

Differential circular RNA expression profiles of invasive and non-invasive non-functioning pituitary adenomas

A microarray analysis

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Abstract

Non-functioning pituitary adenomas (NFPAs) are the most common pituitary tumors, and some exhibit locally invasive or even clinically aggressive behavior. Circular RNAs (circRNAs) are a reinvented class of non-coding RNAs that play important roles in tumor initiation and progression.

CircRNA microarray assays were performed in 4 invasive and 4 non-invasive NFPAs, and 4 typically differential expression circRNAs were selected for validation using quantitative reverse transcription-polymerase chain reaction. The diagnostic and prognostic values of tested circRNAs were further evaluated. Bioinformatics analysis and a literature review of potential miRNAs targets involved in pituitary tumor invasion were performed.

A specific circRNA expression profile was detected between invasive and non-invasive NFPAs, including 91 upregulated and 61 downregulated circRNAs in invasive tumors. The dysregulation of the 4 circRNAs has been confirmed. The expression of hsa_circRNA_102597, a downregulated circRNA, was significantly correlated with tumor diameter ($P < .05$) and Knosp grade ($P < .01$). Hsa_circRNA_102597 alone or in combined with Ki-67 index was able to accurately differentiate invasive from non-invasive NFPAs as well as predict tumor progression/recurrence. Fourteen aberrantly expressed circRNAs might be involved in the invasiveness of pituitary adenomas via seven predicted potential miRNA targets.

CircRNAs are participated in pituitary tumor invasion, and may be used as novel diagnostic and prognostic biomarkers in NFPAs.

Abbreviations: AUC = area under the receiver operating characteristic curve, circRNAs = circular RNAs, circSRY = sex-determining region Y, ciRS-7 = circular RNA sponge for miR-7, HMGA2 = high-mobility group A 2, miRNA = microRNA, mirSVR = miRNA support vector regression, MREs = miRNA response elements, MRI = magnetic resonance imaging, ncRNAs = noncoding RNAs, NFPAs = non-functioning pituitary adenomas, qRT-PCR = real-time quantitative reverse transcription-polymerase chain reaction, ROC = receiver operating characteristic.

Keywords: biomarker, circular RNA, invasive, microRNA, pituitary adenoma

Editor: Kou Yi.

YH and NZ contributed equally to this work and are co-first authors.

Informed consent was obtained from all individual participants included in the study.

This work is supported by Sichuan province science and technology support plan (2015SZ0120, 2016SZ0015, and 2018SZ0179).

All authors declare that they had no conflict of interest.

Supplemental Digital Content is available for this article.

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Medicine (2019) 98:26(e16148)

Received: 28 November 2018 / Received in final form: 24 May 2019 / Accepted: 29 May 2019

<http://dx.doi.org/10.1097/MD.00000000000016148>

1. Introduction

Pituitary adenomas, arising from adenohypophyseal cells, account for ~10% to 15% of all intracranial tumors and have a prevalence of 77.6 cases per 100,000 population.^[1,2] According to clinical and biochemical characteristics, pituitary adenomas are classified into functioning and non-functioning adenomas. The most common pituitary tumors are non-functioning pituitary adenomas (NFPAs), which do not present with symptoms associated with hormone hypersecretion but rather compressive symptoms.^[3] Although the majority of pituitary adenomas are biologically benign, 25% to 55% of them locally invade surrounding structures, such as cavernous sinus and sphenoid sinus, making curative radical surgery difficult.^[2,4] Approximately 50% of patients with NFPAs suffered incomplete surgical resection, and the regrowth of the residual tumor may occur, necessitating adjuvant therapies or additional surgery.^[5,6] Therefore, the identification of biomarkers that reflect the clinicopathological behavior of NFPAs is of great importance in enhancing prognostic predictions as well as guiding early adjuvant treatments after surgery.^[4,7]

Circular RNAs (circRNAs), a novel type of endogenous noncoding RNAs (ncRNAs), represent a recent hotspot in the field of RNA research.^[8,9] CircRNAs were serendipitously discovered and originally misinterpreted as “splicing rubbish” decades ago, but recent advances in RNA sequencing revealed that a fairly large number of circRNAs are stable, evolutionarily conserved and tissue-specific ncRNAs in eukaryotes.^[10] Unlike linear RNAs, circRNAs are produced by back-splice events and characterized by covalently closed loop structures without 5' to 3' polarities and polyadenylated tails.^[11] Therefore, circRNAs are resistant to degradation by RNase R and much more stable than linear RNAs. Although the functions of circRNAs still remain largely unknown, recent studies have demonstrated that circRNAs could function as microRNA (miRNA) sponges to regulate gene expression at post-transcriptional levels, such as ciRS-7 (circular RNA sponge for miR-7) and circSRY (sex-determining region Y).^[8,9] Ongoing studies indicated that circRNAs may play important roles in biological development, and particularly the initiation and progression of various tumors.^[8,9,12,13] Moreover, circRNAs have been increasingly used as novel diagnostic and prognostic markers in colorectal cancer, gastric cancer, laryngeal cancer, and so on.^[14–16]

Considering the emerging roles of circRNAs in cancers, we used microarrays to evaluate the circRNA expression profiles in 8 NFPAs, of which 4 NFPAs were invasive and 4 NFPAs were non-invasive tumors. Among the aberrantly expressed circRNAs, 4 circRNAs were selected and tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Subsequently, a validated downregulated circRNA, hsa_circRNA_102597, was selected to further explore its clinical significance and application in NFPAs. Bioinformatics analysis was performed to seek potential miRNA targets associated with the dysregulated circRNAs.

2. Materials and methods

2.1. Patients and samples

Tumor samples were obtained from patients who underwent transsphenoidal surgery or craniotomy for NFPA resection at West China Hospital. Patients with previous surgical resection or radiation therapy were excluded from this study. NFPA was

diagnosed when there was neither clinical nor biochemical features of hormonal hypersecretion as well as histopathological analysis confirmed a pituitary adenoma. The preoperative pituitary hormone deficiencies were recorded in all participants. Tumor volume was calculated using the length \times width \times height \times 0.5 on the basis of preoperative magnetic resonance imaging (MRI). Tumor invasion was determined by Knosp grading scale (grades 3 or 4) or intraoperative finding (sphenoid sinus invasion). Tumor recurrence was diagnosed when a new growth was documented on follow-up MRI after complete surgical resection, and tumor progression was defined as the regrowth of a tumor remnant. The residual tumor was considered stable if there no signs of tumor progression on follow-up MRI. The clinicopathological characteristics of all patients are detailed in Supplementary Table 1, <http://links.lww.com/MD/D52>. After excision, tumor specimens were preserved in liquid nitrogen within half an hour and then stored in a freezer at -80°C until RNA extraction. Four invasive and 4 non-invasive samples were selected for microarray based circRNA profiling study, and all 46 specimens were used for qRT-PCR validation. This study was approved by the Ethics Committee of West China Hospital and informed consent was obtained from all patients. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

2.2. Total RNA extraction and quality control

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. The purity and concentration of total RNA were evaluated by NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE). Agarose gel electrophoresis was conducted to assess the total RNA integrity.

2.3. RNA labeling and hybridization

In order to enrich pure circRNAs, the total RNA was treated with RNase R (Epicentre, USA) to remove linear RNA. The enriched circRNAs were amplified and transcribed into fluorescent circRNAs by utilizing a random priming method according to Arraystar Super RNA Labeling protocol (Arraystar, USA). Next, the labeled circRNAs were purified using RNeasy Mini Kit (Qiagen, Germany). To assess the labeling efficiency, the concentration and specific activity of the labeled circRNAs were evaluated by NanoDrop ND-1000. The labeled circRNAs were hybridized onto the Arraystar Human circRNA Arrays ($8 \times 15\text{K}$, Arraystar). The slides were incubated at 65°C for 17 hours in an Agilent Hybridization Oven. After washing, the slides were fixed and scanned using the Agilent G2505C Scanner.

2.4. Microarray data analysis

The acquired array images were analyzed using Agilent Feature Extraction software. R software package was used for quantile normalization and subsequent data processing. Low-intensity filtering was performed after normalization of the raw data. The circRNAs with at least 4 out of 8 samples that have flags in Present or Marginal were selected for further differential analysis. Unpaired *t* test was used to analyze the statistical difference. CircRNAs with a fold change in expression ≥ 2.0 (*P* value $< .05$) were considered differentially expressed with statistical significance. The hierarchical clustering analysis was performed to generate an overview of aberrantly expressed circRNAs using Cluster and TreeView program.

2.5. qRT-PCR assay

The extracted RNA was subjected to cDNA synthesis using PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa) according to the manufacturer's instructions. The qRT-PCR was carried out with SYBR Premix Ex Taq (TaKaRa) on a Bio-Rad CFX96 system (Bio-Rad). β -actin was used as an internal control. The Relative level of each circRNA was calculated based on C_T values (corrected for β -actin expression) according to the equation: $2^{-\Delta\Delta CT}$ ($\Delta CT = C_T$ (gene of interest) - C_T (β -actin)). The primers sequences used for qRT-PCR are listed in Supplementary Table 2, <http://links.lww.com/MD/D52>.

2.6. Sanger sequencing

The qRT-PCR products of tissue hsa_circRNA_102597 were purified, and then cloned using PMD18-T Vector Cloning Kit (TaKaRa). Sanger sequencing was performed by Qingkezixi Biotech Co., Ltd (Hangzhou, China).

2.7. Delineation of circRNA-miRNA interactions

Arraystar's homemade software based on miRanda and TargetScan was used to predict potential miRNA targets of differentially expressed circRNAs. The efficiency of the predicted miRNA targets was scored and ranked using the miRNA support vector regression (mirSVR) algorithm. Accordingly, we identified 5 miRNAs with the highest mirSVR score for each circRNA. The circRNA-miRNA network was constructed with the help of Cytoscape 3.01.

2.8. Immunohistochemistry

All tumor samples were fixed in 10% buffered formalin, embedded in paraffin for 5 μ m serial sections, and stained with hematoxylin and eosine routinely. Immunohistochemistry was carried out using specific antibodies against Ki-67 and hormones (GH, PRL, ACTH, β -TSH, β -LH, β -FSH, and α -subunit). All the antibodies were from ZSGB-BIO (China), except for the α -subunit (Bio-Rad, UK). All the procedures were carried out at the pathology department of our hospital.

2.9. Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Data were expressed as mean \pm SD or median (interquartile range), depending on data distribution. The artworks were created by GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Differences in continuous variables between groups were analyzed with independent sample t-test or Wilcoxon signed rank test. The comparison of categorical variables was carried out using the χ^2 test. The sensitivity and specificity of the biomarkers were evaluated using the area under the receiver operating characteristic curve (AUC). A combined biomarker expression score was obtained with logistic regression. $P < .05$ was considered statistically significant.

3. Results

3.1. Differential circRNA expression profiles between invasive and non-invasive NFPA.

In this study, a total of 12,371 circRNA targets were evaluated in 4 invasive and 4 non-invasive NFPA tumor tissues by high-

throughput human circRNA microarray. A box plot revealed that the overall distributions of the tested sample data were essentially identical (Fig. 1A). The difference in circRNA expression between the invasive and the non-invasive tumor samples was displayed in a scatter plot (Fig. 1B). And significantly aberrantly expressed circRNAs between the 2 groups were illustrated in the volcano plot (Fig. 1C). Overall, 152 dysregulated circRNAs (fold change ≥ 2.0 , $P < .05$) were identified (Supplementary Table 3, <http://links.lww.com/MD/D52>). Among them, 91 were upregulated and 61 were down-regulated in the invasive tumor tissues compared with the non-invasive tumor samples. Hierarchical clustering analysis showed distinct circRNA expression profiles between the invasive and the non-invasive tumor tissues (Fig. 1D). Our results indicated that the circRNA expression pattern in the invasive NFPA was different from that in the non-invasive NFPA.

3.2. Differentially expressed circRNAs in human chromosomes

The significant differentially expressed circRNAs were further categorized according to their transcribed from chromosomes. We found that the dysregulated circRNAs were widely distributed among all chromosomes except for Y chromosome (Fig. 2A). The top 3 chromosomes transcribed with upregulated circRNAs were chr2 (13.2%), chr6 (11.0%), and chr10 (10.0%). The top 3 chromosomes transcribed with downregulated circRNAs were chr12 (13.1%), chr2 (9.8%), and chr13 (9.8%). According to the genomic locations, each of the aberrantly expressed circRNAs was classified into 1 of 5 categories (exonic, intronic, antisense, intragenic and intergenic). Most of the dysregulated circRNAs belong to the exonic circRNA category, while a few circRNAs originated from other sources (Fig. 2B).

3.3. Validation of the microarray results by qRT-PCR

To verify the microarray expression data, 4 aberrantly expressed circRNAs were selected and quantified via qRT-PCR in 46 NFPA tumor tissues. Divergent primers were designed and used to determine the expression of the 4 circRNAs. We found that the expression levels of hsa_circRNA_405761 and hsa_circRNA_000992 were significantly higher in the invasive than the non-invasive NFPA (Fig. 3A and 3B, $*P < .05$). Meanwhile, the expression of hsa_circRNA_102598 and hsa_circRNA_102597 were significantly decreased in the invasive as compared to the non-invasive tumors (Fig. 3C and 3D, $*P < .05$, $**P < .01$). These data suggested that the expression trends of the 4 validated circRNAs were consistent with the microarray results, confirming the reliability of the microarray data.

3.4. Downregulation of hsa_circRNA_102597 expression in NFPA

By inquiring from circBase (<http://www.circbase.org/>), we found hsa_circRNA_102597 was located at chromosome 19q13.42 and was composed of 2 exons (Fig. 4A). Sanger sequencing demonstrated that the back splice point of hsa_circRNA_102597 was capable of specifically amplified, which was completely in accordance with the sequence in circBase (Fig. 4B). Furthermore, we explored whether the low expression level of hsa_circRNA_102597 was associated with clinicopathological characteristics in NFPA. As shown in Table 1, the expression level of

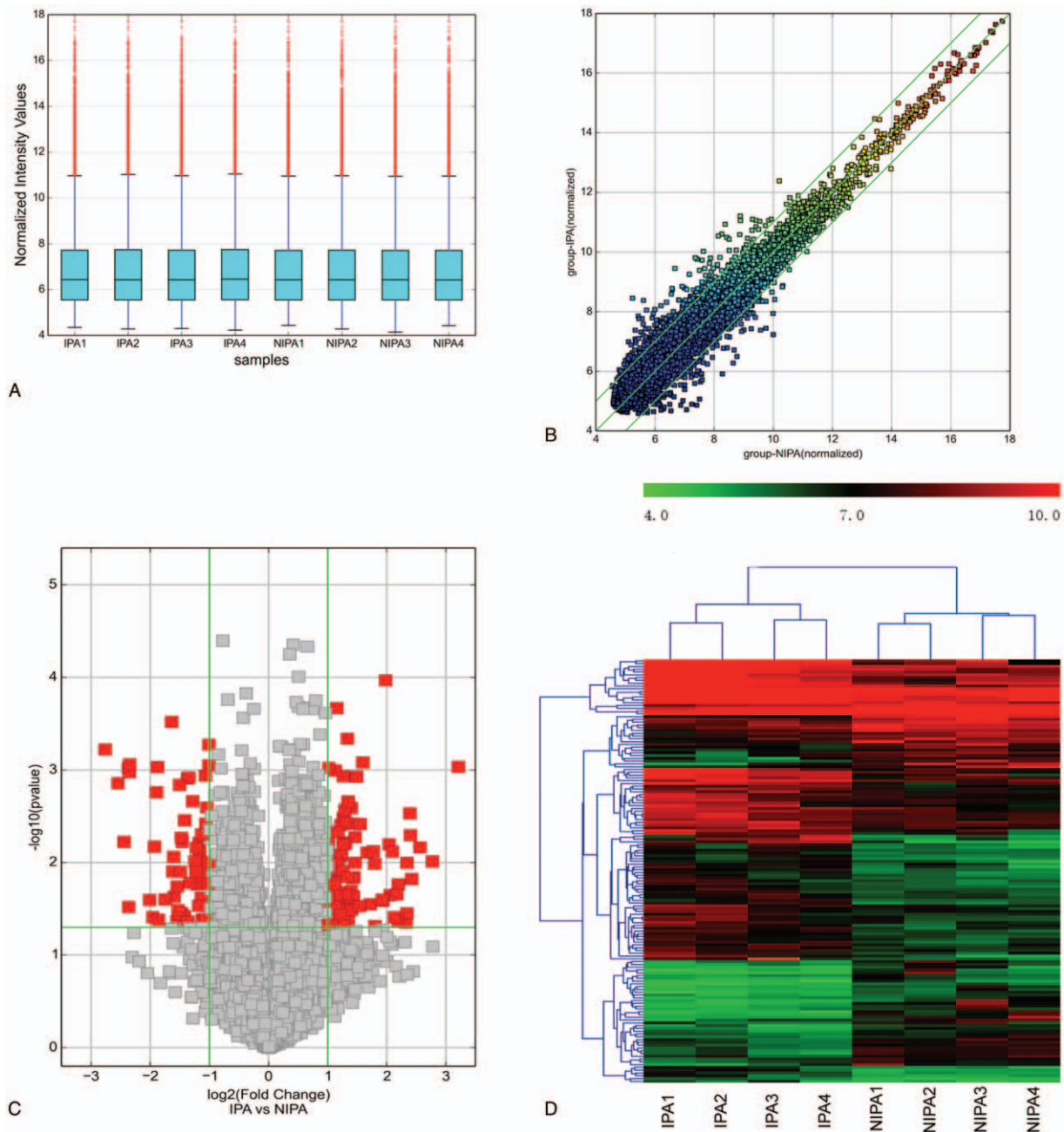


Figure 1. Different expression profiles of circRNAs between invasive and non-invasive NIPA tissues. (A) A box plot shows the distribution of normalized intensities was almost similar in all samples. (B) A scatter plot is used for visualizing the difference in the expression of circRNAs between invasive and non-invasive tumor samples (Points above the top and below the bottom green lines represent more than 2.0 fold changes between the 2 groups). (C) A volcano plot reveals dysregulated circRNAs between 2 different groups. The red points represent circRNAs expressed differentially with statistical significance. (D) Heat map and hierarchical clustering analysis show different circRNA expression profiles between invasive and non-invasive groups. IPA=invasive pituitary adenoma, NIPA=non-invasive pituitary adenoma.

hsa_circRNA_102597 between groups of different genders ($P=.065$), ages ($P=.489$), pituitary dysfunction ($P=.795$), histological types ($p=.078$), or Ki-67 index ($P=.386$) did not reach statistical significance. However, there were significant associations between the expression level of hsa_circRNA_102597 and tumor diameter ($P<.05$) or Knosp grade ($P<.01$).

Since circRNAs have been reported as novel biomarkers of various diseases in numerous studies, we performed a receiver operating characteristic (ROC) curves analysis to investigate the

diagnostic and prognostic values of hsa_circRNA_102597 in NFPAs. A combination of the expression of hsa_circRNA_102597 (AUC=0.777, 95% CI 0.644–0.909, $P<.01$; Fig. 4C) and Ki-67 labeling index (AUC=0.730, 95% CI 0.582–0.878, $P<.01$) distinguished tumor invasiveness better than individual biomarker, as evidenced by AUC=0.839 (95% CI 0.719–0.959, $P<.01$; Fig. 4D). During the follow-up period (25.5, 21.8–30.0), tumor progression/recurrence occurred in 6 (13.0%) out of 46 patients. The ROC analysis revealed that

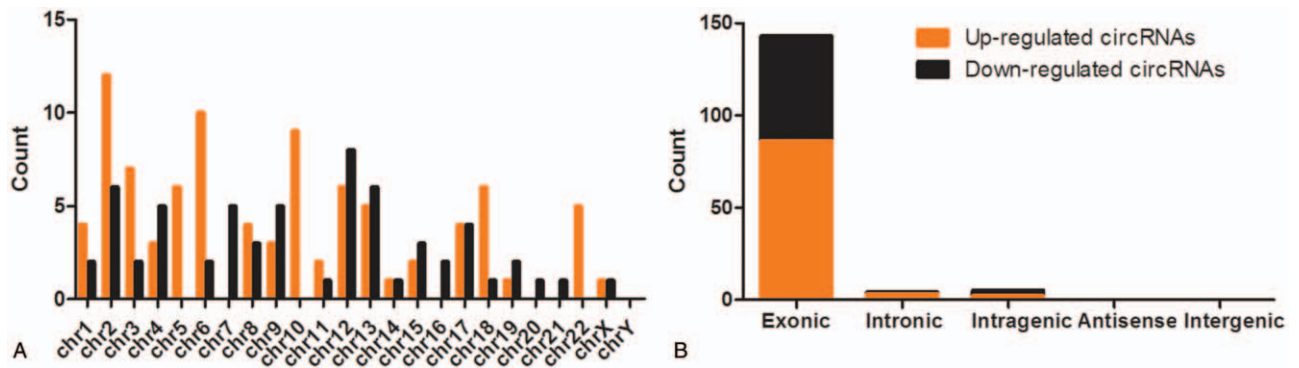


Figure 2. The distribution of dysregulated circRNAs in human chromosomes. (A) The aberrantly expressed circRNAs were distributed among all chromosomes with an exception for Y chromosome. (B) The differentially expressed circRNAs were categorized according genomic origin.

hsa_circRNA_102597 (AUC=0.783, 95% CI 0.518–1.049, $P < .05$) presented a notable discriminatory ability to predict postoperative tumor progression/recurrence (Fig. 4E). Our study demonstrated that a combination of the Ki-67 index (AUC=0.785, 95% CI 0.638–0.932, $P < .05$) and hsa_circRNA_102597 distinguished tumor progression/recurrence even better (AUC=

0.846, 95% CI 0.721–0.971, $P < .05$; Fig. 4F). Other validated circRNAs did not have significant difference in discriminating tumor invasion and progression/recurrence (data no show). Our findings suggest that hsa_circRNA_102597 is a novel potential biomarker, at least partly, for the invasiveness and progression/recurrence of human NFPA.

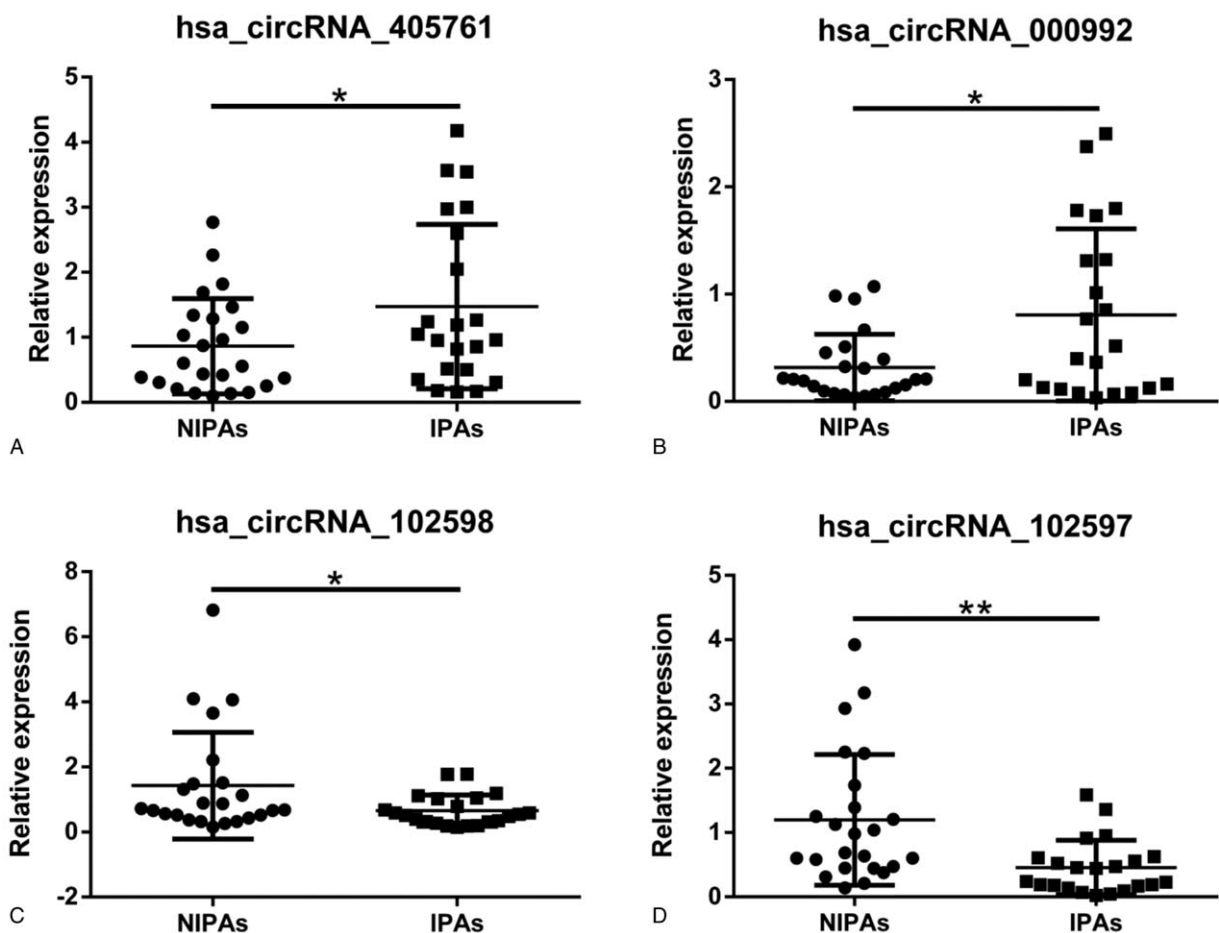


Figure 3. The expression of 4 dysregulated circRNAs was validated by qRT-PCR. (A-B) Hsa_circRNA_405761 and hsa_circRNA_000992 were significantly upregulated in invasive NFPA tissues, while hsa_circRNA_102598 and hsa_circRNA_102597 were significantly downregulated (C-D). Data are presented as mean \pm SD. * $P < .05$, ** $P < .01$.

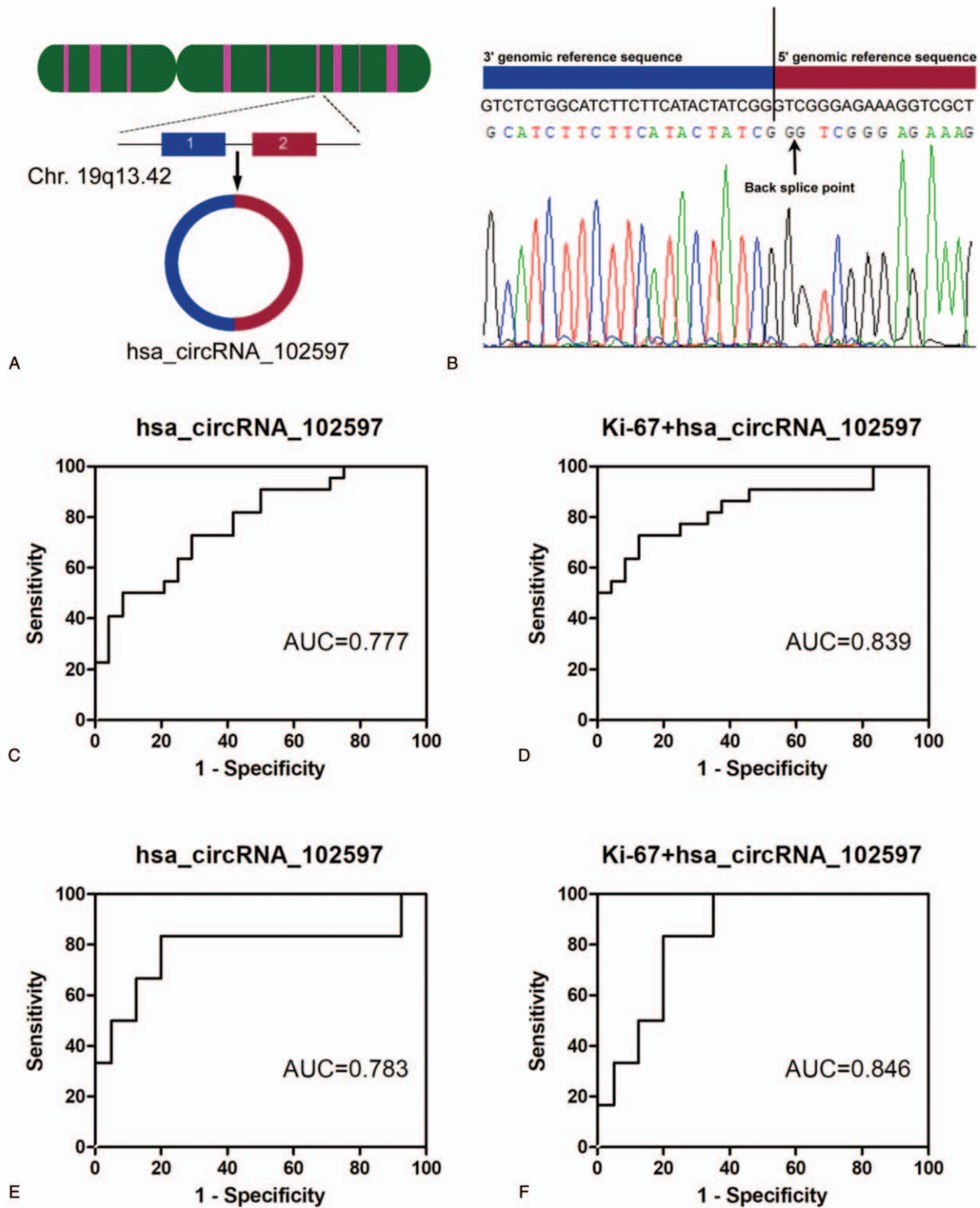


Figure 4. Characterization of hsa_circRNA_102597 and its diagnostic and prognostic capability in NFPAs. (A) Hsa_circRNA_102597 was composed of 2 exons from chromosomal region 19q13.42. (B) Sanger sequencing shows the back splice point of hsa_circRNA_102597. Receiver Operating Characteristic (ROC) curve for the hsa_circRNA_102597 (C), and the combination of ki-67 index and hsa_circRNA_102597 (D) to distinguish non-invasive from invasive NFPAs. Performance of hsa_circRNA_102597 (E), and the combination of ki-67 index and hsa_circRNA_102597 (F) in identifying patients with tumor progression/recurrence.

3.5. CircRNA-miRNA interaction network analysis

Evidence is mounting that circRNAs can sponge miRNAs with miRNA response elements (MREs) to regulate gene expression at post-transcriptional level. Using the miRNA target prediction

software, we preliminarily sought potential miRNA targets associated with dysregulated circRNAs. 515 matched circRNA-miRNA pairs for 152 differentially expressed circRNAs were identified.

Table 1
The associations between the hsa_circRNA_102597 expression level and clinicopathological features of patients with NFPAs.

Characteristics	No. of patients (%)	hsa_circRNA_102597	P value
Gender			.065
Male	24 (47.83)	0.644 (0.445–1.241)	
Female	22 (52.17)	0.423 (0.163–0.736)	
Age (yr)			.489
<60	30 (65.22)	0.602 (0.293–1.148)	
≥60	16 (34.78)	0.465 (0.183–1.417)	
Pituitary dysfunction			.795
Yes	7 (15.22)	0.474 (0.191–1.362)	
No	39 (84.78)	0.600 (0.229–1.128)	
Tumor diameter (cm)			.016
≤3	31 (67.39)	0.635 (0.311–1.389)	
>3	15 (32.61)	0.458 (0.093–0.583)	
Knosp grade			.003
I	12 (26.09)	1.084 (0.611–2.022)	
II	13 (28.26)	0.600 (0.412–1.661)	
III	10 (21.74)	0.451 (0.186–0.568)	
IV	11 (23.91)	0.229 (0.068–0.625)	
Histological types			.078
Gonadotropinoma	22 (47.83)	0.602 (0.448–1.246)	
Null cell adenoma	14 (30.43)	0.593 (0.206–1.287)	
Other types	10 (21.74)	0.161 (0.062–0.945)	
Ki-67 index			.386
<3%	33 (71.74)	0.600 (0.409–1.084)	
≥3%	13 (28.26)	0.240 (0.176–1.811)	

Among the predicted miRNA targets of aberrantly expressed circRNAs, we found several miRNAs have been associated with the invasion of pituitary adenomas after a literature review, such as let-7, miR-15a, and so on (Table 2). These miRNAs are considered promising candidates for further mechanism study.

4. Discussion

In the present study, circRNA microarray was used to determine the circRNA expression profiles in the invasive and non-invasive NFPAs. We identified 152 significantly aberrantly expressed circRNAs, of which 91 were upregulated and 61 were down-

regulated in invasive NFPAs. The qRT-PCR results were consistent with that measured by microarray analysis, confirming the reliability of the microarray data. A validated circRNA, hsa_circRNA_102597, was downregulated in the invasive NFPAs and have a notable discriminatory ability to differentiate pituitary tumor invasion as well as predict tumor progression/recurrence. We also found that several potential miRNA targets of differentially expressed circRNAs have participated in the invasion of pituitary adenomas in literature. These data collectively demonstrated that circRNAs are participated in the invasive behaviors of NFPAs, and may function as promising diagnostic and prognostic biomarkers.

The expression of circRNAs in human tumors is an emerging field of research. Accumulating evidence demonstrates that circRNAs are differentially expressed in several types of human cancer.^[13,17–20] To determine the expression profiles of invasive pituitary adenomas, circRNA microarray analysis was performed in this study. We observed that the circRNA expression profiles were clearly discriminated between the invasive and the non-invasive NFPAs. Our findings are consistent with previous reports that circRNAs are involved in tumor malignant behaviors, such as invasion and metastasis.^[21–23] Xu et al demonstrated that the expression of ciRS-7 was significantly upregulated in hepatocellular carcinoma patients with hepatic microvascular invasion, suggesting that ciRS-7 may be associated with tumor metastasis and poor prognosis.^[21] The expression level of hsa_circ_0000190 in the gastric cancer tissues was negatively correlated with tumor diameter, lymphatic metastasis, and distal metastasis.^[22] Guo et al found hsa_circ_0000069 was significantly upregulated in colorectal cancer and associated with tumor stage. Further loss of function studies demonstrated that the circRNA could promote cell proliferation, invasion, and migration.^[23] These observations, together with the findings of our study, indicate that circRNAs may be implicated in the invasion of pituitary adenomas.

CircRNAs are highly conserved and remarkably stable non-coding RNAs with half-lives more than 48 hours, making them promising biomarkers in various diseases, including cancers.^[13,15,22,24,25] CircCCDC66 was significantly higher in colorectal cancer tissues and negatively correlated with the overall survival rates, suggesting the circRNA may serve as a

Table 2
Dysregulated circRNAs and their potential miRNA targets that associated with pituitary adenoma invasion.

CircRNA ID	MiRNA targets	Regulation	MiRNA target genes	Tumor types	Functions	Author/Years
hsa_circ_0062161	let-7	Down	HMGA2	PRL, ACTH, FSH/LH	Invasion, cell proliferation	Qian et al, 2009;
hsa_circ_0000987						Palmieri et al, 2012
hsa_circ_0075748						
hsa_circ_0043837	miR-15a	Down	Sox5	NA	Invasion, Cell proliferation and migration	Wang et al, 2015
hsa_circ_0017628						
hsa_circ_0001367	miR-16	Down	Sox5	NA	Invasion and Cell proliferation	Wang et al, 2015
hsa_circ_0001369						
hsa_circ_0006667						
hsa_circ_0035360	miR-181b-5p	Up	TCF3, MAX, CYP26A1, MYC, SREBF1,	NFPA	Invasion	Wu et al, 2017
hsa_circ_0006163	miR-383	Down	TCF3, MAX, CYP26A1, MYC, SREBF1	NFPA	Invasion	Wu et al, 2017
hsa_circ_0008179						
hsa_circ_0006509	miR-145	Down	AKT3	NA	Invasion, Cell proliferation and migration	Zhou et al, 2017
hsa_circ_0076931	miR-34a	Down	FGF2, CCNB1, surviving	NA	Invasion	Yu et al, 2017
hsa_circ_0030388						

NA = not available.

novel diagnostic and prognostic biomarker in colorectal cancer.^[13] Zhang et al constructed a 4-circRNA-based classifier that effectively predicted the early recurrence of stage III gastric cancer.^[26] Moreover, they found a combination of the 4-circRNA-based classifier and TNM stages provided better predictive effect power. Our study also suggested that the accuracy of the model can be further enhanced through its combination with clinicopathological features, especially the Ki-67 index. As a slowly growing tumor, a Ki-67 labeling index $\geq 3\%$ is considered as a defining of atypical adenomas according to 2004 WHO classification.^[2] The prognostic value of Ki-67 index in NFPAs has been documented by Ramirez et al in a series of patients with gonadotroph and null cell adenoma.^[27] They found that the Ki-67 index was significantly associated with tumor size as well as tumor recurrence. Our results are consistent with the above observations that the labeling index is associated with tumor progression/recurrence in NFPAs. More importantly, a combination of the Ki-67 index and the expression level of hsa_circRNA_102597 could be a good predictive biomarker for pituitary adenoma invasion detection and prognosis. A larger sample size of patients with longer follow-up period may further improve the accuracy of the biomarker.

MiRNAs, consisting of 18 to 24 nucleotides, is a class of single-stranded, non-coding RNAs, which are known to regulate target genes expression at post-transcriptional level. Some circRNAs can function as miRNA decoys to sequester miRNAs from their target mRNAs, and a circRNA - miRNA - mRNA regulatory axis is increasingly recognized in tumor pathogenesis and progression.^[11,12,17,18,28] In the literature, we found some potential miRNA targets that have been reported to associate with the invasiveness of pituitary adenomas.^[29-33] Let-7 was significantly downregulated in invasive pituitary adenomas, and it could directly regulate the expression of high-mobility group A 2 (HMGA2) that is associated with pituitary tumor invasion.^[29,30] Furthermore, hsa_circ_0062161, hsa_circ_0000987, and hsa_circ_0075748 were predicted upstream regulators of let-7, implying that they might sponge let-7 and then regulate HMGA2 expression. Similarly, miR-15a/16 were less expressed in invasive pituitary adenomas, and upregulated the expression of miR-15a/16 resulted in inhibition of cell proliferation, migration and invasion by directly targeting Sox-5.^[31] Considering hsa_circ_0043837, hsa_circ_0017628, hsa_circ_0001367, hsa_circ_0001369, and hsa_circ_0006667 were upregulated in invasive pituitary adenomas, we therefore speculate those circRNAs might facilitate tumor invasion by targeting a miR-15a/16 - Sox-5 axis. In contrast, miR-181b-5p was significantly overexpressed in invasive NFPAs as compared to non-invasive ones.^[34] Thus, we postulate that the downregulated hsa_circ_0035360 promoted tumor invasion by targeting miR-181b-5p. Further experiments are requested for validating the accurate mechanisms of circRNAs in pituitary tumor invasion.

In this study, bioinformatics analysis revealed that a large number of targeted genes might participate in hsa_circRNA_102597-miRNA-gene network, such as CDKN1A, WNT4, EGFR and so on. Take Wnt4 for an example, it has been reported that Wnt4 could regulate multiple developmental processes of pituitary gland.^[35] The expression of Wnt4 was upregulated in most pituitary adenomas and inversely correlated to the Knosp grade of tumor invasion.^[35] Wnt4 may contribute to the tumorigenesis and progression of pituitary adenomas through canonical Wnt/ β -catenin signaling pathway or β -catenin-independent pathways.^[35,36] However, further research is

necessary to explore the detailed molecular mechanisms by which hsa_circRNA_102597 functions as miRNA sponges to regulate the target genes in pituitary tumor initiation and progression.

5. Conclusions

In conclusion, we identified a distinct circRNA expression profile between invasive and non-invasive NFPAs. A validated circRNA, hsa_circRNA_102597, could be an effective biomarker in differentiating patients with tumor invasion and non-invasion as well as predicting tumor progression/recurrence after surgery. Bioinformatics analysis predicted potential miRNA targets of dysregulated circRNAs, and several miRNA targets are associated with pituitary tumor invasion in literature. Further studies are needed to uncover the detailed mechanisms of circRNAs in the regulation of pituitary adenomas pathogenesis and progression.

Acknowledgments

We are grateful to the patients for donating their tissue for research, and we acknowledge the excellent technical assistance Xuemei Chen at the Research core facility, West China Hospital, Sichuan University.

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