LncRNA-mRNA Expression Pattern in Invasive Pituitary Adenomas: A Microarray Analysis

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Abstract

**Backgrounds:** Long non-coding RNAs (lncRNAs) play important roles in tumorigenesis and progression of various cancer types; however, their roles in the development of invasive pituitary adenomas (PAs) remain to be investigated.

**Methods:** lncRNA microarray was performed in three invasive and three noninvasive PAs. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed, and coexpression networks between lncRNA and mRNA were constructed. Furthermore, three differentially expressed lncRNAs were selected for validation by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) in PA samples. The diagnostic values of these three lncRNAs were further evaluated by receiver operating characteristic (ROC) analysis.

**Results:** A total of 8872 lncRNAs were identified in invasive and paired noninvasive PAs using lncRNA microarray. Among these, the differentially expressed lncRNAs included 81 that were upregulated and 165 that were downregulated. GO enrichment and KEGG pathway analysis showed that these differentially expressed lncRNAs were associated with post-translational modifications of proteins. Furthermore, we performed target gene prediction and coexpression analysis. The interrelationships between the lncRNAs and mRNAs with significant differential expression were identified. Additionally, three differentially expressed lncRNAs were selected for validation in 41 PA samples by qRT-PCR. The expression levels of FAM182B, LOC105371531, and LOC105375785 in the invasive PAs were significantly \( P < 0.05 \) lower than in the noninvasive PAs, and these results were consistent with the microarray data. ROC analysis suggested that FAM182B and LOC105375785 expression levels could be used to distinguish invasive PAs from noninvasive PAs.

**Conclusion:** Our findings demonstrated the lncRNAs expression patterns in invasive PAs. Thus, FAM182B and LOC105375785 may be involved in the invasiveness of PAs and serve as new candidate biomarkers for the diagnosis of invasive PAs.

**Background**

Pituitary adenomas (PAs), one of the most common intracranial tumors, account for 10%–20% of intracranial tumors [1]. According to the tumor biological characteristics, PAs can be divided into noninvasive pituitary adenoma (NIPA), invasive pituitary adenoma (IPA), and pituitary adenocarcinoma [2]. IPAs, characterized by high proliferative and invasive activities, tend to invade vital surrounding structures, such as the cavernous sinus, sphenoid bone, and cranial nerves [3]. The aggressive behavior of IPAs leads to a lower cure rate of complete removal by surgical resection and a higher incidence of recurrence. Therefore, the identification of novel biomarkers for early diagnosis that reflect the clinicopathological behaviors of IPAs is important. Additionally, exploration of the molecular mechanisms involved in the invasiveness of PAs is urgently needed.
IncRNAs, with lengths > 200 nucleotides, are involved in various processes of gene regulation, such as nuclear and cytoplasmic trafficking, chromosome dosage compensation, mRNA splicing, and translation [4]. With the development of high-throughput sequencing technologies, accumulating evidence indicates that the expression of IncRNA is associated with various tumors [5, 6]. Furthermore, IncRNAs have been increasingly identified as novel diagnostic and prognostic markers of various tumors [7, 8]; however, the roles of IncRNAs in PAs remain to be further investigated.

Xue et al.[9] have identified differentially expressed IncRNAs in PAs and revealed the key IncRNAs associated with the progression of PAs. Moreover, Guo et al.[10] showed that critical IncRNAs were associated with the recurrence of non-functioning PAs, while Zhu et al. [11] demonstrated that increased expression levels of the IncRNA maternally expressed 8 promote bone destruction in bone-invasive PAs by regulating miR-454-3p/tumor necrosis factor (TNF)-α. Exploring the expression patterns of IncRNAs in IPAs might confirm the existence of novel potential biomarkers for the diagnosis of IPAs.

This study aimed to investigate the expression pattern of IncRNAs in both invasive and noninvasive PAs. We performed microarray experiments to reveal the expression profiles of IncRNAs in three IPAs and three NIPAs. Then, we identified the expressed IncRNAs in IPAs and constructed the IncRNAs-mRNAs networks. Finally, among the differentially expressed IncRNAs, three IncRNAs were selectively examined by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) in a large sample size for further validation, and they were applied for the diagnosis of IPAs. These findings could provide a novel insight into understanding the mechanisms of the invasive behaviors of PAs. Perhaps, IncRNAs may even be novel biomarkers for the diagnosis of IPAs.

Material And Methods

Patients and samples

Tumor specimens were obtained from patients with PAs, who underwent transsphenoidal surgery at the Department of Neurosurgery of the Guangdong Provincial People's Hospital (Guangzhou, China) between January 2020 and June 2021. The diagnosis of PAs was based on clinical manifestations, biochemical features of hormonal secretion, magnetic resonance imaging (MRI), and histopathological analyses confirmed by two pathologists after surgical resections. NIPA is defined as the limitation of tumor mass within the sellar region, without any compression on peripheral structures (Fig. 1A, B). The definition of IPA is according to Knosp classifications into grades III–IV [12] (Fig. 1C, D). IPAs (n = 3) and NIPAs (n = 3) were selected for IncRNA microarrays. The details of these six PAs are shown in Table 1. Also, another 41 specimens of PAs, including IPAs (n = 21) and NIPAs (n = 16), were used for the validation by qRT-PCR. The clinical characteristics of the 41 patients with PAs are summarized in Table 2. Tumor dimensions were manually obtained from MRI. A microadenoma was defined by a maximal tumor diameter of < 10 mm; a macroadenoma was ≥ 10 mm; a large macroadenoma was ≥ 20 mm; while a giant adenoma was ≥ 40 mm. The dimensional indices of the tumors were measured and recorded in three orthogonal planes, namely: transverse (TR), anteroposterior (AP), and craniocaudal (CC). The tumor
volumes were estimated using the following formula: \( V = \frac{\pi \times [TR \times AP \times CC]}{6} \) [13]. After surgical excision, all tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analyses. All procedures of this study were approved by the Ethics Committee of Guangdong Provincial People's Hospital. Informed consent was obtained from all patients.

**Total RNA extraction and purification**

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Furthermore, the quantification of total RNA was evaluated by Bioanalyzer 2200 (Agilent, California, USA) and kept at −80 °C. The RNA samples with RNA integrity number (RIN) > 6.0 are acceptable for rRNA depletion and subsequent IncRNA purification. The purification of total RNA was validated by gel electrophoresis.

**cDNA library construction**

cDNA libraries were constructed for each pooled RNA sample using the NEBNext® Ultra™ Directional RNA Library Prep Kit (New England BioLabs Inc., MA, US) for Illumina according to the manufacturer’s instructions. The protocol comprises the following steps: mRNA molecules are fragmented into 150–200 bp using divalent cations at 94 °C for 8 min. The cleaved RNA fragments as templates were reverse-transcribed into first-strand cDNA. Subsequently, the second-strand cDNA was synthesized using Polymerase I and RNase H with reaction buffer. Target bands were harvested through AMPure XP Beads (Beckman Coulter). The products were then purified and enriched by PCR to create the final cDNA libraries and quantified by Agilent 2200. The tagged cDNA libraries were pooled in equal ratio and used for 150 bp paired-end sequencing in a single lane of the Illumina HiSeq XTen. The experiments of library construction and RNA sequencing were completed at the Center of NovelBio Lab center of Novelbio lab (Shanghai, China).

**Mapping and identification of differentially expressed genes**

Before read mapping, clean reads were obtained from the raw reads by removing the adaptor sequences, reads with > 5% ambiguous bases (noted as N), and low-quality reads containing more than 20% of bases with qualities of < 20. The clean reads were then aligned to the human genome (version: GRCh38 NCBI) using the HISAT2 [14]. HTSeq was used to count gene and IncRNA, while reads per kilobase per million mapped reads method was used to determine gene expression [15]. We applied DESeq algorithm [16] to filter the differentially expressed genes, after the analysis of the level of significance, i.e, determination of \( P \) value, and false discovery rate (FDR) analysis under the following criteria: 1) fold change > 2 or < 0.5; 2) FDR < 0.05 [17].

**Functional enrichment analysis**

Gene Ontology (GO) analysis was performed to facilitate elucidating the biological implications of unique genes [18]. We downloaded the GO annotations from NCBI (http://www.ncbi.nlm.nih.gov/), UniProt (http://www.uniprot.org/), and the Gene Ontology (http://www.geneontology.org/). Fisher’s exact test was
applied to identify the significant GO categories and FDR was used to correct the $P$ values. The Gene Ontology is structured as a directed acyclic graph, and each term has defined relationships to one or more other terms. GO-Tree was built based on the Gene Ontology Directed Acyclic Graph to provide user-friendly data navigation and visualization. We selected the significant GO-Term ($P$ value < 0.01) in GO analysis based on the differentially expressed genes to construct the GO-Tree for summarizing the function affected in the experiment [19].

Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to clarify intuitively the role and significance of target genes in the overall biological pathways [20]. We selected the genes in enriched biological pathways and using Cytoscape for graphical representations of pathways [21]. The KEGG database was used to build the network of genes according to the relationship among the genes, proteins, and compounds in the database.

**Construction of the IncRNA-miRNA-target gene interaction network**

The role of IncRNAs in IPAs was investigated by a IncRNA-miRNA-target gene interaction network. According to the IncRNA microarray results, the 10 most dysregulated IncRNAs in IPAs were selected and Cytoscape software was performed to map out an interaction network. Putative interactions between IncRNAs and miRNAs were predicted using the online databases Jefferson Computational Medicine Center-RNA22 v2 microRNA target detection (https://cm.jefferson.edu/ma22/Interactive/) and LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?p=r=lncbasev2%2Findex-predicted). Then, the miRNA with the highest target scores were selected, and their target genes were evaluated by TargetScan [22] and miRanda [23]. Finally, miRNAs and their target genes with high targeting-relationship scores were selected to construct the IncRNA-miRNA-mRNA interaction network. The interaction network was delineated using the Cytoscape software.

**qRT-PCR assay for the validation**

Total RNA was extracted using Trizol reagent (Invitrogen) for 41 specimens of PAs. Reverse transcription and qRT-PCR were performed using a Reverse Transcription Kit (Takara, Dalian, China) and PrimeScript RT Reagent Kit (Takara, Dalian, China), respectively, as previously described [24]. The expression of IncRNA was measured by qRT-PCR. The sequences of the primers are listed in Table 3. The gene expression levels were normalized to *actin*. Gene expression levels were determined by the $2^{-\Delta\Delta Ct}$ method and analyzed for statistical significance.

**Statistical analysis**

Measurement data are presented as mean ± standard error of the mean (SEM) and enumeration data are presented as percentages. Comparisons were performed using independent sample t-test between pairs of groups or one-way analysis of variance for more than two groups followed by Dunnett’s multiple comparison test. The receiver operating characteristic (ROC) curves and the area under the curve (AUC) were used to estimate the diagnostic power and accuracy of IncRNAs on invasive/noninvasive PAs. All
statistical analyses were performed on Statistical Product and Service Solutions (SPSS) 25.0 software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant, which is indicated in the figures.

**Results**

**Identification of differentially expressed IncRNAs between IPAs and NIPAs**

To identify differentially expressed IncRNAs in IPAs and NIPAs, we performed a high-throughput human IncRNA microarray in three IPAs and three NIPAs. The correlation plot was used to detect the correlation between microarray samples and to confirm the homogeneity between biological replicates (Fig. 2A). The box plot demonstrated that the distributions of normalized intensities were almost identical among all samples (Fig. 2B). Furthermore, hierarchical clustering was used to illustrate significantly differential IncRNAs expression in the two groups (Fig. 2C), and the volcano plots were used to reveal the variations in IncRNAs expression levels between the two groups (Fig. 2D). Overall, 8872 IncRNAs and 16039 mRNAs were identified in PAs by human IncRNA microarray. Among these, 246 IncRNAs were differentially expressed in IPAs compared with NIPAs, including 81 upregulated IncRNAs and 165 downregulated ones (Fig. 2D). Meanwhile, 566 mRNAs were differentially expressed, including 289 upregulated mRNAs and 277 downregulated ones between the invasive and noninvasive tumor tissues. These results suggested that the expression of IncRNAs in IPAs was different from that matched NIPAs.

**Delineation of GO enrichment and KEGG pathway analysis**

To further investigate the functional roles of the differentially expressed IncRNAs, GO enrichment and KEGG pathway analyses were performed. GO terms are classified into three different domains, namely: biological processes (BP), molecular functions (MF), and cellular components (CC). The top 15 generally changed GO terms were ranked by fold enrichment or enrichment score as listed in Figure 3. The top five identified BP were post-translational protein modification, cellular protein metabolic process, regulation of inhibitory postsynaptic membrane, negative regulation of synapse assembly, and visual perception (Fig. 3A). The top five identified MF terms were RS domain binding, ligand-gated ion channel activity, cyclic guanosine monophosphate (cGMP) binding, uridine diphosphate (UDP) -glycosyltransferase activity, and ion channel binding (Fig. 3B). Furthermore, the top five identified CC terms were slit diaphragm, nuclear speck, dendrite, transmembrane transporter complex, and gamma-tubulin small complex (Fig. 3C).

The KEGG database was used to identify the pathways and molecular interactions associated with the target genes. Our data indicated that the target genes were mostly enriched in neuroactive ligand-receptor interaction, hypoxia-inducible factor 1alpha (HIF-1) signaling pathway, spliceosome, pathways in cancer, and N-glycan biosynthesis (Fig. 4). According to these results, these pathways may contribute significantly to the invasiveness of PAs.

**Construction of the IncRNA-miRNA-mRNA coexpression network**
To reveal the potential functions and mechanisms of differently expressed lncRNAs in IPAs, a lncRNA-miRNA-mRNA coexpression network was subsequently constructed, based on bioinformatics analysis. The most down- and up-regulated lncRNAs in IPAs were selected for the lncRNA-miRNA-mRNA coexpression network, which is delineated using Cytoscape (Fig. 5).

Validation of differentially expressed lncRNAs in PAs by qRT-PCR

To validate the microarray results, three lncRNAs that exhibited significant changes in expression levels were selected for validation using qRT-PCR in 25 IPAs and 16 NIPAs, namely: FAM182B, LOC105371531, and LOC105375785. qRT-PCR results confirmed that all three lncRNAs were significantly \((P < 0.05)\) decreased in IPAs than in NIPAs (Fig. 6A-C). The results of qRT-PCR were consistent with the microarray expression data, confirming the high reliability of the microarray data.

Diagnostic values of three selected lncRNAs for IPAs

ROC curve analyses were performed to determine the diagnostic sensitivity and specificity of the three selected lncRNAs for IPAs (Fig. 6D-F). The AUCs for FAM182B, LOC105371531, and LOC105375785 were 0.798 (95% confidence interval (95% CI): 0.650–0.945), 0.730 (95% CI: 0.557–0.945), and 0.762 (95% CI: 0.604–0.921), respectively. These data suggested that FAM182B and LOC105375785 can be used to distinguish patients with IPAs from patients with NIPA if AUC \(\geq 0.75\) is considered diagnostically significant for the biomarker.

Correlation between FAM182B expression and clinical features of patients with PAs

In consideration of FAM182B with the highest value of AUC, we conducted further investigation on FAM182B. Using median values of FAM182B expression levels of all patients as the boundary line, we further divided the 41 patients with PAs into high-/low-expression subgroups of FAM182B. The analysis demonstrated that FAM182B expression had no association with gender, age, Ki67 percentage, and surgical extent; however, a significant relationship was found between invasive behavior and FAM182B \((P = 0.001)\) (Table 4). The ratio of FAM182B high-expression in IPAs was significantly \((P = 0.001)\) higher than that in patients with NIPA (Table 4).

Discussion

lncRNAs, a class of RNA transcripts longer than 200 nucleotides, can regulate the expression of protein-coding genes at the transcriptional and translational levels [25]. lncRNAs are involved in various processes related to gene regulation [26]. Accumulating evidence indicates that the expression of lncRNAs is associated with various tumors and can be a promising biomarker for the diagnosis of tumors [27]. Pituitary adenoma, one of the most common intracranial tumors, may invade the cranial bone, sphenoid bone, etc [3]. The mechanisms associated with the invasion of PAs and novel biomarkers for the diagnosis of IPAs remain largely unclear. Recently, several lncRNAs, including small nucleolar RNA host gene 1 (SNHG1), H19, colon cancer associated transcript 2 (CCAT2), LINC00473, and antisense non-coding RNA in the INK4 locus (ANRIL), participated in the proliferation, progression, and invasion of
PAs [28-31]. However, the expression pattern of IncRNAs-mRNA and comprehensive analysis for
dysregulated IncRNAs in IPAs remained to be investigated.

In this study, IncRNA microarray was performed to investigate the IncRNA expression pattern in the
invasive and noninvasive PAs. We identified 246 IncRNAs that were differentially expressed at a
significant level; 81 of which were upregulated and 165 were downregulated in IPAs. Then, these
differentially expressed IncRNAs were integrated into hierarchical categories according to the heat maps.
We observed that the IncRNA expression patterns were remarkably different between the invasive and
noninvasive PAs. The results may indicate that IncRNAs are involved in regulating the invasiveness of
PAs. These findings are consistent with previous reports, which reported that IncRNAs are involved in
tumor invasive behaviors [32, 33]. The results of the expression pattern of the differentially expressed
IncRNAs and pathway analysis are different from the previous study analyzed using the GSE26966
database [9]. The basis of this difference may be because pituitary gonadotrope tumors were used for the
microarray experiments in the GSE26966 database.

To further identify the potential function of these differentially expressed IncRNAs, GO enrichment and
KEGG pathway analyses were performed. Notably, the most significant GO terms of IncRNAs were post-
translational protein modifications, which were previously reported to be important in the development of
PAs [34]. KEGG pathway analysis for the differentially expressed IncRNAs revealed that neuroactive
ligand-receptor interaction, HIF-1 signaling pathway may serve pivotal roles in the invasive mechanisms
of PAs, as they were more likely to be identified in the IPAs than in NIPAs. Previous studies demonstrated
that the expression levels of HIF-1 in IPAs were significantly higher than in NIPAs, and HIF-1 signaling
pathway contributed to promoting the invasion of PAs [35, 36]. Hou et al. [37] identified that differentially
expressed genes in pituitary gonadotroph adenomas were enriched in neuroactive ligand-receptor
interaction pathway, which agrees with our results.

Further analysis of three dysregulated IncRNAs from the tissues of 25 IPAs and 16 NIPAs confirmed the
reliability of the IncRNA microarray results. Additionally, the three validated IncRNAs, FAM182B,
LOC105371531, and LOC105375785, were downregulated in IPAs and could distinguish IPAs from NIPAs.
These results collectively demonstrated that IncRNAs may be implicated in the invasive behaviors of PAs.
Accumulating evidence has also shown that IncRNAs can be promising biomarkers in various
cancers [38, 39]. For instance, Liu et al. [40] demonstrated that SNHG16 can be a potential biomarker in
hepatocellular carcinoma, while Teng et al. [41] showed that lung cancer associated transcript 1 acts as a
potential biomarker in gastric cancer. In our study, both FAM182B and LOC105375785 had relatively high
specificity and sufficient sensitivity for the diagnosis of IPAs by ROC analysis. These results collectively
demonstrated that IncRNAs may function as promising novel biomarkers for the diagnosis of IPAs.
Moreover, FAM182B is associated with hepatocellular carcinoma [42]. In our study, FAM182B, with the
highest value of AUC among the three validated IncRNAs, had a significant relationship with the invasive
behavior of PAs.
Notably, there are two main limitations of this study. First, we did not perform the functional confirmation of these differentially expressed lncRNAs to clarify the functions and mechanisms of lncRNAs in the invasiveness of PAs. Second, the number of PAs is relatively small that may limit statistical power. The possible clinical implications of lncRNAs for the diagnosis of IPAs remain to be elucidated using a larger number of samples from patients with IPAs.

In conclusion, our results revealed the expression profile of differentially expressed lncRNAs in IPAs by microarray analysis. Furthermore, GO enrichment and KEGG pathway analyses were performed to identify the potential functions of differentially expressed lncRNAs. Additionally, coexpression networks were constructed for lncRNA-mRNA. Taken together, three validated lncRNAs, namely: FAM182B, LOC105371531, and LOC105375785 can be promising biomarkers in differentiating IPAs with NIPAs. Further investigations are needed to illustrate the detailed functions and mechanisms of lncRNAs in the invasive behaviors of PAs.

**Abbreviations**

lncRNAs: long non-coding RNAs; PAs: pituitary adenomas; NIPA: noninvasive pituitary adenoma; IPA: invasive pituitary adenoma; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; qRT-PCR: real-time quantitative reverse transcription polymerase chain reaction; ROC: receiver operating characteristic; AUC: area under the curve; MRI: magnetic resonance imaging; TR: transverse; AP: anteroposterior; CC: craniocaudal; RIN: RNA integrity number; FDR: false discovery rate; SPSS: Statistical Product and Service Solutions; BP: biological processes; MF: molecular functions; CC: cellular components

**Declarations**

**Ethics approval and consent to participate**

All procedures of this study were approved by the Ethics Committee of Guangdong Provincial People's Hospital. Informed consent was obtained from all patients.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.
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Author contributions
Chao Peng and Shuaikai Wang performed the experiments, prepared figures and tables, and wrote the manuscript. Jinxiu Yu analyzed the data. Xiaoyi Deng, Zhishan Chen, Huiyu Ye, Hongru Yao and Hanjia Cai collected the data and performed the experiments. Yong Yuan and Yanli Li conceived and designed the study. All the authors agreed on the final manuscript.

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**Tables**

Table 1. Details of three IPAs and three NIPAs used for microarray analysis.

| ID    | Sex | Age (years) | Tumor size (mm) | Secretory function | Knosp grade |
|-------|-----|-------------|-----------------|--------------------|-------------|
| IPA-1 | male | 21          | 25*20*38        | Nonfunctioning     | IV          |
| IPA-2 | male | 50          | 24*26*18        | Nonfunctioning     | III         |
| IPA-3 | female | 57          | 23*22*18        | Nonfunctioning     | IV          |
| NIPA-1 | male | 41          | 35*24*25        | Nonfunctioning     | I           |
| NIPA-2 | male | 56          | 29*26*19        | Nonfunctioning     | I           |
| NIPA-3 | female | 38          | 14*18*18        | Nonfunctioning     | II          |

Table 2. Characteristics of 41 patients with PAs.
### Table 3. Oligonucleotide sequences of primer for qRT-PCR analysis.

| Gene symbol | Forward primer | Reverse primer |
|-------------|----------------|----------------|
| Actin       | TGTGGATCGGTGGCTCCATCCT | AAACGCAGCTCAGTAACAGTCCGC |
| FAM182B     | GCACCTCTGGGTCTCTGTTCTC | CACTTTCCCTGCTCTTCACTACAC |
| LOC105371531| CAGGGTTATGAGATCGTC   | GTTTCTGGGTCTTTGGAGT    |
| LOC105375785| ATCATCAGCTGCCCACCAT  | AGTCGGATGACCTCCTCCTTT  |

PRL, prolactin hormone; GH, growth hormone; ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone.
Table 4. Association between FAM182B expression and clinical characteristics

| Variable            | FAM182B expression | Univariate analysis |
|---------------------|--------------------|---------------------|
|                     | Low (n=20)         | High (n=21)         | $c^2$ | $P$ value |
| Gender              |                    |                     |      |           |
| Male                | 11 (55.0%)         | 14 (66.7%)          | 0.586 | 0.444     |
| female              | 9 (45.0%)          | 7 (33.3%)           |      |           |
| Age (years)         |                    |                     |      |           |
| <44                 | 12 (60.0%)         | 8 (38.1%)           | 1.967 | 0.161     |
| ≥44                 | 8 (40.0%)          | 13 (61.9%)          |      |           |
| Ki67 (%)            |                    |                     |      |           |
| <3                  | 15 (75.0%)         | 15 (71.4%)          | 0.067 | 0.796     |
| ≥3                  | 5 (25.0%)          | 6 (28.6%)           |      |           |
| Surgical extent     |                    |                     |      |           |
| Residual            | 3 (15.0%)          | 7 (33.3%)           | 2.012 | 0.156     |
| Gross total         | 17 (85.0%)         | 14 (66.7%)          |      |           |
| Invasiveness        |                    |                     |      |           |
| No                  | 13 (65.0%)         | 3 (14.3%)           | 11.072 | 0.001     |
| Yes                 | 7 (35.0%)          | 18 (85.7%)          |      |           |

Figures
Figure 1

Enhanced magnetic resonance imaging of two patients with noninvasive pituitary adenoma (NIPA) and invasive pituitary adenoma (IPA), respectively. (A, B) Coronal and sagittal scans of NIPAs, respectively. (C, D) Coronal and sagittal scans of IPAs invaded the left cavernous sinus to surround the internal carotid artery (Knosp classification grades IV).
Figure 2

Long non-coding RNAs (IncRNA) profiles based on microarray. (A) The correlation among the six samples based on expression values of differentially expressed IncRNAs. (B) The boxplots of microarray intensity values. (C) Heat map based on the expression values of significantly altered IncRNAs with fold changes > 2 or < 0.5 and P < 0.05. Red and green indicate increased and decreased expression levels, respectively. (D) Volcano plot showing differently expressed IncRNAs between invasive pituitary adenomas and noninvasive pituitary adenomas, with red dots indicating upregulation and green dots indicating downregulation.
Figure 3

Gene ontology (GO) terms from BP (A), MF (B), and CC (C). Functional analysis of differentially expressed IncRNAs and co-expressed mRNAs for GO enrichment. BP, biological processes; MF, molecular functions; CC, cellular components.
Figure 4

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed long non-coding RNAs (lncRNAs) and co-expressed mRNAs. The length of the column indicates the P value. The longer the column and the lower the P value, the more enriched and meaningful the pathway.
Figure 5

The interaction network of long non-coding RNAs (lncRNAs)-miRNA-target genes. The arrows, rhombus, and circle indicate lncRNA, miRNA, and mRNA, respectively. Red and blue colors designate upregulation and downregulation, respectively.
Figure 6

Validation of long non-coding RNAs (lncRNAs) microarray results by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) and receiver operating characteristic (ROC) analysis for the three selected lncRNAs. (A–C) The expression levels of the three selected lncRNAs, including FAM182B, LOC105371531, and LOC105375785 in invasive pituitary adenoma (IPAs) (n = 25) and noninvasive pituitary adenoma (NIPAs) (n = 16). (D–F) ROC curves of the selected three lncRNAs between IPAs and NIPAs. Data is represented as mean ± SEM, *P < 0.05, **P < 0.01, and ***P < 0.001 versus the NIPAs group.