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Retrovirus-Mediated shRNA Knockdown of Mig-7 Inhibits Vasculogenic Mimicry and In Vitro Invasion and Metastasis in Human Hepatocellular Carcinoma Cells (Postprint)

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Date: 2017-12-21T00:00:00+00:00

Abstract

Objective To investigate the effects of retrovirus-mediated short hairpin RNA (shRNA) interference targeting migration-inducing gene 7 (Mig-7) on vasculogenic mimicry (VM) formation and in vitro invasion and metastasis capabilities of hepatocellular carcinoma (HCC) cells. Methods Two Mig-7 mRNA oligonucleotide sequences (Mig-7 shRNA-1, Mig-7 shRNA-2) and one unrelated sequence serving as a negative control (Mig-7 shRNA-N) were designed. Mig-7 shRNA retroviral expression vector plasmids were constructed, and human hepatocellular carcinoma MHCC-97H cells to be transfected were divided into six groups: Mig-7 shRNA-1 transfection group; Mig-7 shRNA-2 transfection group; Mig-7 shRNA-N transfection group; empty vector transfection group (Vector); recombinant human endostatin (ES, trade name: Endostar) group; and MHCC-97H cell control group (Control). Semi-quantitative PCR and Western blot were employed to detect the effects on Mig-7 expression; three-dimensional cell culture was used to observe the effects on VM formation; and cell-cell adhesion assay, Transwell invasion assay, and migration assay were utilized to examine the effects on cell adhesion, invasion, and migration capabilities. Results Following transfection, the expression of Mig-7 mRNA and protein, the VM formation capability of MHCC-97H cells, and cellular invasion and migration capabilities were significantly reduced in the Mig-7 shRNA-1 and Mig-7 shRNA-2 groups ($P<0.05$), whereas cell-cell adhesion capability was significantly enhanced ($P<0.05$). In the Mig-7 shRNA-N, Vector, and ES groups, the expression of Mig-7, VM formation, cell-cell adhesion, migration, and invasion capabilities of MHCC-97H cells exhibited no significant alterations compared with the MHCC-97H cell control group. Conclusion Retrovirus-mediated shRNA can effectively downregulate Mig-7 expression and markedly suppress VM formation

capability and invasion-metastasis capability of HCC cells while enhancing cell-cell adhesion. ES demonstrates no significant effect on Mig-7 expression, VM formation, invasion-metastasis, or adhesion capabilities of HCC cells.

Full Text

Preamble

DOI: 10.3969/j.issn.1673-4254.2016.11.06
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Abstract

Objective: To investigate the effects of retrovirus-mediated short hairpin RNA (shRNA) interference targeting migration-inducing gene 7 (Mig-7) on vasculogenic mimicry (VM) formation and in vitro invasion and metastasis of hepatocellular carcinoma (HCC) cells. **Methods:** Two Mig-7 mRNA oligonucleotide sequences (Mig-7 shRNA-1, Mig-7 shRNA-2) and one negative control sequence (Mig-7 shRNA-N) were designed. Mig-7 shRNA retroviral expression plasmids were constructed and used to transfect human HCC cell line MHCC-97H, which was divided into six groups: Mig-7 shRNA-1 transfection group, Mig-7 shRNA-2 transfection group, Mig-7 shRNA-N transfection group, empty vector transfection group (Vector), recombinant human endostatin (ES, trade name: Endostar) group, and MHCC-97H cell control group (Control). Semi-quantitative PCR and Western blotting were performed to detect Mig-7 expression; three-dimensional cell culture was used to observe VM formation; and intercellular adhesion assay, Transwell invasion assay, and migration assay were conducted to evaluate cell adhesion, invasion, and migration capabilities. **Results:** Following transfection, Mig-7 mRNA and protein expression, VM formation capacity, and cell invasion and migration abilities were significantly decreased in both Mig-7 shRNA-1 and Mig-7 shRNA-2 groups ($P<0.05$), while intercellular adhesion was markedly increased ($P<0.05$). No significant changes in Mig-7 expression, VM formation, adhesion, migration, or invasion were observed in the Mig-7 shRNA-N, Vector, ES, or Control groups. **Conclusions:** Retrovirus-mediated shRNA effectively downregulates Mig-7 expression and significantly inhibits VM formation and invasion-metastasis capacity in HCC cells while enhancing intercellular

adhesion. ES showed no significant effect on Mig-7 expression, VM formation, invasion, metastasis, or adhesion in HCC cells.

Keywords: vasculogenic mimicry; migration-inducing gene 7; hepatocellular carcinoma; invasion; metastasis

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Introduction

Hepatocellular carcinoma (HCC) accounts for 91.5% of primary liver cancer (PLC) and has a poor prognosis, with an overall 5-year survival rate of less than 5% [1-2]. The high mortality is primarily attributed to high metastatic potential and postoperative recurrence rates [3-4]. Consequently, anti-invasion and anti-metastasis strategies have become crucial therapeutic approaches for improving HCC prognosis.

Vasculogenic mimicry (VM) represents a distinct tumor microcirculation pattern that differs from classical tumor angiogenesis and operates independently of vascular endothelial cells, playing a significant role in tumor growth and metastasis [5-6]. However, conventional anti-vascular therapies targeting vascular endothelial cells (VEC) are ineffective against VM [7], and the tumor hypoxia induced by such treatments may paradoxically promote VM formation [8], thereby increasing opportunities for tumor invasion and metastasis through this alternative pathway. Therefore, anti-VM strategies must be integrated into anti-angiogenic therapy, making the identification of therapeutic targets for VM highly significant in HCC treatment.

Migration-inducing gene 7 (Mig-7) encodes a cysteine-rich protein localized to the cell membrane of various tumor cells and plays an important role in tumor plasticity and VM formation [9-10]. Mig-7 is not expressed in normal tissues or low-invasive tumor cells lacking VM capacity but is specifically expressed in highly invasive tumor cells capable of forming VM [11-12]. Current studies have linked Mig-7 to VM formation in gastric cancer [13], yet reports on its influence on VM formation and invasion-metastasis capacity in HCC remain scarce.

Based on preliminary experiments, we hypothesized that Mig-7 serves as a critical regulator of VM formation and invasion-metastasis in HCC, representing an ideal target for malignant tumor therapy. This study constructed specific Mig-7 shRNA retroviral expression vectors to transfect Mig-7-high-expressing human HCC cell line MHCC-97H, aiming to observe the effects on Mig-7 expression, VM formation capacity, and cell adhesion, invasion, and migration abilities.

Materials and Methods

1.1 Experimental Materials

Human HCC cell line MHCC-97H was purchased from the Liver Cancer Institute of Zhongshan Hospital, Fudan University. Rabbit anti-human Mig-7

antibody was obtained from Abcam (USA). Primers were designed and synthesized by Takara Bio. Lipofectamine™ 2000 Reagent was purchased from Invitrogen (USA). Matrigel was from BD Biosciences (USA). Transwell chambers were from Corning (USA). Recombinant human endostatin (ES, trade name: Endostar) was from Simcere-Medgen Bio-Pharmaceutical Co.

1.2 shRNA Design and Synthesis

The human Mig-7 mRNA sequence (GenBank: DQ080207.2) was obtained from GenBank. Based on shRNA design principles [14], sequences targeting the coding sequence (CDS) region of Mig-7 mRNA were designed. BLAST analysis was performed against the human genome database to exclude sequences with significant homology to other genes. Three sequences were designed, including two Mig-7 mRNA oligonucleotide sequences (Mig-7 shRNA-1, Mig-7 shRNA-2) and one negative control unrelated sequence (Mig-7 shRNA-N). Mig-7 shRNAs were designed, synthesized, and fluorescently labeled by Takara Bio. The sequences were as follows:

- **Mig-7 shRNA-1**
  Sense: 5'-GATCCAAAGTTTCATTCTTCGACTTCAAGAGAGTCGAAGAATGAAACTTTTTTTTT
  3'
  Antisense: 3'-GTTTCAAAGTAAGAAGCTGAAGTTCTCTCAGCTTCTTTACTTTGAAAAAAAAAC
  5'
- **Mig-7 shRNA-2**
  Sense: 5'-GGATCCCACAGCTTGAGTGGAATACTTCAAGAGAGTATTCCACTCAAGCTGTGTTTT
  3'
  Antisense: 3'-GGTGTCGAACTCACCTTATGAAGTTCTCTCATAAGGTGAGTTCGACACAAAAAA
  5'
- **Mig-7 shRNA-N**
  Sense: 5'-GGATCCTGATAAACGAACTGCGCGTTTCAAGAGAACGCGCAGTTCGTTTATCATTTT
  3'
  Antisense: 3'-GACTATTTGCTTGACGCGCAAAGTTCTCTTGCGCGTCAAGCAAATAGTAAAAAA
  5'

1.3 Mig-7 shRNA Transfection of MHCC-97H Cells

MHCC-97H cells were routinely cultured in DMEM high-glucose medium containing 10% FBS at 37°C in a 5% CO incubator. Twenty-four hours before transfection, cells were seeded at 4×10 /mL (0.5 mL/well) in 24-well plates and cultured overnight. When cells reached 80-90% confluence, transfection was performed using Lipofectamine™ 2000 according to the manufacturer's instructions. MHCC-97H cells were divided into four groups: (1) Mig-7 shRNA-1 transfection group; (2) Mig-7 shRNA-2 transfection group; (3) Mig-7 shRNA-N transfection group; (4) empty vector transfection group (Vector). At 24 h post-transfection, green fluorescence protein expression was observed under a fluorescence microscope. Ten random fields were selected per group, and transfection efficiency

was calculated as the percentage of green fluorescence-positive cells relative to total cell count.

1.4 Semi-Quantitative PCR Detection of Mig-7 mRNA Expression

Cells were divided into six groups: (1) Mig-7 shRNA-1; (2) Mig-7 shRNA-2; (3) Mig-7 shRNA-N; (4) Vector; (5) ES group (treated with 125 g/mL ES [15]); and (6) MHCC-97H cell control group (Control). Total RNA was extracted and reverse-transcribed into cDNA for target gene amplification. PCR conditions were: 94°C denaturation for 10 s, annealing and extension for 30 s, for 40 cycles. Primer sequences were: -actin (sense: 5' -ATCGTGCGTGACATTAAGGAGAAG-3' , antisense: 5' -AGGAAGGAAGGCTGGAAGAGTG-3') and Mig-7 (sense: 5' -TCTCAGGCAGTCAGTGGG-3', antisense: 5'-GTTGGATGGGATGTCTCG-3').

1.5 Western Blotting

Experimental groups were identical to those in section 1.4. Following transfection, proteins were extracted and separated by 0.1% SDS-10% PAGE, then transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk in TBST for 2 h, incubated overnight at 4°C with primary antibodies (Mig-7, 1:200; -actin, 1:500), washed with TBST, then incubated with HRP-conjugated secondary antibodies (anti-rabbit, 1:1000; anti-mouse, 1:1000) for 2 h at room temperature. After TBST washes, bands were visualized using an ECL chemiluminescence kit.

1.6 Three-Dimensional Cell Culture for VM Formation

Experimental groups were identical to those in section 1.4. Fifty microliters of Matrigel was added to 6-well plates and allowed to solidify for 30 min at room temperature. Subsequently, 100 L of complete culture medium containing cells at 1×10 /mL was added to each well, followed by 2 mL of complete medium. Plates were incubated at 37°C in a 5% CO incubator.

1.7 Cell Adhesion, Invasion, and Migration Assays

1.7.1 Intercellular Adhesion Assay Experimental groups were identical to those in section 1.4. Single-cell suspensions were prepared at 1×10 /mL, and 1 mL aliquots were placed in 1.5 mL EP tubes (5 tubes per group). After incubation at 37°C for 12 h, cell counts were performed at 2, 6, and 12 h. Intercellular adhesion capacity was evaluated as the percentage of remaining single cells relative to total cells; lower percentages indicated stronger cell-cell adhesion.

1.7.2 Transwell Invasion Assay Experimental groups were identical to those in section 1.4. Transwell chambers were placed in 24-well plates, coated

with 10 g/well Matrigel, and incubated at 37°C for 4 h to allow gel solidification. Cells were digested and resuspended at 1×10 /mL, and 400 L of cell suspension was added to the upper chamber, while the lower chamber received DMEM with 10% FBS. After 20 h incubation at 37°C with 5% CO , cells were fixed with methanol and stained with crystal violet. Cells on the underside of the microporous membrane were counted under a light microscope in five random fields.

1.7.3 Migration Assay Experimental groups were identical to those in section 1.4. After transfection, cells were digested and seeded at 1.0×10 cells per well (100 L cell suspension) in the upper chamber, with 20% FBS-containing medium in the lower chamber. Following 24 h incubation, cells on the upper surface of the membrane were carefully removed with a cotton swab, while cells adhered to the lower surface were fixed with methanol and stained with crystal violet. Observation and counting methods were identical to those in section 1.7.2.

1.8 Statistical Analysis

All data were analyzed using SPSS 20.0 software. Measurement data are expressed as mean ± standard deviation. Comparisons among multiple groups were performed using ANOVA followed by pairwise comparisons. $P<0.05$ was considered statistically significant.

Results

2.1 Efficient Retroviral Transfection of MHCC-97H Cells

The retroviral vector system used in this study carries green fluorescence protein, enabling visualization of transfection efficiency under an inverted fluorescence microscope [Figure 1: see original paper]. All groups (Mig-7 shRNA-1, Mig-7 shRNA-2, Mig-7 shRNA-N, and Vector) exhibited >90% stable transfection with green fluorescence-positive cells, demonstrating that the retroviral vector system efficiently and stably infected MHCC-97H cells.

2.2 Mig-7 shRNA Inhibits Mig-7 mRNA and Protein Expression in MHCC-97H Cells

Semi-quantitative PCR [FIGURE:2A, B] revealed that Mig-7 mRNA levels were significantly reduced in both Mig-7 shRNA-1 and Mig-7 shRNA-2 groups, with the most pronounced decrease in the Mig-7 shRNA-1 group. Western blotting [Figure 2C: see original paper] confirmed marked reductions in Mig-7 protein levels in both shRNA groups, again most notably in the Mig-7 shRNA-1 group. These results demonstrate that the constructed Mig-7 shRNA effectively suppressed Mig-7 expression at both mRNA and protein levels.

2.3 Mig-7 shRNA Inhibits VM Formation Capacity in MHCC-97H Cells

Three-dimensional cell culture [Figure 3: see original paper] showed that cells in Mig-7 shRNA-1 and Mig-7 shRNA-2 groups adhered in clusters without forming VM structures. In contrast, VM formation in the Mig-7 shRNA-N group was comparable to that in the Vector, ES, and Control groups. These findings indicate that Mig-7 interference significantly inhibited the VM formation capacity of MHCC-97H cells.

2.4 Mig-7 shRNA Enhances Intercellular Adhesion in MHCC-97H Cells

Intercellular adhesion assay results [Figure 4: see original paper] demonstrated that adhesion capacity was significantly stronger in Mig-7 shRNA-1 and Mig-7 shRNA-2 groups compared to all other groups ($P<0.05$), with Mig-7 shRNA-1 showing greater enhancement than Mig-7 shRNA-2 ($P<0.05$). No significant differences were observed among the remaining four groups ($P>0.05$), indicating that Mig-7 downregulation increases cell-cell adhesion.

2.5 Mig-7 shRNA Reduces Invasive Capacity of MHCC-97H Cells

Transwell invasion assay results [FIGURE:5, FIGURE:6A] showed that the number of cells penetrating the membrane was significantly lower in Mig-7 shRNA-1 and Mig-7 shRNA-2 groups compared to other groups ($P<0.05$). No significant differences were found among Mig-7 shRNA-N, Vector, ES, and Control groups ($P>0.05$), demonstrating that Mig-7 downregulation markedly reduces MHCC-97H cell invasiveness.

2.6 Mig-7 shRNA Reduces Migration Capacity of MHCC-97H Cells

Cell migration assay results [Figure 6B: see original paper] revealed no significant differences among groups at 12 h ($P>0.05$). However, at 24 h, migration capacity was significantly weaker in Mig-7 shRNA-1 and Mig-7 shRNA-2 groups compared to other groups ($P<0.05$), with Mig-7 shRNA-1 showing greater inhibition than Mig-7 shRNA-2 ($P<0.05$). No significant differences were observed among Mig-7 shRNA-N, Vector, ES, and Control groups ($P>0.05$). The ratio of invasive to migratory cell numbers at 24 h [Figure 6C: see original paper] further confirmed that Mig-7 shRNA significantly decreased the invasion-metastasis capacity of MHCC-97H cells.

Discussion

Tumor vasculature plays a crucial role in tumor growth, invasion, and metastasis. However, the discovery of VM has complicated anti-vascular therapy for cancer. As an important supplement to tumor microcirculation, VM provides additional nutrients and invasion-metastasis channels for tumors. Studies have

shown that VM is associated with invasion and metastasis in multiple malignant tumors, and tumors with VM formation often have poor prognosis [9, 12]. Therefore, investigating VM formation mechanisms and exploring VM-specific targets holds significant clinical importance.

Current research on VM formation mechanisms has focused on factors including VE-cadherin [16-17], EphA2 [18], PI3K [19-20], MMPs [9, 12], laminin-5 2 chain [19], HIF-1 [22], and focal adhesion kinase [23]. However, the precise mechanisms underlying VM formation require further investigation.

With the advent of the "Precision Medicine Initiative" driven by DNA sequencing and genomic technologies, the era of genetic applications in life sciences has truly arrived. Identifying genes specifically targeting VM to inhibit VM formation and tumor cell invasion-metastasis represents a current research focus in HCC.

Mig-7 is a newly discovered human gene located at 1p22.1, with a full-length cDNA of 1617 bp encoding a cysteine-rich protein [11]. Studies have shown that increased Mig-7 expression enhances invasiveness in colon cancer, endometrial cancer, lung cancer, gastric cancer, and epithelial ovarian cancer, while its suppression effectively reduces tumor invasion-metastasis capacity [12, 24-25]. Although the specific mechanisms remain unclear, research indicates that Mig-7 is primarily induced by growth factors and the COX-2/PGE2 pathway, regulates EMT through ZEB-1 and Twist, and influences tumor invasion-metastasis [12]. Other studies suggest Mig-7 participates in activating MT1-MMP, which in turn activates MMP-2 to degrade the Ln-5 2 chain into 2' and 2× fragments, thereby increasing VM formation and tumor invasion-metastasis [25]. Importantly, Mig-7 is expressed only in highly invasive tumor cells capable of VM formation and not in normal tissues [11-12], offering a potentially safe and effective target for precise VM-targeted gene therapy. We previously hypothesized [26] that RNAi technology could suppress Mig-7 expression in HCC, thereby inhibiting VM formation and reducing tumor cell invasion-metastasis. This Mig-7-targeted approach holds substantial clinical potential, particularly when combined with conventional therapies such as endothelium-targeted angiogenesis inhibitors (e.g., ES), potentially yielding greater efficacy than any single-agent anti-angiogenic therapy.

RNA interference (RNAi) technology offers a rapid, reliable, and economical alternative to traditional gene knockout and transgenic methods. In this study, shRNA transfection of MHCC-97H cells demonstrated significant inhibitory effects, particularly with the Mig-7 shRNA-1 plasmid, which showed marked differences compared to negative control and parental cell groups. The slight decrease in Mig-7 mRNA in the negative control group was attributed to non-specific effects of exogenous plasmid introduction. The constructed Mig-7 shRNA recombinant retroviral expression vector effectively suppressed Mig-7 expression and significantly reduced VM formation and tumor invasion-metastasis capacity, indicating that Mig-7 plays an important role in VM formation and VM-associated invasion-metastasis in HCC.

Our results demonstrate that Mig-7 shRNA specifically and efficiently downregulates Mig-7 expression at both gene and protein levels in MHCC-97H cells, significantly reducing invasion and VM formation while enhancing intercellular adhesion, thereby partially reversing the malignant phenotype. Additionally, ES showed no effect on VM formation in our experiments, further confirming that conventional anti-vascular therapies targeting VEC do not directly inhibit VM generation.

While Mig-7 represents a promising addition to anti-HCC therapeutic targets, additional related genes must be identified to refine multi-target anti-tumor treatment strategies in the context of precision medicine. A recent study on HCC genotypes [4] revealed significant intra-patient heterogeneity among multiple tumor lesions, with different lesions sharing only 8% to 97% of gene mutations, highlighting the challenge of comprehensive genomic characterization for precision therapy. Future research should identify more specific targets and their key connecting genes.

In summary, RNAi-mediated specific and efficient downregulation of Mig-7 inhibits VM formation and invasion-metastasis capacity in HCC cells, providing a basis for Mig-7 as a specific therapeutic target. Additionally, ES showed no influence on Mig-7 expression, VM formation, or invasion-metastasis. Our findings and hypothesis regarding combined VM-targeted and endostatin therapy for inhibiting HCC invasion-metastasis require further in vivo validation.

References

[1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012 [J]. CA Cancer J Clin, 2015, 65(2): 87-108.
[2] Kobeisy MA, Morsy KH, Galal M, et al. Clinical significance of elevated alpha-foetoprotein(AFP) in patients with chronic hepatitis C without hepatocellular carcinoma in upper EGYPT [J]. Arab J Gastroenterol, 2012, 13(2): 49-53.
[3] Xu L, Kim Y, Spolverato G, et al. Racial disparities in treatment and survival of patients with hepatocellular carcinoma in the United States [J]. Hepatobiliary Surg Nutr, 2016, 5(1): 43-52.
[4] Xue R, Li R, Guo H, et al. Variable intra-tumor genomic heterogeneity of multiple lesions in patients with hepatocellular carcinoma [J]. Gastroenterology, 2016, 150(4): 998-1008.
[5] Sestito R, Cianfrocca R, Rosano L, et al. Macitentan blocks endothelin-1 receptor activation required for chemoresistant ovarian cancer cell plasticity and metastasis [J]. Life Sci, 2016, 159(SI): 77-86.
[6] Tan LY, Mintoff C, Johan MZ, et al. Desmoglein 2 promotes vasculogenic mimicry in melanoma and is associated with poor clinical outcome [J]. Oncotarget, 2016, [Epub ahead of print].
[7] Van Der Schaft DW, Seftor RE, Seftor EA, et al. Effects of angiogenesis inhibitors on vascular network formation by human endothelial and melanoma cells [J]. J Natl Cancer Inst, 2004, 96(19): 1473-7.

[8] Liu K, Sun BC, Zhao XL, et al. Hypoxia promotes vasculogenic mimicry formation by the Twist1-Bmi1 connection in hepatocellular carcinoma [J]. Int J Mol Med, 2015, 36(3): 783-91.
[9] Ren K, Yao N, Wang GY, et al. Vasculogenic mimicry: a new prognostic sign of human osteosarcoma [J]. Hum Pathol, 2014, 45(10): 2120-9.
[10] Ho MY, Hung SW, Liang CM, et al. Recombinant viral capsid protein VP1 suppresses lung cancer metastasis by inhibiting COX-2/PGE2 and MIG-7 [J]. Oncotarget, 2014, 5(11): 3931-43.
[11] Crouch S, Spidel CS, Lindsey JS. HGF and ligation of alpha v beta 5 integrin induce a novel, cancer cell-specific gene expression required for cell scattering [J]. Exp Cell Res, 2004, 292(2): 274-87.
[12] Ho MY, Liang CM, Liang SM. MIG-7 and phosphorylated prohibitin coordinately regulate lung cancer invasion/metastasis [J]. Oncotarget, 2015, 6(1): 381-93.
[13] Liao S, Gao Q. Expressions and clinical significance of vasculogenic mimicry and related protein Mig-7 and MMP-2 in gastric carcinoma [J]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2013, 29(2): 190-3.
[14] Reynolds A, Leake D, Boese Q, et al. Rational siRNA design for RNA interference [J]. Nat Biotechnol, 2004, 22(3): 326-30.
[15] Ling Y, Yang Y, Lu N, et al. Endostar, a novel recombinant human endostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells [J]. Biochem Biophys Res Commun, 2007, 361(1): 79-84.
[16] Tang NN, Zhu H, Zhang HJ, et al. HIF-1 alpha induces VE-cadherin expression and modulates vasculogenic mimicry in esophageal carcinoma cells [J]. World J Gastroenterol, 2014, 20(47): 17894-904.
[17] Yang J, Zhang X, Zhang Y, et al. HIF-2 alpha promotes epithelial-mesenchymal transition through regulating Twist2 binding to the promoter of E-cadherin in pancreatic cancer [J]. J Exp Clin Cancer Res, 2016, 35: 26.
[18] Guo JQ, Zheng QH, Chen H, et al. Ginsenoside Rg3 inhibition of vasculogenic mimicry in pancreatic cancer through downregulation of VE-cadherin/EphA2/MMP9/MMP2 expression [J]. Int J Oncol, 2014, 45(3): 1065-72.
[19] Bai R, Ding T, Zhao J, et al. The effect of PI3K inhibitor LY294002 and gemcitabine hydrochloride combined with ionizing radiation on vasculogenic mimicry of Panc-1 cells in vitro and in vivo [J]. Neoplasma, 2016, 63(1): 80-92.
[20] Wang H, Lin H, Pan J, et al. Vasculogenic mimicry in prostate cancer: the roles of EphA2 and PI3K [J]. J Cancer, 2016, 7(9): 1111-7.
[21] Zhang JT, Sun W, Zhang WZ, et al. Norcantharidin inhibits tumor growth and vasculogenic mimicry of human gallbladder carcinomas by suppression of the PI3-K/MMPs/Ln-5 2 signaling pathway [J]. BMC Cancer, 2014, 14: 193.
[22] Zhou TJ, Huang XH, Gong L, et al. Vasculogenic mimicry and hypoxia-inducible factor-1alpha expression in cervical squamous cell carcinoma [J]. Genet Mol Res, 2016, 15(1): 15017396.
[23] Zang M, Zhang Y, Zhang B, et al. CEACAM6 promotes tumor angiogenesis and vasculogenic mimicry in gastric cancer via FAK signaling [J]. Biochim

Biophys Acta, 2015, 1852(5): 1020-8.
[24] Huang BH, Yin MZ, Li X, et al. Migration-Inducing gene 7 promotes tumorigenesis and angiogenesis and independently predicts poor prognosis of epithelial ovarian cancer [J]. Oncotarget, 2016, 7(19): 27552-66.
[25] Petty AP, Wright SE, Rewers-Felkins KA, et al. Targeting migration inducting gene-7 inhibits carcinoma cell invasion, early primary tumor growth, and stimulates monocyte oncolytic activity [J]. Mol Cancer Ther, 2009, 8(8): 2412-23.
[26] Qu B, Guo L, Ma J, et al. Antiangiogenesis therapy might have the unintended effect of promoting tumor metastasis by increasing an alternative circulatory system [J]. Med Hypotheses, 2010, 74(2): 360-1.

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