnRHa were analyzed by using cDNA microarray technology.
Institutional review board approval was obtained before initiation of this investigation by the Taipei Medical University Hospital (Taipei, Taiwan). Endometric tissues were obtained from endometriosis patient have been treated with or without GnRHa (n= 4). The mRNA was extracted for cDNA microarray analysis. The human cDNA microarray system (9,600 genes, including known regulatory genes and expressed sequence tag, EST) with colorimetric detection system was used to identify the differentially expressed genes. The realtime Q RT-PCR, Immunohistochemistry and Western blotting were used to confirm the microarray data.
According to cDNA microarray analysis, we have identified 75 genes whose expression was down regulated in endometric tissues with GnRHa treatment, and 216 genes were up regulated. The genes related to cell growth (PCNA, CDC2 delta

T, and topoisomerase II alpha), cell transformation (pituitary tumor transforming), and cell invasion (Enolase 1 alpha) were highly expressed in the endometric tissues without GnRHa treatment. Immunohistochemistry was used to confirm the cell proliferating marker, PCNA, was decreased following GnRHa treatment.

According to the results from microarray, realtime Q RT-PCR, and Western blotting, we defined that Enolase 1 alpha, Keratin 19, pituitary tumor-transforming gene (PTTG), and H-cadherin were consisted expressed differentially in endometriotic tissues, PF, and/or serum from patients with or without GnRHa treatment. To identify these differentially expressed genes globally by microarray add to our understanding of the pathological mechanisms about endometriosis- induced infertility and might provide the information to find the diagnostic markers or therapeutic targets for endometriosis.

Part II: To Study the Gene Regulation during Blastocyst Hatching

Blastocyst hatching process is very important for implantation during early embryo development. Several factors are highly regulated and cross-talked with maternal endometrium during this stage. Previous studies were focused on the endometrium and relatively little known about blastocysts. In this study, the global gene expression profiles in blastocysts before and after hatching were analyzed and compared by integrating the technologies of T7-based RNA amplification (in vitro transcription) and cDNA microarray.

ICR-mouse embryos (unhatched and hatched blastocysts) were collected for RNA extraction (twenty-five blastocysts were used in each group in the triplicate experiments). The RNA was amplified by in vitro transcription for microarray analysis. The mouse cDNA microarray system (6,144 genes, including known regulatory genes and expressed sequence tags, ESTs) with colorimetric detection system was used. Realtime Q RT-PCR was used to confirm the gene expression differences.

According to cDNA microarray analysis, we have identified 2,087 genes were detectable during blastocyst stage, 13 genes whose expression was higher in pre-hatched blastocyst, and 85 genes were higher in hatching stage. The differentially expressed genes were further grouped into categories by their putative functions, including: cell adhesion molecules, hormones/cytokines, immuno-regulators, extracellular matrix and related enzymes, and some ESTs. The different expressed ratio greater than 2.5-folds (including, INF- receptor-2, IL-4 receptor, IL-7 receptor, neurotrophin-3, copine III, type II keratin, and some ESTs) were selected to confirm their mRNA expression levels from early blastocyst stage to hatched blastocyst stage by realtime RT-PCR. We find that copine III was up-regulated in hatching stage and was reported to be capable of interacting with

MEK1 and the CDC42 regulated kinase, which indicate that copine III may be involved in cell division and growth in the hatching stage of blastocyst. Using immuno-staining with specific antibodies, IL-4 receptor is highly expressed in hatching blastocyst which may regulate the immunoresponse and blastocysts growth during implantation.

This work adds to our understanding in the mechanisms of blastocyst hatching and provides the information for studying the cross-talk of blastocyst and endometrium by reporting the global gene expression profiles of blastocyst during hatching process.