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ORIGINAL RESEARCH

Targeting eIF3f Suppresses the Growth of Prostate Cancer Cells by Inhibiting Akt Signaling

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Background: Eukaryotic initiation factor 3 (eIF3) is the largest translation initiation factor, and oncogenic roles have been discovered for its subunits, including the f subunit (ie, eIF3f), in various human cancers. However, the roles of eIF3f in the development and progression of prostate cancer (PCa) have not been reported.

Materials and Methods: We performed in silico analysis to screen the expression of eIF3 subunits. Relevant shRNAs were used to knock down eIF3 subunits in 22Rv1 cells and cell proliferation was analyzed. eIF3f expression in PCa specimens was confirmed by immuno-histochemistry. eIF3f knockdown was established to evaluate the effects of eIF3f on cell proliferation in vitro and in vivo. RNA-seq, bioinformatics analysis and Western blotting were applied to explore the molecular details underlying the biological function of eIF3f in PCa cells. shRNA-resistant eIF3f and myristoylated-Akt were used to rescue the effects of eIF3f disturbance on PCa cells.

Results: Functional analyses confirmed that eIF3f is essential for PCa proliferation. Notably, the expression of eIF3f was found to be elevated in human PCa tissues as well as in PCa cell lines. eIF3f silencing significantly suppressed the growth of PCa cells, both in vitro and in vivo. eIF3f expression was positively correlated with Akt signaling activity in RNA-seq profiles and published prostate cohorts. Knockdown of eIF3f markedly reduced the levels of phosphorylated Akt in PCa cells. Exogenous expression of shRNA-resistant eIF3f in eIF3f knockdown cells restored Akt phosphorylation levels and cell growth. Importantly, rescue experiments revealed that ectopic expression of myristoylated-Akt partially alleviated the suppressive effects of eIF3f disturbance with respect to the growth of PCa cells.

Conclusion: These results suggested that eIF3f has an oncogenic role in PCa, mediated at least partially through the regulation of Akt signaling, and that eIF3f represents a potential target for the inhibition of PCa growth and progression.

Keywords: eukaryotic initiation factor 3, eIF3f, prostate cancer, Akt

Introduction

Prostate cancer (PCa) is regarded as the most commonly diagnosed cancer and the second leading cause of cancer-related death among American men, with an estimated 174,650 new cases and 31,620 new deaths in 2019.[1] Its rate in developing countries, such as China, is also increasing.[2,3] Patients with primary prostate tumors typically undergo radical prostatectomy or radiation therapy, and some patients develop recurrent disease. Because the growth of PCa is initially hormone-dependent, most patients with recurrent PCa receive androgen deprivation therapy. However, a large proportion of tumors eventually relapse to castration-resistant PCa within a few years.[4,5] Recently developed next-generation antiandrogens (e.g., abiraterone and

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enzalutamide), as well as specific chemotherapies (e.g., docetaxel), are efficacious for the treatment of this lethal stage of the disease.[5,6] However, inherent or acquired resistance to these therapies remains a major clinical challenge; therefore, it is imperative to develop novel therapeutic approaches.

Translational control, including the regulation of translation factor levels and variations in translation activities, is a key link in the carcinogenic process.[7] Eukaryotic initiation factor 3 (eIF3) is the largest translation initiation factor (800 kDa) in human cells; it consists of 13 unique subunits (a–m). eIF3 is essential for the initiation of protein translation and performance as a docking site for establishment of the 43S preinitiation complex (PIC), which is composed of Met-tRNA$_i^{Met}$, eIF2, and the 40S ribosomal subunit.[8] The 43S PIC is recruited to mRNA via the interaction of eIF3 with eIF4g to form the 48S PIC. In the 48S PIC, eIF3 enables AUG recognition on mRNA.[9] It has been reported that changes in the level of a single eIF3 subunit can affect the expression characteristics of other subunits, resulting in the formation of eIF3 subcomplexes that promote the translation of specific mRNAs.[10] eIF3 targets mRNAs involved in the control of cell growth, via translational activation or repression.[11] It has been reported that some eIF3 subunits are involved in the initiation and development of PCa. For example, the expression of eIF3b mRNA has been associated with tumor grade, stage, and survival in human PCa, and depletion of eIF3b has been shown to reduce cancer cell growth in vitro.[12] eIF3c plays an oncogenic role in PCa through the regulation of PI3K/Akt/NF-κB signaling.[13] eIF3d plays an oncogenic role in the proliferation and invasion of PCa cells.[14] eIF3h is amplified in PCa tissue and is associated with advanced tumor stage;[15–18] downregulation of eIF3h has been shown to reduce the growth of LNCaP and PC-3 cells.[19] The reduction of EGF-induced eIF3i expression by exposure to penta-O-galloyl-β-D-glucose can suppress the invasion of PC-3 cells in vitro.[20] In LNCaP cells, androgen can induce the palmitoylation of eIF3l and is closely associated with the dynamic palmitoylated level of eIF3l.[21] However, the involvement of eIF3f in PCa remains unknown.

The present study showed that eIF3f expression was upregulated in PCa cells and PCa tissues both in The Cancer Genome Atlas (TCGA) PRAD Dataset[22] and prostate cancer specimens. We screened for eIF3 subunits upregulated in PCa tissues in the TCGA PRAD Dataset, which could affect the proliferation of PCa cells. The results indicated that silencing of eIF3f expression by lentiviral transfection significantly repressed tumor growth of PCa cells, both in vitro and in vivo. Disruption of eIF3f in PCa cells markedly inhibited the phosphorylation of Akt, whereas overexpression of sheIF3f-resistant wild-type eIF3f in eIF3f knockdown PCa cells restored both cell viability and Akt phosphorylation. Furthermore, ectopic expression of constitutively activated Akt partially rescued growth inhibition that had been induced by eIF3f knockdown. Taken together, the results of this study suggested that eIF3f plays an oncogenic role in the proliferation of PCa cells.

Materials and Methods
Cell Culture
The PC3 PCa cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The 22Rv1 PCa cell line was kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). The 22Rv1 and PC3 cells were cultured in RPMI 1640 medium (Corning Inc., Corning, NY, USA) with 10% fetal bovine serum (Gemini, Woodland Hills, CA, USA), 1% HEPES (Corning, Inc.), and 1% penicillin/ streptomycin (Gibco, Grand Island, NY, USA). All cells were grown at 37°C in a 5% CO_2 humidified incubator.

Plasmids and Lentivirus Infection
Short hairpin RNA (shRNA) expression sequences were as follows:

sheIF3f#1 (5′-GTGGCTGTTGACATGGAATTT-3′)
sheIF3f#2 (5′CACAATGAGTCAGAAGATGAA-3′)
sheIF3m (5′-CTTCAGATTGGGAGCTGATGAT-3′)
sheIF3g (5′-CGATGTCTCTATGACGTTCAT-3′)
sheIF3j (5′-ACCTCGAATTAGCAAAGGAAA-3′)

These sequences were cloned into the pLKO.1 vector. Plasmids for sheIF3f#1-resistant wild-type eIF3f and constitutively active Akt (ie, myristoylated-Akt) were cloned into the pLVX-IRES-ZsGreen1 vector with a C-terminal 3*Flag tag. Plasmids were transfected into HEK293FT cells using PEI 25K (23966–1; Polysciences, Warrington, PA, USA), in accordance with the manufacturer's instructions. PC3 and 22Rv1 cells were transduced with lentivirus, and stable transformants were isolated with puromycin (5 µg/mL) (Sigma-Aldrich, St. Louis, MO, USA) for 7 days.

Real-Time PCR Analysis
RNA was isolated from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's instructions; RNA was then

reverse transcribed into cDNA using a PrimeScript™ 1st Strand cDNA Synthesis Kit (6110A; TaKaRa, Kyoto, Japan). qRT-PCR was performed using TB Green® Premix ExTaq™ (Tli RNaseH Plus) (RR420; TaKaRa) on an ABI7500 System (Applied Bio Systems, Foster City, CA, USA). The relative expression levels of the assayed genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The following primers were used:

eIF3f forward, 5′-ACGGGCCATGACATCACAG-3′; eIF3f reverse, 5′- AAGTGCTGACGTAGGCTTTGA-3′; eIF3m forward, 5′-TCAGAAGAGAA CTCGGAAGGTG-3′; eIF3m reverse, 5′-ACCACACTGTTCATCACACTTT-3′; eIF3g forward, 5′-TCAAGATTGTCCGCACCTTCA-3′; eIF3g reverse, 5′-CTGGCAGTTCAGGTCCTCTTT-3′; eIF3j forward, 5′-GTCAAGGATAACTGGGATGACG-3′; eIF3j reverse, 5′-CGAGGTCTGACTCTTCCTGTAA-3′. The GAPDH gene was used as an internal control for qRT-PCR with the following primers: GAPDH forward, 5′-TCCTGTTCGACAGTCAGCCGCA-3′; GAPDH reverse, 5′-ACCAGGCGCCCAATACGACCA-3′.

Western Blotting Analysis
Cells were washed twice with phosphate-buffered saline and solubilized in lysis buffer. Aliquots of approximately 40 µg of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes; the membranes were blocked with 5% bovine serum albumin in Tris-buffered saline with Tween, then incubated with primary antibodies at 4°C overnight. Subsequently, the membranes were hybridized with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1.5 hours, then washed in Tris-buffered saline with Tween. The signal density was visualized on FluorChem E (ProteinSimple, San Jose, CA, USA). The following antibodies were used: eIF3f (A7023; ABclonal, Wuhan, China), pAkt (4058S; Cell Signaling Technology, Danvers, MA, USA), Akt (9272S; Cell Signaling Technology), vinculin (ET1705-94; Huaan, Hangzhou, China), and β-actin (sc-47778; Santa Cruz Biotechnology, Dallas, TX, USA).

Cell Growth Assay
Cell growth was assessed using Cell Counting Kit-8 (CK04; Dojindo, Kumamoto, Japan). A total of 1500 cells/well were seeded in 96-well plates; after cells had adhered to the wells, the CCK8 reagent was added to 100 µL of RPMI 1640 medium with 10% FBS in each well and cells were incubated at 37°C for 3 hours. Absorbance was then detected at 450 nm (A_{450}) using a microplate reader (Tecan, Mechelen, Belgium).

Colony Formation Assay
Cells were cultured in 6-well plates (2000–3000 cells per well) in complete medium for 7–12 days, depending on the sizes of the colonies. The cells were then fixed with methanol for 15 minutes and stained using 0.1% crystal violet for 1 hour.

Animal Experiments
Aliquots of approximately 3×10^5 lentivirus-infected 22Rv1 cells were suspended in Matrigel (volume, 1:1; 356234; Corning, Inc.) and implanted subcutaneously into 6-week-old male athymic nude mice (n=9). All mice were killed 37 days later, and the xenografts were dissected and weighed. All animal experiments were approved by the Experimental Animal Ethics Committee of East China Normal University (m20190401), and performed according to the regulations for Laboratory Animal Center, East China Normal University.

Immunohistochemistry (IHC)
The sections were deparaffinized, rehydrated and incubated in 3% hydrogen peroxide at room temperature for 10 minutes. Antigens were retrieved in 0.01 mol/L citric buffer (pH 6.0) at 95°C to 98°C for 25 minutes. Slides were cooled down for 1 hour before blocked with a preferred blocking solution for 30 minutes at room temperature. Staining with primary antibodies was performed at 4°C overnight. Each sample was measured by the intensity (0: negative, 1: weak, 2: moderate, and 3: strong) and the percentage of positive cells (0: 0%, 1: 1%-25%, 2: 26%-50%, 3: 51%-75%, and 4: 76%-100%). The final IHC scores = intensity score × percentage score. The following antibodies were used for IHC: eIF3f (A7023; ABclonal, Wuhan, China) and Ki67 (A2094; ABclonal). From 2018 to 2019, ninety surgical specimens from patients (58 PCa tissues and 32 adjacent normal tissues) were collected by Shanghai Fifth People's Hospital, Fudan University with the donor being informed completely and with their consent. The research protocol was approved by Medical Ethics Committee of Shanghai Fifth People's Hospital, Fudan University (2018LL028). All experiments were conducted following the guidelines and regulations of Medical Ethics Committee of Shanghai Fifth People's Hospital, Fudan University.

RNA Sequencing and Analysis

Total RNA extracted from the indicated groups of 22Rv1 cells was subjected to RNA sequencing (RNA-Seq) performed by Majorbio Biopharm Technology (Shanghai, China). The sequencing reads were analyzed with the free online Majorbio Cloud Platform (www.majorbio. com) to obtain expression profiles. The resulting sequence data have been submitted to the Gene Expression Omnibus database (GEO, GSE140526). Gene set enrichment analysis (GSEA) used the GSEA software provided by the Broad Institute (http://www.broadinstitute.org/gsea/index. jsp), in accordance with the instructions provided by the Broad Institute. Hallmark gene sets were used within the Molecular Signatures Database version 7.0.

Published Datasets and Analysis

TCGA expression profiles were downloaded from UCSC Xena (http://xena.ucsc.edu/). Fred Hutchinson Cancer Research Center[23] expression profiles were downloaded from cBioPortal[24,25] (http://www.cbioportal.org/). GSE62872[26] expression profiles were downloaded from the Gene Expression Omnibus database. When published datasets were assessed by GSEA, the Pearson metric was used for ranking genes and the phenotype permutation type was used; for all other parameters, default settings were used.

Statistical Analysis

Student's *t* test or Mann–Whitney tests were performed to evaluate differences between two or multiple groups. Kaplan-Meier survival analysis was used to analyze the tumor-free survival. Data are presented as the mean ± SD of three independent experiments. All statistical analyses were performed with GraphPad Prism (version 7; GraphPad Software, La Jolla, CA, USA). For all analyses, $P<0.05$ was considered to indicate statistical significance.

Results
eIF3f Was Identified as a Regulatory Factor in PCa

To categorize possible oncogenes in eIF3 subunits, we performed vitro analysis to filter the expression levels of eIF3 subunits in normal or tumor tissues of the prostate (Figure 1A). We screened four eIF3 subunits that were predicted to be highly expressed in human PCa tissues, compared to normal prostate tissues (genes marked red in Figure 1A); notably, the functions of these genes in PCa development and progression have not been confirmed.

We silenced each eIF3 subunit by using a relevant shRNA and the efficiencies of shRNAs were examined by qRT-PCR (Figure 1B). The proliferation rates of shRNA lentivirus-infected PCa cells indicated that eIF3f knockdown markedly decreased the proliferation of PCa cells (Figure 1C). In the TCGA published dataset, the mRNA levels of eIF3f were markedly upregulated in PCa tissues, compared to adjacent non-tumor tissues (Figure 1D). Next, we detected eIF3f expression in normal tissues (n=32) and PCa tissues (n=58) using IHC. The staining of normal prostate tissues was weaker than PCa tissues (Figure 1E). The stain scores of tumor tissues were significantly higher than normal tissues (Figure 1F). Consistent with the results of published datasets, eIF3f expression was elevated in PCa tissues. Western blotting revealed that eIF3f also showed higher expression levels in four types of PCa cell lines, compared to human prostatic RWPE-1 cells (Figure 1G). Taken together, these results suggested that eIF3f may play a role in the development of PCa.

eIF3f Was Essential for Growth of PCa Cells

To examine the function of eIF3f in PCa cell proliferation in vitro, we disrupted eIF3f with two individual shRNAs targeting eIF3f in 22Rv1 and PC3 cells. We confirmed the knockdown efficiency of eIF3f by Western blotting (Figure 2A). CCK-8 assay results showed that the loss of eIF3f markedly affected cell growth in the tested PCa cells (Figure 2B). Consistent with these observations, colony formation assays showed that eIF3f deletion significantly reduced cell clone formation, in comparison to the control group (Figure 2C-D). Furthermore, we found that ectopic expression of sheIF3f#1-resistant wild-type eIF3f rescued the proliferation rate of eIF3f knockdown cells (Figure 2E). These observations ruled out the possibility that the inhibition of proliferation had been caused by off-target effects. Overall, our results support the hypothesis that eIF3f plays an important role in the growth of PCa cells.

eIF3f Knockdown Suppressed the Growth of PCa Tumor Xenografts

To explore the function of eIF3f in the growth of PCa cells in vivo, we subcutaneously injected 22Rv1 cells stably expressing shRNA targeting eIF3f (sheIF3f#1 and sheIF3f#2) or vector control (shCON) into 6-week-old nude mice. Thirty-seven days later, we sacrificed the mice and dissected the
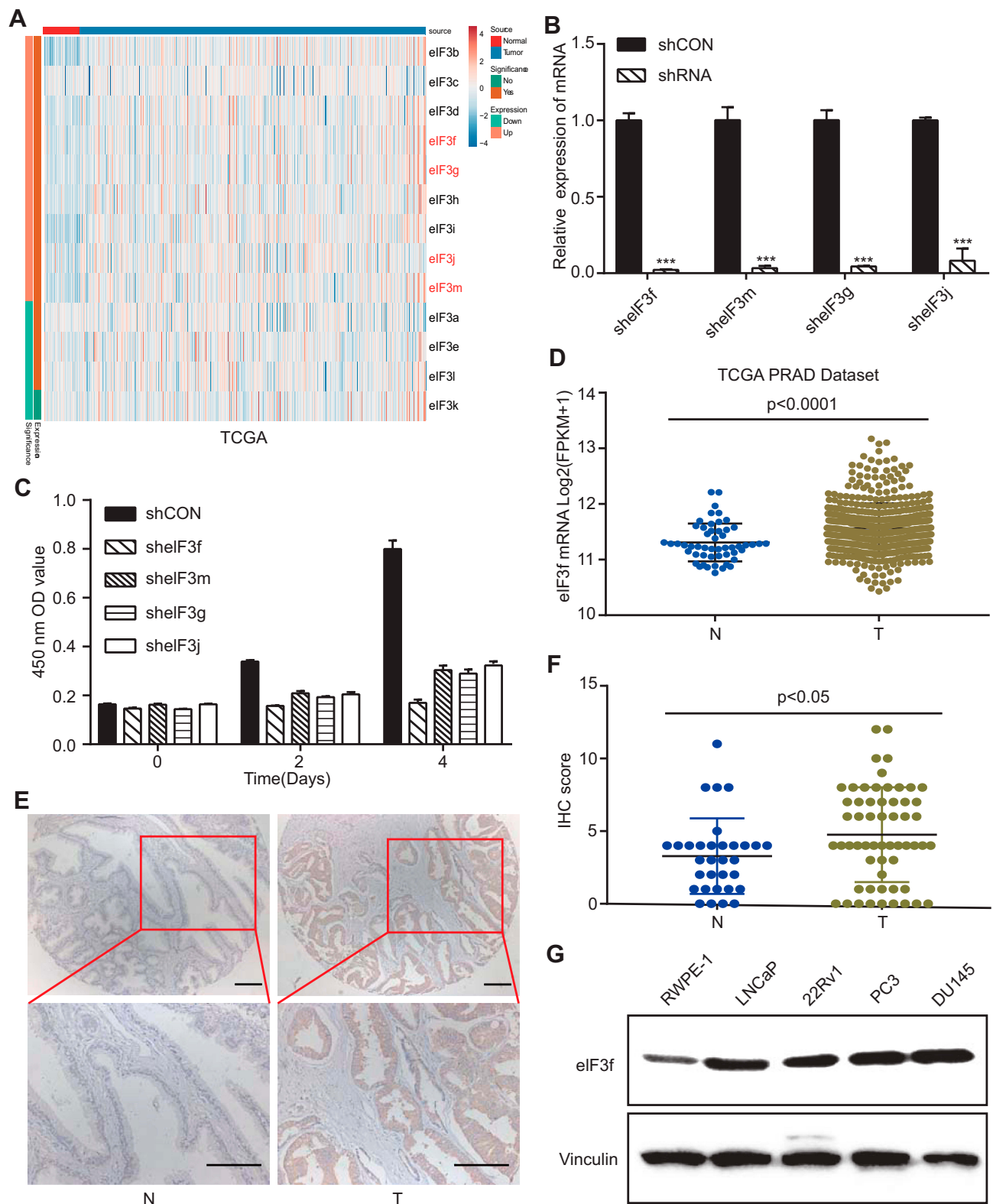
Figure I eIF3f is a potential oncogene in PCa. (**A**) Expression levels of eIF3 subunits of normal prostate tissues and PCa tissues in TCGA are shown in a heat map using differential expression. (**B**) 22RvI cells were transfected with short hairpin RNAs (shRNAs) targeting different eIF3 subunits. The efficiencies of the shRNAs were confirmed by real-time PCR. (**C**) Cell growth was determined by the CCK-8 assay. (**D**) eIF3f expression in PCa tissues from the TCGA prostate adenocarcinoma (PRAD) dataset (normal, n=52; tumor, n=498). (**E**) Representative photographs of normal prostate tissues and PCa tissues (scale bar:200 μm). (**F**) Expression of eIF3f in normal prostate tissues and PCa tissues was examined by immunohistochemistry (Mann–Whitney test). (**G**) Western blotting analysis of eIF3f protein expression in PCa cell lines. Vinculin was chosen as the internal control in Western blotting analysis. Each value represents the mean ± standard deviation of three independent experiments. ***P<0.001.
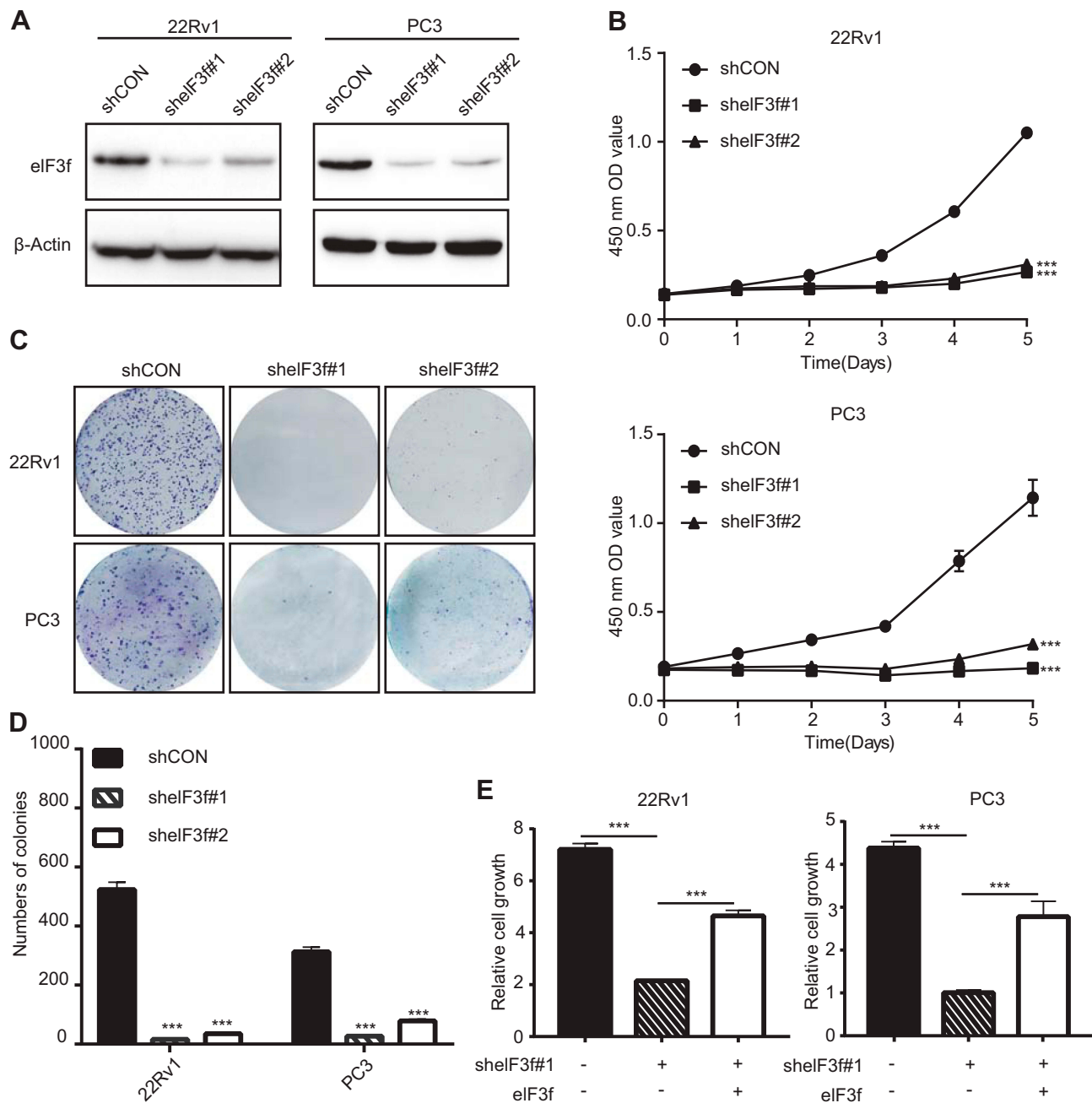
Figure 2 eIF3f knockdown impaired PCa cell proliferation. 22Rv1 and PC3 cells, respectively, transfected with shRNAs against eIF3f (sheIF3f#1 and sheIF3f#2) or vector control (shCON). (**A**) The efficiency of shRNA was verified by Western blotting. β-Actin was used as an internal control in Western blotting. (**B**) The viability of each group of cells was assessed by CCK-8 assay at the indicated time points. (**C**) 22Rv1 and PC3 cells expressing their respective shRNAs were examined by colony formation assay. (**D**) The numbers of colonies for each group are shown. (**E**) 22Rv1 and PC3 cells were infected with control or eIF3f (sheIF3f#1-resistant) lentivirus, as indicated. These cells were transfected with sheIF3f#1or shCON lentivirus. Cell viability was determined by the CCK-8 assay. Each value represents the mean ± standard deviation of three independent experiments. ***$P<0.001$.

xenografts for measurement. The xenografts of the control group were significantly larger and had significantly greater mass than those in the eIF3f knockdown group (Figure 3A and B). In addition, the inhibition of eIF3f expression led to delayed tumor onset and improved tumor-free survival rates

in nude mice (Figure 3C). The IHC analysis of the xenograft tissues exhibited that inhibiting eIF3f reduced Ki67 expression, indicating decreased proliferation of tumor cells (Figure 3D-F). These results confirmed that the inhibition of eIF3f significantly blocked PCa cell growth in vivo.

eIF3f Downregulation Inhibited Phosphorylation of Akt in PCa Cells

To investigate the mechanism underlying the inhibitory effects of eIF3f silencing on PCa cell growth, we performed RNA-Seq analysis of 22Rv1 cells, with or without eIF3f knockdown. GSEA of the gene expression profiles of 22Rv1 cells, with or without eIF3f knockdown, revealed that genes upregulated by the activation of the PI3K/Akt/mTOR pathway were distinctly enriched in the control group (Figure 4A). In clinical specimens, the gene set that reflected activity of the PI3K/Akt/mTOR pathway was also significantly increased in cohorts with high eIF3f expression (Figure 4B and C). To further characterize the role of eIF3f in PCa cell growth, we investigated the expression of proteins involved in the Akt pathway by Western blotting; we found that the level of Akt phosphorylation at Ser473 was downregulated in eIF3f knockdown groups (Figure 4D). Ectopic expression of sheIF3f#1-resistant eIF3f restored Akt phosphorylation in cells expressing sheIF3f#1 (Figure 4E).

Akt Reactivation Partially Restored PCa Cell Proliferation Under Conditions of eIF3f Silencing

To confirm that Akt activation is involved in the regulation of PCa cell proliferation by eIF3f, we overexpressed myristoylated Akt (Myr-Akt) under conditions of eIF3f silencing to explore whether Akt reactivation could counteract the effects of eIF3f knockdown. The expression levels of eIF3f and pAkt were evaluated by Western blotting (Figure 5A). We analyzed cell growth by using CCK-8 and colony formation assays. Although Myr-Akt-expressing cells remained susceptible to eIF3f knockdown, Akt reactivation partially restored the proliferation and colony formation abilities of eIF3f silenced cells (Figure 5B–D). These results indicated that eIF3f knockdown caused inhibition of PCa cell proliferation at least partially through the suppression of Akt phosphorylation.

Discussion

There have been a number of reports regarding the roles of eIF3 subunits in cancer,[27] which indicate that eIF3 subunits are crucial for the development of PCa. Previous studies indicated that eIF3 subunits b, c, d, h, and i are involved in the development of PCa.[12–14,18–20,28] In the present study, we showed that deletion of eIF3 subunits g, j, m, and f resulted in marked suppression of PCa cell proliferation. In silico analyses suggested that eIF3f may promote the progression of PCa.

eIF3f is an indispensable subunit of the eIF3 molecule, which participates in formation of the mammalian eIF3 functional core.[29] Notably, eIF3f regulates skeletal muscle size through interaction with the mTOR/raptor complex, thereby promoting the phosphorylation of S6K1 and regulating downstream effectors of mTOR.[30–32] eIF3f has also been reported to play roles in various cancers. Most investigations have shown that eIF3f acts as a tumor suppressor. Notably, the eIF3f level was reportedly reduced in pancreatic cancer, and overexpression of eIF3f in pancreatic cancer cells resulted in apoptosis;[33] moreover, eIF3f promoted rRNA degradation and inhibited translation.[34] eIF3f has been shown to act as a tumor suppressor in melanoma.[35,36] In patients with gastric cancer, reduced expression of eIF3f was associated with poor prognosis, and eIF3f presumably plays an important role in gastric cancer recurrence.[37–39] However, it remains controversial whether eIF3f acts as an oncogene or a suppressor. eIF3f knockdown in A549 cells was shown to inhibit cell proliferation and induce apoptosis.[40] Esteves et al[41] reported that eIF3f interacted with STAT3 and increased snail2 expression, thereby promoting lung cancer metastasis. Shi et al[42] used eIF3f cDNA as a probe in a cancer profiling array for assessment of eIF3f expression in diverse human tumor samples; they reported that eIF3f was upregulated in colon cancer and rectum cancer, compared to normal tissues. Overall, the results of these studies indicated that eIF3f plays diverse roles in tumors.

Our study is the first to investigate the role of eIF3f in prostate cancer. According to the shRNA-based screen, disturbing eIF3f remarkably affected PCa cells proliferation. We found that eIF3f was significantly upregulated in PCa tissues compared to normal prostate tissues in TCGA PRAD dataset and clinical specimens. eIF3f was highly expressed in PCa cell lines compared to normal prostate epithelial cells RWPE-1. We confirmed that the loss of eIF3f reduced the proliferation of PCa cells, both in vitro and in vivo. The RNA-seq data using 22Rv1 cells and published datasets both revealed that the disruption of eIF3f suppressed PI3K/Akt/mTOR signaling. Previous studies[43–45] have shown that the Akt pathway is frequently activated during PCa progression; the efficacy of PI3K/Akt inhibitors has been confirmed in PCa preclinical models. Using Western blotting, we confirmed that phosphorylated Akt levels were reduced in eIF3f knockdown cells. Furthermore,
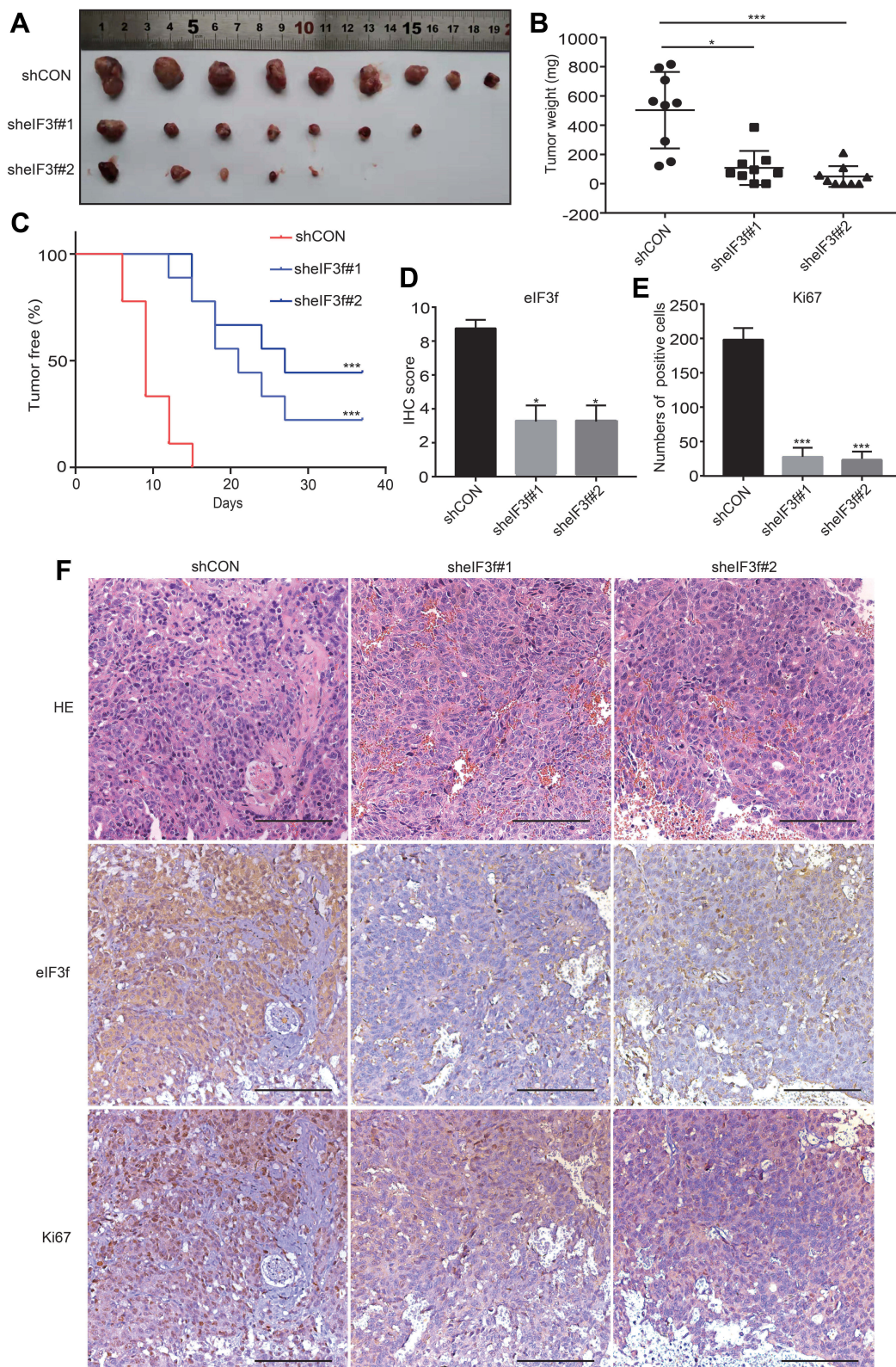
Figure 3 Knockdown of eIF3f impeded PCa tumor growth in vivo. 22Rv1-shCON, 22Rv1-sheIF3f#1, and 22Rv1-sheIF3f#2 cells (3×10^5) were suspended in Matrigel (volume, 1:1) and subcutaneously implanted into nude mice (n=9). (**A**) The mice were killed 37 days later, and the volumes of the xenograft tumors were determined. (**B**) The weights of the xenograft tumors are shown. Error bars represent mean ± standard deviation (Mann–Whitney test; n=9). (**C**) Kaplan–Meier analysis of tumor onset (log-rank). (**D**) eIF3f expression is shown as an IHC score (Mann–Whitney test). (**E**) Ki67 expression is shown as number of positive cells. (**F**) Hematoxylin-eosin (HE) staining and eIF3f and Ki67 immunohistochemical staining in tumor xenografts (scale bar: 200μm). *$P<0.05$; ***$P<0.001$.
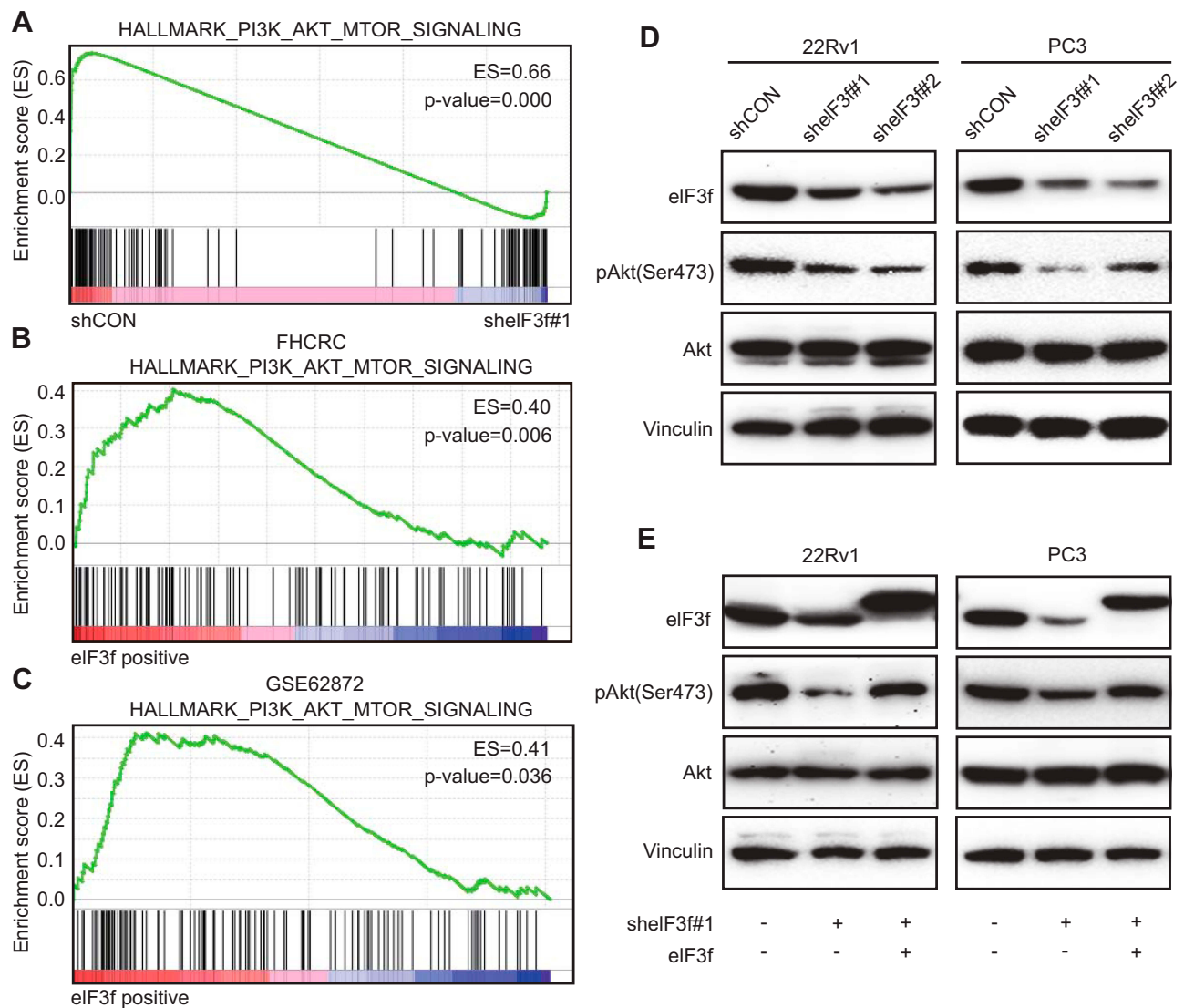
Figure 4 Interference with eIF3f expression inhibited Akt activation. (**A**) GSEA of the dataset following inhibition of eIF3f expression in 22Rv1 cells, based on the PI3K/Akt/mTOR signaling gene sets in the control (left) and sheIF3f#1 (right). (**B** and **C**) GSEA of the Fred Hutchinson Cancer Research Center (FHCRC) and GSE62872 datasets based on the PI3K/Akt/mTOR signaling gene set and eIF3f transcription levels. (**D**) The protein levels of phosphorylated Akt (pAkt), Akt, and eIF3f were determined by Western blotting in 22Rv1 and PC3 cells expressing shRNA targeting eIF3f or vector control. (**E**) sheIF3f#1-resistant eIF3f was expressed under conditions of eIF3f interference. pAkt, Akt, and eIF3f protein levels were detected in each group.

ectopic expression of shRNA-resistant eIF3f in eIF3f knockdown cells restored Akt phosphorylation levels and cell growth. We found that ectopic expression of constitutively active Akt partially rescued the obstructed proliferation caused by eIF3f knockdown. Interestingly, eIF3f expression was elevated by ectopic expression of constitutively active Akt in eIF3f knockdown cells. This results indicated that Akt signaling might upregulate the expression level of eIF3f in PCa cells. We guessed that the regulating relation between Akt and eIF3f might influence the growth of PCa cells and the restoration of the effects of eIF3f knockdown by overexpressing Myr-

Akt might partially depend on upregulating eIF3f. Additional work is required to establish if Akt signaling activates eIF3f expression in prostate cancer. Besides, further studies are required to identify other molecular mechanisms underlying the roles of eIF3f in the progression of PCa.

In summary the findings of this study suggested that eIF3f may play important roles in the development of PCa. The mechanisms underlying the effects of eIF3f in the progression of PCa, as well as the precise role of eIF3f in Akt signaling, require further investigation, and the findings of future studies may facilitate the development
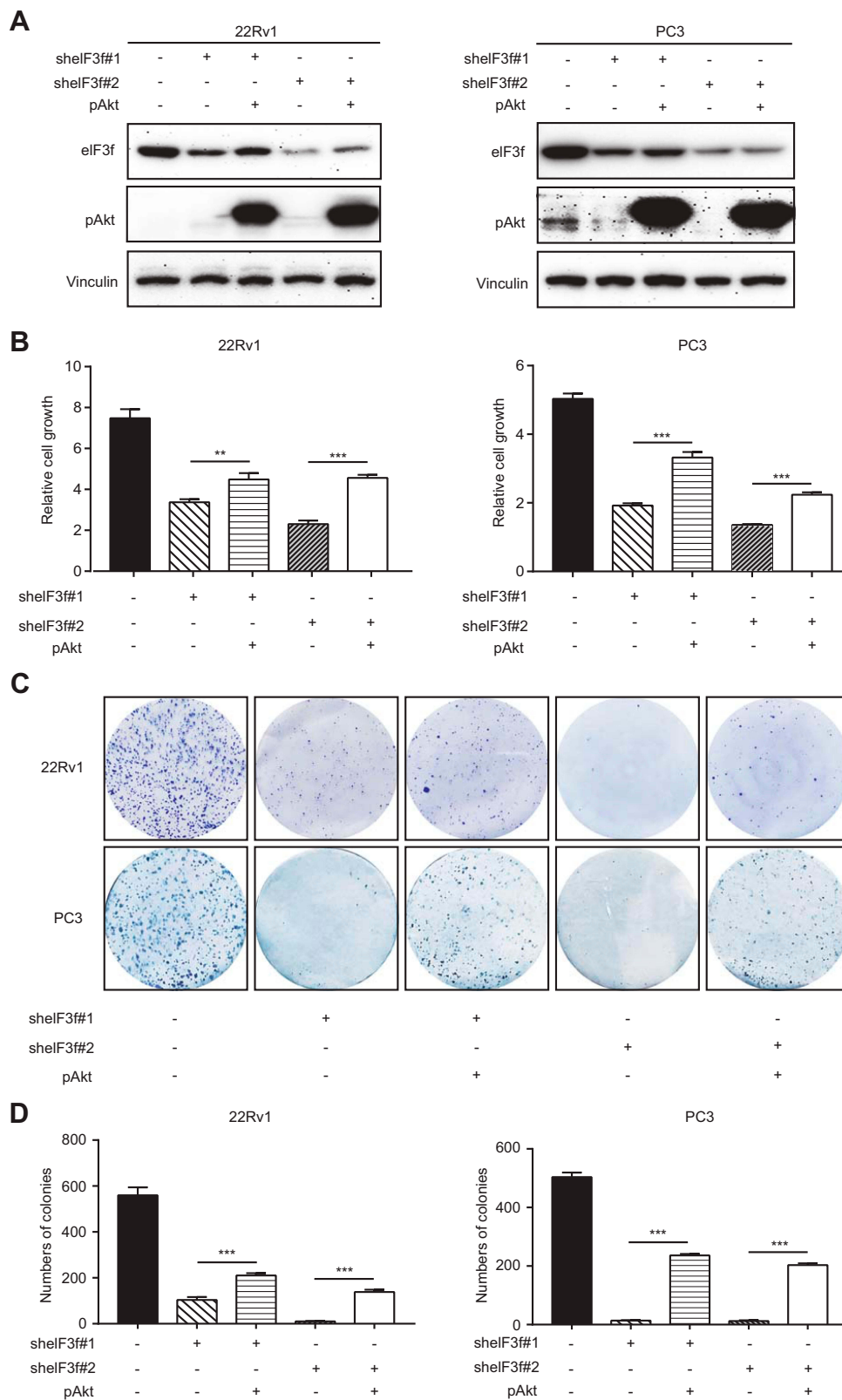
Figure 5 Ectopic expression of Myr-Akt alleviated PCa cell proliferation of eIF3f knockdown cells. 22Rv1 and PC3 cells were infected with vector control or Myr-Akt lentivirus and then transfected with eIF3f shRNAs or shCON lentivirus, respectively. (**A**) The expression efficiencies of pAkt and eIF3f were confirmed by Western blotting. (**B**) Cell viability was determined by the CCK-8 assay. (**C**) Each group was examined by colony formation assay. (**D**) The numbers of colonies for each group were counted. Each value represents the mean ± standard deviation of three independent experiments. **$P<0.01$, ***$P<0.001$.

of novel therapeutic approaches against the progression of PCa.

Disclosure

The authors declared that they have no conflicts of interest to this work.

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