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RESEARCH

Galectin-3 Accelerates the Progression of Oral Tongue Squamous Cell Carcinoma via a Wnt/β-catenin-Dependent Pathway

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Abstract The purpose of this study was to elucidate the clinicopathological significance and mechanism of action of galectin-3 in oral tongue squamous cell carcinoma (OTSCC). Here, the expression of galectin-3 was quantified in OTSCC (n=68) and paired OTSCC and normal surrounding tissues (n=10) using immunohistochemical staining. Tca8113 OTSCC cells were transfected with a plasmid expressing galectin-3 cDNA or siRNA against galectin-3. Cell proliferation, migration and invasion were measured using the MTT assay, Matrigel-coated Transwell migration assay and wound healing assay. The effect of galectin-3 on the Wnt/β-catenin signaling pathway and epithelial mesenchymal transition (EMT) were investigated using a plasmid expressing the Wnt antagonist dickkopf 1 (DKK1) and Western blotting. Galectin-3 was expressed at significantly higher levels in OTSCC than the normal adjacent tissues; galectin-3 expression correlated strongly with pathological stage, pathological grade and lymph node invasion in OTSCC. Overexpression of galectin-3 promoted Tca8113 cell proliferation, migration and invasion, upregulated Wnt protein expression, activated β-catenin and induced the EMT; knockdown of galectin-3 had the opposite effects. Co-transfection of Tca8113 cells overexpressing galectin-3 with the Wnt antagonist DKK1 reduced the ability of galectin-3 to increase cell proliferation, migration and invasion, reduced upregulation of Wnt, inhibited β-catenin activation and abrogated the EMT, demonstrating that the Wnt/β-catenin signaling pathway mediated the effects of galectin-3. Galectin-3 plays an important role in the progression of OTSCC via activation of the Wnt/β-catenin signaling pathway.

Keywords Galectin-3 · Oral tongue squamous cell carcinoma · Wnt/β-catenin · Clinicopathological significance · Mechanism

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common types of oral malignant neoplasm, accounting for more than 95 % of all oral cancers. In recent years, the incidence of oral cavity cancers in younger patients has increased [1, 2]. Forty to 50 % of patients with early stage oral tongue carcinoma develop neck lymph node metastasis, which correlates highly with relapse, poor prognosis and death [3–5].

Galectins are a family of lectins which bind to β-galactosides via a carbohydrate recognition domain containing many conserved sequence elements [6]. Galectins also contain a core sequence of approximately 130 amino acids, and many of these sequences are also highly conserved. There are currently 15 known mammalian galectins [7] which are involved in a variety of biological processes, including morphogenesis, regulation of cell apoptosis and the immune response [8]. Galectin-3, a 31 kDa member of the β-galactoside-binding protein family, is an intracellular and extracellular lectin which interacts with intracellular glycoproteins, cell surface

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molecules and extracellular matrix proteins. The carbohydrate-binding properties of galectin-3 constitute the basis for cell-cell and cell-matrix interactions [9]. Overexpression of galectin-3 occurs in many types of cancer, including thyroid, prostate, colon and laryngeal tumors, and galectin-3 has been strongly associated with the initiation and progression of cancer [10–14].

Galectin-3 serves as a prognostic factor in oral tongue carcinoma and is involved in the neoplastic progression of this disease [15, 16]; however, the role and mechanism of action of galectin-3 in OTSCC have not yet been clarified. The present study was designed to determine how galectin-3 accelerates the progression of oral tongue squamous cell carcinoma (OTSCC). Our data demonstrates that galectin-3 may accelerate the initiation and progression of OTSCC via a Wnt/β-Catenin-dependent signaling pathway.

Table 1 Clinicopathological features and prognosis of the 68 OTSCC patients

Variable	No. of patients	%
Pathological stage		
I	34	50.0
II	18	26.5
III	10	14.7
IV	6	8.8
Pathological garde		
I	50	73.5
II	13	19.1
III	5	7.4
Disease outcome		
Recurence	18	26.5
Death	13	19.1
Lymph node metastases		
Absent	52	76.5
Present	16	23.5

Materials and Methods

Cell Culture

The U937 cells and the immortalized human OTSCC cell line Tca8113 (Chinese Life-Science Academy, Shanghai, China) was grown in RPMI 1640 supplemented with 10 % fetal bovine serum (Gibco BRL, Grand Island, NY, USA), 2 mM glutamine, 100 U/ml penicillin and streptomycin at 37 °C in 5 % CO_2. All studies were carried out using 90–100 % confluent cells which had been serum-deprived overnight.

Patient Tissue Samples

The 68 paraffin-embedded OTSCC tissue samples and ten paired OTSCC and adjacent normal tissue samples used in this study were histopathologically and clinically diagnosed at Sun Yat-sen University Cancer Center between 2006 and 2009. None of the patients had received radiotherapy or chemotherapy prior to surgery. The International Union against Cancer TNM classification was used to categorize the OTSCC patients (48 males and 30 females; median age, 53 years; age range, 20–81 years; Table 1). The patients' consent and approval from the Institutional Research Ethics Committee were obtained prior to the study.

Plasmid Construction and Transfection

A galectin-3 overexpression plasmid was constructed as previously described [17]. Briefly, total RNA was extracted form U937 cells and first-strand cDNA synthesis was performed using Superscript III First-Strand Synthesis Supermix (18080-051, Invitrogen, CA, USA) following the manufacturer's instructions. Oligonucleotide primers flanking the 5′ end of the galectin-3 coding sequence (5′-GGCTAGAAGCTTATGGCAGACAATTTTTCGCTG-3′; including an artificial *Hind* III site 5′ of the galectin-3 ATG start codon) and 3′ end of the galectin-3 coding sequence (5′-GGCGAATTCTTATATCATGGTATATGA-3′, including an *EcoR* I site 3′ of the galectin-3 stop codon) were used to generate full-length galectin-3 cDNA by PCR. The cDNA was inserted into the *BamH* I and *Hind* III sites of the basic pcDNA3 plasmid (V790-20, Invitrogen, CA, USA), transformed into *Escherichia coli* (strain DH5α), plasmid DNA was isolated by the alkaline lysis method and positive transformants were confirmed by dideoxy DNA sequencing (Invitrogen, Carlsbad, CA, USA) of their inserts.

Galectin-3 siRNA was used as previously described for knockdown experiments [17]. Briefly, oligonucleotide siRNA duplexes were synthesized by Shanghai Gene Pharma (Shanghai, China). The human galectin-3 siRNA sequence was 5′-GAAGAAAGACAGTCGGTTT-3′ and the scrambled siRNA sequence was 5′-UUCUCCGAACGUGUCACGUTT-3′.

The purified plasmid or siRNAs were transfected into Tca8113 cells using Lipofectamine 2000 (11668, Invitrogen, CA, USA). To block Wnt signaling, the cells were concomitantly transfected with the empty control vector pFLAG or the DKK1 expression plasmid pFlag-DKK1 (provided by Dr. Xi He, Harvard Medical School, Boston, MA, USA).

Immunostaining

OTSCC paraffin-embedded tissue sections were prepared at 5 μm thickness and stained via a routine indirect immunohistochemistry protocol using a galectin-3 antibody (Abcam, Research Diagnostics Inc., MA, USA). Briefly, 5-mm tissue sections were deparaffinized in xylene, rehydrated in ethanol, inhibit endogenous peroxidase activity with freshly prepared 3 % hydrogen peroxide. After blocking with 5 % normal

rabbit serum in PBS buffer, the slides were incubated with the primary galectin-3 antibody in PBS containing 1 % BSA overnight at 4 °C. Slides were then incubated at room temperature for 30 min with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) and then avidin-biotin complex reagent, (Vector Laboratories). Subsequently, diaminobenzidine (Abbott Laboratories, Abbott Park, IL) was used to visualize the bound antibody. Adjacent normal tissue samples used as positive, internal control were also analyzed. As a negative control, the primary antibody was omitting. The stained slides were viewed under an Olympus BX51 microscope equipped with a digital camera (Nikon, Melville, NY, USA). Galectin-3 staining was semi-quantitatively scored by computer-aided morphometric analysis, as previously described [18]. Briefly, a grid containing 117 (13×9) sampling points was superimposed onto high-power field images (x400). The number of grid points overlying galectin-3-positive areas was determined and expressed as a percentage of the total number of sampling points. For each tissue, ten randomly selected non-overlapping fields were analyzed in a blinded manner.

MTT Cell Proliferation Assay

The 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed to measure cell proliferation. Briefly, Tca8113 cells (7500 cells/well) were cultured in 96-well plates for 24 h, transfected with galectin-3 and/or DKK1 expression plasmids, or galectin-3 siRNA, and cell proliferation was determined 24 h later. MTT solution (5 mg/ml in PBS) was added to each well, incubated at 37 °C for 4 h, the media was removed and 100 μl dimethyl sulfoxide was added to each well. The absorbance of each well was measured at 570 nm using an automated microplate reader (Type Elx800, Bio-Tek Instruments, Winooski, ST, USA).

Migration Assay

Cells were seeded into 35 mm plates, transfected with galectin-3 and/or DKK1 expression plasmids, or galectin-3 siRNA, and grown to confluence. Two parallel wounds were created in each confluent monolayer using a yellow pipette tip, as previously described [19]. The cells were washed twice with PBS, and the wounds were photographed 24 h later.

Invasion Assay

Invasion assays were performed using Transwell culture plates (Corning Life Sciences, Acton, MA, USA) according to the manufacturer's instructions using filters precoated with Matrigel (Becton Dickinson Labware, Franklin Lakes, NJ, USA). Cells transfected with galectin-3 and/or DKK1 expression plasmids, or galectin-3 siRNA (4×10^4 cells/insert) were placed into the upper chamber in 0.5 ml of RPMI 1640 serum-free medium. RPMI 1640 supplemented with 10 % fetal bovine serum was placed in the lower chamber as a chemo-attractant. After incubation for 20 h, cells which had migrated to the lower surface of the filters were fixed in 0.1 % paraformaldehyde followed by 4 % paraformaldehyde in PBS for 10 min, stained with hematoxylin and counted. Cell invasion was expressed as the average number of cells per microscopic field, from at least four fields of view per filter. All experiments were performed in triplicate.

Western Blotting

Western blotting analysis was performed using standard procedures. Briefly, cells were washed with PBS, whole-cell lysates were prepared, and protein content was quantified using Coomassie Brilliant Blue. The proteins (20 ug) were separated by SDS-PAGE, transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, ST, USA) and subjected to Western blotting using rabbit polyclonal anti-Galectin-3 (1: 150; Abcam), mouse polyclonal anti-Vimentin (1:250; ab-2 clone V9, Neomarker, CA, USA), rabbit polyclonal anti-Wnt1 (1:400; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-active β-catenin (1:100; 4270, Cell Signaling, MA, USA) and rabbit polyclonal anti-E-cadherin (1:100; 3195, Cell Signaling, MA, USA). Band intensity was quantified using Gel-pro Image analysis software (Media Cybernetics, Inc., Bethesda, MD, USA).

Statistical Analysis

All data are expressed as the mean ± SD. Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, IL, USA). Comparisons between groups were made using one-way analysis of variance, followed by the Student-Newman-Keuls test. P values<0.05 were considered significant.

Results

Galectin-3 is Upregulated and Correlates with Pathological Stage in OTSCC

Expression of galectin-3 was quantified using immunohistochemistry in 68 paraffin-embedded OTSCC tissues. Galectin-3-positive area increased significantly in advanced

pathological stage (Fig. 1a) and poorly differentiated OTSCC. Higher magnification images indicated that galectin-3 was mainly expressed in the cytoplasm (89.7 %). One-way ANOVA and the Student-Newman-Keuls test demonstrated that expression of galectin-3 showed a significant correlation with pathological stage (Fig. 1b) and pathological grade.

Immunohistochemical analysis of 10 paired OTSCC and adjacent normal tissues demonstrated that galectin-3 was expressed at significantly higher levels in OTSCC than the surrounding normal tissues (Fig. 2).

Overexpression of Galectin-3 Promotes OTSCC Cell Proliferation and Migration

The immortalized Tca8113 cell line was used to examine the effects of galectin-3 in vitro. A galectin-3 overexpressing plasmid was constructed and Tca8113 cells were transfected with empty pcDNA, pCDNA3-galectin-3, scrambled siRNA or galectin-3 siRNA. Western blotting was used to confirm the overexpression and knockdown of galectin-3. As shown in Fig. 3a, both the galectin-3 overexpression plasmid and galectin-3 siRNA were effective.

The MTT assay and wound healing assay were used to investigate the effect of galectin-3 on OTSCC cell proliferation and migration. As shown in Fig. 3b and c, overexpression of galectin-3 promoted both Tca8113 cell proliferation and migration, compared to control and empty vector-transfected cells. Conversely, knockdown of galectin-3 inhibited Tca8113 cell proliferation and migration, compared to control and scrambled siRNA-transfected cells.

Overexpression of Galectin-3 Increases the Invasion of OTSCC Cells

To explore the relationship between galectin-3 and invasion in OTSCC, the expression of galectin-3 was compared in five pairs of OTSCC tissues at the same tumor (T) stage with or without lymph node invasion. Both the galectin-3-positive staining area and intensity were significantly higher in OTSCC tissues with lymph node invasion than tissues without lymph node invasion (Fig. 4a).

Next, we examined the effect of galectin-3 on the invasive ability of OTSCC cells using the Matrigel-coated Transwell migration assay. Overexpression of galectin-3 increased the number of migrating Tca8113 cells, compared to control and empty vector-transfected cells (Fig. 4b). Conversely, knockdown of galectin-3 using siRNA inhibited Tca8113 cell invasion, compared to control and scrambled siRNA-transfected cells (Fig. 4b).

Galectin-3 Activates Wnt-β-catenin Signaling and Induces the Epithelial-mesenchymal Transition in OTSCC Cells

Recent studies have suggested that the Wnt/β-catenin signaling plays a critical role in the initiation and progression of OTSCC [20–22]. To provide further insights into the mechanism by which galectin-3 stimulated the proliferation, migration and invasion of OTSCC cells, we investigated the effect of galectin-3 on Wnt/β-catenin signaling in Tca8113 cells. As shown in Fig. 5a, overexpression of galectin-3 promoted upregulation of Wnt 1, whereas galectin-3 siRNA inhibited the expression of Wnt 1 in Tca8113 cells. As β-catenin is the principal mediator of canonical Wnt signaling [23], we also examined the effect of galectin-3 on β-catenin expression. Western blotting analysis revealed that overexpression of galectin-3 upregulated β-catenin, whereas galectin-3 siRNA inhibited β-catenin expression (Fig. 5a).

Wnt/β-catenin signaling plays a central role in regulation of the epithelial-mesenchymal transition (EMT), a phenotypic conversion which frequently occurs during cancer development and metastasis [24, 25]. Vimentin and E-cadherin are two key regulators of the EMT. Interestingly, overexpression of galectin-3 induced expression of the mesenchymal marker vimentin, and inhibited expression of the epithelial marker E-cadherin (Fig. 5b). Taken together, these results indicated that galectin-3 may increase proliferation, migration and invasion, and induce the EMT in OTSCC cells via activation of the Wnt/β-catenin signaling pathway.

Wnt/β-catenin Signaling Mediates the Effects of Galectin-3 in OTSCC Cells

To confirm that Wnt/β-catenin signaling mediated the effects of galectin-3 in OTSCC cells, we blocked endogenous Wnt signaling using a plasmid expressing DKK1 (pFlag-DKK1). DKK1 is a natural antagonist of canonical Wnt signaling by virtue of its ability to bind and block the low-density lipoprotein receptor-related protein 5/6 (LRP5/6) co-receptor [26, 27]. OTSCC cells were transfected with pCDNA-Galectin-3 with or without pFlag-DKK1. Co-transfection of DKK1 reduced the ability of galectin-3 to stimulate cell proliferation, migration and invasion in OTSCC cells, as demonstrated by the MTT assay (Fig. 6a), would healing assay (Fig. 6b) and Matrigel-coated Transwell migration assay (Fig. 6c).

To confirm whether DKK1 inhibited the ability of galectin-3 to induce Wnt/β-catenin signaling activation and the EMT in OTSCC cells, we performed Western blotting to quantify the expression of Wnt 1, activated β-catenin, vimentin and E-cadherin. As shown in Fig. 7a, co-transfection of OTSCC cells with galectin-3 and pFlag-DKK1 inhibited the expression of

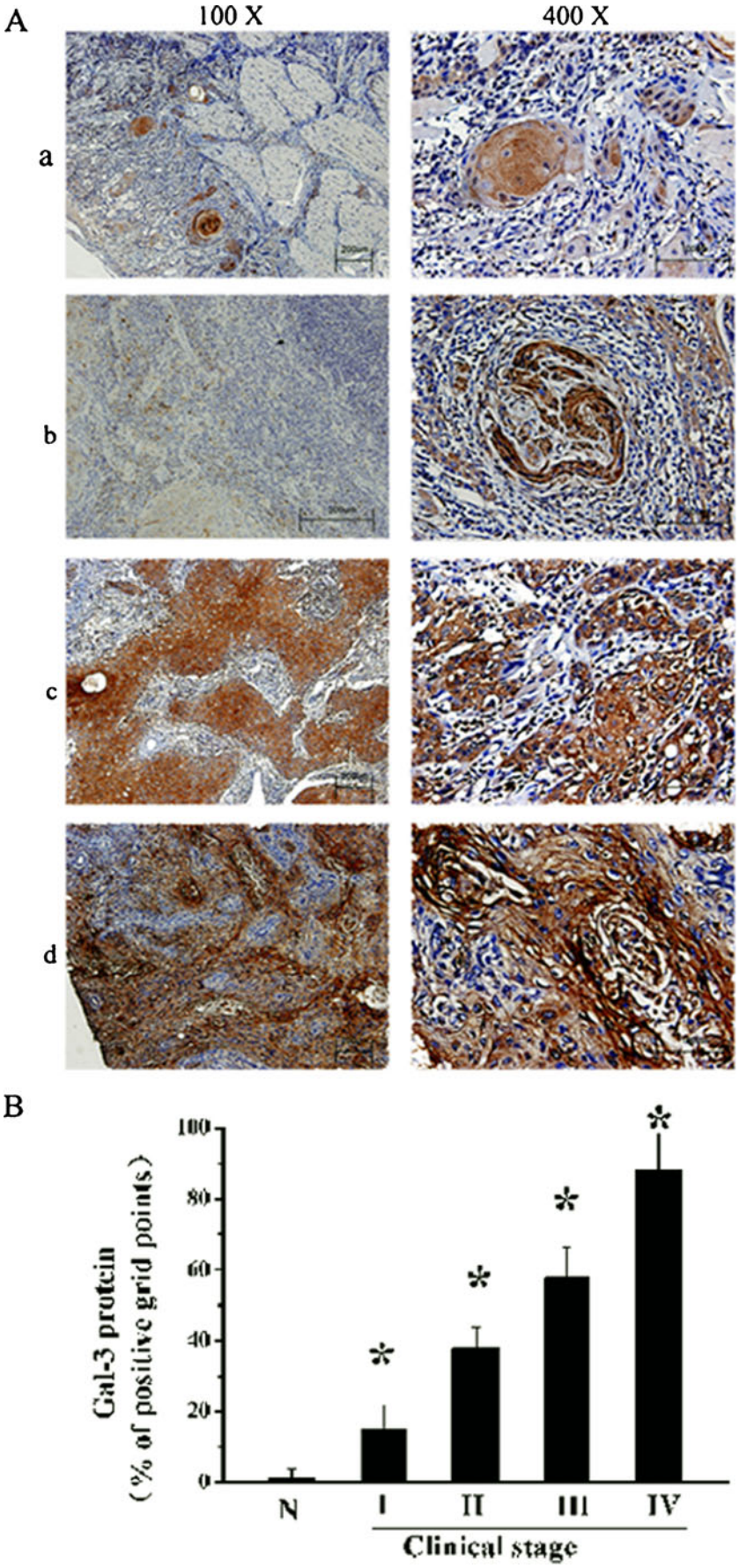

Fig. 1 Upregulation of galectin-3 correlates with pathological stage in OTSCC. **a** Expression of galectin-3 was analyzed by immunohistochemistry in different pathological stages of OTSCC using a galectin-3 antibody. *a*, pathological stage I; *b*, pathological stage II; *c*, pathological stage III; *d*, pathological stage IV. **b** Quantification of the galectin-3-positive area in different pathological stages of OTSCC. Data are expressed as mean ± SD of 68 OTSCC samples; # $P<0.001$, *$P<0.05$ vs. adjacent normal tissues (one-way ANOVA)

Wnt 1 and activation of β-catenin, compared to cells transfected with only galectin-3. Interestingly, DKK1 also reduced the expression of vimentin and restored the expression of E-cadherin in galectin-3 overexpressing cells, compared to cells transfected with only galectin-3 (Fig. 7b). Taken together, these results provide direct evidence for the involvement of Wnt signaling in the effects of galectin-3 in OTSCC cells.

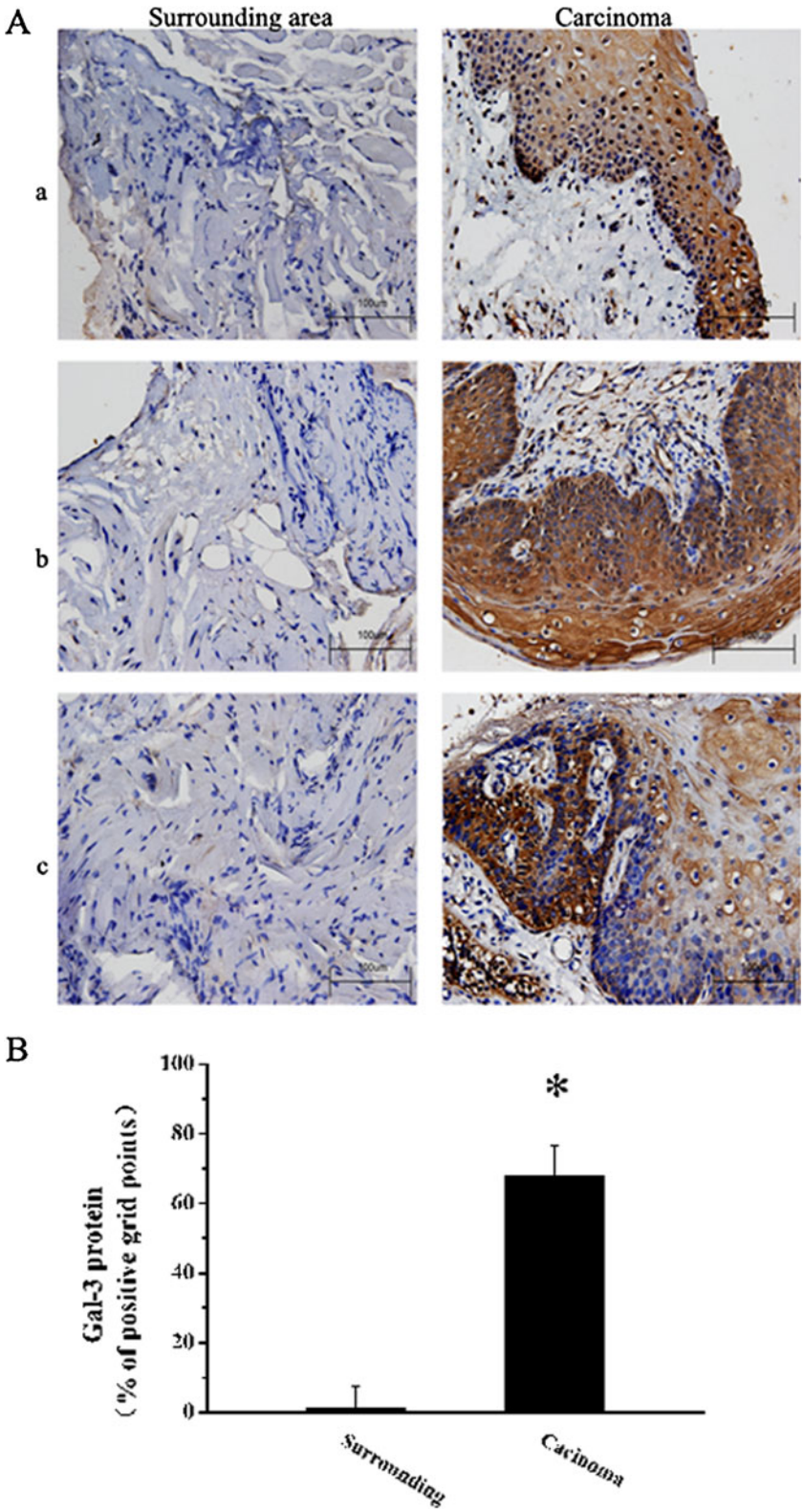

Fig. 2 Galectin-3 is upregulated in OTSCC compared to the adjacent normal tissues from the same patient.
a Immunohistochemical staining demonstrating that galectin-3 is upregulated in OTSCC tissues, compared to the paired adjacent normal tissues from the same patient. Representative images from three different patients (*a*, *b*, *c*) are shown. **b** Quantification of the galectin-3-positive area in OTSCC and the paired adjacent normal tissues. Data are expressed as the mean ± SD of 10 patients; **P*<0.05 vs. adjacent normal tissues (one-way ANOVA)

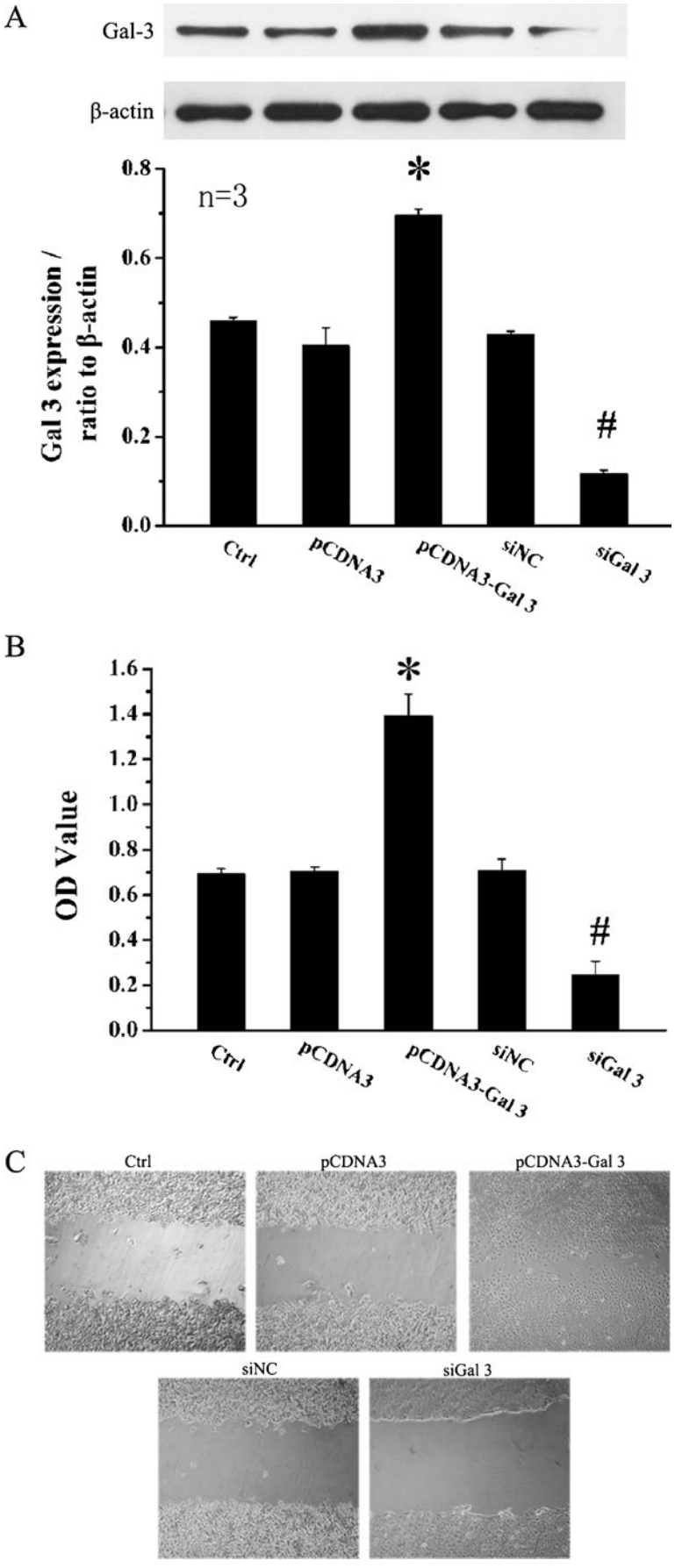

Fig. 3 Overexpression of galectin-3 promotes OTSCC cell proliferation and migration. **a** Western blotting analysis of galectin-3 protein expression in Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA. **b** MTT cell proliferation assay of Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA; #$P<0.001$, *$P<0.05$ vs. control cells (one-way ANOVA). **c** Representative images of cell migration by Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA in the wound healing assay

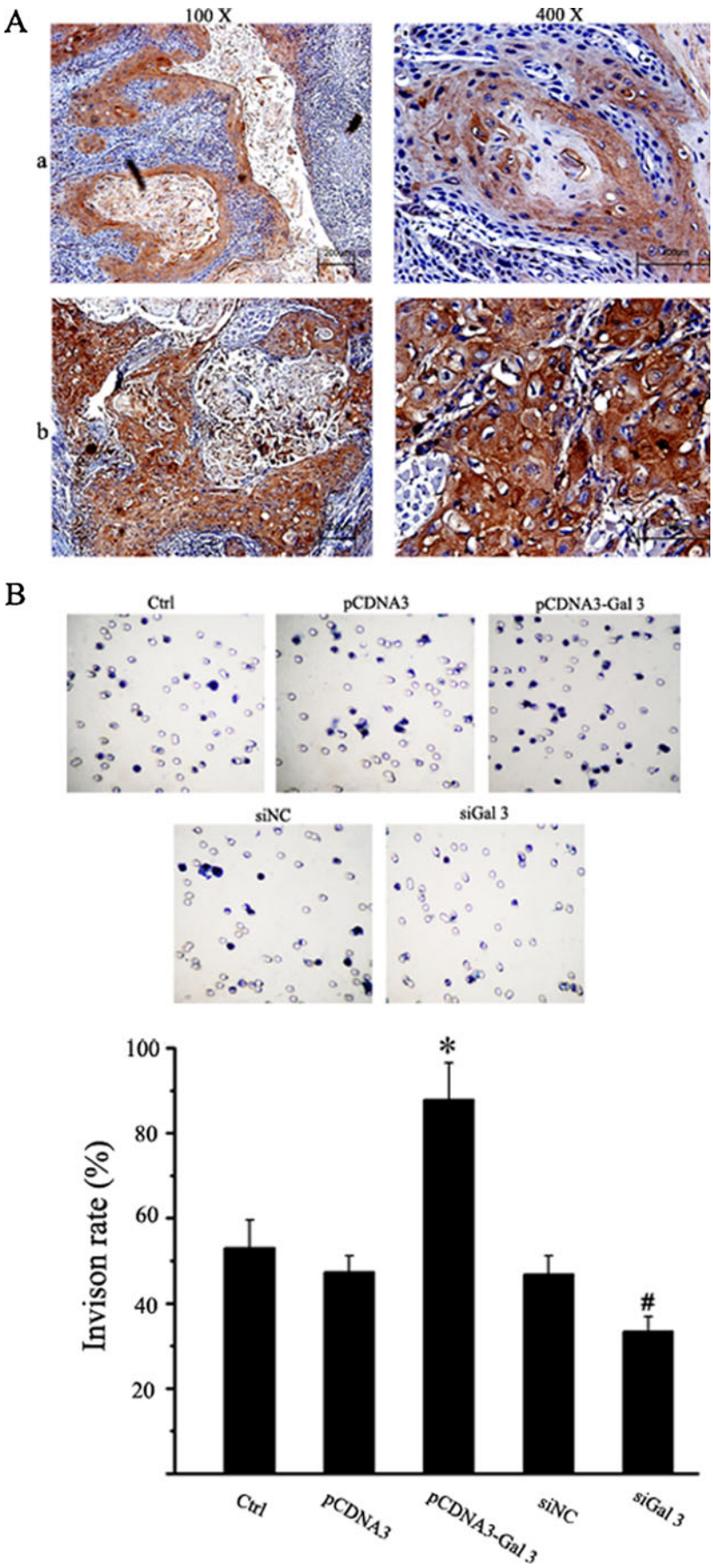

Fig. 4 Overexpression of galectin-3 increases the invasion of OTSCC cells. **a** Representative images of immunohistochemical staining demonstrating that galectin-3 was expressed at higher levels in OTSCC tissues with lymph node invasion (*b*) than OTSCC tissues without lymph node invasion (*a*). **b** Representative images and quantification of invasion by Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA in the Matrigel-coated Transwell migration assay. Data are expressed as the mean ± SD of three independent experiments; #$P<0.001$, *$P<0.05$ vs. control cells (one-way ANOVA)

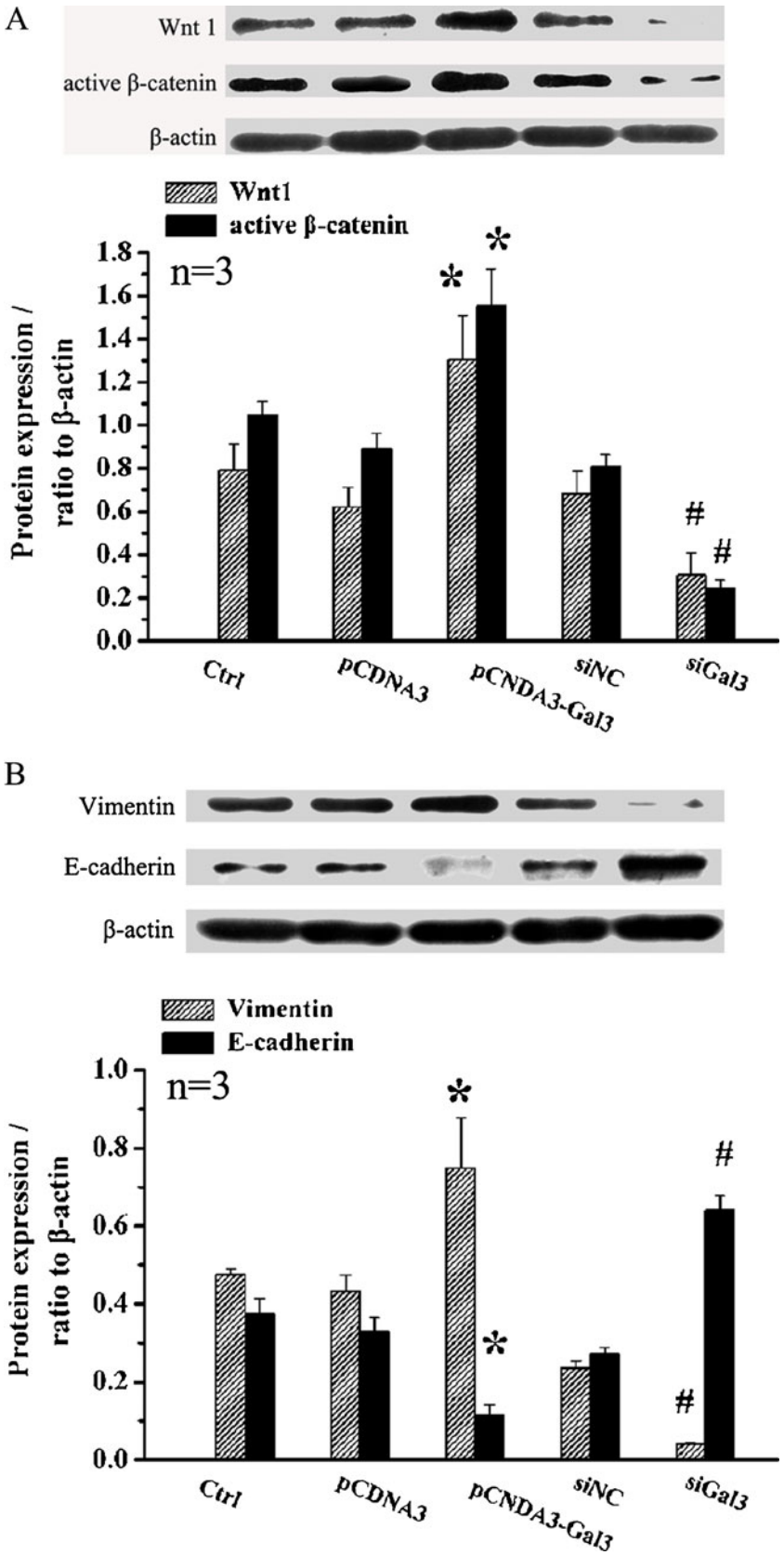

Fig. 5 Galectin-3 activates Wnt-β-catenin signaling and promotes the EMT in OTSCC cells. **a** Western blotting and quantification of Wnt 1 protein expression and activated β-catenin in Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA. **b** Western blotting and quantification of vimentin and E-cadherin expression in Tca8113 cells transfected with pcDNA, pcDNA3-Galectin-3, scrambled siRNA (#NC) or galectin-3 siRNA. Data are expressed as the mean ± SD of three independent experiments; #$P<0.001$, *$P<0.05$ vs. control (one-way ANOVA)

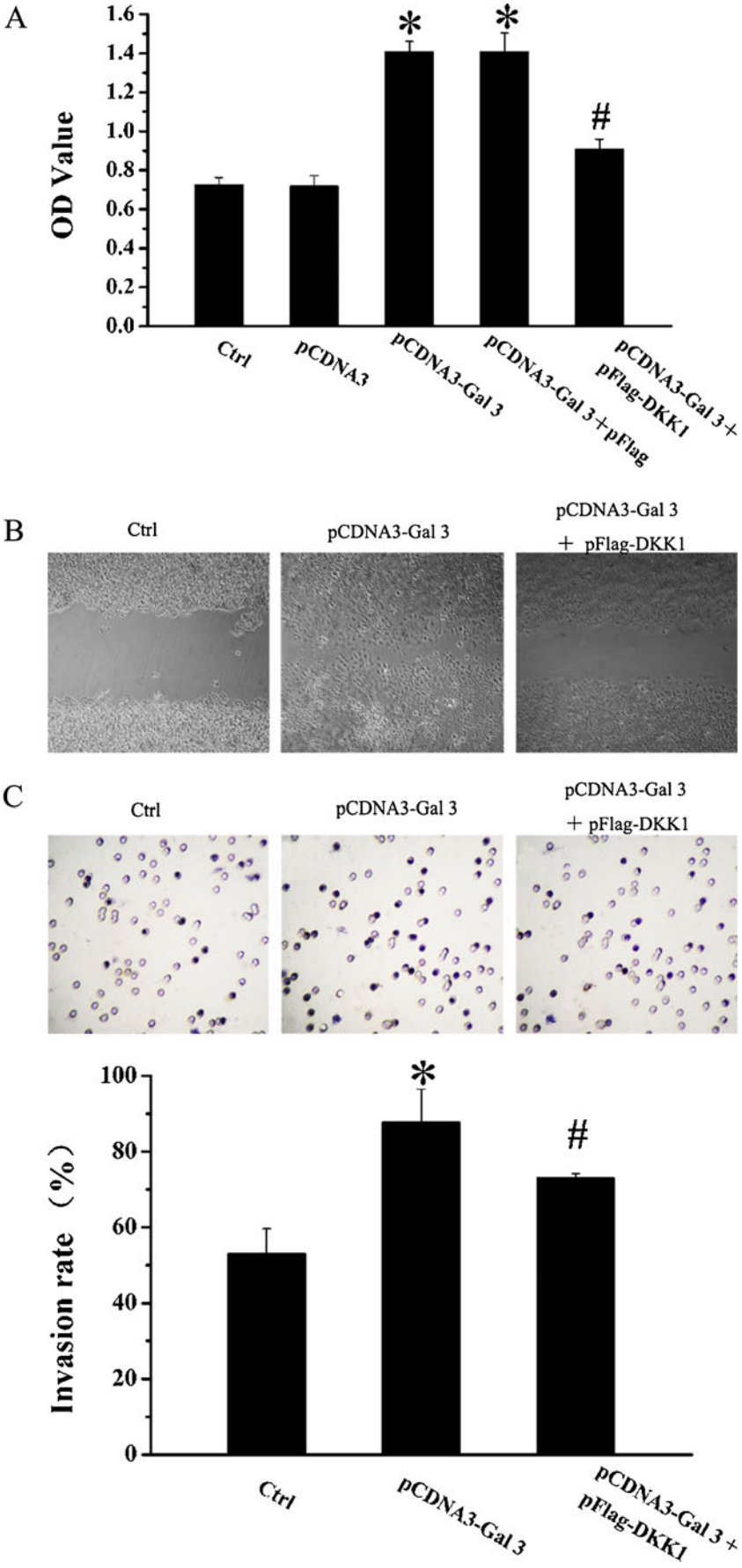

Fig. 6 Galectin-3 induces proliferation, migration and invasion in OTSCC cells via activation Wnt/β-catenin signaling. **a**–**c** Co-transfection of a plasmid expressing the Wnt antagonist DKK1 inhibited the ability of galectin-3 to induce Tca8113 cell proliferation, migration and invasion, as indicated by the MTT assay (**a**), would healing assay (**b**) and Matrigel-coated Transwell migration assay (**c**). Data are expressed as the mean ± SD of three independent experiments; # $P<0.001$, *$P<0.05$ vs. control cells (one-way ANOVA)

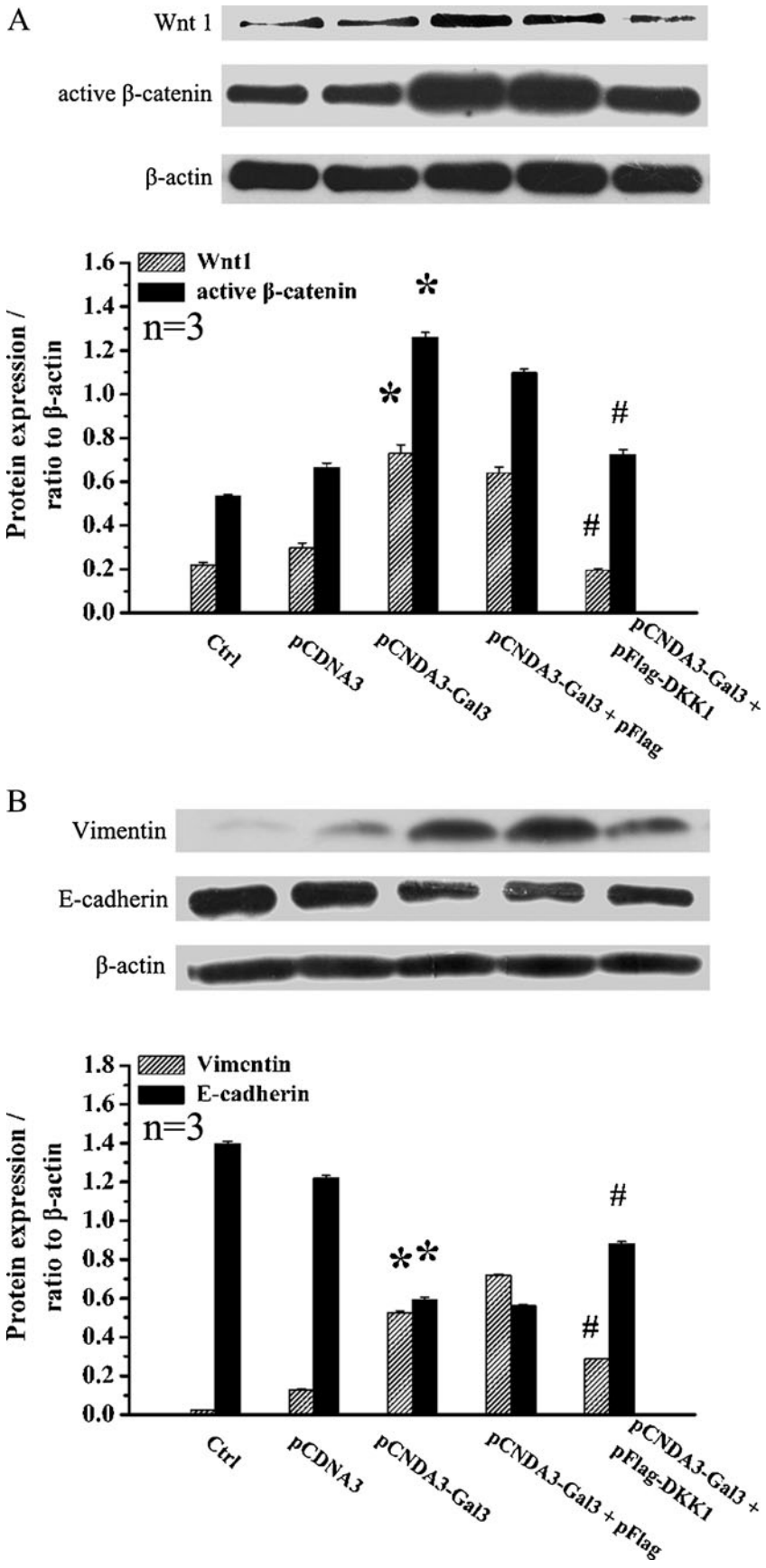

Fig. 7 Galectin-3 induces the EMT in OTSCC cells via activation of Wnt/β-catenin signaling. **a** Western blot demonstrating that co-transfection of a plasmid expressing the Wnt antagonist DKK1 inhibited the ability of galectin-3 to induce upregulation of Wnt 1 and activate β-catenin in Tca8113 cells. **b** Western blot demonstrating that co-transfection of a plasmid expressing the Wnt antagonist DKK1 attenuated the ability of galectin-3 to induce upregulation of vimentin and restored expression of E-cadherin in Tca8113 cells. Data are expressed as the mean ± SD of three independent experiments; # $P<0.001$, * $P<0.05$ vs. control (one-way ANOVA)

Discussion

Galectin-3 is expressed widely in epithelial and immune cells and its expression correlates with aggressiveness and metastasis in cancer [28]. Galectin-3 has been recognized as a diagnostic/prognostic marker in specific types of cancer, such as thyroid and prostate cancer. Galectin-3 has been shown to have a number of functions in cancer cell biology, including regulation of growth, apoptosis, angiogenesis and mRNA splicing [8]; however, the functions of galectin-3 in OTSCC are poorly characterized.

Galectin-3 has been reported to play a critical role in the pathogenesis of OTSCC [29]. Alves et al. [16] found that galectin-3 immunostaining was significantly correlated with metastasis and histological grade of tongue SCC, with an increased expression being observed in cases developing metastases to the cervical lymph nodes and in cases of high-grade malignancy. Thirty-five (61.4 %) of cases positive for galectin-3, exhibited cytoplasmic staining only, and 19 (33.3 %) showed nuclear and cytoplasmic staining. Honjo et al. [15] analyzed galectin-3 expression in 77 tongue specimens (including 54 SCC and 23 normalmucosa specimens). They observed that an average of 84.9 % of tumor cells expressing cytoplasmic galectin-3 and the levels of cytoplasmic expression of galectin-3 markedly iecreased during the progression from normal to cancerous states. In the present study, similar results were also found. Firstly, galectin-3 was upregulated in OTSCC, and a significant correlation was observed with pathological garde. Additionally, the galectin-3 positive-area significantly increased as the pathological stage increased , indicating that galectin-3 may play a role in the progression of OTSCC. Secondly, galectin-3 was expressed at higher levels in OTSCC compared to the adjacent normal tissues, indicating that galectin-3 may promote the initiation of OTSCC. Thirdly, the expression levels of galectin-3 in OTSCC samples with lymph node invasion were higher than OTSCC samples without lymph node invasion, indicating that galectin-3 may promote metastasis in OTSCC.

We used an immortalized OTSCC cell line to further investigate the function of galectin-3 during the initiation and progression of OTSCC. Overexpression of galectin-3 significantly increased OTSCC cell proliferation, migration and invasion, and induced the EMT, whereas knockdown of galectin-3 using siRNA inhibited OTSCC cell proliferation, migration and invasion, and reversed the EMT. These results further indicate that galectin-3 may promote the initiation and progression of OTSCC.

Recent studies have revealed a correlation between galectin-3 and Wnt signaling in a variety of tumor types. Galectin-3 can interact with the β-catenin/TCF complex in breast [30], thyroid [31] and colon cancer [13]. Downregulation of galectin-3 decreased β-catenin protein expression, increased AKT and glycogen GSK-3β dephosphorylation and increased GSK activity, which lead to increased phosphorylation and degradation of β-catenin, implying that galectin-3 can modulate the function of β-catenin [32]. However, the relationship between galectin-3 and Wnt signaling in OTSCC remained unknown. Our results demonstrate that overexpression of galectin-3 activated Wnt signaling in OTSCC.

Wnt/β-catenin signaling is known to play a central role in the determination of cell fate and Wnt/β-catenin signaling is highly evolutionarily conserved [33]. Wnt signaling inhibits the degradation of β-catenin, which regulates the transcription of a number of genes, including genes associated with cancer. Wnt signaling induces accumulation of β-catenin in the nucleus and activates the T cell factor (TCF) transcription factor, which activates transcription of downstream genes such as cyclin D1 and c-myc, which play important roles in the initiation and progression of carcinogenesis [22, 34–37]. Wnt signaling also activates MAPK and JNK signaling. Two distinct pathways, the β-catenin/TCF and JNK/c-Jun pathway physiologically cooperate during tumorigenesis to enhance the transcriptional activity of promoters containing activator protein (AP-1) and TCF-binding sites [38–41]. The expression of these genes generates a positive feedback loop which further enhances the expression of many Wnt-target genes, consistent with the function of Wnt/β-catenin signaling in human tumors [23, 32, 42].

DKK proteins are unique Wnt antagonists as they can specifically block the binding of Wnt ligands to LRP5/6, the obligatory co-receptors for transmission of canonical Wnt/β-catenin pathway signaling. We observed that DKK1 reduced the ability of galectin-3 to promote OTSCC cell proliferation, migration, and invasion, and induce the EMT. It should be noted that DKK1 inhibited the ability of galectin-3 to upregulate expression of Wnt 1 and activate β-catenin, but could not completely inhibit the effects of galectin-3 in OTSCC cells, implying that galectin-3 may also act via another signaling pathway in OTSCC. Other studies have revealed that galectin-3 can act as a selective binding partner of activated K-Ras, as co-transfection of K-Ras and galectin-3 enhanced and prolonged EGF-stimulated Ras and PI3-K activity [43, 44].

In summary, our examination of clinical samples and in vitro experiments demonstrate that galectin-3 may play an important role in the initiation and progression of OTSCC. Furthermore, we mechanistically correlated the effects of galectin-3 in OTSCC with the activation Wnt/β-catenin signaling, as the native Wnt antagonist DKK1 almost completely inhibited the ability of galectin-3 to promote

OTSCC cell proliferation, migration and invasion, and induce the EMT. Therefore, targeting the galectin-3-Wnt/β-catenin signaling pathway may represent a novel strategy for the development of therapeutic modalities for OTSCC, which devastates the lives of millions of people worldwide.

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