

Original Article

GY II represses proliferation and metastasis and promotes apoptosis in laryngeal squamous cell carcinoma

Yunfei Bai^{1,2}, Ru Wang¹, Xiaobo Cui², Lin Feng¹, Qian Shi¹, Hongzhi Ma¹, Meng Lian¹, Xixi Shen¹, Yifan Yang¹, Chen Tan¹, Jugao Fang¹

¹Department of Otorhinolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing, China; ²Department of Otorhinolaryngology, Inner Mongolia Medical University Affiliated Hospital, Huhhot, China

Received December 7, 2018; Accepted May 7, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: Traditional Chinese Medicine (TCM) has been considered to provide certain advantages in the treatment of cancer. Guben Yiliu II (GY II) has been widely used for clinical treatment of cancer in China. The current study investigated the effects of GY II on proliferation, apoptosis, invasion, and metastasis of laryngeal squamous cell carcinoma via MTT assays, Transwell assays, and flow cytometry, respectively, in Hep-2 and cells from clinical samples. Results of MTT assays revealed that GY II inhibited the growth of cells via arresting the cell cycle at G0/G1 phase. The addition of GY II significantly suppressed cell migration and invasion. Additionally, GY II treatment significantly promoted apoptosis of tumor cells. Present findings have significant implications regarding the understanding of mechanisms and targets of GY II in terms of tumors.

Keywords: Guben Yiliu II (GY II), laryngeal squamous cell carcinoma, traditional Chinese medicine (TCM).

Introduction

Laryngeal carcinomas are the second-most common type of head and neck cancer. Prevalence of laryngeal cancer is as high as 72.7 per 100,000 individuals [1]. In clinical treatment, main treatments for laryngeal cancer include surgery, radiotherapy, and chemotherapy. Malnutrition of patients, usually due to surgical trauma, immune suppression caused by chemotherapy drugs, inadequate nutrient intake, metabolic disorders, and a high metabolic status caused by the disease, seriously deteriorate treatment effects and prognosis [2]. In contrast, Traditional Chinese Medicine (TCM) provides certain advantages in the treatment of cancer. Some active compounds have been isolated from TCM and regarded as valuable drugs against cancer [3]. For example, β -elemene, a compound isolated from Curcuma, has been used as an anti-cancer drug in China [4, 5]. According to the theory of TCM, laryngeal carcinomas are caused by stagnation of blood and phlegm, resulting from the defi-

ciency of Zang-Fu organs [6]. In fact, cancer sufferers, especially in later stages, generally have symptoms with blood stagnation and/or blood stagnation in Qi deficiency conditions. Regarding treatment, Qi should be strengthened, protecting the body and activating the blood to eliminate pathogenic factors, re-establishing a new balance [7].

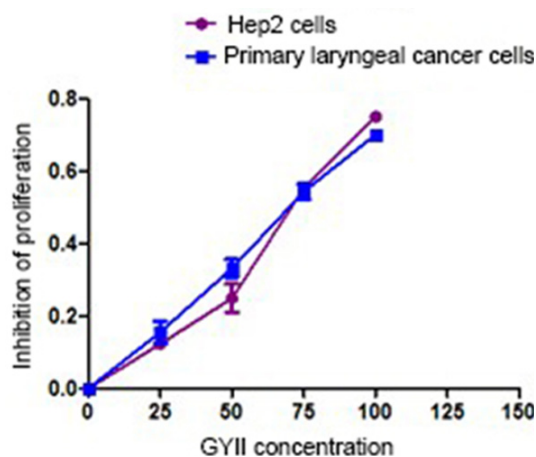
In recent years, for patients with Chinese backgrounds, Chinese Herbal Medicine (CHM) has been widely used to reduce adverse events (AEs), enhance the body's immunity and the quality of life, and prevent metastasis. It has also been applied in adjuvant treatment during chemo-and-radiotherapy. Furthermore, CHM has been used as an alternative treatment for cancers, including breast cancer, lung cancer, and pancreatic cancer [8].

Guben Yiliu II (GY II), created by Rencun Yu and other masters majoring in TCM, is an important component. It may promote the synthesis of benefiting Qi, activating blood circulation and detoxifying. It is a prescription widely used in

Table 1. Inhibitory rates on primary laryngeal carcinoma cells and Hep-2 cells (%) ($\bar{x} \pm s$)

Drug concentration	IC ₅₀ (primary laryngeal carcinoma cells)	IC ₅₀ (Hep-2 cells)
Control group (0%)	1	1
25%	15.5±5.4*	12.3±0.9*
50%	33.2±4.2 [#]	25.0±5.6 [#]
75%	54.4±3.8 [#]	55.3±1.1 [#]
100%	70.1±1.1 [#]	75.1±2.6 [#]
F	194.23	275.48
P	<0.0001	<0.001

Compared with the control group, *P<0.05, [#]P<0.001.

**Figure 1.** Effects of GY II on primary laryngeal carcinoma cells and Hep-2 cells proliferation *in vitro*. MTT assays were performed to determine the proliferation of cells.

China. Based on clinical experiences, accumulative clinical trials have demonstrated the anti-cancer properties of GY II [9].

Exploring the antitumor functions of GY II and possible mechanisms, the current study detected influences of GY II-containing serum from mice on the function of Hep-2 cells and primary human laryngeal carcinoma cells.

Materials and methods

Cell culture

Hep-2 cell lines were purchased from China Center for Type Culture Collection (CCTCC) of Wuhan University. Primary laryngeal cancer cells were isolated from laryngeal cancer tissues of patients. Informed consent was obtained from all patients and the current study

was approved by the Ethics Committee of Beijing Tongren Hospital. All cells were cultured in fresh PRMI1640 (Gibco) medium, supplemented with 10% fetal bovine serum (FBS, HyClone) in an incubator (Thermo) with 5% CO₂ at 37°C. The cells were then sub-cultured once 90% of the cells were fused.

Preparation of GY II aqueous extract

GY II was obtained from Beijing Hospital of Traditional Chinese Medicine. It was extracted from 13 different species of herbs used in TCM, including Codonopsis, Poria, Atractylodes, Radix Astragali, Rhizoma Chuanxiong, and Caulis Spatholobi, etc. According to requirements of the current study, 7.88 Kg of raw materials produced 250 g of clear ointment containing 2.42 g crude drug (GY II). Products of the extraction were stored at -80°C.

Preparation of GY II-containing serum

Thirty male Wistar rats, with body weights of 180~190 g, were purchased from Vital-Lee Company. The rats were fed with standard laboratory food and water in individual cages. The 30 rats were randomly divided into two groups, with 20 rats in the experimental group. They were washed using GY II. Ten rats made up the control group, washed using normal saline. For GY II treatment, the dosage was 20 g crude drug per Kg of body weight. Each rat was given treatment two times a day for six days. After the last treatment, the rats were sacrificed, collecting blood from the abdominal aorta. After 2 hours of incubation at room temperature, the blood was centrifuged for 20 minutes at 3,500 r/min. The serum was obtained followed incubation at 56°C for 30 minutes to induce inactivity. The serum was then diluted with the cell culture medium before application. All animal experiments were approved by the Animal Use and Management Committee of University.

MTT assays

Viability of Hep-2 cells and primary laryngeal carcinoma cells was determined by methyl thiazolyl tetrazolium (MTT) assays. Hep-2 cells or primary laryngeal carcinoma cells were seeded into 96-well plates at a density of 5,000 cells/well. They were incubated overnight until the cells were adherent. They were then exposed to various concentrations of GY II-conta-

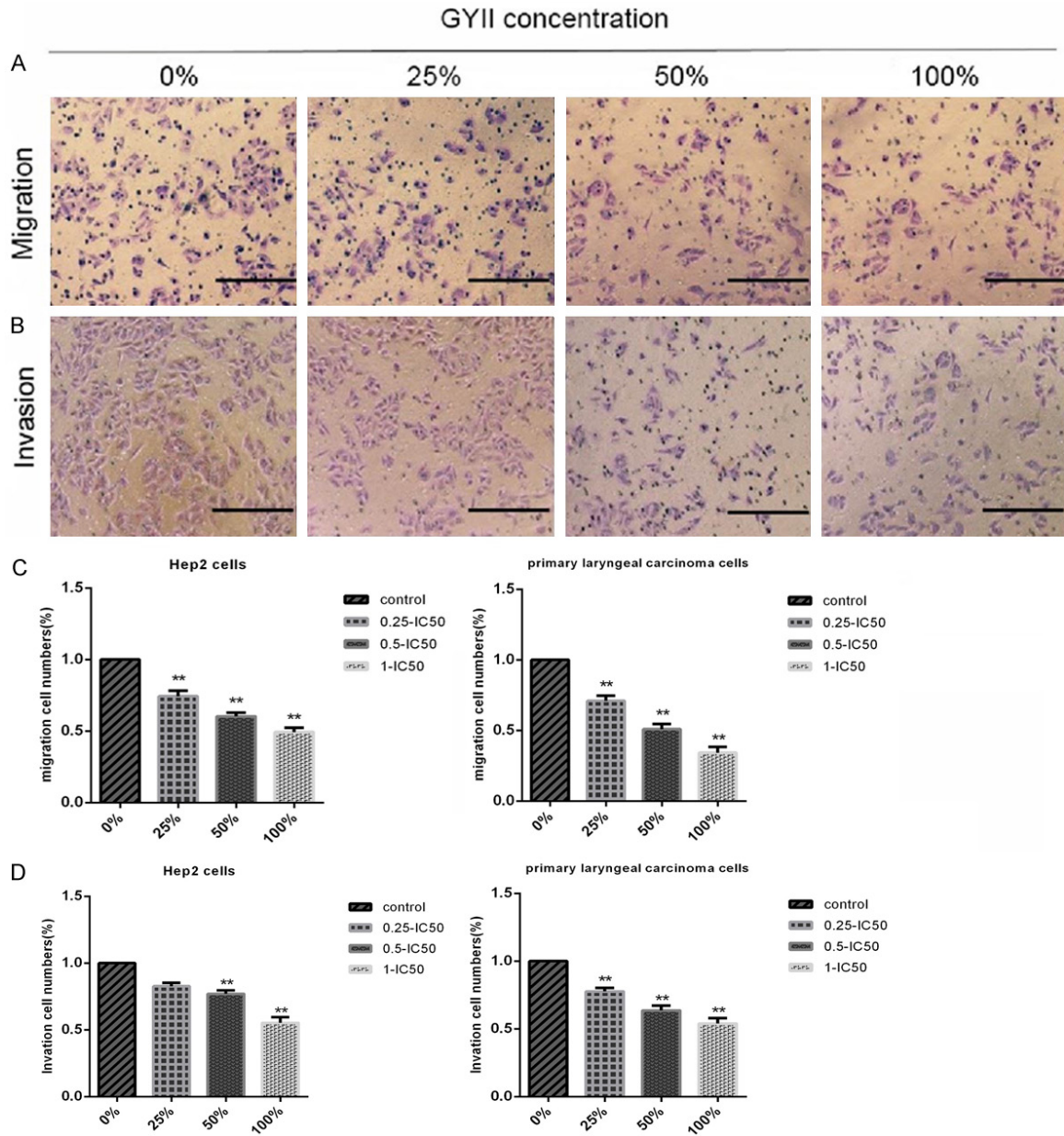


Figure 2. GY II attenuates cell migration and invasion. (A) Migration of primary laryngeal carcinoma cells and Hep-2 cells was assayed for indicated times by Transwell assays in the presence of different concentrations of GY II-containing serum; (B) Invasion of primary laryngeal carcinoma cells and Hep-2 cells was assayed for indicated times by Matrigel Transwell assays in the presence of different concentrations of GY II-containing serum; (C, D) Statistical graphs of relative cell migration and invasion changes of results are shown. All data are presented as means \pm SD of 3 independent experiments. ** $p < 0.01$ by one-way ANOVA vs untreated control. Bar=100 μ m.

ining serum (0%, 25%, 50%, 75%, and 100%) for 24 hours. Cell proliferation in each group was detected using MTT kits (E606334, Sangon Biotech). After adding MTT solution at an amount of 10% of the culture medium, the cells were cultured for 4 hours. After removal of the solution, 200 μ l DMSO was added into each

well, with a 10-minute oscillation to completely dissolve the formazan crystals. Absorbance values of each well at OD540 were measured, calculating cell inhibitory rates and the IC_{50} . Three repeated wells were set for each group. The experiment was repeated, independently, 3 times.

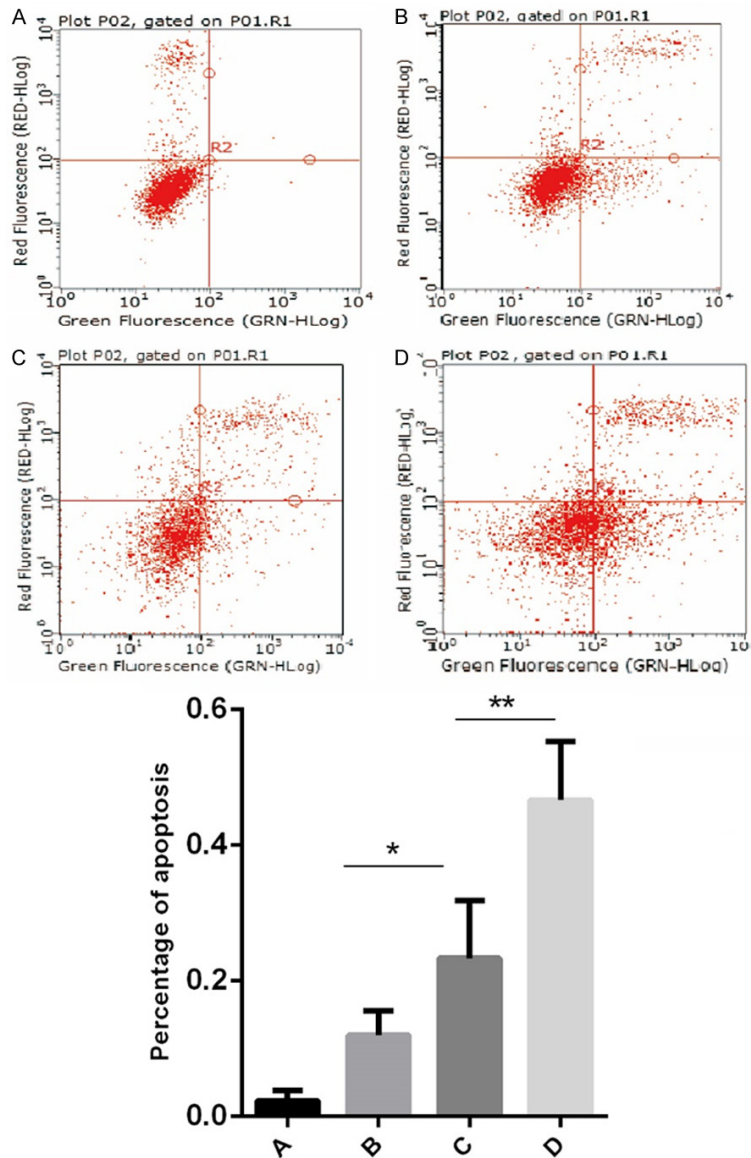


Figure 3. GY II enhances apoptosis of Hep-2 cells. Flow cytometry was conducted to determine the apoptosis of Hep-2 cells. (A) Control group (0%); (B) 25% GY II-containing serum group; (C) 50% GY II-containing serum group; (D) 100% GY II-containing serum group. Statistical graphs of relative cell apoptosis are shown.

Table 2. Apoptosis index of primary laryngeal carcinoma cells and Hep-2 cells ($\bar{x} \pm s$)

Drug concentration	primary laryngeal carcinoma cells (%)	Hep-2 cells (%)
Control group (0%)	2.41±3.32	0.57±0.14
25%	6.31±0.41 [#]	10.43±0 [*]
50%	12.8±0.18 [#]	15.46±5.04 [*]
100%	9.83±7.64 [#]	25.98±0.92 [*]
F	34.05	577.21
p	0.03	<0.001

Compared with the control group, ^{*}P<0.05, [#]P<0.001.

Cell invasion assays

Cell invasion assays were performed in 24-well Transwell chambers, containing polycarbonate membrane with 10 mm diameter and 8 μ m pores. Briefly, Matrigel (Corning, 354248) was first diluted with an incomplete culture medium. Next, 60 l dilution was added to each Transwell upper chamber and 5×10^4 Cells in 200 μ l of GY II-containing serum were seeded into the upper chamber. The lower chamber was filled with 500 μ l of preheated medium containing 10% FBS. After 16 hours of incubation at 37°C, the cells were fixed with 4% paraformaldehyde for 10 minutes. Non-invasive cells were removed with cotton swabs. The upper chamber was washed with PBS two times. The number of cells that invaded through the basement membrane were counted, following staining with 0.2% crystal violet/20% methanol (wt/vol) solution for 15 minutes. Invaded cells were visualized under a microscope and averaged in three random fields (magnification of 10 \times).

Cell migration assays

Cell migration assays were performed in 24-well Transwell chambers (Corning, 3458) with 10 mm diameter and 8

μ m pores, according to manufacturer instructions. Briefly, the cells were resuspended in 200 μ l GY II-containing serum and seeded into the upper chamber. Moreover, 500 μ l of medium containing 10% FBS was added into the lower chamber. After 16 hours of incubation at 37°C, migrated cells were dyed with 0.2% crystal violet/20% methanol (wt/vol) solution for 15 minutes and counted under a microscope. Three fields of view were randomly selected for each chamber.

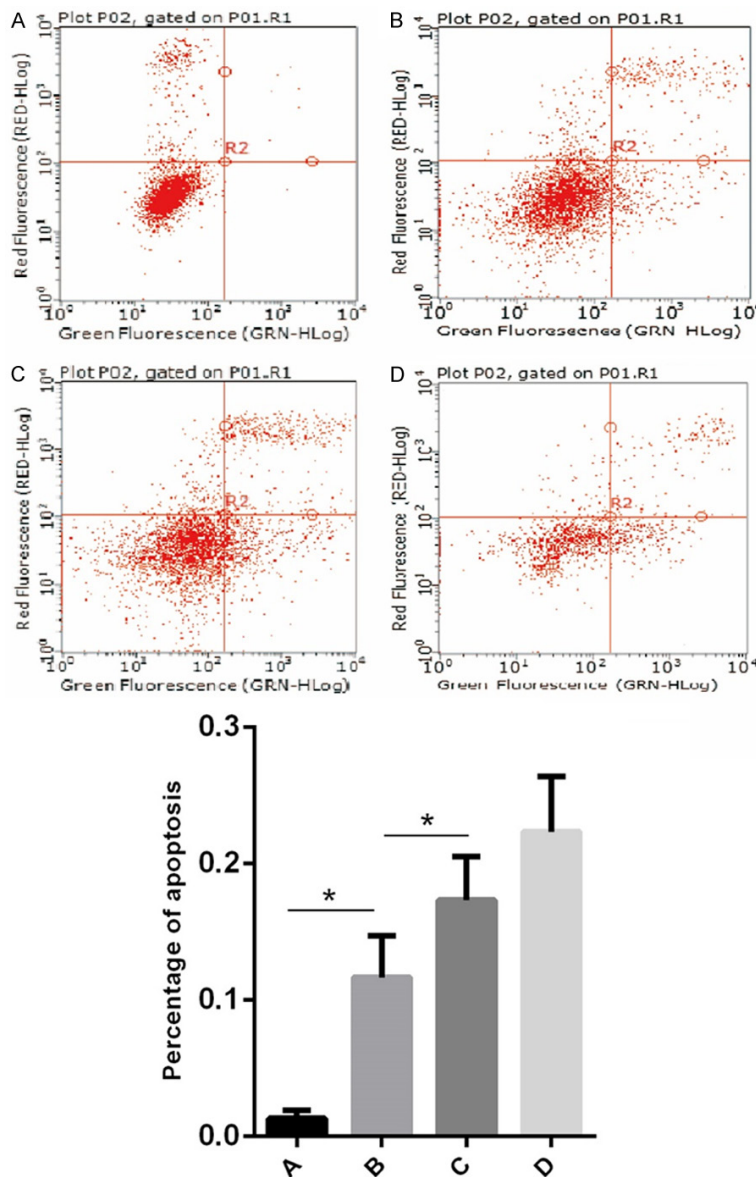


Figure 4. GY II enhances apoptosis of primary laryngeal carcinoma cells. Flow cytometry was conducted to determine the apoptosis of primary laryngeal carcinoma cells. (A) Control group (0%); (B) 25% GY II-containing serum group; (C) 50% GY II-containing serum group; (D) 100% GY II-containing serum group. Statistical graphs of relative cell apoptosis are shown.

Flow cytometry for analysis of apoptosis and cell cycle

For apoptosis analysis, the cells (5×10^5 cells/well) were seeded in 6-well plates and treated with GY II-containing serum for 24 hours. They were then digested by trypsin without EDTA. The cells were transferred to micro-centrifuge tubes and washed twice with PBS. According to manufacturer instructions, the cells were resuspended with 500 μ l binding buffer and

incubated with 5 μ l Annexin V-FITC and 5 μ l PI solution for 5 minutes. The percentage of apoptotic cells was analyzed using flow cytometry.

Regarding cell cycle analysis, cell suspension was mixed with PBS (according to the concentration of the drug) and fixed in 70% ethanol at 4°C overnight. After overnight incubation at 4°C, the immobilized cells were collected by centrifugation and suspended in PBS. This was followed with PI staining for 30 minutes. Cellular DNA content levels of each phase were quantified with flow cytometry.

Data statistics and processing

Significance of the data was analyzed using SPSS 20.0 statistical software. Data are presented as mean \pm standard deviation ($\bar{x} \pm s$). Unpaired Student's t-tests were applied for comparisons between two groups and one-way or two-way ANOVA was used for comparisons among groups. Statistical significance is set as $P < 0.05$.

Results

GY II inhibited the growth of Hep-2 and primary laryngeal carcinoma cells

To investigate the effects of GY II on tumor cell growth, MTT assays were performed, calculating proliferation rates. As shown in **Table 1** and **Figure 1**, GY II-containing group significantly repressed the proliferation of primary laryngeal cancer cells and Hep-2 cells. Furthermore, the repression effects were displayed in a dose-dependent manner (**Figure 1**). The inhibitory rate and IC_{50} level, respectively, were 54.4% and 67.59 mg/mL in primary laryngeal cancer cells when the concentration of added GY

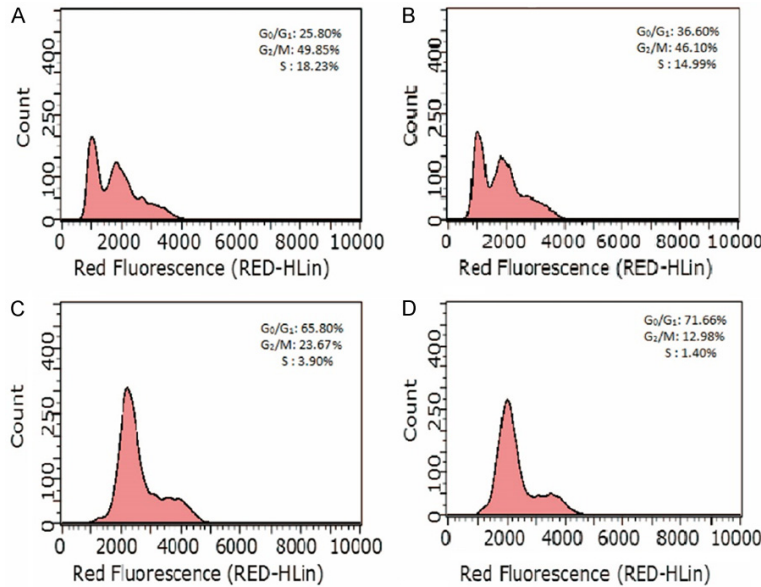


Figure 5. GY II treatment contributes to cell arrest of Hep-2 cells. At 24-hours post-treatment, cells were stained with PI and cellular DNA content was quantified by flow cytometry. The percentages of G₀/G₁, G₂/M, and S phase are presented. (A) Control group (0%); (B) 25% GY II-containing serum group; (C) 50% GY II-containing serum group; (D) 100% GY II-containing serum group.

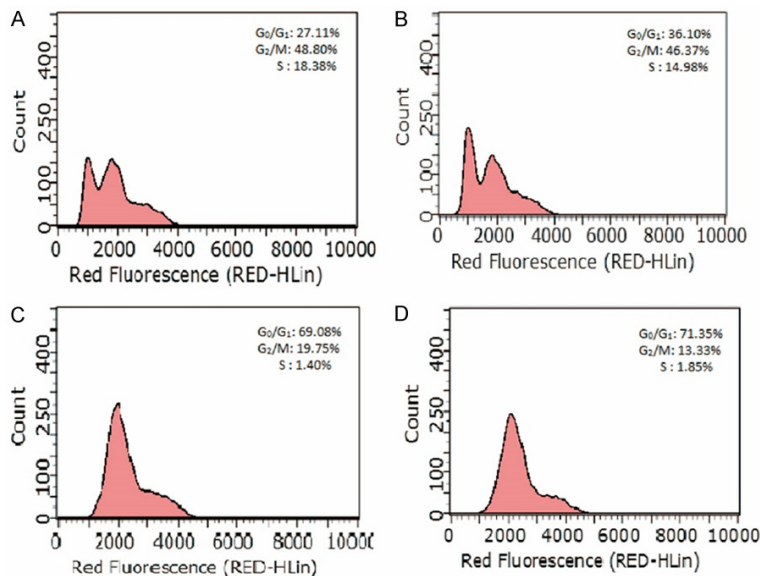


Figure 6. GY II treatment contributes to cell arrest of primary laryngeal carcinoma cells. At 24-hours post-treatment, cells were stained with PI and cellular DNA content was quantified by flow cytometry. The percentages of G₀/G₁, G₂/M, and S phase are presented. (A) Control group (0%); (B) 25% GY II-containing serum group; (C) 50% GY II-containing serum group; (D) 100% GY II-containing serum group.

II-containing serum was 75% (**Figure 1** and **Table 1**). Results showed a 55.3% inhibitory rate and 69.57 mg/ml IC₅₀ when concentration

of added GY II-containing serum was 75% in Hep-2 cells (**Figure 1** and **Table 1**).

GY II prevented laryngeal carcinoma cell migration and invasion

Invasion and metastasis are important features of tumors. Therefore, the effects of GY II on invasion and metastasis of laryngeal carcinoma cells and Hep-2 cells were further analyzed using Transwell migration assays. After the addition of GY II in the serum, the number of cells passing through the filter membrane in the lower chamber was reduced in both Hep-2 cells and primary laryngeal carcinoma cells. Results were displayed in a dose-dependent manner (**Figure 2A-C**). Moreover, invasion assays were performed. Results were consistent with those of the migration assay (**Figure 2B-D**). Current results indicate that GY II addition was able to inhibit cell migration and invasion.

GY II promoted Hep-2 cell and primary laryngeal carcinoma cell apoptosis

To determine the effects of GY II on apoptosis of Hep-2 cells and primary laryngeal carcinoma cells, apoptotic cells were stained with Annexin V-fluorescein isothiocyanate (FITC). The apoptosis rate of the GY II-containing group was significantly higher than that of the control group in Hep-2 cells (**Figure 3** and **Table 2**). Results were consistent in primary laryngeal carcinoma cells (**Figure 4** and **Table 2**). Present evidence indicates that

the addition of GY II could induce apoptosis of human laryngeal carcinoma Hep-2 cell lines and primary laryngeal carcinoma cells.

Table 3. Effects of serum containing GY II on Hep-2 and primary laryngeal carcinoma cell cycle (N=3 $\bar{X} \pm S$)

		G0/G1	S	G2/M	PI
Primary laryngeal carcinoma	0% (Control)	27.11±2.07	48.80±1.89	18.38±0.47	80.50%
	25%	36.10±1.70*	46.37±3.45	14.98±1.71	84.63%
	50%	69.08±2.11*	19.75±1.91*	1.40±2.33*	98.45%
	100%	71.35±2.14*	13.33±1.02*	1.85±1.27*	97.86%
Hep-2 cells	0% (Control)	25.80±2.87	49.85±2.23	18.23±1.03	80.58%
	25%	36.60±2.30*	46.10±3.23	14.99±1.34	84.66%
	50%	65.80±2.21*	23.67±2.98*	3.90±2.23*	95.82%
	100%	71.66±3.72*	12.98±2.11*	1.40±3.12*	98.37%

Compared with the control group, * $P < 0.05$.

GY II affected Hep-2 and primary laryngeal carcinoma cell cycle

To further investigate the mechanisms of GY II in inhibiting the proliferation of cancer cells, cell cycles were further detected via flow cytometry. Intriguingly, the addition of GY II-containing serum exhibited a great influence on cell cycle. GY II treatment dramatically caused G0/G1 phase arrest in Hep-2 and primary laryngeal carcinoma cells (**Figures 5, 6 and Table 3**). Present results suggest that GY II treatment could inhibit cell proliferation via arresting tumor cells at the G0/G1 phase.

Discussion

According to Traditional Chinese Medicine, malignant tumors are in the category of “accumulation”, in which the onset is caused by a deficiency of the vital-Qi [10]. The imbalance of Yin and Yang Qi, further developed from pathological products, such as stagnation of Qi and blood stasis, phlegm, and poison accumulate, are latent in vivo. In the view of TCM, common approaches applied in the treatment of malignant tumors, such as chemotherapy and radiation, can damage health and elevate deficiencies of Qi and blood stasis [11, 12]. Fuzheng Peiben therapy in clinical treatment of TCM has been often utilized to promote blood circulation, aiming to remove blood stasis. This will eventually mitigate the side effects of radiotherapy and chemotherapy and lengthen the patient's cycle of treatment [13]. Based on the principles of supplementing Qi, activating blood circulation, and detoxification, GY II is a critical component of a long-term and widely used prescription for treatment of Qi deficiencies, blood stasis, and toxin accumulation in cancer patients [14].

Guben Yiliu is rich in Astragalus, angelica, big-head atractylodes rhizome, Chinese yam, Poria, and suberect. Yam can tonify the kidneys to arrest spontaneous emission. Chinese Angelica, prepared rehmannia root, and Caulis Spatholobi can nourish blood and nourish yin [15]. Chinese angelica is the most useful component for supplementation of the blood. It can complement the blood without leaving blood stasis, invigorating the circulation of blood at the same time. Zedoary turmeric, trigone, and Corydalis can relieve pain. Notably, modern pharmacology studies have confirmed that curcuma zedoary, angelica, and Astragalus mongholicus can improve human immunity, even killing tumor cells [16].

Although TCM has been used for thousands of years, the underlying mechanisms are not well-understood. Certain herbs are able to inhibit the migration and invasion of cancer cells in vitro, while others trigger apoptosis in cancer cells [17]. In previous studies, the combination of chemotherapy and GY II displayed good short- and long-term effects, including improved quality of life for patients receiving chemotherapy via reducing the toxic and side effects of chemotherapy. Furthermore, it impaired the occurrence of Qi deficiency and blood stasis syndrome [18]. The current study shows that GY II provides certain inhibitory effects on the migration and invasion of the primary laryngeal cancer cells and Hep-2 cells. Indeed, the inhibitory effects of GY II were displayed in a dose-dependent manner. When the concentration of GY II is 75%, the inhibition rate of high dose GY II (75%) is 54.4% (IC_{50} =67.59 mg/mL) of primary laryngeal cancer cells and 55.3% (IC_{50} =69.57 mg/mL) of Hep-2 cells.

Regulating cell apoptosis and/or cell cycle has become an important strategy for treatment of malignant tumors. Most TCM herbs have been shown to exhibit effects on cell apoptosis [19, 20]. Current results indicate that GY II can induce cell apoptosis, with effects displayed in a dose-dependent manner.

Overall, the current study demonstrates that GY II inhibits cancer cell proliferation via arresting the cell cycle. Cell invasion and migration abilities are also inhibited by GY II. Moreover, GY II can significantly induce cell apoptosis. Current findings have significant implications regarding understanding the mechanisms and targets of TCM herb GY II in terms of curing tumors.

Acknowledgements

This work was supported by the National Science Foundation of China (NO. 81473499).

Disclosure of conflict of interest

None.

Address correspondence to: Jugao Fang, Department of Otorhinolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, No.1 Dong Jiao Min Xiang, Eastern District, Beijing 100730, China. Tel: 86-01058269206; E-mail: fangjg19651110@163.com

References

- [1] Jones TM, De M, Foran B, Harrington K and Mortimore S. Laryngeal cancer: United Kingdom national multidisciplinary guidelines. *J Laryngol Otol* 2016; 130: S75-S82.
- [2] Klatka J, Grywalska E, Klatka M, Wasiak M, Andrzejczak A and Rolinski J. Expression of selected regulatory molecules on the CD83+ monocyte-derived dendritic cells generated from patients with laryngeal cancer and their clinical significance. *Eur Arch Otorhinolaryngol* 2013; 270: 2683-2693.
- [3] Jin, Huang WJ, Meng X, Yang F, Zheng YJ, Bao Q, Zhang MZ, Yang YN, Ni Q, Lian FM and Tong XL. Chinese herbal medicine TangBi formula treatment of patients with type 2 diabetic distal symmetric polyneuropathy disease: study protocol for a randomized controlled trial. *Trials* 2017; 18: 631.
- [4] Aldini R, Budriesi R, Roda G, Micucci M, Ioan P, D'Errico-Grigioni A, Sartini A, Guidetti E, Marocchi M, Cevenini M, Rosini F, Montagnani M, Chiarini A and Mazzella G. Curcuma longa extract exerts a myorelaxant effect on the ileum and colon in a mouse experimental colitis model, independent of the anti-inflammatory effect. *PLoS One* 2012; 7: e44650.
- [5] Hong SL, Lee GS, Syed Abdul Rahman SN, Ahmed Hamdi OA, Awang K, Aznam Nugroho N and Abd Malek SN. Essential oil content of the rhizome of *Curcuma purpurascens* Bl. (Temu Tis) and its antiproliferative effect on selected human carcinoma cell lines. *ScientificWorldJournal* 2014; 2014: 397430.
- [6] Kang N, Cao SJ, Zhou Y, He H, Tashiro S, Onodera S, Qiu F and Ikejima T. Inhibition of caspase-9 by oridonin, a diterpenoid isolated from *Rabdosia rubescens*, augments apoptosis in human laryngeal cancer cells. *Int J Oncol* 2015; 47: 2045-2056.
- [7] Shi Z, Song T, Wan Y, Xie J, Yan Y, Shi K, Du Y and Shang L. A systematic review and meta-analysis of traditional insect Chinese medicines combined chemotherapy for non-surgical hepatocellular carcinoma therapy. *Sci Rep* 2017; 7: 4355.
- [8] Jiao L, Dong C, Liu J, Chen Z, Zhang L, Xu J, Shen X, Che J, Yang Y, Huang H, Li H, Sun J, Jiang Y, Mao Z, Chen P, Gong Y, Jin X and Xu L. Effects of Chinese medicine as adjunct medication for adjuvant chemotherapy treatments of non-small cell lung cancer patients. *Sci Rep* 2017; 7: 46524.
- [9] Zhang Y, Zhang GL, Sun X, Cao KX, Shang YW, Gong MX, Ma C, Nan N, Li JP, Yu MW, Yang GW and Wang XM. Gubenyliliu II inhibits breast tumor growth and metastasis associated with decreased heparanase expression and phosphorylation of ERK and AKT pathways. *Molecules* 2017; 22.
- [10] Wang N, Feng Y, Cheung F, Wang X, Zhang Z and Feng Y. A Chinese medicine formula Gegen Qinlian decoction suppresses expansion of human renal carcinoma with inhibition of matrix metalloproteinase-2. *Integr Cancer Ther* 2015; 14: 75-85.
- [11] Huang ST, Pang JH and Yang RC. Anti-cancer effects of *phyllanthus urinaria* and relevant mechanisms. *Chang Gung Med J* 2010; 33: 477-487.
- [12] Tian Y, Liu SY and Jiang WJ. The effects of Qizhenfuzheng medical soup combined with the Chemotherapies on the treatment of non-small cell lung cancer patients after operation. *Chinese Journal of Surgery of Integrated Traditional & Western Medicine* 2016.
- [13] Shufeng AN, Shen Y and Zhang Y. Clinical Observation on JianPi YiShen Tang combined with NP regimen in treating advanced non-small cell lung cancer. *Western Journal of Traditional Chinese Medicine* 2016.

- [14] Zhang GL, Wang XM and Ping LI. Inhibitory effect of Guben Yiliu Formula II and its combinative effect with chemotherapeutants on mouse Lewis lung carcinoma. *Journal of Beijing University of Traditional Chinese Medicine* 2007.
- [15] Lin PC, Chiou TW and Harn HJ. An evidence-based perspective of angelica sinensis (Chinese Angelica) for cancer patients. Springer Netherlands, 2011.
- [16] Liu Y, Roy SS, Nebie RH, Zhang Y and Nair MG. Functional food quality of curcuma caesia, curcuma zedoaria and curcuma aeruginosa endemic to Northeastern India. *Plant Foods Hum Nutr* 2013; 68: 72-77.
- [17] Lin YR, Wu MY, Chiang JH, Yen HR and Yang ST. The utilization of traditional Chinese medicine in patients with dysfunctional uterine bleeding in Taiwan: a nationwide population-based study. *BMC Complement Altern Med* 2017; 17: 427.
- [18] Zhang Q, Chi HC and Jie YU. Clinical study of Guben Yiliu II combined with chemicals arterial perfusion for advanced hepatoma of 58 cases. *Journal of traditional Chinese Medicine* 2007.
- [19] Zendedel E, Butler AE, Atkin SL and Sahebkar A. Impact of curcumin on sirtuins: a review. *J Cell Biochem* 2018; 119: 10291-10300.
- [20] Lin L, Li L, Chen X, Zeng B and Lin T. Preliminary evaluation of the potential role of beta-elemene in reversing erlotinib-resistant human NSCLC A549/ER cells. *Oncol Lett* 2018; 16: 3380-3388.