**Cyclopamine Suppresses Human Esophageal Carcinoma Cell Growth by Inhibiting Glioma-Associated Oncogene Protein-1, a Marker of Human Esophageal Carcinoma Progression**

**Background:** Esophageal carcinoma is a common gastrointestinal tumor in humans. Cyclopamine, a Hedgehog (Hh)-pathway-specific inhibitor, is an effective chemotherapeutic drug for suppressing tumor cell differentiation, with unclear mechanisms. We investigated glioma-associated oncogene protein-1 (Gli-1) expression in human esophageal carcinoma tissue and the inhibition of cyclopamine on EC9706 esophageal carcinoma cell growth.

**Material/Methods:** Gli-1 in tumor tissue was measured by immunohistochemistry (IHC). EC9706 cells were treated with different concentrations of cyclopamine and incubated for different times. MTT method, flow cytometry, and Acridine orange/ethidium bromide (AO/EB) double-fluorescence staining were applied to detect cell proliferation and apoptosis. Western blot (WB) analysis was performed to assess Gli-1 expression.

**Results:** Gli-1 was associated with patient age, gender, lymphatic metastasis, tumor recurrence, and stage, with significantly (P<0.05) positive correlations with age, lymphatic metastasis, tumor recurrence, and stage. At 12 h (F=214.57), 24 h (F=76.832), 48 h (F=236.90), and 72 h (F=164.55), the higher the concentration of cyclopamine, the higher the inhibition rate of suppressing EC9706 proliferation, and this effect was significant (P<0.05). The number of early-apoptosis cells increased as the concentration of cyclopamine increased. Morphology of EC9706 cells appeared as round with rough edges, karyopyknosis, and karyorrhexis. After 48 h, apoptosis rates of EC9706 cells treated with different concentrations of cyclopamine were (7.73±1.25)% at 2.5 μM, (13.37±1.42)% at 5.0 μM, (22.3±2.92)% at 10.0 μM, and (33.57±1.75)% at 20.0 μM, and the effect was dose-dependent. Gli-1 was obviously reduced after cyclopamine treatment and the effect was dose-dependent.

**Conclusions:** Gli-1 is highly expressed in human esophageal carcinoma, and could be a marker for use in assessing tumor stage and the deciding on treatment target.

**MeSH Keywords:** Apoptosis • Cell Proliferation • Esophageal Neoplasms • Glioma • Hedgehogs

**Full-text PDF:** https://www.medscimonit.com/abstract/index/idArt/912858
Background

Esophageal cancer arises from the esophagus and is prevalent worldwide [1,2]. In 2012, esophageal cancer was the eighth most prevalent malignant tumor, with 456,000 new cases per year, causing 400,000 deaths, up from 345,000 in 1990 [3]. Causes of esophageal cancer include tobacco, alcohol, long-term stimulation by hot drinks, poor diet, and chewing betel nut [4]. Treatments of esophageal cancer depend on the cancer stage and the tumor location, as well as general condition and individual preferences of the patient [5]. Small localized tumors can be treated with surgery alone [5], but in other cases, chemotherapy, along with radiotherapy or not, is used as the adjuvant therapy [5]. However, larger tumors can continue to grow slowly during chemotherapy or radiotherapy [6].

Signaling pathways are abnormally activated in esophageal carcinoma. Peng et al. revealed that the MMP-/PAR-1 pathway plays an important role in esophageal tumorigenesis [7]. According to Watanabe et al., the EGF-STAT1 pathway influences the progression of esophageal carcinoma [8]. The Hh signaling pathway plays the critical role in embryo development, and is inactive in normal adults. Hh can be activated in the process of development in many kinds of carcinomas, as well as promoting cells proliferation, survival, and differentiation [9]. Activated Hh in esophageal squamous cell carcinoma is associated with the progression and occurrence of esophageal cancer [10–13]. This association frees Gli in Hh, which can translocate to the nucleus, and then triggers the expression of diverse transcription factors during cell growth. The defect of Hh leads to continuously activating Gli, which is associated with certain types of cancers.

Cyclopamine is a type of steroidal alkaloid extracted from herb species. It can specifically restrain Hh [14]. Recent studies show that cyclopamine has no toxic effect on mammals, which provides good prospects for its application against cancer [15]. Based on this, cyclopamine, as an antitumor drug, is already in phase one clinical trials.

In this study, we observed Gli-1 expression in human esophageal carcinoma tissue, and then investigated the effect of cyclopamine on morphology, proliferation, and apoptosis of EC9706 cells by suppressing Hh, as well as elucidating the basic mechanisms underlying possible treatment of esophageal carcinoma by targeting Hh.

Material and Methods

Patients

Clinical characteristics and tumor samples were collected from 70 patients – 49 males and 21 females – from January of 2011 to December of 2014, who were diagnosed with esophageal cancer according to the criteria of the American Joint Committee on Cancer [16] and the Union Internationale Contre le Cancer [17]. All the patients had received resection and were hospitalized in the First Affiliated Hospital of Shantou University Medical College. All patients were followed for 36 months. Informed consent was obtained from each patient. The study was approved by the First Affiliated Hospital of Shantou University Medical College (the First Affiliated Hospital of SUMC-Scientific Research-No. 2011 008).

Gli-1 detection in human esophagus carcinoma tissue

IHC was used for detecting Gli-1 in human esophagus carcinoma tissues and the adjacent tissues. Tissue slices were made and estimated by 2 pathologists, with consensus. Slices were dried at 68°C for 20 min. Regular de-waxing and gradual ethanol hydration were performed, followed by incubation with 3% H2O2 at 37°C for 10 min. Washing with phosphate-buffered solution (PBS) (Solarbio Life Sciences, Inc. Beijing, China) was followed by boiling the slices with citrate buffer solution (0.01 M) (Boster Biological Technology Co., Wuhan, China) at 95°C for 20 min. After cooling to 25°C, we blocked the slices with normal goat serum (Beijing China Ocean Co.) at 37°C for 10 min. Incubation was performed with rabbit anti-Gli-1 polyclonal antibody (1: 500) (ab151796, Abcam, Shanghai, China) at 4°C overnight. After washing with PBS, slices were incubated with goat anti-Rat H&L (1: 200) (Abcam) at 37°C for 30 min. DAB (Beyotime Biotechnology, Shanghai, China) was used for staining at 25°C for 3–30 min until coloring. Hematoxylin-eosin (HE) (Beyotime) was for staining at 25°C for 2 min. Regular dehydration was carried out, and we sealed the slices with neutral resin (Bioway Biotechnology Co., Beijing, China). We observed the results under a microscope (Olympus Corporation, Beijing, China).

EC9706 culture

The human esophage cancer cell line EC9706 was obtained from the American Type Culture Collection (ATCC). EC9706 cells were cultured in RPMI1640 (Thermo Fisher Scientific, Inc. Shanghai, China) containing 10% fetal bovine serum (FBS) (Thermo), 100 U/ml of penicillin (Sigma), and 100 μg/ml of streptomycin (Sigma), and maintained at 37°C, 5% CO2, and saturated humidity. EC9706 cells were subcultured (1: 2–1: 3) at 90% confluence after digestion with trypsin (Thermo). EC9706 cells in logarithmic growth phase were used for the following experiments.

EC9706 cell proliferation detection with MTT

EC9706 cells were seeded in 96-well plates (Corning, Shanghai, China) at 6×104 cells/ml. Cyclopamine (Sigma) was dissolved in DMSO (Sigma) and the well plate was incubated at 37°C for 48 hours.
in dimethyl sulphoxide (DMSO) (Sigma), adjusted to 5 mg/ml, and stored at 4°C. Before using, we adjusted cyclopamine in RPMI1640 (2.5 μM, 5.0 μM, 10.0 μM, 20.0 μM), added it to EC9706 cells, and incubated at 37°C, 5% CO$_2$, and saturated humidity for 12 h, 24 h, 48 h, and 72h, separately. We added 10 μl MTT solution (5 mg/ml) (Sigma) to each well and incubated at 37°C for 4 h. We discarded the supernatant and added 150 μl DMSO to each well. Cells were incubated with a microplate oscillator (Scientific Industries, Inc., NY, USA) for 20~30 min. Optical density (OD) was read at 492 nm and 630 nm with a microplate reader (Synergy HTX, Biotek Instrument, Inc., Beijing, China). Inhibition rate was calculated as: inhibition rate (%)=(average OD of control group–average OD of dosing group)/(average OD of control group) ×100%. DMSO without cyclopamine was taken as control.

**AO/EB double-fluorescence staining**

EC9706 cells were seeded in 6-well plates and treated with cyclopamine as mentioned before. We washed the cells with pre-cooled PBS, added 20 μl AO/EB (1: 1) (Solarbio) in 2 ml medium, and incubated the cells at 25°C for 2~5 min. We observed results under a fluorescence microscope (TE2000-U, Nikon, Japan).

**Apoptosis detection by flow cytometry**

EC9706 cells were seeded in 6-well plates and treated with cyclopamine as mentioned before. Cells were collected by centrifuging at 1000×g for 3 min. We washed the cells twice with pre-cooled PBS and adjusted the concentration to 5×10$^5$~10$^6$ cells. An Annexin V-FITC/PI kit (Nanjing KeyGen Biotech Co., Nanjing, China) was used for detecting apoptosis. Cells were resuspended with 500 μl binding buffer, mixed with 5 μl Annexin V-FITC, and then mixed with 5 μl propidium iodide (PI), followed by incubation at 25°C in the dark for 5~15 min. Cell apoptosis was measured by flow cytometry (Attune® Nxt, Thermo) within 1 h.

**Figure 1.** Gli-1 expression in human esophageal carcinoma tissue (A Negative Gli-1; B Positive Gli-1 (+); C Positive Gli-1 (++); D Positive Gli-1 (+++)).
EC9706 cells were seeded in 6-well plates and treated with cyclopamine as mentioned before. Total protein was extracted from the cells with RIPA buffer (Thermo) and quantified by a BCA kit (Beyotime Biotechnology, Shanghai, China). We analyzed 40 μg proteins by 10% separating gel and 5% stocking gel with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred them to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Shanghai, China). After blocking with 5% skim milk (Millipore) at 25°C for 2 h, protein bands were separately incubated with rabbit anti-Gli-1 polyclonal antibody (1: 2000) (ab151796, Abcam) and β-actin polyclonal antibody (1: 1,000) (Santa Cruz Biotechnology) at 4°C overnight. Then, the bands were incubated with goat anti-rabbit antibody (1: 3000) (Jackson ImmunoResearch Laboratories, Inc. Shanghai, China) at 25°C for 1 h. An electrochemiluminescence (ECL) kit (Millipore) was used for analyzing the proteins.

### Statistical analysis

Quantitative data are shown as mean ± standard deviations (χ±SDs) and were analyzed by SPSS 13.0 software. One-Way ANOVA was used for statistical evaluation. Multi-variate analysis was used to determine independent risk factors. *P*<0.05 was considered significant.

### Results

**Gli-1 is highly expressed in human esophagus carcinoma tissue**

As shown in Figure 1, positive Gli-1 expressions were found in human esophagus carcinoma tissues (Figure 1B–1D). Some esophagus carcinoma tissues had negative Gli-1 (Figure 1A).

### Table 1. Gli-1 expression is associated with human esophagus carcinoma progression.

|                | Age | N | – | + | ++ | +++ | F   | Adjusted R² | P       |
|----------------|-----|---|---|---|----|-----|------|------------|---------|
| Age            |     | 70 | – | – | –  | –   | 8.346| 0.242      | <0.001* |
| Gender         |     |    |   |   |    |     |      |            |         |
| Male           |     | 49 | 5 | 17| 12 | 15  | 3.055| 0.082      | 0.034*  |
| Female         |     | 21 | 8 | 7 | 2  | 4   |      |            |         |
| Lymphatic      |     |    |   |   |    |     |      |            |         |
| metastasis     |     |    |   |   |    |     |      |            |         |
| No             |     | 26 | 10| 15| 1  | 0   | 18.575| 0.433      | <0.001* |
| Yes            |     | 44 | 3 | 9 | 13 | 19  |      |            |         |
| Recurrence     |     |    |   |   |    |     |      |            |         |
| No             |     | 19 | 8 | 11| 0  | 0   | 11.706| 0.318      | <0.001* |
| Yes            |     | 51 | 5 | 13| 14 | 19  |      |            |         |
| Stage          |     |    |   |   |    |     |      |            |         |
| I              |     | 25 | 12| 13| 0  | 0   | 65.853| 0.738      | <0.001* |
| II             |     | 15 | 1 | 9 | 5  | 0   |      |            |         |
| III            |     | 23 | 0 | 2 | 9  | 12  |      |            |         |
| IV             |     | 7  | 0 | 0 | 0  | 7   |      |            |         |

CI – confidence interval; * *P*<0.05.

### Table 2. Effect of cyclopamine on EC9706 proliferation (χ±SDs).

| Cyclopamine concentration (μM) | 12 h Inhibition rate (%) | 24 h Inhibition rate (%) | 48 h Inhibition rate (%) | 72 h Inhibition rate (%) |
|-------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| DMSO                          | 0.322±0.02               | 0.461±0.03               | 0.632±0.02               | 0.942±0.03               |
| 2.5                           | 0.318±0.05               | 1.24                     | 0.444±0.07               | 3.56                     | 0.5977±0.06               | 5.46                       | 0.8912±0.06               | 5.46                       |
| 5.0                           | 0.308±0.04               | 4.38                     | 0.4257±0.04              | 7.70                     | 0.5558±0.08               | 12.08                     | 0.7713±0.06               | 18.18                     |
| 10.0                          | 0.3033±0.08              | 5.87                     | 0.4868±0.03              | 11.41                    | 0.6023±0.03               | 23.00                     | 0.6023±0.03               | 36.11                     |
| 20.0                          | 0.2831±0.03              | 12.15                    | 0.3677±0.01              | 20.27                    | 0.4326±0.02               | 31.57                     | 0.5213±0.01               | 44.70                     |
| F value                       | 214.57                   | 76.832                   | 236.90                   | 164.55                   |
| P value                       | <0.001                   | <0.001                   | <0.001                   | <0.001                   |
Gli-1 is associated with human esophagus carcinoma progression

As shown in Table 1, Gli-1 expression in cancer tissue of human esophagus carcinoma was significantly (P<0.05) associated with patient age, gender, lymphatic metastasis, tumor recurrence, and tumor stage. Males had a higher incidence of human esophagus carcinoma than females. A higher expression of Gli-1 in tumor tissue was associated with greater probability of lymphatic metastasis, as well higher recurrence rate and cancer progression rate.

Multivariate analysis showed that Gli-1 expression in cancer tissue of human esophagus carcinoma patients was positively and significantly (P<0.05) correlated with patient age ($R^2=0.242$), gender ($R^2=0.082$), lymphatic metastasis ($R^2=0.433$), tumor recurrence ($R^2=0.318$), and tumor stage ($R^2=0.738$).

Cyclopamine suppresses EC9706 proliferation

As shown in Table 2 and Figure 2, the inhibition rate of cyclopamine suppressing EC9706 proliferation increased as the concentration of cyclopamine increased after 12 h ($F=214.57, P<0.001$), 24 h ($F=76.832, P<0.001$), 48 h ($F=236.90, P<0.001$), and 72 h ($F=164.55, P<0.001$), and this effect was significant. These results indicate that high-concentration cyclopamine can effectively suppress EC9706 proliferation.

![Figure 3. Cyclopamine induced EC9706 apoptosis by AO/EB double-fluorescence staining. (A Blank; B DMSO; C 2.5 μM; D 5.0 μM; E 10.0 μM; F 20.0 μM)](image-url)
Cyclopamine promotes EC9706 apoptosis

Fluorescence staining results showed that cyclopamine promoted EC9706 cell apoptosis (Figure 3). The treatment time was 48 h. Apoptotic EC9706 cells appeared rounded with rough edges, karyopyknosis, and karyorrhexis. As the concentration of cyclopamine increased, the number of apoptotic cells increased, showing less green fluorescence and more red fluorescence (Figure 3).

Flow cytometry results showed cyclopamine promoted EC9706 cell apoptosis (Figure 4). The treatment time was 48 h. The apoptosis rate of normal EC9706 cells was (0.81±0.07)%, and those with DMSO treating were (1.05±0.13)%. Apoptosis rates of EC9706 cells treated with different concentrations of cyclopamine were (7.73±1.25)% at 2.5 μM, (13.37±1.42)% at 5.0 μM, (22.3±2.92)% at 10.0 μM, and (33.57±1.75)% at 20.0 μM. These results indicate that high-concentration cyclopamine can effectively promote EC9706 cell apoptosis.

Cyclopamine and downregulation of Gli-1 expression

The expression of Gli-1 after exposure to different concentrations of cyclopamine is shown in Figure 5. There was no significant difference in Gli-1 expression between normal EC9706 cells and in EC9706 cells treated with DMSO. With cyclopamine treatment, Gli-1 expression was obviously reduced as the concentration of cyclopamine increased, compared to normal EC9706 cells and EC9706 cells with DMSO treatment. These results indicated that cyclopamine can downregulate Gli-1 expression and suppress the Hh pathway.

Discussion

First, we investigated Gli-1 expression in human esophageal carcinoma tissue. Second, we used cyclopamine to inhibit Hh and downregulate Gli, and then measured the proliferation and apoptosis of EC9706 cells, representing human esophageal carcinoma. Results showed that Gli-1 was highly expressed in...
Cyclopamine is a nonsteroidal alkaloid existing in North American black false hellebore, Indian pyrola, Veratrum grandiflorum, and Fritillariae pallidiflorae. Cyclopamine has been confirmed as the inhibitor of Hh in many investigations [24–26]. Although some research proved the abnormally active Hh in esophageal carcinoma, there have been few studies focusing on the effect of cyclopamine in suppressing Hh or downregulating Gli-1 in esophageal carcinoma. In our study, we used cyclopamine to inhibit Hh activity as assessed by detecting Gli-1 expression in EC9706 cells. WB results confirmed the successful inhibition of Gli-1 expression, suggesting inhibition of the Hh pathway. Though measuring EC9706 cell proliferation and apoptosis, we found that inhibiting the Hh pathway significantly suppressed EC9706 proliferation but promoted EC9706 apoptosis, in a dose-dependent manner. These results indicate that high-concentration cyclopamine could be used as a drug to prevent esophageal carcinoma development and to promote apoptosis.

Some researchers showed a positive correlation of the expression levels of Smo and Gli-1 with invasion and lymphatic metastasis in esophageal squamous cell carcinoma [10,27,28]. Mori et al. found that silencing Gli-1 obviously suppressed EC9706 proliferation and promoted cell apoptosis, which indicated that the expression of Gli-1 influences the growth of esophageal cells [10]. In our study, we found that downregulation of Gli-1 suppresses EC9706 proliferation and promotes cell apoptosis, which agrees with previous research. However, we did not directly silence Gli-1 or investigate the metastasis of EC9706 cells after downregulating Gli-1, which are limitations of our study. Further investigations should focus on the metastasis of esophageal carcinoma in animal experiments.

**Conclusions**

Gli-1 expression is a marker of human esophageal carcinoma progression, and cyclopamine might be an effective drug for treating esophageal carcinoma by suppressing the Hh pathway. These results may assist with clinical diagnosis and treatment of esophageal cancer, and elucidate the underlying molecular mechanisms.

**Acknowledges**

We thank the First Affiliated Hospital of Shantou University Medical College for support.

**Conflict in this paper**

None.
References:

1. Afonso LA, Moysés N, Cavalcanti SMB: Human papillomavirus detection and p16 methylation pattern in a case of esophageal papilloma. Braz J Med Biol Res, 2010; 43(7): 694–96

2. Kennedy KW: Esophageal cancer prevention. Gastroenterol Hepatol (N Y), 2016; 12(12): 780–82

3. Duda M, Adamcik L, Dusek L et al: [Malignant tumors of the esophagus in the Czech Republic]. Rozhledy, 2012; 91(3): 132 [in Chech]

4. Akhtar S: Areca nut chewing and esophageal squamous-cell carcinoma risk in Asians: A meta-analysis of case-control studies. Cancer Causes Control, 2013; 24(2): 257–65

5. Stahl M, Budach W, Meyer H-J, Cervantes A, ESMO Guidelines Working Group: Esophageal cancer: Clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol, 2010; 21 (Suppl. 5): v46–49

6. Rice TW, Rusch VW, Allen MS et al: Worldwide esophageal cancer collaboration. Dis Esophagus, 2010; 22(1): 1–8

7. Peng HH, Zhang X, Cao PG: MMP-1/PAR-1 signal transduction axis and its chemoradiotherapy sensitivity and survival in esophageal squamous cell carcinoma. Jpn J Clin Oncol, 2011; 41(3): 386–93

8. Watanabe G, Kaganoi J, Imamura M et al: Progression of esophageal carcinoma by loss of EGF-STAT1 pathway. Cancer J, 2002; 7(2): 132–39

9. Xie K, Abbruzzese JL: Developmental biology informs cancer: The emerging role of the hedgehog signaling pathway in upper gastrointestinal cancers. Cancer Cell, 2003; 4(4): 245–47

10. Mori Y, Okamura T, Tsuchida S et al: Gli-1 expression is associated with lymph node metastasis and tumor progression in esophageal squamous cell carcinoma. Oncology, 2006; 70(5): 378–89

11. Yang L, Wang LS, Chen XL et al: Hedgehog signaling activation in the development of squamous cell carcinoma and adenocarcinoma of esophagus. Int J Biochem Mol Biol, 2012; 3(1): 46–57

12. Zhao Z, Zhang L, Zhang J, Wang J: [Expressions of Sonic hedgehog and matrix metalloproteinase 2 in human esophageal squamous cell carcinoma and the clinicopathological implications]. Nan Fang Yi Ke Da Xue Xue Bao, 2013; 33(7): 1008–11 [in Chinese]

13. Zhu W, You X, Li T et al: Correlation of hedgehog signal activation with chemoradiotherapy sensitivity and survival in esophageal squamous cell carcinomas. Jpn J Clin Oncol, 2011; 41(3): 386–93

14. Cooper MK, Porter JA, Young KE, Beachy PA: Teratogen-mediated inhibition of target tissue response to Shh signaling. Science, 1998; 280(5369): 1603–7

15. Taijale J, Chen JK, Cooper MK et al: Effects of oncogenic mutations in Smoothened and Patchedcan be reversed by cyclopamine. Nature, 2000; 406(6799): 1005–9

16. Edge SB, Compton CC: The American Joint Committee on Cancer: The 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. Ann Surg Oncol, 2010; 17(6): 1471–74

17. Sobin LH, Fleming ID: TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer, Springer-Verlag, 1997; 1803–4

18. Pasca di Maglano M, Hebrok M: Hedgehog signaling in cancer formation and maintenance. Nat Rev Cancer, 2003; 3(12): 903–11

19. Ogden SK, Ascano M Jr., Stegman MA, Robbins DI: Regulation of Hedgehog signaling: A complex story. Biochem Pharmacol, 2004; 67(5): 805–14

20. Varjosalo M, Taijale J: Hedgehog: Functions and mechanisms. Genes Dev, 2008; 22(18): 2454–72

21. Kata Y, Kata M: Hedgehog signaling pathway and gastric cancer. Cancer Biol Ther, 2005; 4(10): 1050–54

22. Von Hoff DD, Lorusso PM, Rudin CM et al: Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Eng J Med, 2009; 361(12): 1164–72

23. Sui G, Bonde P, Dhara S et al: Epidermal growth factor receptor and hedgehog signaling pathways are active in esophageal cancer cells from rat reflux model. J Surg Res, 2006; 134(1): 1–9

24. Kim SK, Melton DA: Pancreas development is promoted by cyclopamine, a Hedgehog signaling inhibitor. Proc Natl Acad Sci USA, 1998; 95(22): 13036–41

25. Mireault M, Moore E, Moniaux N et al: Cytotoxic effects induced by a combination of cyclopamine and gefitinib, the selective hedgehog and epidermal growth factor receptor signaling inhibitors, in prostate cancer cells. Int J Cancer, 2006; 118(4): 1022–31

26. Mukherjee S, Floreva N, Sadlonoa A et al: Hedgehog signaling and response to cyclophosphamide differs in epithelial and stromal cells in benign breast and breast cancer. Cancer Biol Ther, 2006; 5(6): 674–83

27. Jun-Ping LI, Yang JP, Cui L et al: [Expression and significance of Smo and Gli1 mRNA in esophageal squamous cell carcinoma tissue.] Clinical Medicine, 2013 [in Chinese]

28. Sun B, Li YX, Fu ZB et al: [Clinicopathological significance of expression of Smo and Gli1 in esophageal squamous cell carcinoma.] World Chinese Journal of Digestology, 2011; 19(5): 483–87 [in Chinese]