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Targeting eIF5A2 reduces invasion and reverses chemoresistance in SCC-9 cells *in vitro*

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Summary. Background and Aims. Eukaryotic translation initiation factor 5A2 (EIF5A2) has been reported to be involved in metastasis and chemotherapy resistance in many human cancers. However, the effect and mechanism of EIF5A2 in oral cancer cells are unknown. Here, we investigated the effects of targeting EIF5A2 on chemotherapy resistance in oral cancer cells *in vitro*.

Methods. By using a lentiviral system, we investigated the effects of targeting EIF5A2 on the invasion, migration, growth, and chemosensitivity of SCC-9 cells to CDDP *in vitro*. Through the method of gene intervention, we explore the role of pro-apoptotic Bim and epithelial and mesenchymal marker E-cadherin protein in this process and the regulation of EIF5A2 on Bim and E-cadherin.

Results. Targeting EIF5A2 reduces invasion and migration in SCC-9 cells partly through upregulation of E-cadherin expression; Targeting EIF5A2 promotes cell apoptosis and inhibits cell survival as well as increasing chemosensitivity in SCC-9 cells through upregulation of Bim expression.

Conclusion. EIF5A2 may be a novel potential therapeutic target for oral cancer by upregulation of Bim and E-cadherin.

Key words: Eukaryotic translation initiation factor 5A2, Bim, E-cadherin, Invasion, Apoptosis, Chemotherapy resistance, Oral cancer

Introduction

Oral cancer (OC) is one of the most common cancers, with approximately 50-60% reported survival rate after treatment (Chi et al., 2015). Cervical lymph node metastases (LNMs) is an essential malignancy criterion in oral cancer, and nearly 40% of patients with oral cancer suffer from lymph node metastatic tumors (Wenzel et al., 2004). Epidemiological data indicated that the 5-year survival rates of oral cancer patients were 80%, 70%, 56.9%, and 36.8% with stage I, II, III, and IV, respectively (Liao et al., 2015). The prevalence of OC-related distant metastases (DM) in the lungs and bones ranges from 52% to 83% Al-Othman et al., 2003). The mean time between symptom onset and detection of the first DM is ~16 months. Once DMs have been identified, the mean survival time is ~5 months (Dedivitis et al., 2009).

Early lymph node metastasis is the main factor for poor prognosis of oral cancer. Surgical resection assisted with or without chemotherapy and radiotherapy is a curative procedure and can have a serious impact on a patient's quality of life. Cisplatin and 5-fluorouracil are commonly used in combination, but the survival rate remains poor. Serious side effects of common chemotherapy drugs are unavoidable, thus limiting their use in oral cancer patients (Luo et al., 2019). Therefore, there is an urgent need to explore novel therapeutic agents against metastatic oral cancer.

Eukaryotic initiation factor 5A (EIF5A) is a eukaryotic translation initiation factor that participates in the initiation and elongation process in protein synthesis. Eukaryotic translation initiation factor 5A2 (EIF5A2) was first discovered in the primary ovarian cancer cell line in 2001, and later found to be aberrantly amplified or upregulated in various cancers, such as gallbladder cancer (Zheng et al., 2020), oral squamous cell carcinoma (Lin et al., 2020), thyroid cancer (Hao et al., 2020; Zhang et al., 2020), cervical cancer (Yang et al., 2016), gastric cancer (Meng et al., 2015), colorectal cancer (Zhu et al., 2012), esophageal squamous cell carcinoma (Li et al., 2014) and hepatocellular carcinoma (Tang et al., 2010; Bao et al., 2020), cute myeloid leukemia cells (Liu et al., 2019) and nasopharyngeal carcinoma (Huang et al., 2016).

Accumulating evidence shows that EIF5A2 plays important roles in enhancing anchorage-independent growth, xenograft tumor growth, increasing cancer cell metastasis, and promoting treatment resistance through multiple pathways (Tang et al., 2010; Zhu et al., 2012). Epithelial-to-mesenchymal transition (EMT) facilitates cancerous cell metastasis. EIF5A2 regulates EMT in

several cancers, and contributes to invasiveness and chemo-resistance of tumors (Yang et al., 2014; Zheng et al., 2021). EIF5A2 positively regulates stemness in ovarian cancer cells in CSCs-targeted therapy via the E2F1/KLF4 pathway (Wang et al., 2021).

EIF5A2 upregulation in cancer tissues is associated with poor prognosis in these patients. In esophageal squamous cell carcinoma (Li et al., 2014), EIF5A2 is overexpressed by gene amplification or hypoxia, and is associated with up-regulation of HIF1α, metastasis, and shorter survival times of patients. Increased expression of eIF5A2 increases metastasis and angiogenesis via the HIF1α-mediated signaling pathway. In cervical cancer patients (Yang et al., 2016), the increased EIF5A2 expression was correlated with higher FIGO stage, deep cervical stromal invasion, lymphovascular space involvement, pelvic lymph node metastasis and postoperative recurrence. Patients with tumours showing high EIF5A2 expression had a poorer survival time than those with normal EIF5A2 expression, especially the patients with negative pelvic lymph nodes and FIGO stage II (Yang et al., 2016). In breast cancer cells, higher expression levels correlated with decreased doxorubicin sensitivity. Silencing of EIF5A2 significantly improved doxorubicin toxicity in breast cancer cell lines (Liu et al., 2015), suggesting that EIF5A2 plays an important role in doxorubicin chemoresistance in breast cancer cells. In papillary thyroid cancer (PTC) cells, EIF5A2 inhibition prevented PTC cell growth, invasiveness and migration and induced cell apoptosis *in vitro*. Furthermore, eIF5A2 depletion inhibited tumor growth and metastasis *in vivo* (Zhang et al., 2020).

It has recently been reported that EIF5A2 overexpression was observed in head and neck squamous cell carcinoma(HNSCC) and was linked to poor progression-free survival and overall survival time (Ye et al., 2023). Furthermore, EIF5A2 overexpression may be a risk factor for prognosis in HNSCC and may be regulated by the SNHG16/miR-10b-5p/EIF5A2 axis (Ye et al., 2023). Lin et al. (2020) has found that high EIF5A2 expression is associated with advanced LNMs and EMT markers (E-cadherin and Beta-catenin) and may serve as a marker for an unfavorable prognosis in patients with oral squamous cell carcinoma (OSCC). EIF5A2 was also related with cell growth and apoptosis. For example, miR-9 improved the anti-tumor effects of Dnr by inhibiting myeloid cell leukemia-1 (MCL-1) expression, which was dependent on downregulation of EIF5A2 expression (Liu et al., 2019). In hepatocellular carcinoma (HCC) cells, EIF5A2 knockdown sensitized HCC cells to doxorubicin (Dox) (Tu et al., 2019). EIF5A2 overexpression decreases DOX sensitivity by increasing EMT, and EIF5A2 inhibition can induce apoptosis via pro-apoptotic molecule (Guan et al., 2019). Targeting eIF5A2 was associated with E-cadherin upregulation and decreased expression of N-cadherin, which affects the cell migration, invasion and metastasis (Zhang et al., 2022).

Induction of cancer cell apoptosis is integral to the success of targeted cancer therapy (Hata et al., 2015). Bim is one of the most prominent BH3-only proteins, which is expressed in all tissues investigated, and the deletion of Bim perturbs homeostasis in the immune system as well as the apoptotic response to many stimuli (Kuroda et al., 2006). Bim can directly activate Bax and Bak, initiating cytochrome c release as well as inhibiting the anti-apoptotic Bcl-2 proteins (Bhola and Letai, 2016). How the activation of BH3-only proteins, including Bim itself, is regulated is less clear. Yang et al. reported that EIF5A2 could regulate the expression of CDK4, Cyclin D1, Bcl-2 and Bad, which affected the cell proliferation, cell cycle and apoptosis in medulloblastoma cells (Yang et al., 2019). However, the exact mechanism by which EIF5A2 acts is unclear.

Here we studied the effect of EIF5A2 on cell invasion and metastasis as well as chemoresistance in oral cancer cells *in vitro*; we then studied the effect of EIF5A2 on pro-apoptotic molecule Bim and EMT E-cadherin, and explored whether Bim and E-cadherin were related to the EIF5A2-induced effect on oral cancer cells.

Materials and methods

Cell culture

Oral cancer cell lines SCC-9 were purchased from ATCC (Shanghai, China). They were all cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO) in a humidified atmosphere of 5% CO_2 at 37°C. The cell lines were validated by short tandem repeat profiling analysis and were free of mycoplasma contamination.

Construction vectors harboring EIF5A2 shRNA and infection

The plasmid EIF5A2 shRNA1-3 and control NC-shRNA1-3 were purchased from Santa Cruz (Shanghai City, P.R. China). All of the constructs were confirmed by both DNA sequencing and diagnostic digestion. SCC-9 cells were cultured in Dulbeccos Modified EagleMedium (DMEM) (Gibco, Carlsbad, CA, USA) supplemented with 10% (vol/vol) FBS, 1% penicillin/streptomycin (P/S), and 1% l-glutamine (l-Glu) (all reagents from PAA). The cells were cultured under standard growth conditions [5% (vol/vol) CO_2, 37°C] and passaged upon confluency. For transfections, SCC-9 cells were grown to 90-95% confluency and transfected using Lipofectamine 2000 for 24-72h (Invitrogen) according to the manufacturer's guidelines. For stable expression, lentiviral plasmids harbouring the desired gene were first transfected into 293T cells together with the packaging plasmids pSPAX2 and pMD2.G at a ratio of 5:3:2. HEK293 cells were placed into a 10-cm plate and cultured as previously described. After reaching 70-80% confluence, the cells were

transfected with 6 μg transfer vector using Lipofectamine 2000 reagent. Forty-eight hours after transfection, the supernatants of each group were collected and used to infect scc-9 cells for another 48 h. Puromycin-tolerant SCC-9 cells were picked. Subsequent western blotting and PCR were applied to confirm the correct expression of the stable cell lines.

siRNA (Small interfering RNA) transfection

siRNA guide sequences targeting BIM (Bim siRNA) and E-cadherin (E-cadherin siRNA) were obtained from CST (Cell Signaling Technology, Shanghai, China). Nontargeting siRNA was purchased from Dharmacon (Beijing, China) and served as a negative control. The stable SCC-9 cells (SCC-9/EIF5A2 shRNA or control shRNA) were seeded 24h before transfection at 50-60% confluence in growth medium without antibiotics. 100 nM siRNAs were then transfected into the culture's cells using Lipofectamine 2000 reagent (Invitrogen, Shanghai, China) folowing the manufacturer's instructions. The cells were harvested 48h after transfection for Western blot analyses.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

SCC-9 cells were plated at a density of 5000 cells/well in a 96-well plate for 24h, then transfected with EIF5A2 shRNA and its respective negative controls for 16 h, then treated with 10 μM CDDP (Sigma-Aldrich, Madrid, Spain). After 72h of treatment, MTT was added to a final concentration of 0.5 mg/ml and cells were incubated for four additional hours. Cells were then dissolved in DMSO and the produced formazan was measured at 520 nm with a Bio-Rad iMark spectrometer, and the relative cell proliferation was expressed as a percentage of the control.

Colony formation assay

The stable EIF5A2 shRNA transfected SCC-9 cells (SCC-9/EIF5A2 shRNA) cells (1×10^3) were seeded into 6-well plates and cultured for 10 days. To explore the effect of CDDP on colony formation, SCC-9/EIF5A2 shRNA cells were seeded (1×10^3) in six well plates and treated with vehicle (DMSO) or CDDP for 5-7 days. Fresh media without CDDP was then added to the cells and cultured for another 3 days. Cell colonies consisting of >50 cells were counted and used for comparison of colony formation ability.

Cell apoptosis assay

SCC-9 cells (3×10^5) were seeded into 6-well plates for 24h, then transfected with EIF5A2 shRNA and its respective negative controls for 16h, then treated with 10 μM CDDP for 72h. The stable EIF5A2 shRNA transfected SCC-9 cells (SCC-9/EIF5A2 shRNA) were

transfected with Bim siRNA and its respective negative controls for 16h, then treated with 10 μM CDDP for 72h. Subsequently, the cells were centrifuged at a low rpm to obtain pellets and residual alcohol was aspirated. Cells were then digested with DNase-free RNase A (2 mg/ml) for 30 min at 37°C. For Annexin V-FITC/PI double staining, cells were processed as per the manufacturer's instructions (BD Bioscience, San Jose, CA, USA), and then analyzed on the Epics XL-MCL Flow Cytometer (Beckman Coulter, Fullerton, CA, USA). We considered Annexin V-FITC negative and PI positive cells (quadrant A1) as mechanically injured during the experiment; both Annexin V-FITC and PI positive cells (quadrant A2) as late necrotic; both Annexin V-FITC and PI negative cells (quadrant A3) as normal; and Annexin V-FITC positive and PI negative cells (quadrant A4) as early apoptotic. The flow cytometric results of apoptotic cells (Annexin V-FITC positive and PI negative cells in quadrant A4) from different treatments were then analyzed for statistical significance.

Invasion assay

SCC-9/EIF5A2 shRNA or SCC-9/EIF5A2 shRNA/ E-cadherin siRNA and its respective negative controls were cultured for 24h. After that, 100 mL cell suspension (1×10^4) was added into the upper chamber and 500 mL complete culture solution was added into the lower chamber. After 24h of incubation in a 5% CO_2 humidified chamber at 37°C, noninvading cells were removed by wiping the upper surface of the membrane. The invaded cells in the bottom side were stained with Crystal Violet dye.

Wound healing assay

Cell migration was assessed by the wound healing assay. Cells were seeded into 6-well plates at a density of 1×105 cells/well and incubated at 37°C in 5 % CO_2 in humidified air. When the cells reached 80% confluency, the cells were scraped using a 1 mL sterile pipette tip to generate a wound, and washed twice with phosphate-buffered saline. Cells were cultured in a serum-free medium and photographed at 0 and 24h following generation of the wound using an inverted phase microscope.

Western blot assay

The extracted SCC-9 cells were lysed with protease inhibitor cocktail and clarified. Whole-cell proteins were electrophoresed under conditions in 12% polyacrylamide gels for immunoblotting using standard Western blotting method. The primary antibodies were obtained from Cell Signaling Technologies (Guangzhou, Guangdong, China): EIF5A2 (1 : 200 diluted), E-cadherin (1 : 200 diluted), Bim (1 : 200 diluted) and GAPDH (1 : 1500 diluted). Protein bands were detected using chemi-luminescence reagents.

Statistical analysis

The statistical comparisons of the two groups were performed using the Student's t-test. The results are presented as mean ± standard deviation (SD) of three replicate assays. Differences of P<0.05 were considered statistically significant.

Results

EIF5A2 knockdown in SCC-9 cells

In preliminary experiments, it was found that SCC-9 cells are rich in EIF5A2 protein expression. We detected EIF5A2 protein expression in stable EIF5A2 shRNA1-3 transfected SCC-9 cells using western blot assay and to see whether the EIF5A2 protein can be effectively knocked out by EIF5A2 shRNA. The results showed that EIF5A2 protein expression was decreased in the stable EIF5A2 shRNA transfected SCC-9 cells (SCC-9/ EIF5A2 shRNA) compared with the empty vector shRNA (SCC-9/NC shRNA) transfected SCC-9 cells (Fig. 1). EIF5A2 expression had the most obvious decline in EIF5A2 shRNA3, so the study took EIF5A2 shRNA3 as the research object. Therefore, EIF5A2 shRNA3 clones were used for subsequent experiments.

Targeting EIF5A2 reduces invasion and migration in SCC-9 cells in vitro

We determined whether EIF5A2 knockdown influenced the migration and invasion ability of SCC-9 cells *in vitro*. Eight fields were counted in each plate and 3 experiments were conducted. The results showed that invading cell numbers of metastatic SCC9 through matrigel were 62.4±20.3 (n=3), the empty vector shRNA (SCC-9/NC shRNA) transfected cells were 59.7±22. (n=3), whereas the invading cells of EIF5A2 shRNA transfected SCC-9 cells (SCC-9/ EIF5A2 shRNA) through matrigel were 38.6±10.08 %(n=3) (p=0.003). In the migration assay, a gap was generated in the cell layer using micropipette tip, then the cells were allowed to grow and migrate for 24h. After 24h, the gap width of SCC-9/EIF5A2 shRNA cells was reduced by

70.4±12.6%, whereas the gap width of SCC-9 cells and SCC-9/NC shRNA cells was reduced only by 14.6±2.56% and 13.48±3.06%, which was significantly decreased compare to the SCC-9/ EIF5A2 shRNA cells (p=0.002).

EIF5A2 is required for survival of SCC-9 cells in vitro

We determined whether EIF5A2 knockdown influenced cell survival and apoptosis in SCC9 cells. SCC-9 cells were transfected with EIF5A2 shRNA or NC shRNA for 72h. Cell viability and cell apoptosis of both cells were analyzed using MTT assay and flow cytometry using FITC Annexin V staining. The results showed that the cell survival rate was 73.6±12.4% (n=3) in the SCC-9/ EIF5A2 shRNA cells, which was lower compared to the SCC-9/NC shRNA cells (96.3±14.8 %) (p=0.02) (Fig. 2A). To confirm the role of EIF5A2 on cell growth, we carried out *in vitro* colony formation assays. Eight fields were counted in each plate and 3 experiments were conducted. The results showed that the number of colonies was 178.6±46.83 in the SCC-9/ EIF5A2 shRNA cells, which was fewer than the SCC-9/NC shRNA cells (258.8±50.2) (p=0.03) (Fig. 2B). We further investigated the effect of targeting EIF5A2 on apoptosis in SCC9 cells. The results showed that the cell apoptotic rate was 18.6±4.3% (n=2) in the SCC-9/ EIF5A2 shRNA cells, which was higher than the SCC-9/NC shRNA cells (5.2±1.4%) and SCC-9 cells (4.4±1.8%) (p=0.03) (Fig. 2C).

Targeting EIF5A2 promotes chemosensitivity in vitro

To determine whether EIF5A2 is involved in chemoresistance, the EIF5A2 shRNA transfected SCC-9 cells or their controls were treated with 10 μM CDDP for 72h. Cell apoptosis was analyzed using flow cytometry using FITC Annexin V staining. The results showed that CDDP treatment only induced (5.3±1.6%) cell apoptosis, combined NC shRNA and CDDP treatment induced (7.2±2.3%) cell apoptosis, and combined EIF5A2 shRNA and CDDP treatment induced (24.6±7.4%) cell apoptosis (n=3, p=0.01). To confirm the role of EIF5A2 on CDDP-induced cell growth, we carried out *in vitro* colony formation assays. Eight fields were counted in each plate and 3 experiments were conducted. The results showed that the number of colonies was 104.56±14.17 in the SCC-9/ EIF5A2 shRNA/CDPP cells, which was fewer than the SCC-9/NC shRNA/CDDP cells (176.6±20.4) (p=0.01).

EIF5A2 deletion is critical for CDDP induced apoptosis by upregulating Bim

CDDP upregulated pro-apoptotic factor Bim, which contributed to CDDP -induced apoptosis in oral cancer cells (Wu et al., 2012). In our study, Bim protein (Bim/GAPDH: 0.19) was less enhanced at 24h after CDDP treatment. EIF5A2 deletion alone moderately
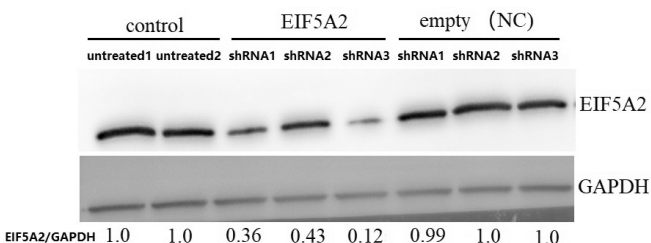

Fig. 1. EIF5A2 expression in stable EIF5A2 shRNA transfected SCC-9 cells. EIF5A2 expression in stable EIF5A2 shRNA transfected SCC-9 cells by western blot assay. EIF5A2 expression has the most obvious decline in EIF5A2 shRNA3.
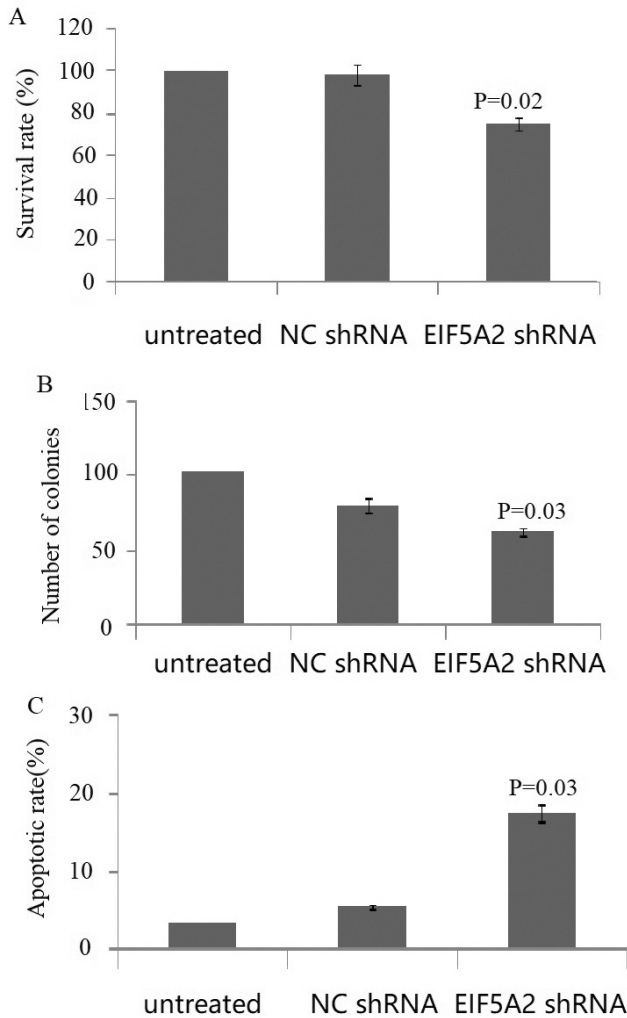
Fig. 2. Targeting EIF5A2 inhibits cell survival and induces cell apoptosis in SCC-9 cells *in vitro*. SCC-9 cells were transfected with EIF5A2 shRNA or NC shRNA for 72 h. A, Cell viability was determined by MTT assay; B, cell survival was detected by Colony formation assay; Cell apoptosis was detected using an Annexin V-FITC apoptosis detection kit.

upregulated Bim expression (Bim/GAPDH: 0.45). Transfection with EIF5A2 shRNA significantly promoted CDDP -induced Bim protein expression in SCC9 cells (Bim/GAPDH: 0.84) (0.84 vs 0.45, t=4.16, p=0.26; 0.84 vs 0.19, t=6.13, p=0.001). (Fig. 3A) We therefore determined whether Bim upregulation was related to the systematic pro-apoptotic effect. Transfection with Bim siRNA significantly inhibited the upregulation of Bim in the SCC9 cells with combined EIF5A2 deletion and CDDP treatment (Bim/GAPDH: 0.38) compared to the control siRNA transfected SCC9 cells with combined EIF5A2 deletion and CDDP treatment (Bim/GAPDH: 0.89) (0.89 vs 0.38, t=4.67, p=0.008) (Fig. 3A).

We next examined the effects of the deletion of Bim alone or the combined deletion on cell apoptosis. The cell apoptotic rate was 6.3±2.4% in the Bim siRNA transfected SCC9 cells with combined EIF5A2 deletion and CDDP treatment, and 19.4±4.6% in the control siRNA transfected SCC9 cells with combined EIF5A2 deletion and CDDP treatment. Deletion of Bim significantly attenuated EIF5A2 deletion in combination with CDDP induced cell apoptosis in the SCC9 cells by flow cytometric assay (t=5.14, p=0.002) (Fig. 3B).

E-cadherin is involved in EIF5A2-induced cell migration and invasion in SCC9 cells

SCC9 cells had poor E-cadherin expression (E-cadherin/GAPDH: 0.19) in the stable control shRNA; in the stable control shRNA and EIF5A2 shRNA transfected SCC9 cells, the relative E-cadherin expression (E-cadherin/GAPDH) was 0.21 and 0.96 confirmed by western blot analysis. The results showed that targeting EIF5A2 significantly promoted E-cadherin expression in the SCC9 cells (0.21 vs 0.96, t=5.39, P=0.007). There was no significant change in E-cadherin expression between the untreated SCC9 cells and control shRNA transfected SCC9 cells (0.18 vs 0.96, t=1.46, p=0.607) (Fig. 4).
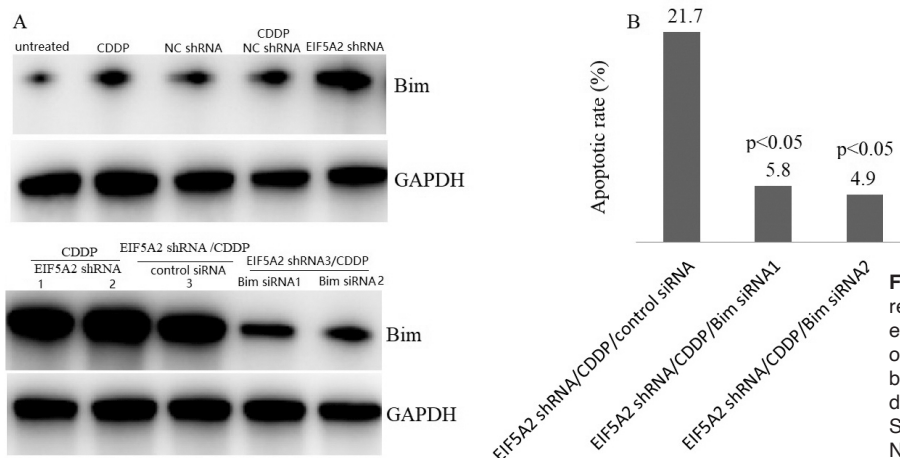
To evaluate the functional role of E-cadherin in

Fig. 3. Targeting EIF5A2 upregulates Bim and reduces CDDP resistance in SCC-9 cells. **A.** Bim expression was detected in SCC-9 cells with CDDP or/and EIF5A2 shRNA or NC shRNA transfection by western blot assay. **B.** Cell apoptosis was detected by Annexin V-FITC apoptosis detection in SCC-9 cells with CDDP or/and EIF5A2 shRNA or NC shRNA transfection.

SCC9 cells, we next investigated the effect of E-cadherin on migration ability of SCC9 cells *in vitro*. The stable control shRNA and EIF5A2 shRNA transfected SCC9 cells were transiently transfected into E-cadherin siRNA or control siRNA for 48h. The E-cadherin expression was significantly decreased in the E-cadherin siRNA transfected SCC9/EIF5A2 shRNA cells (E-cadherin/GAPDH: 0.12) compared to the control siRNA transfected SCC9/EIF5A2 shRNA cells (E-cadherin/GAPDH: 0.82) (0.12 vs 0.82, t=5.26, p=0.008) (Fig. 4).

We determined whether EIF5A2 knockdown influenced the migration and invasion ability of SCC-9 cells by E-cadherin upregulation. Eight fields were counted in each plate and 2 experiments were conducted. The results showed that invading cell numbers of E-cadherin siRNA transfected SCC9/EIF5A2 shRNA cells was 54.6±18.7 (n=2), which was higher than the control siRNA transfected SCC9/EIF5A2 shRNA cells (31.9±14.6) (t=5.22, p=0.06). In the migration assay, a gap was generated in the cell layer using micropipette tip, then the cells were allowed to grow and migrate for 24h. After 24h, the gap width of E-cadherin siRNA transfected SCC9/EIF5A2 shRNA cells was reduced by 29.8 ±17.3%, whereas the gap width of control siRNA transfected SCC9/EIF5A2 shRNA cells was reduced only by 56.4±11.8% (t=5.21, p=0.037). The results showed EIF5A2-induced invasion may be mediated at least in part through E-cadherin.

Discussion

EIF5A2 has been identified as a powerful gene in tumor invasion and metastasis. Through the intervention of EIF5A2 gene expression, the biological behavior of malignant tumor cells can be regulated (Zhu et al., 2012; Li et al., 2014). In the present study, EIF5A2 is overexpressed in the SCC9 cells, and silencing the expression of EIF5A2 could effectively reduce *in vitro* cell invasion and migration. The detailed mechanism by which EIF5A2 affects cell metastatic behavior is still unclear.

The E-cadherin epithelial cell adhesion protein is a tumor suppressor which is involved in collective cell migration behaviors that facilitate invasion and metastasis. Loss of E-cadherin expression during epithelial-mesenchymal transition (EMT) is generally thought to promote metastasis by allowing dissociation and invasion of cancer cells (Valastyan and Weinberg, 2011). EIF5A2 can enhance intra-variation through induction of EMT in many cancers. Our study found that SCC9 cells contained a low level of background E-cadherin protein expression. When EIF5A2 was disturbed, the expression of E-cadherin protein was significantly increased, accompanied by a significant decrease in cell invasive ability; After the expression of E-cadherin protein was disturbed, the decrease of cell invasion ability caused by targeting EIF5A2 was obviously reversed. Therefore, the regulation of cell metastasis ability by targeting EIF5A2 was achieved by up-regulating E-cadherin.

It has recently been found that EIF5A2 plays an important role in regulating cell proliferation, cell apoptosis and chemoresistance (Chen et al., 2016). We also found in the study that silencing the expression of EIF5A2 promoted cell apoptosis and reduced cell proliferation and enhanced the chemosensitivity to CDDP in SCC-9 cells *in vitro*. Therefore siRNA-mediated suppression of EIF5A2 may represent a novel therapeutic strategy for the adjuvant treatment of SCC-9. Tumor resistance to chemotherapy relies on cancer cells bypassing programmed cell death by apoptosis. Poor apoptotic response was related to low expression of the pro-apoptotic gene Bim in the cells (Hatal et al., 2015). In the present study, targeting EIF5A2 upregulated Bim expression in the SCC-9 cells; additionally, when Bim expression was knocked down by Bim siRNA, the effect of targeting EIF5A2 on cell apoptosis and CDDP treatment was significantly reduced; the synergistic effects of targeting EIF5A2 and CDDP on SCC9 cells were also greatly reduced. Therefore, targeting EIF5A2 promotes cell apoptosis and overcome CDDP resistance by Bim signal.

Conclusion

In summary, we showed that targeting EIF5A2 reduces invasion and enhances the cytotoxicity of CDDP in SCC9 cells by upregulation of Bim and E-cadherin. These studies highlight the importance of combining EIF5A2 suppression with chemotherapy for the treatment of oral cancer.
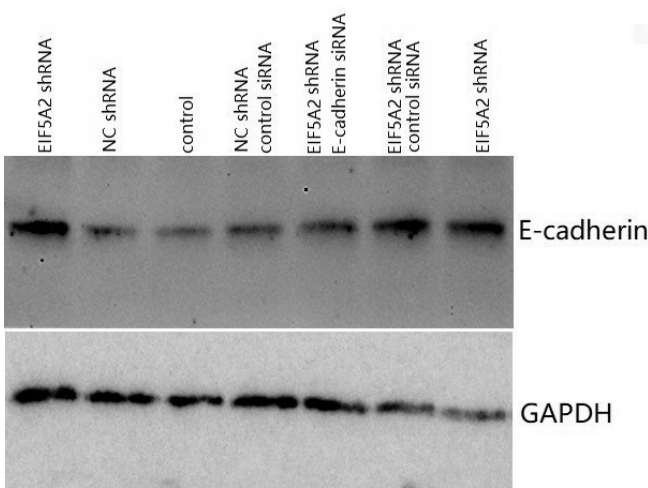

Fig. 4. E-cadherin is regulated by EIF5A2 in SCC9 cells. E-cadherin was detected in SCC-9 cells with EIF5A2 shRNA or/and E-cadherin siRNA transfection by western blot assay.

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