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ORIGINAL ARTICLE

# Analysis of differential gene expression by fiber-optic BeadArray and pathway in prolactinomas

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**Abstract** Prolactinomas are the most common secretory pituitary tumors; however, their pathogenesis is unclear. In order to explore the pathogenesis of prolactinomas, we used fiber-optic BeadArray to examine gene expression profiles in five prolactinomas compared with three normal pituitaries. Three down-regulated genes and one up-regulated gene were chosen for validation by quantitative real-time reverse-transcription polymerase chain reaction. We then performed pathway analysis on the identified differentially expressed genes using the Kyoto Encyclopedia of Genes and Genomes. Array analysis showed significant increases in the expression of 27 genes and 3 expressed sequence tags (ESTs), and decreases in 182 genes and 9 ESTs, including HIG1 domain family, member 1B, S100 calcium binding protein A9, angiopoietin 2, interleukin 8, hydroxyprostaglandin dehydrogenase 15-(NAD), suppression of tumorigenicity18, and WNT inhibitory factor 1. Pathway analysis showed that the P53 and GnRH signaling pathways may play an important role in tumorigenesis of prolactinomas. Our data suggest fiber-optic BeadArray combined with pathway analysis of differential gene expression profile appears to be a valid approach for investigating the pathogenesis of tumors.

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## Introduction

Prolactinomas are the most common secretory pituitary tumors, accounting for ~40% of all pituitary adenomas [1]. The symptoms of prolactinoma arise because of excessive prolactin in the blood (hyperprolactinemia) or because of the pressure that the tumor exerts on surrounding tissues. These eventually lead to menstrual disturbances (usually amenorrhea), galactorrhea, loss of libido in both genders and in patients harboring macroprolactinomas and symptoms related to the tumor mass effect, such as visual disturbances and headache.

The pathogenetic mechanisms that underlie pituitary adenomas are complex and remain unclear [2]. Genetic mutations that are characteristic of other human neoplasms are rarely observed in human pituitary tumors [3]. Several studies examining X-chromosome inactivation have shown that primary pituitary adenomas are monoclonal [4, 5]. In the past years, several candidate factors have been implicated in the genesis and progression of prolactinomas. For example, overexpression of protooncogenes, that include cell cycle progression molecules, growth factors or receptors, such as pituitary tumor transforming gene (PTTG), high-mobility group A nonhistone chromosomal proteins 2 (HMGA2), epidermal growth factor receptor type 4, has been observed in prolactinomas, although at present it is unknown whether these changes have a causative role or are representative of a secondary event. Similarly, the low expression of tumor suppressor genes has been implicated in lactotroph proliferation, although genetic mutations of

candidate genes have not yet been identified, despite the frequent occurrence of LOH (loss of heterozygosity) in several loci [6]. However, the molecular pathogenesis of prolactinomas has resisted elucidation, and, with the exception of a RAS (rat Sarcoma) mutation in a single aggressive prolactinoma, no mutational changes have been identified to date.

Microarray technology is being applied to investigate the pathogenesis of cancers. It is becoming more evident that better diagnostic methods and increased understanding of disease mechanisms can be achieved by a careful analysis of microarray measurements of gene expression profiling [7]. There are reports which detail the use of microarray technology to study the differential gene expression profiles of prolactinoma [8–10]. In order to explore the possible pathogenesis of prolactinoma, we applied the human genome-wide fiber-optic BeadChip (Illumina HumanWG-6 v3.0), which has high density, repeatability, and sensitivity, to identify the differential expression of genes between prolactinomas and normal pituitary tissues. We then analyzed the differential gene expression profiles by bioinformatic and pathway analysis to search for new candidate genes that may be implicated in the pathogenesis of prolactinoma.

## Materials and methods

### Tissue specimens

Five prolactinomas were obtained from patients at TianTan Hospital following transsphenoidal surgery. The clinical and pathological characteristics of these tumors are presented in Table 1. Sections of the surgical specimens were immediately frozen in liquid nitrogen and stored at −80°C within 1 year. Three normal pituitary glands, which were obtained within 12 h of death from three adult males who had died from accidents, were used as controls in the study. Informed consent was obtained from family of the deceased and from patients recovering from transsphenoidal surgery and the study was approved by the local Ethics Committee.

### RNA extraction

Total RNA was extracted from normal pituitaries (50–100 mg) and prolactinomas (50–100 mg) using the Unizol reagent protocol (Shanghai Biostar, Shanghai, China). The integrity of the total RNA of each sample was determined by denaturing agarose gel electrophoresis, and the mRNA was quantified by spectrophotometry.

### RNA amplification procedure

RNA was amplified using the TotalPrep RNA Amplification Kit protocol (Ambion, Austin, TX). In brief, with RNA serving as a template, T7 oligonucleotide was used as a primer to synthesize, via reverse transcription, the first strand cDNA; then, the first cDNA served as a template in a reaction that employed DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize the second strand cDNA. cDNA purification then removed RNA, primers, enzymes, and salts that would otherwise inhibit in vitro transcription. In vitro transcription to synthesize cRNA generated multiple copies of biotinylated cRNA from the double-stranded cDNA templates; this was the amplification and labeling step. cRNA purification then removed unincorporated NTPs, salts enzymes, and inorganic phosphate. After purification, the cRNA was used with direct hybridization array kits (Illumina, San Diego, CA).

### Hybridization illumina

A total of 1.5 μg of cRNA was used for hybridization in each array. Sample labeling and hybridization on Illumina WG6 v3.0 Sentrix Bead Chips (Illumina, San Diego, CA), which contains over 48,000 transcripts, a total of 37,804 genes which cover 25,158 well-characterized genes from the human genome and 12,646 ESTs, were performed for all arrays in this study according to the manufacturer's instructions (http://www.illumina.com) at a single Illumina BeadStation facility.

**Table 1** Clinical and pathological characteristics of patients

| Patient | Sex, age (years) | PRL (μg/l serum) and tumor size (cm) | Immunohistochemistry |
|---|---|---|---|
| 228 | M, 23 | 3.81 μg/ml, 3.1 × 2.1 × 4.9 | PRL3+ |
| 231 | F, 19 | 49.65, 2.0 × 1.5 × 2.2 | PRL3+ |
| 214 | F, 49 | 25.87, 2.4 × 1.6 × 2.0 | PRL3+ |
| 226 | M, 47 | 300, 3.5 × 2.8 × 1.8 | PRL3+ |
| 249 | F, 54 | 200, 2.8 × 1.2 × 1.8 | PRL3+ |
| 1(1) | M, 34 | 3.53, 1.3 × 1.0 × 0.6 | Normal |
| 1(2) | M, 36 | 5.35, 1.1 × 0.9 × 0.5 | Normal |
| 3(1) | M, 31 | 4.26, 1.2 × 1.1 × 0.4 | Normal |

All samples were analyzed by fiber-optic BeadChip and RT-qPCR

### Image scanning and data analysis

The bead chips were scanned by image analysis software Illumina BeadArray Reader (Illumina, San Diego, CA), and the original signal value for each probe was obtained. In order to reduce random errors generated by the experiment and to improve the comparability between the different samples, we used quantile normalization to correct these data. Data were then exported into the SAM (significance analysis of microarrays) software to calculate the genes differential expression fold and false discovery rates (FDR) between tumor and normal tissues. Filtering was performed to identify genes over- or under-expressed by at least 2.0-fold and the $q$ value (similar to the $P$ value, i.e., the significant genetic differences between the two sets of data when the control of FDR is 5% or less) <5% in tumors compared with normal pituitary. Further analysis regarding the potential relevance of differentially expressed genes in tumorigenesis was performed using the Genbank database (http://www.ncbi.nlm.nih.gov/Genbank/). In order to further elucidate tumorigenesis, we also analyzed the differentially expressed gene profiling using the Pathway analysis methods by R language and Bioconductor package. Hypergeometric tests were used to classify the enrichment of pathway, and FDR was calculated to correct the $P$ value.

### Reverse transcriptase-real time quantitative polymerase chain reaction

In order to verify the microarray analysis results, we randomly selected four down- or up-regulated genes which were differentially expressed in microarray analysis and measured their expression levels in a blinded fashion in five prolactinomas and three normal pituitary glands using RT-qPCR with SYBR GreenIdye detection (Applied Biosystems, Foster City, CA). $\beta$-Actin was used as an internal control. The four genes analyzed were as follows: PREDICTED-Homo sapiens UDP glucuronosyltransferase 2 family, polypeptide B7 (UGT2B7 GenBank accession no. XM_943434.1), growth arrest and DNA-damage-inducible, beta (GADD45B GenBank accession no. NM_015675.2), hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD GenBank accession no. NM_000860.3), S100 calcium binding protein A9 (calgranulin B) (S100A9 GenBank accession no. NM_002965.2). The primers of these genes were selected with Primer5.0 and synthesized by Generay Biotech Co. (Shanghai China). The primers used are listed in Table 2.

RT-qPCR was performed as described [10]. The reactions and analysis were carried out using the ABI Prism 7900 Sequence Detection System (Applied Biosystems). The fold-change of each gene was calculated using the $2^{-\Delta\Delta C_t}$ method, and the algorithm has been previously described in detail in the literature [11].

## Results

### Expression profile of prolactinomas

In the prolactinoma array, 27 genes and 3 ESTs were over-expressed ≥2.0-fold and 182 genes and 9 ESTs were under-expressed ≥2.0-fold. The highest over- and under-expressed genes are listed in Table 3. Two genes, HIGD1B, FAP and one EST were selectively and highly expressed in prolactinomas, 44 genes and two ESTs were only expressed in the normal pituitary tissues.

### Validation of differentially expressed genes by RT-qPCR

The microarray analysis showed that the genes UGT2B7, GADD45B, and HPGD decreased 55.7-, 6.4-, and 143.4-fold, respectively, whereas S100A9 increased 23.6-fold in all prolactinomas compared with normal pituitary controls. RT-qPCR analysis of these four genes confirmed the changes in mRNA levels that were observed by expression profiling. The mean expression level of four genes is shown in Fig. 1a. Figure 1b shows the relative expression level of four genes mRNA in microarray analysis compared with the RT-qPCR analysis.

### Pathway analysis

The KEGG pathways were compiled from multiple literature sources and integrated individual components into a

**Table 2** PCR primers used for real-time RT-PCR

| Gene | Forward primer | Reverse primer |
|---|---|---|
| UGT2B7 | 5′-GGTTCCTGCTGGTCTGTGTG-3′ | 5′-TCATTTTTTCCCTTCTTTGCTT-3′ |
| GADD45B | 5′-ATGGAGCAGAAGCCGGAGTG-3′ | 5′-GGGAGTTCATGGGTACAGAGCA-3′ |
| HPGD | 5′-CTCTTTGCAAGCAGTAATTAACACTA-3′ | 5′-CAGACAGGATGAGTCACTTATACGAT-3′ |
| S100A9 | 5′-GGCACCCCCTAAGACCACAG-3′ | 5′-GCCCCACAGCCAAGACAGTT-3′ |
| Beta-actin | 5′-GACTTAGTTGCGTTACACCCTTTC-3′ | 5′-TGCTGTCACCTTCACCGTTC-3′ |

**Table 3** The highest over-expressed and under-expressed genes in the prolactinomas

| Gene | Gene bank ID | Definition | Fold change |
|---|---|---|---|
| HIGD1B | NM_016438.2 | HIG1 domain family, member 1B | 91.7 |
| S100A9 | NM_002965.2 | S100 calcium binding protein A9 (calgranulin B) | 23.6 |
| EST | BX105747 | BX105747 Barstead colon HPLRB7 Homo sapiens cDNA clone IMAGp998G155786, mRNA sequence | 15.6 |
| LOC646043 | XM_929003.1 | PREDICTED: Homo sapiens similar to CG32346-PB, isoform B (LOC646043), mRNA | 13.5 |
| ANGPT2 | NM_001147.1 | Angiopoietin 2 | 11.7 |
| HPGD | NM_000860.3 | Hydroxyprostaglandin dehydrogenase 15-(NAD) | −143.4 |
| VSTM1 | NM_198481.3 | V-set and transmembrane domain containing 1 | −75.1 |
| TSHB | NM_000549.3 | Thyroid stimulating hormone, beta | −59.0 |
| GJB7 | NM_198568.1 | Gap junction protein, beta 7, 25 kDa | −57.0 |
| UGT2B7 | XM_943434.1 | PREDICTED: Homo sapiens UDP glucuronosyltransferase 2 family, polypeptide B7 (UGT2B7), mRNA | −55.7 |

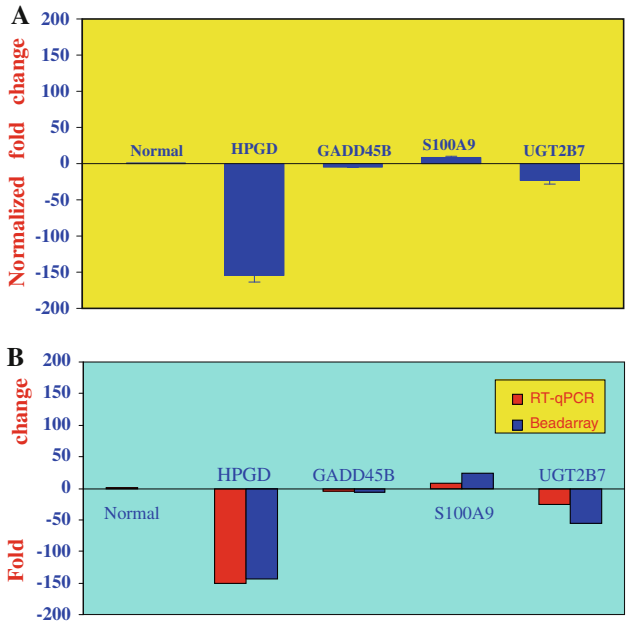


**Fig. 1** **a** RT-qPCR of relative mean expression of HPGD (−150.3-fold), GADD45B (−5.0-fold), S100A9 (8.8-fold) and UGT2B7 (−25.7-fold) mRNAs in prolactinomas compared with normal pituitaries. *Columns* represent mRNA expression of each gene in prolactinoma adenomas or normal pituitary glands and *bars* represent standard deviation (SD). Decreased expression for HPGD, UGT2B7 (see Table 3) and GADD45B (6.4-fold) were all confirmed by RT-qPCR. Increased expression for S100A9 (see Table 3) was also confirmed. **b** The relative expression level of four genes mRNA in microarray analysis compared with the RT-qPCR analysis. In right, *blue bars* represent the levels of gene expression by fiber-optic BeadArray Illumina WG6 v3.0 and, in left, *red bars* represent the RT-qPCR

unified pathway. Therefore, the KEGG pathway database was used to characterize the enrichment of specific pathway components into functionally regulated gene groups. We also analyzed the differentially expressed gene profiling and the genes expressed solely in normal pituitary glands using the pathway analysis methods by R language and Bioconductor package. Seven KEGG pathways were significantly enriched in the genes associated with prolactinomas ($P < 0.05$; see Table 4). Of the significant signaling pathways, two were selected for further analysis, the p53 signaling pathway and the GnRH signaling pathway.

## Discussion

### Analysis of the differentially expressed gene profiling

We used the NCBI database, Genbank (http://www.ncbi.nlm.nih.gov/Genbank/), to analyze the differentially expressed gene profiling in prolactinomas compared with normal pituitaries, the genes only expressed in the normal pituitary tissues and tumor tissues. We identified the genes HIGD1B, S100A9, ANGPT2, IL18, HPGD, ST18, and WIF1 as potential candidate genes involved in the pathogenesis of prolactinoma.

### HIGD1B (HIG1 domain family, member 1B)

In our BeadArray research, HIGD1B expression levels were significantly up-regulated and only expressed in prolactinoma tissues, but its functions were unknown. Denko et al. [12] reported that in cultured cells, *HIG1* and *HIG2* expressions are induced by hypoxia and by glucose deprivation. In addition, tumor xenografts derived from human cervical cancer cells display increased expression of *HIG1* and *HIG2* when they are deprived of oxygen. The role of HIGD1B in the pathogenesis of prolactinoma is not clear, but we believe that it may be associated with increasing tumor hypoxia tolerance, and it may promote

**Table 4** Seven highly significant ($P < 0.05$) pathways in prolactinomas

| Pathway ID | Pathway title | Genes | $P$ value |
|---|---|---|---|
| hsa00040 | Pentose and glucuronate interconversions | UGT2B11 UGT2B7 UGT2B17 | 0.001 |
| hsa00980 | Metabolism of xenobiotics by cytochrome P450 | UGT2B11 AKR1C2 UGT2B7 UGT2B17 | 0.003 |
| hsa00860 | Porphyrin and chlorophyll metabolism | UGT2B11 UGT2B7 UGT2B17 | 0.006 |
| hsa00150 | Androgen and estrogen metabolism | UGT2B11 UGT2B7 UGT2B17 | 0.013 |
| hsa04115 | p53 signaling pathway | PMAIP1 RPRM GADD45B | 0.022 |
| hsa00500 | Starch and sucrose metabolism | UGT2B11 UGT2B7 UGT2B17 | 0.037 |
| hsa04912 | GnRH signaling pathway | GNRHR LHB FSHB | 0.050 |

angiogenesis and drug resistance, but the specific mechanisms require further study.

S100A9 (S100 calcium binding protein A9, calgranulin B)

In our BeadArray and RT-qPCR research, the expression of S100A9 was significantly overexpressed. S100A9, also known as calgranulin B and MRP-14, belongs to the S100 protein family and has two $Ca^{2+}$-binding helix–loop–helix motifs (EF-hand motifs) [13]. Recent experimental data have suggested that changes in the expression and/or function of S100 proteins represent a key step during cancer development or progression [14].

More recently, an association of S100 protein expression with adenocarcinomas in humans has emerged. Immunohistochemical investigations have shown that S100A9 protein is expressed in hepatocellular carcinomas [15], pulmonary adenocarcinomas [16], and invasive ductal carcinomas of the breast [17]. In these tumors, elevated expression of S100A9 correlated with poor differentiation. The study showed that enhanced expression of S100A8, S100A9, and RAGE is an early event in prostate tumorigenesis and may contribute to development and progression or extension of prostate carcinomas [18]. S100A8 and S100A9 overexpressions should therefore be considered as markers of poor prognosis in invasive ductal carcinoma of the breast [19].

Our study showed that the expression of S100A9 was significantly overexpressed; it may play an important role in promoting the tumorigenesis, progression, and invasion of prolactinomas, but further study of the specific mechanisms is warranted.

ANGPT2 (angiopoietin 2)

Angiopoietins are members of another novel family of angiogenic factors that participate in the formation of blood vessels. Of the four currently known family members, the best characterized angiopoietins are angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), both of which function as ligands for the endothelium-specific tyrosine kinase receptor, Tie-2 [20]. Ang-1 reduces endothelial permeability of non-cerebral vessels and plays a major role in vascular stabilization and maturation and therefore in maintaining vessel integrity. Ang-2, which is believed to be an endogenous antagonist of Ang-1, competes for binding to the Tie-2 receptor and blocks the Ang-1-induced Tie-2 autophosphorylation during vasculogenesis, subsequently leading to a reduction in cell–matrix and cell–cell contacts which in turn allows access to angiogenic inducers [21].

Nag et al. [22] reported the localization of both Ang-1 and Ang-2 in scattered PAS positive adenohypophysial cells rather than in endothelial cells. There is also accumulating evidence that implicates Ang-2 in tumor progression [23]. The overexpression of Ang-2 by colon or gastric cancer cells enhanced tumor angiogenesis and growth in mice, and implicated Ang-2 in tumor invasion or metastases [23]. Our study showed that the Ang-2 was overexpressed by up to 11.7-fold in prolactinomas, and thus we considered that Ang-2 may play an important role as a promoter of angiogenesis, progression, and invasion of the tumor.

IL8 (interleukin 8)

IL-8 is a multifunctional chemokine, involved in inflammation mediated neutrophil infiltration and chemotaxis [24]. IL-8, a member of the Cysteine-X-Cysteine (CXC) motif chemokines, is one of the most promiscuous mediators of immune and cellular functions, including motility, invasion, and activation of survival and proliferative pathways in cells of mesenchymal lineage and in aggressive tumor cells [25, 26].

Green et al. [27] used the reverse transcriptase-linked polymerase chain reaction (PCR) to identify the presence of cytokine mRNA within human pituitary adenomas. All 17 adenomas expressed IL-8 transcripts: four somatotrophinomas, seven non-functional adenomas, four prolactinomas, one case of Cushing's disease, and one case of Nelson's syndrome. However, Suliman et al. [28] reported that IL-8 mRNA is present in the neoplastic cells of only a

small proportion (12% in this study) of human pituitary adenomas. It was identified in three pituitary adenomas, of which two were null-cell adenomas and one was gonadotrophinoma. In each of these tumors, more than 90% of cells were clearly positive for IL-8 mRNA. In the remaining 22 adenomas, IL-8 mRNA was not identified. Our studies showed that IL-8 was over-expressed in the prolactinomas, by up to 6.8-fold. The pathophysiological role of the cytokines in pituitary adenomas is not apparent; however, cytokines in general have many actions, including stimulation or inhibition of growth and proliferation, cellular differentiation, apoptosis, and hormone secretion [28].

### HPGD (hydroxyprostaglandin dehydrogenase 15-PGDH)

15-PGDH, a prostaglandin degrading enzyme, catalyses the oxidization of the 15(S)-hydroxyl group of PGE2 to yield an inactive 15-keto PGE2. PGE2 is related to carcinogenesis through immunosuppression, inhibition of apoptosis, increase of metastatic potential of epithelial cells, and promotion of angiogenesis [29]. The genetic deletion of 15-Pgdh in mice leads to increased tissue levels of PGE2 [30].

Recent studies identified a tumor suppressor activity of 15-PGDH in colon, lung, bladder cancers, and breast carcinomas, and suggested epigenetic silencing of the enzyme by DNA methylation and histone modification [31]. The expression of 5-PGDH in benign tumors is not clear. Our study shows that the expression of 5-PGDH in the prolactinoma was significantly down-regulated, by up to 143.4-fold, suggesting that the gene in prolactinomas may function as a tumor suppressor gene, playing an important role in the tumorigenesis and progression of this tumor. However, the specific mechanisms require further study.

### ST18 suppression of tumorigenicity18 (breast carcinoma) (zinc finger protein)

ST18 was first isolated from rat brain tissue. Human ST18 is 86% homologous with neural zinc-finger protein-3 (NZF3) [32]. ST18/NZF3 is a member of the myelin transcription factor 1 (MyT1) family of transcription factors that contain zinc-finger DNA-binding domains [33]. ST18 is expressed at relatively high levels in the brain and at lower levels in other tissues under normal conditions. However, its function has not yet been elucidated [34]. In breast cancer cell lines and the majority of primary breast tumors, ST18 mRNA is significantly downregulated [35]. Yang et al. [34] reported that ST18 plays an important role in stimulating proapoptotic and proinflammatory gene expression and that its overexpression significantly enhances apoptosis.

Our study shows that the expression of ST18 in the prolactinoma was significantly down-regulated, by up to 47.2-fold. The role of ST18 in the pathogenesis of prolactinoma is not clear, but it may play an important role in the tumorigenesis and progression of this type of tumor. However, the specific mechanisms require further elucidation.

### WIF1 (WNT inhibitory factor 1)

Wnt inhibitory factor-1 (WIF-1) is a secreted antagonist of Wnt signaling and functions by directly binding to Wnt ligands in the extracellular space. Elston et al. [36] used microarray analysis to compare pituitary tumors with normal pituitary gland and demonstrated that *WIF1* mRNA expression is markedly under-expressed in all pituitary tumor subtypes; they also confirmed via immunohistochemistry that WIF1 protein expression was reduced in 76% of tumors. Their data suggest that *WIF1* mRNA down-regulation in pituitary tumors is an early event because it is reduced in all tumor subtypes. Finally, they concluded that *WIF1* may be a tumor suppressor, specifically in nonfunctioning pituitary tumors, and that the Wnt pathways are important in pituitary tumorigenesis. Consistent with these data, our study showed that the expression of wif1 in the prolactinoma was significantly down-regulated, by up to 47-fold.

### Pathway analysis

Single-gene analysis may miss important effects on pathways. Cellular processes often affect sets of genes acting in concert. An increase of 20% in all genes encoding members of a metabolic pathway may dramatically alter the flux through the pathway and may be more important than a 20-fold increase in a single gene [37]. Therefore, we used the KEGG pathway database (http://www.genome.jp/kegg/pathway.html) to analyze the $P$ value $<0.05$ pathways (Table 4). The study suggested that the p53 signaling pathway and GnRH signaling pathway may play an important role in the pathogenesis of prolactinoma.

### P53 signaling pathway

In our study, the P53 signaling pathway in prolactinomas is inactivated. The p53 pathway is composed of a network of genes and their products are targeted to respond to a variety of intrinsic and extrinsic stress signals that impact cellular homeostatic mechanisms involved in monitoring DNA replication, chromosome segregation, and cell division [38]. In response to a stress signal, the p53 protein is activated in a specific manner by post-translational modifications, and this leads to cell cycle arrest, a program that

induces cellular senescence or cellular apoptosis [39]. Abrogation of the negative growth regulatory functions of p53 occurs in many, perhaps all, human tumors [40].

GADD45 is a family of proteins involved in the DNA damage response and cell growth arrest. They can also inhibit cell proliferation at different stages, including G1-S and G2-M checkpoints, and can induce cellular apoptosis [41]. Our study showed that the expression of GADD45B decreased suggesting that it may promote the proliferation and progression of prolactinomas.

Ohki et al. [42] reported that Reprimo (RPRM) induces cell cycle arrest by inhibiting Cdc2 activity and nuclear translocation of the Cdc2/cyclin B1 complex. Our study showed that the expression of RPRM decreased, suggesting that it may promote the proliferation and progression of prolactinomas.

The PMAIP1 gene is a tumor suppressor gene that is an important mediator of p53-related apoptosis. Recent studies have shown that altered expression of the PMAIP1 gene is involved in the development of a number of systemic malignancies in addition to pancreatic cancer [43]. Our study showed that the expression of PMAIP1 decreased suggesting that it may promote the proliferation and progression of prolactinomas.

From the above analysis, since it was observed that the P53 signaling pathway in prolactinomas is inactivated, thus it may play an important role in promoting the growth of this tumor.

GnRH signaling pathway

In our study, the GnRH signaling pathway is inactivated in prolactinoma. Gonadotropin-releasing hormone (GnRH) is the hypothalamic factor that mediates reproductive competence. Intermittent GnRH secretion from the hypothalamus acts upon its receptor in the anterior pituitary to regulate the production and release of LH and FSH. LH and FSH then stimulate sex steroid hormone synthesis and gametogenesis in the gonads to ensure reproductive competence. In our study, the genes GnRHR, LHB, and FSHB were involved in this pathway.

GnRHRs (gonadotropin-releasing hormone receptor) are members of the family of rhodopsin-like G protein-coupled receptors characterized by seven transmembrane domains. GnRH receptors are located in the anterior pituitary on the surface of the pituitary gonadotrope cells (representing 8–15% of anterior pituitary cells), where their activation by GnRH results in the biosynthesis and release of the gonadotropins luteinizing hormone (LH) and follicle-stimulated hormone (FSH) [44]. As a central component of the reproductive axis, it is not surprising that defects in the GnRH/GnRHR system have been proposed as a major cause of reproductive failure. Genetic mutations have been increasingly recognized as a cause of reproductive disorders such as idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome [45]. These disorders are characterized by absent or incomplete pubertal development and infertility associated with inappropriately low circulating levels of gonadotropins. Our study showed that the GNRHR was significantly under-expressed in prolactinoma, by up to 31.6-fold, and that it may be related to infertility.

The gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), play a pivotal role in controlling reproductive development and function in mammals [46]. These hormones are heterodimeric proteins composed of the $\alpha$-glycoprotein subunit, which is common to all members of the glycoprotein class of hormones, and a unique $\beta$ subunit (LH$\beta$, product of the Lh$\beta$ gene, or FSH$\beta$, product of the Fsh$\beta$ gene) that confers biological specificity. Once released from the gonadotroph, LH and FSH activate their receptors in the gonads, enhancing steroidogenesis and gametogenesis. Numerous mouse models have shown that the absence of LHB, LH signaling, and the inability of gonadotrophs to function normally impacts reproductive function [47]. Our study showed that the expression of LHB and FSHB was significantly under-expressed in prolactinoma, by up to 38.5- and 33.8-fold, respectively.

From the above analysis, we can see that the GnRH signaling pathway is inactivated in prolactinoma and it may be closely related to menstrual disorders or amenorrhea and infertility in this type of tumor.

In conclusion, through this study, we have identified a number of genes and pathways, which may play important roles in the tumorigenesis and progression of prolactinoma. Expression profiling analysis of tumors using the fiber-optic BeadArray appears to be a valid method of identifying genes that may be important in tumor pathogenesis. Moreover, the further analysis of gene expression profiling via the pathway methods may play a vital role in elucidating the pathogenesis of tumors.

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