Abstract. The Human Genome Project revealed that >90% of the human genome was found to transcribe non-coding RNAs (lncRNAs). lncRNAs have been identified to play a crucial role in cancer progression. Thyroid cancer (TC) is a common type of endocrine cancer; however, the functional roles of lncRNAs in TC have yet to be fully elucidated. The present study investigated whether LINC01420 was upregulated in TC tissues, compared with normal thyroid tissues, and the results suggested that LINC01420 may play a regulatory role in TC. Bioinformatics analysis demonstrated that LINC01420 was associated with translation, rRNA processing, mRNA splicing, regulation of transcription, DNA repair and double-strand break repair. Furthermore, the exact role of LINC01420 in TC was explored by performing a loss-of-function assay, which revealed that the knockdown of LINC01420 inhibited TC cell proliferation and cell cycle progression. The findings of the present study provide a novel insight into the molecular mechanisms underlying TC development. Moreover, they suggest that LINC01420 may serve as a potential therapeutic target for the treatment of TC, and that increased LINC01420 expression levels show potential as a prognostic marker for the disease.

Introduction

The completion of the Human Genome Project illustrated that >90% of the human genome transcribes non-coding RNAs (ncRNAs). It has been demonstrated that ncRNAs [including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs)] play a key role in cancer progression. lncRNAs (non-coding RNA molecules >200 nucleotides in length) are dysregulated and may function as potential biomarkers of various malignancies, such as breast (1), gastric (2), lung (3) and prostate cancer (4). Moreover, lncRNAs may act as oncogenes in certain types of cancer. For example, small nucleolar RNA host gene 15 promotes colon cancer progression by interacting with, and stabilizing snail family transcriptional repressor 2 (5) and differentiation antagonizing non-protein coding RNA (6), increasing the proliferative and invasive capacities of gastric cancer cells. However, lncRNAs can also act as tumor suppressors; for instance, lncRNA overexpressed in colon carcinoma-1 suppressed colorectal cancer-cell proliferation by destabilizing HuR protein (7), and maternally expressed 3 suppressed liver cancer-cell proliferation through the inhibition of β-catenin (8). Therefore, investigation into the functional roles of lncRNAs in cancer may provide new insights into the identification of novel diagnostic and therapeutic targets.

Thyroid cancer (TC) is one of the most common endocrine malignancies. In previous years, the functional roles of several lncRNAs in TC have been revealed. Pvt1 oncogene (PVT1) was the first lncRNA to be reported as having a functional role in TC (9). Zhou et al (9) found that PVT1 contributed to TC tumorigenesis through the recruitment of enhancer of zeste 2 polycomb repressive complex 2 subunit and the regulation of thyroid stimulating hormone receptor expression. Furthermore, lncRNA CDKN2B antisense RNA 1 was identified as a tumor suppressor gene in TC (10). However, lncRNAs were also found to be involved in the prognosis of TC; lncRNA papillary thyroid carcinoma susceptibility candidate 3 was identified as a tumor suppressor gene in TC (11). Moreover, low expression levels of growth arrest specific 5 were found to be associated with poor prognosis in patients with TC (12). High expression levels of LINC01420 have been associated with poorer overall survival (OS) in patients with nasopharyngeal carcinoma, and
LINC01420-knockdown inhibited nasopharyngeal carcinoma cell migration. However, the functions and underlying molecular mechanisms of LINC01420 in TC progression remain largely unknown.

The present study investigated whether LINC01420 was of value as a biomarker for TC. The expression of LINC01420 was evaluated by analyzing a dataset containing TC patient information retrieved from The Cancer Genome Atlas (TCGA). Additionally, bioinformatics analysis was performed to reveal the functional roles of LINC01420 in TC progression. Finally, the effect of LINC01420 on cell proliferation and cell cycle progression was investigated. Improved understanding of the role of LINC01420 in TC progression may indicate its use as either a biomarker, or a potential therapeutic target.

Materials and methods

TCGA dataset retrieval and analysis. The TCGA Thyroid carcinoma (THCA) dataset containing specimens from both TC patients and disease-free subjects was retrieved from TCGA (http://www.cbioportal.org/study/summary?id=thym_tcgca), and the LINC01420 expression levels were determined (13). In total, 502 PTC tissue samples and 58 normal thyroid tissue samples were included in the TCGA-THCA dataset. Clinical information regarding LINC01420 was downloaded using cBioPortal (http://www.cbioportal.org/). The tumor-node-metastasis classification system (detailed in the American Joint committee on Cancer Manual) was used to determine disease stage. The inclusion criteria have been previously reported in TCGA.

Co-expression network, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Correlation between the expression of LINC01420 in cancerous and disease-free tissues was calculated using the Pearson's correlation coefficient in cBioPortal (http://www.cbioportal.org/). The co-expressed LINC01420-mRNA pair with an absolute Pearson's correlation coefficient ≥0.3 was selected for analysis. GO and KEGG pathway analysis were used to predict the biological functions of LINC01420 using the STRING database (http://string.embl.de/) (14). A confidence score >0.4 was considered as the criterion of judgment.

Cell culture and transfection. All cell lines were obtained from the American Type Culture Collection. CAL62 and SW579 cells were cultured in L-15 medium supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) in a 37°C incubator with 5% CO2. The short interfering (si)RNA for LINC01420 (5'-CAUCUCAGGUCUCUGCUU GGCUU GCCA-3') and an siRNA negative control were purchased from Guangzhou Ribobio Co., Ltd. Cells were transfected with siRNAs (10 nM) using Lipofectamine™ 3000 reagent (Thermo Fisher Scientific, Inc.). Reverse transcription-quantitative (RT-q)PCR analysis. Total RNA was extracted from transfected CAL62 and SW579 cells using TRIzol® (Sigma-Aldrich; Merck KGaA) reagent, according to the manufacturer's protocol. Total RNA was then reverse transcribed into cDNA using the PrimeScript RT Master Mix (Takara Bio, Inc.), according to the manufacturer's protocol. qPCR was performed using the AceQ qPCR SYBR® Green Master Mix (Vazyme) on a Roche LightCycler 480 according to the manufacturer's protocol. The C value of β-actin was used as an internal control to calibrate the Cq values of the genes of interest, in order to determine the differential expression levels. Relative RNA expression was calculated using the 2-ΔΔCq method (16), and each sample was run in triplicate.

Cell proliferation and cell cycle distribution. The Cell counting kit (CCK)-8 assay (Dojindo Molecular Technologies, Inc.) was used to detect cell proliferation following transfection according to the manufacturer's protocol. After 48 h transfection, cells were seeded in 96 well plate at the density of 4x10³ cells/100 µl per well, and proliferation was detected at 0, 1, 2, 3 and 4 days. CCK-8 reagent (10 µl medium/well) was added prior to detection and after incubation for 1.5 h at 37°C, and the absorbance was measured at 450 nm using a microplate reader. Absorbance at 630 nm was used as the control.

For the cell cycle assays, 3x10⁵ cells/well were harvested from a 6 well plate and fixed in ice-cold 70% (v/v) ethanol for 24 h at 4°C. The cell pellet was collected following centrifugation at 500 x g for 10 min at 4°C and resuspended in PBS. Cells were then stained with a mixture of RNase (10 µg/ml) and propidium iodide (50 µg/ml; Beyotime Institute of Biotechnology) in sodium citrate containing 0.5% Triton X-100 for 20 min, in the dark and at room temperature. The cells were subsequently analyzed using a flow cytometer (Gallios, Beckman Coulter, Inc.). The ModFit software version 4.0 (Verity Software House, Inc.) was used for data analysis.

Statistical analysis. Statistical analysis was performed using the SPSS software package, version 15.0 (SPSS, Inc.). Significant differences between two groups were compared using two-tailed Student's t-test. Statistical comparisons between two paired groups was performed using a paired t-test. For comparison of >2 groups, one-way ANOVA was used, followed by the Newman-Keuls post hoc test. Kaplan-Meier and Cox regression analyses were used to evaluate the association between LINC01420 and disease-free survival (DFS), as well as the prognosis of patients with TC. P<0.05 was considered to indicate a statistically significant difference.

Results

LINC01420 is overexpressed in TC tissue samples. The TCGA-THCA dataset was analyzed to evaluate the expression levels of LINC01420 in TC. The data suggested that the expression of LINC01420 was significantly higher in TC tissues, compared with that in normal tissue samples (Fig. 1A). Furthermore, LINC01420 expression was analyzed using 50 paired TC samples from TCGA dataset. Of these paired samples, 80% exhibited a higher expression level...
Co-expression analysis of LINC01420 in TC. Co-expression analysis is widely used to explore the potential roles of lncRNAs in human disease. Considering that LINC01420 is a novel lncRNA implicated in TC, a LINC01420-mediated co-expression network was constructed to identify its potential mechanism of action. A Pearson’s correlation coefficient value of ≥0.50 was selected as the cut-off for the identification of reliable LINC01420-mRNA pairs. A total of 948 mRNAs were positively co-expressed and 568 mRNAs were negatively co-expressed in this network. The two largest hub networks of positively and negatively co-expressed mRNAs are shown in Fig. 2A and C, respectively.

Furthermore, key positively- and negatively-related gene-mediated protein-protein interaction networks were identified using the Search Tool for the Retrieval of Interacting Genes/Proteins database. GO analysis revealed that genes positively related to LINC01420 were significantly associated with ‘translation’, ‘rRNA processing’, ‘translational initiation’, ‘mRNA splicing’, ‘regulation of cellular amino acid metabolic processes’, ‘NIK/NF-κB signaling’, ‘mitochondrial translation’, ‘regulation of mRNA stability’, ‘Wnt signaling pathway’, ‘stimulatory C-type lectin receptor signaling pathway’, ‘protein targeting to mitochondrion’, ‘spliceosomal snRNP assembly’, ‘protein
polyubiquitination’, ‘TNF-α-mediated signaling pathway’ and ‘T-cell receptor signaling pathway’ (Fig. 2B). Genes negatively associated with LINC01420 were significantly involved in ‘regulation of transcription’, ‘peptidyl-serine phosphorylation’, ‘DNA repair’, ‘double-strand break repair’, ‘protein polyubiquitination’, ‘stem cell population maintenance’, ‘protein import into nucleus’, ‘protein K48-linked deubiquitination’, ‘telomere maintenance’ and ‘cytoplasmic microtubule organization’ (Fig. 2D).

**Knockdown of LINC01420 inhibits cell proliferation in TC.** The CCK-8 was used to evaluate the functional roles of LINC01420 in TC. Following transfection, the expression of LINC01420 was found to be significantly downregulated in the LINC01420-knockdown group compared with that in the control group, in both CAL62 (Fig. 3A) and SW579 cells (Fig. 3C). The results of the CCK-8 assay indicated that compared with the control group, LINC01420 silencing significantly suppressed CAL62 (Fig. 3B) and SW579 (Fig. 3D) cell proliferation at 72 h.

**LINC01420-knockdown inhibits cell cycle progression in TC.** The effect of LINC01420 on the cell cycle was also investigated. Flow cytometry demonstrated that suppressing LINC01420 expression modulated the cell cycle by inducing G0/G1 arrest (compared with the control groups) in CAL62 (Fig. 4A-B) and SW579 cells (Fig. 4C-D).
Figure 3. LINC01420-knockdown inhibits the proliferation of thyroid cancer cells. Relative expression levels of LINC01420 in (A) CAL62 and (C) SW579 cells. LINC01420-knockdown significantly suppresses the proliferation of (B) CAL62 and (D) SW579 cells compared with the control-transfected group. Comparison between two groups was performed using two-tailed Student’s t-tests. ***P<0.001 vs. control-transfected group. NC, negative control; si, small interfering RNA.

Figure 4. Knockdown of LINC01420 inhibits cell cycle progression in thyroid cancer. Silencing of LINC01420 in TC cells modulated the cell cycle by inducing G0/G1 arrest in (A and B) CAL62 and (C and D) SW579 cells. *P<0.05, **P<0.01 and ***P<0.001. TC, thyroid cancer; NC, negative control; si, small interfering RNA.
Discussion

IncRNAs have been identified as important regulators of cancer progression, binding to DNA, RNA and proteins to regulate epigenetic modification and protein and gene post-transcriptional regulation (17). IncRNAs act as both oncogenes and tumor suppressor genes; for example, epigenetically-induced IncRNA1 was reported to be oncogenic, promoting cell cycle progression by interacting with the MYC proto-oncogene (18). Conversely, testis associated oncogenic IncRNA promotes cancer progression and mRNA stabilization by interacting with insulin-like growth factor 2 mRNA binding protein 1 (19). Moreover, certain IncRNAs, such as X-inactive specific transcript, nuclear paraspeckle assembly transcript 1 and HOX transcript antisense RNA, were found to act as miRNA molecular sponges, which are implicated in human cancer progression (20-24). The current study investigated the role of LINC01420 in TC progression. LINC01420 expression was previously reported to be upregulated in nasopharyngeal carcinoma, and LINC01420-knockdown inhibited nasopharyngeal carcinoma-cell migration (25). In the present study, a loss-of-function assay was performed and revealed that LINC01420-knockdown inhibited TC cell proliferation by arresting cell cycle progression. Taken together, these findings provide evidence to support the oncogenic nature of LINC01420.

TC is a common endocrine malignancy; however no sensitive biomarkers are currently available for TC diagnosis. Previous studies have primarily focused on identifying diagnostic and therapeutic targets for TC. For example, Read et al (26) reported that higher PTTG1 regulator of sister chromatid separation, securin and zinc finger protein 395 expression predicted poor patient outcomes in TC. Downregulation of serum Dickkopf WNT signaling pathway inhibitor 1 was also found to be associated with a poor prognosis in PTC patients (27). Furthermore, IncRNAs are often dysregulated in TC; NEAT1_2 (28), TNRC6C-ASI (29), CNALPTC1 (30) and AFAPI-ASI (31) were found to be overexpressed in TC. However, GAS8-ASI (32), GAS5 (12) and BANCR (33) were all found to be downregulated. To the best of our knowledge, the present study is the first to demonstrate the upregulation of LINC01420 in TC compared with normal tissues, and to determine a correlation between the aforementioned upregulation and poor prognosis. Furthermore, high LINC01420 expression was also associated with shorter DFS time. Thus the results of the present study suggest that LINC01420 may play a regulatory role in TC development, and consequently, may be a useful biomarker.

The functional roles and underlying molecular mechanisms of LINC01420 in TC progression remain largely unclear. Consequently, GO and KEGG pathway analysis were also performed in the present study. The findings demonstrated that LINC01420 was significantly associated with translation, rRNA processing, translational initiation, mRNA splicing, regulation of transcription, DNA repair and double-strand break repair. Therefore, the results of the present study indicate that LINC01420 may serve as a potential therapeutic target, and that increased LINC01420 levels may be used as a novel prognostic biomarker for TC.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA-THCA repository (https://www.cbioportal.org/study/summary?id=thym_tcg).

Authors’ contributions

Experimental conception and design were conducted by JZL and LJZ. LJZ, LQ and LJZ developed the methodology, and analyzed and interpreted the data. JZL, LQ and LJZ wrote, reviewed and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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