MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 3863-3872
DOI: 10.12659/MSM.907889

Received: 2017.11.03
Accepted: 2018.01.08
Published: 2018.06.08

Upregulation of *KIF26B*, Cell Migration and Proliferation of Human Ovarian Cancer Cell Lines *In Vitro*, and Patient Outcomes from Human Bioinformatic Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDE 1 Xingshuang Yang
ABCDE 2 Lan Zhang
ABCDEF 3 Lei Xie

1 Department of Gynecology and Obstetrics, Linyi Central Hospital, Linyi, Shandong, P.R China
2 Department of Gynecology, Taian City Central Hospital, Taian, Shandong, P.R. China
3 Department of Gynecology, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei, P.R. China.

Corresponding Author: Lei Xie, e-mail: leixiepro@foxmail.com
Source of support: Departmental sources

Background: The aim of this study was to assess the expression profile of the *KIF26B* gene, its effects on ovarian cancer cell behavior *in vitro*, the clinical prognostic value of expression of the *KIF26B* gene and associated signaling pathways, from bioinformatics data analysis.

Material/Methods: The Cancer Genome Atlas-Ovarian Cancer (TCGA-OV) database and the online Kaplan-Meier plotter for ovarian cancer were analyzed. Human ovarian cancer cell lines A2780 and SKOV3 were used to study the *in vitro* effects of *KIF26B* gene expression on cell proliferation and cell invasion. Genes that interacted with, co-localized to, and were co-expressed with the *KIF26B* gene (Pearson's r ≥0.6) were identified and underwent Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results: Increased expression of the *KIF26B* gene increased the proliferation and migration of A2780 and SKOV3 cells *in vitro*. KIF26B protein expression was upregulated in ovarian cancer tissue and was associated with lymphatic and venous invasion. TCGA-OV data analysis showed that increased expression of the *KIF26B* gene was significantly associated with reduced 3-year, 5-year, and 10-year overall survival (OS), progression-free survival (PFS), and post-progression survival (PPS). The genes that interacted with, co-localized to, and were co-expressed with *KIF26B* were enriched in several KEGG pathways; upregulation of the *KIF26B* gene was associated with TGF-β signaling pathway.

Conclusions: Upregulation of *KIF26B* enhanced proliferation and migration of ovarian cancer cells *in vitro*. Bioinformatics analysis supported the association between increased expression of the *KIF26B* gene and reduced clinical outcome in patients with ovarian carcinoma.

MeSH Keywords: Lymphatic Metastasis • Ovarian Neoplasms • Prognosis

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/907889

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Epithelial ovarian cancer is a common gynecological malignancy and is one of the leading causes of cancer-related death among women [1]. Ovarian cancers can be derived from the ovarian surface epithelium, inclusion cysts, or the Fallopian tube and are a group of heterogeneous tumors [2–4]. The molecular processes that underlie the onset and progression of ovarian cancer remain poorly understood [2,5,6]. Therefore, to support future development of individualized treatment regimens, it is necessary to explore the genetic and epigenetic dysregulations involved in ovarian carcinoma [7].

The human kinesin family member 26B (*KIF26B*) gene, which maps to 1q44, encodes a member of the kinesin family protein [8]. In a mouse model of embryogenesis, the *KIF26B* gene is specifically involved in the development of limbs, face, and somites [8]. In embryonic kidney development, *KIF26B* plays a critical role by regulating the adhesion of mesenchymal cells in contact with ureteric buds [9]. Some recent studies also found that upregulated the *KIF26B* gene contribute to oncogenesis and confers malignant behaviors of some cancers. Increased expression of *KIF26B* was correlated with larger tumor size, higher risk of metastases and poor prognosis of breast cancer [10], gastric cancer [11], and colorectal cancer [12]. Overexpression of the *KIF26B* gene can also increase chemoresistance in osteosarcoma [13].

In ovarian cancer, the role of some kinesin family members has been gradually revealed. For example, high *KIF14* expression in ovarian cancer might be an independent predictor of worse outcomes [14]. Increased expression of the *KIFC1* gene has been proposed to be a potential prognostic biomarker for metastatic disease, of increased stage, in patients with ovarian cancer [15]. However, the expression profiles, prognostic value, and functional role of the *KIF26B* gene in ovarian cancer are still poorly characterized.

The aim of this study was to assess the expression profile of the kinesin family member 26B (*KIF26B*) gene, and its effects on ovarian cancer cell behavior *in vitro*, and the clinical prognostic value the *KIF26B* gene, and the possible signaling pathways involved in its effects, from bioinformatics data analysis.

Material and Methods

Data analysis from the Human Protein Atlas

The kinesin family member 26B (*KIF26B*) gene expression at the RNA and protein level in normal and malignant ovarian tissue was reviewed by data mining in the Human Protein Atlas (*http://www.proteinatlas.org*) [16,17]. Images of KIF26B protein immunohistochemistry (IHC) staining in normal ovarian tissues and ovarian cancer tissues were downloaded from this database.

Data analysis from The Cancer Genome Atlas-Ovarian Cancer (TCGA-OV)

Secondary analysis of the data from The Cancer Genome Atlas-Ovarian Cancer (TCGA-OV) was performed using the University of California Santa Cruz (UCSC) Xena Browser (*http://xena.ucsc.edu*). Expression of the *KIF26B* gene mRNA in normal ovarian tissues and in ovarian cancer tissues was compared. Online Kaplan-Meier plotting data of overall survival (OS) of patients with primary serous ovarian cancer were generated. The patients were classified into high and low *KIF26B* expression groups according to the median *KIF26B* gene expression levels.

Bioinformatics analysis using the online Kaplan-Meier plotter

The association between the expression levels of the *KIF26B* gene and overall survival (OS), first progression-free survival (FPS), or post-progression survival (PPS) in ovarian cancer patients were investigated by using the online Kaplan-Meier plotter, which is an online database for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data of 1,287 ovarian cancer patients (*http://kmplot.com/analysis/*) [18]. Median *KIF26B* mRNA expression was used as the cutoff. The hazard ratio (HR) with 95% confidence intervals (CI), log-rank p-value, as well as median survivals, were calculated.

Cell culture and transfection

Human ovarian cancer cell lines A2780 and SKOV3 were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin, in a humidified incubator with 5% CO_2 at 37°C.

Full-length *KIF26B* cDNA was cloned into a pcDNA3.1 vector, and the recombinant plasmids were termed as pcDNA3.1-KIF26B. The A2780 and SKOV3 cells were transfected with the recombinant plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Lentiviral *KIF26B* shRNA was obtained from OriGene (Rockville, MD, USA). The A2780 and SKOV3 cells were infected with the lentiviral particles, or the empty controls, in the presence of polybrene, 24 hours after transfection or infection, and the cells then underwent cell counting kit-8 (CCK-8) or transwell assays.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Western blot analysis

Cell samples were lysed using RIPA buffer containing protease and phosphatase inhibitors. Protein concentrations were determined with a BCA assay (Cat. No. 23227) (Thermo Scientific, Waltham, MA, USA). Then, 30 µg of protein was separated in a 10% SDS-PAGE gel and was transferred to nitrocellulose membranes. The membranes were blocked, washed, and incubated overnight with primary antibodies, including anti-KIF26B (1: 200) (ab121992) (Abcam, Cambridge, UK), anti-p-SMAD2 (1: 1000) (ab53100) (Abcam, Cambridge, UK), anti-SMAD2 (1: 2000) (ab40855) (Abcam, Cambridge, UK), or anti-β-actin (1: 1000) (sc-47778) (OriGene, Rockwell, MD, USA). After 24 hours, the membranes were washed three times with TBST, incubated with appropriate secondary antibody conjugated to horseradish peroxidase (HRP) for 30 min at room temperature. The membranes were then washed three times with TBST. The signals were developed using SuperSignal West Femto Maximum Sensitivity Substrate, which is an ultra-sensitive enhanced chemiluminescent (ECL) substrate (Thermo Scientific).

CCK-8 assay of cell proliferation

The cell counting kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) was used to assess cell proliferation, according to the manufacturer's instruction. Briefly, transfected cells (1×10^4 cells/well) were seeded onto a 96-well plate and incubated for 24, 48, 72, or 96 hours, respectively. At the indicated time points, 10 µl of the CCK-8 solution was added to each well and incubated for another 4 hours. Absorbance at 450 nm was measured on a microplate reader.

Transwell assay of cell invasion

Transwell assay of cell invasion was conducted using standardized conditions with BD BioCoat Matrigel invasion chambers (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol. Briefly, 20% FBS in the lower chamber was used as the chemoattractant. Non-invading cells on the upper surface of the membrane were removed, while the invading cells were fixed and stained with 0.1% crystal violet, 24 hours after seeding. The cells that invaded through the filter were quantified by counting the entire area of each filter using a grid and a light microscope at ×20 magnification.

Bioinformatic analysis using Genemania, cBioPortal for Cancer Genomics, and ClueGo

Data mining was performed using Genemania (*http://genemania.org/*) to identify the known proteins with interactions or co-localization with *KIF26B*.The genes strongly co-expressed with the *KIF26B* gene (Pearson's r ≥0.6) in TCGA-OV were identified by using cBioPortal for Cancer Genomics (*www.cbioportal.org*). Then, the genes were loaded into the ClueGo in Cytoscape plug-in [19], to analyze their enrichment in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways ($p\leq0.05$).

Statistical analysis

Continuous variables were reported as the mean ± standard deviation (SD). Comparisons were performed by two-tailed Student's t-test. The log-rank test was used to assess the significance of the difference between survival curves. A p-value <0.05 was considered as statistically significant.

Results

The *KIF26B* gene was not expressed in normal ovarian tissues but was upregulated in ovarian cancer

Using data in the Human Protein Atlas, the expression of the kinesin family member 26B (*KIF26B*) gene at both RNA and protein levels in normal human tissues were characterized. The expression of *KIF26B* RNA was low, and there was no or minimal expression of the KIF26B protein in normal ovarian tissues (Figure 1A–1C; red arrows).

Of the cases of ovarian cancer from the Human Protein Atlas, that were 11 cases that showed mildly positive immunohistochemical staining for expression of the KIF26B protein product, nine cases of that showed medium immunohistochemical staining for expression of the KIF26B protein product, and three cases that showed strong immunohistochemical staining for expression of the KIF26B protein product (Figure 2). These findings suggest that the *KIF26B* gene was upregulated in ovarian cancer compared with normal ovarian tissue.

Upregulation of the *KIF26B* gene was associated with lymphatic and venous invasion

Data mining and analysis of the Cancer Genome Atlas-Ovarian Cancer (TCGA-OV) included the clinicopathologic features of patients with primary ovarian serous cystadenocarcinoma, which is the major type of ovarian cancer. *KIF26B* gene expression was significantly greater in cases with lymphatic invasion (N=133) compared with those without lymphatic invasion (N=77) (p=0.01) (Figure 3A). Also, cases with venous invasion were associated with significantly increased *KIF26B* gene expression compared with cases without venous invasion (p=0.03) (Figure 3B). These findings might provide support for the role of the *KIF26B* gene in vascular invasion of ovarian cancer cells, which is required for tumor metastasis to occur.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

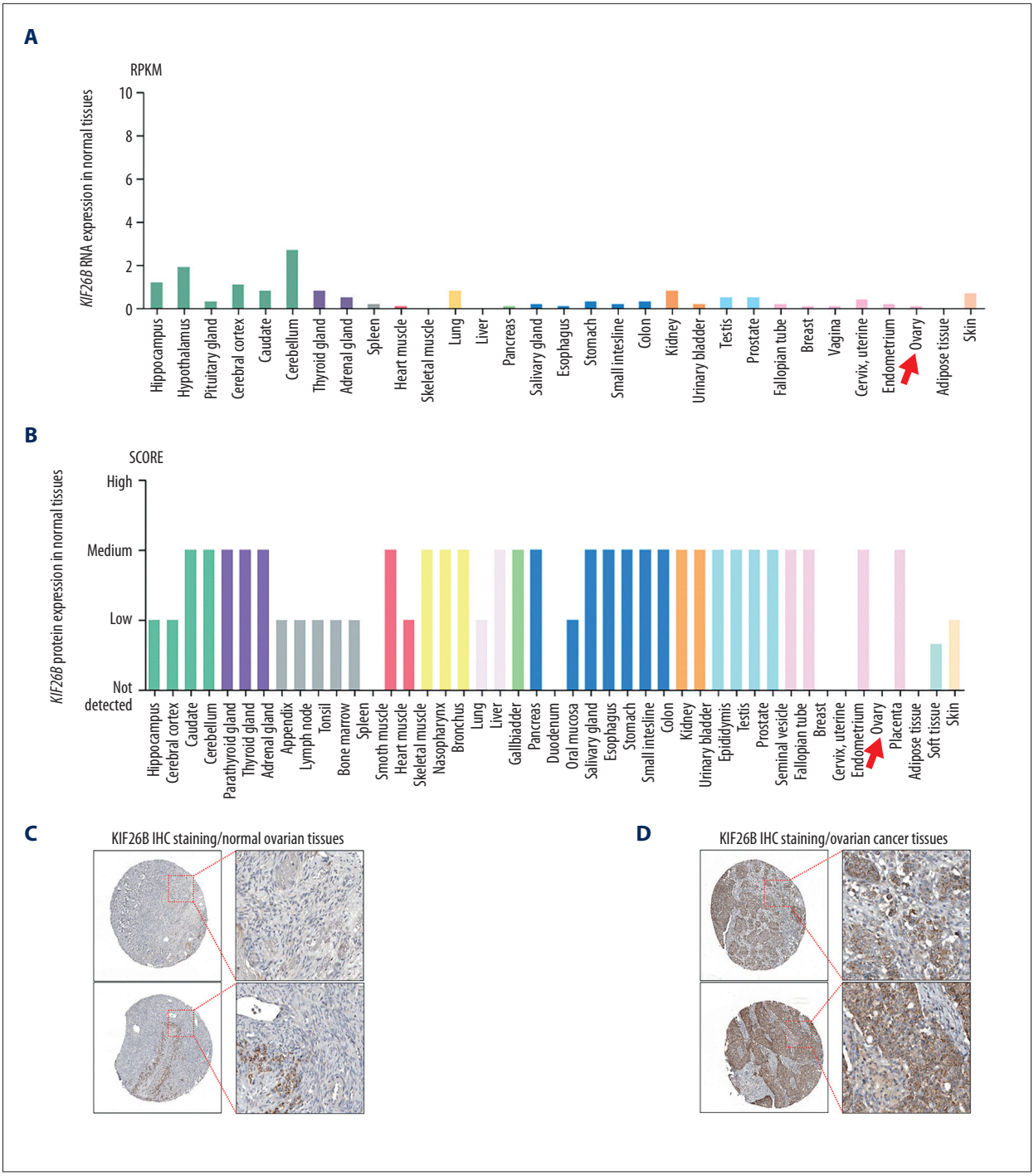

Figure 1. The *KIF26B* gene is not expressed in normal ovarian tissues, but is upregulated in ovarian cancer. (**A, B**) The expression of the *KIF26B* gene at the mRNA (**A**) and protein (**B**) levels in all human tissues. RNA-seq data are reported as median reads per kilobase per million mapped (RPKM) reads, and were generated by the Genotype-Tissue Expression (GTEx) project. (**C, D**) Representative images of immunohistochemistry (IHC) staining of KIF26B protein in normal ovarian tissues (**C**), and ovarian cancer tissues (**D**). Data were obtained from the Human Protein Atlas: (*www.proteinatlas.org/ENSG00000162849-KIF26B/tissue*) and (*www.proteinatlas.org/ENSG00000162849-KIF26B/pathology/tissue/ovarian+cancer*).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

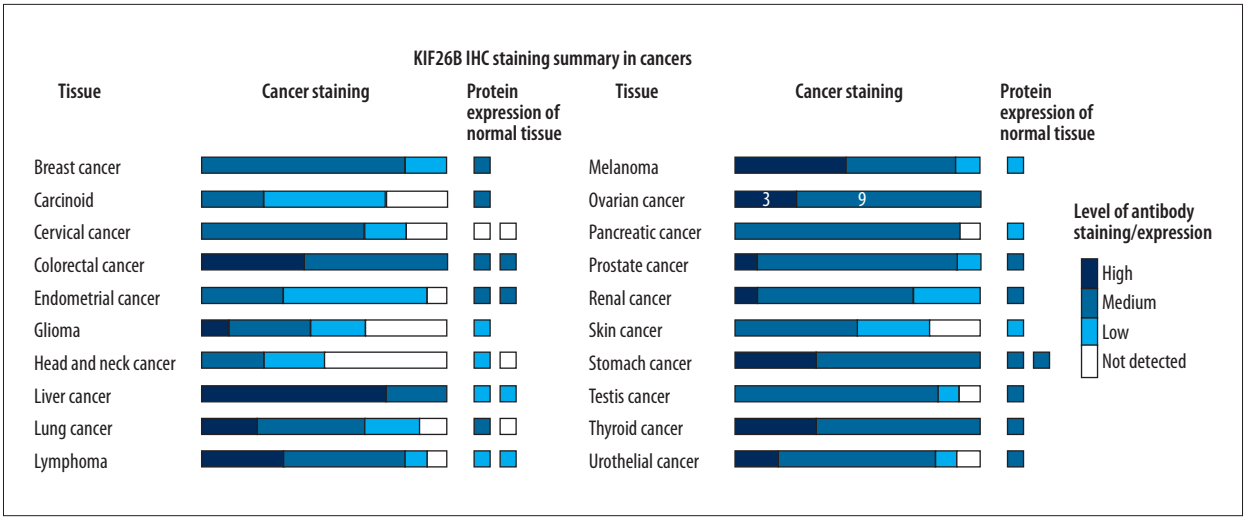

Figure 2. Immunohistochemistry for detection of KIF26B protein product staining in ovarian cancer tissue. Of the cases of ovarian cancer from the Human Protein Atlas, that were 11 cases that showed mild immunohistochemical staining for expression of the KIF26B protein product, nine cases of that showed medium immunohistochemical staining for expression of the KIF26B protein product, and three cases that showed strong immunohistochemical staining for expression of the KIF26B protein product. Data were obtained from the Human Protein Atlas: (*www.proteinatlas.org/ENSG00000162849-KIF26B/pathology*).

Upregulation of the *KIF26B* gene enhanced cell proliferation and migration of ovarian cancer cells *in vitro*

To investigate the regulatory effect of the *KIF26B* gene on cancer cell behavior, the A2780 and SKOV3 ovarian cancer cells were transfected with the pcDNA3.1-*KIF26B* expression vector, or lentiviral short hairpin RNA (shRNA) particles, respectively (Figure 3.C). After confirmation of transfection, the cells underwent the cell counting kit-8 (CCK-8) assay.

Overexpression the *KIF26B* gene significantly increased proliferation of both A2780 and SKOV3 cells (Figure 3D, 3E). In comparison, knockdown of the *KIF26B* gene significantly reduced the proliferation of cells *in vitro* (Figure 3D, 3E). Also, the results of transwell assay showed that increased *KIF26B* gene expression significantly enhanced the invasion of both A2780 and SKOV3 cells, while *KIF26B* gene knockdown reduced the invasive potential of the ovarian cancer cells *in vitro* (Figure 3F–3I).

Increased expression levels of the *KIF26B* gene were associated with reduced overall survival (OS) in patients with ovarian cancer

The expression levels of the *KIF26B* gene and survival outcomes in ovarian cancer patients were analyzed, including overall survival (OS), progression-free survival (PFS), and post-progression survival (PPS). Data mining was performed in both TCGA-OV and the online Kaplan-Meier plotter. In 534 patients with the *KIF26B* gene expression measured by Agilent RNA array in TCGA-OV, high *KIF26B* expression was associated with a significantly reduced 3-year, 5-year, and 10-year OS (p=0.002, p=0.002, and p=0.006, respectively) (Figure 4A–4C).

Data from the online Kaplan-Meier plotter also showed that increased *KIF26B* gene expression was associated with a reduced OS (HR: 1.25; 95% CI, 1.08–1.44; p=0.0021), FPS (HR: 1.36; 95% CI, 1.19–1.56; p<0.001), and PPS (HR: 1.31; 95% CI, 1.11–1.55; p=0.0016) in patients with ovarian cancer (Figure 4D–4F). The median OS, FPS, and PPS in the low *KIF26B* expression group were 46.6 months, 22 months, and 44.7 months, respectively (Figure 4D–4F), compared with median OS, FPS, and PPS in the high *KIF26B* expression group of 37.47 months, 16 months, and 37.43 months, respectively (Figure 4D–4F).

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of genes that interacted with, co-localized to, or were co-expressed with the *KIF26B* gene

Using Genemania, the proteins that interacted with and co-localized to the *KIF26B* gene were identified, and included: *MYH10*, *EPYC*, *ZC3H13*, *BSDC1*, *MAP4K4*, *ANKLE2*, *MPP7*, *CASK*, *LIN7C*, *DLG1,* and *CBLL1* (Figure 5A).

Using cBioPortal for Cancer Genomics, there were 199 identified genes that were co-expressed with the *KIF26B* gene in TCGA-OV (Pearson's r ≥0.6). To provide further insights to provide direction for future exploration of the regulative network of the *KIF26B* gene in ovarian cancer, the genes that interacted with, co-localized to, and co-expressed with *KIF26B* were subjected to the KEGG pathway analysis (Table 1).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

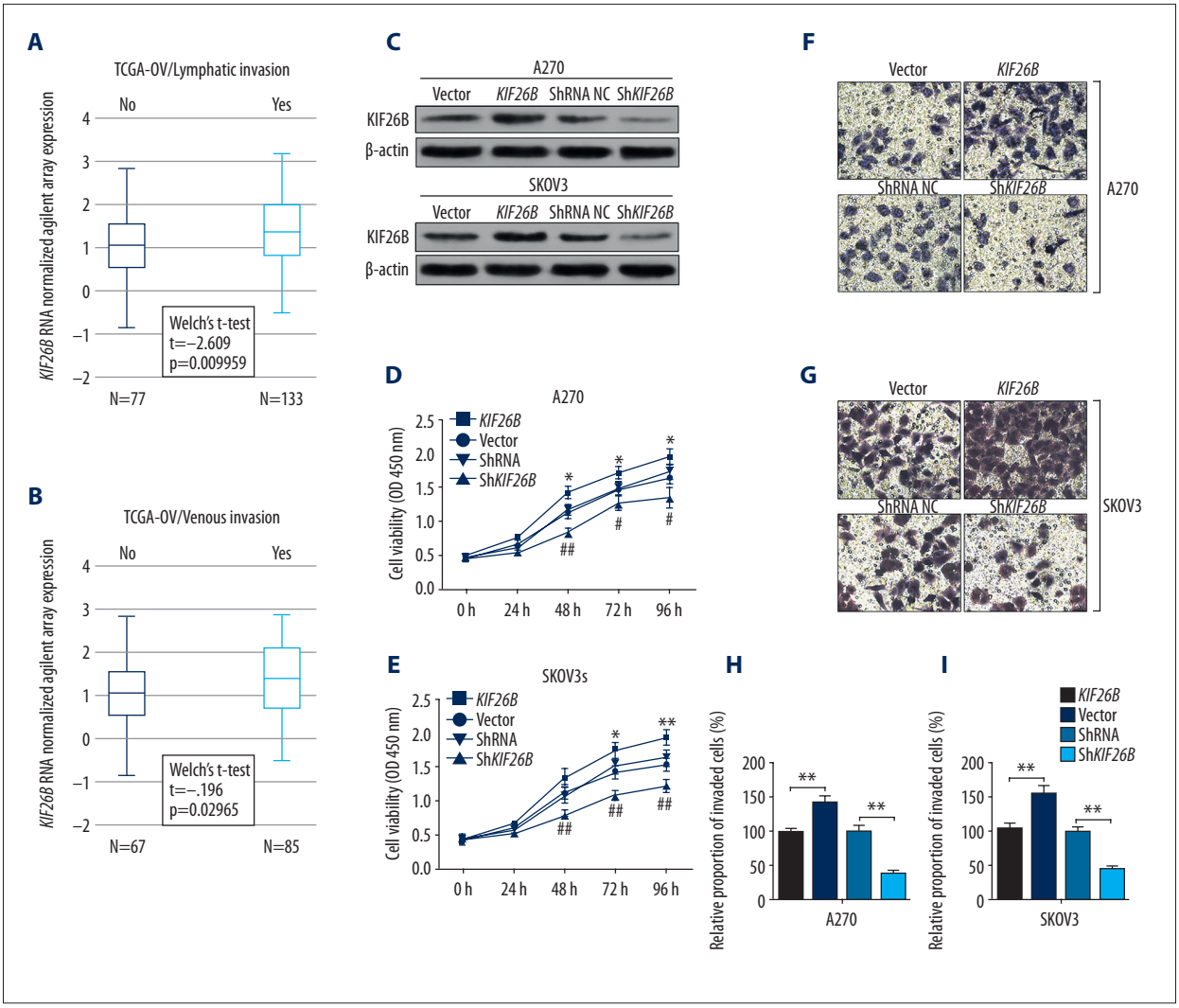

Figure 3. Upregulation of the *KIF26B* gene is associated with lymphatic and venous invasion. (**A, B**) Box plots of expression of the *KIF26B* gene in patients with or without lymphatic invasion (**A**) or venous invasion (**B**). (**C**) Western blot analysis of *KIF26B* expression in A2780 and SKOV3 cells, 24 hours after transfection of *KIF26B* gene expression vectors or *KIF26B* short hairpin RNA (shRNA). (**D, E**) Cell proliferation of A2780 (**D**) and SKOV3 (**E**) cells after transfection, was evaluated using the cell counting kit-8 (CCK-8) assay. * p<0.05 and ** p<0.01 versus the vector control group, # p<0.05 and ## p<0.01 versus the shRNA control group. (**F–I**) Representative images (F, G) and quantitation (H, I) of transwell assay of invasion capability of A2780 (**F, H**) and SKOV3 (G and I) cells after transfection. * p<0.05 and ** p<0.01.

Some of the identified pathways were recognized to be important for cancer initiation and progression and included extracellular matrix (ECM)-receptor interaction, focal adhesion, PI3K-Akt signaling pathway, proteoglycans in cancer, the TGF-beta signaling pathway, the Wnt signaling pathway and p53 signaling pathway (Figure 5B). *COL1A1*, *COL1A2*, *COL4A1*, *COL6A2, COL6A3*, *HSPG2*, *ITGA11* and *ITGA5*, *LAMB1*, *THBS1,* and *THBS2* were enriched in the ECM-receptor interaction pathway. *COL1A1*, *COL1A2*, *COL4A1*, *COL6A2*, *COL6A3*, *IGF1*, I*TGA11*, *ITGA5*, *LAMB1*, *PDGFRA*, *PDGFRB, THBS1,* and *THBS2* were enriched in the focal adhesion pathway. *COL1A1*, *COL1A2*, *COL4A1*, *COL6A2*, *COL6A3*, *CREB3L1*, *FGF7*, *GNG2*, *IGF1*, *ITGA11*, *ITGA5*, *LAMB1*, *PDGFRA*, *PDGFRB*, *THBS1* and *THBS2* were enriched in the PI3K-Akt signaling pathway. *GDF6*, *INHBA*, *NBL1*, *TGFB3* and *THBS1* were enriched in the TGF-beta signaling pathway. *CAMK2A*, *FZD1*, *NFATC2*, *SERPINF1*, *SFRP2* and *SFRP4* are enriched in the Wnt signaling pathway. *IGF1*, *SERPINE1,* and *THBS1* were enriched in the p53 signaling pathway (Table 1).

The *INHBA* gene was the only gene that was co-localized and co-expressed with the *KIF26B* gene in ovarian cancer. Because the *INHBA* gene activates the TGF-β signaling pathway in ovarian cancer [20], an assessment was undertaken as to whether the *KIF26B* gene had a similar function to *KIF26B*. Western

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

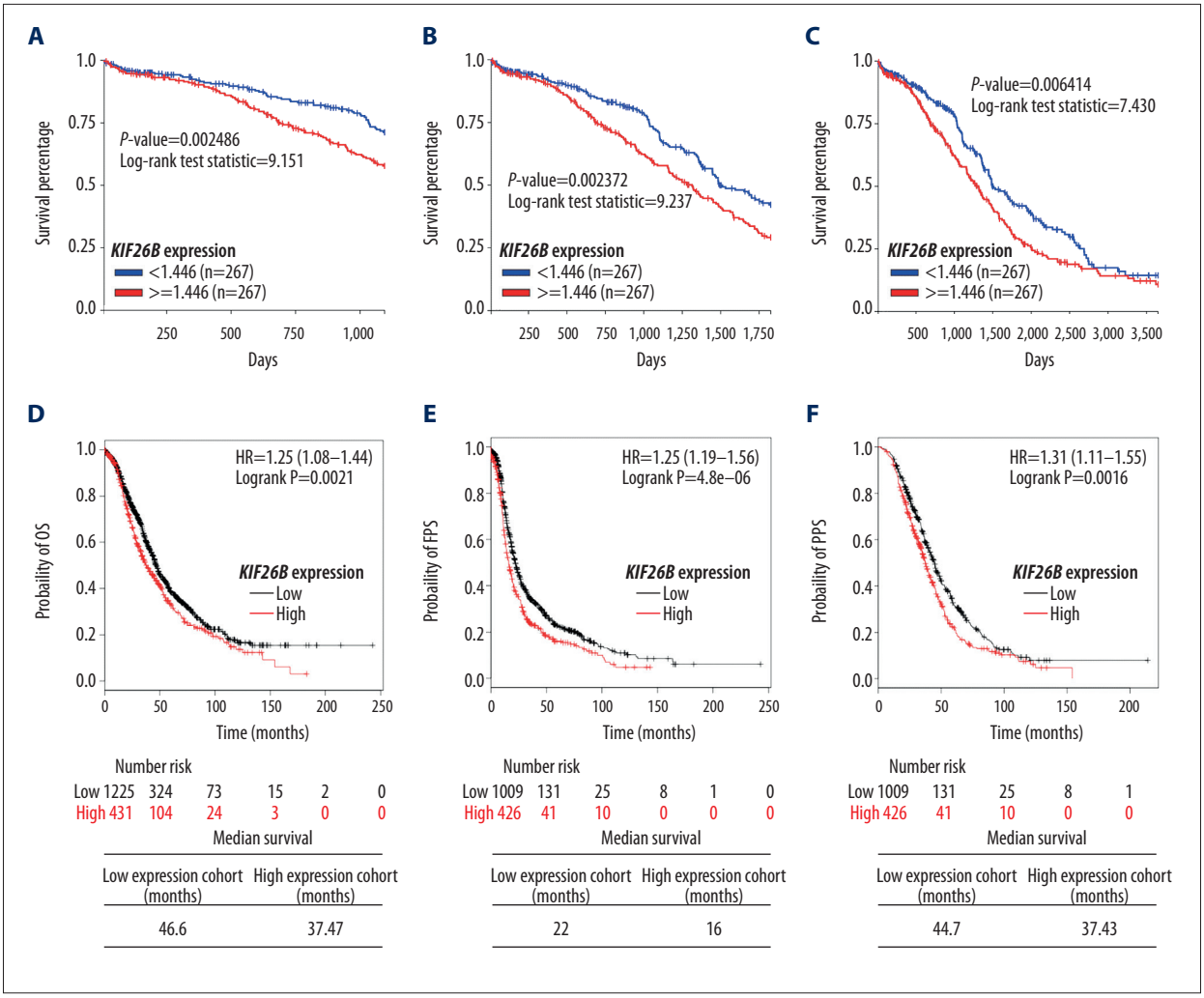

Figure 4. High *KIF26B* gene expression is associated with poor overall survival (OS), progression-free survival (PFS), and post-progression survival (PPS) in patients with ovarian cancer. (**A–C**) Online Kaplan-Meier plotting of 3-year (**A**), 5-year (**B**), and 10-year (**C**) overall survival (OS) in ovarian cancer patients with high or low *KIF26B* gene expression. Data were obtained from The Cancer Genome Atlas-Ovarian Cancer (TCGA-OV) database. (**D–F**) Online Kaplan-Meier plotting (upper) of OS (**D**), FPS (**E**), and PPS (**F**) in patients with ovarian cancer with high/low *KIF26B* gene expression. Median survival was calculated (lower). Data were obtained from online Kaplan-Meier plotting.

blot data showed that overexpression of the *KIF26B* gene induced upregulation of p-SMAD2 levels, while knockdown of the *KIF26B* gene decreased p-SMAD2 levels, in both A2780 and SKOV3 cells *in vitro* (Figure 5C).

Discussion

The kinesin family member 26B (*KIF26B*) gene has been recognized to be an oncogene in several human cancers, including breast cancer [10], gastric cancer [11], and colorectal cancer [12]. In these human cancers, upregulation of the *KIF26B* gene is consistently associated with malignant clinicopathologic features, including increased tumor size, increased tumor grade, lymph node metastasis, and distant metastasis, all of which are associated with reduced tumor prognosis [10–12].

In this study, with the use of bioinformatics data mining in two large databases, the expression of the *KIF26B* gene and KIF26B protein was not present in normal ovarian tissues, but was upregulated in ovarian cancer. Upregulation of the *KIF26B* gene was significantly associated with lymphatic and venous invasion, based on data from Cancer Genome Atlas-Ovarian Cancer (TCGA-OV). In *in-vitro* cell models using the ovarian carcinoma cell lines, A2780 and SKOV3, cells with overexpression of the *KIF26B* gene had an increased rate of proliferation and increased cell migration and invasion properties. In comparison, knockdown of the *KIF26B* gene impaired cell proliferation

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

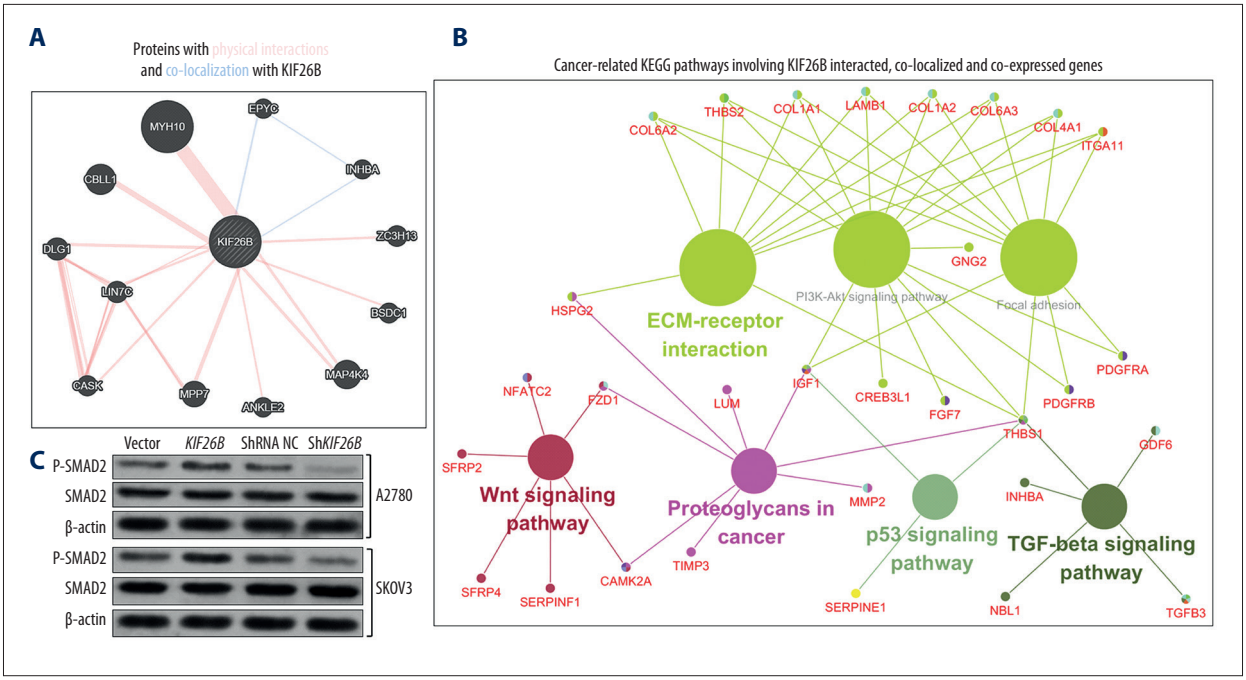

Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of KIF26B and its co-expressed genes in The Cancer Genome Atlas-Ovarian Cancer (TCGA-OV) database. (**A**) The proteins that interacted and co-localize with KIF26B. (**B**) Cancer-related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways involving genes that interacted with, co-localized to, and were co-expressed with the *KIF26B* gene. (**C**) Western blot analysis of p-*SMAD2* and *SMAD2* expression in A2780 and SKOV3 cells, 46 hours following transfection of the *KIF26B* expression vectors or *KIF26B* shRNA.

and invasion *in vitro*. These findings suggest that the *KIF26B* gene might be a key gene in the regulation of the metastatic potential of ovarian cancer. The prognostic value of the *KIF26B* gene in ovarian cancer was further investigated in this study using the online Kaplan-Meier plotter survival analysis. This analysis method showed that high levels of expression of the *KIF26B* gene were associated with reduced overall survival (OS), progression-free survival (PFS), and post-progression survival (PPS) in patients with ovarian cancer.

Although the findings of this study identified the expression profile and potential prognostic value of expression of the *KIF26B* gene in ovarian cancer, the signaling pathways involved remain unknown. In gastric cancer, expression of *KIF26B* has been shown to enhance the vascular endothelial growth factor (VEGF) signaling pathway, via inducing the expression of *VEGFA*, *PXN*, *FAK*, *PIK3CA*, *BCL2*, and *CREBI* [11]. Another study found that expression of the *KIF26B* gene was positively correlated with increased tissue expression of the proliferation marker, Ki67, in colorectal cancer cells [12].

This study used bioinformatics analysis using Genemania and cBioPortal for Cancer Genomics, which identified the genes that interacted with, co-localized to, and were co-expressed with the *KIF26B* gene, and analyzed their enrichment in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Among the identified pathways, the ECM-receptor interaction, the focal adhesion, the PI3K-Akt signaling pathway, the proteoglycans in cancer, the TGF-beta signaling pathway, the Wnt signaling pathway, and the p53 signaling pathway were closely related to the pathological development of ovarian cancer. This study showed that the key genes involved in these pathways included: *COL1A1*, *COL1A2*, *COL4A1*, *COL6A2*, *COL6A3*, *HSPG2*, *ITGA11*, *ITGA5*, *LAMB1*, *THBS1*, *THBS2*, *IGF1*, *PDGFRA*, *PDGFRB*, *CREB3L1*, *FGF7*, *GNG2*, *GDF6*, *INHBA*, *NBL1*, *TGFB3*, *CAMK2A*, *FZD1*, *NFATC2*, *SERPINF1*, *SFRP2*, *SFRP4* and *SERPINE1*. Previous studies have shown that the genes *COL1A1*, *COL1A2*, *LAMBI*, and *FZD1* were significantly upregulated in multi-drug resistant ovarian cancer cells when compared with untreated cells [21,22]. Also, *COL6A2* and *THBS2* are in a ten-gene signature that is associated with poor OS in patients with high-grade serous ovarian cancer [23].

DNA duplication of the *FGF7* gene has been reported to occur in ovarian cancer and to have a significant role in the development of ovarian cancer [24]. The *SFRP4* gene is a Wnt gatekeeper modulating epithelial-mesenchymal transition (EMT), cell migration, and downstream Wnt signaling in serous ovarian cancer cells [25]. The *INHBA* gene was the only gene that was found to be co-localized and co-expressed with the KIF26B gene in ovarian cancer in this study. The upregulation of the *INHBA* gene has previously been shown to contribute to EMT

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the *KIF26B* gene and the interactive, co-localized, and co-expressed genes in breast cancer.

GO ID	GO term	Term P value	% associated genes	Nr. genes	Associated genes found
GO: 0004512	ECM-receptor interaction	1.5E-9	13.41	11.00	[COL1A1, COL1A2, COL4A1, COL6A2, COL6A3, HSPG2, ITGA11, ITGA5, LAMB1, THBS1, THBS2]
GO: 0004974	Protein digestion and absorption	57.0E-9	11.11	10.00	[COL11A1, COL1A1, COL1A2, COL3A1, COL4A1, COL5A1, COL5A2, COL6A2, COL6A3, ELN]
GO: 0004510	Focal adhesion	310.0E-9	6.53	13.00	[COL1A1, COL1A2, COL4A1, COL6A2, COL6A3, IGF1, ITGA11, ITGA5, LAMB1, PDGFRA, PDGFRB, THBS1, THBS2]
GO: 0004151	PI3K-Akt signaling pathway	1.1E-6	4.68	16.00	[COL1A1, COL1A2, COL4A1, COL6A2, COL6A3, CREB3L1, FGF7, GNG2, IGF1, ITGA11, ITGA5, LAMB1, PDGFRA, PDGFRB, THBS1, THBS2]
GO: 0004933	AGE-RAGE signaling pathway in diabetic complications	120.0E-6	7.07	7.00	[COL1A1, COL1A2, COL3A1, COL4A1, MMP2, SERPINE1, TGFB3]
GO: 0004390	Hippo signaling pathway	340.0E-6	5.19	8.00	[DLG1, FRMD6, FZD1, GDF6, LATS2, SERPINE1, SNAI2, TGFB3]
GO: 0004270	Vascular smooth muscle contraction	420.0E-6	5.79	7.00	[ACTA2, ACTG2, CALD1, EDNRA, MRVI1, PRKG1, PTGIR]
GO: 0005205	Proteoglycans in cancer	460.0E-6	4.43	9.00	[CAMK2A, FZD1, HSPG2, IGF1, ITGA5, LUM, MMP2, THBS1, TIMP3]
GO: 0005146	Amoebiasis	740.0E-6	6.25	6.00	[COL1A1, COL1A2, COL3A1, COL4A1, LAMB1, TGFB3]
GO: 0005144	Malaria	2.2E-3	8.16	4.00	[LRP1, TGFB3, THBS1, THBS2]
GO: 0004350	TGF-beta signaling pathway	2.5E-3	5.95	5.00	[GDF6, INHBA, NBL1, TGFB3, THBS1]
GO: 0004360	Axon guidance	3.6E-3	4.00	7.00	[CAMK2A, CXCL12, NFATC2, SEMA3D, SEMA6B, SEMA7A, SLIT2]
GO: 0004392	Hippo signaling pathway -multiple species	4.2E-3	10.34	3.00	[DCHS1, FRMD6, LATS2]
GO: 0004310	Wnt signaling pathway	5.6E-3	4.20	6.00	[CAMK2A, FZD1, NFATC2, SERPINF1, SFRP2, SFRP4]
GO: 0005214	Glioma	5.9E-3	6.25	4.00	[CAMK2A, IGF1, PDGFRA, PDGFRB]
GO: 0005218	Melanoma	7.7E-3	5.80	4.00	[FGF7, IGF1, PDGFRA, PDGFRB]
GO: 0005414	Dilated cardiomyopathy	19.0E-3	4.44	4.00	[IGF1, ITGA11, ITGA5, TGFB3]
GO: 0004115	p53 signaling pathway	43.0E-3	4.35	3.00	[IGF1, SERPINE1, THBS1]

in both normal and ovarian cancer cells, and also promotes the invasive behavior of ovarian cancer cells [26]. The *INHBA* gene has also been previously reported to be part of the metastasis-associated gene expression signature of ovarian cancer, together with the THBS2 gene [20].

The TGF-β signaling pathway plays a critical role in the pathogenesis of ovarian cancer, including in DNA methylation during the EMT associated with ovarian cancer cells [27]. The findings of this study showed that upregulation of the *KIF26B* gene enhanced the TGF-β signaling pathway in ovarian cancer cells, which might be one of the mechanisms contributing to the oncogenic properties of the *KIF26B* gene. However, current understanding of the association between the expression of *KIF26B* and other genes in ovarian cancer remains unclear. Further future studies are needed to explore the effects of *KIF26B* gene expression in the regulatory and signaling networks in human breast cancer.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Conclusions

The findings of this study showed that, in ovarian carcinoma, upregulation of the *KIF26B* gene enhanced cell proliferation, cell migration, and cell invasion of ovarian cancer cells *in vitro*, and was associated with poor survival outcomes when bioinformatics data were analyzed.

References:

1. Qiu HL, Deng SZ, Li C et al: High expression of KIF14 is associated with poor prognosis in patients with epithelial ovarian cancer. Eur Rev Med Pharmacol Sci, 2017; 21: 239–45
2. Mccluggage WG: Morphological subtypes of ovarian carcinoma: A review with emphasis on new developments and pathogenesis. Pathology, 2011; 43: 420–32
3. Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. Nature, 2011; 474: 609–15
4. Dong L, Che H, Li M, Li X: Sam68 is overexpressed in epithelial ovarian cancer and promotes tumor cell proliferation. Med Sci Monit, 2016; 22: 3248–56
5. Dong L, Hui L: HOTAIR promotes proliferation, migration, and invasion of ovarian cancer SKOV3 cells through regulating PIK3R3. Med Sci Monit, 2016; 22: 325–31
6. Yiwei T, Hua H, Hui G et al: HOTAIR Interacting with MAPK1 regulates ovarian cancer skov3 cell proliferation, migration, and invasion. Med Sci Monit, 2015; 21: 1856–63
7. Yao Y, Yu L, Su X et al: Synthesis, characterization and targeting chemotherapy for ovarian cancer of trastuzumab-SN-38 conjugates. J Control Release, 2015; 220: 5–17
8. Marikawa Y, Fujita TC, Alarcon VB: An enhancer-trap LacZ transgene reveals a distinct expression pattern of Kinesin family 26B in mouse embryos. Dev Genes Evol, 2004; 214: 64–71
9. Uchiyama Y, Sakaguchi M, Terabayashi T et al: KIF268, a kinesin family gene, regulates adhesion of the embryonic kidney mesenchyme. Proc Natl Acad Sci USA, 2010; 107: 9240–45
10. Wang Q, Zhao ZB, Wang G et al: High expression of KIF26B in breast cancer associates with poor prognosis. PLoS One, 2013; 8: e61640
11. Zhang H, Ma RR, Wang XJ et al: KIF26B, a novel oncogene, promotes proliferation and metastasis by activating the VEGF pathway in gastric cancer. Oncogene, 2017; 36(40): 5609–19
12. Wang J, Cui F, Wang X et al: Elevated kinesin family member 26B is a prognostic biomarker and a potential therapeutic target for colorectal cancer. J Exp Clin Cancer Res, 2015; 34: 13
13. Pu Y, Yi Q, Zhao F et al: MiR-20a-5p represses multi-drug resistance in osteosarcoma by targeting the KIF26B gene. Cancer Cell Int, 2016; 16: 64
14. Theriault BL, Pajovic S, Bernardini MQ et al: Kinesin family member 14: An independent prognostic marker and potential therapeutic target for ovarian cancer. Int J Cancer, 2012; 130: 1844–54
15. Pawar S, Donthamsetty S, Pannu V et al: KIFCI, a novel putative prognostic biomarker for ovarian adenocarcinomas: Delineating protein interaction networks and signaling circuitries. J Ovarian Res, 2014; 7: 53
16. Uhlen M, Oksvold P, Fagerberg L et al: Towards a knowledge-based Human Protein Atlas. Nat Biotechnol, 2010; 28: 1248–50
17. Thul PJ, Akesson L, Wiking M et al: A subcellular map of the human proteome. Science, 2017; 356(6340): pii: eaal3321
18. Gyorffy B, Lanczky A, Szallasi Z: Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. Endocr Relat Cancer, 2012; 19: 197–208
19. Bindea G, Galon J, Mlecnik B: CluePedia Cytoscape plugin: Pathway insights using integrated experimental and in silico data. Bioinformatics, 2013; 29: 661–63
20. Kim H, Watkinson J, Varadan V, Anastassiou D: Multi-cancer computational analysis reveals invasion-associated variant of desmoplastic reaction involving INHBA, THBS2 and COL11A1. BMC Med Genomics, 2010; 3: 51
21. Januchowski R, Swierczewska M, Sterzynska K et al: Increased expression of several collagen genes is associated with drug resistance in ovarian cancer cell lines. J Cancer, 2016; 7: 1295–310
22. Januchowski R, Zawierucha P, Rucinski M et al: Extracellular matrix proteins expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. Biomed Res Int, 2014; 2014: 365867
23. Cheon DJ, Tong Y, Sim MS et al: A collagen-remodeling gene signature regulated by TGF-beta signaling is associated with metastasis and poor survival in serous ovarian cancer. Clin Cancer Res, 2014; 20: 711–23
24. Yasuhara T, Okamoto A, Kitagawa T et al: FGF7-like gene is associated with pericentric inversion of chromosome 9, and FGF7 is involved in the development of ovarian cancer. Int J Oncol, 2005; 26: 1209–16
25. Ford CE, Jary E, Ma SS et al: The Wnt gatekeeper SFRP4 modulates EMT, cell migration and downstream Wnt signalling in serous ovarian cancer cells. PLoS One, 2013; 8: e54362
26. Basu M, Bhattacharya R, Ray U et al: Invasion of ovarian cancer cells is induced byPITX2-mediated activation of TGF-beta and Activin-A. Mol Cancer, 2015; 14: 162
27. Cardenas H, Vieth E, Lee J et al: TGF-beta induces global changes in DNA methylation during the epithelial-to-mesenchymal transition in ovarian cancer cells. Epigenetics, 2014; 9: 1461–72

Conflict of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]