**Abstract.** Epsin 3 (EPN3) expression is limited to gastric parietal cells and wounded or pathological tissue rather than normal brain tissue, and although it has been identified as an oncogene in estrogen receptor-positive breast cancer and non-small cell lung cancer, its function in cancer is poorly understood. The present study aimed to investigate the association of EPN3 expression with the clinicopathological features of patients with glioma, as well as the effects of EPN3 on glioblastoma cells and the potential molecular mechanisms for its effects on glioblastoma cell behavior. EPN3 expression was assessed by immunohistochemistry in tissue samples from 167 patients with glioma, as well as by western blotting in 5 glioblastoma cell lines. The U87 and U251 glioblastoma cell lines were used to investigate the effects of EPN3 on glioblastoma cell invasion and migration through gain and loss of EPN3 expression experiments; expression levels were further investigated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analyses. The results demonstrated that EPN3 expression levels were upregulated in high-grade glioma tissues compared with low-grade tissues, and there were varying expression levels of EPN3 in the five glioblastoma cell lines. No significant differences were observed in EPN3 expression in relation to patient age, sex or tumor size. Overexpression of EPN3 promoted glioblastoma cell migration and invasion, which we hypothesized was through affecting epithelial-mesenchymal transition (EMT). RT-qPCR and western blotting revealed that EPN3 upregulation increased the expression of Notch1 intracellular domain, β-catenin, Slug, Twist and zinc-finger E-box-binding homeobox (ZEB)-1. These results suggested that EPN3 enhances the migration and invasion of glioblastoma cells by activating the transcription factors Slug, Twist and ZEB1, but not Snail 1 or ZEB2, to induce EMT in glioma cells; EPN3 involvement in the Notch and WNT/β-catenin signaling pathways may contribute to this process.

**Introduction**

Glioblastoma is the most common malignant tumor of the nervous system; it is highly invasive, resistant to treatment and relapses easily (1). Numerous studies have focused on glioblastoma in relation to methylation status of a panel of genes (2), angiogenesis and DNA repair pathways (3,4), epidermal growth factor receptor, p53, isocitrate dehydrogenase 1 and MDM2 mutation status (5), and various therapeutic strategies (6,7); however, the overall survival of patients remains poor, with a median survival time of 12-15 months (8). Therefore, further investigations of the mechanisms and potential therapeutic strategies for glioblastoma are necessary.

Epsin (EPN)-3 is a member of the endocytosis adapter protein gene family, which contains several different interaction motifs, and is involved in clathrin-mediated endocytosis (9,10). The Epsin protein family is conserved between yeast, *Drosophila melanogaster* and mammals. In mammals, the Epsin family includes EPN1, EPN2 and EPN3. EPN1 and EPN2 are ubiquitously expressed, to various degrees, in different tissues, particularly in the brain (11,12). Previous studies have reported that EPN1 and EPN2 may be upregulated in a variety of cancer types and are associated with tumor cell proliferation, migration and invasion (11,12), whereas EPN3 expression is normally limited to gastric parietal cells (13), as well as wounded or pathological tissue, rather than normal brain tissue (14). It has been reported that EPN3 is overexpressed in basal cell carcinoma and ulcerative colitis (13,14). In addition, several studies have suggested that Epsins, as adapter proteins in clathrin-mediated endocytosis, are involved in the regulation of various signal receptors, including receptor tyrosine

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*Contributed equally

**Abbreviations:** BMP, bone morphogenetic protein; EMT, epithelial-mesenchymal transition; EPN3, Epsin 3; NICD1, Notch1 intracellular domain

**Key words:** EPN3, glioma, Notch pathway, WNT/β-catenin, EMT
kinases, Notch ligands and receptors, and WNT receptors;
defective regulation of these signaling ligands and receptors is
associated with tumorogenesis (15-17).

Notch signaling regulates tissue renewal and development, as
well as serving various roles in cell differentiation, proliferation
and apoptosis in a tissue context-dependent manner (18).
Aberrant loss or gain of Notch signaling components is associ-
ated with multiple human diseases (19-21). Activation of Notch
signaling requires its receptors to bind their specific ligands,
resulting in receptor proteolysis and the release of the active
Notch intracellular domain (NICD), which translocates to the
nucleus to initiate downstream target gene transcription (22).
A previous study has suggested that Epsins are required for
Notch signaling pathway activation (9).

WNT/β-catenin signaling is also important in tumor
development. WNT signaling is activated through endocy-
tosis (17,23,24), and Epsins are required for the stability of the
downstream protein Dishevelled and WNT signaling activa-
tion in colon cancer development (17).

Notch and WNT signaling pathways are associated with
epithelial-mesenchymal transition (EMT) (25-27). EMT is a
vital mechanism in embryonic development and tissue repair,
and is an essential mechanism of cancer cell metastasis (28).
The characteristics of EMT include the loss of the expression
or function of epithelial (E)-cadherin with a concomitant
increase in mesenchymal markers, including Vimentin (VIM)
and Neural-cadherin (29), potentially leading to the spread and
dissemination of cancer cells. EMT may be induced by several
transcription factors, including Snail1, Slug and Twist (25).

In the present study, EPN3 was revealed to be highly
expressed in high-grade glioma tissues compared with
low-grade samples. The effects of EPN3 on glioblastoma cell
migration and invasion were observed by inducing the gain
and loss of EPN3 expression in U87 and U251 glioblastoma
cells; the results revealed that the overexpression of EPN3
promoted glioblastoma cell migration and invasion, and
increased the expression of NICD1, β-catenin, VIM, Slug,
Twist and zinc-finger E-box-binding homeobox (ZEB)-1,
whereas the expression level of E-cadherin was decreased.
These results suggested that EPN3 may promote glioblastoma
cell migration and invasion by inducing EMT, and that EPN3
may be a potential therapeutic target in glioblastoma.

Materials and methods

Tumor samples and cell culture. Glioma tissues from four
different World Health Organization (WHO) grades were
collected from 167 patients at the Department of Neurosurgery
at Tianjin Huanhu Hospital (Tianjin, China), and included 71
women and 96 men (age 1-73 years; Table I); human colon carci-
noma tissue was donated by Professor Li Qi (Tianjin Medical
University Cancer Institute and Hospital). Informed consent
was obtained from all patients or their guardian, and the use
of patient tissue was approved by the ethics committee of
Tianjin Huanhu Hospital (2016-3). Glioblastoma cell lines
A172, LN229, LN308, U251 and U87 were purchased from the
American Type Culture Collection (Genetices ExCell
Technology, Inc., Shanghai, China). Cells were cultured in
Dulbecco’s modified Eagle’s medium (DMEM; Gibco; Thermo
Fisher Scientific, Inc., Waltham, MA, USA) supplemented
with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher
Scientific, Inc.), and maintained in a humidified atmosphere
with 5% CO₂ at 37°C.

Plasmid vectors and transfection. All plasmids were purchased
from OriGene Technologies, Inc. (Rockville, MD, USA).
The pGFP-C-shLenti vector encoding short hairpin (sh)RNA
against EPN3 (shEPN3; cat. no. TL304750; 5'-CACAATAC
TCCGAGGCAGAAATCAAGGT-3') was used to silence
EPN3 expression; Scrambled-shRNA (cat. no. TR30021;
5'-GCACTAACAGAGCTACTCAGATAGTACT-3') was
used as negative control. pCMV6-EPN3 (cat. no. RC212297)
was used for the upregulation of EPN3; pCMV6-Entry
(cat. no. PS100001) was used as negative control. Cells were
seeded at a density of 2x10⁵ cells/well in 6-well culture plates,
and plasmid transfections (2,500 ng) were performed using
Lipofectamine® 2000 transfection reagent (Invitrogen;
Thermo Fisher Scientific, Inc.), according to the manufactur-
er's instructions, when cell cultures reached 90% confluency;
cells were incubated in a humidified atmosphere with 5% CO₂
at 37°C for 48 h. Gene expression levels were evaluated by
reverse transcription-quantitative polymerase chain reaction
(RT-qPCR) and western blot analysis at 48 h post-transfection.

RT-qPCR. Total RNA was extracted from the transfected cells
(5x10⁵) using 1 ml TRIzol reagent (Invitrogen; Thermo Fisher
Scientific, Inc.), according to the manufacturer's protocol.
cDNA was synthesized using a cDNA Reverse Transcription
Kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to
the manufacturer's protocol. qPCR was performed using Power
SYBR Green PCR master mix (Applied Biosystems; Thermo
Fisher Scientific, Inc.) and an ABI 7500 series Real-Time PCR
machine (Applied Biosystems; Thermo Fisher Scientific, Inc.).
The thermocycling condition was as follows: Initial denatur-
ation at 95°C (30 sec), followed by 40 cycles of denaturation
at 95°C (20 sec), annealing at 60°C (20 sec) and elongation
at 72°C (20 sec). mRNA expression levels were normalized to
GAPDH and were calculated using the 2^ΔΔCt method (30). The
primers used for PCR are listed in Table II.

Antibodies. Anti-EPN3 was purchased from Sigma-Aldrich
(cat. no. SAB1303019; Merck KGaA, Darmstadt, Germany);
anti-GAPDH was purchased from ProteinTech Group, Inc.
(cat. no. 10494-1-AP; Chicago, IL, USA); anti-E-cadherin
(cat. no. sc-8426), anti-Slug (cat. no. sc-166902), anti-Snail
1 (cat. no. sc-393172), anti-ZEB2 (cat. no. sc-271984)
and anti-Twist (cat. no. sc-81417) were from Santa Cruz
Biotechnology, Inc. (Dallas, TX, USA); anti-β-catenin
was from BioLegend, Inc. (cat. no. 677801; San Diego, CA,
USA); and anti-NICD1 (cat. no. WL03097a), anti-ZEB1
(cat. no. WL01657) and anti-β-catenin (cat. no. WL0962a)
were from Wanleibio Co., Ltd. (Shanghai, China).
The horseradish peroxidase (HRP)-conjugated goat anti-mouse
(cat. no. ZB-2305) and HRP-conjugated goat anti-rabbit
(ZB-2301) secondary antibodies were purchased from
ZSGB-BIO (OriGene Technologies, Inc.).

Western blot analysis. Total protein was extracted from cells
(1x10⁶) using Radioimmunoprecipitation Assay lysis buffer
(Beyotime Institute of Biotechnology, Shanghai, China) on
ice. Protein concentrations were determined by Bicinchoninic Acid assay (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Equal amounts of protein (30 µg) were separated using SDS-PAGE and transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% skim milk in TBST (1X TBS + 0.1% Tween-20) buffer for 2 h. Following washing 3 times for 5 min in TBST, membranes were incubated with primary antibodies against EPN3, E-cadherin, Slug, Twist (all at 1:500), VIM (1:5,000), NICD1 (1:500), β-catenin (1:1,000) and GAPDH (1:10,000) overnight at 4˚C. Membranes were washed 3 times (5 min each) in TBST, and subsequently incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (1:1,000) for 1 h at room temperature. Protein bands were visualized using an Enhanced Chemiluminescence Western Blot Detection kit, and the intensity of protein bands were normalized to GAPDH and quantified by densitometric analysis using Quantity One software (version 4.6.9; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Immunohistochemistry (IHC).** Human colon carcinoma tissues were used as a positive control. Colon carcinoma and glioma tissue samples were fixed using 10% formalin at room temperature for 2 h, following washing 3 times for 5 min in TBST, membranes were incubated with primary antibodies against EPN3, E-cadherin, Slug, Twist (all at 1:500), VIM (1:5,000), NICD1 (1:500), β-catenin (1:1,000) and GAPDH (1:10,000) overnight at 4˚C. Membranes were washed 3 times (5 min each) in TBST, and subsequently incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (1:1,000) for 1 h at room temperature. Protein bands were visualized using an Enhanced Chemiluminescence Western Blot Detection kit, and the intensity of protein bands were normalized to GAPDH and quantified by densitometric analysis using Quantity One software (version 4.6.9; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Table I. Relationship between EPN3 expression and clinicopathological features in patients with glioma.**

<table>
<thead>
<tr>
<th>Clinicopathological feature</th>
<th>EPN3 expression</th>
<th>Positive rate (%)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>49 Low 41 High 8</td>
<td>17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>47 Low 38 High 9</td>
<td>52.1</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>36 Low 19 High 17</td>
<td>35.4</td>
<td>0.322</td>
</tr>
<tr>
<td>IV</td>
<td>35 Low 15 High 20</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>96 Low 62 High 34</td>
<td>31.1</td>
<td>0.715</td>
</tr>
<tr>
<td>Female</td>
<td>71 Low 51 High 20</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>90 Low 62 High 28</td>
<td>31.1</td>
<td>0.963</td>
</tr>
<tr>
<td>≥45</td>
<td>77 Low 51 High 26</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4.5</td>
<td>121 Low 82 High 39</td>
<td>32.2</td>
<td></td>
</tr>
<tr>
<td>≥4.5</td>
<td>46 Low 31 High 15</td>
<td>32.6</td>
<td></td>
</tr>
</tbody>
</table>

aP-values were calculated by χ². EPN3, Epsin 3; WHO, World Health Organization.

**Table II. Primer sequences used for reverse transcription-quantitative polymerase chain reaction.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPN3</td>
<td>F: CTTGGGCTGACATCTTCGTACCT R: TGTGGTCGCCCTAAACACCTG</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>F: CAGCAGCTACAGAGGCTAA R: ACCCTGAGCTTGGACATCTT</td>
</tr>
<tr>
<td>Vimentin</td>
<td>F: AGATGAGGGCAGTGAGATTTGAG R: TGGAAGAGGCAGAGAATTC</td>
</tr>
<tr>
<td>Notch1</td>
<td>F: ACCAAATACAACCTCCTGGCGG R: GGCCCTTGATAGTTGTCATAC</td>
</tr>
<tr>
<td>Notch2</td>
<td>F: GGAGGCCACCTGATATTGACCT</td>
</tr>
<tr>
<td>Notch3</td>
<td>F: CTGGGCCCCCTCATGTTATCCT</td>
</tr>
<tr>
<td>Notch4</td>
<td>F: TTCCCAAGACCTGTGCAAT R: AACTGGACCTTCCACCCAG</td>
</tr>
<tr>
<td>Snail1</td>
<td>F: CCTCCCTGTCAGATGAGGAC R: GTCCTTCTGAGGTGCAGAC</td>
</tr>
<tr>
<td>Slug</td>
<td>F: GGAGAAGACGTTTCTTG</td>
</tr>
<tr>
<td>Twist</td>
<td>F: GAGGCTCCAGCTGTCAGAGG</td>
</tr>
<tr>
<td>ZEB1</td>
<td>F: GCACAAACAGGTCGAAAG</td>
</tr>
<tr>
<td>ZEB2</td>
<td>F: AATGCAAGATGATGCGAAC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: CATGGACCCCTTCATGCA</td>
</tr>
</tbody>
</table>

E-cadherin, epithelial cadherin; EPN3, Epsin 3; F, forward; R, reverse; ZEB zinc-finger E-box-binding homeobox.

Human colon carcinoma tissues were used as a positive control. Colon carcinoma and glioma tissue samples were fixed using 10% formalin at room temperature for 2 h, embedded in paraffin and sectioned (4 µm), slides were deparaffinized with xylene and hydrated in a decreasing ethanol series. Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide for 15 min. Following heat-induced antigen retrieval at 100°C for 10 min, sections were incubated with 5% rabbit serum (ZSGB-BIO; OriGene Technologies, Inc.) for 10 min to eliminate non-specific binding. Anti-EPN3 (1:200) was added and the sections were incubated overnight at 4°C. Sections were washed 3 times (5 min each) using PBS, and the corresponding optimized secondary antibodies (Bond Polymer refine detection; cat. no. DS9800; Leica Microsystems, Ltd., Milton Keynes, UK) were added to the sections and incubated for 1 h at room temperature, followed by incubation with the streptavidin-HRP complex (Santa Cruz Biotechnology, Inc.). Immunoreactivity was visualized by incubating with 3,3'-diaminobenzidine (Sigma-Aldrich; Merck KGaA) for 3-15 min, and the sections were counterstained with hematoxylin. Two pathologists blinded to the clinical data independently scored the percentage of positive-staining cells and the intensity of the EPN3 staining. A three-tiered scale was used to grade the staining intensity (1, negative or weak; 2, medium; 3, strong), whereas the percentage of EPN3-positive tumor cells was scored as follows: 0, ≤10% positive tumor cells; 1, 11-24%; 2, 25 -50%; 3, 51-75% and 4, >75%. The product of the intensity and positive percentage score of the cells was used as a staining index (negative, 4; positive, ≥4).
Overexpression of EPN3 enhances migration and invasion of U87 and U251 glioblastoma cells. Varying EPN3 protein expression levels were detected among the five glioblastoma cell lines examined (A172, LN229, U251, LN308 and U87), with the highest expression levels detected in U251 and U87 cells, and the lowest level in A172 cells (Fig. 2A). U251 and U87 cells were selected for further experiments. Notably, although U87 is different from the original glioblastoma cells, Marie Allen et al have reported that U87 cell line is of CNS origin and is likely to be a bona fide human glioblastoma cell line with unknown patient origin (31). U251 and U87 cells transfected with shEPN3 exhibited reduced EPN3 mRNA and protein expression levels compared with cells transfected with scrambled-shRNA (Fig. 2B and C). In addition, cells transfected with EPN3 overexpression vector exhibited increased levels of EPN3 mRNA and protein expression compared with expression levels empty vector-transfected cells (Fig. 2B and C).

The effects of EPN3 on glioblastoma cell migration and invasion were examined using Transwell migration and invasion assays. The results demonstrated that the reduction of EPN3 expression significantly inhibited U87 and U251 glioblastoma cell migration and invasion compared with the control cells (P<0.001; Fig. 3A and B, respectively). Conversely, the overexpression of EPN3 significantly promoted U87 and U251 cell migration and invasion compared with control cells (P<0.01, P<0.001; in fig. 3). These results indicated that EPN3 overexpression enhanced glioblastoma cell migration and invasion in vitro.

Overexpression of EPN3 induces EMT in U87 and U251 glioblastoma cells. EMT serves an important role in the process of tumor cell migration and invasion (32); therefore, expression levels of key molecular markers of EMT, E-cadherin and VIM, were analyzed by RT-qPCR and western blotting in transfected U87 and U251 cells. U87 and U251 cells transfected with shEPN3 exhibited increased levels of E-cadherin mRNA and protein expression (Fig. 4A and B), whereas the expression levels of VIM decreased. Conversely, EPN3-overexpressing U87 and U251 cells exhibited decreased E-cadherin mRNA and protein expression levels (Fig. 4A and B, respectively), whereas the mRNA and protein expression levels of VIM were increased. These results indicated that EPN3 may be associated with EMT, which may contribute to the migratory and invasive ability of glioblastoma cells.

Overexpression of EPN3 increases Notch1 and β-catenin expression in U87 and U251 glioblastoma cells. The Notch pathway is activated in glioblastoma cells and promotes their migration and invasion (33,34); EPN1 and EPN2 were reported to be adaptor proteins in clathrin-mediated endocytosis, which is necessary for the activation of the Notch
pathway (9,35-37). Therefore, whether EPN3 was involved in the activation of the Notch pathway in glioblastoma cells was examined. RT-qPCR analysis was used to identify the effects on Notch receptor expression following the overexpression of EPN3 in U87 cells. The results suggested that Notch1 expression increased when EPN3 was overexpressed, whereas no significant differences were observed in Notch2, Notch3 or Notch4 expression (Fig. 5A). As Notch1 is expressed in primary human gliomas and glioblastoma cell lines (18), the expression of the activated NICD1 was examined by western blotting in transfected U87 and U251 glioblastoma cells. It was demonstrated that the expression levels of NICD1 decreased...
when EPN3 was downregulated and increased when EPN3 was overexpressed in U87 and U251 cell lines compared with the respective control-transfected cells (Fig. 5B). WNT/β-catenin signaling is also activated via endocytosis (23,24), and it was previously reported that Epsin is required for WNT signaling activation in colon cancer development (17). In the present study, the expression of β-catenin was decreased when EPN3 was downregulated (Fig. 5C). In conclusion, EPN3 may function to activate Notch1 and WNT signaling pathways to promote tumorigenesis in glioblastoma.

**Overexpression of EPN3 increases Slug, Twist and ZEB1 expression levels in U87 and U251 glioblastoma cells.** Notch activation induces EMT through the Slug-induced repression of E-cadherin (38) and the upregulation of EMT-inducing transcription factors, including Snail1, Slug, ZEB1, ZEB2 and Twist (25). Whether EPN3 activated these EMT transcription factors was assessed using RT-qPCR and western blotting (Fig. 6). The results demonstrated that Slug, Twist and ZEB1 mRNA expression levels were increased in cells overexpressing EPN3 compared with the respective levels in cells transfected with empty vector (Fig. 6A); Snail1 and ZEB2 mRNA expression levels remained unaffected. Conversely, protein expression levels of Slug, Twist and ZEB1 were decreased in shEPN3-transfected U87 and U251 cells, compared with Scramble shRNA-transfected cells, whereas their expression levels were increased when EPN3 was overexpressed (Fig. 6B-D) Conversely, Snail1 and ZEB2 expression levels were unaltered (Fig. 6E and F, respectively). These results suggested that EPN3 may increase the
transcription of the EMT transcription factors Slug, Twist and ZEB1, rather than Snail-1 and ZEB2, to induce EMT in glioblastoma cells.

**Discussion**

The Epsin protein family may promote oncogenesis and cancer progression (11); EPN1 and EPN2 have been associated with various types of tumor (39-41), whereas studies regarding EPN3 are limited. A recent study reported that the high expression levels of EPN3 were associated with an increased risk of metastasis in estrogen receptor-positive breast cancer and non-small cell lung cancer (42). Other studies have suggested that EPN3 promotes cellular senescence and is associated with the p53 pathway (43,44). The present study demonstrated that EPN3 was expressed in glioma tissues and glioblastoma cell lines. The results also revealed that the overexpression of EPN3 promoted glioblastoma cell migration and invasion, which indicated that EPN3 may serve as a potential target for glioblastoma therapy.

Relatively little is known about the role of EPN3 in the endosome. Howe and Mobley proposed the signaling endosome hypothesis as a cellular mechanism for distance communication (45); they suggested that activated Tyrosine kinase receptor A and other signaling proteins were internalized into an endocytic organelle, which was then retrograde-transported from the axon tip to the neuron cell body. In addition, Vanlandingham *et al* reported that EPN1...
serves an important endosomal role in the efficient retrograde transport of bone morphogenetic protein (BMP) signaling endosomes into motor neuron nuclei, and that EPN1 may negatively regulate BMP signaling at the plasma membrane of the neuromuscular junction, while serving a positive role in nuclear accumulation of phosphorylated Mothers against decapentaplegic (46). These data suggested that EPN3, as an endocytosis adapter protein, may serve different roles in different subcellular localization; however, additional studies to determine these roles are required.

Epsin family proteins are required for Notch pathway activation (9); Notch signaling plays vital roles in glioblastoma cell survival and proliferation (18), migration and invasion (38), radioresistance (21) and EMT (25,26). Similarly, WNT/β-catenin signaling has been reported to be implicated in the proliferation of neural stem cells (47), and the activation...
of canonical WNT/β-catenin signaling enhances the migration and invasion of glioblastoma cells via activating ZEB1 and other activators of EMT (27). Consistently, the results of the present study demonstrated that EPN3 enhanced the migration and invasion of glioblastoma cells, which may have been due to the activation of Notch and WNT/β-catenin signaling. It has been reported that the Notch and WNT pathways interact in breast tumorigenesis (48), and that Notch signaling upregulates the expression of Snail 1 and Slug (49). Slug is important for Notch-mediated EMT as it functions to repress E-cadherin expression, which in turn induces β-catenin activation in human breast cancer (38). However, in colorectal cancer, Notch1 counteracts WNT/β-catenin signaling through chromatin modification (50). Nevertheless, the present study hypothesized that Notch signaling and WNT signaling may act synergistically to promote glioblastoma cell migration and invasion.

The present study results indicated that overexpression of EPN3 may activate EMT in glioblastoma cells and increase the expression of Slug, Twist and ZEB1. In addition, previous studies have reported that E-cadherin may be removed through clathrin-mediated endocytosis, which EPN3 is involved in, and may lead to E-cadherin degradation and the consequent disassembly of adherens junctions (51). Taken together, EPN3
may promote glioblastoma cell EMT, which may be dependent on activating EMT.

In conclusion, the present study results demonstrated the oncogenic role of EPN3 in glioblastoma and its induction of EMT; the activation of Notch and WNT signaling pathways may also have enhanced the migration and invasion of glioblastoma cells induced by EPN3 overexpression. However, there are some limitations in the present study. First, whether the effects of EPN3 on Notch and WNT pathway are related to EPN3-enhanced glioblastoma cells migration and invasion, as well as EMT, remain to be investigated. Second, whether transcription factors are involved in EPN3-induced Notch and WNT pathways also needs further research. However, further investigations are needed to clarify the precise molecular mechanisms of the effects on glioblastoma migration and invasion induced by EPN3 overexpression.

Acknowledgments

We thank Dr Li Qi for donating the human colon carcinoma tissue (Tianjin Medical University Cancer Institute and Hospital, Tianjin, China). We also thank Dr Qiaoli Wu and Mrs. Xiuhua Yao (Clinical Laboratory, Tianjin Key Laboratory of Cerebral Vascular and Neurodegenerative Diseases, Tianjin Neurosurgical Institute, Tianjin Huanhu Hospital) for their technical support and assistance in the study.

Funding

This work was supported by a grant from The Tianjin Health and Family Planning Commission (grant no. 14KG118) and The National Nature Science Foundation of China (grant no. 81501035).

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YW performed most experiments. WS analyzed the patient data regarding the glioma tissues. PK, CH and ZM drafted the manuscript. XY and QW revised the manuscript critically for important intellectual content; BZ gave final approval of the version to be published. These authors were also involved in the conception of the study. Each author has participated sufficiently in the study to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Informed consent was obtained from all patients, and the use of patient tissue was approved by the Ethics Committee of Tianjin Huanhu Hospital (Tianjin, China; 2016-3).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


