Vigilin is overexpressed in hepatocellular carcinoma and is required for HCC cell proliferation and tumor growth

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Abstract. Vigilin contains multiple KH domains and is an evolutionarily conserved RNA-binding protein from yeast to the human. Its reported roles in human carcinogenesis are controversial in different types of human cancers. To obtain the specific expression profiles of vigilin in human hepatocellular carcinomas (HCCs), we examined vigilin protein levels in normal human liver, liver cirrhosis, adjacent non-tumor liver and HCC tumor tissues as well as in several HCC cell lines. We discovered that vigilin expression increased progressively from the liver cirrhosis tissue to adjacent non-tumor liver tissue and then to HCC tumor cells. Vigilin protein was also overexpressed in all three HCC cell lines examined, HepG2, BEL7402 and SMMC7721, when compared with the vigilin expression level in the L-02 human embryonic hepatocyte cell line. We further investigated the impact of vigilin knockdown on HCC cell proliferation, survival, motility, tumor growth and sensitivity to chemotherapy. We found that knockdown of vigilin in the BEL7402 HCC cells significantly inhibited their proliferation, colony formation and migration, but largely enhanced the cisplatin treatment-induced growth inhibition of these cells in culture. We also found that vigilin knockdown effectively inhibited the growth of BEL7402 cell-derived xenograft tumors in nude mice by decreasing the proliferation and increasing the apoptosis of the BEL7402 HCC cells. Taken together, these results suggest that progressively upregulated vigilin may serve as a molecular risk marker for HCC development, and targeting vigilin may help to inhibit HCC cell growth, survival and migration.

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Introduction

Human hepatocellular carcinoma (HCC) is highly malignant and is the second cause of cancer-related death in China. The incidence rates of HCCs are high in Western countries, central Africa and eastern and southeastern Asia. However, the molecular mechanisms underlying HCC pathogenesis remain poorly understood. The clinical efficacy of current chemotherapies and available targeted therapies for HCC is also limited (1). Thus, basic, translational and clinical studies are urgently needed to identify new therapeutic targets for the treatment of HCCs.

Vigilin, a multi-KH domain protein, is highly conserved from yeast to the human. As an RNA-binding protein, vigilin is localized in the nuclear envelope (2), nucleus (3,4) and rough endoplasmic reticulum (ER) where it associates with the polysome (5,6), translation elongation factor 1A and WD-repeat protein Asc1p (2,5,7,8). Vigilin is involved in translational control (9), nuclear export of tRNA (10), cytoplasmic transport of RNA (4,11), metabolism of specific mRNAs (12) and RNAi-mediated vigilin silencing (13). Human vigilin was initially characterized as a high density lipoprotein binding protein (HDLBP; HBP) (14), and was subsequently shown to play an important role in the cellular sterol metabolism in human atherogenesis (15). However, the role of vigilin in human types of cancers remains controversial. Studies showing vigilin overexpression in the TOV112D human ovarian cancer cells and human prolactinomas and gastric cancer suggest that vigilin may play a promotive role in carcinogenesis (16-19). In contrast, vigilin can bind to the 3' untranslated mRNA region of the c-fms proto-oncogene encoding the receptor of

macrophage colony stimulating factor in breast cancer cells to destabilize this mRNA and inhibit its translation, suggesting vigilin may have a tumor-suppressor role in these cells (20). Similarly, the role of vigilin in human HCC cell growth is also poorly understood.

The present study was designed to examine the effects of vigilin on HCC cells in culture and in mouse models. We showed that vigilin expression was upregulated in a subset of human HCCs and vigilin was required for HCC cell proliferation, survival, migration and tumor growth. Knockdown of vigilin sensitized cells to cisplatin, a widely used drug for cancer chemotherapy, to inhibit HCC cell growth.

Materials and methods

Clinical specimens and cell lines. Thirty-three human primary hepatocellular carcinomas (HCCs) and paired adjacent non-tumor tissues were collected at the Department of Surgery, West China Hospital of Sichuan University. Demographic patient data and clinicopathological characteristics were also obtained from the same hospital. The criteria for grading HCCs were based on the classification of the World Health Organization. Seven normal liver tissues were obtained as controls from subjects affected by hepatic hemangioma, intrahepatic stones, liver cysts, other non-cancerous or non-cirrhosis liver diseases. Eight cirrhosis liver tissues were obtained from subjects with liver cirrhosis other than HCC. Informed consent was obtained from each patient, and the study was approved by the appropriate institutional review committees. Cold cup biopsies of tumor tissues were snap frozen and stored in liquid nitrogen. The remaining tissues were fixed in 10% formalin in saline for 24 h, followed by dehydration in ethanol and embedding in paraffin for diagnostic assessment.

The L-02 human embryonic liver cell line and the three human HCC cell lines including HepG2, SMMC7721 and BEL-7402 were obtained from the Chinese Type Culture Collection (CTCC). Cells were cultured in standard media at 37˚C in 5% CO_2.

Immunohistochemistry (IHC). For detection of vigilin protein by IHC, clinically collected human HCC specimens and BEL7402 xenograft tumor tissues collected from nude mice were fixed in 10% formalin in saline for 24 h, dehydrated and embedded in paraffin. Tissue sections were prepared, deparaffinized, rehydrated and treated with 3% hydrogen peroxide in a citric acid buffer (pH 6.0) at 95˚C for 40 min. The vigilin antibody was made in our laboratory and its specificity was validated using lymph cells, cancer cells and tumor tissues with known high and low vigilin protein expression as described previously (21). Primary antibodies against vigilin (1:200), Ki67 (1:50) and activated caspase 3 (1:50) (both from Abcam, UK) were diluted with phosphate-buffered saline (PBS) containing 10% normal goat serum. Pretreated tissue sections were incubated with the diluted primary antibodies in a humidified chamber at 37˚C for 45 min. Tissue sections were subsequently inoculated with the Dako Rapid EnVision secondary antibody system (Dako, USA) and developed using the 3,3'-diaminobenzidine (DAB) substrate. Sections were further counterstained with hematoxylin. A blank control was obtained by excluding the primary antibody in the staining. Stained sections were examined under an Olympus BX41 microscope and imaged using a CCD camera. The intensity of vigilin immunoreactivity was scored using a four-scale system (22): 0, no expression; 1, weak expression; 2, medium expression and 3, high expression. Cells positive for Ki67 or activated caspase-3 immunostaining were counted against the total number of cells in the viewing fields.

Western blotting. Cultured cells or xenograft tumors were lysed in an ice-cold lysis buffer (KaiJi, China). The lysates were cleared by centrifugation at 16,000 x g at 4˚C for 10 min. Supernatants were collected, and their protein concentrations were determined using the Bradford protein assay reagent (KaiJi). Sixty micrograms of total protein for each sample was analyzed by western blotting. Antibodies used for western blotting were against vigilin (1:100) and β-actin (1:1,000 dilution; Santa Cruz, USA). The bound primary antibodies were visualized using the enhanced chemiluminescence detection system (Pierce Chemical, USA).

Lentiviral-mediated delivery of shRNA and generation of vigilin stable knockdown cell lines. Human vigilin shRNAs were constructed into the pLKO.1-puro vector from Sigma-Aldrich (23). The targeting sequence of vigilin mRNA was 5'-UCCCAACACAAGUAUGUCAUU-3' (24). A non-targeting shRNA, 5'-CGCUGAGUACUUCGAAAUGUC, from Sigma-Aldrich was used as a negative control. The shRNA vectors were co-transfected with the lentiviral packaging plasmids psPAX2 and pMD2.G (Addgene, Cambridge, MA, USA) into HEK293T cells using Lipofectamine 2000 (Invitrogen, USA). The media containing lentivirus particles were collected, filtered and overlaid onto BEL7402 cells in the presence of 8 μg/ml polybrene for 24 h. The lentivirus expressing the luciferase-specific shRNA was used to generate the control BEL7402 cell line. Subsequently, the infected cells were selected with 3 μg/ml of puromycin (Acros, Belgium). The resulting stable control and vigilin knockdown cell lines were termed BEL7402-Ctrl and BEL7402-KD cells, respectively.

Cell growth assay. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay was employed to measure cell growth in culture. BEL7402-Ctrl and BEL7402-KD cells ($2x10^3$ in 150 μl of medium) were plated in 96-well plates and cultured at 37˚C under 5% CO_2. MTT assays were performed at 24, 48, 72 and 96 h after cells were seeded. For the MTT assay, 20 μl of MTT reagent (5 mg/ml in stock solution) was added to each well, and the cells were incubated for another 4 h under normal culture condition. After removing the culture medium, DMSO was added to each well and the plate was shaken horizontally for 10 min at room temperature to dissolve the formazan crystals formed in live cells. Absorbance at 570 nm was read with a micro-ELISA reader (Bio-Rad, USA). Each assay was carried out in 6 replicates.

Colony formation assay. BEL7402-Ctrl and BEL7402-KD cells were seeded in 6-well plates (200 cells/well) and cultured in RPMI-1640 medium with 3 μg/ml puromycin for 2 weeks to allow colony formation. The cell colonies were fixed with methanol, stained with Giemsa, photographed under a

microscope and counted manually at high-power magnification. Only colonies containing ≥50 number of cells were counted. Assays were repeated 3 times.

Wound-healing assay. BEL7402-Ctrl and BEL7402-KD cells were cultured in RPMI-1640 medium with 3 μg/ml puromycin in a 6-well plate. The monolayer of cell culture at 60% confluency was carefully scratched with a 200-μl pipette tip as described previously (25). Non-adherent cells and cellular debris were washed away with PBS. Cells migrated into the scratched area were monitored and photographed at 24, 48 and 72 h after cells were scratched. The scratch width was measured at 5 random points using ImageJ 1.43 software. Cell migration distance (in μm) was calculated by the formula (original width - current width) (25). Experiments were carried out in triplicates and repeated 3 times.

Xenograft tumor growth assay. All animal procedures were approved by the Sichuan University Animal Care and Use Committee. Nude mice were maintained in a special animal facility for immune-defective mice. Male BALB/c-nu/nu mice (4-6 weeks of age, 16-18 g) were obtained from the Laboratory Animal Center of Sichuan University. The BEL7402-Ctrl and BEL7402-KD cells were collected in PBS and inoculated subcutaneously into the left and right dorsal flanks of the nude mice. A total of $2x10^6$ cells in 200 μl of PBS were injected to each site. The tumor size was measured weekly with a caliper, and tumor volume was calculated by the formula: Tumor volume (mm^3) = [width (mm)]^2 x [length (mm)]/2, as described previously (26). Five weeks later, the mice were sacrificed and tumors dissected from the mice were weighed. Part of every tumor was snap-frozen in liquid nitrogen, and used for western blotting and semi-quantitative RT-PCR assays. The rest of the tumor was fixed for 24 h in 10% phosphate-buffered formalin and paraffin-embedded. Then, 5-μm sections were prepared for IHC staining.

RT-PCR. Total RNA was extracted from BEL7402 cells using the TRIzol reagent (Invitrogen). One microgram of RNA was reversely transcribed using the reverse transcriptase kit (Promega, USA) according to the manufacturer's instructions. The reaction without adding the transcriptase was used as a negative control for RT-PCR. PCR analysis was performed using the β-actin as an internal control. Vigilin cDNA levels were analyzed using the following primers: vigilin-F, 5'-CGTTATTGGGCAGAAAGGAA and vigilin-R, 5'-CTCTGTGGGAAGCGAATGTC; β-actin-F, 5'-TCATCACCATTGGCAATGAG and β-actin-R, 5'-CACTGTGTTGGCGTACAGGT. PCR products were separated on agarose gel and visualized under UV light after being stained with ethidium bromide.

Cisplatin treatment. BEL7402-Ctrl and BEL7402-KD cells were seeded in a 96-well plate at a density of $2x10^3$ cells/well and were cultured for 24 h. After removing the initial medium, the cells were treated with culture medium containing different concentrations of cisplatin for 72 h. Six-wells were used for each concentration of cisplatin treatment. Relative cell number was measured by MTT assay. Experiments were independently repeated 3 times.

Statistical analysis. The data are expressed as means ± SEM of independent measurements. Statistical analysis was performed based on the 3 repeated tests by using the Student's t-test with SPSS 13.0 software. Statistical significance was set at $p<0.05$ (95% confidence level).

Results

Vigilin is overexpressed in human HCCs. To explore the link between vigilin and HCC, we performed immunohistochemistry to compare vigilin expression in normal human liver, liver cirrhosis, adjacent non-tumor liver and HCC tissues (Fig. 1A). Six out of 7 normal livers exhibited low levels (score=1) of vigilin staining and the other one had a medium (score=2) level of vigilin staining. Among the 8 liver cirrhosis samples examined, 3 showed low vigilin staining and 5 exhibited medium vigilin staining (Fig. 1B). Among the 33 samples containing HCC and adjacent non-tumor liver tissues, 19 and 10 adjacent non-tumor hepatocytes exhibited medium and high (score=3) levels of vigilin expression, respectively, and only one showed low level vigilin expression. Notalby, as many as 29 of these 33 (88%) HCCs had high levels of vigilin expression and the other 4 exhibited medium level of vigilin expression (Fig. 1B). Statistical analysis revealed that vigilin expression in normal liver and liver cirrhosis had no significant difference ($p>0.05$). The level of vigilin expression in the adjacent non-tumor liver hepatocytes was significantly higher than that in the normal liver ($p<0.001$), but was not significantly different from that in liver cirrhosis ($p>0.05$). The level of vigilin expression in HCCs was significantly higher than the level in all other 3 types of liver tissues ($p<0.01$). These results suggest that vigilin is expressed in an increasing gradient from normal liver to cirrhosis, to adjacent non-tumor hepatocytes and to HCC. Vigilin is overexpressed in most human HCCs.

We also compared the levels of vigilin protein in an embryonic hepatocyte cell line (L-02) and 3 HCC cell lines (HepG2, BEL7402 and SMMC7721). We found that vigilin was expressed at much higher levels in the 3 HCC cell lines vs. the L-02 non-tumor hepatocellular cell line (Fig. 1C). These results were consistent with the elevated vigilin expression found in the human HCC specimens. Taken together, our results suggest that increased vigilin expression may play an important role in HCC progression.

Knockdown of vigilin decreases HCC cell proliferation and clonogenicity. In order to assess the biological role of vigilin in HCC cells, we generated a stable BEL7402-KD cell line with vigilin knockdown. The effective knockdown of vigilin in this cell line was confirmed by RT-PCR and western blotting (Fig. 2A). Knockdown of vigilin in BEL7402-KD cells significantly decreased their proliferation vs. BEL7402-Ctrl cells bearing an empty vector. After 4 days of culture, the number of BEL7402-KD cells was 33% less than the number of BEL7402-Ctrl cells (Fig. 2B). Furthermore, knockdown of vigilin also drastically reduced the number of colonies formed from the culture with a low density of BEL7402-KD cells vs. control cells. The mean numbers of colonies developed from 200 cells/well in a 6-well plate were 143 from BEL7402-KD cells and 52 from the control cells (Fig. 2C). These data indicate that vigilin is required for HCC cell proliferation and survival.

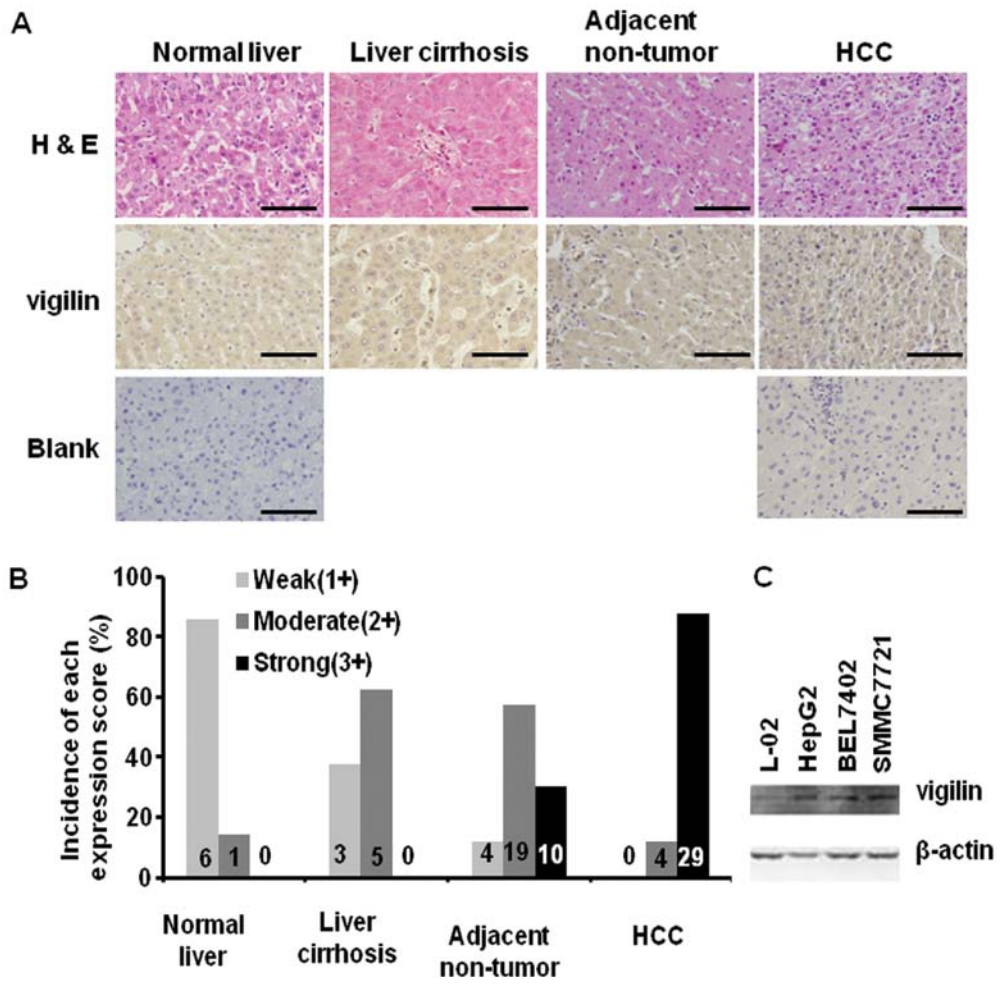

Figure 1. Increased vigilin expression in human HCC specimens. (A) Vigilin protein expression was analyzed by immunohistochemistry in normal liver, liver cirrhosis, adjacent non-tumor and HCC tissue sections. Representative images are presented. Negative control was performed by replacing the vigilin antibody with non-immune IgG. Scale bar, 100 μm. (B) Semi-quantitative analysis of vigilin expression levels using the 4-scale (0-3) scoring system. For each sample group, the numbers of specimens with different vigilin expression scores are indicated in each bar. Each of the 3 bars for each sample group represents the percentage of specimens with the indicated expression scores. Statistical analysis, p=0.0000 between HCC and normal liver and between HCC and the liver cirrhosis; p=0.014 between HCC and the adjacent non-tumor tissues; p=0.0002 between normal liver and the adjacent non-tumor tissues; p=0.087 between the liver cirrhosis and the adjacent non-tumor tissues; p=0.084 between normal liver and the liver cirrhosis. (C) Western blot analysis of vigilin protein in non-cancer L-02 cells and HCC cells including HepG2, BEL7402 and SMMC7721. The β-actin serves as a loading control. HCC, hepatocellular carcinoma.

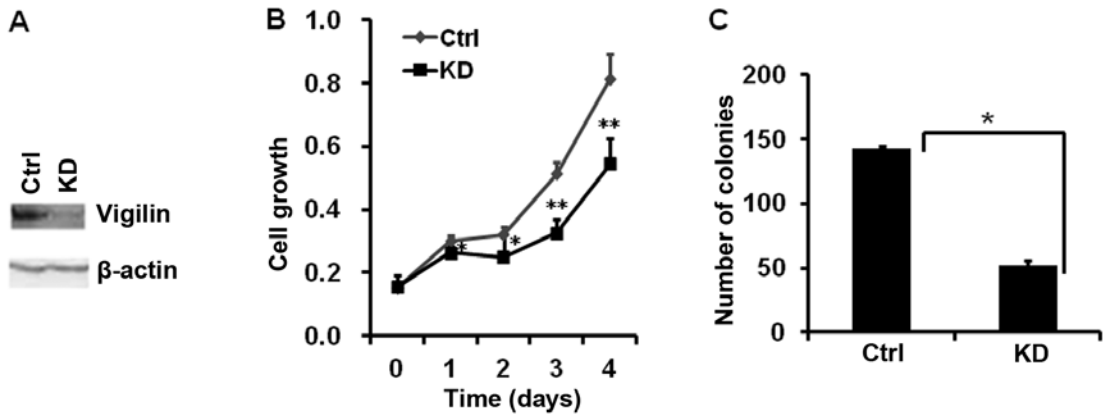

Figure 2. Knockdown of vigilin inhibits BEL7402 cell proliferation and colony formation. (A) Western blot analysis showing vigilin protein levels in control (Ctrl) and vigilin knockdown (KD) BEL7402 cells. β-actin served as a loading control. (B) Growth analysis of BEL7402-Ctrl and BEL7402-KD cells. Equal numbers of BEL7402-Ctrl and BEL7402-KD cells were seeded at day 0. Relative cell growth was measured by MTT assay on days 1-4. (C) Colony formation assay of BEL7402-Ctrl and BEL7402-KD cells. Equal numbers of BEL7402-Ctrl and BEL7402-KD cells were cultured at low density for 2 weeks. Formed colonies were stained with Giemsa and counted. Data in panels B and C are presented as means ± SEM (n=3). *p<0.05 and **p<0.01 by Student's t-test.

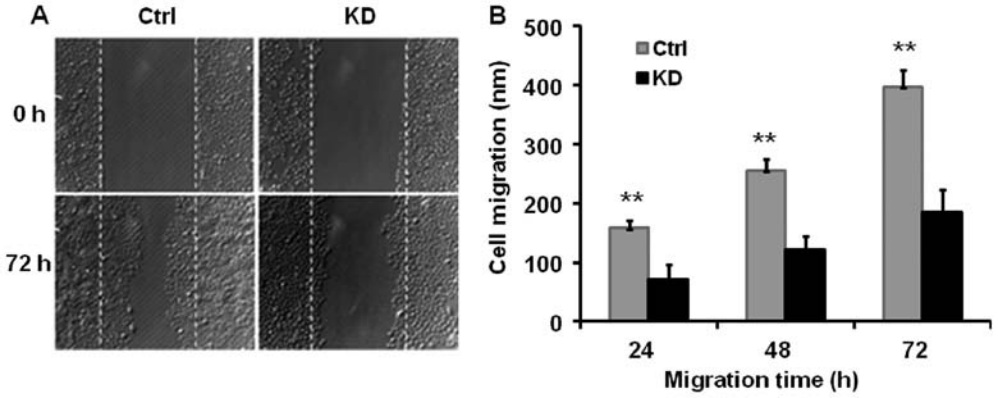

Figure 3. Vigilin knockdown decreases BEL7402 cell motility. (A) Images captured from the wound healing assay of BEL7402-Ctrl and BEL7402-KD cells at the starting time point (0 h) and at 72 h. The area between the 2 dotted lines indicates the wounded area at 0 h. (B) Quantitative cell migration data were collected at 24, 48 and 72 h after the wounding scratch. Relative cell migration distances in pixel were measured on electronic images by using computer software. Data are presented as means ± SEM (n=5). $^{**}p<0.01$ by Student's t-test.

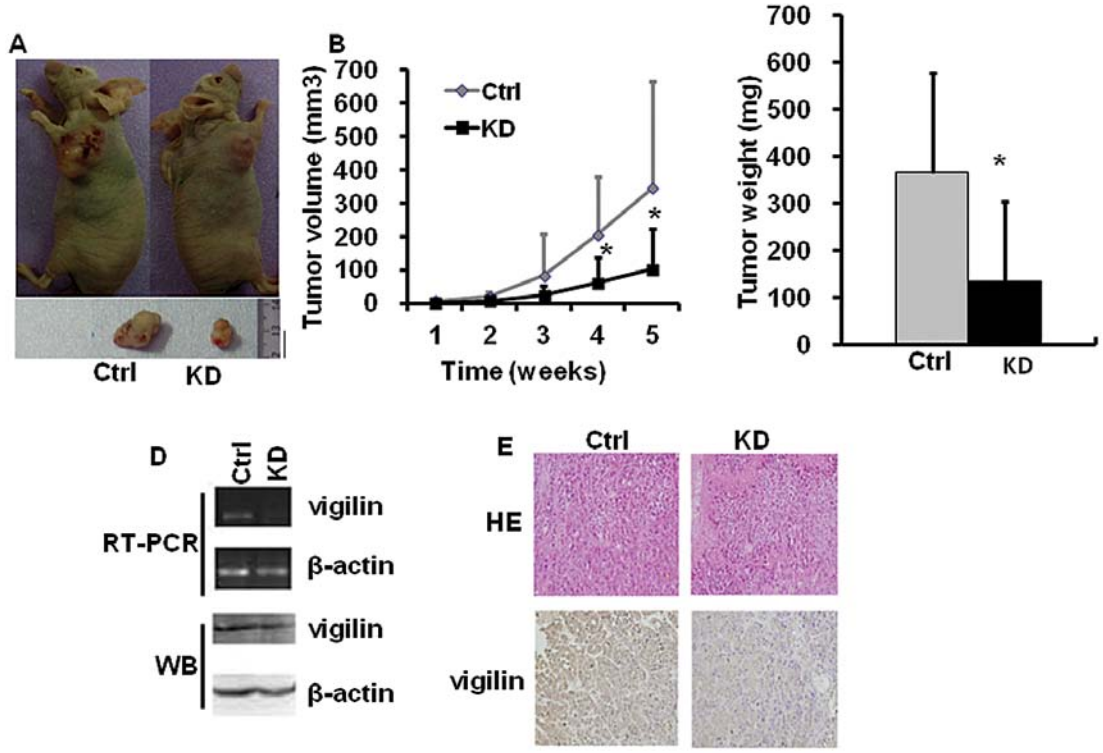

Figure 4. Vigilin knockdown inhibits the growth of BEL7402 cell-derived tumors in nude mice. (A) Representative image of subcutaneous xenograft tumors derived from BEL7402-Ctrl and BEL7402-KD cells in nude mice. (B) The growth curves of BEL7402-Ctrl and BEL7402-KD cell-derived tumors in nude mice (n=6). Tumor volumes are presented as means ± SEM. $^{*}p<0.05$ by Student's t-test. (C) The average weights of BEL7402-Ctrl and BEL7402-KD cell-derived tumors at the experimental end point (week 5). $^{*}p<0.05$ by Student's t-test. (D) RT-PCR and western blot analyses of vigilin mRNA and protein levels in BEL7402-Ctrl and BEL7402-KD cell-derived tumors at the experimental end point. β-actin served as a loading control. (E) H&E-stained and vigilin IHC-stained sections of BEL7402-Ctrl and BEL7402-KD cell-derived tumors in mice at the experimental end point. HE, hematoxylin and eosin; IHC, immunohistochemistry.

Knockdown of vigilin decreases HCC cell migration. We performed a wound-healing assay to estimate the effect of vigilin knockdown on HCC cell migration. We found that knockdown of vigilin in BEL7402-KD cells reduced their migration capability by ~52% vs. BEL7402-Ctrl cells as measured at 24, 48 and 72 h after performing the scratch wound (Fig. 3). These data indicate that vigilin plays a promotive role in HCC cell migration.

Knockdown of vigilin inhibits HCC xenograft tumor growth in nude mice. To determine the role of vigilin in HCC tumor growth, we subcutaneously injected BEL7402-KD cells with vigilin knockdown and BEL7402-Ctrl cells with vigilin expression into nude mice, and monitored their tumorigenesis. Vigilin knockdown significantly attenuated the growth of BEL7402-KD cell-derived tumors as compared to the growth of BEL7402-Ctrl cell-derived tumors in nude

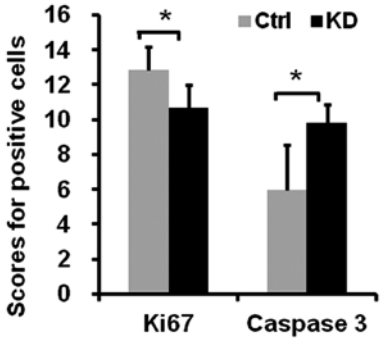

Figure 5. Vigilin knockdown reduces the proliferation rate but increases the apoptosis rate of BEL7402-Ctrl and BEL7402-KD tumor cells in nude mice. Immunohistochemical staining for Ki67 and activated caspase 3 was performed with tissue sections prepared from BEL7402-Ctrl and BEL7402-KD cell-derived tumors (n=6) at the experimental end point. Positive cells were counted and normalized to total tumor cells. *p<0.05 by Student's t-test.

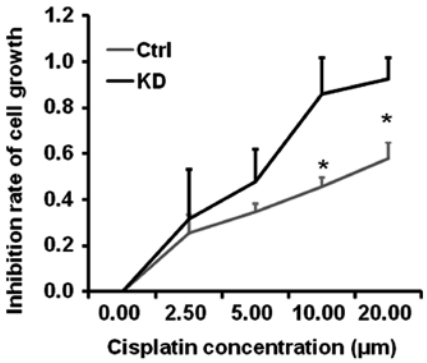

Figure 6. Vigilin knockdown enhances cisplatin-inhibited HCC cell growth. BEL7402-Ctrl and BEL7402-KD cells were treated with different concentrations of cisplatin as indicated for 3 days. Relative cell numbers were measured by MTT assays. The relative inhibition rate of cell growth for BEL7402-KD cells treated with 20 μM of cisplatin was set to 1 (100%) and other data points were accordingly normalized to this setting. Data are presented as means ± SEM (n=6). *p<0.05 by Student's t-test. HCC, hepatocellular carcinoma.

mice (Fig. 4A). The average size of BEL7402-Ctrl tumors reached ~400 mm^3 within 35 days, while the average size of BEL7402-KD tumors was only ~110 mm^3 within the same period (Fig. 4B). At the experimental end point, the average weight of BEL7402-KD tumors was only 37% of the weight of BEL7402-Ctrl tumors (Fig. 4C). RT-PCR, western blotting and IHC analyses revealed that the levels of vigilin expression remained low in the BEL7402-KD tumors vs. BEL7402-Ctrl tumors (Fig. 4D and E). These results demonstrate that vigilin is required for the growth of BEL7402 HCC cell-derived tumors in mice.

To identify the cellular mechanisms responsible for vigilin knockdown-suppressed tumor growth in mice, we performed IHC for Ki67 to detect proliferative cells and IHC for cleaved caspase 3 to detect apoptotic cells. The number of Ki67-positive cells was decreased 16.8%, while the number of apoptotic cells was increased 63.8% in the BEL7402-KD tumors vs. the BEL7402-Ctrl tumors (Fig. 5). These results suggest that knockdown of vigilin suppresses the growth of BEL7402-KD tumors in mice by inhibiting cell proliferation and promoting cell apoptosis.

Knockdown of vigilin enhances cisplatin-mediated inhibition of HCC cell growth. Cisplatin is a commonly used drug for cancer chemotherapy. To examine whether vigilin knockdown enhances cisplatin-inhibited HCC cell growth, we treated BEL7402-Ctrl and BEL7402-KD cells with different concentrations of cisplatin for 72 h. We found that BEL7402-KD cells exhibited much higher sensitivity than BEL7402-Ctrl cells in response to cisplatin treatment. The EC_{50} of cisplatin was ~6 μM for BEL7402-Ctrl cells and ~15 μM for BEL7402-KD cells (Fig. 6). These results suggest that targeting vigilin may sensitize HCC cells to cisplatin to promote cell killing.

Discussion

In the present study, we demonstrated that the expression of vigilin is increased in human HCC tissues. The frequency and degree of vigilin overexpression were also increased in a gradient from benign lesions, liver cirrhosis to HCC, and each step showed a significant elevation in vigilin expression. This suggests that vigilin may promote HCC progression and its overexpression may also serve as a molecular marker for HCC progression. Knockdown of vigilin in HCC cells suppressed their proliferation, clonogenic ability and mobility, suggesting that vigilin is an important factor that promotes HCC cell proliferation and tumorigenesis.

Our results demonstrating that vigilin is overexpression in human HCC specimens and cell lines and that vigilin promotes HCC cell proliferation and tumorigenesis are consistent with previous studies showing vigilin overexpression in other types of cancers. For example, it has been reported that vigilin is upregulated in TOV112D ovarian cancer cells (16,17), prolactinomas, gastric cancer (18,19), Hep-2 larynx carcinoma, HeLa cervix carcinoma, MG63 osteosarcoma, U937 and HL60 leukemia (27), pancreatic carcinoma (28) and LNCaP prostate cancer cells (29). Together, these results suggest a detrimental role of vigilin in the development and progression of multiple human types of cancers, including human HCC. Targeting vigilin in these cancer cells may have therapeutic value.

Intriguingly, other studies have suggested that vigilin may be a tumor suppressor in other types of human cancers. For example, vigilin was recently shown to accelerate the degradation and inhibit the translation of the c-fms proto-oncogene mRNA in breast cancer cells (20). These observations suggest that vigilin may either inhibit or promote carcinogenesis, depending on different cancer types and cellular and tissue contexts.

Carcinogenesis, including HCC development, consists of multiple steps. Most HCCs developed in Chinese patients undergo a progression from HBV hepatitis infection, followed by liver cirrhosis and finally to carcinoma (30). However, there are few biomarkers to screen patients at risk for liver cirrhosis. Notably, the present study revealed that vigilin expression is frequently and progressively increased from benign lesions to liver cirrhosis, and then to HCC, suggesting that vigilin may serve as a potential molecular marker for evaluating the risk of HCC development. This assumption is coincident with evidence indicating that vigilin is a cancer-related antigen in the sera of breast cancer patients (31) and the existence of vigilin antibody in the sera of melanoma patients (32).

In conclusion, our findings indicate that vigilin is frequently overexpressed in human HCCs and may play a crucial role in HCC cell proliferation, survival, migration and tumor growth. Since knockdown of vigilin inhibits HCC cell growth, survival and tumorigenesis, vigilin may be a potential therapeutic target for HCC treatment, alone or combined with chemotherapeutic agents such as cisplatin.

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