Gelsolin regulates proliferation, apoptosis, migration and invasion in human oral carcinoma cells

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Abstract. Gelsolin (GSN) is one of the most abundant actin-binding proteins, and is involved in several pathological processes, including Alzheimer's disease, cardiac injury and cancer. The aim of the present study was to assess the effect of GSN on the growth and motility of oral squamous cell carcinoma Tca8113 cells. The overexpression vector pcDNA3.1-GSN was transfected into Tca8113 cells and the stable GSN overexpression cell line was identified based on G418 antibiotic selection. The effect of GSN overexpression on the proliferation, apoptosis, migration and invasion of Tca8113 cells was examined using a cell counting kit-8 assay, flow cytometry and Transwell assays. The results revealed that GSN overexpression significantly promoted the cell proliferation and apoptosis of Tca8113 cells. In addition, Transwell assays demonstrated that the migration and invasion abilities of Tca8113 cells were enhanced by GSN overexpression. Therefore, the upregulation of GSN promotes cell growth and motility, indicating that it may perform a vital function in the progression of human oral cancers.

Introduction

In eukaryotic species, the actin cytoskeleton is essential for numerous cellular functions, including maintenance of morphology, motility, division, adhesion, endocytosis, intracellular transport and signal transduction (1-5). The varied and complex activities of the actin cytoskeleton are dynamically regulated by actin-binding proteins (ABPs) (2-4,6,7). Gelsolin (GSN) is one of the most abundant ABPs, and has been found to be a multifunctional regulator of physiological and pathological cellular processes (7,8).

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Previous studies have indicated that GSN may be a tumor suppressor that exerts a crucial role in the carcinogenic process (7,9). However, biphasic expression of GSN in oral precancerous lesions and oral cancers has been observed, which revealed a downregulation in GSN between oral precancerous lesions and oral cancers, and demonstrated upregulation of GSN in the stages of oral cancer progression (8). The biphasic expression indicated that GSN may perform a more complicated role in oral cancer biology.

In order to study the biological roles in oral cancer development in the present study, GSN was overexpressed in oral cancer Tca8113 cells, and the effect of GSN on the proliferation, apoptosis, cell cycle, migration and invasion of these cells was investigated, which may contribute to the present understanding of the biological actions of GSN.

Materials and methods

Tca8113 cell culture. The human oral squamous cell carcinoma Tca8113 cell line was provided by the Shanghai Ninth People's Hospital, Medical School of Shanghai Jiao Tong University (Shanghai, China). The cells were cultured in RPMI 1640 medium (Gibco-BRL, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 units/ml penicillin, at 37˚C in a 5% CO_2 atmosphere.

Stable transfection. To transiently transfect GSN into the Tca8113 cells, $0.5x10^5$ cells/well were seeded into a 24-well plate (Corning, Inc., Corning, NY, USA) one day prior to transfection with 0.5 μg plasmid DNA, using the GBfectene-Elite Transfection Reagent (Genebank Biosciences, Inc., Zhangjiagang, Jiangsu, China). The transfection rate was monitored by a fluorescence microscope (Eclipse Ti; Nikon Corporation, Tokyo, Japan). A total of 400 μg/ml G418 (Invitrogen Life Technologies, Carlsbad, CA, USA) was applied to the stable transfectants, and the level of GSN expression was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting. β-actin and glyceraldehyde 3-phosphate dehydrogenase were used as reference genes for RT-qPCR and western blotting, respectively. Cells transfected with the empty pcDNA3.1 vector served as negative controls. The Tca8113 cells transfected with the pcDNA3.1-GSN and pcDNA3.1 vectors were respectively termed Tca8113-GSN and Tca8113-Vec.

Proliferation assay. The cell counting kit-8 (CCK-8) proliferation assay (Dojindo Molecular Technologies, Kumamoto, Japan) was used to analyze the growth of Tca8113 cells. According to the manufacturer's instructions, 100 μl RPMI 1640 medium, containing 1x10^3 cells/well, was added to 96-well plates (Corning, Inc.). The plates were incubated for 24, 48, 72 or 96 h in a humidified incubator. Subsequently, 10 μl CCK-8 solution was added to the wells and five replicate wells were used for each time point. Following incubation for 1 h, the absorbance was measured using the EnSpire Multimode Plate Reader (PerkinElmer, Inc., Waltham, MA, USA) at a wavelength of 450 nm.

Cell apoptosis assay. Cell apoptosis was analyzed using the Annexin V-phycoerythrin (PE)/7-amino-actinomycin D (7-AAD) Apoptosis Detection Kit (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. Briefly, the cells were digested with trypsin and washed twice with phosphate-buffered saline (PBS) and 5x10^5 cells were resuspended in 500 μl binding buffer (Nanjing KeyGen Biotech Co., Ltd.). The suspension was stained with 1 μl Annexin V-PE in the dark for 10 min, at room temperature. Next, 5 μl 7-AAD was added to the suspension and left for 10 min at room temperature, in the dark. Cell apoptosis was analyzed using the BD FACSAria II cell sorter (BD Biosciences, Franklin Lakes, NJ, USA).

Cell cycle assay. The cell cycle was analyzed using the propidium iodide (PI) Detection kit (Nanjing KeyGen Biotech Co., Ltd.) according to the manufacturer's instructions. The cells were collected and washed once in 1X buffer A (Nanjing KeyGen Biotech Co., Ltd.). The cells were resuspended at a concentration of 1x10^6 cells/ml in 1X buffer A. The suspension was mixed with 70% ethanol at a ratio of 1:9 and was left for ≥12 h at -20˚C. The cells were then centrifuged at <500 x g at room temperature and washed with 1X buffer A and resuspended in 500 μl 1X buffer A. RNase A (0.25 mg/ml) was added and allowed to react at 37˚C for 30 min. A total of 5μl PI was added in the dark and allowed to react for 30 min at room temperature. The cell cycle data were analyzed by flow cytometry (BD FACSAria II; BD Biosciences).

Cell migration and invasion assay. A 3422 Transwell chamber (Corning, Inc.) was used to perform the cell invasion assay. Each Transwell chamber was coated with Matrigel (BD Biosciences) 24 h prior to use. The cells were harvested and resuspended at a concentration of 1x10^6 cells/ml in RPMI 1640 medium containing 1% bovine serum albumin. Subsequently, 100 μl of the suspension was added to the upper chamber, while 500 μl of the RPMI 1640 medium containing 20% FBS was added to the lower chamber. After 48-h incubation, the cells on the upper surface of the chamber were completely removed. The lower surface was washed gently with PBS and fixed in 4% formaldehyde polymerisatum for 20 min. The chamber was stained with DAPI (Sigma-Aldrich, St. Louis, MO, USA) and quantified under a microscope (Eclipse Ti; Nikon Corporation). The cell migration assay was the same as the invasion assay, with the exception of the Matrigel coating.

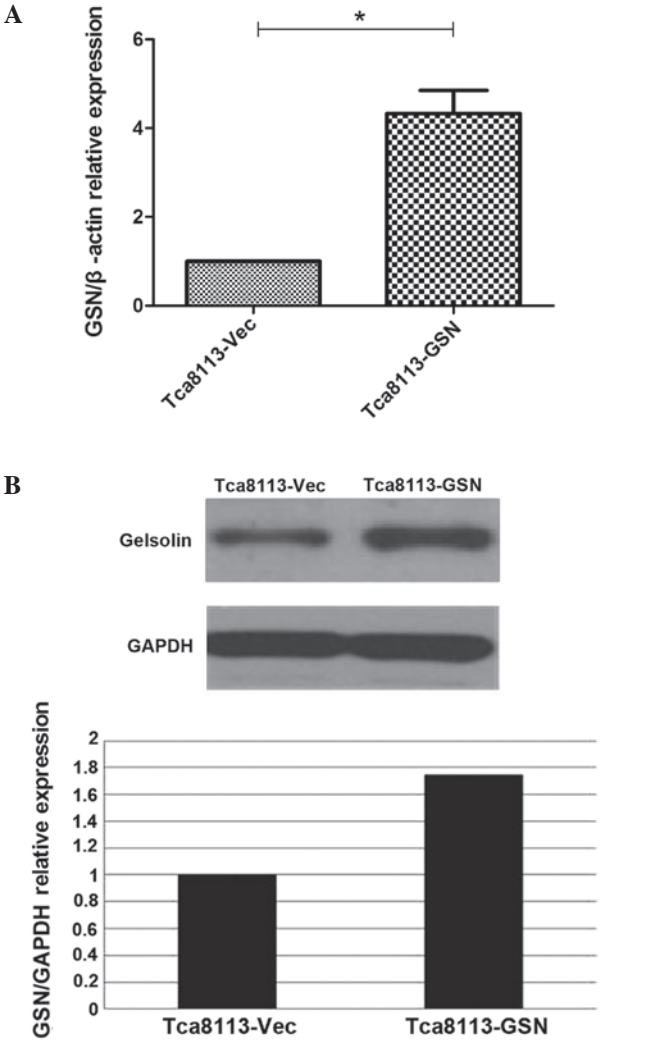

Figure 1. GSN overexpression in Tca8113 cells at the (A) mRNA and (B) protein levels. (A) GSN mRNA expression was four-fold higher in the Tca8113-GSN cells when compared with the Tca8113-Vec cells. (B) GSN protein expression was increased by 1.75 times in the Tca8113 cells when compared with the negative control cells *P<0.05 vs. Tca8113-Vec. GSN, gelsolin; Vec, vector; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

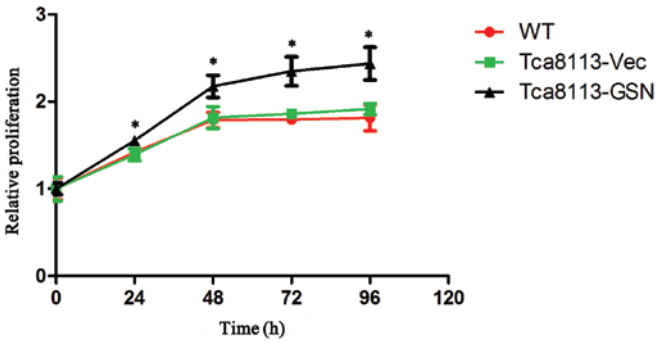

Figure 2. Upregulation of GSN expression promotes Tca8113 cell proliferation. The growth of Tca8113-GSN cells was significantly increased when compared with Tca8113-Vec and wild type Tca8113 cells. *P<0.05 vs. Tca8113-Vec or Tca8113-WT. WT, wild type; Vec, vector; GSN, gelsolin.

Results

Overexpression of GSN in transfected Tca8113 cells.

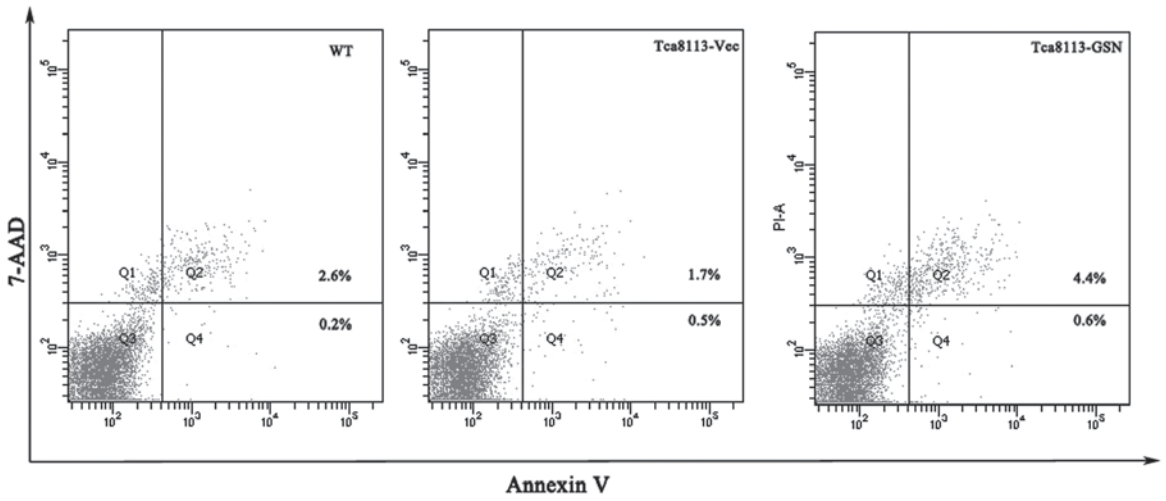

Figure 3. Upregulation of GSN expression promotes Tca8113 cell apoptosis. 7-AAD, 7-amino-actinomycin; WT, wild type; Vec, vector; GSN, gelsolin.

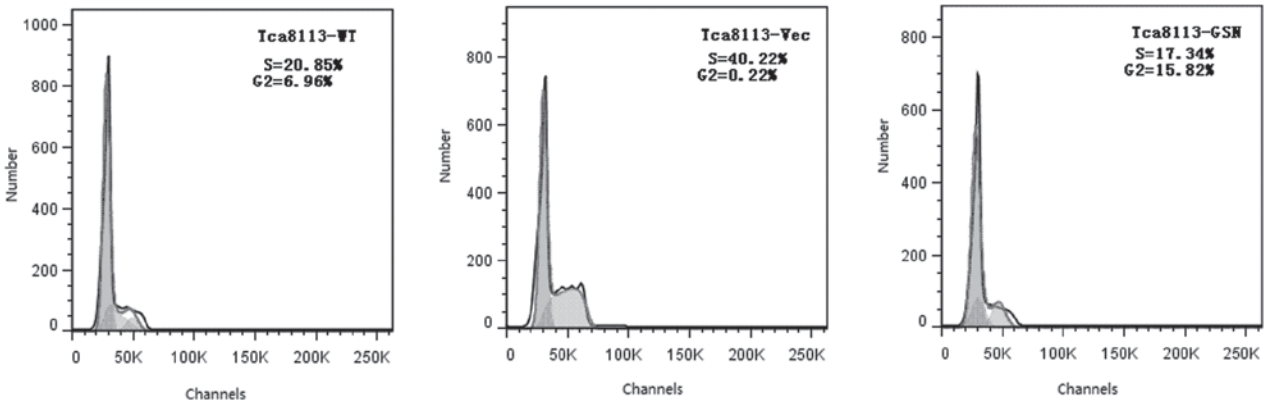

Figure 4. GSN overexpression exerted no significant effect on the cell cycle of Tca8113 cells. WT, wild type; GSN, gelsolin; Vec, vector.

Subsequent to the stable transfectants being established, RT-qPCR revealed that GSN mRNA expression in the Tca8113-GSN cells was 4-fold higher compared with the Tca8113-Vec cells (Fig. 1A). GSN protein expression was increased by 1.75 times compared with the negative control cells (Fig. 1B).

Effect of GSN overexpression on the proliferation of Tca8113 cells. CCK-8 assays were performed to examine the proliferation of wild type Tca8113 (Tca8113-WT), Tca8113-GSN and Tca8113-Vec cells *in vitro*. As shown in Fig. 2, the growth of Tca8113-GSN cells was significantly increased when compared with Tca8113-Vec or wild type Tca8113 cells.

Effect of GSN on the apoptosis and cell cycle of Tca8113 cells. The cellular apoptosis of Tca8113-WT, Tca8113-GSN and Tca8113-Vec cells was analyzed using an Annexin V-fluorescein isothiocyanate assay. The results demonstrated that the apoptosis rate of Tca8113-GSN cells was 5.0%, compared with 2.2% in Tca8113-Vec cells and 2.8% in Tca8113-WT cells (Fig. 3). Thus, these results indicated that GSN may promote apoptosis in Tca8113 cells. However, cell cycle analysis revealed that the number of cells in the S and G_2 phases in the Tca8113-WT and Tca8113-GSN groups was lower compared with the Tca8113-Vec group (Fig. 4).

GSN overexpression correlates with the promotion of migration and invasion in Tca8113 cells. Increased migration and invasion was closely associated with carcinoma progression. To determine the role of GSN in oral cancer cell migration and invasion, Transwell assays were performed. The results revealed that Tca8113-GSN cells exhibited significantly increased migratory and invasive abilities (Figs. 5 and 6).

Discussion

Oral cancer is the eighth most common cancer globally with ~270,000 novel cases and 130,000 mortalities annually, and a five-year survival rate of ~63% (10-14). Oral cavity cancer is characterized by high aggression, early recurrence and frequent lymph node metastasis (15).

The early diagnosis of oral cancer is extremely important as, early stage oral cancer treated with surgery or radiation is usually associated with a good prognosis. Advanced cancers are routinely treated by surgery and post-operative radiotherapy, but the prognosis is often poor (14.) However, numerous patients possess advanced-stage disease at the time

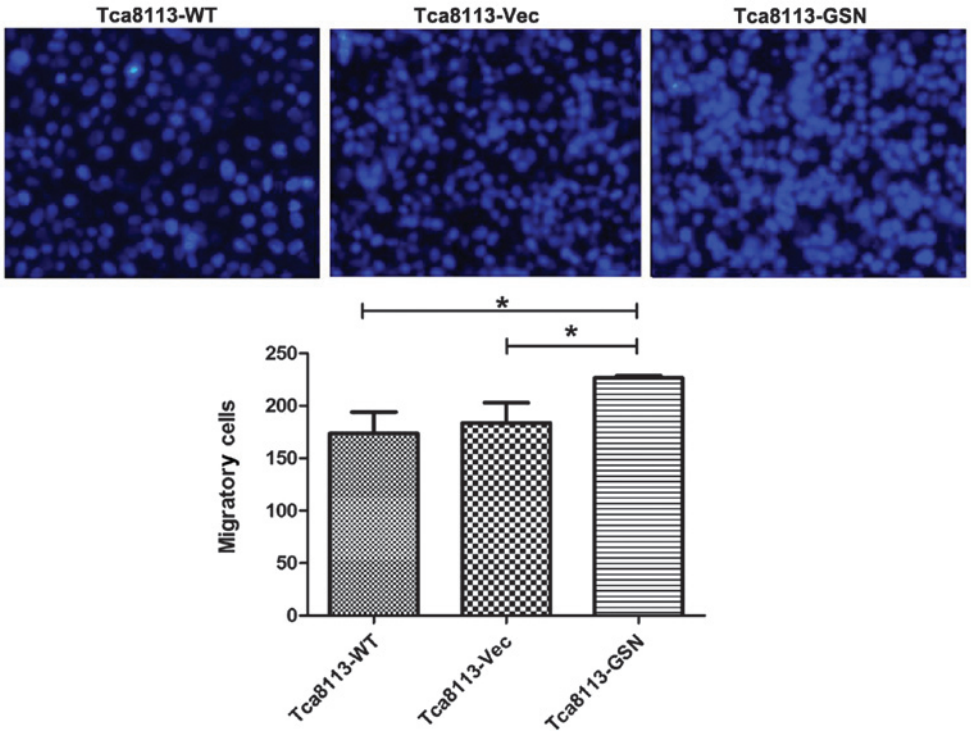

Figure 5. GSN overexpression enhances the migration of Tca8113 cells. *P<0.05. WT, wild type; Vec, vector; GSN, gelsolin.

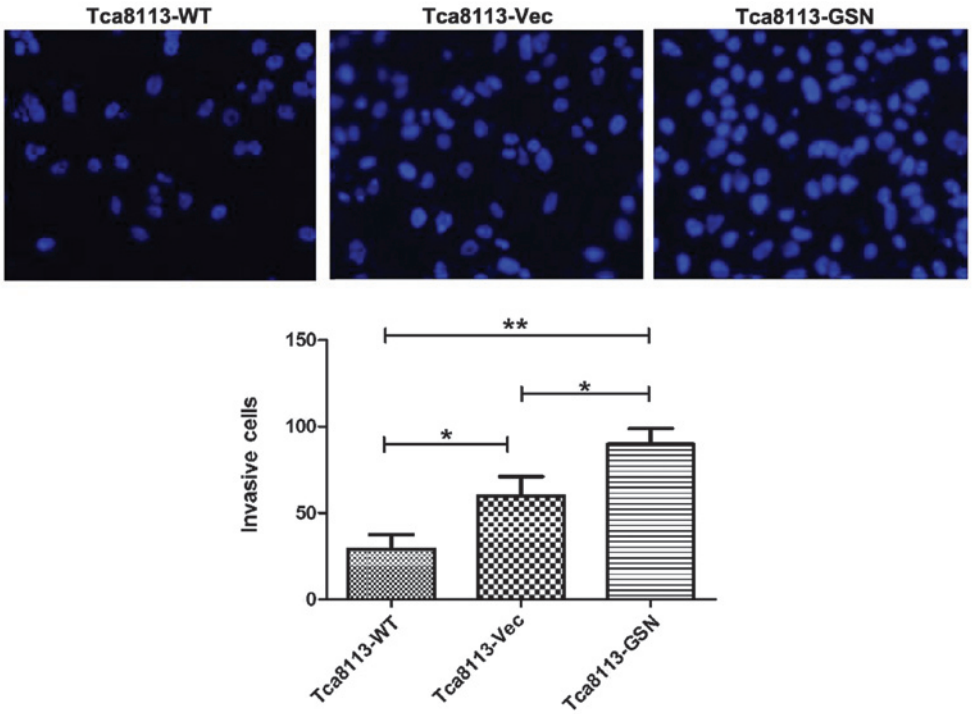

Figure 6. GSN overexpression enhances the invasion of Tca8113 cells. *P<0.05 vs. controls. **P<0.01 vs. Tca8113-WT. WT, wild type; Vec, vector; GSN, gelsolin.

of diagnosis. The five-year survival rates are 41 and 9% for patients with stage III and IV disease, respectively, which are significantly lower compared with patients possessing stage I or II disease, with a five-year survival rate of 85 and 66%, respectively (16). It has been shown that early diagnosis is critical for improving oral cancer treatment. A number of studies have been devoted to identifying appropriate biomarkers for the early diagnosis, which may increase understanding of the pathogenesis and may aid in the identification of therapeutic targets (12,15).

In eukaryotic species, it has been found that ABPs are not only involved in normal cell metabolism, but also perform an extremely important role in numerous pathological processes (1-3,5,7,17). As an extremely abundant eukaryotic

ABP, GSN severs, caps and nucleates actin filaments, and then dynamically regulate the cytoskeleton. Therefore, GSN exerts numerous physiological effects, including modulation of cell apoptosis, mediation of signal transduction and regulation of transcriptional coactivation (7).

The importance of GSN in the initiation and progression of malignancies remains unclear. To date, the altered expression of GSN has been identified in specific cancers, including human bladder, ovarian, lung, breast, gastric, pancreatic, cervical and oral carcinomas (8,18-25). The majority of carcinomas exhibited downregulation of GSN, but GSN overexpression was observed in cervical carcinoma (8,18-25). In contrast to other tumors, a biphasic expression of GSN in oral precancerous lesions and cancers has been observed (8). The downregulation of GSN was observed between oral normal mucosa and precancerous lesions, whereas GSN upregulation in oral cancer predicated an increased tumor size and invasive growth (8). The dual effects indicate that GSN may serve as a tumor suppressor in oral cancer, while serving as a promoter in oral cancer progression.

In the present study, GSN expression was increased in oral squamous cell carcinoma cells, which was demonstrated by GSN protein expression in Tca8113-GSN cells being double that of Tca8113-Vec cells. In this study, the overexpression of GSN promoted the proliferation and apoptosis of Tca8113 cells. However, the mechanism of the promotion of apoptotic activity in Tca8113 cells was not investigated and thus requires additional investigation. However, previous studies have indicated that GSN functions as a growth promoter and apoptosis inhibitor (26). GSN overexpression may inhibit the activation of caspases-3, -8 and -9 by preventing the release of cytochrome *c* from mitochondria, and thus exert its function in anti-apoptotic mechanisms. Additional studies have revealed that GSN may exert anti-apoptotic effects by blocking actin-dependent voltage-dependent anion-selective channels in mitochondrial-dependent cell death, and repress the p53-mediated apoptosis in HepG2 cells (7,27-34).

The results of the present study revealed that GSN was involved in cell motility. Increased cell motility is important in tumor progression, particularly in the multistep process of invasion and metastasis (35). A previous study found that the expression of GSN was closely associated with oral carcinoma progression (8). The current study indicated that the upregulation of GSN increased Tca8113 cell migration and invasion *in vitro*, which caused enhanced motility. In addition, it has been indicated that GSN may affect motility in human colon cancer and melanoma cells (30,36-40). In human colon cancer cells, the overexpression of GSN was accompanied by the promotion of migration capacity. In addition, the decreased expression of GSN decreased the migratory potential of melanoma cells (30,36-40). Therefore, GSN overexpression may result in positive effects that promote invasion and metastasis in oral cancer progression.

In conclusion, the results of the present study demonstrate that GSN significantly promotes oral squamous cell carcinoma Tca8113 cell proliferation, migration and invasion in human oral cancer. GSN may exert an important role in cell growth and motility, which may provide novel insights for understanding the potential mechanisms of oral cancer progression.

Acknowledgements

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