

Original Article

Short interfering RNA directed against the GOLPH3 gene enhances the effect of chemotherapy against oral squamous cell carcinoma by regulating Caspase3, Bcl2 and cytochrome-c expression

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Abstract: Growing evidence reported that Golgi phosphoprotein 3 (GOLPH3) was involved in the progression of several human cancers. To determine whether knockout of GOLPH3 enhances the effect of Chemotherapy against cell growth of oral squamous cell carcinoma *in vitro*. OSCC cells were transfected with Golph3 plasmid, Golph3-RNAi and the relative control plasmids. Transfected Tca-8113 cells treated with cis-Dichlorodiamineplatinum (DDP; 0, 0.05, 0.25, 1.25, 6.25 and 31.25 μ g/ml) or Paclitaxe (0, 2, 10, 50, 250 and 1250 nM) or Adriamycin (0, 0.25, 0.5, 1, 2 and 4 μ g/ml) for 24 h, respectively, was determined using MTT assay. Apoptosis-related protein expression Cytochrome-C, Caspase3 and Bcl-2 was analyzed by RT-PCR and western blots. Result of MTT showed that Golph3-RNAi transfected Tca-8113 cells enhanced the effect of chemotherapy, and the effect was strengthened with the increasing concentration of drugs, and the Golph3 plasmid transfected Tca-8113 cells showed the opposite effect. RT-PCR and western blots assays revealed that expression of cytochrome-C and caspase3 were up-regulated, while Bcl-2 expression was down-regulated in Golph3-RNAi transfected Tca-8113 cells. Taken together, this study demonstrated that GOLPH3 had potent pro-tumor growth and decreased the effect of Chemotherapy, and its mechanism is primarily *via* cell anti-apoptosis, down-regulating the expression of cytochrome-C and caspase3, up-regulating Bcl-2 expression.

Keywords: GOLPH3, cell growth, cell apoptosis, oral squamous cell carcinoma

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumor with a rising incidence and mortality [1]. In the past years, although advanced in surgery, radiation therapy and chemotherapy, OSCC is still a serious health issue to be treated [2, 3]. OSCC is a complex molecular disease, which is related to multiple interrelated pathways. It is urgent to understand the mechanisms in responding to the cell proliferation and apoptosis of OSCC.

Previous studies have demonstrated that Golgi phosphoprotein 3 (GOLPH3) acted as a novel oncogene involved in the progression of many kinds of cancers [4-8]. However, the underlying

mechanisms of GOLPH3 in cell proliferation, apoptosis and the response to chemotherapy in OSCC remain largely unclear. Herein, we found that GOLPH3 is significantly upregulated in OSCC and correlated with cell proliferation. To understand how GOLPH3 affects OSCC cell proliferation, apoptosis and the response to chemotherapy, we investigated apoptosis-related protein expression Cytochrome-C, Caspase3 and Bcl-2. In sum, we provided evidence that GOLPH3 acted as a tumor promoter in OSCC.

Material and methods

OSCC cell culture and transfection

The oral squamous cell carcinoma cell lines SCC-9, TSCCa, SCC-25, Tca-8113 and tongue

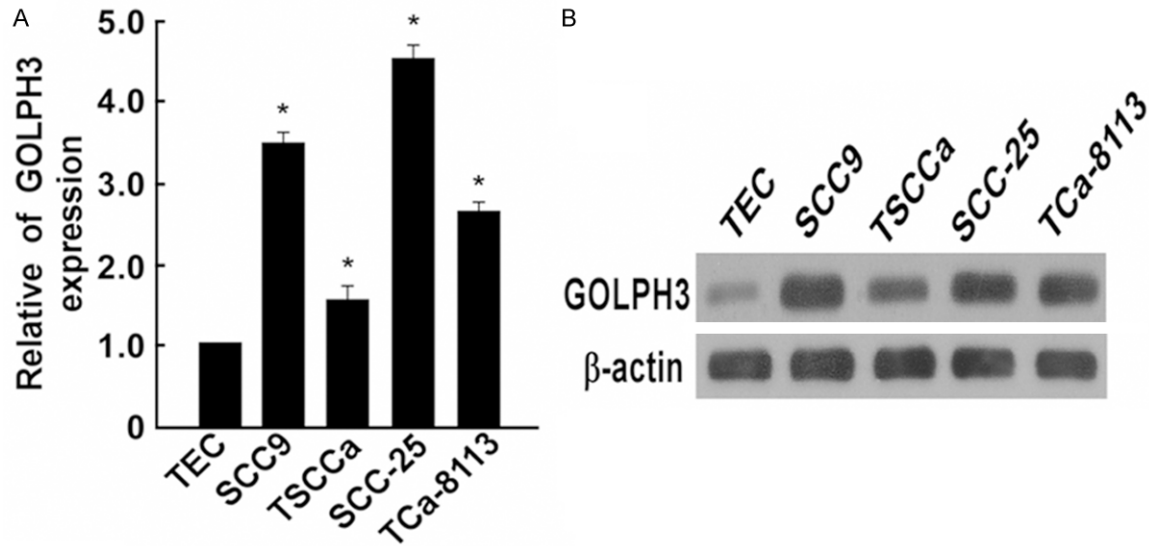


Figure 1. GOLPH3 expression in OSCC cell lines. A. The mRNA levels of GOLPH3 in TEC cells and 4 OSCC cell lines, by PCR analysis. B. Western blot was performed to detect GOLPH3 expression in TEC cells and 4 OSCC cell lines. β-Actin was used as a loading control.

epithelial cell TEC were obtained from the American Type Culture Collection and grew in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS; HyClone; Thermo Scientific) at 37°C in an atmosphere of 5% CO₂ and 95% air.

Tca-8113 cells were transfected with lentiviral vectors encoding short hairpin RNA targeting human Golp3 for Golp3 knockdown (Golp3-RNAi) or a scrambled shRNA as control (Scramble) (Sigma-Aldrich). Multiplicity of infection was 10. Cells were cultured for 72 h after transfection. Cells were grown to 80% confluency in 60-mm dish. 10 ng of Golp3 plasmid (Golp3) or empty plasmid (Vector) was transfected using lipofectamine 2000 (Invitrogen, USA). Cells were cultured for 48 h after transfection.

RNA extraction and real-time quantitative PCR

Total RNA was extracted from culture cells TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and then cDNA was synthesized from 5 ng of total RNA according to the manufacturer's instructions (SYBR PCR Kit, Qiagen, China). Quantitative PCR was performed by SYBR Kit (Qiagen, China) using a Light Cycler system. The primers which were synthesized by GeneCopoeia Co. (Guangzhou, China) were used as follows: Golp3 (HQP016768), Cytochrome-C (HQP002812),

Caspase3 (HQP020297) and Bcl-2 (HQP016-211). Expression data were normalized to the geometric mean of GAPDH (HQP006940) to control the variability in expression levels and calculated as $2^{-[(Ct \text{ of Golp3, Cytochrome-C, Caspase3 and Bcl-2}) - (Ct \text{ of GAPDH})]}$.

MTT assays

For MTT assay, Tca-8113 cells after transfection were seeded in 96-well plates in medium containing 10% FBS at approximately 3,000 cells/well. After 1, 2, 3, 4 and 5 day, 20 μl of 5 mg/ml MTT solution (Sigma-Aldrich) was added to each well and incubated at 37°C for 4 h. The generated formazan was dissolved in DMSO, and the absorbance was recorded.

To detect the effect of DDP, Paclitaxel and Adriamycin on cell proliferation, cells in each group were treated with different concentration of cis-Dichlorodiamineplatinum (DDP; 0, 0.05, 0.25, 1.25, 6.25 and 31.25 μg/ml) or Paclitaxel (0, 2, 10, 50, 250 and 1250 nM) or Adriamycin (0, 0.25, 0.5, 1, 2 and 4 μg/ml) for 24 h, respectively. MTT assay was used to detect as describe above.

Western blotting

Protein lysates were prepared, the equivalent aliquots (50 μg) of proteins were resolved by 10% SDS/polyacrylamide gel and transferred

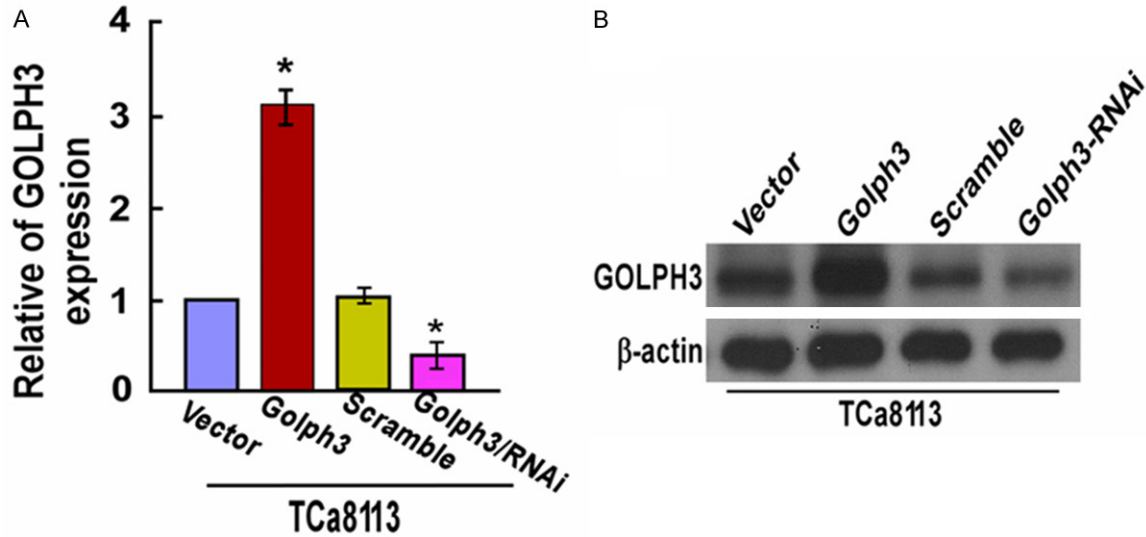


Figure 2. Overexpression of Golph3 decreased and downexpression of Golph3 enhanced the effect of chemotherapy in Tca-8113 cells. **A.** The mRNA levels of GOLPH3 in TCa8113 OSCC cell line after transfection with Golph3 plasmid, Golph3-RNAi and the relative control plasmids, by PCR analysis. **B.** Validation of GOLPH3 protein levels after transfection by Western blot analysis. β -Actin was used as a loading control.

to PVDF membrane. The membrane was incubated overnight at 4°C with anti-GOLPH3, anti-Cytochrome-C, anti-Caspase3, anti-Bcl-2 and anti- β -actin (Cell Signaling Technology) diluted according to the manufacturer's recommendations. Following incubated with secondary antibody (horseradish peroxidase-conjugated) for 2 h at room temperature. The protein bands were visualized using ECL chemiluminescence (Beyotime Biotechnology, China).

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 (SPSS Inc, Chicago, IL, USA). Statistical analyses were done by analysis of variance (ANOVA) or Student's t test. A $P < 0.05$ was thought to be significantly different for two groups of data.

Result

GOLPH3 expression in OSCC cell lines and human OSCC tissues

To detect the role of GOLPH3 on the pathogenesis of OSCC, we examined the expression of GOLPH3 in OSCC cell lines and human OSCC tissues. GOLPH3 was significantly up-regulated at both the mRNA and protein levels in tested OSCC cell lines compared with normal tongue epithelial cell (TEC) (**Figure 1**).

Overexpression of Golph3 decreased and downexpression of Golph3 enhanced the effect of chemotherapy in Tca-8113 cells

To determine whether GOLPH3 impacted the effect of chemotherapy on OSCC cells, Tca-8113 cells were transfected with Golph3 plasmid, Golph3-RNAi and the relative control plasmids. Result of RT-PCR and Western blots revealed that expression of GOLPH3 was decreased in Tca-8113 cells after transfection with Golph3-RNAi, while GOLPH3 expression was increased in Golph3 plasmid transfected Tca-8113 cells (**Figure 2A** and **2B**). As showed in **Figure 3A**, GOLPH3 upregulation promoted cell growth and a significant decrease in proliferation was observed in Tca-8113 cells transfected with Golph3-RNAi compared with the relative controls in day 2, 3, 4 and 5. But cell growth of transfected Tca-8113 cells in each group had no different after incubating for 24 h, so Tca-8113 cells were incubated with various concentrations of DDP or Paclitaxe or Adriamycin for 24 h, and cell proliferation were evaluated by MTT assay. As showed in **Figure 3B-D**, cell proliferation of transfected Tca-8113 cells was decreased the increasing concentration of DDP or Paclitaxe or Adriamycin. Taken together, our data demonstrated that Golph3-RNAi transfected Tca-8113 cells enhanced the effect of chemotherapy on cell proliferation.

GOLPH3 decreased chemotherapy effect against OSCC

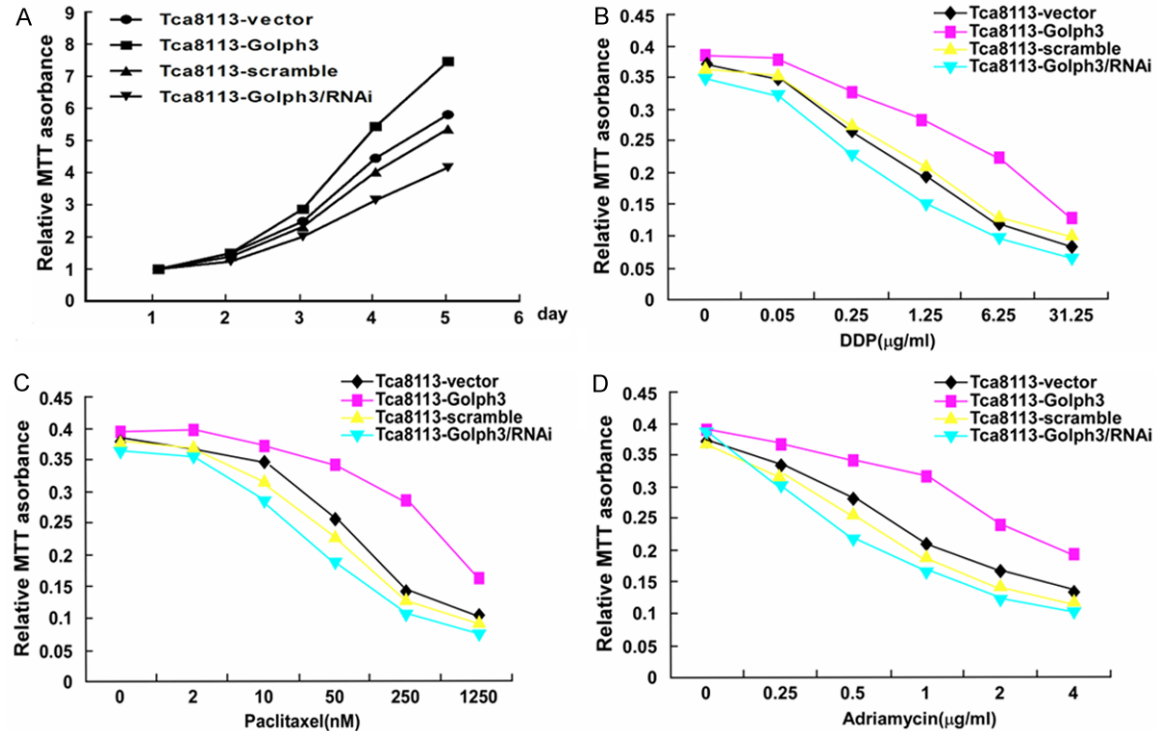


Figure 3. AGK expression overexpression promotes and its depletion inhibits cell proliferation. (A) MTT assay showed that GOLPH3 overexpression promoted proliferation rate and its depletion inhibited proliferation (Day 2, 3, 4 and 5, $P < 0.05$). Tca-8113 cells after transfection were incubated with various concentrations of DDP (B) or Paclitaxel (C) or Adriamycin (D) for 24 h, and cell proliferation were evaluated by MTT assay.

GOLPH3 regulates cell proliferation by regulating of apoptosis-related protein expression Cytochrome-C, Caspase3 and Bcl-2

Apoptosis-related protein expression Cytochrome-C, Caspase3 and Bcl-2 was analyzed by RT-PCR and western blots. Result of RT-PCR and western blots assays revealed that expression of cytochrome-C and caspase3 were up-regulated, while Bcl-2 expression was down-regulated in Golph3-RNAi transfected Tca-8113 cells. At the opposite, up-regulation of Golph3 resulted in decreased expression of cytochrome-C and caspase3 (mRNA and protein) whereas Bcl-2 expression was increased (Figure 4).

Discussion

The effects of GOLPH3 have been mostly described in multiple kinds of cell lines [9-11]. GOLPH3 is a phosphorylated protein that involved in the maintenance of the Golgi [12]. Finding of JH Wang *et al.* revealed that high expression of GOLPH3 may play a vital role in the progression of ESCC tumorigenesis and

high expression of GOLPH3 in ESCC patients is positively associated with poor prognosis [13]. In the current study, we examined GOLPH3 expression in OSCC cell lines and normal tongue epithelial cell TEC. Our results revealed that compared with TEC cells, GOLPH3 expression (mRNA and protein) was significantly upregulated in OSCC cell lines, suggesting that GOLPH3 is a molecule associated with development of OSCC.

To gain insight into the biological roles of GOLPH3 increased in OSCC tumorigenesis, we knocked down and upregulated GOLPH3 expression in Tca8113 cells. Result of MTT revealed that GOLPH3 upregulation increased while GOLPH3 depletion inhibited cell proliferation rate at day 2, 3, 4 and 5, no different after incubating for day 1. To investigate the effect of GOLPH3 expression and DDP or Paclitaxel or Adriamycin on OSCC cell proliferation. Tca-8113 cells after transfection were incubated with various concentrations of DDP or Paclitaxel or Adriamycin for 24 h, the results showed that Golph3-RNAi transfected Tca-8113 cells enhanced the effect of chemotherapy on cell pro-

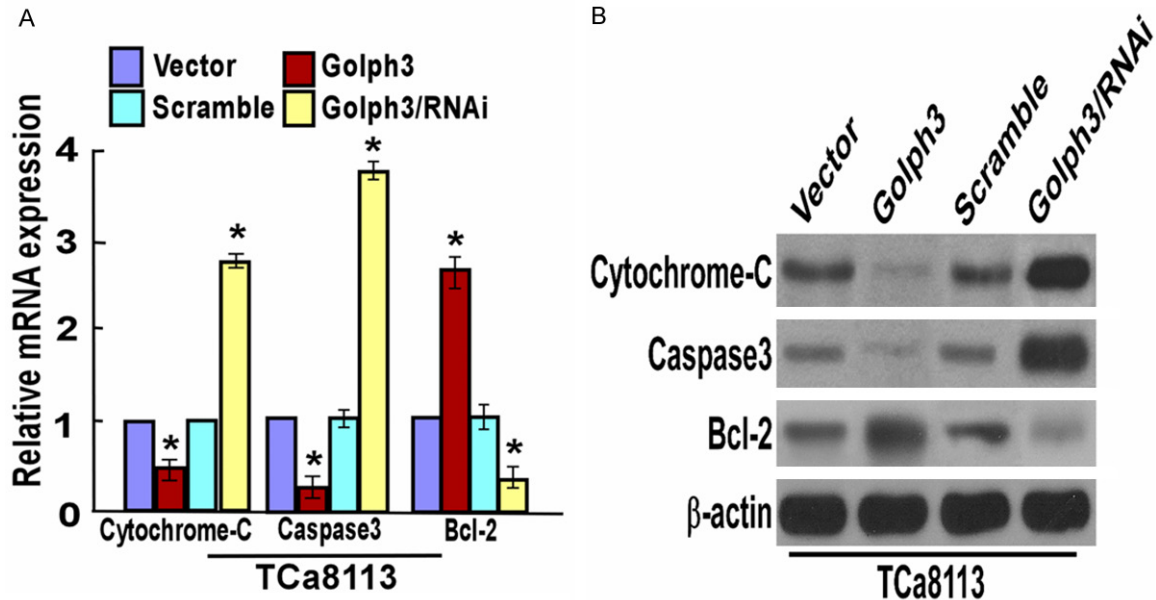


Figure 4. GOLPH3 regulates cell proliferation by regulating of apoptosis-related protein expression Cytochrome-C, Caspase3 and Bcl-2. A. PCR analysis was used to detect Cytochrome-C, Caspase3 and Bcl-2 in Tca-8113 cells after transfection. B. Cytochrome-C, Caspase3 and Bcl-2 were measured by western blot in Tca-8113 cells after transfection. β-actin was used as a loading control.

liferation. However, the underlying mechanisms remain unclear. To find out the potential mechanism, we checked the expression of several cell apoptosis-related proteins Cytochrome-C, Caspase3 and Bcl-2. It is known that Cytochrome C, caspase3 and Bcl-2 gene function as regulators of the apoptotic pathway [14-17]. We found that GOLPH3 upregulation caused a significant decreased while knockdown of GOLPH3 increased the mRNA and protein levels of Cytochrome C and caspase3, and upregulation of GOLPH3 increased while GOLPH3-RNAi inhibited the mRNA and protein level of Bcl-2.

Taken together, our data above revealed GOLPH3 was upregulated in OSCC and acted as a potential tumor promoter. GOLPH3 upregulation promoted and its depletion inhibited cell proliferation. Downregulated expression of GOLPH3 enhanced the effect of chemotherapy on cell proliferation in OSCC and this may be related to increasing the mRNA and protein levels of Cytochrome C and caspase3 and decreasing the expression of Bcl-2. Our results provided an encouraging basis elucidation of the functional importance of GOLPH3 in the progression of OSCC and this implied GOLPH3 might be developed as a new therapeutic target in OSCC.

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Disclosure of conflict of interest

None.

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