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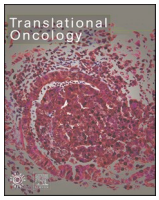

Original Research

# TCF19 promotes cell proliferation and tumor formation in lung cancer by activating the Raf/MEK/ERK signaling pathway

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ABSTRACT

*Objective:* This study aimed to investigate TCF19's role in lung cancer development, specifically its involvement in the RAF/MEK/ERK signaling pathway.
*Methods:* Lung cancer tissue analysis revealed significant TCF19 overexpression. *In vitro* experiments using A549 and Hop62 cells with TCF19 overexpression demonstrated enhanced cell growth. Transgenic mouse models confirmed TCF19's role in primary tumor development. Transcriptome sequencing identified altered gene expression profiles, linking TCF19 to RAF/MEK/ERK pathway activation. Functional assays elucidated underlying mechanisms, revealing increased phosphorylation of Raf1, MEK1/2, and ERK1/2. Inhibiting RAF1 or ERK through shRaf1 or ERK inhibitor reduced cell cycle-related proteins and inhibited TCF19-overexpressing cell growth.
*Results:* TCF19 was identified as an oncogene in lung carcinoma, specifically impacting the RAF/MEK/ERK pathway. Elevated TCF19 levels in lung cancer suggest targeting TCF19 or its associated pathways as a promising strategy for disease management.
*Conclusion:* This study unveils TCF19's oncogenic role in lung cancer, emphasizing its modulation of the RAF/MEK/ERK pathway and presenting a potential therapeutic target for TCF19-overexpressing lung cancers.

## Introduction

Cancer poses a significant risk to human well-being, with lung cancer remaining among the most fatal malignancies globally [1–6]. The World Health Organization's International Agency for Research on Cancer released the most recent global cancer burden statistics in 2020, revealing a total of 9.96 million deaths caused by cancer. Among these, lung cancer accounted for 1.8 million deaths, significantly exceeding other types of cancer and claiming the highest number of lives. We have invested heavily in lung cancer in basic and clinical research and obtained some effective treatment methods for lung cancer, such as targeted therapy and immunotherapy, and these treatments have certain therapeutic effects [7]. Nevertheless, the survival rate for individuals with lung cancer remains exceedingly poor [8,9]. Hence, further comprehensive investigations are required to examine the mechanism behind the onset, progression, and management of lung carcinoma.

Among the various MAPKs signals, the RAF-MEK-ERK pathway is most deeply studied, and it is mainly activated by the orderly phosphorylation of Raf, MEK, and ERK kinases [10]. Cell cycling was promoted by the Ser/Thr-kinases ERK1 and ERK2 in the cytoplasm, while the cytosolic kinases MEK1/2 increased the activity of ERK1/2 and phosphorylated the Thr/Tyr-in the activation loop of ERK1/2. Additional examination of the kinase cascade uncovered that RAF1 serves as the upstream kinase responsible for phosphorylating MEK1 at Ser222

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and MEK2 at Ser218, thereby controlling the functionality of MEK. This process enables the transmission of MAPK signaling from RAS, RAF, MEK, and ultimately to ERK [11–14]. The RAF-MEK-ERK signaling pathway is strongly associated with the development of different types of cancers, and several drugs that target important components of this pathway have been effectively commercialized [13,15–18]. The importance of this pathway in controlling the malignant development of tumors can be demonstrated by the proportion of its marketed drugs and the current research situation. Nevertheless, the precise connections between TCF19 and the Raf/MEK/ERK signaling pathway, which facilitates the onset and progression of lung cancer, are yet to be determined.

The TCF19 gene (Transcription factor 19), alternatively referred to as SC1, was initially discovered as a growth modulator in human, mouse, and hamster cells [19]. Early studies have shown that TCF19 is associated with the occurrence of type I diabetes and type II diabetes [20], and is also a risk-related site for chronic hepatitis B [21]. In the past few years, scientists have discovered that TCF19 inhibits WWC1 in colon cancer, leading to increased cell growth and movement, exhibiting oncogenic properties [19]. Researchers discovered that TCF19 enhances the advancement of the G1/S phase in the cell cycle in liver cancer by activating AKT/FOXO1, consequently stimulating cell proliferation [22]. Zi-Hao Zhou et al. The study demonstrated that TCF19 hinders FOXO1 to enhance the growth of SPC-A1 and SK-MES-1 cells [23]. And our findings suggest that TCF19 drives its oncogene function through an entirely new mechanism in lung cancer. TCF19 promotes lung cancer tumor progression and cell cycle at least in part by activating Raf1/-MEK/ERK signaling pathway. The findings presented herein provide preliminary evidence supporting the notion that the overexpression of TCF19 is instrumental in the abnormal activation of the Raf1/MEK/ERK signaling pathway. At the same time, we discovered that blocking ERK or using shRaf1 can suppress the growth of lung cancer cells induced by TCF19, offering a solid scientific foundation for treating lung cancer patients with elevated TCF19 levels.

## Materials and methods

### *Plasmid*

The pLVX-TetOne-puro plasmid was purchased from Clontech, and the pLVX-TetOne-human-TCF19-Flag-tagged-puro plasmid was constructed using standard molecules. The pCDH-EF1a Plasmid was provided by Dr. Liang Chen (Jinan University, Guangzhou, China), pCDH-EF1a-human-TCF19–3 × Flag and pCDH-EF1a-GFP × Flag were constructed from standard molecules. Addgene was the source of the acquisitions of psPAX2 and pMD2. G. Standard subcloning protocols were used to construct the shTCF19#1 (TRCN0000015410), shTCF19#2 (TRCN0000015411) [24], shTCF19#3 (TRCN0000015412), and shRaf1 (TRCN0000001068) [25] in either the pLKO-vector (Addgene) or pLKO-tet-zeocin (Addgene) vectors.

### *Cell lines and cell culture*

Dr. Liang Chen (Jinan University, Guangzhou, China) supplied NCI-H322, A549, NCI-H460, EKVX, Hop62, PC-9, NCI-H466, NCI-H1650, and HEK293T. Lung cancer cells were grown in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS, Gibco, 42G9274K) and 1 % penicillin/streptomycin/glutamine (Gibco, 15,140,122). HEK293T was grown in DMEM medium supplemented with 10 % fetal bovine serum (FBS, Gibco, 42G9274K) and 1 % penicillin/streptomycin/glutamine (Gibco, 15,140,122).

HEK293T cells were transfected with pCDH-EF1a-human-TCF19-3 × Flag/pCDH-EF1a-GFP × Flag/pLKO-shTCF19/ pLKO-tet-shRaf1-zeocin using psPAX2 and pMD2. G, along with Lipofectamine 3000 (Invitrogen) as the transfection reagent. Fresh media replaced the culture 6–8 h following transfection, and then the supernatant was collected after 48 h and filtered using a 0. 45 μm sterilization filter. The medium containing the modified virus was introduced to the specified cells while polybrene (8 μg/mL) was present. The infected cells were selected with 1 μg/mL puromycin or 3 mg/mL zeocin for 7 days before additional experiments were performed.

### *Human tissues*

Approval for the human lung cancer clinical samples collection was granted by the Sunshine Union Hospital in Shandong province, China. All the patients provided written consent. Pathologists from the Department of Pathology at Sunshine Union Hospital reviewed all cases to verify the histology and content of the tumors. The research materials received approval from the Human Ethics Committee at Weifang Medical University. All work was performed following the approved protocol.

### *Western blot analysis*

The Western blot analysis in this investigation was conducted according to the methods described earlier [26,27]. RIPA lysis buffer (Santa Cruz) along with Protease and phosphatase inhibitor cocktail (Biotech Roche) were used to lyse cells or clinical samples of lung cancer. In this study, the antibodies used included anti-TCF19 (diluted 1:1000, YT5078, Immunoway), anti-MEK1/2 (diluted 1:500, sc-81,504, Santa Cruz, USA), anti-p-MEK1/2 (diluted 1:500, sc-81,503, Santa Cruz, USA), anti-ERK1/2 (diluted 1:1000, #4695, Cell Signaling Technology), anti-p-ERK1/2 (diluted 1:1000, #4370, Cell Signaling Technology), anti-Raf-1 (diluted 1:500, sc-7267, Santa Cruz, USA), anti-p-Raf-1 (diluted 1:500, sc-271,919, Santa Cruz, USA), anti-FLAG (diluted 1:1000, #14,793, Cell Signaling Technology), CyclinA1 (diluted 1:1000, CY1027, Abways), CyclinD1 (diluted 1:10,000, ab228528, Abcam), CyclinE1 (diluted 1:1000, #20,808, Cell Signaling Technology), CDK2 (diluted 1:10,000, ab228528, Abcam), anti-GAPDH (diluted 1:1000, #5174, Cell Signaling Technology), and anti-β-actin (diluted 1:1000, A5316, Sigma). We bought the anti-rabbit and anti-mouse IgG conjugated with horseradish peroxidase from sigma (A6154, 1 1000; A0168, 1 1000). The results were obtained using Western blotting substrate (Millipore).

### *Real-time PCR*

Trizol reagent (Invitrogen) was employed for total RNA extraction, followed by quantitative reverse transcription PCR with gene-specific primers (5′−3′) as Table 1.

### *Cell proliferation assay*

A total of one thousand and three cells were placed in 96-well plates and grown in RPMI medium 1640 supplemented with 10 % FBS. The cells were treated with or without Doxycycline hyclate (DOX, 0. 5 μg/mL, Sigma) for a duration of 1 to 5 days. Cell proliferation was assessed

**Table 1**
Primers for detecting gene transcription.

| Gene (Human) | Primers (5′−3′) |
|---|---|
| TCF19-F | TCAGCCTGGAAGACCACAGCAG |
| TCF19-R | CCAAAGGTCAGGAGGTCTCCAT |
| CDK2-F | ATGGATGCCTCTGCTCTCACTG |
| CDK2-R | CCCGATGAGAATGGCAGAAAGC |
| Cyclin A1-F | GCACACTCAAGTCAGACCTGCA |
| Cyclin A1-R | ATCACATCTGTGCCAAGACTGGA |
| Cyclin E1-F | TGTGTCCTGGATGTTGACTGCC |
| Cyclin E1-R | CTCTATGTCGCACCACTGATACC |
| Cyclin D1-F | TCTACACCGACAACTCCATCCG |
| Cyclin D1-R | TCTGGCATTTTGGAGAGGAAGTG |
| β-actin-F | CACCATTGGCAATGAGCGGTTC |
| β-actin-R | AGGTCTTTGCGGATGTCCACGT |

by employing the Cell Counting Kit-8 (CCK8, Dojindo Molecular Technologies) according to the instructions provided by the manufacturer.

*Colony-forming assay*

For the colony forming experiment, $0.5 \times 10^3$ cells were placed in 6-well dishes using RPMI medium 1640 supplemented with 10 % FBS. Following a period of 14 days, the cells underwent rinsing using 1 × PBS, subsequent fixation with formaldehyde for a duration of 10 min, and finally staining with 0.05 % crystal violet for a period of 30 min. The software Image J was used to tally the colonies.

*Soft agar assay*

Soft agar was carried out as described in our previous report [28]. Base agar (0.6 %, 2 × DMEM + 20 % FBS + 2 % PS + 1.2 % agar) and top agar (0.35 %, 2 × DMEM + 20 %FBS + 2 % PS + 0.7 % agar) were prepared respectively. 1 mL base agar was added into each well of 6-well plates and left for 1 h. $1 \times 10^4$ cells were mixed with 2 mL top agar with or without DOX (0.5 μg/ml), and then the mixture was seeded into 6-well plates. Every 4 days, 0.2 mL of 1 × liquid medium was applied onto the top gel, with or without DOX (0.5 μg/ml) on its surface. Following incubation in a moist incubator at a temperature of 37 °C for a period of 3 to 4 weeks, the number of colonies was determined using an optical microscope placed upside down.

*Cell cycle analysis*

At 48 h post-DOX treatment, cells were gathered and subsequently preserved in 70 % ethanol for an overnight period at 4 °C. The cell cycle analysis was conducted by employing the cell cycle kit (Beyotime) according to the instructions provided by the manufacturer. Subsequently, the analysis was carried out using a BD AccuriTM C6 flow cytometer.

*RNA sequencing*

After a 48 h incubation period, cells were subjected to treatment with or without DOX, followed by extraction of total RNA using Trizol reagent. The Institute of Genomics conducted RNA sequencing and created RNA libraries.

*In vivo xenograft model and KrasG12D/CC10rtTA transgenic mice model*

Every mouse was kept in a sterile setting at Weifang Medical University, and all research procedures adhered to the Ethical Guidelines for Laboratory Animal Usage and were sanctioned by the Ethics Committee of Weifang Medical University. All animal research was conducted in strict compliance with authorized procedures. All surgery was performed under 2-3 % isoflurane, and every effort was made to minimize suffering.

Xenograft assay was performed as described in our previous report [28,29]. A total of twelve 6-week-old female BALB/c nude mice were subcutaneously implanted with $2 \times 10^6$ cells suspended in 100 μL of Matrigel (Corning) on their right flank. Once the tumors reached a size of approximately 80 mm3, the mice were divided into two groups using a pair comparison method. This involved carefully matching the animals based on factors such as age, sex, weight, and others. Essentially identical animals were paired up, and each pair was then randomly assigned to one of the two groups. The mice fed with DOX food as experiment group($n = 6$), fed with normal food as control group($n = 6$). The tumor's size was observed every second day for 1–2 weeks until the experiment concluded [28,29]. At the conclusion of the experiment, the mice were euthanized, and the tumors were gathered, photographed, and measured.

A transgenic mice experiment was performed as described in our previous report [29]. TetO-KrasG12D/CC10rtTA transgenic mice were provided by Dr. Liang Chen (Jinan University, Guangzhou, China). After consuming food containing doxycycline for a period of 2 months, the mice developed lung adenocarcinoma. Lentivirus overexpression Tetone-TCF19-FLAG($n = 6$) or control virus($n = 6$) was administered intranasally to TetO-KrasG12D/CC10rtTA mice on a diet containing doxycycline. Following a 2-month period, the mice underwent computed tomography (CT) scans using the PINGSENG Healthcare recorder SNC-100. Subsequently, they were euthanized. Lung tissues were gathered for hematoxylin & eosin (H&E) staining.

*Statistical analysis*

Statistical analyses were carried out using GraphPad Prism 8.0 software. Statistical significance was determined by analyzing all experimental data using the two-tailed Student's *t*-test, with a significance level of $p < 0.05$. All error bars represent SEM.

## Results

In lung cancer, TCF19 is a significant and medically pertinent tumor oncogene. The role of TCF19 as a tumor oncogene has been documented in different types of cancer, although its specific mechanism in lung cancer remains unknown. Hence, we examined the effect of TCF19 on the progression of lung carcinoma. By analyzing the TCGA database (http//kmplot.com/analysis), it was found that the prognosis of lung cancer paitents in all stages was significantly associated with the expression level of TCF19 (Fig. 1A, left). Additionally, this correlation was also observed in stage I of lung cancer patients (Fig. 1A, right). According to the UCSC website (https//xenabrowser.net/), Fig. 1B demonstrates a notable increase in TCF19 mRNA expression in lung tumor tissues compared to Para-tumor tissues. Then we confirmed that TCF19 was expressed at higher mRNA and protein levels in lung cancer tumors than in their Para-tumor tissues (Fig. 1C, D). The findings indicate a potential association between TCF19 expression and the onset and progression of lung cancer, highlighting its significance in clinical research.

*The ectopic expression of TCF19 promotes the proliferation and tumorigenesis of lung cancer cells in vitro*

To explore the function of TCF19, we examined its expression in different lung cancer cell lines (see Supplementary Fig. 1A and B). Our findings revealed that TCF19 expression was comparatively low in two lung cancer cell lines (H460 and Hop62), while two other lung cancer cell lines (H466 and PC-9) exhibited relatively high TCF19 expression. Subsequently, we generated stable cell lines that exhibited elevated levels of TCF19 (H460- TCF19 and Hop62-TCF19), as depicted in Supplementary Fig. 1C and D, and reduced TCF19 (H466-shTCF19 and PC9-shTCF19), as illustrated in Supplementary Fig. 1E–H). The proliferation and viability of lung cancer cell lines modified with TCF19 were assessed using colony-formation and CCK8 assay. The colony-formation experiment demonstrated that the excessive expression of TCF19 enhances the growth of H460 and Hop62 cells (Fig. 2A and C). The CCK8 test demonstrated that the increased expression of TCF19 improved the survival of H460 and Hop62 cells (Fig. 2B and D). However, according to the colony-formation and CCK8 assay experiments, the stable lines with TCF19 knockdown grew much more slowly than the control cells (Fig. 2E–H). The findings from all these experiments indicated that the TCF19 protein has the ability to enhance the growth of lung cancer cells in a laboratory setting.

The soft agar experiment can reflect the malignant degree of tumor cells. Then, we performed soft agar experiments according to the operating procedures of the previous articles [28,29]. Fig. 2I demonstrated that the elevated TCF19 expression greatly enhanced the formation of soft agar colonies in H460 and Hop62 cells. Moreover, the knockdown of TCF19 inhibited the tumorigenic ability of PC9 and H446 lung cancer

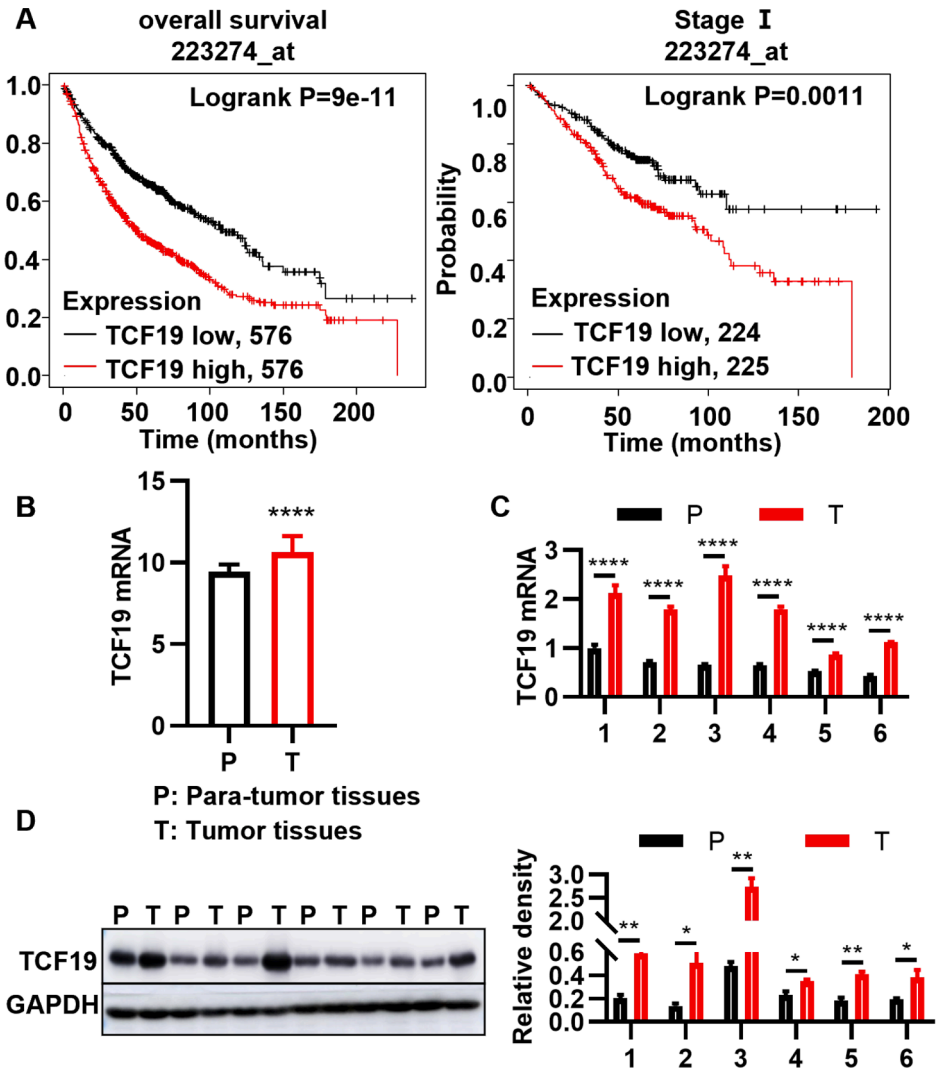


**Fig. 1.** TCF19 is an important tumor gene in lung cancer. A. Kaplan-Meier survival analysis of TCF19-overexpression and TCF19-underexpression lung cancer patients. B. Expression level of TCF19 in lung tumor tissues and Para-tumor tissues (TCGA database). C, D. TCF19 mRNA and protein levels in lung cancer patient tissue samples (P: Para-tumor tissues, T: Tumor tissues). Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

cells (Fig. 2J). These results suggested that TCF19 can significantly promote the tumorigenic ability of lung cancer cells and has an oncogene phenotype.

*TCF19 promotes the growth of xenograft and primary lung cancer in vivo*

The effect of TCF19 was also tested *in vivo*. H460-TCF19 cells were injected subcutaneously into nude mice and then divided into two groups, namely the control group and the TCF19 group, as depicted in Fig. 3A. Notably, the control group (Con) exhibited a comparatively slower rate of tumor growth in contrast to the TCF19 group (Fig. 3B, C, Supplementary Fig. 2A). Additionally, the tumor weight in the control group was markedly lesser than that of the TCF19 group (Fig. 3D). Subcutaneously, we injected PC9-shTCF19 cells into nude mice and then randomly separated them into two groups: the shGFP group and the shTCF19 group. Based on the test findings, it was observed that the tumors in the shGFP cohort exhibited accelerated growth compared to those in the shTCF19 cohort (as depicted in Fig. 3E and F, Supplementary Fig. 2B). Additionally, the suppression of TCF19 resulted in a decrease in tumor weight (as shown in Fig. 3G).

To further verify the function of TCF19 *in vivo*, we tested the tumor formation following TCF19 overexpression in a transgenic lung cancer mouse model. The DOX-induced KrasG12D/CC10rtTA mouse model was utilized for studying lung cancer. After being fed DOX food for 2–3 months, this mouse model acquired lung cancer, whereas it remained free from lung cancer when given regular food [28,29]. By adhering to this procedure, we administered lentivirus-overexpressing Teto-TCF19-Flag or a control virus through the nasal route to TetO-KrasG12D/CC10rtTA mice. Consequently, the mice began expressing KrasG12D and TCF19 or control in their lung epithelial cells upon consumption of a DOX diet (Fig. 3H). The CT scan and HE staining findings indicated that the lung tumor load was greater in transgenic mice exhibiting elevated TCF19 expression compared to the control group (Fig. 3I, J, Supplementary Fig. 2C). Therefore, our data revealed a potent oncogene function of TCF19 *in vivo*.

*TCF19 promotes the progression of the lung cancer cell cycle*

Our results *in vitro* and vivo confirmed that TCF19 could promote the growth process of lung cancer, and we usually think that the change in cell proliferation is closely related to cell cycle regulation [30]. Therefore, we used flow cytometry to detect the difference in cell cycle between lung cancer cells overexpressing TCF19 and the control group. In our findings, it was observed that the excessive expression of TCF19

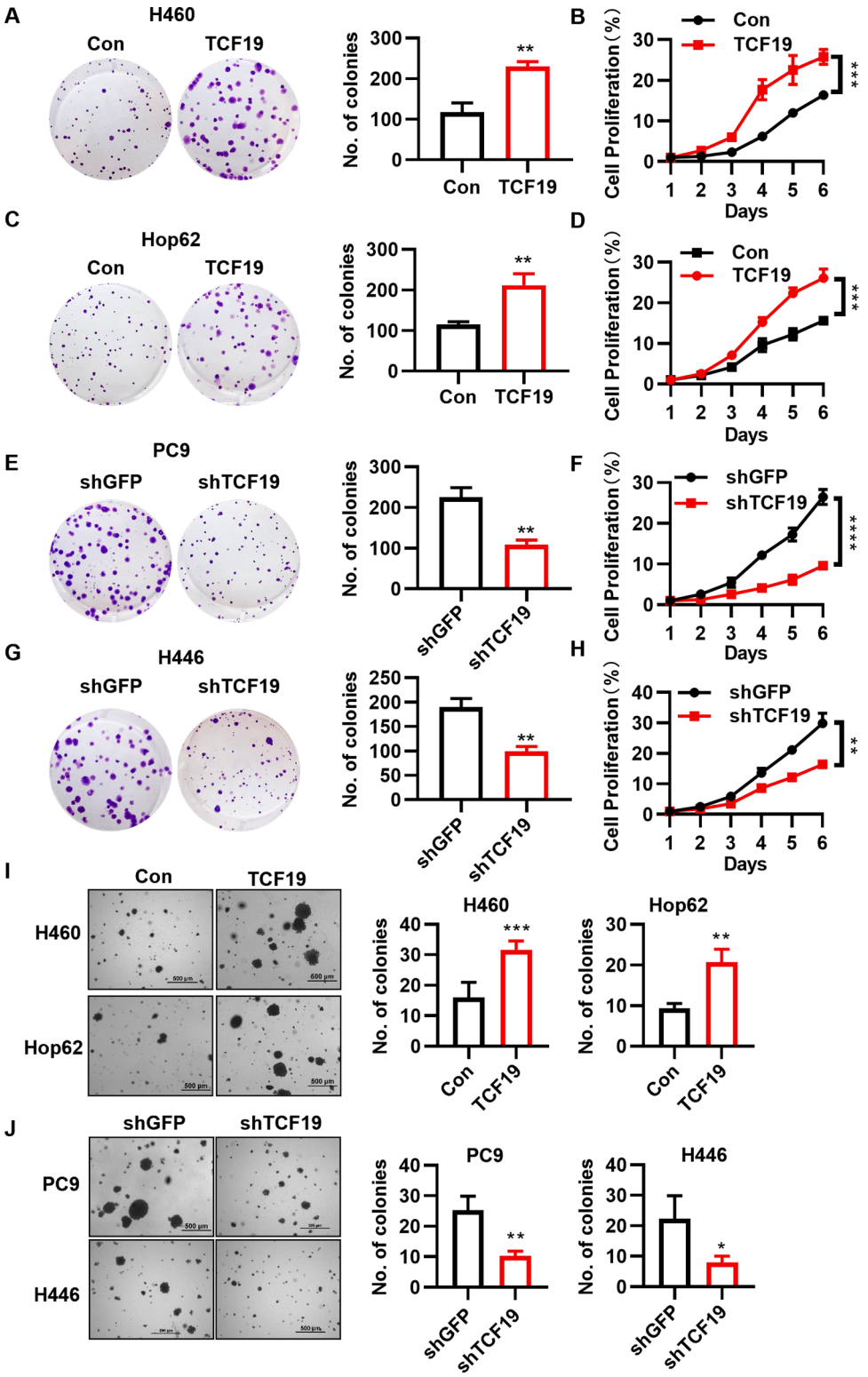


**Fig. 2.** TCF19 overexpression promotes lung cancer cell growth and tumorigenicity *in vitro*. A. Colony-forming evaluated the effects of TCF19 overexpression on the growth and proliferation of H460. B. CCK8 evaluated the effects of TCF19 overexpression on the growth and proliferation of H460. C. Colony-forming evaluated the effects of TCF19 overexpression on the growth and proliferation of Hop62 cells. D. CCK8 evaluated the effects of TCF19 overexpression on the growth and proliferation of Hop62 cells. E. Colony-forming evaluated the effects of TCF19 knock-down on the growth and proliferation of PC-9 cells. F. CCK8 evaluated the effects of TCF19 knock-down on the growth and proliferation of PC-9 cells. G. Colony-forming evaluated the effects of TCF19 knock-down on the growth and proliferation of H446 cells. H. CCK8 evaluated the effects of TCF19 knock-down on the growth and proliferation of H446 cells. I. Soft-agar assay of H460 and Hop62 cells in the presence of TCF19. **J.** Soft-agar assay of PC-9 and H446 cells in the absence of TCF19.
Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

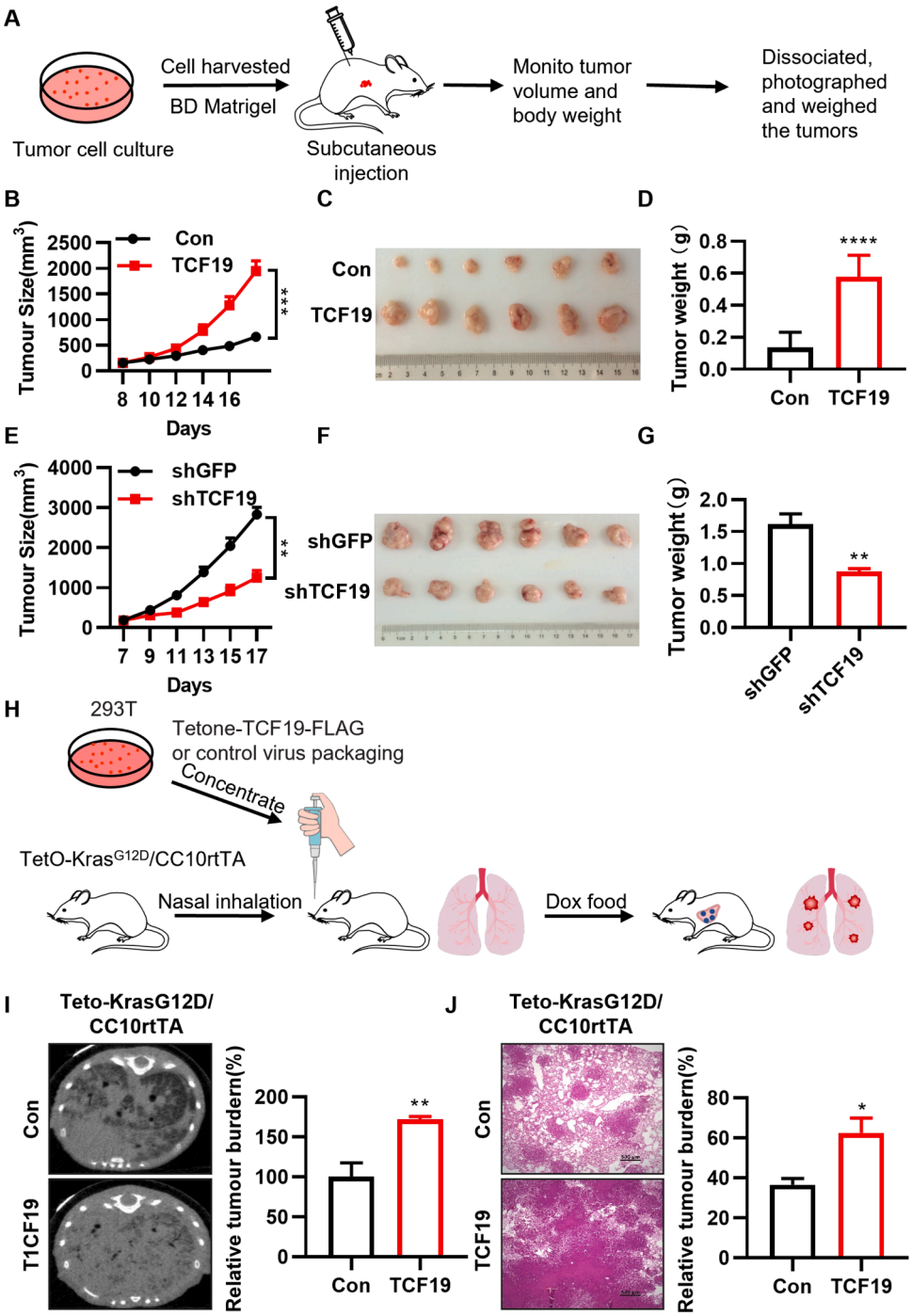


**Fig. 3.** TCF19 promoted tumorigenesis *in vivo*. A. Schematics for the xenograft treatments of nude mice. $2 \times 10^6$ cells were subcutaneously inoculated into the right flank of 8-wk-old female nude mice fed with normal food or DOX food. B–D. Tumor growth curves (B) were calculated based on the monitoring data every other day after subcutaneously injecting Hop62-TCF19 cells into female nude mice. The xenograft tumors were dissociated, photographed (C), and weighed (D) at the end of the experiments. ($n = 6$). E–G. Tumor growth curves (E) were calculated based on the monitoring data every other day after subcutaneously injecting H446-shTCF19 cells into female nude mice. The xenograft tumors were dissociated, photographed (F), and weighed (G) at the end of the experiments. ($n = 6$, shGFP as control group and shTCF19 as TCF19 knock-down group). H. Schematics of intranasal instillation of retrovirus for forced expressing TCF19 in Dox inducible Tet-KRAS$^{G12D}$/CC10rtTA lung cancer mouse model. ($n = 6$; mice fed with normal food as the control group, mice fed with DOX to induce TCF19 overexpression as TCF19 overexpression group). I. Ectopic expression of TCF19 promotes lung cancer formation in TetO-Kras$^{G12D}$/CC10rtTA mouse model. J. Representative images of Hematoxylin and eosin (H&E) staining of the lung tissues obtained from lenti-control and lenti-TCF19 treated Kras$^{G12D}$/CC10rtTA mice.
Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

greatly enhanced the progression of the S phase and G2/M phase in the cell cycle, whereas the G0/G1 phase experienced a notable decrease in H460-TCF19 and Hop62-TCF19 cells (as shown in Fig. 4A, B). The Western blotting and RT-PCR findings indicated that the upregulation of TCF19 in H460 and Hop62 cells enhanced the levels of proteins associated with the cell cycle (Fig. 4C, D). All these data indicated that the promotion of the cell cycle in TCF19-overexpression cells must be responsible for its effect on the proliferation of lung cancer cells.

**Lung cancer experienced activation of the Raf/MEK/ERK pathway due to TCF19**

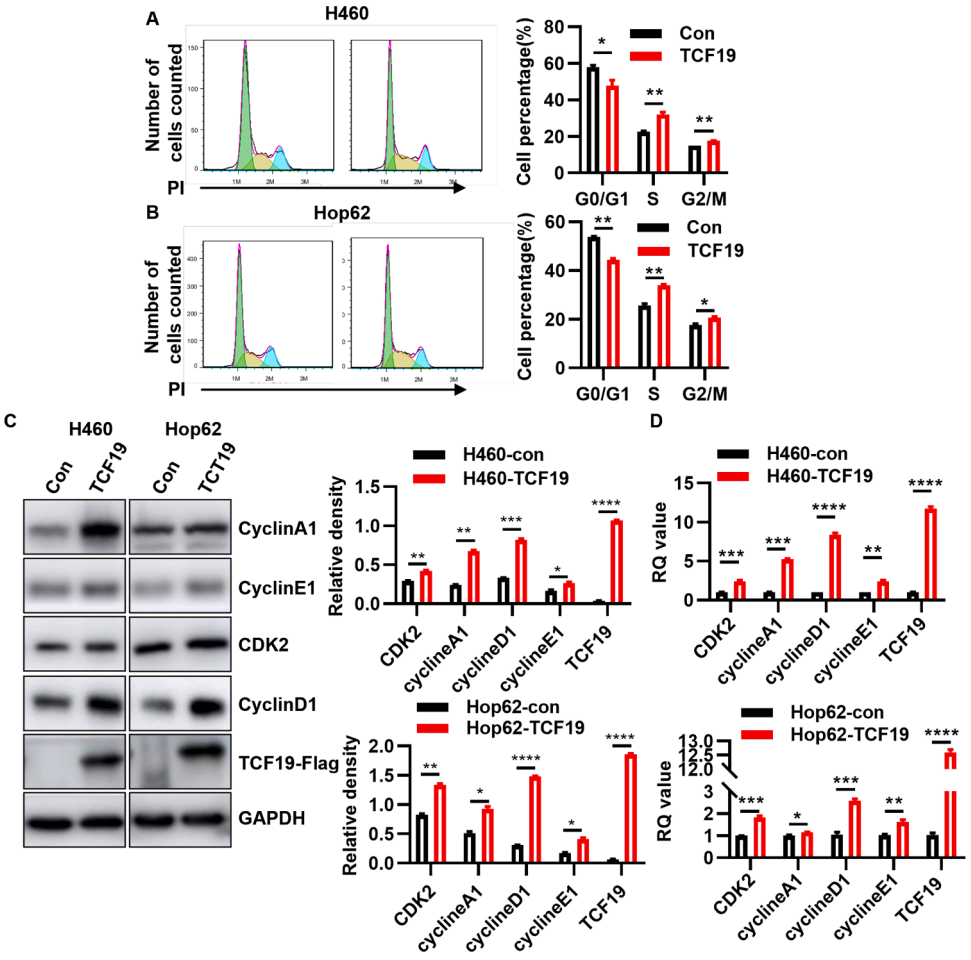


**Fig. 4.** TCF19 promoted lung cancer cell cycle progression. A. The cell cycle of H460-TCF19 cells was analyzed by flow cytometry. B. The cell cycle of Hop62-TCF19 cells was analyzed by flow cytometry. C, D. The expression of cyclin A1, cyclin E1, cyclin D1, CDK2, and TCF19 in H460- TCF19 and Hop62-TCF19 cells was detected by western blotting.
Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

In order to further investigate the potential mechanism through which TCF19 enhances the progression of lung cancer, transcriptome sequencing was conducted (Fig. 5A). The Ras and MAPK signaling pathway in the TCF19-overexpression group were enriched by KEGG enrichment analysis (Fig. 5B). It is common knowledge that Raf1 plays a crucial role as a protein in the Ras signaling pathway, and the conventional Raf/MEK/ERK signaling pathway is strongly associated with the role of Raf1 in the progression of cancer [25]. Surprisingly, it was discovered that the phosphorylation status of Raf1, MEK1/2, and ERK1/2 proteins exhibited a notable rise in H460-TCF19 and Hop62-TCF19 cells (Fig. 5C). In addition, we observed a rise in proteins related to the cell cycle and the elevated levels of protein phosphorylation linked to the Raf/MEK/ERK signaling pathway (Fig. 5D). The findings indicated that TCF19 triggers the activation of the Raf/MEK/ERK pathway in lung carcinoma.

### *The Raf1/MEK/ERK signaling pathway has a significant impact on the TCF19-driven growth of lung cancer cells*

The findings indicated that TCF19 has the ability to enhance the cell cycle progression and elevate the phosphorylation status of crucial proteins within the Raf/MEK/ERK signaling pathway. Thus, our hypothesis suggests that TCF19 has the ability to enhance the advancement of the cell cycle via the Raf/MEK/ERK pathway, consequently fostering the growth of lung cancer cells. To verify this hypothesis, we used shRaf1 to knock down Raf1 in Hop62-TCF19 cells (Supplementary Fig. 3A). From the results, we found that shRaf1 could significantly inhibit the cell proliferation caused by TCF19 in Hop62 by Colony-formation and CCK8 (Fig. 6A, B). shRaf1 has the ability to suppress the phosphorylation of proteins such as Raf1, MEK1/2, and ERK1/2. In addition, cell cycle-related proteins caused by TCF19 were also inhibited by shRaf1 in Hop62 cells (Fig. 6C).

Regulating the expression of cell cycle-related proteins is a crucial function of ERK activation during the cell cycle process [31]. To validate the role of activated ERK1/2 in promoting cell cycle advancement in lung cancer cells exhibiting elevated TCF19 levels, we employed an ERK inhibitor (ERKi, GDC-0994) to hinder the phosphorylation of ERK1/2 induced by TCF19 in lung cancer cells (Supplementary Fig. 3B). When Hop62-TCF19 cells were treated with ERKi, cell proliferation was significantly inhibited (Fig. 6D and E), and the protein and mRNA level of cell cycle-related proteins was also significantly down-regulated (Fig. 6F). The findings from these experiments indicate that TCF19 enhanced the growth of lung cancer cells and the development of tumors in both laboratory conditions and living organisms by increasing the levels of cyclin D1, cyclin E1, cyclin A1, and CDK2 through the Raf/-MEK/ERK signaling pathway. Notably, we analyzed the TCGA database and found that TCF19 was positively correlated with CCNA1, CCNE1, CDK2, RAF1, MAPK3, and MAPK1 in lung cancer tissue samples (supplementary Fig. 4A–F).

In summary, these results further confirmed that Raf/MEK/ERK cascade is required for TCF19 to function in lung cancer cells.

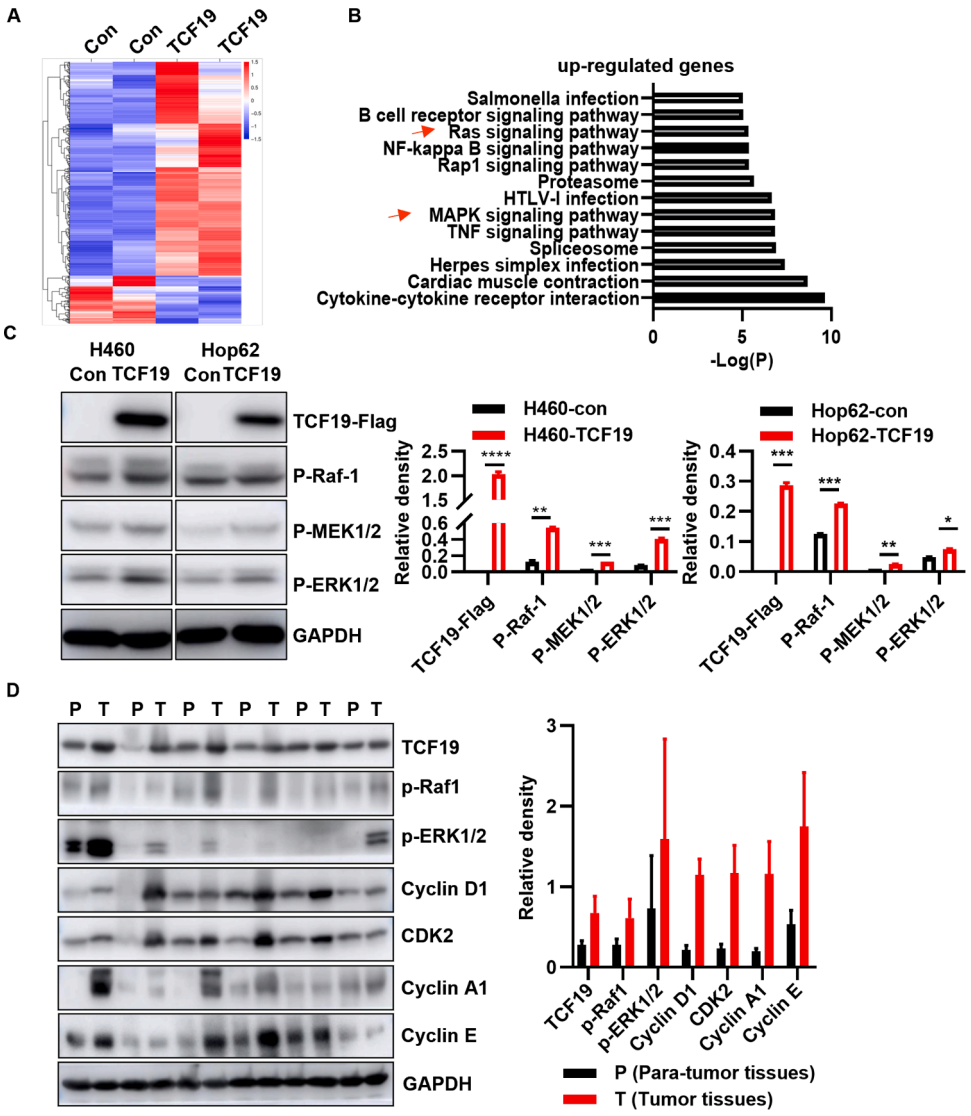


**Fig. 5.** TCF19 activated Raf/MEK/ERK pathway. A. Heatmap of mRNA expression of Hop62-TCF19 cells. Cells were extracted for RNA-sequencing analysis. B. KEGG enrichment analysis of signal pathways affected by TCF19. C. The effect of TCF19 on the key proteins of Raf/MEK/ERK signaling pathway in H460 and Hop62 cells by western blotting. D. The relationship between TCF19 and proteins related to Raf/MEK/ERK signaling pathway or cell cycle in clinical lung cancer tissue samples. Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

## Discussions

From the start of this century, the progress in the clinical management of lung cancer has transitioned from 'identical medication for various patients' to 'a single medication for each patient', progressing towards a precise and personalized approach of 'a unique prescription for every patient'. Therefore, precisely targeted therapy strategies for oncogenes are urgently needed to continuously update and improve the prognosis of patients. Within this investigation, TCF19 exhibited significant upregulation in certain individuals diagnosed with lung cancer, and this elevated expression of TCF19 demonstrated a direct association with unfavorable outcomes in lung cancer patients. In lung adenocarcinoma cells H460 and Hop62, TCF19 exhibited significant expression. The proliferation and malignancy of lung adenocarcinoma cells were enhanced by the overexpression of TCF19, as demonstrated by Cck8, plate clone formation, and Soft Agar experiments. Indeed, we showcased the growth-enhancing impact of TCF19 not just in laboratory settings but also in live organisms by utilizing a xenograft tumor model and a transgenic mouse model for lung cancer that occurs naturally.

Malfunctioning of the Ras-ERK pathway serves as a primary catalyst for the formation of the majority of cancer types. The ERK cascade is activated in nearly all cancer types, making mutations that activate this pathway the most prevalent oncogenic elements across all cancer types [32–34]. The MAPK signaling pathway is crucial in regulating a range of physiological processes including cellular growth, development, proliferation, and apoptosis within the network of signaling pathways. ERK belongs to the MAPK group, and the ERK/MAPK signaling pathway plays a central role in controlling cell growth, development, and division [32]. This statement also confirmed our findings. In terms of mechanism, we discovered that TCF19 stimulates the rise in cyclins by amplifying the phosphorylation of crucial proteins in the Raf/MEK/ERK signaling pathway, resulting in the proliferation of cancer cells. Notably, TCF19 was found to be positively correlated with CCNA1, CCNE1, CDK2, RAF1, MAPK3, and MAPK1 in lung cancer tissue samples in the TCGA database. These findings suggest TCF19 upregulation affects the prognosis of lung cancer patients by promoting RAF/MEK/ERK signaling pathway.

Collectively, these findings suggest that shRaf1/ERKi synergistically blocks TCF19-induced lung cancer cell proliferation and promotes downregulation of cell cycle checkpoints. Therefore, our results indicate

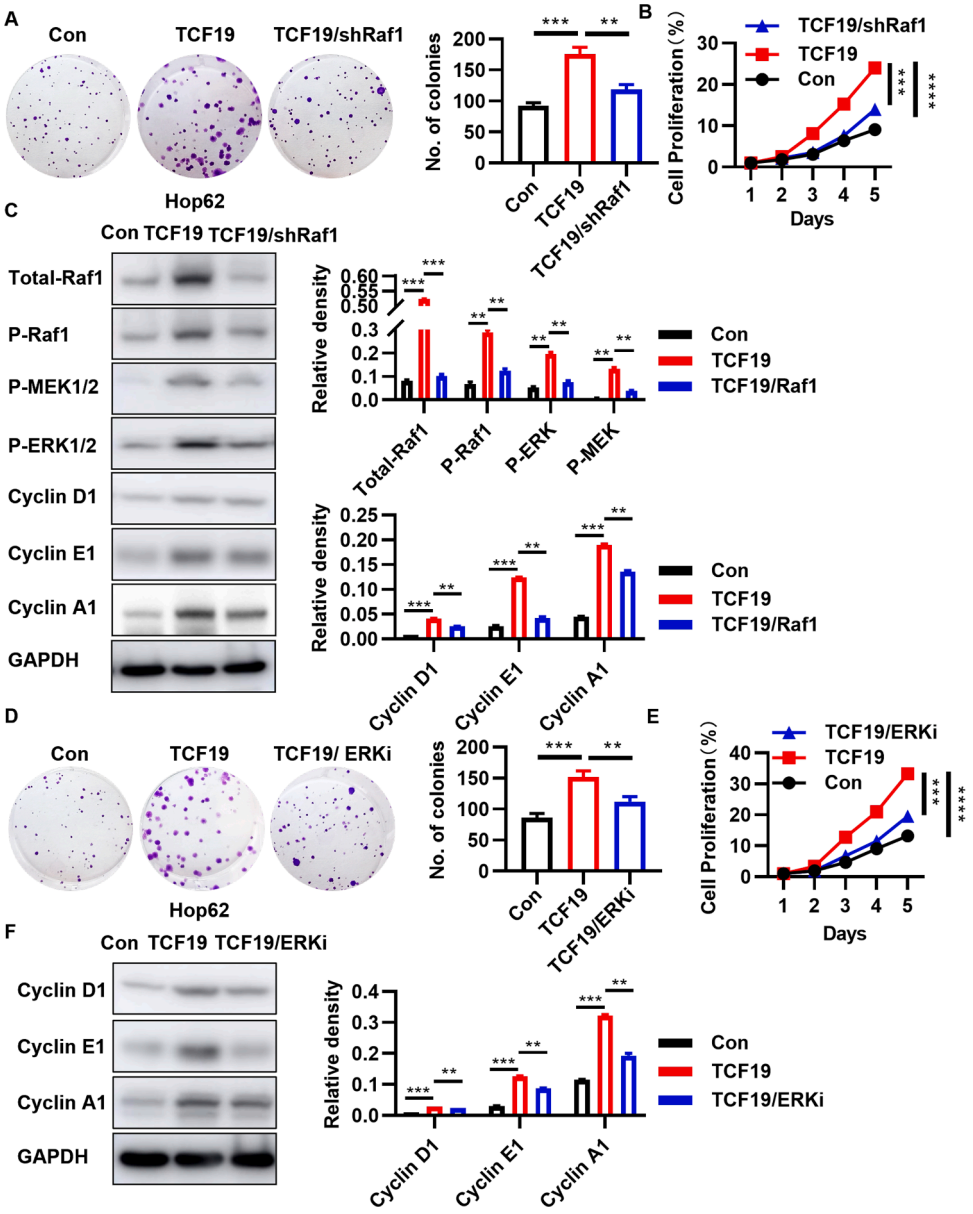


**Fig. 6.** TCF19 promoted cell proliferation through the Raf/MEK/ERK signaling pathway. A, B. TCF19 promoted the growth of lung cancer cells through Raf1 by colony-forming and CCK8 experiments. C, D. TCF19 promoted the growth of lung cancer cells by activating ERK1/2. shRaf1 inhibited the promoting effect of TCF19 on the lung cancer cell Hop62 by western blotting. E. ERKi inhibited the promoting effect of TCF19 on lung cancer cells Hop62 by western blotting. F. The schematic diagram of TCF19 promoting lung cancer cell proliferation and tumor growth. Data are means ± SEM of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test).

that targeting Raf1 or ERK may help suppress the malignant progression of TCF19-overexpressing lung cancer.

## The accessibility of information and resources

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

## Additional information

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## CRediT authorship contribution statement

**Yahui Tian:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shaowei Xin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation. **Zitong Wan:** Software, Resources, Project administration, Formal analysis, Data curation, Conceptualization. **Honghong Dong:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Lu Liu:** Software, Formal analysis, Data curation. **Zhenzhen Fan:**

Investigation, Funding acquisition. **Tian Li:** Software, Investigation. **Fujun Peng:** Resources, Methodology, Investigation, Funding acquisition. **Yanlu Xiong:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision. **Yong Han:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors did not report any conflicts of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2024.101978.

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