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VRK1 promotes proliferation, migration, and invasion of gastric carcinoma cells by activating β-catenin

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Vaccinia-related kinase 1 (VRK1) is a member of the VRK subfamily belonging to the casein kinase superfamily, and it regulates the proliferation and survival of cells both in normal and malignant tissues. A variety of transcription factors including c-Jun can also be specifically phosphorylated and stimulated by VRK1. However, the regulatory mechanism of VRK1 in gastric carcinoma (GC) remains unclear. This research aimed to determine the function of VRK1 during tumor progression in GC. The mRNA and protein expression of the VRK1 and other genes were evaluated in GC cell lines using real-time RT-PCR and western blotting. Cell proliferation was analyzed using the cell count kit-8 (CCK-8) assay, and cell migration and invasion were monitored by the Transwell assay. The downregulated genes in shVRK1 cells compared with shCtrl were assessed using RNA-seq. The interactions of VRK1 with β-catenin or c-Jun were detected by co-IP. We found that VRK1 was overexpressed in gastric cancer cells, conversely, knockdown of VRK1 inhibits GC cells' proliferation, migration, and invasion. Moreover, VRK1 might regulate the expression of β-catenin (CTNNB1) at the transcriptional level by phosphorylating c-Jun, the transcriptional factor of β-catenin. VRK1 changes the subcellular location and decreases the nuclear aggregation of c-Jun by phosphorylating the Ser243 site. To conclude, VRK1 can affect migration and invasion by regulating the expression of β-catenin at the transcriptional level in GC cells.

Key words: VRK1, gastric cancer, β-catenin, proliferation, migration, invasion

Gastric cancer (GC) is one of the most common malignant tumors of the digestive tract [1]. It is the sixth most common cancer worldwide, but the third leading cause of cancer-related deaths [2]. The prognosis of GC is dismal, with the five-year survival rates of below 20%, because the early stages are clinically silent [3]. Therefore, there is an urgent need to discover new molecular diagnostic and therapeutic targets suitable for GC patients. Vaccinia-related kinase 1 (VRK1) is a member of the VRK subfamily belong to the casein kinase superfamily [4]. VRK1 contains a C-terminals nuclear localization signal [5] and is one of the three subtypes of VRKs. VRK1 plays an important role in cell cycle progression, transcriptional activation, DNA repair, and histone modification [6, 7]. A lot of studies have reported that VRK1 expression regulates the proliferation and survival of cells both in normal or malignant tissues [7–9]. It has been shown that the high expression of VRK1 in several types of cancers including head and neck squamous cell carcinoma, glioma, lung carcinomas, breast carcinomas, and hepatocellular carcinoma [9–13]. VRK1 regulates cells growth through multiple intracellular signal transduction cascades [14]. In addition to p53, a variety of transcription factors can also be phosphorylated and stimulated by VRK1, resulting in stress response [5, 15, 16].

Wnt signaling pathways include β-catenin dependent canonical (classical Wnt/β-catenin signaling pathway) and β-catenin independent non-canonical pathways [17]. The classical Wnt/β-catenin signaling pathway is involved in plentiful biological processes, including cell growth, immune function, differentiation, and apoptosis [18]. Dysregulation of the Wnt/β-catenin signaling pathway is associated with many hereditary disorders, cancer, and other diseases [19, 20]. β-catenin is a crucial molecule in the classical Wnt/β-catenin signaling pathway. The Wnt/β catenin signaling pathway affects cellular functions by regulating both β-catenin levels and subcellular localization [21].

c-Jun (JUN) belongs to the AP-1 transcription factor family. Studies have shown that c-Jun plays an important role in liver cell survival and liver regeneration, and controls the initiation of tumors in the occurrence of hepatocarcinogenesis [22–26]. c-Jun can transactivate a large number of genes involved in cell proliferation and tumor transformation. Therefore, overexpression of c-Jun was implicated functionally in many types of cancers [27]. In addition, c-Jun was reported to participate in the modulation of tumor-initiating cells (TICs) [28, 29]. c-Jun is directly involved in the proliferation and subsequent invasion/metastasis of cells as an oncogene. In particular, c-Jun is essential for the transition beyond G1/S and G2/M checkpoints [30].

We found that VRK1 was overexpressed in GC cells. Downregulation of VRK1 expression declines the proliferation, invasion, and migration of GC cells. The expression of β-catenin (CTNNB1) in shVRK1 cell lines was decreased significantly, and the expression of its downstream genes was also reduced accordingly. Furthermore, our results indicate that VRK1 can regulate the expression of β-catenin, the key protein in the Wnt/β-catenin pathway, at the transcriptional levels by phosphorylating the transcription factor c-Jun.

Materials and methods

Cell culture. Normal human gastric epithelial cell line, GES-1, and two gastric cancer cell lines (low-differentiated cell line MNK45 and undifferentiated cell line HGC-27) were obtained from Bena Culture Collection (Beijing Beina Chuanglian Biotechnology Research Institute). The cells were cultured in the Roswell Park Memorial Institute 1640 (RPMI 1640) medium, culture media were supplemented with 10% fetal bovine serum (ProSpec-Tany TechnoGene, Ltd., Rehovot, Israel), and 100 mg/ml penicillin/streptomycin (ProSpec-Tany TechnoGene, Ltd.), and the cells were cultured in a humidified 5% CO_2 atmosphere at 37 °C.

Cell transfection. The human VRK1-targeting small hairpin RNA (VRK1 shRNA) sequence was designed as 5'-ATAATAACTGACATGGCAA-3' [31]. The human VRK1-targeting small hairpin RNA (VRK1 shRNA2) was purchased from Sigma (TRCN0000010366). The human c-Jun-targeting small hairpin RNA (c-Jun shRNA) and human JNK-targeting small hairpin RNA (JNK shRNA) were obtained from Sigma (TRCN0000010366 for c-Jun and TRCN0000352709 for JNK). Then, we generated recombinant lentiviral particles, and the cells were transfected with VRK1, c-Jun, JNK, or negative control recombinant lentivirus (shRNA-VRK1, shRNA-c-Jun, shJNK, or shRNA-NC, respectively). ATF2 small interfering siRNA (cat. No. 106604), CUX1 siRNA (cat. No. 110461), TCF3 siRNA (cat. No. 114338), SOX9 siRNA (cat. No. 106059), c-Jun siRNA (cat. No. 115273), and control siRNA (cat. No. 131905) were purchased from Invitrogen, Carlsbad, CA. Transfection experiments were performed using Lipofectamine 2000 according to the manufacturer's instructions. Transfection efficiency was detected by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR).

Table 1. The primers of gene for qRT-PCR.

Gene	Forward	Reverse
VRK1	TGGCAAATTGGACCTCAGTG	TGGTTCTTGAACGGGTCTGT
CTNNB1	CTTACACCCACCATCCCACT	CCTCCACAAATTGCTGCTGT
MYC	CTTTCCTCCACTCTCCCTGG	AACCCTCTCCCTTTCTCTGC
CCND1	TTTGTTGTGTGTGCAGGGAG	TTTCTTCTTGACTGGCACGC
ATF2	AGTACAAGTGGTCGTCGGAG	TGACTTCACTCTGCAGCTGA
CUX1	CTGACCACCTGCACAAGTTC	CAGCGGATAAGGGCAGTTTC
TCF3	GATGCTGCCTTTGGTCTCTG	CTCAGGTTTACACGGGGTCT
SOX9	TTGAGCCTTAAAACGGTGCT	CTGGTGTTCTGAGAGGCACA
c-Jun	TTTCAGGAGGCTGGAGGAAG	CTGCCACCAATTCCTGCTTT
GAPDH	ATCACTGCCACCCAGAAGAC	TTTCTAGACGGCAGGTCAGG

RNA extraction and qRT-PCR. GES-1 and gastric cancer cells were harvested and collected, and TRIzol reagent (Thermo Fisher Scientific Inc., USA) was used to extract total RNA. A NanoDrop spectrophotometer (NanoDrop program 1000 version 3.8.2; Thermo Fisher Scientific, Inc.) was used to measure the quality and quantity of RNA. For qRT-PCR, a reverse transcription kit (cat. no. RR036A; Takara Bio, Inc.) was employed to reverse transcribe 1,000 ng total RNA to cDNA in a final volume of 20 µl. qRT-PCR was performed with SYBR Select Master Mix (cat. no. 4472908; Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers used for VRK1, CTNNB, MYC, CCND1, ATF2, CUX1, TCF3, SOX9, c-Jun, and GADPH genes are listed in Table 1. QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Inc.) was used to collect qRT-PCR data. The qRT-PCR reaction included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 92 °C for 15 s, and 60 °C for 1 min. Each sample was carried out in triplicate, and relative expression was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GADPH.

Nuclear and cytoplasmic fractionation of proteins. A nuclear and cytoplasmic protein extraction kit (Beyotime Biotechnology Co., Ltd.) was used to extract cytosolic and nuclear proteins and operated according to the operation manual. All isolated fractions were analyzed by western blotting.

Western blotting. Cells were harvested and processed in lysis buffer (Tris-HCl, sodium dodecyl sulfate [SDS], β-mercaptoethanol, and glycerol) on ice; a BCA kit (Nanjing KeyGen Biotech Co., Ltd.) was utilized to quantitate the protein concentrations. Equal amounts of protein were separated via SDS-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked in 5% skim milk powder in Tris-buffered saline/Tween 20 (TBS-T) for 1 h and subsequently incubated overnight at 4 °C with primary antibodies against VRK1 (ab171933, 1:1,000), β-catenin (ab227499, 1:1,000), c-Jun (ab40766, 1:3,000), Cyclin D1 (ab134175, 1:20000), Myc (ab32072, 1:1,000), β-tubulin

(ab210797, 1:1,000), Lamin B1 (ab133741,1:2,000), β-actin (ab179467, 1:5,000), or GAPDH (ab181602, 10,000). After washing with TBS-T, the membrane was incubated with a goat anti-rabbit secondary antibody (ab7090, 1:10,000) at room temperature for 2 h. The primary and secondary antibodies were purchased from Abcam, USA. The blots were visualized by enhanced chemiluminescence detection (Thermo Fisher Scientific Inc.). All experiments were independently repeated at least three times.

Proliferation, migration, and invasion assays *in vitro*. Cell proliferation was examined using Cell Count Kit-8 (CCK-8) assay. Cells were plated at a density of 1,000 cells/well in 96-well plates, and cultured at 37 °C in a humidified 5% CO_2 atmosphere. Prior to the analysis of proliferation, 10 µl of CCK-8 reagent (Beijing Solarbio Science & Technology, Beijing, China) was added to each well and incubated at 37 °C for an additional 1.5 h. The optical density was measured at 450 nm using an ELx-800 universal microplate reader (BioTek, CA, USA). The cell number was analyzed and a cell growth curve was generated. Each experiment was repeated three times. Cell migration and invasion assays were performed in a 6.5 mm Transwell (#3422, Corning, NY, USA). Cells (2×10^5) suspended in 100 µl of serum-free medium were added to the upper chamber, and the lower chamber was filled with a complete medium with 10% serum. The cells were allowed to migrate following the incubation at 37 °C for 18 h. After removing the non-migrated cells, the membranes were fixed in methanol and stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution. The migrated cells were photographed and quantified in five random fields per membrane. Each sample was assayed in triplicate. For the invasion assay, the Transwell were first coated with Matrigel (BD Biosciences, NY, USA) and cells were allowed to invade for 24 h.

Flow cytometry analysis. The cell cycle distribution and apoptosis were detected by flow cytometry. For the cell cycle distribution, cells were harvested and fixed with 70% ethanol at 20 °C for 24 h, and subsequently, propidium iodide solution was added into the cell suspensions at a final concentration of 100 µg/ml. After incubation of 10 min in dark, cells were washed with PBS containing 0.5% BSA. The samples were analyzed using a FACSCalibur flow cytometer. The percentage of cells phases was evaluated and compared. For apoptosis analysis, cells were washed and resuspended, and the Annexin V-FITC cell apoptosis detection kit was used according to the manufacturer's instructions (BD Biosciences, USA). After incubation at room temperature in the dark for 20 min, the cells were immediately analyzed by flow cytometry using a FACScan. All samples were detected in triplicate.

Transcriptome sequence. RNA was extracted using QIAzol lysis reagent (Qiagen GmbH) and subsequently purified with an RNeasy mini kit (Qiagen GmbH). Purified RNA was digested with DNase I (New England Biolabs, Inc.) to remove residual genomic DNA. RNA quality and quantity were analyzed using a NanoDrop and Bioanalyzer (Agilent Technologies, Inc.), respectively. RNA-seq library preparation and sequencing were conducted at the Beijing Novogene Co., Ltd. Libraries were constructed using an Ovation human FFPE RNA-seq multiplex system kit (NuGEN Technologies, Inc.). Directional mRNA-seq was conducted using the HiSeq 2000 system (Illumina, Inc.), using the single-read 100 cycles option. The resulting raw data were transformed into sequenced reads by base calling. Raw reads were filtered to remove reads containing adapters or those of low quality.

Co-immunoprecipitation assay. The cells were harvested with 1% NP40 lysis buffer containing protease and phosphatase inhibitors (150 mM NaCl, 50 mM Tris-HCl [pH 7.4], and 1% Nonidet P-40). The supernatant was removed by centrifugation with 1,000×g for 1 min. VRK1, β-catenin, or c-Jun antibodies bind to the protein A/G agarose using Protein G immunoprecipitation kit (Sigma-Aldrich, cat. No. 11719386001). The agarose slurry (20 µl) was washed twice with 200 µl PBS buffer solution and incubated with 100 µl antibody (10 µl antibody + 85 µl H_2O + 5 µl 20× PBS) in PBS for 30 minutes at 25 °C on a mixer. In parallel, 100 µl rabbit serum with the same IgG concentration or anti-rabbit IgG peroxidase secondary antibody was prepared as a negative control. Agar glycoprotein and 10 µl antibody were incubated in a mixer for 1 h at 25 °C and then incubated with 600 µl pre-clarified lysate overnight. The immunoprecipitation products were washed with washing buffer five times and eluted with 2× Laemmli buffer at 100 °C for 10 min. Three independent experiments were carried out.

Statistical analysis. All statistical analyses were carried out using the SPSS 18.0 statistical software package (SPSS Inc., USA). All values are depicted as mean ± standard deviation. All data were statistically analyzed using a one-way analysis of variance (ANOVA) with a Bonferroni correction. LSD test was used for the statistical analysis of two groups of independent data. A value of $p<0.05$ was considered statistically significant.

Results

Overexpression of VRK1 in GC cells. The protein and mRNA expression of VRK1 was analyzed in normal human gastric epithelial cell line GES-1, and two gastric cancer cell lines (MNK45 and HGC-27) using western blotting and qPCR. The results showed that both mRNA (Figure 1A) and protein (Figure 1B) levels of VRK1 were significantly higher in the GC cells than those in VRK1. The mRNA and protein levels of VRK1 were ~6–7 times and ~3–6 times higher, in the two GC cell lines compared to GES-1 cells, respectively.

Knockdown of VRK1 inhibits proliferation, migration, and invasion of GC cells. Two packaging the virus including shRNA lentiviral vector that specifically targeted VRK1 were constructed and used to infect MNK45 and HGC-27 cells. The relative levels of mRNA and protein of VRK1 in the two shRNA cell lines were significantly ($p<0.01$)

reduced (Figures 2A, 2B). The VRK1 mRNA expression in the shVRK1 groups was 40–50% lower than that observed in the MNK45 and HGC-27 shCtrl group (Figure 2A), and the protein level was <20% than that in the shCtrl group (Figure 2B). Analysis of cell proliferation using CCK-8 assay showed that the proliferation ability of MNK45 and HGC-27 cells was significantly reduced (Figure 2C) following the downregulation of VRK1. The number of shVRK1 cell lines was only approximately half of that in the control group at 72 h. We measured the cell cycle distribution and cell apoptosis of GC cells using flow cytometry analysis. VRK1 knockdown altered the periodic distribution of MNK45 and HGC-27 cells. In comparison to shCtrl cells, cells with

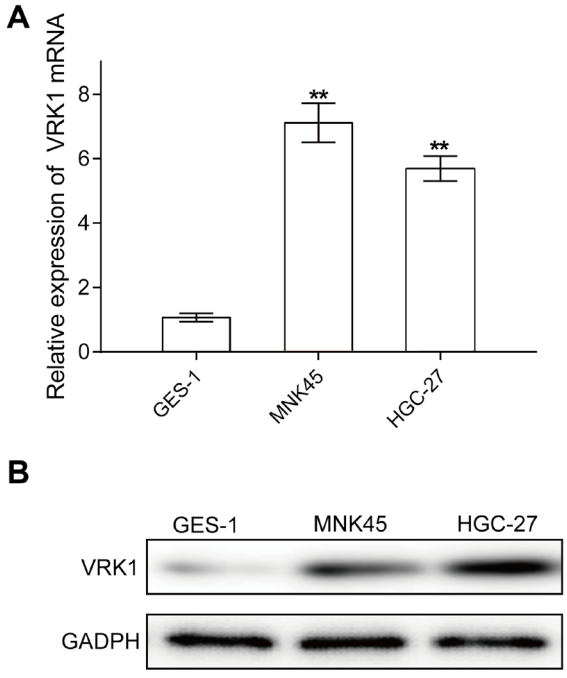

Figure 1. VRK1 is upregulated in gastric cancer cells. A) mRNA and B) protein expressions of VRK1 are higher in gastric cancer cell lines than in GES-1 cells. The mRNA expression of VRK1 in the gastric cancer cell lines, MNK45 and HGC-27, is notably higher than that in GES-1 was assessed by RT-qPCR (GADPH was a reference gene, n=3). The protein expression patterns were consistent with the mRNA results. **p<0.01

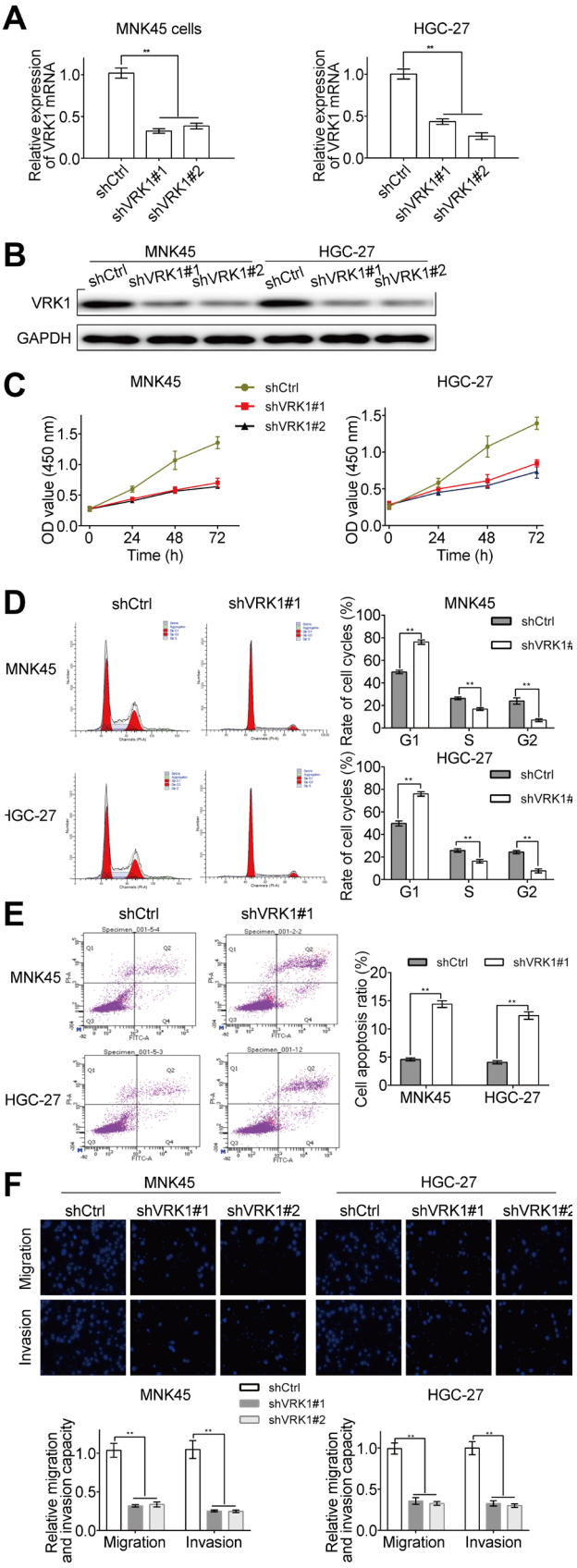

Figure 2. Knockdown of VRK1 inhibits the proliferation, migration, and invasion of gastric cancer cells. A) mRNA and B) protein expression of VRK1 decreased in the shVRK1 cell lines. C) Cell count curve of MNK45 and HGC-27 cells. D) Distributions of cell cycle phases for shCtrl and shVRK1#1 cells, determined by flow cytometry. In comparison to shCtrl cells, VRK1 knockdown induced a significant increase in cells in the G1 phase and decreased the percentage of cells in S and G2 phase. E) The proportion of apoptotic cells in shCtrl and shVRK1#1 cell lines. The apoptosis rate was 11.7–14.9% in shVRK1#1 cells and 3.8–4.5% in shCtrl cells. F) Transwell assays and relative migration and invasion activities of shCtrl and shVRK1 cell lines. Knockdown of VRK1 impairs migration and invasion ability of MNK45 and HGC-27 cell lines. LSD test was used for the statistical analysis. The migration and invasion ability of shVRK1 cells was 35–47% and 28–42% that in shCtrl cells, respectively. **p<0.01

VRK1 knockdown showed a significant increase in the G1 phase cells and a decrease in the percentage of S and G2 phase (Figure 2D). This result indicated that downregulation of VRK1 induced G1/S phase arrest in GC cells. Further analysis indicated that downregulation of VRK1 expression promoted GC cell apoptosis (Figure 2E). Transwell assay was performed to analyze the effect of VRK1 on cell migration and invasion. As shown in Figure 2F, migration and invasion

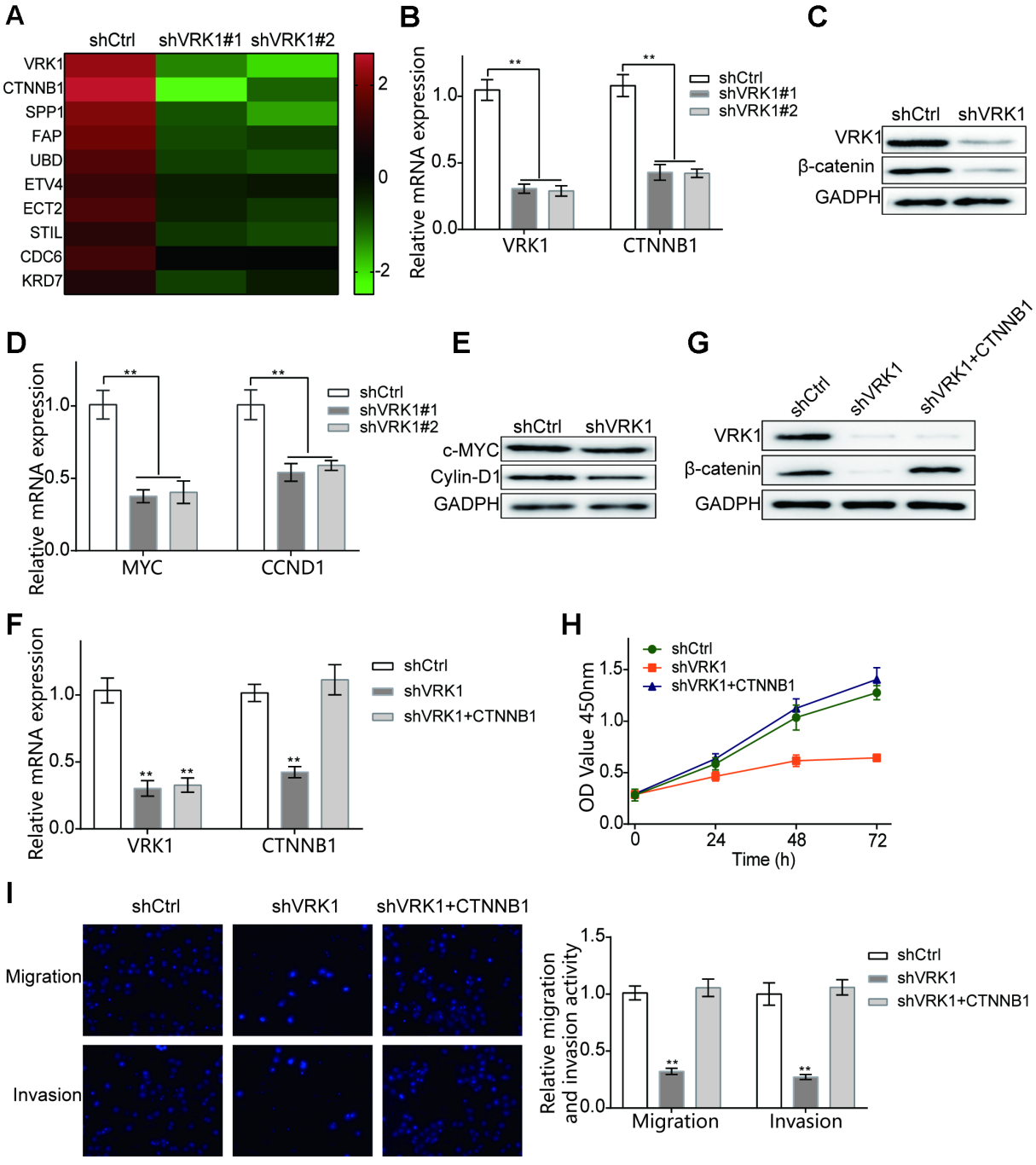

Figure 3. Knockdown of VRK1 decreases the expression of β-catenin. A) Top 10 downregulated genes in both of two shVRK1 cell lines. B) mRNA and C) protein expression of VRK1 and β-catenin. D) mRNA and E) protein expression of the downstream genes of CTNNB1, Myc, and cyclin D, in the shCtrl and shVRK1 cell lines. F) mRNA and G) protein expression of VRK1 and β-catenin in shCtrl, shVRK1, and VRK1+CTNNB1 (CTNNB1 overexpressed in shVRK1 cells) cell lines. H) CCK-8 analysis of shCtrl, shVRK1, and shVRK1+CTNNB1 cell lines. The proliferation of shVRK1+CTNNB1 cells was recovered. I) Transwell assays of shCtrl, shVRK1, and VRK1+CTNNB1 cell lines. Overexpressed CTNNB1 in shVRK1 cell lines restored the activities of migration and invasion. LSD test was used for the statistical analysis. **p<0.01

of the shVRK1 groups of MNK45 and HGC-27 cell lines were significantly repressed, and the migratory and invasive abilities were <50% of shCtrl cells. These results point out that VRK1 is important for the proliferation, invasion, and migration of GC cells.

Knockdown of VRK1 affects the expression of β-catenin. To investigate the mechanism of VRK1 in the progression of GC, differential genes between shCtrl and shVRK1 cells were detected by RNA-seq. CTNNB1 is the top 1 among the 10 genes downregulated in both two shVRK1 lines (shVRK1-1 and shVRK1-2 of MNK45 cells) (Figure 3A). Expression of CTNNB1 in shVRK1 cells was examined with qRT-PCR and western blotting analyses. In agreement with the results of the enrichment analyses, the knockdown of VRK1 resulted in lower mRNA (Figure 3B) and protein (Figure 3C) levels of CTNNB1 than shCtrl treatment. The expression of c-MYC, and cyclin D1, downstream genes of CTNNB1 (β-catenin), was detected subsequently, and the mRNA (Figure 3D) and protein (Figure 3E) levels were also significantly downregulated in shVRK1 cells. Overexpressed CTNNB1 in shVRK1 cell lines (Figures 3F, 3G) resulted in the recovery of proliferation (Figure 3H), migration, and invasion (Figure 3I) activities. These results indicated that VRK1 in GC cells may regulate the expression of β-catenin, a key member of the Wnt/β-catenin pathway.

VRK1 regulates the expression of β-catenin at the transcriptional level. Co-immunoprecipitation (co-IP) assay showed no direct interaction between VRK1 and β-catenin, indicating that VRK1 could not regulate the expression of β-catenin directly (Figure 4A). Through bioinformatics analysis (http://alggen.lsi.upc.es/cgibin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3 and http://gene-regulation.com/pub/databases.html) and literature review [32, 33], it was found that ATF2, CUX1, TCF3, SOX9, and c-Jun may be transcription factors of β-catenin. When we used siRNAs to decrease the expression levels of these transcription factors (Figure 4B), the mRNA (Figure 4C) and protein (Figure 4D) expression levels of β-catenin in the c-Jun siRNA group showed downregulation. An interaction was observed between VRK1 and c-Jun, as so did between c-Jun and β-catenin (Figure 4E). We also found that the expression of c-Jun was decreased significantly in the shVRK1 cell line (Figure 4F).

The Ser243 site of c-Jun phosphorylated by VRK1 determines the nuclear distribution of c-Jun. As a kinase, VRK1 could cooperate with the N-terminal kinase of c-Jun (JNK) to activate c-Jun via phosphorylating the Ser63 and Ser73 of c-Jun [15]. We found that the Ser243 of c-Jun could be phosphorylated by VRK1 while not by JNK (Figure 5A). Subsequently, it was identified that Ser243 phosphorylation

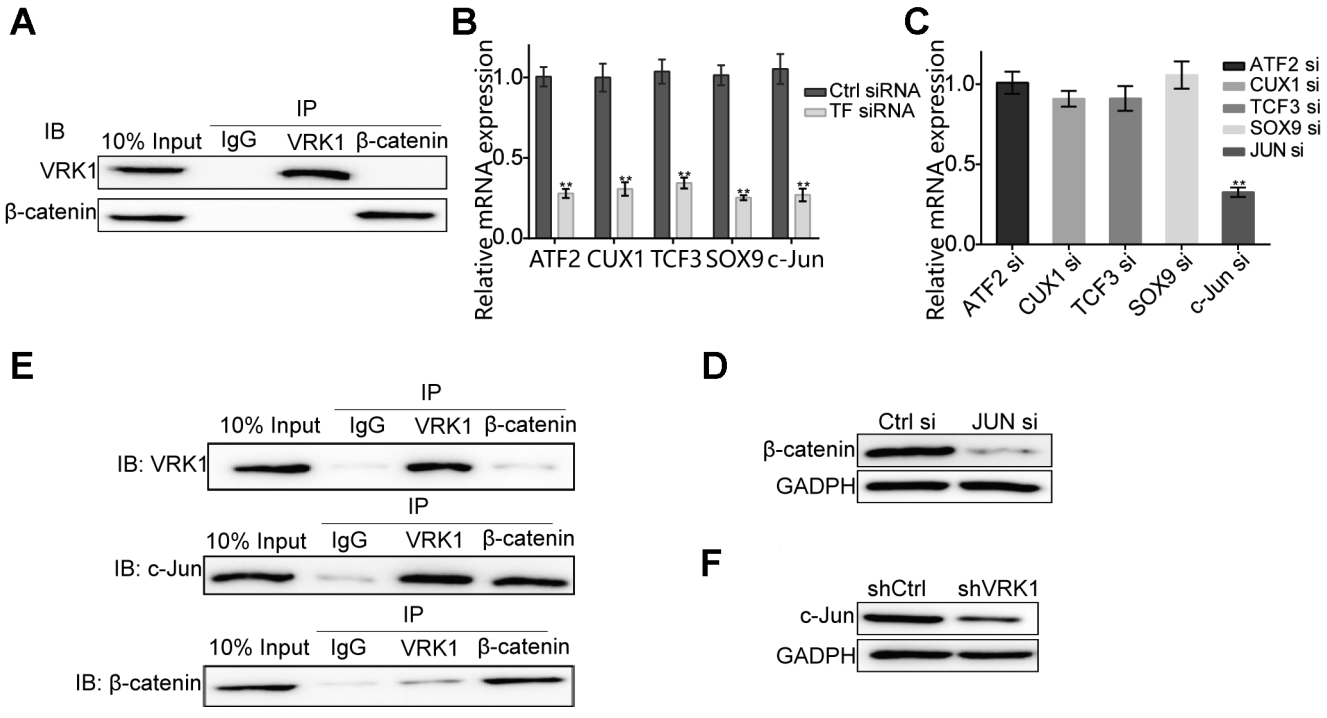

Figure 4. VRK1 regulates the expression of β-catenin at the transcriptional level via c-Jun. A) The interaction between VRK1 and β-catenin. There is no interaction between these two proteins. B) mRNA expression of TFs that may be the transcription factor of CTNNB1, as analyzed by qPCR. C) mRNA expression of CTNNB1 in five TF siRNA and Ctrl siRNA cell lines. Downregulation of c-Jun affected the mRNA level of CTNNB1. D) Protein expression of β-catenin in Ctrl si and c-Jun siRNA cell lines. E) The interaction between VRK1 and c-Jun or β-catenin. F) Protein expression c-Jun in shCtrl and shVRK1 cell lines. **p<0.01

of c-Jun could determine its subcellular localization, c-Jun protein in shCtrl, shJNK, and shJUN+Ser243 cell lines was mainly located in the nucleus, while c-Jun was only detected in the cytoplasm of the shVRK1 and shJUN+A243 cell lines (Figure 5B). Meanwhile, the expression level of c-Jun was reduced both in shJNK and shVRK1 cell lines (Figure 5C). The results showed that both JNK and VRK1 affected the expression of c-Jun, while only VRK1 was crucial for the

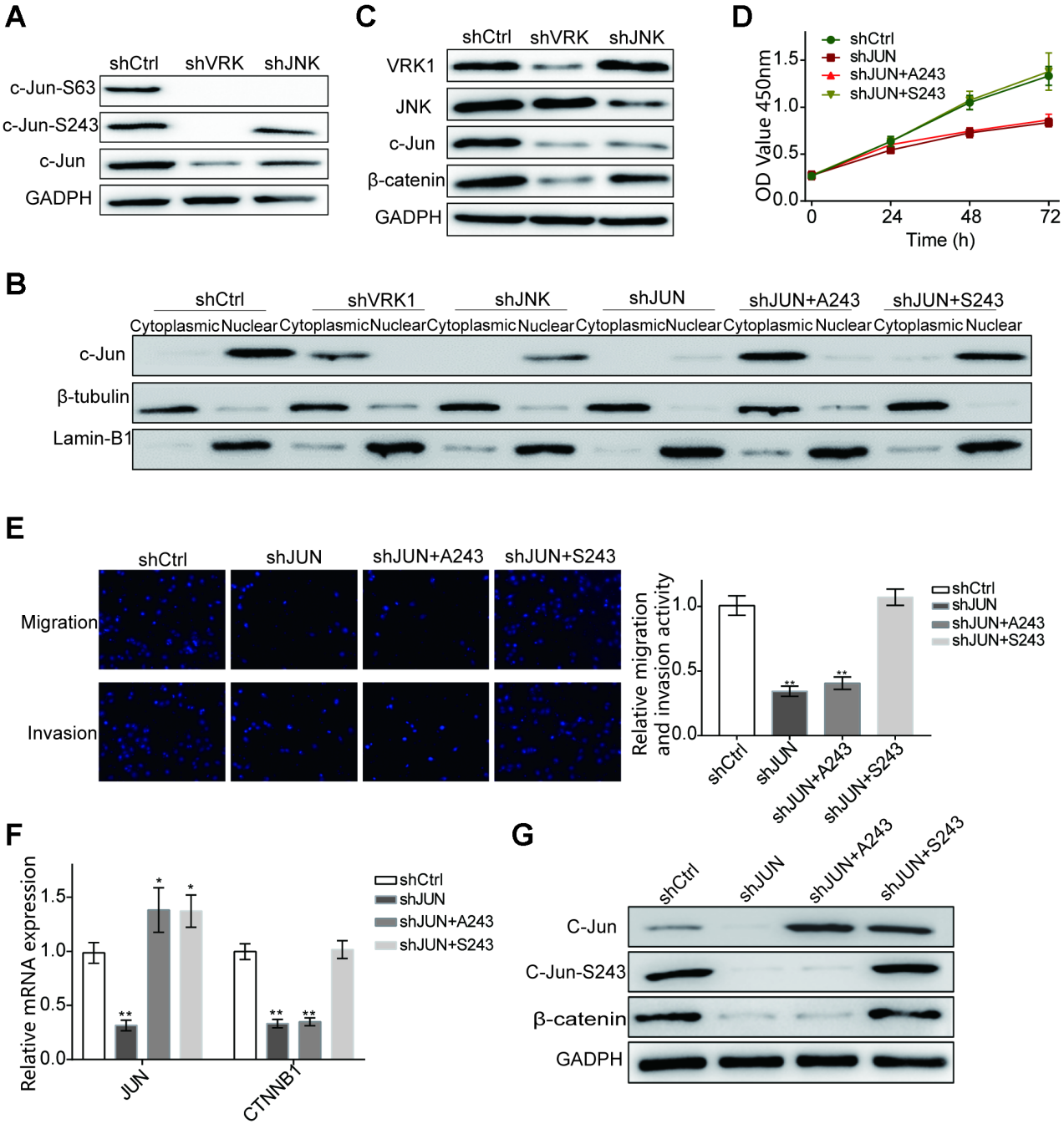

Figure 5. VRK1 determines the nuclear distribution of c-Jun by phosphorylating the Ser243 site of c-Jun. A) Western blot analysis of phosphorylation sites of c-Jun in shCtrl, shVRK1, and shJNK cell lines. B) Protein expression of c-Jun in cytoplasmic and nuclear fractions of shCtrl, shVRK1, shJNK, shJUN, shJUN+A243, and shJUN+S243 cell lines. C) Protein expression of VRK1, JNK, c-Jun, and β-catenin in shCtrl, shVRK, and shJNK cell lines. D) CCK-8 analysis of shCtrl, shJUN, shJUN+A243, and shJUN+S243 cell lines. The proliferation of shJUN+S243 cells was recovered. E) Transwell assays of shCtrl, shJUN, shJUN+A243, and shJUN+S243 cell lines. Migration and invasion abilities of shJUN+S243 cell line (Expression of wildtype c-Jun in shJUN cells) rather than shJIN+A243 cell line (Ser243 was replaced by Ala243) was restored. LSD test was used for the statistical analysis. F) mRNA and G) protein expression of c-Jun and β-catenin in shCtrl, shJUN, shJUN+A243, and shJUN+S243 cell lines. *p<0.05. **p<0.01

nuclear aggregation of c-Jun because of phosphorylated Ser243 of c-Jun. The cell proliferation (Figure 5D), migration, and invasion (Figure 5E) of the shJUN+S243 cell lines were higher significantly compared to the shJUN and shJUN+A243 cell lines, and with a similar level as the shCtrl cell line. Followed by the analysis of β-catenin in mRNA and protein expression level, we found that both mRNA (Figure 5F) and protein (Figure 5G) expression of β-catenin in shJUN+S243 were higher than that in shJUN and shJUN+A243, comparable with shCtrl cell lines.

Discussion

VRK1 is a serine/threonine kinase with a primarily nuclear localization [6, 34], it is highly expressed in proliferative tissues, tumors, and cancer-derived cell lines [8, 9, 12, 34–38], especially, VRK1 has been proposed to exert action in cancer progression as a kinase. In this work, VRK1 mRNA and protein were highly expressed in both the GC cell lines, MNK45 and HGC-27. Further analysis showed that the proliferation, migration, and invasion abilities of GC cells were significantly decreased following the downregulation of VRK1. Therefore, VRK1 plays an important role in GC cells progression.

To explore the specific mechanism underlying the tumor-promoting effect of VRK1 on GC cells, we used microarray analysis to detect the differences in transcription before and after VRK1 knockdown in GC cells. We found that CTNNB1, a key gene involved in the Wnt pathway, was downregulated significantly accompanied by the decreasing of VRK1, which was confirmed by qPCR and western blotting. We found that the downstream genes of CTNNB1 were downregulated accordingly in the shVRK1 cell lines. CTNNB1 overexpression resulted in the recovery of the phenotype of shVRK1 cells, such as inhibition of proliferation, migration, and invasion. These results suggested that the function of VRK1 in GC cells may be related to the activation of the Wnt/β-catenin pathway. The imbalances of the Wnt/β-catenin signaling pathway in the intracellular was involved in lots of diseases including malignant tumors. β-catenin (CTNNB1), a marker of Wnt signal activity and a core factor transmitting Wnt signals, is highly expressed in almost all types of tumorigenesis [39, 40]. Consequently, the activity of the Wnt signaling pathway is abnormally activated, thus, promoting cell colonization and migration, affecting the normal cell cycle, and inducing tumor deterioration [19, 20, 41]. Our research showed that VRK1 affected the expression of β-catenin in GC cells, however, there was not an interaction between VRK1 and β-catenin. Given the downregulation of VRK1 affects the expression of β-catenin in the transcription level, then we speculate that the transcriptional activity of the transcription factor of β-catenin was influenced by VRK1.

We found that the expression of β-catenin was downregulated in the c-Jun siRNA cell line, which is consistent with the results of RNA-seq. Further analysis revealed an interaction between VRK1 and c-Jun, and the expression of β-catenin was recovered when c-Jun was overexpressed in the shVRK1 cells. c-Jun, as a transcription factor and a member of the AP-1 transcription factor family, is responsible for the transcription of a large number of genes that are involved in multiple signaling pathways [27], its levels are in a state of continuous turnover in cells [42]. In the recent past, using ChIP-seq as the tool, the role of c-Jun in multiple diverse physiological pathways has been characterized [43–45]. Many transcriptional factors 1, such as ATF2 [16], CREB1 [46], c-Jun [15], and histone proteins such as mitotic histone H3, can be phosphorylated by VRK1. The JNK can bind to c-Jun and phosphorylate the residues Ser63, Ser73, Ser91, and Ser93 amino acids [47, 48]. Both VRK1 and JNK can phosphorylate c-Jun at the residues Ser63 and Ser73, and then activate the c-Jun transcriptional activity. There is a key difference between JNK and VRK1. JNK is activated in the cytosol while VRK1 is in the nucleus like c-Jun [15]. VRK1 rather than JNK affects the degree of nuclear aggregation of c-Jun. We found that the residues Ser243 can be phosphorylated by VRK1, but not JNK. Expression of wildtype c-Jun in shJUN cells can increase nuclear aggregation of c-Jun, however, the nuclear localization of c-Jun did not restore in the shJUN+A243 cell lines (Ser243 was replaced by Ala243). These results indicate that VRK1 determines the nuclear localization of c-Jun via phosphorylating the residues Ser243 of c-Jun.

Taken together, our results indicated that VRK1 was overexpressed in gastric cancer cells, and VRK1 promoted the proliferation, migration, and invasion of gastric cancer cells. The downregulation of VRK1 was found to decrease the expression of β-catenin. Concurrently, VRK1 regulates the expression of β-catenin at the transcriptional level by affecting the degree of nuclear aggregation of c-Jun through phosphorylating c-Jun at Ser243 residue, the transcription factor can regulate the mRNA expression of β-catenin. These findings might help us to understand gastric cancer development and to identify potential new targets for gastric cancer therapy.

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