

Original Article

The small Rho GTPase Rif promotes cancer progression through ERK2-mediated epithelial-mesenchymal transition

Haile Zhao¹, Man Wu¹, Xiaozhou Bai¹, Jingna Wang¹, Morigen Morigen^{1,*}, and Lifei Fan^{1,2,*}

¹Inner Mongolia Key Laboratory for Molecular Regulation of the Cell, State Key Laboratory of Reproductive Regulation & Breeding of Grassland livestock, School of Life Sciences, Inner Mongolia University, Hohhot 010020, China, and ²Southern University of Science and Technology, Shenzhen 518055, China

*Correspondence address. E-mail: morigenm@hotmail.com (M.M.); lifei.fan@imu.edu.cn (L.F.)

Received 9 September 2025 Accepted 5 January 2026 Published 15 May 2026

Abstract

Cancer remains one of the most formidable global health challenges, demanding a comprehensive exploration of its molecular underpinnings to advance therapeutic innovation. Tumorigenesis is driven by interrelated pathological processes such as metastatic dissemination, therapy resistance, and immune evasion, which are intricately linked to epithelial-mesenchymal transition (EMT). Rif, a member of the Rho GTPase family, has been implicated as an important modulator of oncogenic transformation. Through systematic investigation across various solid tumors, we identify Rif overexpression as a biomarker of histopathological aggressiveness and poor survival outcomes in breast, ovarian, and stomach tumors. Rif facilitates oncogenic progression by suppressing apoptosis and promoting cell proliferation, metastasis, and EMT induction. Integrated multiomics and functional profiling reveal ERK2 as a key downstream effector of Rif's protumorigenic effects. While engaging with tumor-specific signaling contexts (e.g., AHR, TGF- β /SMAD3), the Rif-ERK2 axis may represent a conserved and actionable target across various solid tumors. Targeted disruption of pathological signaling pathways may provide a novel approach to impede cancer aggressiveness and improve clinical outcomes in oncology.

Key words Rif, ERK2, EMT, cancer metastasis, MAGI2

Introduction

Phenotypic plasticity, a defining feature of neoplastic evolution, enables cancer cells to acquire different malignant phenotypes that enhance adaptability under dynamic environmental pressures [1]. Central to this adaptive capability is epithelial-mesenchymal plasticity (EMP), a paradigmatic manifestation wherein cancer cells undergo reversible phenotypic shifts across a spectrum spanning hybrid epithelial-mesenchymal intermediates to fully mesenchymal phenotypes [2]. This process includes phenomena such as epithelial-mesenchymal transition (EMT), partial EMT (pEMT), and mesenchymal-epithelial transition (MET), all of which are regulated by microenvironmental factors, including biochemical gradients and mechanical stimuli, which drive dynamic cellular reprogramming [3,4]. EMT is an intricate and multifaceted process that is activated by various intrinsic and extrinsic factors and is finely mediated by a series of signaling pathways [5]. Clinically, elevated EMT activity is strongly associated with adverse outcomes, reflecting its role in promoting metastatic dissemination and

conferring resistance to chemotherapeutic and targeted therapies [6,7]. Despite advances in characterizing EMT-related pathways, delineating their spatiotemporal regulation and functional interplay with cancer evolution remains a critical frontier in oncology research.

Extracellular signal-regulated kinase 1 and 2 (ERK1/2), key members of the mitogen-activated protein kinase (MAPK) family, regulate critical physiological and pathological processes [8–10]. Notably, ERK2 exhibits superior tissue abundance and stability compared to ERK1 [11], ranking among the top 400 proteins in NIH3T3 fibroblasts with a 53-h half-life [12]. Its broad substrate diversity (hundreds of phosphorylation targets) highlights the devastating consequences of its dysregulation [13]. ERK2 silencing markedly reduces global ERK activity, disrupting senescence [14], fibrosis diseases [15], embryogenesis [16], cardiac remodeling [17,18], and cardiac contractility [19]. In oncological contexts, ERK2 silencing suppresses EMT across breast [20], pancreatic [21], and lung cancer [22], affects colitis-associated colorectal tumor-

igenesis [23], mediates hepatocyte growth factor (HGF)-induced motility in lung carcinoma [24], and blocks metabolic adaptation in hepatocellular carcinoma [25]. ERK2 overexpression also potentiates the cancer stem cell (CSC) phenotype and metastasis in triple-negative breast cancer (TNBC) [26], while its inhibition triggers DNA damage response cascades and apoptotic signaling in melanoma and ovarian cancer [27–29]. More importantly, the discovery of ERK small-molecule inhibitors has gradually become a research hotspot in cancer pharmacology [30]. Amplification of the *ERK2* gene has been observed in tumors from patients treated with anti-EGF receptor kinase inhibitors, which has been proposed to contribute to treatment resistance [31]. A deeper mechanistic exploration of ERK2 may facilitate the development of antitumor drugs aimed at overcoming drug resistance.

Rho guanosine triphosphatase (GTPase) serves as a molecular switch for cytoskeletal organization, migratory behavior, and adhesive dynamics through its spatiotemporal regulation of actin networks [32–34]. Ras homolog family member F (Rif), a member of the Rho GTPase family, plays an important role in multiple cell functions, such as vesicular trafficking, membrane protrusion formation, and cell adhesion [33,35]. Rif mainly promotes filopodium formation on the dorsal surface of cells [36,37]. In hepatocellular carcinoma, Rif has been shown to trigger the Warburg effect to withstand metabolic stress via RAB3D [38]. In pancreatic cancer, Rif promotes Snail1 lactylation by promoting PKM2-induced glycolysis [39,40]. Another study revealed that Rif mediates the Wnt5a-Ror1-Dvl2 axis to form polarized filopodia in lung adenocarcinoma cells cultured on 3D matrices [34]. These findings suggest that Rif regulation may be an early event in the carcinogenesis process. However, further studies and preclinical trials are essential to understand Rif's oncogenic effects.

In this study, we found a significant association between Rif overexpression and adverse clinical outcomes across multiple malignancies. Rif facilitates oncogenic progression in breast, ovarian, and stomach tumors through the activation of the ERK2 signaling pathway. Our data highlight a functional role for the Rif-ERK2 axis in cancer pathogenesis and propose its therapeutic targeting for multimodal anticancer interventions.

Materials and Methods

Two-dimensional (2D) cell culture

Human normal breast epithelial cell line MCF-10A was maintained in DMEM F12 (1:1) medium (11320033; Gibco, Carlsbad, USA) supplemented with 5% horse serum (26050088; Gibco), 1% L-glutamine (25030081; Gibco), 1% penicillin-streptomycin (PS; 15140122; Gibco), 10 µg/mL insulin (CI6561; Coolaber, Beijing, China), 0.5 µg/mL hydrocortisone (SL4150; Coolaber), 20 ng/mL human EGF (PHG0313; Gibco), and 100 ng/mL cholera toxin (227036; Sigma, St Louis, USA). Human breast cancer cell line MCF-7 was maintained in DMEM medium (12430054; Gibco) supplemented with 5% fetal bovine serum (FBS; DCF-101-0500; DCELL, Shanghai, China), 1% L-glutamine, and 1% PS. Human breast cancer cell line SUM159 was maintained in DMEM F12 (1:1) medium supplemented with 5% FBS, 1% L-glutamine, 1% PS, 10 µg/mL dexamethasone (SL4070; Sigma), and 5 µg/mL insulin. Human ovarian cancer cell line OVCAR5 was maintained in RPMI 1640 medium (C11875500BT; Gibco) supplemented with 10% FBS, 1% L-glutamine, 1% PS, and 5 µg/mL insulin. Human stomach adenocarcinoma cell lines (MKN28, MKN45, SGC-7901, and BGC-

803), and ovarian cancer cell line HO-8910 were maintained in RPMI 1640 medium supplemented with 10% FBS, 1% PS, and 1% L-glutamine. Human gastric mucosal epithelial cell line GES-1, normal ovarian epithelial cell line IOSE-80, ovarian cancer cell lines (A2780 and SKOV3 cells) were maintained in DMEM medium supplemented with 10% FBS, 1% PS, and 1% L-glutamine. All cells were obtained from the National Collection of Authenticated Cell Culture (Shanghai, China) and incubated at 37°C in a humidified 5% CO₂ atmosphere.

Three-dimensional (3D) cell culture

Cells were cultured using the CERO cell culture simplified (OMNI Life Science, Bremen, Germany) as previously described [41,42]. For each experiment, 1×10^7 cells were seeded into each Falcon tube. The cultivation process involved two distinct phases: an initial inoculation period and a subsequent culture period. During the inoculation phase, all cells were cultured in suspension under the following conditions: rotation pause 0 s, rotation period 1 s, agitation pause 40 min, agitation period 2 min, and rotation speed 50 rpm. This phase lasted for 4 h. For the culture phase, the parameters were adjusted as follows: rotation pause 0 s, rotation period 1 s, agitation pause 0 s, agitation period 2 min, and rotation speed 80 rpm. This phase was maintained indefinitely (∞). Each Falcon tube was filled with 40 mL of complete growth medium. After 72 h, 30 mL of medium was carefully removed and replaced with an equivalent volume of fresh complete growth medium to ensure optimal nutrient availability and waste removal.

Reagents and antibodies

Reagents used in this study included: RIPA (SL1020; Coolaber), 4',6-diamidino-2-phenylindole (DAPI; D3571; Invitrogen, Carlsbad, USA), BSA (A6003; Sigma), DMSO (D2650; Sigma), propidium iodide (PI; P4170; Sigma), paraformaldehyde (PFA; P6148; Sigma), and FR180204 (S7524; Selleck, Shanghai, China).

Antibodies used in this study included: anti-Rif antibody (ab81024; abcam, Cambridge, UK); Alexa Fluor® 594 Phalloidin (A12381; Invitrogen); anti-Cdk2 (M2) (sc-163; Santa Cruz Biotechnology, Dallas, USA); anti-NF-κB p65 (D14E12) XP® Rabbit mAb (8242; CST, Danvers, USA); anti-PTEN Monoclonal antibody (60300-1-Ig), anti-AHR Monoclonal antibody (67785-1-Ig), anti-CYP1A1 Polyclonal antibody (13241-1-AP), anti-E2F7 Polyclonal antibody (24489-1-AP), anti-GSTP1 Polyclonal antibody (15902-1-AP), anti-P27/KIP1 Polyclonal antibody (25614-1-AP), and anti-MYC Tag Monoclonal antibody (60003-2-Ig) (Proteintech, Wuhan, China); anti-p-AKT1 (abs140184) and anti-Phospho-ERK1/2 (Thr202/Tyr204) Polyclonal antibody (abs130614) (absin, Shanghai, China); anti-Erk1/2 (AF0155), anti-E-Cadherin (AF0131), anti-Vimentin (AF7013), anti-MAGI2 (AF0492), and anti-AKT1 (AF0836) (Affinity Biosciences, Liyang, China); anti-CyclinE (BA0774; BOSTER; Wuhan, China); ProteinFind® Anti-β-Tubulin Mouse Monoclonal antibody (HC101), ProteinFind® Anti-GAPDH Mouse Monoclonal antibody (HC301-02), ProteinFind® Goat Anti-Mouse IgG(H+L) HRP Conjugate (HS201), and ProteinFind® Goat Anti-Rabbit IgG(H+L) HRP Conjugate (HS101) (TransGen Biotech, Beijing, China).

Cell transfection

The transfections were performed using Lipofectamine 2000 Reagent (11668019; Invitrogen) following the guidelines provided

by the manufacturer. Cell transfection was performed on 1×10^6 cells using one of the following: 50 nM negative control siRNA (siNC), 50 nM small interfering RNA (siRNA), 2.5 µg control plasmid pcDNA3.0, 2.5 µg overexpression plasmid pcDNA3.0-Rif (Rif), 2.5 µg control plasmid pcDNA3.1, or 2.5 µg overexpression plasmid pcDNA3.1-ERK2 (ERK2). Subsequently, the cells were cultured for another 24 h or 48 h to allow gene expression modulation before proceeding with subsequent analyses. In multiple transfection experiments, the second transfection was performed 24 h after the first transfection. After another 24 h of culture, samples were collected for analysis. The transfection efficiency was measured by real-time quantitative-PCR (RT-qPCR) and western blot analysis. The siNC and siRNA sequences, designed and synthesized by Sangon Biotech (Shanghai, China), are detailed in [Supplementary Table S1](#). Both the control plasmid (pcDNA3.0 and pcDNA3.1) and the overexpression plasmid (pcDNA3.0-Rif and pcDNA3.1-ERK2) were maintained in the laboratory under standard storage conditions. This approach ensured consistent and reliable transfection efficiency for downstream experimental applications.

RT-qPCR

RNA extraction was conducted using TRIzol reagent (ET111; TransGen Biotech), adhering to guidelines provided by the manufacturer. Complementary DNA (cDNA) synthesis was carried out with the HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper) (R212; Vazyme Biotech, Nanjing, China) on a Light Cycler 96 thermal cycler (SENSOQUEST, Goettingen, Germany), following an established method [42]. Target genes were amplified via PCR using cDNA templates and gene-specific primers. qPCR was performed using PerfectStart® Green qPCR SuperMix (AQ601; TransGen Biotech) on a LightCycler 480 II instrument (Roche, Basel, Switzerland). Relative gene expression was quantified using the comparative $\Delta\Delta C_q$ method [43]. The information of primer sequences, designed and supplied by Sangon Biotech, is provided in [Supplementary Table S2](#).

Immunofluorescence assay

The immunofluorescence assay was processed following established protocols [41]. Briefly, cells plated onto glass coverslips were allowed to adhere for 24 h prior to experimental treatments. Following 48 h of Rif expression modulation, the cells were fixed using 4% PFA for 15 min, permeabilized with 0.1% Triton X-100 for 5 min, and subsequently treated with sodium borohydride for 10 min. To label F-actin, adherent cells were incubated with Alexa Fluor® 594 Phalloidin (diluted 1:200 in 1% BSA) for 1 h. The nuclei were counterstained with DAPI (1:5000 dilution in H₂O). Fluorescence imaging was performed using a laser scanning confocal microscope 710 (Carl Zeiss, Wetzlar, Germany).

Western blot analysis

The assay was performed following established protocols [44], and appropriate modifications were made. The treated cells were extracted on ice with RIPA buffer. The total protein concentration was quantified via a Pierce™ BCA Protein Assay Kit (23227; Thermo Fisher Scientific, Waltham, USA). Proteins were then separated by 12% SDS-PAGE and electrotransferred onto PVDF membranes (Millipore, Billerica, USA). Following transfer, the membranes were blocked with 5% Difco™ Skim Milk prepared in 1% Tween 20 in PBS (PBST) for 1 h at room temperature, followed by overnight

incubation at 4°C with primary antibodies (1:1000 diluted in PBST) under gentle rotation. After three washes with PBST, membranes were probed with a species-specific secondary antibody (1:20,000 diluted in PBST) at room temperature for 1 h with mild shaking. Protein bands were detected using Pierce ECL Western Blotting Substrate (32106; Thermo Fisher Scientific). Relative protein levels were quantified using the ChemiDoc XRS system (Bio-Rad, Hercules, USA) and analyzed with Image Lab (Version 5.2.1).

DNA ploidy assay

DNA ploidy was determined as previously described [41]. Following treatment, cells were harvested using 0.05% or 0.25% trypsin, rinsed twice with ice-cold PBS, and fixed in 70% ethanol overnight at -20°C. The following day, the cells were washed with PBS and stained with a solution containing 50 µg/mL PI and 50 µg/mL RNase A in PBS. The staining process was conducted on ice for 30 min in the absence of light. Cell cycle profiling was performed using a FACSCalibur flow cytometry system with CELLQuest v3.3 software (BD Biosciences, San Jose, USA). Data analysis and quantification of cell cycle phases were quantified using ModFit LT v3.0 software (Verity Software House, Topsham, USA).

Apoptosis assay

After 48 h post transfection, cells were harvested and subjected to apoptosis detection using the FITC Annexin V Apoptosis Detection Kit I (556547; BD Biosciences) as per the provided guidelines [45]. Apoptosis analysis was performed using a FACSCalibur flow cytometry system, and data quantification was performed with CELLQuest v3.3 software.

Cell proliferation assay

Twenty-four hours post-transfection, 2×10^3 cells were seeded onto a 96-well plate and allowed to adhere overnight. Cell viability was determined using a 10% Cell Counting Kit-8 (BS360A; Biosharp, Beijing, China) for 2 h in the dark within a cell culture incubator (Thermo Fisher Scientific). Absorbance was measured at 450 nm using a spectrophotometric microtiter plate reader (BioTek Epoch; Agilent Technologies, Santa Clara, USA).

Cell migration assay

At 24 h post-transfection, 4×10^5 cells were seeded onto a 6-well plate and allowed to adhere overnight. Uniform wounds were created in the confluent cell monolayer using a sterile 200-µL pipette tip. Cells were then rinsed 3 times with PBS and incubated in medium containing 1% horse serum or FBS, with or without antibiotics and lysozyme. Wound closure progression was monitored using an inverted fluorescence microscope (Nikon, Tokyo, Japan), and images were acquired and analyzed using Nikon NIS-Element imaging software.

Cell invasion assay

Twenty-four hours post-transfection, 1×10^5 cells were seeded into the upper compartment of a Transwell chamber (3422; Corning, Corning, USA) containing serum-free medium. The lower chamber was filled with 600 µL of medium supplemented with 5% horse serum or FBS to serve as a chemoattractant. Following a 24-h incubation period, the cells were fixed with 4% PFA for 20 min and subsequently stained with 3% crystal violet (prepared in methanol absolute) for 60 min at room temperature in the dark. Images were

acquired and quantitatively analyzed using ZEN 2012 software (Carl Zeiss).

Cell clonogenicity assay

Twenty-four hours post-transfection, 2×10^3 cells were plated on a 6-well culture dish and incubated until distinct colonies were visible. Upon colony formation, cells were fixed with 4% PFA for 20 min, stained with 1.5% crystal violet (prepared in methanol absolute) for 20 min at room temperature, and shielded from light. The colonies were imaged, and the average colony counts were determined for quantitative analysis.

RNA-sequencing

MCF-7, A2780, and SGC-7901 cells were transfected with either 50 nM siNC or 50 nM siRif according to the manufacturer's guidelines. After transfection, RNA sequencing analysis was performed on these cells. The RNA-sequencing library was prepared by the BioMarker Technologies Company (Beijing, China). Raw sequencing reads were processed using the BMKCloud bioinformatics platform (www.biocloud.net). Differential gene expression analysis was conducted using DESeq2, with thresholds set at a false discovery rate (FDR) of < 0.05 and a fold change of ≥ 1.8 . To identify statistically significant biological pathways, KOBAS software [46] was employed to analyze the enrichment of differentially expressed genes (DEGs) in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Set Enrichment Analysis (GSEA).

Bioinformatics methods and data source

The gene expression profiles for patients with breast cancer, ovarian cancer, and stomach cancer were obtained from the TCGA database (<https://portal.gdc.cancer.gov>) via the Xena platform [47]. The immunohistochemistry (IHC) data were obtained from The Human Protein Atlas (<https://www.proteinatlas.org>) [48]. RNA expression and the co-expression analyses were conducted using the GEPIA database [49], with Spearman's method applied to determine correlation coefficients. Additionally, Kaplan-Meier survival plots were generated using GEPIA. The protein-protein interaction (PPI) networks were constructed using the STRING database [50]. The RNA expression levels of *Rif* in lymph node metastasis were extracted from the UALCAN platform [51]. The Rap1 signaling pathway map (KEGG PATHWAY: map04015) was generated using the KEGG database [52].

Statistical analysis

In general, at least three independent biological replicates were included for each experiment, and the data are presented as the mean $\pm$ SEM. All statistical analyses were conducted using GraphPad Prism version 9.5. Significance was determined using either Student's unpaired *t* test or one-way or two-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison Test. Thresholds for statistical significance were set at $P < 0.05$.

Results

Rif is frequently elevated in human cancers and associated with poor prognosis

To comprehensively explore genetic alterations in the *Rif* gene across human cancers, we analyzed publicly available datasets from TCGA and HPA. *Rif* mRNA expression was significantly

upregulated in malignant compared to normal tissues across multiple cancer types in the TCGA dataset (Figure 1A). Whole-genome sequencing data from TCGA identified 30 somatic mutations in the *Rif* gene among 3233 cases, including frameshift, missense, splice acceptor, and synonymous mutations (Figure 1B and Supplementary Table S3). These mutations were distributed across 91 primary sites, including the brain, colon, breast, ovary, and stomach (Figure 1C), highlighting the conserved and functional importance of the *Rif* protein.

Breast cancer (BRCA) and stomach adenocarcinoma (STAD) are among the leading causes of cancer-related death, while ovarian cancer (OVCA) exhibits one of the fastest-growing incidence rates [53,54]. To assess *Rif*'s clinical significance, we evaluated its expression patterns in a multicancer cohort with clinical annotation. Using an automated optimal cutoff point selection method [55], patients were stratified into *Rif*-high-expression and *Rif*-low-expression groups. Intriguingly, elevated *Rif* mRNA levels correlated with poor overall survival (OS) in the OVCA and STAD cohorts (Figure 1D), although no pathological stage-dependent variations in *Rif* transcript levels were detected (Supplementary Figure S1). IHC analysis revealed significantly higher *Rif* expression in cancer tissues than in normal tissues, where only weak signals were detected (Figure 1E). Additionally, *Rif* protein levels were significantly upregulated in 1 of 2 BRCA cell lines, 1 of 4 OVCA cell lines, and 3 of 4 STAD cell lines compared to immortalized normal cell lines (Figure 1F). TCGA data implied a significant correlation between *Rif* upregulation and lymph node metastasis in BRCA (Figure 1G). These findings implicate *Rif* overexpression as a prognostic biomarker associated with aggressive disease phenotypes in cancers.

Rif inhibits cancer apoptosis and promotes malignant progression

Cancer cells are characterized by their ability to sustain proliferative signaling, evade growth-inhibitory mechanisms, and resist apoptosis [56,57]. To explore the role of *Rif* in these processes, we produced cell lines with varying *Rif* expression levels. Cell lines exhibiting high *Rif* expression (MCF-7, A2780, SGC-7901, and MKN45) were subjected to siRNA-mediated knockdown, while those with low *Rif* expression (SUM159, HO-8910, SKOV3, and MKN28) were used for overexpression experiments. The transfection efficiency was detected by western blot analysis (Figure 2A,B and Supplementary Figure S2). The results showed that altering *Rif* expression level significantly changed the proportion of cells in the S or G2/M phase (Figure 2C and Supplementary Table S4), suggesting that *Rif* can regulate cancer cell cycle progression. Our data revealed that *Rif* knockdown resulted in a significant increase in apoptosis rates compared to controls, whereas *Rif* overexpression reduced apoptosis (Figure 2D and Supplementary Table S5). Additionally, *Rif* depletion notably reduced cancer cell proliferation, while *Rif* overexpression enhanced it (Figure 3A,B). Colony formation assays supported *Rif*'s role in promoting cancer cell survival (Figure 3C,D and Supplementary Figure S3A). In 3D culture models, *Rif* inhibition reduced tumor spheroid size, whereas *Rif* overexpression increased it (Figure 3E,F). Wound-healing and transwell migration assays demonstrated that *Rif* depletion significantly reduced cancer cell migration and invasion. Conversely, *Rif* overexpression markedly enhanced cell motility

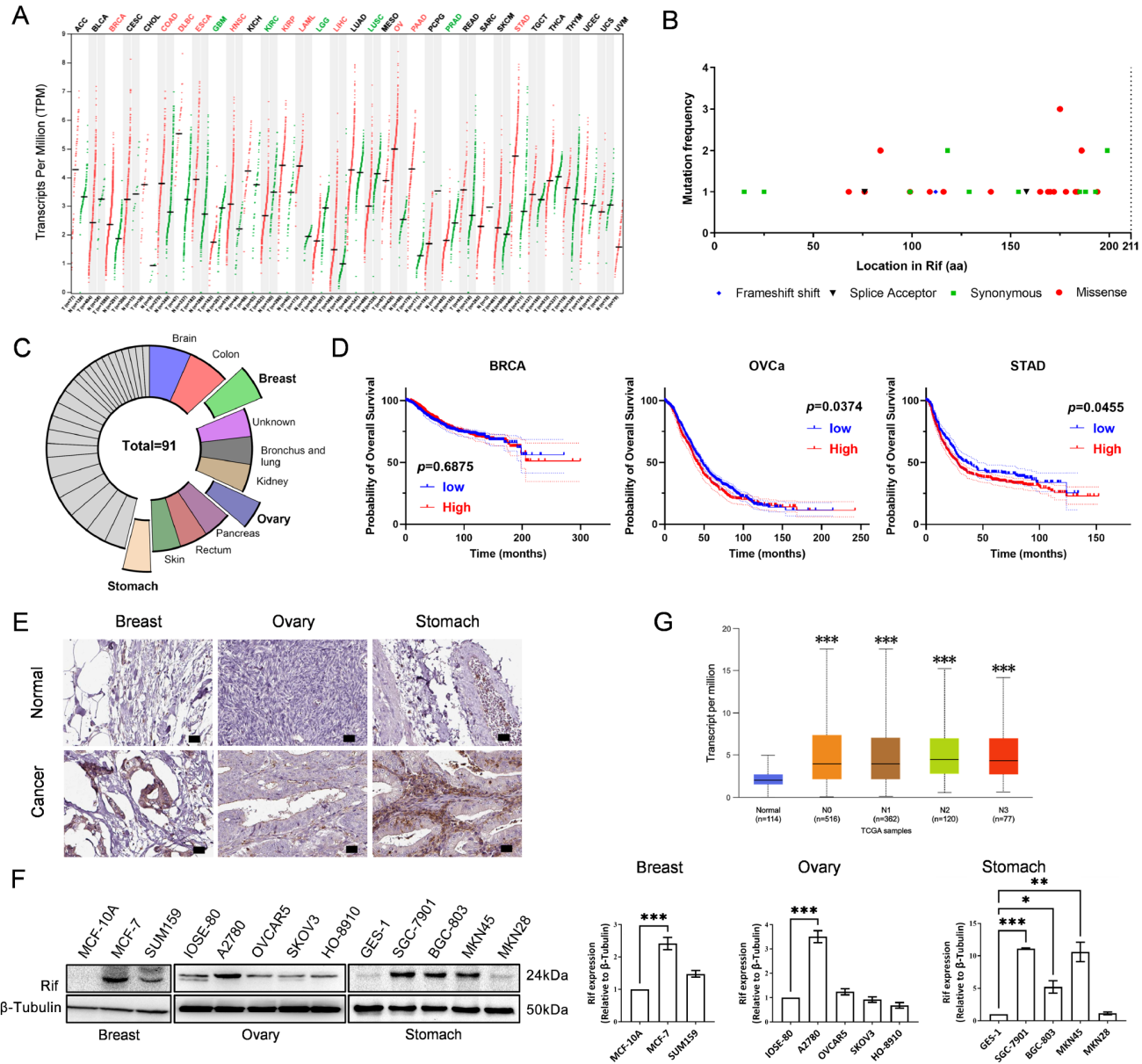


Figure 1. Rif is associated with poor prognosis (A) The *Rif* gene expression profile in all tumor samples and paired normal tissues. Data are analyzed with GEPIA website, matched TCGA normal and GTEx data, using $\log_2(\text{TPM}+1)$ for log-scale. ANOVA methods. (B) Location of mutation sites in *Rif* gene across different tumor types from TCGA. (C) Pie chart of the distribution of the primary sites where *Rif* gene undergoes mutation. Data were extracted from TCGA. (D) Kaplan-Meier curves are made to show the survival rate of the patients with high and low *Rif* mRNA expression levels. (E) IHC staining showing *Rif* protein expression. Data were extracted from HPA. Scale bar: 10 μ m. (F) The *Rif* protein expression in human breast, ovary, and stomach cells, normalized to β -Tubulin, and expressed as a ratio to the normal cell line. Data are presented as the mean $\pm$ SEM, $n = 3$, one way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (G) The RNA expression of *Rif* in lymph node metastasis in BRCA. Data were extracted from UALCAN.

(Figure 3G–J) and Supplementary Figure S3B,C). These findings indicate that *Rif* suppresses apoptosis and promotes cell cycle progression, thereby contributing to cancer cell survival, proliferation, and metastasis.

Rif activates the ERK2 signaling pathway to mediate EMT in cancer cells

To explore the molecular mechanisms underlying the function of *Rif*, RNA-sequencing analysis of *Rif*-knockdown cells was used to predict its downstream pathway. GSEA showed that *Rif* expression

was associated with many pathways, such as the MAPK, TGF- β and PI3K/AKT signaling pathways (Figure 4A), which were invariably able to activate EMT [58]. A previous study showed that EMT programs across various biological contexts, whether in developmental stages or disease states, exhibit numerous common hallmarks. In cells undergoing EMT, the actin cytoskeleton is rearranged, resulting in a mesenchymal shape that resembles a spindle and increased motility through membrane protrusion formation [58]. To explore the effect of *Rif* on EMT, we assessed morphological changes in cancer cells. Immunofluorescence stain-

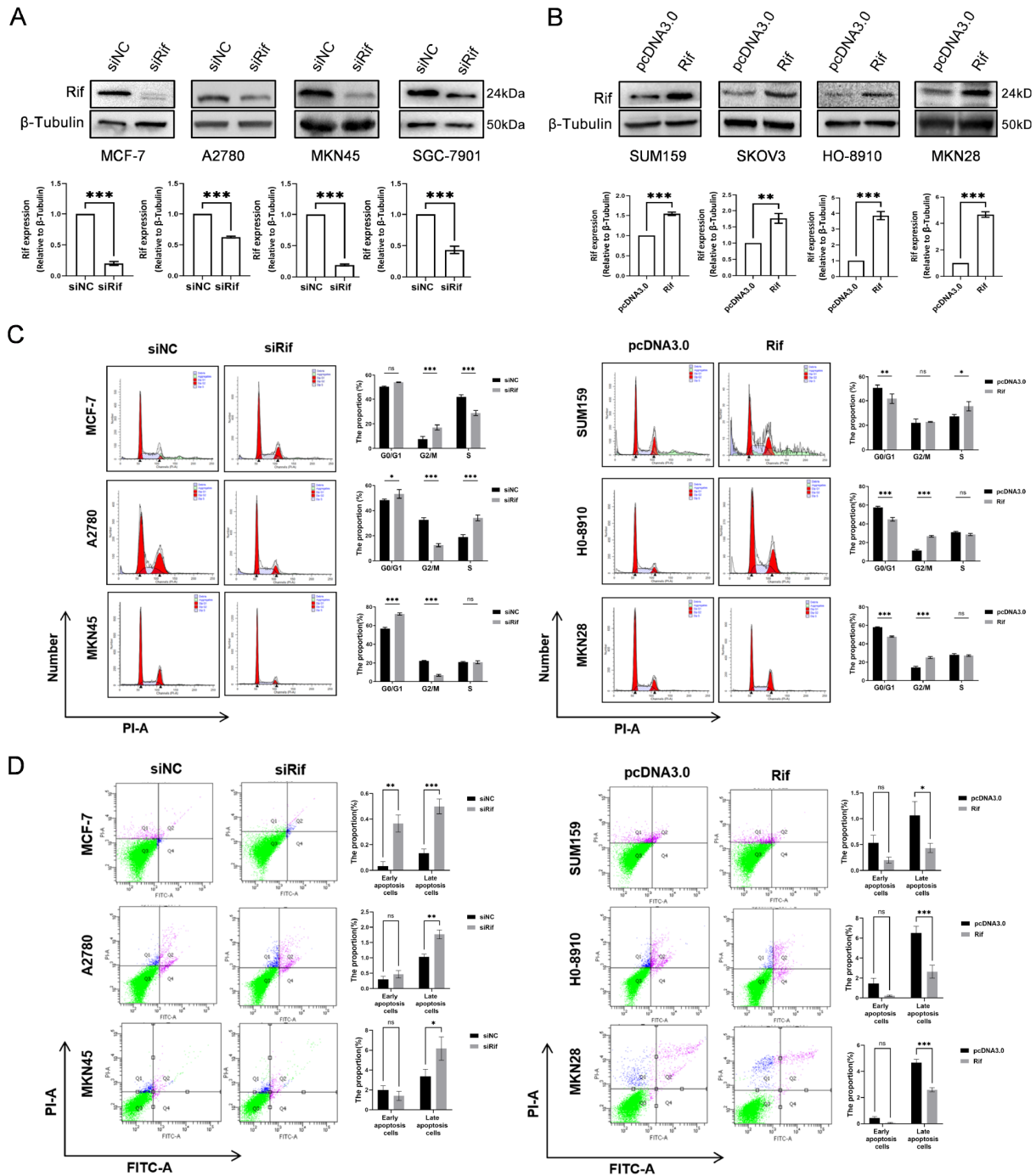


Figure 2. Rif inhibits apoptosis and promotes cell cycle progression in cancer cells (A,B) The protein levels of Rif in human breast, ovary, and stomach cells transfected with 50 nM siRif, 50 nM siNC, 2.5 μ g pcDNA3.0, or 2.5 μ g pcDNA3.0-Rif (Rif). (C) The FACS assay of cells stained with PI to analyze the quality of DNA content. (D) The apoptosis analysis of the indicated cells under the treatment. The percentage of apoptotic cells was analyzed by flow cytometry. Data are presented as the mean $\pm$ SEM, $n = 3$, one-way ANOVA in (A,B) and two-way ANOVA in (C,D). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant.

ing revealed that *Rif*-depleted cells displayed a cobblestone-like appearance with diminished F-actin fibers, whereas cells overexpressing *Rif* exhibited an elongated, spindle-like morphology with prominent F-actin fibers (Figure 4B,C). Further analyses demonstrated that *Rif* depletion suppressed EMT-like alterations, characterized by E-cadherin upregulation, Vimentin inhibition, and the suppression of a group of core EMT transcription factors (EMT-

TFs), including *SIP1*, *Slug*, *Snail*, *Twist*, and *Zeb1*. Conversely, *Rif* overexpression reversed these changes (Figure 4D,E and Supplementary Figure S4A). Consistent findings were obtained based on Immunofluorescence assay (Supplementary Figure S4B).

In addition, bioinformatics analysis of public data from the UCSC Xena platform was conducted to validate gene expression RNA-seq across BRCA, OVCA, and STAD tissues. A combination of the six-

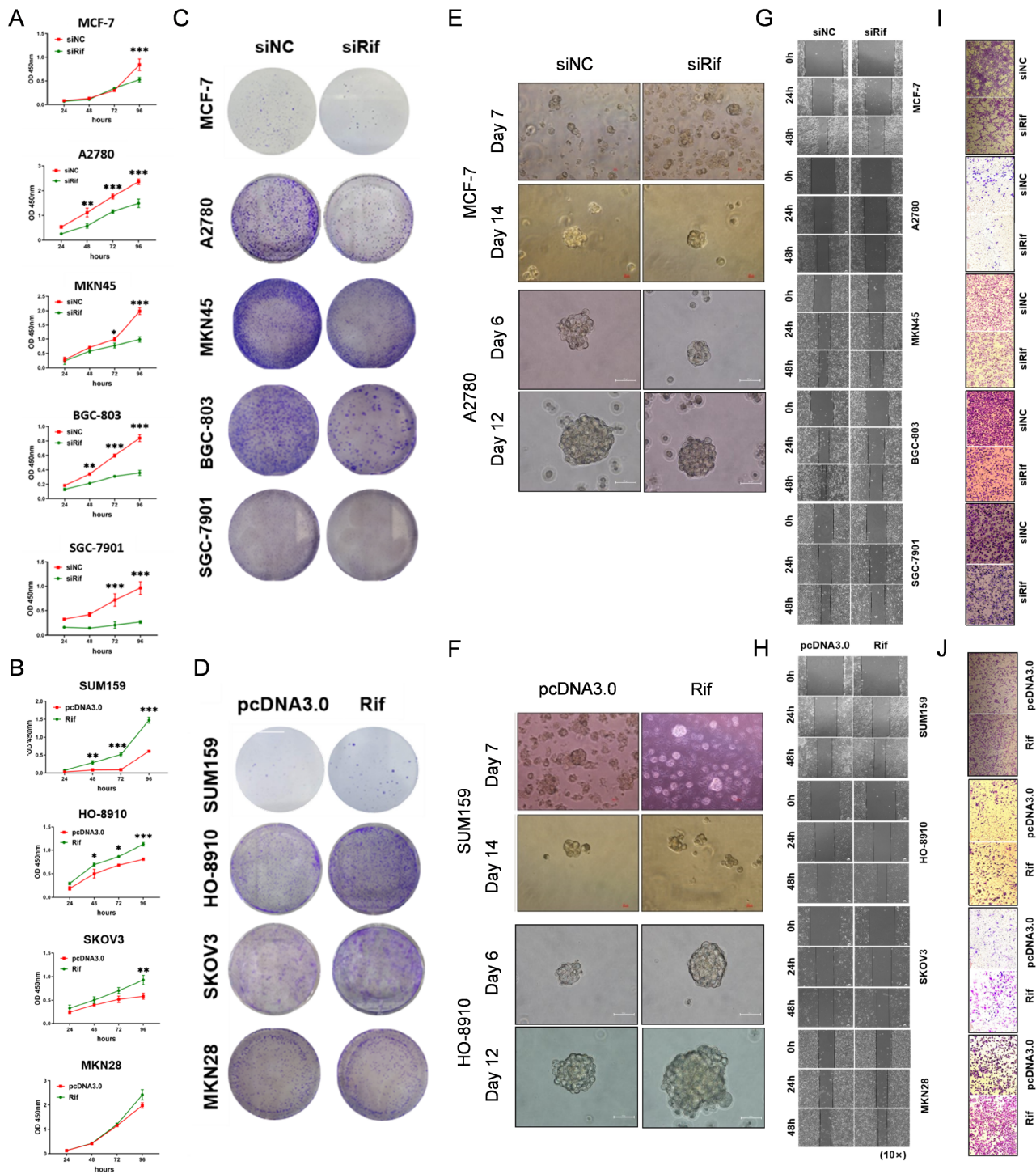


Figure 3. Rif promotes cancer cell survival, proliferation, migration and invasion (A–J) The cell proliferation assay (A,B), colony formation assays (C,D), 3D culture assay (E,F), wound healing assays (G,H), and transwell invasion assays (I,J) in human breast, ovary, and stomach cells transfected with 50 nM siRNA, 50 nM siNC, 2.5 μ g pcDNA3.0, or 2.5 μ g pcDNA3.0-Rif (Rif). All assays were performed in three independent replications. Data are presented as the mean $\pm$ SEM, (A) and (B) were analyzed using two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

screening dataset showed that ERK2 emerged as a potential downstream target of Rif (Figure 5A). Similar to previous reports [20,27], ERK2 overexpression is associated with cancer progression in BRCA, OVCA, and STAD (Supplementary Figure S5). Although

the relationship between ERK2 and Rif remains underexplored, PPI network analysis suggested a potential interaction between the two, supported by coexpression and interaction of their putative homologs in other organisms (Supplementary Figure S6 and

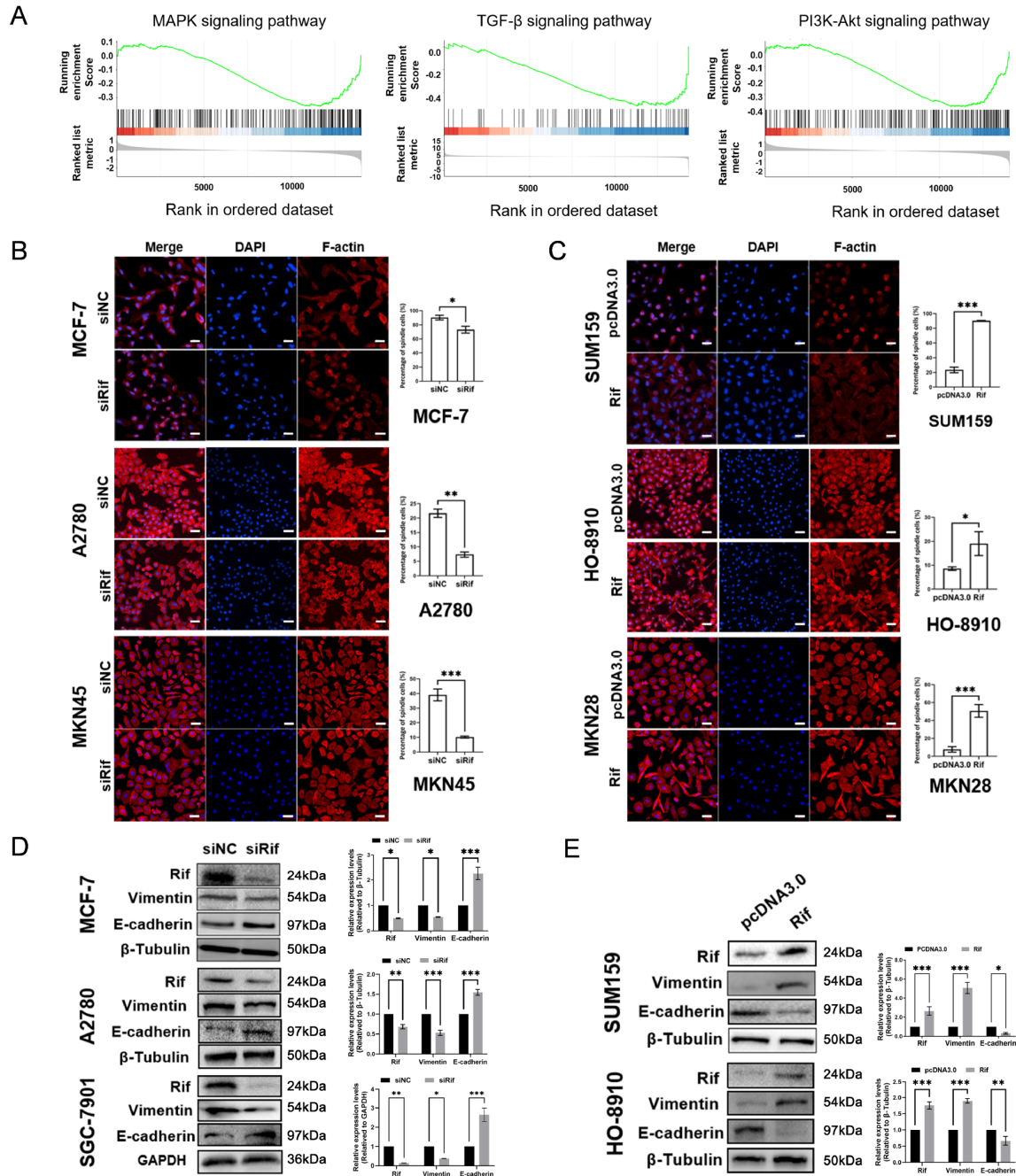


Figure 4. Rif promotes EMT phenotypes in cancer cells (A) The GESA analysis of the functional pathway in *Rif*-knockdown cells. (B,C) Representative images of immunofluorescence staining with anti-F-actin (red) and DAPI/nuclei (blue), visualized by LSCM 710. Scale bar: 50 μ m. Cells were transfected with 50 nM siRif, 50 nM siNC, 2.5 μ g pcDNA3.0, or 2.5 μ g pcDNA3.0-Rif (Rif). The proportion of spindle cells was quantified. Data are presented as the mean $\pm$ SEM, $n = 3$, Student's unpaired *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (D,E) The protein levels in the indicated cells. Data are presented as the mean $\pm$ SEM, $n = 3$, two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Table S6). The RT-qPCR assay demonstrated that silencing of *Rif* inhibited *ERK2* mRNA expression (Figure 5B). Further experimental validation revealed that both *ERK2* and p-*ERK2* protein levels were notably downregulated in *Rif*-silenced cells and upregulated in *Rif*-overexpressing cells (Figure 5C,D). We further examined whether *ERK2* is involved in *Rif*-induced EMT in the absence or presence of the *ERK2* inhibitor FR180204. Pharmacological inhibition of *ERK2* with FR180204 significantly

reduced p-*ERK2* level and inhibited EMT alteration, as evidenced by Vimentin inhibition and E-cadherin upregulation (Figure 5E). In addition, overexpression of *ERK2* completely abrogated the EMT-like changes induced by *Rif* depletion (Figure 5F,G). Taken together, these results indicate that *ERK2* is a key downstream regulator of *Rif*. Moreover, *Rif* is also able to activate other factors downstream of *ERK*, including *ATF2*, *c-Jun*, *c-fos*, *ELK1*, *NF- κ B*, *c-Myc*, and *MMPs* (Supplementary Figure S7). Our findings demon-

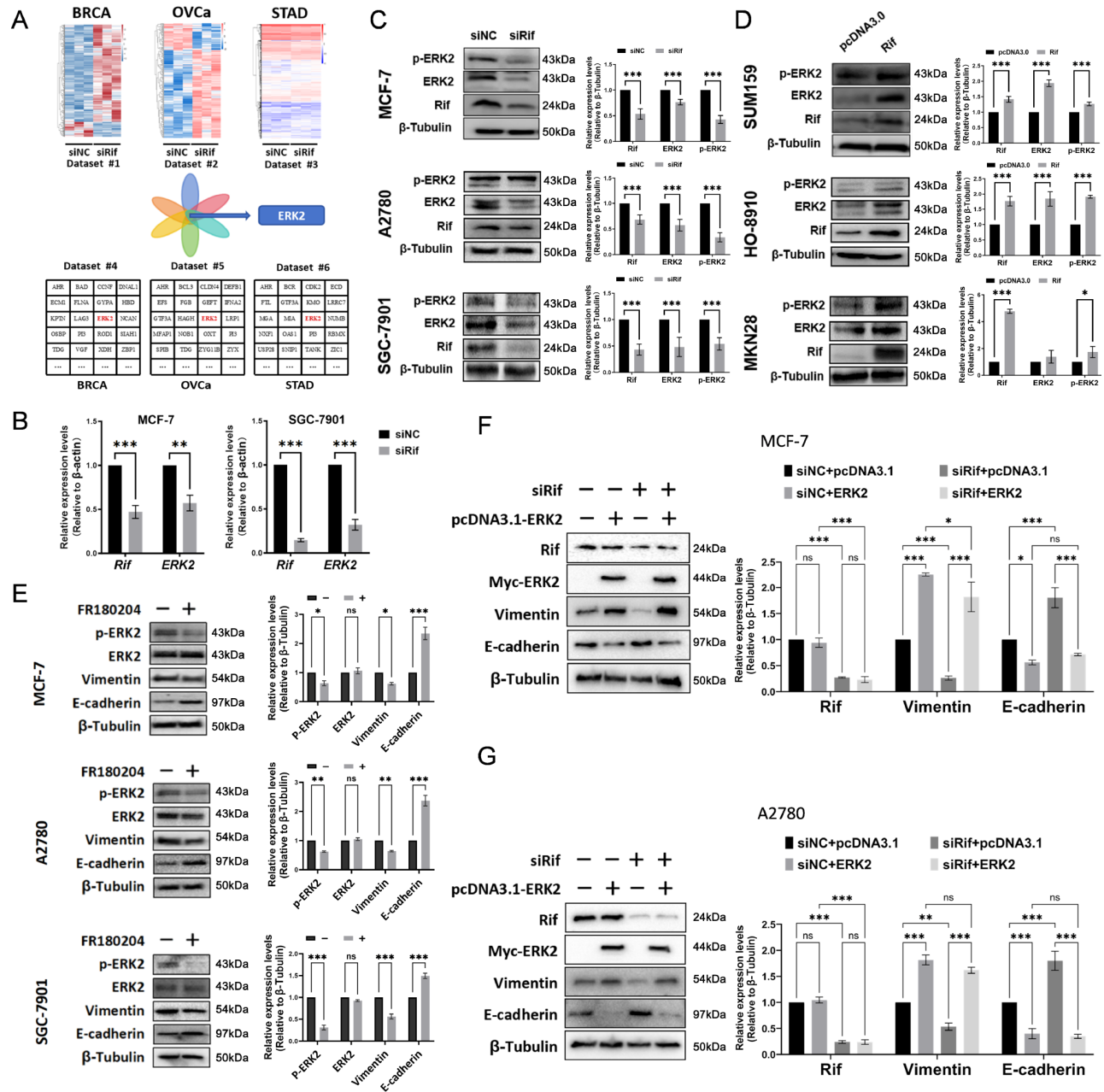


Figure 5. Rif activates ERK2 signaling to promote cancer cell progression (A) ERK2 was identified as a candidate target of Rif. The upper shows the heat maps of DEGs in *Rif*-depleted BRCA, OVCA, and STAD cells (datasets 1-3). The lower panel shows a partial list of DEGs in BRCA, OVCA, and STAD tissues (datasets 4-6). (B) The expression levels of *Rif* and *ERK2* mRNA. (C,D) The expression levels of ERK2, p-ERK2 in indicated cells. (E) Cells were treated with FR180204 (20 μ M). The relative protein expression was measured by western blot analysis. (F,G) Cells were transfected with 50 nM siRif or siNC for 24 h and then transfected 2.5 μ g pcDNA3.1, or 2.5 μ g pcDNA3.1-ERK2 (ERK2) for 24 h before harvesting. The relative protein expression was measured by western blot analysis. All assays were performed in three independent replications. Data are presented as the mean $\pm$ SEM, two-way ANOVA. * P < 0.05, ** P < 0.01, *** P < 0.001. ns, not significant.

strate that Rif induces EMT in multiple solid tumor types through activation of the ERK2 signaling pathway.

Rif induces EMT via the MAGI2 signaling pathway in cancer cells

Bioinformatics analyses revealed significant enrichment of small GTPase Rap1-associated signaling networks, including the MAPK, PI3K-Akt, cAMP, and Ras signaling pathways (Figures 4A and

Supplementary Table S7). Notably, MAGI2, a member of the MAGI family which serves as a classical upstream regulator of Rap1 [59–61], exhibited upregulated transcriptional upregulation among DEGs (Supplementary Figure S8A and Supplementary Table S7). Clinical profiling exhibited significantly reduced MAGI2 expression in OVCA patients compared to normal subjects (Figure 6A), corroborated by IHC showing reduced MAGI2 protein levels in cancer compared to normal tissues (Figure 6B). However, based on

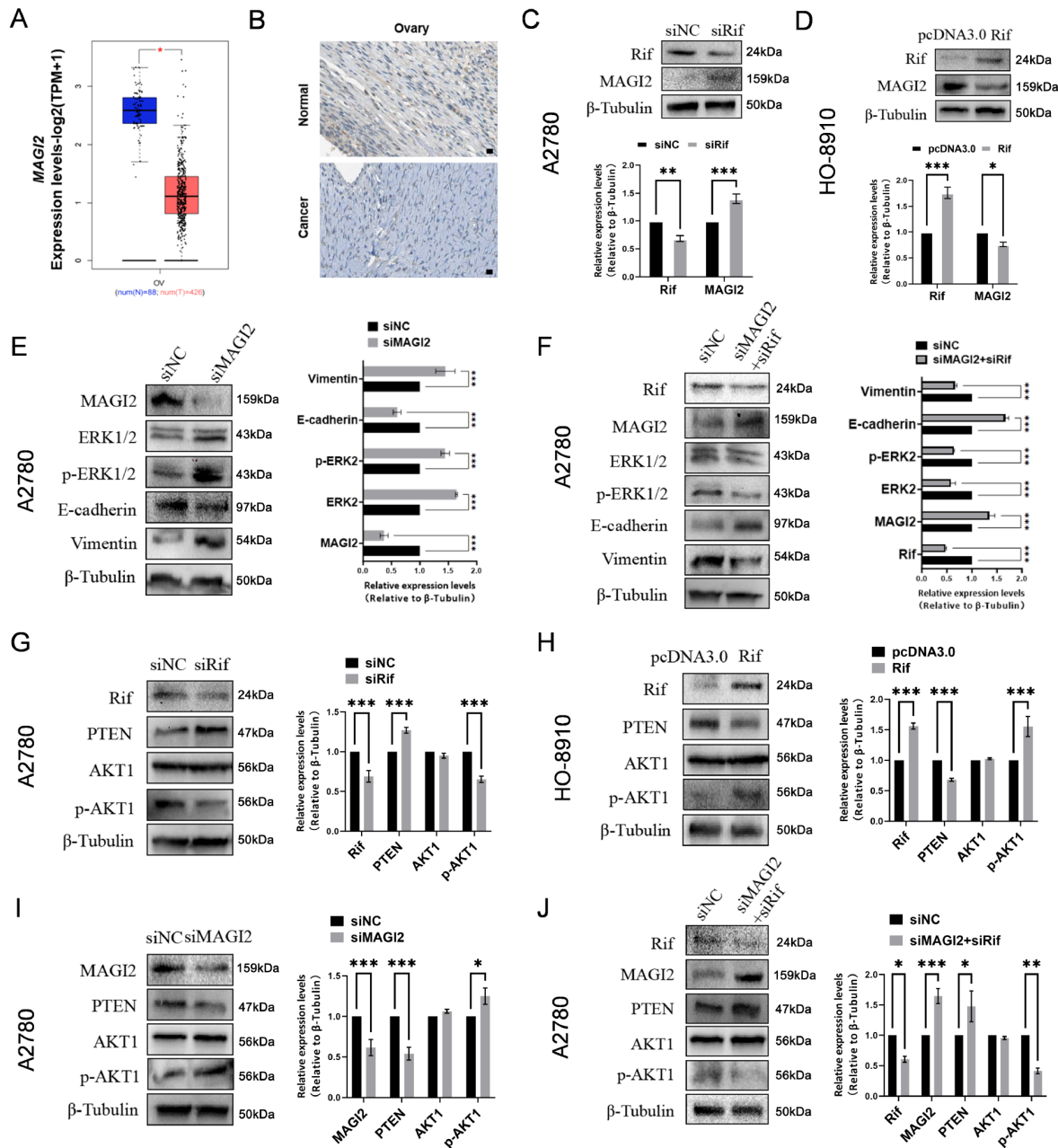


Figure 6. Rif activates the *MAGI2*-ERK2/PTEN/AKT1 signaling pathway to mediate cancer cell EMT (A) The gene expression profile of *MAGI2* in human ovary tumor tissues and normal tissues. Data are analyzed with GEPIA website, matched TCGA normal and GTEx data, using log2(TPM+1) for log-scale. ANOVA methods. (B) IHC staining showing *MAGI2* expression levels. Data were extracted from HPA. Scale bar: 50 μ m. (C–J) The protein expression levels of *MAGI2*, *Rif*, *E-cadherin*, *Vimentin*, *ERK2*, *p-ERK2*, *PTEN*, *AKT1*, and *p-AKT1* in OVCa cells transfected with 50 nM siRNA, 50 nM siNC, 2.5 μ g pcDNA3.0, or 2.5 μ g pcDNA3.0-Rif (Rif). Data are presented as the mean $\pm$ SEM, $n = 3$, two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the current limited clinical data, *MAGI2* transcript levels did not differ significantly across OVCa pathological stages (Supplementary Figure S8B), with no significant prognostic correlation to survival outcomes (Supplementary Figure S8C). Our data suggested that *Rif* depletion upregulated *MAGI2* protein levels, while *Rif* overexpression induced the opposite result (Figure 6C,D). In addition, *MAGI2* depletion elevated *ERK2*, *p-ERK2*, and *Vimentin* levels while downregulating *E-cadherin* level (Figure 6E). Interestingly, *Rif* depletion in *MAGI2*-silenced cells reversed these effects (Figure 6F).

These findings confirm that *MAGI2* is an essential mediator of *Rif*/ERK2-driven EMT.

Rif activates the *MAGI2*/PI3K/AKT1 signaling pathway to promote cancer progression

Bioinformatics analysis of DEGs identified the PI3K/AKT signaling pathway as one of the top-enriched pathways (Figure 4A). This pathway plays a critical role in modulating essential cellular processes, including growth regulation, proliferation, cellular

motility, metabolism and survival [62]. We found that *Rif* knockdown decreased the level of p-AKT1 in A2780 cells, while *Rif* overexpression amplified the p-AKT1 level in HO-8910 cells (Figure 6G,H). Moreover, *MAGI2* depletion similarly induced elevated level of p-AKT1 (Figure 6I). Interestingly, *Rif* depletion in *MAGI2*-silenced cells led to a restoration of *MAGI2* expression level, accompanied by a downregulation of p-AKT1 (Figure 6J). The cancer suppressor PTEN, which is frequently inactivated in both heritable and sporadic malignances, including brain cancer, breast cancer, and prostate cancer [63,64], induces overactivation of the PI3K/AKT signaling pathway due to impaired lipid phosphatase activity [65,66]. In OVCa cells, *Rif* depletion upregulated PTEN protein level, while *Rif* overexpression suppressed it (Figure 6G,H). *MAGI2* knockdown similarly reduced PTEN expression (Figure 6I). In addition, *Rif* silencing in *MAGI2*-deficient OVCa cells restored PTEN level (Figure 6J). These findings imply that *Rif* activates the *MAGI2*/PI3K/AKT1 signaling pathway to promote cancer progression.

Rif activates AHR signaling and the TGF- β /SMAD3 signaling pathway to promote cancer progression

The aryl hydrocarbon receptor (AHR), a basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) transcription factor, regulates diverse cellular processes through interactions with structurally varied ligands [67]. While ligand-activated AHR suppresses BRCA proliferation via cell cycle arrest [68], recent evidence has demonstrated its endogenous role in modulating apoptotic pathways by the transcriptional regulation of apoptosis-associated genes, particularly during malignant transformation [69,70]. Clinical analyses detected no significant differences in *AHR* mRNA expression between BRCA patients and healthy controls (Figure 7A), but there was a significant difference in *AHR* mRNA expression at different pathological stages (Figure 7B). Moreover, survival analysis showed no association between *AHR* expression and clinical outcomes (Figure 7C). We further identified the functional crosstalk between *Rif* and AHR signaling in BRCA pathogenesis. RT-qPCR assay demonstrated that *Rif* knockdown significantly reduced *AHR* transcript level (Figure 7D). Concordantly, our data confirmed that *Rif* inhibition significantly decreased AHR protein level alongside a concomitant downregulation of its downstream effectors, including p27, CDK2, CyclinE, and E2F7, while upregulating the effectors CYP1A1 and GSTP1 (Figure 7E). Conversely, *Rif* overexpression induced opposing effects (Figure 7F). In addition, *Rif* overexpression failed to restore the expression of AHR and other downstream factors in *AHR*-depleted cells (Figure 7G), confirming AHR dependency in *Rif*-mediated oncogenesis.

Transcriptomic profiling also revealed a significant enrichment of TGF- β signaling (Figure 4A), a pathway known to synergize with Ras-MAPK cascade during EMT [71–73]. We found that *Rif* depletion significantly reduced p-SMAD3 level, while *Rif* overexpression amplified them (Figure 7H). These results establish *Rif* as a modulator of EMT via activation of the TGF- β /SMAD3 axis.

Discussion

Malignant neoplasms remain a major global health challenge, marked by persistently high morbidity and mortality rates despite advances in treatment [53,54]. While tumor heterogeneity complicates therapeutic strategies, consistent evidence highlights that conserved oncogenic pathways drive progression through over-

lapping mechanisms. Targeting these common pathways may reveal pancancer therapeutic vulnerabilities, enabling the development of therapies with broad applicability to improve clinical outcomes.

Rho GTPases are critical regulatory molecules that modulate the actin cytoskeleton and regulate microtubule dynamics. In humans, 20 canonical members have been identified: RHO (RHOA, RHOB, and RHOC); RAC (RAC1, RAC1B, RAC2, RAC3, and RHOG); CDC42 (CDC42, G25K, TC10, TCL, WRCH1, and WRCH2); RHOD (RHOD and RIF); RND (RND1, RND2, and RND3); and RHOH [32]. Rho GTPases, represented by RhoA, promote malignant proliferation and metastasis [34,38–40,74–80], but recent studies indicate that RhoA can protect against tumorigenesis by preventing chromatin bridge breakage in cytokinesis [81]. Given that Rho GTPases are potential therapeutic targets in oncology, a comprehensive understanding of their context-dependent functions is crucial for designing effective treatments. In this study, we explored the expression profile of *Rif* across BRCA, OVCa, and STAD. The analysis of TCGA data revealed significant associations between *Rif* upregulation and adverse clinicopathological features, including pathological stages, lymph node metastasis, and OS. These associations were validated in the HPA cohort, reinforcing *Rif*'s prognostic utility. Previous studies have indicated that *Rif* overexpression drives metastatic dissemination in diverse cancers [38–40]. Here, we revealed that *Rif* knockdown suppressed cancer cell viability, proliferation, and motility while inducing apoptosis in BRCA, OVCa, and STAD cells. Conversely, *Rif* overexpression enhanced these oncogenic phenotypes. These results underscore *Rif*'s significant role in cancer progression.

EMT drives malignancy by enhancing invasiveness, stem-like properties, and therapy resistance [6,82,83]. In this process, E-cadherin and vimentin play a central role. E-cadherin, an epithelial marker and cancer suppressor, regulates cell migration, differentiation, and polarity [84], while vimentin, a mesenchymal marker, facilitates invasive behavior and cancer expansion [85]. In this study, we demonstrated that *Rif* knockdown enhanced the expression of E-cadherin and reduced Vimentin expression, whereas *Rif* overexpression induced the opposite alterations. These results suggest that *Rif* can mediate EMT.

EMT represents a highly complex and diverse biological process triggered by various intrinsic and extrinsic stimuli and is subject to precise temporal and spatial regulation [5]. Coordination of this process involves the activation of a series of core EMT-TFs, complemented by epigenetic modifiers, post-transcriptional controllers, and post-translational modulators. Multiple signaling pathways can activate these factors and induce transcriptional networks driving EMT-associated phenotypes [86]. In this study, GSEA revealed that *Rif* regulates several pathways, such as the AHR, MAPK, TGF- β /SMAD3, and PI3K/AKT signaling pathways. Functionally, *Rif* drives EMT in BRCA, OVCa, and STAD via coordinated activation of the *MAGI2*/ERK2 axis and TGF- β /SMAD3 signaling pathway. Pathological *Rif* overexpression activates EMT and ultimately confers a range of malignant behaviors on cancer cells. Therapeutic targeting of EMT-associated epigenetic regulators may improve treatment efficacy.

Normal cells activate the proliferative state only upon receiving mitogenic growth signals coupled with appropriate microenvironmental conditions. In contrast, cancer cells autonomously produce growth signals, diminishing reliance on external microenviron-

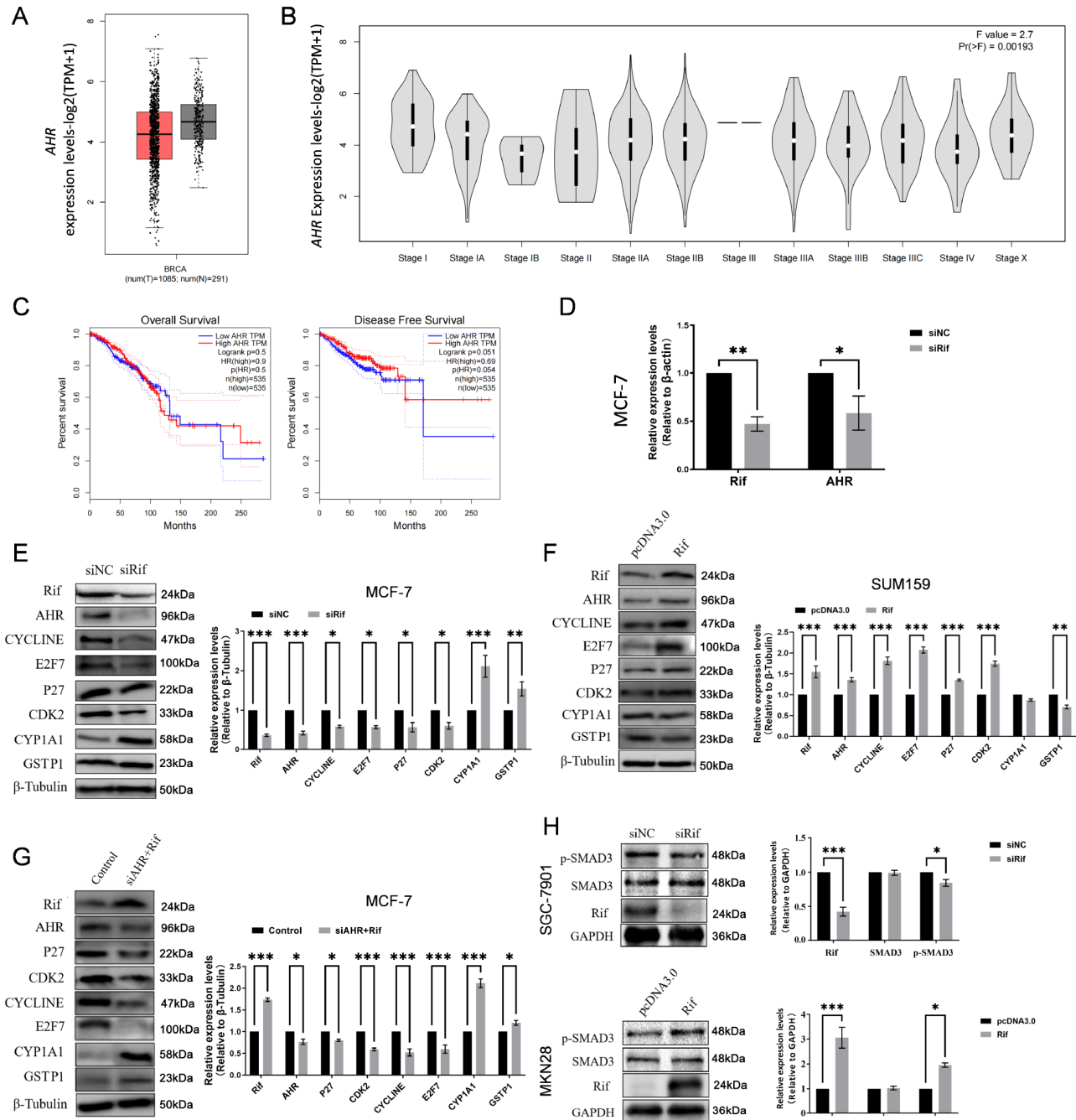


Figure 7. Rif activates the AHR signaling pathway and TGF- β /SMAD3 signaling pathway to mediate cancer cell progression (A) The gene expression profile of *AHR* in human breast tumor tissues and normal tissues. Data are analyzed with GEPIA website, matched TCGA normal and GTEx data, using log₂(TPM+1) for log-scale. ANOVA was used. (B) The RNA expression of *AHR* in different stages of BRCA. Data were analyzed with GEPIA website. (C) Kaplan-Meier curves are made to show the survival rate of BRCA patients with high and low *AHR* mRNA expression levels. Data were analyzed with GEPIA 2 website. (D) The mRNA expression levels of *Rif* and *AHR*. (E–H) The protein expression levels of *Rif*, *AHR*, CyclinE, E2F7, P27, CDK2, CYP1A1, GSTP1, SAMD3, and p-SAMD3 in cells transfected with 50 nM siRNA, 50 nM siNC, 2.5 μ g pcDNA3.0, or 2.5 μ g pcDNA3.0-Rif (Rif). Data are presented as the mean $\pm$ SEM, $n = 3$, two-way ANOVA was used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mental stimuli [56,57]. In osteosarcoma, RHOA modulates apoptosis via AMPK signaling [87]. In cervical cancer, RHOC controls radio resistance through ROCK2-dependent modulation of DNA repair pathways [88]. In hepatocellular carcinoma, Rif promotes metastasis by activating AMPK [38]. In this study, our KEGG pathway analysis implied that *Rif* depletion led to alterations in various

signaling pathways, including the AHR, MAPK, cAMP, Rap1, Ras, TGF- β , and PI3K-Akt signaling pathways. Beyond EMT induction, Rif promotes proliferation via MAGI2-dependent ERK2 and AKT1 activation. Functional studies further demonstrated that Rif facilitates the G1/S checkpoint transition through AHR and its downstream factors P27, CyclinE, cdk2, and E2F7, enabling sustained

tumor growth. The activation of the Rif/AHR axis additionally promotes cancer progression by activating chemical carcinogenesis via CYP1A1 and GSTP1, a mechanism requiring deeper investigation. In conclusion, these findings highlight Rif as a key oncogenic factor, making it a potential therapeutic target for improving clinical efficacy in cancer and a biomarker for patient prognosis.

This study has several limitations. First, previous research reported that different EMT-TFs and EMT dynamics have distinct contributions to cancer progression and metastasis. Therefore, although this experiment utilized multiple cell lines, expanded cellular models encompassing diverse genetic backgrounds and cancer types are still needed to ensure generalizability across heterogeneous malignancies. Second, the reliance on *in vitro* systems creates a translational gap. The absence of *in vivo* validation in biologically complex systems represents a key constraint for evaluating Rif's therapeutic potential. Most importantly, Rif's clinical relevance as a proliferative driver remains to be rigorously established. This necessitates the implementation of large-scale, well-controlled clinical trials with stratified patient cohorts.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Author contributions

Haile Zhao: Conceptualization, Investigation, Writing-original draft, Writing-review & editing; Man Wu: Conceptualization, Investigation, Formal analysis, Methodology; Xiaozhou Bai: Conceptualization, Investigation, Formal analysis; Jingna Wang: Conceptualization, Investigation, Formal analysis; Morigen Morigen: Conceptualization, Supervision, Writing-review & editing; Lifei Fan: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing-original draft, Writing-review & editing.

Data Availability

Data can be accessed upon reasonable request from the corresponding authors.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No. 31960162) and the Central Government Guides Projects Funded by Local Science and Technology Development Funds (No. 2022ZY0208).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Subhadarshini S, Markus J, Sahoo S, Jolly MK. Dynamics of epithelial-mesenchymal plasticity: what have single-cell investigations elucidated so far? *ACS Omega* 2023, 8: 11665–11673
- Tripathi S, Levine H, Jolly MK. The physics of cellular decision making during epithelial-mesenchymal transition. *Annu Rev Biophys* 2020, 49: 1–18
- Brabletz S, Schuhwerk H, Brabletz T, Stemmler MP. Dynamic EMT: a multi-tool for tumor progression. *EMBO J* 2021, 40: e108647
- Wang X, Xue X, Pang M, Yu L, Qian J, Li X, Tian M, *et al.* Epithelial-mesenchymal plasticity in cancer: signaling pathways and therapeutic targets. *MedComm* 2024, 5: e659
- Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014, 15: 178–196
- Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol* 2019, 20: 69–84
- Qu S, Huang X, Guo X, Zheng Z, Wei T, Chen B. Metastasis related epithelial-mesenchymal transition signature predicts prognosis and response to chemotherapy in acute myeloid leukemia. *Drug Des Devel Ther* 2023, 17: 1651–1663
- Boulton TG, Yancopoulos GD, Gregory JS, Slaughter C, Moomaw C, Hsu J, Cobb MH. An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. *Science* 1990, 249: 64–67
- Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD, DePinho RA, *et al.* ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 1991, 65: 663–675
- Lavoie H, Gagnon J, Therrien M. ERK signalling: a master regulator of cell behaviour, life and fate. *Nat Rev Mol Cell Biol* 2020, 21: 607–632
- Frémin C, Saba-El-Leil MK, Lévesque K, Ang SL, Meloche S. Functional redundancy of ERK1 and ERK2 MAP kinases during development. *Cell Rep* 2015, 12: 913–921
- Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, *et al.* Global quantification of mammalian gene expression control. *Nature* 2011, 473: 337–342
- von Kriegsheim A, Baiocchi D, Birtwistle M, Sumpton D, Bienvenut W, Morrice N, Yamada K, *et al.* Cell fate decisions are specified by the dynamic ERK interactome. *Nat Cell Biol* 2009, 11: 1458–1464
- Shin J, Yang J, Lee JC, Baek KH. Depletion of ERK2 but not ERK1 abrogates oncogenic Ras-induced senescence. *Cell Signal* 2013, 25: 2540–2547
- Li F, Zeng B, Chai Y, Cai P, Fan C, Cheng T. The linker region of Smad2 mediates TGF- β -dependent ERK2-induced collagen synthesis. *Biochem Biophys Res Commun* 2009, 386: 289–293
- Krens SFG, He S, Lamers GEM, Meijer AH, Bakkers J, Schmidt T, Spalink HP, *et al.* Distinct functions for ERK1 and ERK2 in cell migration processes during zebrafish gastrulation. *Dev Biol* 2008, 319: 370–383
- Miao R, Lu Y, Xing X, Li Y, Huang Z, Zhong H, Huang Y, *et al.* Regulator of G-protein signaling 10 negatively regulates cardiac remodeling by blocking mitogen-activated protein kinase-extracellular signal-regulated protein kinase 1/2 signaling. *Hypertension* 2016, 67: 86–98
- Yuan Y, Yan L, Wu QQ, Zhou H, Jin YG, Bian ZY, Deng W, *et al.* Mnk1 (mitogen-activated protein kinase-interacting kinase 1) deficiency aggravates cardiac remodeling in mice. *Hypertension* 2016, 68: 1393–1399
- Szokodi I, Kerkelä R, Kubin AM, Sármán B, Pikkariainen S, Kónyi A, Horváth IG, *et al.* Functionally opposing roles of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase in the regulation of cardiac contractility. *Circulation* 2008, 118: 1651–1658
- Shin S, Dimitri CA, Yoon SO, Dowdle W, Blenis J. ERK2 but not ERK1 induces epithelial-to-mesenchymal transformation via DEF motif-dependent signaling events. *Mol Cell* 2010, 38: 114–127
- Yan Z, Ohuchida K, Fei S, Zheng B, Guan W, Feng H, Kibe S, *et al.* Inhibition of ERK1/2 in cancer-associated pancreatic stellate cells suppresses cancer-stromal interaction and metastasis. *J Exp Clin Cancer Res* 2019, 38: 221
- Wang K, Ji W, Yu Y, Li Z, Niu X, Xia W, Lu S. FGFR1-ERK1/2-SOX2 axis promotes cell proliferation, epithelial-mesenchymal transition, and metastasis in FGFR1-amplified lung cancer. *Oncogene* 2018, 37: 5340–5354

23. Tan G, Huang C, Chen J, Zhi F. HMGB1 released from GSDME-mediated pyroptotic epithelial cells participates in the tumorigenesis of colitis-associated colorectal cancer through the ERK1/2 pathway. *J Hematol Oncol* 2020, 13: 149
24. Radtke S, Milanovic M, Rossé C, de Rycker M, Lachmann S, Hibbert A, Kermorgant S, *et al.* ERK2 but not ERK1 mediates HGF-induced motility in non small cell lung carcinoma cell lines. *J Cell Sci* 2013, 126: jcs.115832
25. Dimri M, Humphries A, Laknaur A, Elattar S, Lee TJ, Sharma A, Kolhe R, *et al.* NAD(P)H quinone dehydrogenase 1 ablation inhibits activation of the phosphoinositide 3-kinase/Akt serine/threonine kinase and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways and blocks metabolic adaptation in hepatocellular carcinoma. *Hepatology* 2020, 71: 549–568
26. Gagliardi M, Pitner MK, Park J, Xie X, Saso H, Larson RA, Sammons RM, *et al.* Differential functions of ERK1 and ERK2 in lung metastasis processes in triple-negative breast cancer. *Sci Rep* 2020, 10: 8537
27. Steinmetz R, Wagoner HA, Zeng P, Hammond JR, Hannon TS, Meyers JL, Pescovitz OH. Mechanisms regulating the constitutive activation of the extracellular signal-regulated kinase (ERK) signaling pathway in ovarian cancer and the effect of ribonucleic acid interference for ERK1/2 on cancer cell proliferation. *Mol Endocrinol* 2004, 18: 2570–2582
28. Qin J, Xin H, Nickoloff BJ. Specifically targeting ERK1 or ERK2 kills melanoma cells. *J Transl Med* 2012, 10: 15
29. Buscà R, Pouyssegur J, Lenormand P. ERK1 and ERK2 map kinases: specific roles or functional redundancy? *Front Cell Dev Biol* 2016, 4: 53
30. Ullah R, Yin Q, Snell AH, Wan L. RAF-MEK-ERK pathway in cancer evolution and treatment. *Semin Cancer Biol* 2022, 85: 123–154
31. Ercan D, Xu C, Yanagita M, Monast CS, Pratilas CA, Montero J, Butaney M, *et al.* Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. *Cancer Discov* 2012, 2: 934–947
32. Hanna S, El-Sibai M. Signaling networks of Rho GTPases in cell motility. *Cell Signal* 2013, 25: 1955–1961
33. Jansen S, Gosens R, Wieland T, Schmidt M. Paving the Rho in cancer metastasis: Rho GTPases and beyond. *Pharmacol Ther* 2018, 183: 1–21
34. Nishita M, Kamizaki K, Hoshi K, Aruga K, Nishikaku I, Shibuya H, Matsumoto K, *et al.* Rho family small GTPase Rif regulates Wnt5a-Ror1-Dvl2 signaling and promotes lung adenocarcinoma progression. *J Biol Chem* 2023, 299: 105248
35. Gad AKB, Aspenström P. Rif proteins take to the RhoD: Rho GTPases at the crossroads of actin dynamics and membrane trafficking. *Cell Signal* 2010, 22: 183–189
36. Ellis S, Mellor H. The novel Rho-family GTPase Rif regulates coordinated actin-based membrane rearrangements. *Curr Biol* 2000, 10: 1387–1390
37. Sudhaharan T, Sem KP, Liew HF, Yu YH, Goh WI, Chou AM, Ahmed S. The Rho GTPase Rif signals through IRTKS, Eps8 and WAVE2 to generate dorsal membrane ruffles and filopodia. *J Cell Sci* 2016, 129: 2829–2840
38. Li S, Liu Y, Bai Y, Chen M, Cheng D, Wu M, Xia J. Ras homolog family member F, filopodia associated promotes hepatocellular carcinoma metastasis by altering the metabolic status of cancer cells through RAB3D. *Hepatology* 2021, 73: 2361–2379
39. Yang RM, Zhan M, Xu SW, Long MM, Yang LH, Chen W, Huang S, *et al.* miR-3656 expression enhances the chemosensitivity of pancreatic cancer to gemcitabine through modulation of the RHOF/EMT axis. *Cell Death Dis* 2017, 8: e3129
40. Zhao R, Yi Y, Liu H, Xu J, Chen S, Wu D, Wang L, *et al.* RHOF promotes Snail1 lactylation by enhancing PKM2-mediated glycolysis to induce pancreatic cancer cell endothelial-mesenchymal transition. *Cancer Metab* 2024, 12: 32
41. Zhao H, Jia P, Nanding K, Wu M, Bai X, Morigen M, Fan L. Lysophosphatidic acid suppresses apoptosis of high-grade serous ovarian cancer cells by inducing autophagy activity and promotes cell-cycle progression via EGFR-PI3K/Aurora-A^{Thr288}-geminin dual signaling pathways. *Front Pharmacol* 2022, 13: 1046269
42. Zhao H, Feng L, Cheng R, Wu M, Bai X, Fan L, Liu Y. miR-29c-3p acts as a tumor promoter by regulating β -catenin signaling through suppressing DNMT3A, TET1 and HBP1 in ovarian carcinoma. *Cell Signal* 2024, 113: 110936
43. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- $\Delta\Delta$ CT} method. *Methods* 2001, 25: 402–408
44. Zhao H, Zhang L, Du D, Mai L, Liu Y, Morigen M, Fan L. The RIG-I-like receptor signaling pathway triggered by *Staphylococcus aureus* promotes breast cancer metastasis. *Int Immunopharmacol* 2024, 142: 113195
45. Bhutia SK, Dash R, Das SK, Azab B, Su Z, Lee SG, Grant S, *et al.* Mechanism of autophagy to apoptosis switch triggered in prostate cancer cells by antitumor cytokine melanoma differentiation-associated gene 7/interleukin-24. *Cancer Res* 2010, 70: 3667–3676
46. Mao X, Cai T, Olyarchuk JG, Wei L. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. *Bioinformatics* 2005, 21: 3787–3793
47. Goldman MJ, Craft B, Hastie M, Repčeka K, McDade F, Kamath A, Banerjee A, *et al.* Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol* 2020, 38: 675–678
48. Uhlén M, Björling E, Agaton C, Szigarty CA-K, Amini B, Andersen E, Andersson AC, *et al.* A Human Protein Atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics* 2005, 4: 1920–1932
49. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 2017, 45: W98–W102
50. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, *et al.* The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021, 49: D605–D612
51. Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, Netto GJ, *et al.* UALCAN: an update to the integrated cancer data analysis platform. *Neoplasia* 2022, 25: 18–27
52. Kanehisa M. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000, 28: 27–30
53. Qi J, Li M, Wang L, Hu Y, Liu W, Long Z, Zhou Z, *et al.* National and subnational trends in cancer burden in China, 2005–20: an analysis of national mortality surveillance data. *Lancet Public Health* 2023, 8: e943–e955
54. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin* 2024, 74: 12–49
55. Lánckzy A, Györfy B. Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. *J Med Internet Res* 2021, 23: e27633
56. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011, 144: 646–674
57. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000, 100: 57–70
58. Nieto MA, Huang RYJ, Jackson RA, Thiery JP. EMT: 2016. *Cell* 2016, 166: 21–45
59. Sakurai A, Fukuhara S, Yamagishi A, Sako K, Kamioka Y, Masuda M, Nakaoka Y, *et al.* MAGI-1 is required for rap1 activation upon cell-cell contact and for enhancement of vascular endothelial cadherin-mediated cell adhesion. *Mol Biol Cell* 2006, 17: 966–976
60. Ni J, Bao S, Johnson RI, Zhu B, Li J, Vadapampil J, Smith CM, *et al.* MAGI-1 interacts with nephrin to maintain slit diaphragm structure

- through enhanced rap1 activation in podocytes. *J Biol Chem* 2016, 291: 24406–24417
61. Zhu B, Cao A, Li J, Young J, Wong J, Ashraf S, Bierzynska A, *et al.* Disruption of MAGI2-RapGEF2-Rap1 signaling contributes to podocyte dysfunction in congenital nephrotic syndrome caused by mutations in MAGI2. *Kidney Int* 2019, 96: 642–655
 62. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol Cancer* 2019, 18: 26
 63. Liaw D, Marsh DJ, Li J, Dahia PLM, Wang SI, Zheng Z, Bose S, *et al.* Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 1997, 16: 64–67
 64. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, *et al.* PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997, 275: 1943–1947
 65. Papa A, Wan L, Bonora M, Salmena L, Song MS, Hobbs RM, Lunardi A, *et al.* Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. *Cell* 2014, 157: 595–610
 66. Haddadi N, Lin Y, Travis G, Simpson AM, Nassif NT, McGowan EM. PTEN/PTENP1: ‘regulating the regulator of RTK-dependent PI3K/Akt signalling’, new targets for cancer therapy. *Mol Cancer* 2018, 17: 37
 67. Iizuka T, Yin P, Zuberi A, Kujawa S, Coon V JS, Björvang RD, Damdimopoulou P, *et al.* Mono-(2-ethyl-5-hydroxyhexyl)phthalate promotes uterine leiomyoma cell survival through tryptophan-kynurenine-AHR pathway activation. *Proc Natl Acad Sci USA* 2022, 119: e2208886119
 68. Tomblin JK, Salisbury TB. Insulin like growth factor 2 regulation of aryl hydrocarbon receptor in MCF-7 breast cancer cells. *Biochem Biophys Res Commun* 2014, 443: 1092–1096
 69. Ronnekleiv-Kelly SM, Nukaya M, Díaz-Díaz CJ, Megna BW, Carney PR, Geiger PG, Kennedy GD. Aryl hydrocarbon receptor-dependent apoptotic cell death induced by the flavonoid chrysin in human colorectal cancer cells. *Cancer Lett* 2016, 370: 91–99
 70. Frauenstein K, Sydlik U, Tigges J, Majora M, Wiek C, Hanenberg H, Abel J, *et al.* Evidence for a novel anti-apoptotic pathway in human keratinocytes involving the aryl hydrocarbon receptor, E2F1, and checkpoint kinase 1. *Cell Death Differ* 2013, 20: 1425–1434
 71. David CJ, Huang YH, Chen M, Su J, Zou Y, Bardeesy N, Iacobuzio-Donahue CA, *et al.* TGF- β tumor suppression through a lethal EMT. *Cell* 2016, 164: 1015–1030
 72. Su J, Morgani SM, David CJ, Wang Q, Er EE, Huang YH, Basnet H, *et al.* TGF- β orchestrates fibrogenic and developmental EMTs via the RAS effector RREB1. *Nature* 2020, 577: 566–571
 73. Lee JH, Sánchez-Rivera FJ, He L, Basnet H, Chen FX, Spina E, Li L, *et al.* TGF- β and RAS jointly unmask primed enhancers to drive metastasis. *Cell* 2024, 187: 6182–6199.e29
 74. Kakiuchi M, Nishizawa T, Ueda H, Gotoh K, Tanaka A, Hayashi A, Yamamoto S, *et al.* Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet* 2014, 46: 583–587
 75. Kumar RD, Swamidass SJ, Bose R. Unsupervised detection of cancer driver mutations with parsimony-guided learning. *Nat Genet* 2016, 48: 1288–1294
 76. Li D, Wang H, Ding Y, Zhang Z, Zheng Z, Dong J, Kim H, *et al.* Targeting the NRF-2/RHOA/ROCK signaling pathway with a novel aziridonin, YD0514, to suppress breast cancer progression and lung metastasis. *Cancer Lett* 2018, 424: 97–108
 77. Cannon AC, Uribe-Alvarez C, Chernoff J. RAC1 as a therapeutic target in malignant melanoma. *Trends Cancer* 2020, 6: 478–488
 78. Lam CRI, Tan C, Teo Z, Tay CY, Phua T, Wu YL, Cai PQ, *et al.* Loss of TAK1 increases cell traction force in a ROS-dependent manner to drive epithelial-mesenchymal transition of cancer cells. *Cell Death Dis* 2013, 4: e848
 79. Okada T, Sinha S, Esposito I, Schiavon G, López-Lago MA, Su W, Pratilis CA, *et al.* The Rho GTPase Rnd1 suppresses mammary tumorigenesis and EMT by restraining Ras-MAPK signalling. *Nat Cell Biol* 2015, 17: 81–94
 80. Bellovin DI, Simpson KJ, Danilov T, Maynard E, Rimm DL, Oettgen P, Mercurio AM. Reciprocal regulation of RhoA and RhoC characterizes the EMT and identifies RhoC as a prognostic marker of colon carcinoma. *Oncogene* 2006, 25: 6959–6967
 81. Balafouti S, Kabouraki M, Zachos G, Petsalaki E. Tension-sensitive LINC-RhoA signaling prevents chromatin bridge breakage in cytokinesis. *EMBO J* 2025, 44: 5834–5859
 82. Nieto MA. Epithelial-mesenchymal transitions in development and disease: old views and new perspectives. *Int J Dev Biol* 2009, 53: 1541–1547
 83. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009, 139: 871–890
 84. Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Mol Cancer* 2016, 15: 18
 85. Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. *Cell Mol Life Sci* 2011, 68: 3033–3046
 86. Lu W, Kang Y. Epithelial-mesenchymal plasticity in cancer progression and metastasis. *Dev Cell* 2019, 49: 361–374
 87. Kamel WA, Sugihara E, Nobusue H, Yamaguchi-Iwai S, Onishi N, Maki K, Fukuchi Y, *et al.* Simvastatin-induced apoptosis in osteosarcoma cells: a key role of RhoA-AMPK/p38 MAPK signaling in antitumor activity. *Mol Cancer Ther* 2017, 16: 182–192
 88. Lv C, Fu S, Dong Q, Yu Z, Zhang G, Kong C, Fu C, *et al.* PAGE4 promotes prostate cancer cells survive under oxidative stress through modulating MAPK/JNK/ERK pathway. *J Exp Clin Cancer Res* 2019, 38: 24