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Thyroid hormone receptor interactor 13 (TRIP13) overexpression associated with tumor progression and poor prognosis in lung adenocarcinoma

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ABSTRACT

Thyroid hormone receptor interactor 13 (TRIP13) is an AAA^{+}-ATPase that plays a key role in mitotic checkpoint complex inactivation and is associated with the progression of several cancers. However, its role in lung adenocarcinogenesis remains unknown. Here, we report that TRIP13 is highly overexpressed in multiple lung adenocarcinoma cell lines and tumor tissues. Clinically, TRIP13 expression is positively associated with tumor size, T-stage, and N-stage, and Kaplan-Meier analysis revealed that heightened TRIP13 expression is associated with lower overall survival. TRIP13 promotes lung adenocarcinoma cell proliferation, clonogenicity, and migration while inhibiting apoptosis and G2/M phase shift in vitro. Accordingly, TRIP13-silenced xenograft tumors displayed significant growth inhibition in vivo. Bioinformatics analysis demonstrated that TRIP13 interacts with a protein network associated with dsDNA break repair and PI3K/Akt signaling. TRIP13 upregulates Akt^{Ser473} and downregulates Akt^{Thr308}/mTOR-^{Ser2448}activity, which suppresses accurate dsDNA break repair. TRIP13 also downregulates pro-apoptotic Bad^{Ser136} and cleaved caspase-3 while upregulating survivin. In conclusion, heightened TRIP13 expression appears to promote lung adenocarcinoma tumor progression and displays potential as a therapeutic target or biomarker for lung adenocarcinoma.

1. Introduction

Non-small cell lung cancer (NSCLC), the leading cause of cancer death worldwide, accounts for 80–85% of all lung cancer cases [1,2]. NSCLC includes lung adenocarcinomas, large cell carcinomas, and squamous cell carcinomas (SCCs), with lung adenocarcinoma being the most common NSCLC type [3,4]. Although SCC and SCLC rates are declining, lung adenocarcinoma incidence continues to rise globally, particularly among females [4]. Unfortunately, the overall prognosis of lung adenocarcinoma patients remains poor, with an overall five-year survival rate of only 15% [5]. Studies have shown that oncogene activation, mutations in tumor suppressor genes, somatic mutations, and/or genomic rearrangements underlie lung adenocarcinogenesis [6]. Therefore, elucidating the functions of abnormally-expressed genes specific to lung adenocarcinoma is of great importance to revealing the disease's pathogenesis and developing more efficacious treatments.

The thyroid hormone receptor interacting protein 13 (TRIP13) is expressed in a variety of adult tissues, most notably in the meiotically-active testis and oocytes [7]. TRIP13 encodes a 432-residue AAA^{+}-ATPase that plays a key role in mitotic checkpoint complex (MCC) inactivation [8]. TRIP13 gain-of-function causes premature MCC inactivation, while TRIP13 loss-of-function delays the metaphase-to-anaphase cell cycle transition [8]. In recent years, TRIP13 has become associated with the occurrence and development of malignant tumors. TRIP13 overexpression produces malignant transformation in non-malignant SCC cells in vitro [9]. Clinically, TRIP13 expression is significantly upregulated in 12

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cancer types, and heightened TRIP13 expression signals inferior prognosis in breast, liver, gastric, and NSCLC patients [10].

That being said, TRIP13's role in the development of NSCLC is still poorly understood. A comparative genomic hybridization analysis of NSCLC tumors revealed that the gain of chromosomal region 5p15.33 (which contains the *TRIP13* gene) was the most common early molecular event in NSCLC [11]. The foregoing evidence suggests that TRIP13 may be potent oncogene underlying the development of lung adenocarcinoma. Therefore, the aim of this study was to elucidate the role of TRIP13 in lung adenocarcinoma cells and elucidate the mechanism(s) by which TRIP13 affects lung adenocarcinoma cells.

2. Materials and methods

A full version of these methods has been provided in the Supplementary Methods.

2.1. Ethics statement

The study was approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (Bengbu, China). The patient's informed consent was obtained in writing prior to specimen collection.

2.2. Cell lines and lung specimens

The human lung adenocarcinoma cell lines H1299, A549, SK-LU-1, and H1975 and the human bronchial epithelial cell line BEAS-2B were all purchased from the Shanghai Cell Bank at the Chinese Academy of Sciences (Shanghai, China). Lung adenocarcinoma specimens with adjacent normal tissue were collected from the Department of Pathology at the First Affiliated Hospital of Bengbu Medical College.

2.3. Analysis of TRIP3 expression in lung specimens

Paraffinized lung adenocarcinoma tissue samples (150 point OD-CT-RsLug01-008, 75 lung adenocarcinoma cases, Shanghai Core Super Biological Technology Co., Ltd.) were scored for TRIP13 expression.

2.4. Quantitative reverse transcription-PCR (qRT-PCR) of TRIP13 mRNA expression

Total RNA was extracted, reverse-transcribed, and relative mRNA expression levels were measured by real-time PCR assay relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

2.5. Analysis of TCGA-LUAD cohort

Level 3 transcriptome expression data for the TCGA-LUAD cohort ($n = 517$ cancerous specimens with 59 adjacent normal tissue specimens) were downloaded from the TCGA database. We found a total of 6123 significant DEGs in lung adenocarcinoma tumors, including TRIP13.TCGA-LUAD cohort patients were then divided into two groups (high and low TRIP13 expression) based on median TRIP13 expression for further analysis.

2.6. Western blotting of TRIP13 protein expression

Following protein extraction, equal amounts of protein (30 μg) were subjected to 8−12% SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Protein band optical density was analyzed with AlphaView image analysis software (ProteinSimple).TRIP13 expression was normalized against GAPDH expression.

2.7. Immunofluorescent detection of TRIP13 expression

Immunofluorescence staining for TRIP13 was performed. The double-blind method was employed to count the foci. The average number of foci was calculated from more than 50 cells per sample.

2.8. TRIP13 shRNA lentiviral infection

Three TRIP13-shRNA sequences (shTRIP13-1, shTRIP13-2, and shTRIP13-3, Easy siRNA Tool) and one scrambled non-coding control sequence were independently cloned into the pGV112 vector. Following 72 h post-infection, green fluorescent protein (GFP) expression was examined by fluorescence microscopy (Olympus). Flow cytometry was applied to enrich GFP-positive cells. These cells were cultured for three days in standard medium prior to further experiments.

2.9. CRISPR/Cas9-mediated TRIP13 knockout in the H1299 cell line

A CRISPR-Cas9-mediated genome editing system was used to target the *TRIP13* gene in H1299 cells.

2.10. Transwell assay

Migration assays were performed via Transwell chamber system (Sigma-Aldrich) using an 8-μm membrane filter. Cells were dyed with Giemsa. The double-blind method was employed to count the cells with five visual fields per sample.

2.11. Colony formation assay

Cells were plated in six-well plates, and cell status was periodically observed. Cells were stained with Giemsa dye. The colony formation rate was calculated as follows: number of colonies formed/number of cells seeded × 100%.

2.12. MTT cell viability assay

Following 24 h, 48 h, 72 h, 96 h, or 120 h of incubation, MTT was added into each well. Absorbance was measured in a microplate reader at 490 nm.

2.13. Celigo cell counting assay

Following 24 h, 48 h, 72 h, 96 h, or 120 h of incubation, green fluorescent cells were counted using a Celigo test plate (Nexcelom) with five visual fields per sample.

2.14. Flow cytometric cell cycle assay

Cells were treated with propidium iodide (PI) and were examined by flow cytometry (FACSCalibur, BD Biosciences).

2.15. Flow cytometric apoptosis assay

Cells were treated with annexin V-APC stain and were examined by flow cytometry (FACSCalibur, BD Biosciences).

2.16. Xenograft experiments

H1299 cells were inoculated into the right axilla of female BALB/

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