Biomedicine & Pharmacotherapy 102 (2018) 639–644

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Increased expression of circRNA_102231 in lung cancer and its clinical significance

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ARTICLE INFO

Keywords:
Circular RNAs
circRNA_102231
Lung cancer
Kaplan-Meier analysis
Receiver operating characteristic

ABSTRACT

Circular RNAs (circRNAs) are a type of endogenous non-coding RNAs which implicated in the progression of cancers. However, the role of circRNA_102231 in lung cancer remains unclear. Gene Expression Omnibus (GEO) datasets were used to investigate aberrantly expressed circRNAs in lung cancer. CircRNA_102231 expression in lung adenocarcinoma (LAC) tissues was dertermined by qRT-PCR. Furthermore, we explored the functions of circRNA_102231 on lung cancer cells progression. In the present study, circRNA_102231 was found to be one of the most significantly upregulated circRNAs in the GEO datasets analysis (GSE101586). QRT-PCR showed that circRNA_102231 expression was significantly upregulated in LAC tissues and associated with the advanced TNM stage, lymph node metastasis, and poor overall survival of lung cancer patients. The area under the receiver operating characteristic curve (ROC) was 0.897. in addition, function assays showed that circRNA_102231 inhibition significantly suppressed lung cancer cells proliferation and invasion ability in vitro. In conclusion, our study demonstrated that circRNA_102231 could act as a potential biomarker and therapeutic target for lung cancer patients.

1. Introduction

Lung cancer is the top leading cause of cancer-related death worldwide, particularly, non-small cell lung cancer (NSCLC) which accounts for 85% of newly diagnosed lung cancer cases [1,2]. NSCLC is further histologically sub-divided into four categories, and lung adenocarcinoma (LAC) is the most common type [3]. Despite the diagnosis and therapeutic advances of NSCLC, the 5-year survival rates and recurrence rate remains dismal [4]. Therefore, understanding the carcinogenesis and identifying the target for the early detection and therapy of lung cancer are urgently needed.

Circular RNAs (circRNAs) are a novel type of endogenous non-coding RNAs featuring stable structure and high tissue-specific expression [5,6]. Unlike linear RNAs, circRNAs form a covalently closed continuous loop without 5′ to 3′ polarity and polyadenylated tail, and it highly represented in the eukaryotic transcriptome [7]. Further evidence suggested that circRNAs could sequester miRNAs to terminate regulation of their target genes, which play an important role in the regulation of gene expression and the development and progression of the disease [8]. CircRNAs might act as potential targets and biomarkers of cancers. For example, Yang et al. found that silencing of cZNF292 circular RNA suppressed human glioma tube formation via the Wnt/β-catenin signaling pathway [8]. Xie et al. showed that hsa_circ_001569 acted as a positive regulator in cell proliferation and invasion of colorectal cancer by targeting miR-145 [9]. Han et al. found that circular RNA circMTO1 acted as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression [10]. However, the roles of circRNAs in tumor carcinogenesis remain unclear.

In the present study, the expression of circRNA_102231 in lung cancer tissues was determined by qRT-PCR. The association between circRNA_102231 expression and LAC patients' clinical features was further explored. Kaplan-Meier survival analysis and receiver operating characteristic (ROC) curve were used to evaluate the prognostic and diagnostic value of circRNA_102231 in LAC patients. In addition, in vitro function assays were used to explore the roles of circRNA_102231 in lung cancer progression. Our results indicated that circRNA_102231 could act as a therapeutic target for the treatment of lung cancer.

2. Materials and methods

2.1. Tissue samples

A total of 57 pairs of primary LAC tissues and adjacent non-tissues (ANT) were collected from lung cancer patients who received treatment

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https://doi.org/10.1016/j.biopha.2018.03.084
Received 10 January 2018; Received in revised form 14 March 2018; Accepted 14 March 2018

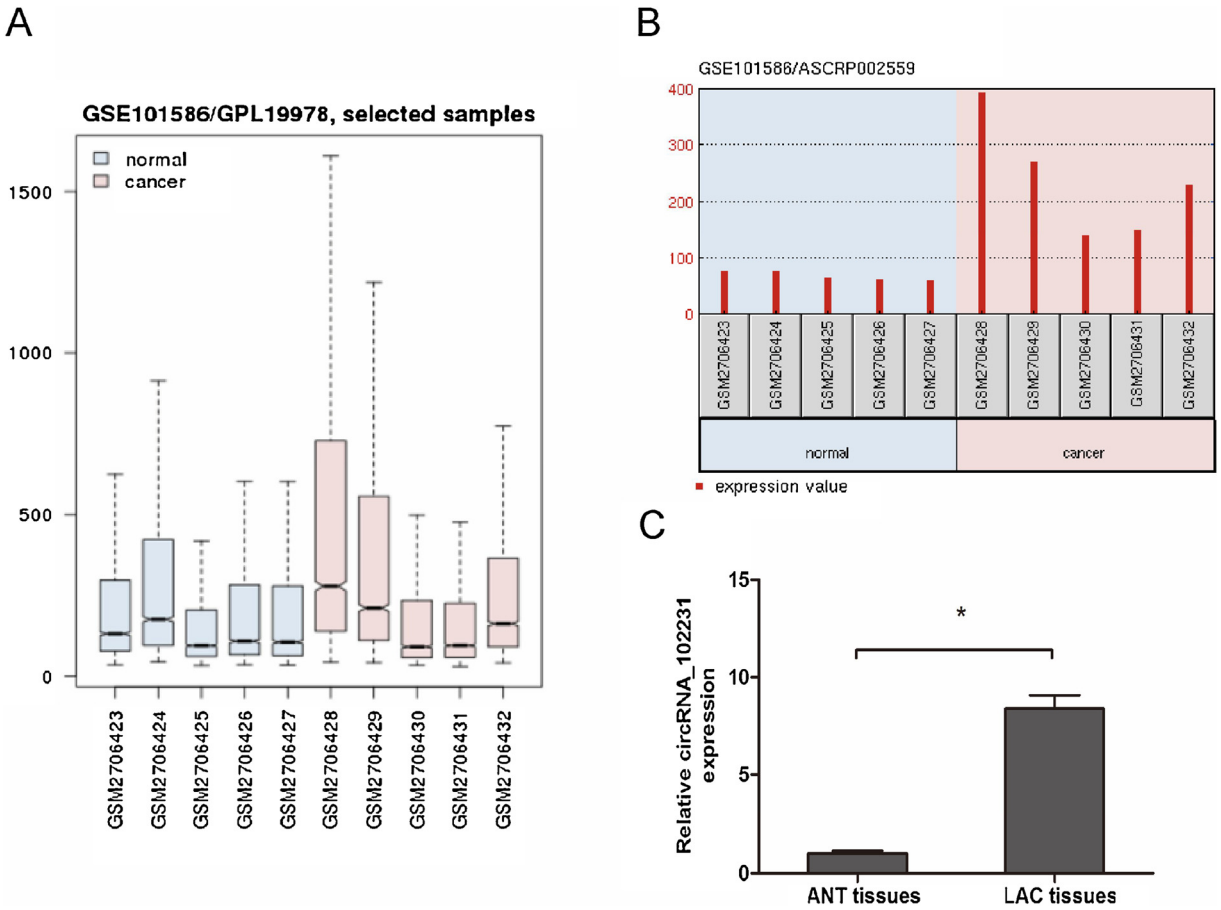

Fig. 1. CircRNA expression profile in lung cancer tissues. (A) Box plot showed the normalized intensities from the tumor and non-tumor tissue samples. (B) GEO dataset (GSE101586) showed that circRNA_102231 was significantly increased in tumor tissues compared to non-tumor tissues. (C) qRT-PCR verification of the expression of circRNA_102231 in LAC tissues and adjacent non-tumor tissues. *P < 0.05.

in The First Affilliated Hospital of Xinjiang Medical University. None of the patients have been received any chemotherapy or radiation before surgery. All patients were staged based on the International Association for the Study of Lung Cancer (IASLC) Tumor-Node-Metastasis (TNM) classification, 7th edition [11]. Written informed consent was obtained from all participants and the study was approved by the Board and Ethics Committee of The First Affilliated Hospital of Xinjiang Medical University. The tissues were immediately frozen in liquid nitrogen following surgery and stored at −80 °C until use.

2.2. Total RNA extraction and reverse transcription

Total RNA was extracted from paired LAC tissues and adjacent non-tumor tissues using the TRIzol™ Reagent and reverse-transcribed into cDNA by using commercial kits according to the manufacturer's instructions (Invitrogen). The RNA quantification and quality was examined spectrophotometrically by absorbance measurements at 260 and 280 nm.

2.3. Quantitative RT-PCR

Quantitative RT-PCR (qRT-PCR) of circRNAs and the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was performed on a ABI PRISM7500 system (Bio-Rad) using an RT Kit (TakaRa) and PCR Master Mix (TakaRa) in accordance with the manufacturer's instructions. Briefly, 500 ng of total RNA was reverse transcribed into cDNA with random primers in a total volume of 20 μL. The reactions were initiated in a 96-well optical plate at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. The levels of circRNA_102231 was normalized by GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method.

2.4. Bioinformatics analysis

The microarray data of circRNA profiles in lung cancer tissues and paired non-tumor tissues were retrieved in NCBI GEO Datasets (http://www.ncbi.nlm.nih.gov/gds, No. GSE101586). Normalized microarray data were analyzed using GEO2R after applying log 2 transformation. GEO2R, an interactive web tool, allows for comparison of original submitter-supplied processed data (https://www.ncbi.nlm.nih.gov/geo/geo2r/).

2.5. Cell culture and transfection

Human lung cancer cell lines BEAS-2B, and A549 were purchased from American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) in a humidified incubator at 37 °C with 5% CO_2. Small interfering RNAs (siRNAs) targeting the circRNA_102231 (si-circRNA_102231) were provided by GenePharma and shown as follows: si-circRNA_102231, 5'-GAGAGGCUGAUGACAU CGU-3'; Cell transfection was performed with lipofectamine 2000 (Invitrogen) according to the manufactures' instructions.

2.6. CCK-8 assay

Cells were incubated in 10% CCK-8 diluted in normal culture medium at 37°C until visual color conversion occurred. Proliferation rates were determined at 24, 48, and 72 h after transfection. The