+ MODEL

Genes & Diseases (2024) 11, 100990

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

RIOK1 synergizes with TRIP13 by regulating the E2F–Rb signaling pathway to promote the proliferation of esophageal cancer cells

Esophageal cancer is one of the leading causes of cancer death in the world, with approximately half of the new cases occurring in China every year.[1] Esophageal squamous cell carcinoma (ESCC) is the main subtype, accounting for more than 90%, and the five-year survival rate is less than 10%. Using large-scale genome analysis, many driver mutations and key pathways associated with ESCC have been identified. However, these genomic signatures have not improved the clinical management of ESCC patients, or established effective targeted therapy.[2] Esophageal cancer still lacks representative molecular markers.

Thyroid hormone receptor interacting protein 13 (TRIP13) is a member of the ATPase family associated with various cellular activities, and is involved in an array of cellular processes, including the checkpoint signaling, DNA damage, DNA repair and recombination, and chromosome synapsis.[3] Several studies have shown that TRIP13 is involved in the spindle assembly checkpoint, an evolutionarily conserved cell-cycle checkpoint supervising the fidelity of chromosome separation in mitosis. In the last decade, the oncogenic roles of TRIP13 have attracted considerable attention. Accumulating studies have indicated that TRIP13 is overexpressed in various cancers and is usually associated with poor survival.[4] Nonetheless, research on TRIP13 in esophageal cancer is still lacking. The expression characteristics, clinical significance, and molecular mechanisms of TRIP13 in esophageal cancer are still unclear.

We checked the public Gene Expression Omnibus (GEO) database, as well as the transcriptome and proteome data[5] of esophageal cancer previously published by our laboratory, and found that TRIP13 was abnormally high at both the protein and RNA levels (Fig. 1A; Fig. S1A). By Western blotting, we again verified that the expression level of TRIP13 in esophageal cancer tissues was higher than that in paired non-tumor esophageal tissues (Fig. S1B). Through survival analysis of 124 patients with esophageal cancer, we found that TRIP13 overexpression was only associated with poor prognosis in patients with early-stage esophageal cancer (pTNM I + pTNM II) ($P < 0.05$); although TRIP13 had a trend of poor prognosis in both overall survival (OS) and tumor-free survival (DFS), its statistical significance was not significant ($P > 0.05$) (Fig. S1C, D).

A series of functional assays showed that knockdown of TRIP13 markedly suppressed the ESCC cell proliferation *in vitro* (Fig. 1B–D; Fig. S2A–D). Moreover, re-expression of TRIP13-WT could restore growth (Fig. S2E–I). Gene set enrichment analysis (GSEA) enrichment analysis of TRIP13 showed that it might participate in the cell cycle (Fig. S3A, B). However, after flow cytometry detection, no significant change was found in the proportion of the cell cycle phases, whether in KYSE150 (Fig. S3C) or KYSE30 (Fig. S3D) cells. After cell cycle synchronization, we found that TRIP13 was highly expressed in the G1 and G2/M phases. In addition, TRIP13 knockout cells showed significant retention in the G1 and G2/M phases (Fig. 1E; Fig. S3E, F). Collectively, these results indicate that TRIP13 enhances the proliferation of esophageal cancer cells by promoting cell cycle progression.

To explore the specific mechanism of TRIP13 function, we queried the interacting proteins of TRIP13 in the BioGRID database (Fig. S4A). After intersecting with our esophageal cancer proteomics data[5] (PXD021701), we conducted a series of bioinformatics analyses on the obtained proteins, and identified the possible interacting protein RIOK1 (right open reading frame protein kinase 1) (Fig. S4B–F). IP experiments showed that an interaction between RIOK1 and TRIP13 (Fig. 1F; Fig. S5A). Further detailed IP results showed that the interaction between the two depended on the N-terminal domain of TRIP13 (Fig. S5B, C).

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.04.024

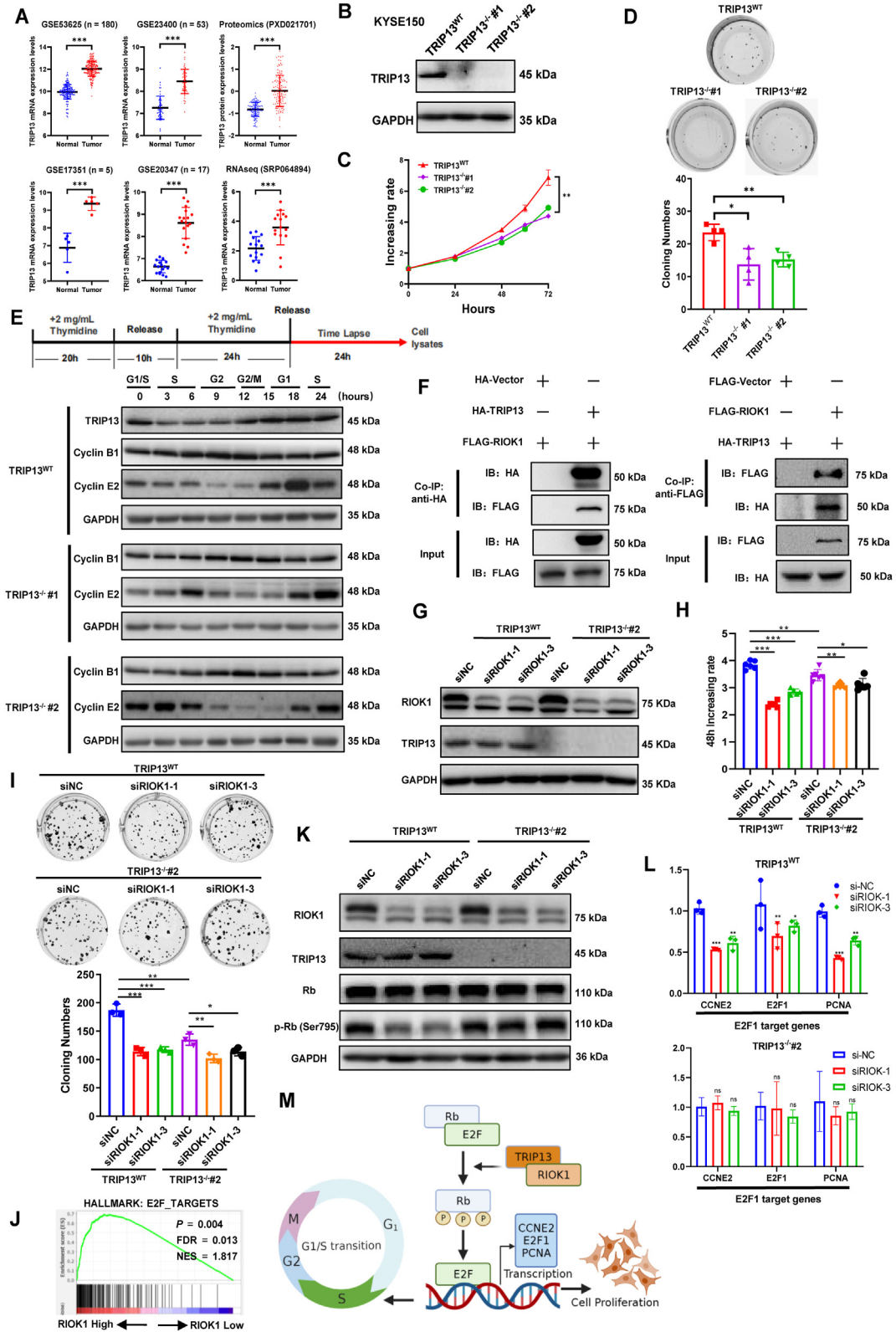

Figure 1 RIOK1 interacts with TRIP13 to phosphorylate Rb and accelerate the proliferation of esophageal cancer cells. **(A)** TRIP13 levels were abnormally high in esophageal cancer tissues at both the transcriptional and protein levels compared with normal esophageal tissues. Data source: GEO database, PXD021701, SRP064894. **(B)** TRIP13 knockout by CRISPR-Cas9 in KYSE150 cells. **(C, D)** Cell proliferation was measured using MTS and colony formation assays in KYSE150 cells. The results are expressed as mean ± SD. **(E)** Double thymidine block flowchart (upper). Double thymidine block was used to synchronize the cell cycle. Western blotting was used to detect the changes in related protein expression levels in the experimental group and the control group. The cycle changes

noted are only in the TRIP13^{WT} cell line. **(F)** The interaction between HA-TRIP13 and Flag-RIOK1 was detected by IP after transient transfection of HA-TRIP13 and Flag-RIOK1 into HEK293T cells. **(G)** In KYSE150 cells, RIOK1 was knocked down in TRIP13 knockout cells and control cells. Western blotting was used to detect knockdown efficiency. **(H)** RIOK1 was knocked down, and MTS data were obtained 48 h after cell seeding. **(I)** Typical graph and statistical results of colony formation. **(J)** GSEA enrichment analysis of RIOK1 showed that RIOK1 correlated with E2F targets. **(K)** Western blotting was used to detect changes in protein levels in related signaling pathways. **(L)** Reverse transcription-polymerase chain reaction was used to measure E2F downstream target gene mRNA levels. **(M)** Model of TRIP13&RIOK1-mediated p-Rb phosphorylation promoting the G1/S transition and cell proliferation in esophageal cancer. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

As an atypical kinase, RIOK1 plays an important role in ribosome biogenesis, but its function in esophageal cancer is still unclear. We found that RIOK1 is also abnormally highly expressed in esophageal carcinoma (Fig. S6A, B). Survival analysis showed that high expression of RIOK1 was associated with poor prognosis in both overall survival and disease-free survival (Fig. S4G). Moreover, high expression of RIOK1 was found to be associated with poor prognosis in patients with early-stage esophageal cancer (Fig. S4H), similar to TRIP13. A series of functional assays showed that knockdown of RIOK1 markedly suppressed the proliferation of ESCC cells *in vitro* (Fig. S6C—F).

To explore the relationship between the two, we either knocked down or overexpressed RIOK1 in the previously constructed KYSE150 cell line with TRIP13$^{+/+}$/TRIP13$^{-/-}$ (Fig. 1G; Fig. S7A). The functional experiments showed that both knockdown and overexpression of RIOK1 significantly affected cell behavior only in cell lines expressing TRIP13 (Fig. 1H, I; Fig. S7B—D). Cell cycle synchronization experiments showed that RIOK1 could affect the transition from G1 phase to S phase only in the presence of TRIP13 (Fig. S8A—C). This is consistent with the correlation of RIOK1 with E2F targets in the GSEA enrichment results (Fig. 1J). Then, we detected the expression of the E2F upstream protein Rb and its phosphorylation. Western blotting showed that RIOK1 knockout only in TRIP13$^{+/+}$ cells could reduce p-Rb levels (Fig. 1H). In addition, reverse transcription-polymerase chain reaction results showed that the mRNA levels of E2F downstream target genes were significantly down-regulated after RIOK1 knockdown in TRIP13$^{+/+}$ cells (Fig. 1L). Thus, our results demonstrate that RIOK1 interacts with TRIP13 to phosphorylate Rb and accelerate the transition from G1 to S phase in esophageal cancer cells. An interpretation of our main findings is represented in the schematic diagram in Figure 1M.

In summary, studies from this work show that TRIP13 is abnormally overexpressed in esophageal cancer and is an indicator of poor prognosis in patients with early esophageal cancer. In addition, another indicator of poor prognosis in esophageal cancer, RIOK1, is dependent on TRIP13 to regulate the proliferation of esophageal squamous cancer cells through the E2F—Rb signaling pathway. These results suggest that TRIP13 may be an early diagnostic marker and could serve as a potential therapeutic target for esophageal cancer.

Author contributions

Cheng-Yu Li: conceptualization, methodology, formal analysis, writing-original draft. De-Yuan Pan: validation, methodology. Wan Lin: validation, investigation. Dan-Xia Deng: software, data curation. Ying-Xin Zhou: resources, investigation. Hui Zhao: resources, investigation. Feng Pan: resources, funding acquisition. Lin Long: resources, investigation. Li-Yan Xu, En-Min Li and Lin Long: writing, review, editing, supervision, project administration, funding acquisition.

Conflict of interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by grants from National Natural Science Foundation of China (No. 82002975 and No.82173011), and Special Fund for Science and Technology of Guangdong Province (No. 200105215896551).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.024.

References

1. Zhao P, Dai M, Chen W, Li N. Cancer trends in China. *Jpn J Clin Oncol*. 2010;40(4):281—285.
2. Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. *Lancet*. 2013;381(9864):400—412.
3. Vader G. Pch2(TRIP13): controlling cell division through regulation of HORMA domains. *Chromosoma*. 2015;124(3):333—339.
4. Tao Y, Yang G, Yang H, et al. TRIP13 impairs mitotic checkpoint surveillance and is associated with poor prognosis in multiple myeloma. *Oncotarget*. 2017;8(16):26718—26731.
5. Liu W, Xie L, He YH, et al. Large-scale and high-resolution mass spectrometry-based proteomics profiling defines molecular subtypes of esophageal cancer for therapeutic targeting. *Nat Commun*. 2021;12(1):4961.

Chengyu Li [a,b,c], Deyuan Pan [a,c], Wan Lin [b,c], Danxia Deng [b], Yingxin Zhou [b,c], Hui Zhao [b,c], Feng Pan [d], Liyan Xu [b,c,**], Enmin Li [a,c,***], Lin Long [a,b,c,*]

[a] *The Key Laboratory of Molecular Biology for High Cancer Incidence Coastal Chaoshan Area, Department of Biochemistry and Molecular Biology, Shantou University Medical College, Shantou, Guangdong 515041, China*
[b] *Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Institute of Oncologic Pathology, Shantou University Medical College, Shantou, Guangdong 515041, China*
[c] *Institute of Basic Medical Science, Cancer Research Center, Shantou University Medical College, Shantou, Guangdong 515041, China*
[d] *Clinical Research Center, Shantou Central Hospital, Shantou, Guangdong 515041, China*

*Corresponding author. Institute of Basic Medical Science, Cancer Research Center, Shantou University Medical College, Shantou, Guangdong 515041, China.

**Corresponding author. Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Institute of Oncologic Pathology, Shantou University Medical College, Shantou, Guangdong 515041, China.

***Corresponding author. The Key Laboratory of Molecular Biology for High Cancer Incidence Coastal Chaoshan Area, Department of Biochemistry and Molecular Biology, Shantou University Medical College, Shantou, Guangdong 515041, China.
E-mail addresses: lyxu@stu.edu.cn (L. Xu), nmli@stu.edu.cn (E. Li), llong@stu.edu.cn (L. Long)

22 September 2022
Available online 24 June 2023