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cDNA microarray reveals signaling pathways involved in hormones expression of human pituitary ☆

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Abstract

Pituitary, a master gland of neuroendocrine system, secretes hormones that orchestrate many physiological processes, under the regulation of multiple signaling pathways. To investigate the genes involved in hormones expression of human pituitary, homemade cDNA microarray containing 14,800 human genes/ESTs were used to profile the gene expression in both fetal and adult pituitaries. Seven hundred and twelve known genes changed over 2-fold between the both tissues. Of which, 23 genes were changed with hormones expression in aging were confirmed by RT-PCR, not only the known regulators such as Pit1, GATA4, ESRRA, GABA-A, and EMK, but also LOC55884, DUSP3, PNN, and RCL, which had not been reported to be involved in the hormones expression. Correspondingly, the mRNAs of GH, PRL, POMC, TSH-β, FSH-β, and LH-β, was increased as much as 6- to 20-fold in adult pituitary than those in fetal pituitary, by real-time quantitative RT-PCR assay. In addition, the mRNAs of signaling pathways, such as cAMP-PKA-CREB, PI3K-Akt, and PKA-ERK were further investigated. Of them, it was only cAMP-PKA-CREB pathway, but not PI3K-Akt and PKA-ERK have the same expressing pattern as hormones. It suggested that cDNA microarray is highly advantages to profile the differential expressed genes that were involved in hormones expression of human pituitary, but it might ignore some responding proteins regulated posttranscriptionally.

Keywords: Hormone; Functional genomics; Microarray; Signaling transduction

1. Introduction

Pituitary, as master gland in whole endocrine system, secretes hormones that chiefly orchestrate many physiological processes such as growth, sexual development, metabolism, and the response to stress. Meanwhile, hypothalamic hormones and other factors regulate the expression and release of pituitary hormones. In the past 10 years, several transcription factors and signaling molecules, such as dopamine (Ben-Jonathan and Hnasko, 2001), estrogen receptor α (Lindzey et al., 1998), galanin (Asa et al., 2000), cAMP-PKA-CREB pathway (Yu and Melmed, 2001), PI3K-Akt pathway (Hayakawa et al., 2002), ERK signaling (Liu et al., 2002), and calcium signaling (Vasilyev et al., 2002; Villalobos et al., 2002), had been demonstrated to regulate hormone expression in transgenic animals or tumor cell lines. It is necessary to be pointed out that the understanding of pituitary development and regulatory programs controlling the hormone expression was derived from animal experi-

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ments, in particular mouse. However, the increasing evidences suggested that there could be vital differences in gene expression profiles with development between mouse and human (Fougerousse et al., 2000; Gou et al., 2001). Actually, whether the signaling pathways naturally work in the processes of human development are largely unknown.

With the advance of the human genome project, huge genome information is available for surveying the physiological and pathological processes of human at global levels. Hormonal genomics has been proposed to develop a reconstructionistic approach substituting gene-by-gene ones to reveal the subfamilies and pathways for genes involved in the hormones signaling (Leo et al., 2002). EST sequencing, series analysis of gene expression (SAGE) and cDNA microarray or gene chip are the most efficient strategies for profiling the gene expression at transcriptome level. We hypothesized that the genes involved in hormones expression had the same expression pattern as hormones in the development. Employing transcriptome approaches and pituitary samples from fetal, adult, and old human beings, we intended to establish a connection between the differentially expressed genes/ESTs obtained from microarray and the hormones, so that we can have a more profound understanding of the molecular mechanisms involved in hormones expression of human pituitary. As expected, the expression pattern of most the genes can attribute to the pituitary function at the specific period. Thus, several key signaling pathways were investigated in further more details.

2. Materials and methods

2.1. Specimens

Two male and two female pituitaries were obtained from fetuses with 16, 18, 16, and 20 weeks old, which were aborted accidentally. Two adult pituitaries were derived from 20 and 35 years old male, who died of traffic accidents. All tissues were removed within 4 h after death and frozen in liquid nitrogen then stored at −80 °C. In addition, total RNA of pituitaries from two elders, who were 69 years old male and 60 years old female, respectively, were obtained from Biocat Company (Heidelberg). All human subjects were done according to related Ethical criteria.

2.2. Extraction of RNA

Total RNA was extracted using TRIZOL Reagent (Life Technologies) and treated with DNase I at 37 °C for 30 min, and then purified with phenol–chloroform. After extraction, the quality of the total RNA was evaluated by electrophoresis on 1% agarose gel containing ethidium bromide, and the ratio of absorbance at 260/280 nm by spectral photometer (Beckman). Total RNA of fetal pituitary with same sex was made a pool, respectively.

2.3. cDNA microarray analysis

cDNA array construction was the same as the previously described (Xu et al., 2001). A cDNA array was assembled with 14,000 cDNA clones, which were from hypothalamus–pituitary–adrenal libraries and liver and hepatocellular carcinoma cell lines or purchased from Research Genetics (Huntsville, AL, USA), representing the same number of independent cDNA clusters, of which 7565 clusters were homologous to that in the UniGene Database. All cDNA fragments were amplified using PCR. The PCR products were precipitated in isopropanol, redissolved in 10 ml of denaturing buffer (1.5 M NaCl, 0.5 M NaOH), and spotted on two 8–12 cm Hybond-N nylon membranes (Amersham-Pharmacia, Buckinghamshire, United Kingdom) using an arrayer (BioRobotics, Cambridge, United Kingdom). Each spot carried 100 nl in volume and was 0.4 mm in diameter, and each cDNA fragment was placed in two different spots (double-offset). Lambda phage and pUC18 vector DNA were spotted as negative controls.

Eight housekeeping genes encoding ribosomal protein S9 (RPS9), β-actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1, M_r 23,000 highly basic protein (RPL13A), ubiquitin C, phospholipase A2, and ubiquitin thiolesterase (UCHL1) were evenly distributed, each in 12 places, on each 8–12 cm array as an intramembrane control. Hybridization data were considered invalid if among the 12 spots representing the same gene, the intensity of the darkest spot exceeded 1.5-fold of that of the weakest spot.

Total RNA was extracted using standard Trizol RNA isolation protocol. Six micrograms of total RNA from each RNA pool derived from two male fetal and two adult pituitaries, respectively, was labeled in a reverse transcription reaction in the presence of 10 μCi of [α-^{33}P]d-ATP (MEN). The reverse transcriptase (RT) reactions were performed with SuperScript II RNase H$^-$ reverse transcriptase and random primers (Life Technologies), and then purified with Micro Spin G-50 Columns (Amersham).

Prehybridization was carried out in 20 ml of prehybridization solution (63 SSC, 0.5% SDS, 53 Denhardt's, and 100 mg/ml denatured salmon sperm DNA) at 68 °C for 3 h. Overnight hybridization with the ^{33}P-labeled cDNA in 6 ml of hybridization solution (63 SSC, 0.5% SDS, and 100 mg/ml salmon sperm DNA) was followed by stringent washing (0.13 SSC, 0.5% SDS, at 65 °C for 1 h). Membranes were exposed to Phosphor Screen overnight and scanned using an PhosphorImager