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ORIGINAL ARTICLE

Genome-wide analysis of differentially expressed lncRNA in sporadic parathyroid tumors

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Abstract

Summary Diagnosis of parathyroid carcinoma on histological examination is challenging. Thousands of differentially expressed lncRNAs were identified on the microarray data between parathyroid cancer and adenoma samples. Four lncRNAs were significantly dysregulated in further validation. The "lncRNA score" calculated from these lncRNAs differentiated parathyroid carcinomas from adenomas. LncRNAs serve as biomarkers for parathyroid cancer diagnosis.

Introduction Diagnosis of parathyroid carcinoma (PC) on histological examination is challenging. LncRNA profile study was conducted to find diagnostic biomarkers for PC.

Methods LncRNA arrays containing 91,007 lncRNAs as well as 29,857 mRNAs were used to assess parathyroid specimen (5 carcinomas and 6 adenomas). Bioinformatics analyses were also conducted to compare the microarray results between parathyroid carcinomas and adenomas (PAs). Differentially expressed lncRNAs of 11 PCs and 31 PAs were validated by real-time quantitative PCR.

Results On the microarray data between PC and PA samples (fold change ≥ 2, $P < 0.05$), 1809 differentially expressed lncRNAs and 1349 mRNAs also were identified. All carcinomas were clustered in the same group by clustering analysis using dysregulated lncRNAs or mRNAs. Four lncRNAs (LINC00959, lnc-FLT3-2:2, lnc-FEZF2-9:2, and lnc-RP11-1035H13.3.1-2:1) identified were significantly dysregulated in further RT-PCR validation. The global "lncRNA score" calculated from the lncRNAs above also differentiated parathyroid carcinomas from adenomas.

Conclusions LncRNA profiling shows distinct differentially expressed lncRNAs in parathyroid neoplasm. They may play a key role in parathyroid cancer and serve as potential biomarkers to distinguish parathyroid cancers from parathyroid adenomas.

Keywords diagnosis · hyperparathyroidism · lncRNAs · parathyroid tumors

Introduction

Parathyroid adenoma (PA) accounts for 85% of sporadic (non-familial) primary hyperparathyroidism (PHPT) whereas parathyroid carcinoma (PC), an exceedingly rare endocrine malignancy, accounts for 0.5 to 5% PHPT [1, 2]. It is a challenge to diagnose parathyroid tumors on histology alone. The pathological distinction of parathyroid carcinoma from

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adenoma in PHPT is still problematic in the absence of local invasion, distant metastasis, or recurrence [2].

Most of the studies published focus on the genetic mutations involved in sporadic PHPT aiming at the identification of the molecular mechanism. Multiple endocrine neoplasia type 1 (MEN1) gene and CDC73/HRPT2 were identified as critical tumor suppressor genes specifically in adenoma and carcinoma respectively [3–5]. Whole-exome sequencing analysis of DNA outlined the genetic landscape of sporadic PC. Apart from MEN1 and CDC73, recurrent somatic mutations in prune homolog 2 (Drosophila) (PRUNE2) PRUNE2 was found in 18% (4/22) of PCs, while only one rare missense polymorphism (Asp1677Asn) was detected in 40 PAs [6].

Long non-coding RNAs (lncRNAs) are RNAs longer than 200 nt. They cannot code proteins [7]. Genome-wide studies have identified thousands of LncRNAs [8]. LncRNA has been found to be a critical regulator in a variety of tumor growth or metastasis processes by influencing at diverse molecular levels, chromatin structure, transcriptional activity, mRNA stability, mRNA post-transcriptional processing, or mRNA translation [9]. LncRNAs also act as regulators in the endocrine system [10]. Differently expressed LncRNAs have been found to be associated with diabetes mellitus and several endocrine cancers, including thyroid, prostate, ovarian cancers, and pituitary tumors [10]. However, the role of lncRNAs in parathyroid tumors is needed to study.

To further understand the mechanisms involved in parathyroid tumors and improve the clinical diagnosis of patients with sporadic primary hyperparathyroidism, we performed lncRNA expression profiling in patients with parathyroid carcinoma and parathyroid adenoma.

Materials and methods

Patients and tissue samples

A case-control study was conducted under an investigation protocol approved by the Institutional Ethics Committees of Beijing Shijitan Hospital. Eleven parathyroid carcinomas (including distance metastasis) and 31 parathyroid adenomas were obtained from 42 patients with primary hyperparathyroidism (sporadic) undergoing parathyroidectomy. The patients with hyperparathyroidism had a mean serum calcium level of 2.9 ± 0.4 mmol/L, while the normal intact parathyroid hormone (PTH) level was 650.0 ± 668.3 pg/mL. The 11PC patients include 7 males and 4 females with a mean age of 58.0 ± 11.6 years, while the 31 PA patients included 6 males and 25 females with a mean age of 56.3 ± 14.3 years. No patient had any anti-cancer treatment pre-operatively. The diagnosis of parathyroid cancer and adenoma was established by two pathologists independently. Cases with parathyroid carcinoma included in this study had a local invasion, lymph node metastasis, or distant metastasis. Clinical and pathologic data was also collected for all the patients. All the tissues were immediately frozen in liquid nitrogen and stored at − 80 °C.

RNA extraction

Total RNA was extracted from the 50-mg tissue sample using 1 mL Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. After homogenizing the tissue, the samples were incubated for 5 min at 15–30 °C. Two hundred microliter chloroform was then added. The mixture was agitated vigorously by hand for 15 s and incubated for 3 min at 15–30 °C. Then, the sample was centrifuged at 12,000×*g* for 15 min at 4 °C. The supernatant was transferred to a new tube and 500 μL isopropanol was added. After mixing and incubating at 15–30 °C for 10 min, the mixture was centrifuged at 12,000×*g* for 10 min at 4 °C. The RNA pellet was washed with 1 mL ethanol (75%) after removal of the supernatant. Then, the samples were subjected to vortex mixing and centrifugation at 7500×*g* for 5 min at 4 °C. The pellet was dissolved in RNase-free water and incubated at 55–60 °C for 10 min. The total RNA quality was confirmed using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to ensure that the OD A260/A280 ratio was close to 2.0 (from 1.9 to 2.1). RNA integrity was evaluated with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) with RIN ≥ 7.0 and 28 s/18 s ≥ 0.7. The total RNAs were reverse transcribed into cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

LncRNA microarray preparation

RNAs extracted from 5 parathyroid carcinomas and 6 parathyroid adenomas samples were included in the microarray study. LncRNA microarray profiling was conducted using SBC Agilent Human lncRNA microarray v.6.0 (074348, Shanghai Biotechnology Corporation, Shanghai, China). This array could detect 91,007 lncRNAs as well as 29,857 mRNAs. Amplified total RNAs were labeled by Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies, Santa Clara, CA, USA), according to the instructions of the manufacturer. Labeled RNAs were immediately purified using RNeasy mini kit (QIAGEN, GmbH, Germany). Hybridization with 1.65 μg labeled RNAs on each slice for 17 h was conducted in a hybridization oven (Agilent Technologies, Santa Clara, CA, USA) following the instruction of Expression Hybridization Kit (Agilent Technologies, Santa Clara, CA, USA). After washing with Gene Expression Wash Buffer Kit (Agilent Technologies, Santa Clara, CA, USA), in staining dishes (Thermo Shandon, Waltham, MA, USA), each slide was scanned by Agilent Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA). Raw data was

extracted with Feature Extraction software 10.7 (Agilent Technologies, Santa Clara, CA, USA) and normalized by Quantile algorithm Gene Spring Software 11.0 (Agilent Technologies, Santa Clara, CA, USA).

Gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis

The cellular component, molecular function, and biological process of the dysregulated mRNAs and target mRNAs were identified by gene ontology (GO) analysis (http://www.geneontology.org) [11]. We also performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (http://www.genome.jp/kegg/) to reveal the important pathways. The analysis was conducted using the cluster Profiler, an R package for comparing biological themes among gene clusters. The criteria for significant GO or KEGG pathway selection were differentially expressed gene ≥ 2 and $P < 0.05$. "Enrichment factor" = (number of dysregulated mRNAs in the term/number of total dysregulated mRNAs in this study)/(number of the total genes in the term in the database/number of the total genes in the database).

LncRNA target prediction

Different algorithms were utilized to involve and identify the cis- (which regulates the transcription of neighboring mRNAs located in the same chromosome) and trans- (which modify the expression of a distant gene with complementary or similar nucleotide sequence located on other chromosomes) regulation target genes of dysregulated lncRNAs. The first algorithm was programmed for target genes in cis. LncRNAs and potential target genes were paired and visualized through the UCSC genome browser (http://genome.ucsc.edu/). The genes transcribed within a 10-kb fragment upstream or downstream of interesting lncRNAs were considered as cis-targets. Another algorithm was based on mRNA sequence complementarity and RNA duplex energy prediction, predicting the effects of lncRNAs binding on complete mRNA molecules. BLAST software was used for the first screening, while RNAplex software was used to decide on the trans-acting targets.

Quantitative real-time PCR

Six lncRNAs were validated with a reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The RT-qPCR was achieved using the 2 × PCR master mix (Arraystar) on a ViiA 7 real-time PCR system (Applied Biosystems) following the manufacturer's instruction. The primers for target lncRNAs and glyceraldeyde-3-phosphate dehydrogenase (GAPDH) involved in the RT-qPCR are listed in Table S1. The reactions were conducted under a 95 °C denaturation for 10 min, followed by 40 cycles at 95 °C for 10 s, 60 °C for 60 s, and 95 °C for 10 s. The relative expression levels were analyzed using the $2^{-\Delta CT}$ method. Each experiment was performed in sample triplicates.

LncRNA and mRNA co-expression analysis

We constructed general coding-noncoding gene co-expression networks in parathyroid cancers with all differentially expressed lncRNAs and mRNAs when compared to parathyroid adenomas. Those lncRNAs and mRNAs that had Pearson correlation coefficients (PCC) ≥ 0.99 were selected, and from that a network was constructed in each of the mentioned groups using Cytoscape program. The analyses were performed by Shanghai Biotechnology Corporation (Shanghai, P.R. China).

Statistical analysis

Statistical analysis was performed using SPSS (Version 17.0 SPSS, Chicago, IL, USA). The Mann-Whitney U test or independent Student's t tests were used to compare the continuous variables between the two groups. Quantitative data were expressed as mean ± standard deviation (mean ± SD). Qualitative data were shown as numbers or percentages. LncRNA genes in the microarray with an adjusted P value < 0.05 and fold change ≥ 2 were selected as differentially expressed when compared between PA and PC. A global score of four lncRNAs (lnc-CASD1-2:2, LINC00959, lnc-FLT3-2:2, and lnc-FEZF2-9:2) was calculated, following formula referenced from microRNA score in parathyroid cancer [12]:

$$\begin{aligned}\text{lncRNA score} = {} & (\text{LINC00959}-\text{RQ}) \\ & \times (\text{lnc}-\text{FLT3}-2:2-\text{RQ}) \\ & \times (\text{lnc}-\text{FEZF2}-9:2-\text{RQ}) \\ & \times (\text{lnc}-\text{RP11}-1035\text{H13.3.1}-2:1-\text{RQ})^{-1}\end{aligned}$$

RQ (relative quantity) represents log2-transformed relative quantity from the fold of change (−ΔCT). Fisher's exact test and P value were applied for detection in GO and KEGG analysis. Receiver operating characteristic curve (ROC) was constructed to evaluate whether lncRNA score could predict parathyroid cancer in hyperparathyroidism patients. $P < 0.05$ was considered statistically significant.

Results

Differentially expressed lncRNAs and mRNAs in microarrays

As differentiation between PC and PA is difficult on histology, we further performed a genome-wide analysis of lncRNAs expression comparison between the two groups. Using 2162 lncRNA probes, 1809 differentially expressed lncRNAs (fold change ≥ 2 and $P < 0.05$) were identified from the microarray data between PC and PA tissue (Fig. 1a). Among them, 912 lncRNAs were upregulated, while 897 lncRNAs were downregulated. Lnc-SNX29-3:1 (fold change 96.2) was the most elevated lncRNA, and ENST00000618339 (fold change 243.5) was the most downregulated one. In addition, the specimens were categorized into groups by hierarchical clustering analysis according to their different lncRNA expression levels (Fig. 1b). Chromosome one was associated with the greatest differentially expressed lncRNAs between PCs and PAs (Fig. 1c) and amounted to 9.9% of lncRNAs. Meanwhile, we found that 1349 mRNAs were differentially expressed (fold change ≥ 2 and $P < 0.05$) between PC and PA (Fig. 1d, e), 710 mRNAs were upregulated among which and 639 mRNAs were downregulated. WT1 (fold change 250.8) was the most elevated mRNA, while PCDH11Y (fold change 79.7) was the most reduced one. Chromosome one was associated with the greatest differentially expressed mRNAs between PCs and PAs (Fig. 1f) and amounted to 10.23%.

Function analysis of differentially expressed genes

GO analyses were performed for all 1349 differentially expressed mRNAs in PCs and PAs. The most mRNAs were enriched in "positive regulation of cellular protein metabolic process"(GO: 0032270; biological process, $P = 0.5$). The most enriched GOs we discovered were "intra-membranous ossification" (GO: 0001957; biological process, $P = 0.00$), and the top 30 GOs were shown in Fig. S1. KEGG pathway analysis was also applied in potential functions prediction of these genes. The results indicated that these differently expressed genes are mainly associated with 21 pathways ($P < 0.05$), as listed in Fig. 2a. Many of the enriched pathways are associated with cancer, such as, "bladder cancer" (associated with 8 genes) and "p53 signaling pathway" (associated with 16 genes).

Target analysis for dysregulated lncRNAs

To delineate whether the differentially expressed lncRNAs were involved in the regulation of genes related to PC, target prediction programs were used to predict potential targets of dysregulated lncRNAs between PCs and PAs. To have target genes, 1443 lncRNAs were found. In addition, 1362 of them targeted cis-genes, 328 lncRNAs acted in trans-regulation, and 247 lncRNAs had both cis and trans-targets. Thirty-one of the predicted cis-target genes and 186 of the predicted trans-target genes were identified as differentially regulated genes in the mRNA array-scan data. The associated signaling pathways of these integrated target genes above were enriched in some pathways (Fig. 2b), such as "p53 signaling pathway" (associated with 9 genes), and "cell cycle" (associated with 11 genes). Those pathways might play a key role in PC development regulated by lncRNAs (Table S2).

Co-expression profile for lncRNAs and mRNAs

To predict the potential roles of differentially regulated lncRNAs in PC and PA, the correlations between 1809 lncRNAs and 1349 dysregulated protein-coding mRNAs in PCs compared with PAs were studied. 1446 links (PCC ≥ 0.99) were identified between 431 of the 1349 protein-coding genes and 546 of 1809 lncRNAs, which indicate that 30.2% lncRNAs were probably associated with mRNAs in PC patients. In addition, a network was integrated with several important mRNAs and co-expressed lncRNAs in PC patients (Fig S2). "Degree" of genes stood for the number of related genes. The top ten genes with the largest "degree" in PCs were shown in Table S3. Lnc-KIAA0101-1:3, ENST00000451766, and NR111952 were lncRNAs with the largest degree. However, there was no related mRNA for Lnc-KIAA0101-1:3 and NR111952 in PAs (co-expression network and degree table were not shown). There were only 3 mRNAs for ENST00000451766 in PAs. These lncRNAs may play an important role in PCs.

Validation of lncRNA expression profile by RT-qPCR

To further investigate the mechanism of lncRNAs in parathyroid tumors, six differentially expressed genes (lnc-DIS3-4:1, lnc-CASD1-2:2, LINC00959, lnc-FLT3-2:2, lnc-FEZF2-9:2, and lnc-RP11-1035H13.3.1-2:1) were chosen for RT-PCR validation. Target mRNAs of those selected lncRNAs were found to be differentially expressed in the microarray analysis. For instance, collagen type I alpha 2 chain (COL1A2) was the target gene of lnc-CASD1-2:2 predicted above, and it was also dysregulated in microarray (fold change 4.27 and $P = 0.00$) (Table S4). These lncRNAs were used to validate the lncRNA array expression data by RT-qPCR. Results of all six lncRNAs (Fig. 3a–f) were similar to our microarray data (Table S1) in PC compared to PA. The expression profiles of LINC00959, lnc-FLT3-2:2, and lnc-FEZF2-9:2 in parathyroid carcinomas were significantly decreased compared to those of adenoma patients (Fig. 3c–e). The expression level of lnc-RP11-1035H13.3.1-2:1 in PCs was significantly increased compared to those in PAs (Fig. 3f).

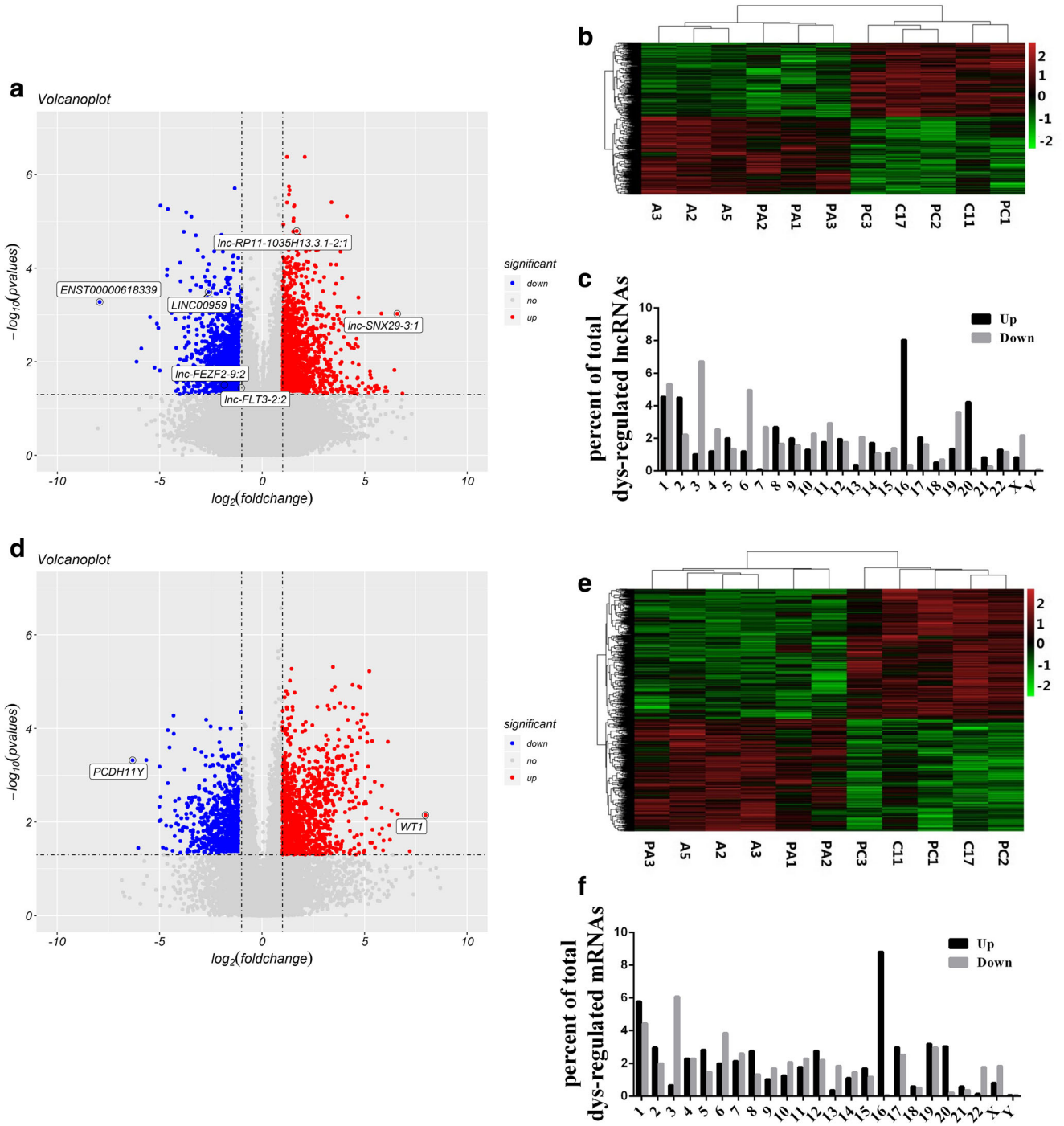

Fig. 1 Dys-expressed lncRNAs and mRNAs in parathyroid carcinomas (PCs) as compared with parathyroid adenomas (PAs). **a** Volcano plot of differentially expressed lncRNAs in PCs when compared with PAs. Red dots represent overexpressed lncRNAs (fold change ≥ 2 and $P < 0.05$), while blue dots stand for reduced ones. **b** LncRNA expression signature discriminated PCs (PC or C) from PAs (PA or A). Hierarchical clustering with significantly different expressed lncRNAs distinguished parathyroid carcinomas from adenomas. LncRNAs were listed vertically, and samples were displayed horizontally. Increased lncRNAs are indicated in red and decreased ones are in green. **c** Percentage of dysregulated lncRNAs on different chromosomes. **d** Volcano plot of differentially expressed mRNAs in PCs when compared with PAs. Red dots represent overexpressed mRNAs (fold change ≥ 2 and $P < 0.05$), while blue dots stand for reduced ones. **e** Coding mRNA expression signature discriminated PCs (PC or C) from PAs (PA or A). Hierarchical clustering with significantly different expressed mRNAs distinguished parathyroid carcinomas from adenomas. Coding mRNAs were listed vertically, and samples were displayed horizontally. Increased mRNAs are indicated in red and decreased ones are in green. **f** Percentage of dysregulated mRNAs on different chromosom

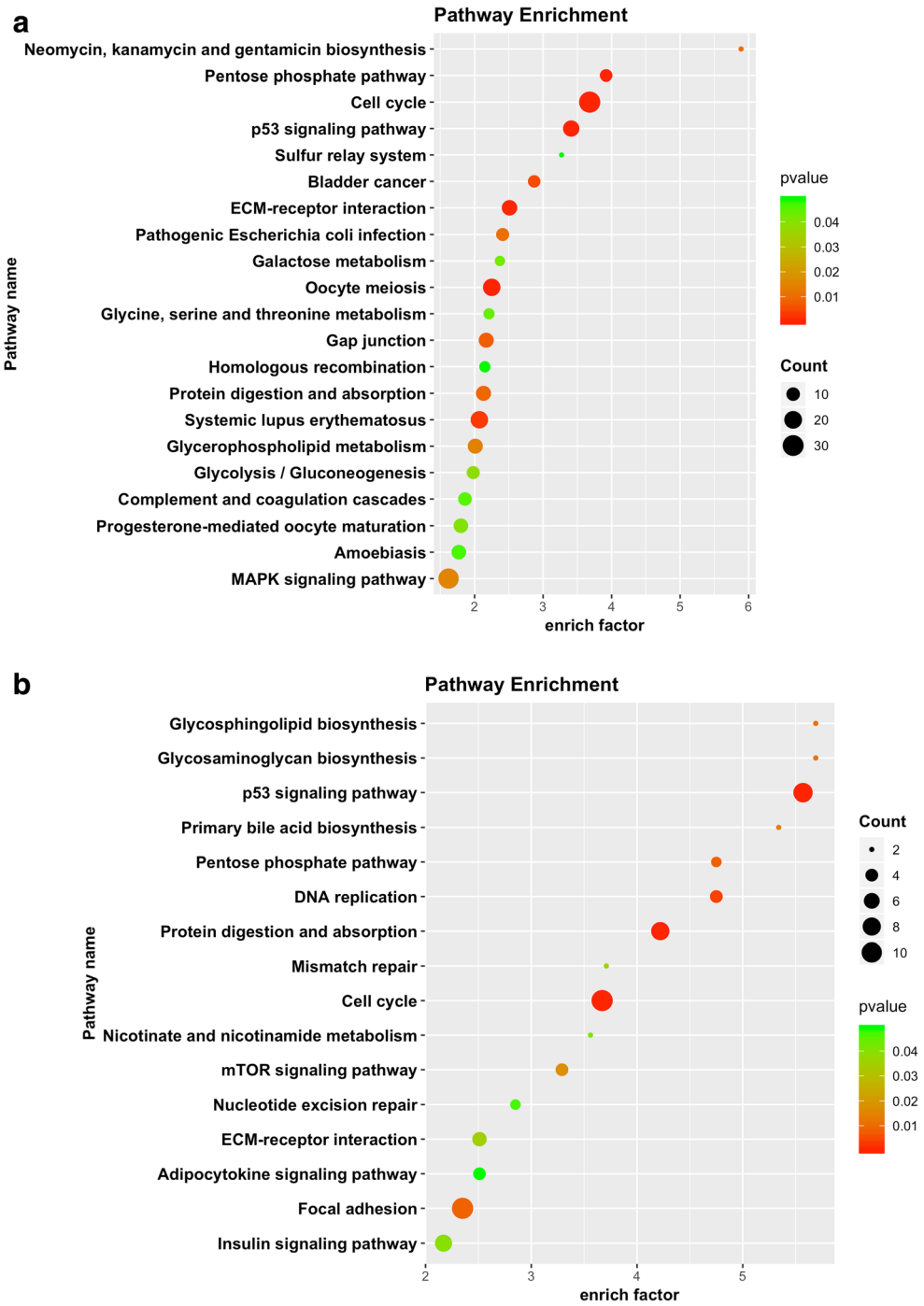

Fig. 2 Function analysis of differentially expressed genes in parathyroid carcinomas (PCs) as compared with parathyroid adenomas (PAs). **a** Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis using differentially expressed mRNAs ($P < 0.05$). **b** KEGG analysis for target mRNAs from differentially expressed lncRNAs ($P < 0.05$). Red dots show smaller P values, while green dots stand for greater P values. The larger size of dots presents the more genes were enriched

However, there was no significant difference in the transcription level of lnc-DIS3-4:1 and lnc-CASD1-2:2 in PC patients with respect to parathyroid adenomas (Fig. 3a, b). Hence, lnc-DIS3-4:1 and lnc-CASD1–2:2 were excluded from the global "lncRNA score" calculation. The global "lncRNA score" discriminated parathyroid adenomas from carcinomas (Fig. 3g). ROC was generated to evaluate the role of "lncRNA score" in PC prediction (Fig. 3h), and the area under the curve (AUC) was up to 88.0% (95% CI 72.0–100.0%, $P = 0.00$). When cutoff value was selected at 48.6, the greatest Youden index was obtained (sensitivity 81.8%, specificity 83.9%).

Discussion

In this paper, we tried to decide whether parathyroid tumors have distinct lncRNA signatures. Parathyroid carcinomas were arranged into the same group by hierarchical clustering analysis according to their different lncRNA

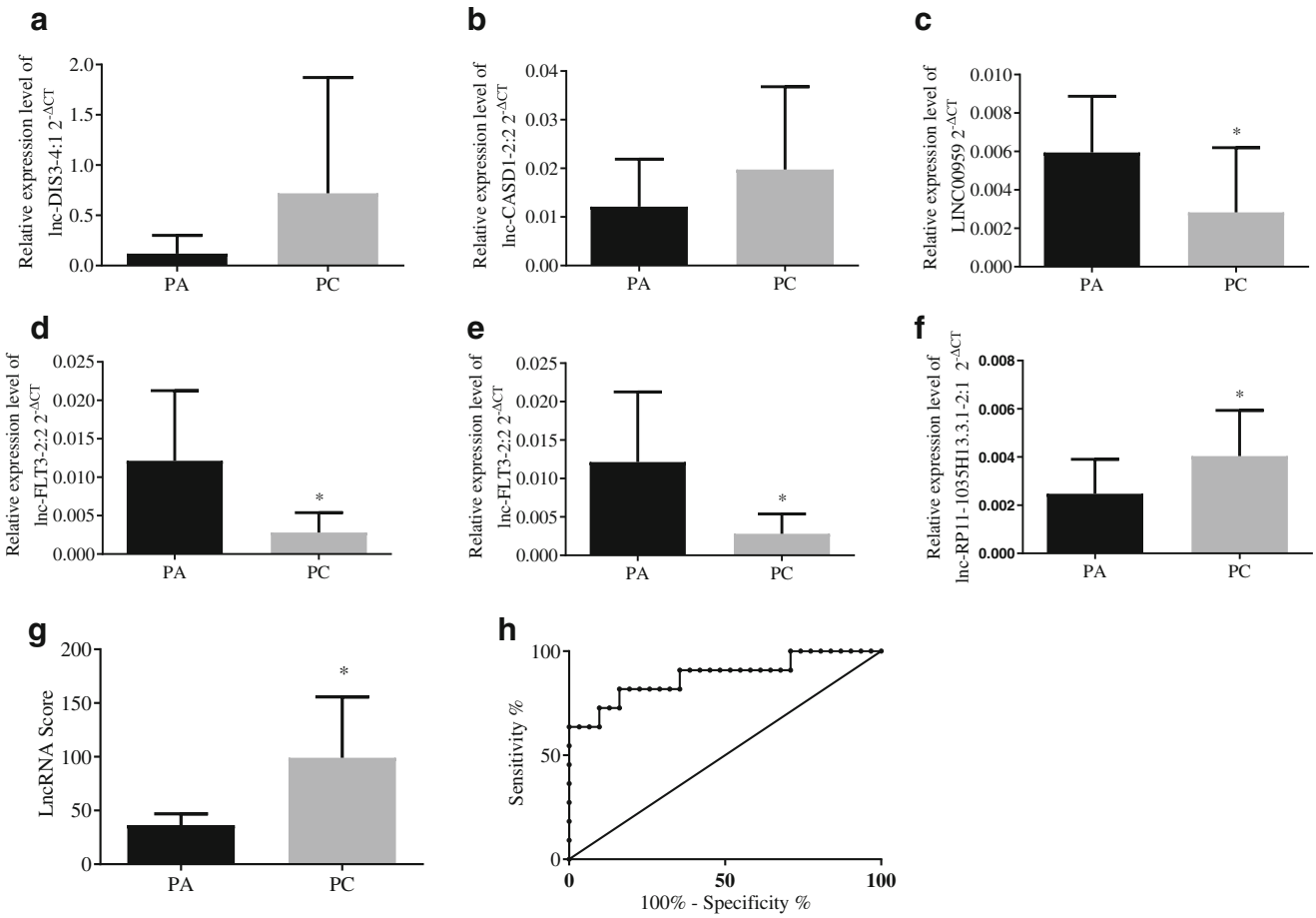

Fig. 3 RT-qPCR validation of target lncRNAs and ROC curve of lncRNA score in parathyroid cancer (PC) diagnosis. Expression levels of lnc-DIS3-4:1 (**a**), lnc-CASD1-2:2 (**b**), LINC00959 (**c**), lnc-FLT3-2:2 (**d**), lnc-FEZF2-9:2 (**e**), and lnc-RP11-1035H13.3.1-2:1 (**f**) in parathyroid adenomas (PAs) and PCs; LncRNA scores in PAs and PCs (**g**) and ROC to evaluate the diagnostic performance of lncRNA score to discriminate PCs from PAs (**h**). $^{*}P < 0.05$

expression levels. We further validated microarray results by RT-qPCR. All lncRNA expression levels correlate similarly to the array data. In addition, LINC00959, lnc-FLT3-2:2, lnc-FEZF2-9:2, and lnc-RP11-1035H13.3.1-2:1 were significantly dysregulated in parathyroid carcinomas compared to adenoma. The global lncRNA scores in parathyroid adenoma were also distinct from that of carcinoma samples.

It is exceedingly difficult to differentiate parathyroid cancers from benign parathyroid tumors in many cases [13]. Histopathological characteristics are always similar between parathyroid carcinoma and adenoma. Patients with presumed PAs were often diagnosed as cancers when local recurrence or distant metastases existed [14]. Only in case of vascular invasion, local invasion, lymph node, or distant metastasis carcinoma can be diagnosed [13]. Diagnostic biomarkers are needed for parathyroid cancer. The absence of parafibromin (protein product of CDC73), elevated galectin-3, protein gene product 9.5 (PGP9.5), and Ki-67 may play a role in PC diagnosis [15–18]. The absence of parafibromin has been shown to have 29 to 100% sensitivity and 61 to 100% specificity in PC diagnosis [15–17, 19]. However, these immunohistochemical biomarkers have only been verified via small cohorts of cancer samples. Unfortunately, no single molecular marker above has been recommended for clinical use in parathyroid carcinoma diagnosis guidelines [20]. LncRNAs have been used as potential diagnostic biomarkers for several types of endocrine cancers, such as thyroid [21] and prostate cancer [22]. Prostate cancer gene 3 (PCA3), the first discovered prominent biomarker among lncRNAs, outperformed PSA in prostate cancer diagnosis [23]. It was exciting that some lncRNAs were not only expressed in cancer tissue but also detected in plasma [24], which may make them more useful than immunohistochemical indexes in diagnosis decision. In the present study, expression profiles of LINC00959, lnc-FLT3-2:2, lnc-FEZF2-9:2, and lnc-RP11-1035H13.3.1-2:1 in parathyroid adenomas were significantly different from those of carcinoma patients. Furthermore, the global lncRNA scores in parathyroid adenoma were also distinct from that from carcinoma samples, and the AUC was up to 88.0%

with sensitivity 81.8% and specificity 83.9%. It suggests that lncRNAs might act as potential tumor markers or play important roles in parathyroid cancer development.

GO and KEGG analyses were also used in the current study to demonstrate the biological functions in all deregulated coding genes. Many different biological pathways were shown in the enrichment results, such as "p53 signaling pathway" and "bladder cancer," which are involved in cancer regulation. Moreover, 31 of the cis-target genes and 186 of the trans-target ones were detected to be dysregulated in the microarrays and might be regulated by ectopic expressed lncRNAs. In addition, in this paper, these mRNAs targeted from dysregulated lncRNAs are enriched in many cancer-related pathways, such as "MAPK signaling pathway" [25], "small cell lung cancer," "non-small cell lung cancer," "p53 signaling pathway," and "cell cycle". Meanwhile, "ECM-receptor interaction" [26] and "Focal adhesion" [27] signaling pathways involved in this differentially expressed lncRNAs in this research were also reported to be associated with cancer invasion or metastasis. In addition, p53 dysfunction and mutation played an important role in the susceptibility to a malignant tumor and to cancer progression [28, 29]. Somatic mutation in the CDC73 locus has been found in the majority of sporadic parathyroid carcinoma patients [3, 4]. Moreover, loss of CDC73 staining has been reported as a driving factor in parathyroid carcinoma. It was observed that p53 also played a novel role in the cancer-induced effect of CDC73 mutation [30]. Of great interest, similar to our study, recent researches showed that lncRNAs acted as p53 regulators or effectors in lots of other cancers [31]. Taken together, those pathways, such as "p53 signaling pathway," might be involved in parathyroid cancer regulated by lncRNAs. On the other hand, chromosome 1 is associated with the greatest differentially expressed lncRNAs between PCs and PAs accounts for 9.9% in this cohort. Interestingly, it is also where the CDC73 gene is located. It has been reported that CDC73 expression is also regulated by epigenetic modifications, such as microRNA [32] and CpG island methylation [33]. The relationship between lncRNAs and CDC73 mutation should be further investigated.

There are limitations to this study. Additional functional studies are still needed to demonstrate the role of lncRNAs in the carcinogenesis in parathyroid cancer. We also plan to investigate the diagnostic values of lnc-CASD1-2:2, LINC00959, lnc-FLT3-2:2, and lnc-FEZF2-9:2 in cancer tissue and plasma samples from patients with parathyroid tumors in a larger cohort study.

In summary, lncRNAs are dysregulated in parathyroid tumors. LncRNA expression profiles are distinct between PC and PA. These lncRNAs may serve as potential biomarkers to distinguish parathyroid cancers from adenomas.

Author contributions TJ, BJW, DXZ, and LL designed research; ZWC, GLQ, and XAY conducted acquisition of data; technical/material supports were provided by ZWC, XL, and XXD; technical/material support TJ, BJW, DXZ, and LL performed research and statistical analysis; TJ, BJW, DXZ, and LL wrote the paper; and BJW, DXZ, and LL conducted a critical revision of the manuscript.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Abbreviations PC, parathyroid carcinoma; PA, parathyroid adenoma; lncRNA, long non-coding RNA; mRNA, messenger RNA; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; RT-qPCR, reverse-transcription quantitative polymerase chain reaction

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