Research Article

Prescription of *Sageretia hamosa* Brongn Relieved Goiter through Promoted Apoptosis of Thyroid Cells via miR-511-3p and PTEN/PI3K/Akt Pathway

Yang-lin Deng,1,2,3 Su Chen,4 Hong-tu Wang,5 Bo Wang,6 and Kai Xiao 1,2

1Precision Medicine Research Center and National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu 610041, China
2National Chengdu Center for Safety Evaluation of Drugs, West China Hospital, Sichuan University, Chengdu 610041, China
3Department of Pharmacy, First Affiliated Hospital of Kunming Medical University, Kunming 650032, China
4Department of Outpatient, First Affiliated Hospital of Kunming Medical University, Kunming 650032, China
5Department of Infections Disease, First Affiliated Hospital of Kunming Medical University, Kunming 650032, China
6Department of Neurosurgery, First Affiliated Hospital of Kunming Medical University, Kunming 650032, China

Correspondence should be addressed to Kai Xiao; 2017324020105@stu.scu.edu.cn

Received 16 July 2021; Revised 5 August 2021; Accepted 8 September 2021; Published 29 September 2021

Academic Editor: Enas Abdulhay

Copyright © 2021 Yang-lin Deng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Goiter is thyroid enlargement, in China, *Sageretia hamosa* Brongn (SHB) can be used to treat goiter, but it has not been reported. Therefore, data analytics of SHB prescription on thyroid were explored in this study to provide a theoretical support for SHB in the treatment of goiter. In this study, rat in goiter model was constructed by using propylthiouracil (PTU) and treated with SHB prescription. Thyroid function about the triiodothyronine (T3), free thyroxine (T4), free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured by ELISA; thyroid coefficient was calculated after weighed thyroid; and HE staining was applied to assess the morphology of thyroid tissue. miRNA microarrays were employed to detect miRNA expression in thyroid tissue of rats. Expression of miR-511-3p was measured by RT-qPCR; expression of proteins (PTEN and apoptosis-related proteins) was tested by western blotting; relationship between miR-511-3p and PTEN was investigated by dual luciferase reporter gene assay; cell viability rate was determined by CCK-8; and cell cycle distribution and apoptosis rate were detected by flow cytometry. The results showed that SHB prescription ameliorated goiter and downregulated miR-511-3p. miR-511-3p targeted PTEN in thyroid cells and PTEN negatively regulated the activation of PI3K/Akt pathway. Furthermore, the inhibition of apoptosis in thyroid cells caused by the overexpression of miR-511-3p or the activation of PI3K/Akt pathway was reversed by treatment of SHB prescription, inhibition of miR-511-3p, or overexpression of PTEN. In conclusion, SHB prescription promoted apoptosis of thyroid through decreased miR-511-3p and regulated PTEN/PI3K/Akt pathway, it might suggest possible medical applications.

1. Introduction

Goiter is thyroid enlargement, which is caused by a large number of agents in the environment or medication, some kinds of goiter with obvious hyperthyroidism or hypothyroidism [1]. Most patients present with benign disease and are euthyroid at presentation, but without effective treatment in time, and it will lead to more serious thyroid pathological changes [2]. Nowadays, the main treatments are thyroidectomy, partial resection, and single or repeated radioiodine therapy [3]. Thyroidectomy is a standard therapy for young and otherwise healthy patients, radioiodine therapy is an attractive alternative to surgery in older patients with cardiopulmonary disease, and thyroxin therapy may be tried in young patients with small diffuse goiters [4], and all have their limitations. In addition, goiter can be cured by many kinds of traditional Chinese medicine (TCM) as well [5].
In many regions of China, a lot of TCM show great curative effect on goiter [6]. For example, Xing Qi Hua Ying Tang ameliorated clinical symptoms of patients that were accompanied with reduction in the size of goiter [7]; besides, Haizao Yuhu Decoction alleviated iodine-deficient goiter via regulating thyroid hormone synthesis [8, 9]. Sageretia hamosa Brongn (SHB) is one of the TCM [10], it is known as Que Mei Teng in China, which has been used to treat many different kinds of disease among the people in Yunnan for a long time. According to folk processing methods and purposes, SHB can be used to treat goiter as well, but it has not been reported. Therefore, in this study, the function of SHB prescription on thyroid and its mechanism were explored.

The study found that the proliferation and apoptosis of thyroid cells are involved in the development of goiter [11, 12]. There was also a study that found that TCM named Kang-Jia-Wan played a therapeutic role via apoptosis induction in the goitrous glands [13]. The research found that TCM take effect through microRNA (miRNA)-target network [14, 15]. Moreover, miRNA deregulation observed in human goiter influences thyroid diseases [16]. All of those reports inspired us to focus on the miRNA that SHB regulates in thyroid cells, we used to figure out whether SHB prescription affects the proliferation and apoptosis of thyroid cells through miRNA, in order to explore a possible mechanism.

Here, we aimed to explore the underlying mechanism that SHB prescription promotes apoptosis and inhibits proliferation of thyroid cells in goiter, provided theoretical support for SHB in the treatment of goiter.

2. Materials and Methods

2.1. Construction of Rat in Goiter Model. 48 SD rats (weigh 250–300 g, half male, healthy) were randomly classified into six groups (8 rats in each group), Group 1 was the normal group without any disposal. Groups 2–6 were administered intragastrically by of propylthiouracil (PTU, #Js30643-5g; YOYOBIO, Shanghai, China) 1 mL · 100 g−1 · 1 one day for 28 days in order to induce goiter. Each rat was fed with 20 g one day and drank freely during the experiment. The behavior and hair color of rats were observed, and each of them was weighed once a week.

2.2. Prescription of Sageretia hamosa Brongn. Prescription of SHB was a compatibility agent including Sageretia hamosa Brongn (SHB), Scutellaria barbata D. Don (SBD), and Hedyotis diffusa Willd. (HDW) (purchased from Kunming traditional Chinese Medicine Factory Co., Ltd.), abbreviated as SHB prescription in this study. Soaked 30 g SHB, 10 g SBD, and 10 g HDW into 750 g water for 30 min and then boiling for 1 h, extracted twice and combined all the extracts, subsequently, filtered with 200 mesh, concentrated the filtrate, then collected the extract (55–60°C, specific gravity of 1.058–1.06), granulating and drying, respectively.

2.3. Treatment. Two days after discontinuation of construction of rat in goiter model, Group 2–6 were treatment with Levothyroxin Sodium Tablets (LST, #B14202007312; Merck KGaA, Germany) or SHB prescription for 28 d. Blank control group, rats in goiter model without any treatment; LST group, rats in goiter model treated with LST 0.07 mg kg−1 one day; light concentration of SHB prescription, rats in goiter model treated with SHB prescription in 63 mg·100 ml−1·100 g−1; moderate of SHB prescription, rats in goiter model treated with SHB prescription in 126 mg·100 ml−1·100 g−1; high concentration of SHB prescription, rats in goiter model treated with SHB prescription in 252 mg·100 ml−1·100 g−1.

2.4. Thyroid Function Observation. Serum samples of rat in each group were collected to measure the triiodothyronine (T3, # KL-E12896; Kanglang), thyroxine (T4, # KL-E1937 R; Kanglang), free triiodothyronine (FT3, # KL-E1640 R; Kanglang), free thyroxine (FT4, # KL-E1006 R; Kanglang), and thyroid-stimulating hormone (TSH, #KL16995; Kanglang) by using ELISA purchased from Shanghai Kanglang Biotechnology Co., Ltd according to the manufacturer’s instructions.

2.5. Thyroid Coefficient Calculation. Thyroid of rats in each group was taken and weighed, and the thyroid coefficient was calculated by the formula (weight of thyroid/body weight of rat) × 100%.

2.6. Hematoxylin-Eosin (HE) Staining. After weighed, thyroid of rats in each group was used for hematoxylin-eosin (HE) staining to observe morphology of thyroid. To be brief, thyroid tissues in each group were embedded in paraffin wax to prepare 5 μm wax pieces, followed by HE staining. Tissue sections were stained by using hematoxylin and eosin staining kit (#GV358430; Yiji, Shanghai, China) according to the manufacturer’s instructions. After washed for 10 min, stained slices were checked and photographed under a 200-fold light microscope.

2.7. RNA Extraction and miRNA Microarrays. Total RNA in rat thyroid sample of each group was extracted by using Trizol Total RNA Extractor (#KL-14770; Kanglang) according to the manufacturer’s instructions. Then, the extracted RNA was further purified by ammonium acetate/ethanol precipitation. Reverse transcription followed by PCR is used to create cDNA constructs. Subsequently, PCR is performed with two primers that anneal to the ends of the adapters. TruSeq Small RNA Sample Preparation Kits (#RS-122-2001; Illumina, San Diego, USA) were used according to the manufacturer’s instructions.

2.8. Cell Culture. Rat thyroid cells (FRTL-5) (#FE325; ATCC, USA) and human thyroid cells (HTori-3) (#FE744; ATCC, USA) were purchased from Shanghai Qiming Biotechnology Co., Ltd. and cultured in Dulbecco’s Modified
Eagle Medium/Nutrient Mixture F-12 (DMEM-F12, #SH30023.01; YOYOBI, Shanghai, China) containing 5% fetal bovine serum (FBS, #12657-029-OJN; Gibco, USA), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine at 37°C and 5% CO₂. When the thyroid cells reached 70–80% confluence, they were passaged in accordance with standard procedures.

2.9. Drug-Contained Serum. 30 SD rats (weigh 250–300 g, half male, healthy) were randomly divided into two groups, 15 rats which used to produce drug-contained serum were treated with SHB prescription in 252 mg, 100 ml⁻¹, 100 g⁻¹, and the other 15 rats which used to produce blank serum received the same dose of normal saline for 10 d. After the last vagalagost for 1 h, blood samples from femoral artery were collected, then coagulation for 2 h, and carefully collected the supernatant after centrifugation at 1500 rpm for 10 min, and placed in −80°C refrigerator. The samples were filtered through 0.22 μm membrane before being used, filtrates were drug-contained serum and blank serum, respectively.

2.10. Cell Transfection and Treatment. miR-511-3p mimics, miR-511-3p inhibitor, si-PTEN, and pcDNA-PTEN were designed and produced by Guangzhou Ruibo Biotechnology Co., Ltd. (China). Invitrogen™ Lipofectamine™ 2000 Transfection Reagent (#11668027; Invitrogen, USA) was used to perform FRTL-5 and Htori-3 transfection according to the manufacturer's instructions. After transfected with miR-511-3p mimics, FRTL-5 and Htori-3 cultured with 1% blank serum as blank group, and 1%, 5%, and 10% drug-contained serum as SHB prescription light, moderate, and high dose groups, respectively. According to the experiment, 50 μg/mL 740 Y–P (#TQ0003; TargetMo, USA) was used to treat FRTL-5 and Htori-3, and FRTL-5 and Htori-3 which cultured with 10% drug-contained serum or transfected with miR-511-3p inhibitor/pcDNA-PTEN for 48 h.

2.11. Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from FRTL-5 and Htori-3 in each group by using TRIzol reagent (#ZC-0021A; ZCIBIO, Shanghai, China). After reversed extracted total RNA into cDNA by using reverse transcription kit (#DXT-218061; Qiagen, USA), RT-qPCR was performed by using SYBR® Premix Ex Taq™ II (#HRR820A-1; Takara, Japan). The reaction conditions were as follows: 95°C for 7 min, 45 cycles of 95°C for 1 min, 60°C for 35 s, and 72°C for 30 s, and finally at 72°C for 2 min. The forward and reverse primers were as follows: miR-511-3p F: 5'-ACACCCATCGTGGCTTTTGC-3' and R 5'-CATTGGACCCCACCTCGTCT-3'; U6 F: 5'-CTCGCTTCGGCA GCACA-3' and R 5'-AACGCTTCAGAATTTCGGT-3'. The concentration of isolated total protein by using BCA protein quantification kit (#GV357593; Yiji), they were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), subsequently, transferred onto polyvinylidene difluoride (PVDF) membrane and blocked with 5% skim milk, then incubated with primary antibodies against Bcl-2 (1:1000, #ER1802-97; HUAIBIO, Hangzhou, China), anti-Bax (1:1000, #ER0907; HUAIBIO), anti-caspase-3 (1:1000, #ET1602-39; HUAIBIO), anti-PTEN (1:1000, #RT1519; HUAIBIO), anti-P13K (1:1000, #bs-0128R; Bioss, Beijing, China), anti-p-P13K (1:1000, #bs-5587R; Bioss), anti-Akt (1:1000, #bs-2056R; Bioss), anti-p-Akt (1:1000, #bs-5188R; Bioss), and anti-GAPDH (1:1000, #ER1901-65; HUAIBIO) overnight. The next day, the PVDF membrane was treated with secondary antibody (1:1000, #HA1024; HUAIBIO) and visualized by using SuperBrite™ ECL Western Blot Substrate/Detection Kit (#BIV-K823-200; BioVision, USA). The gray values of bands were analyzed by using ImageJ used to software.

2.12. Western Blotting. Total protein was isolated from FRTL-5 and Htori-3 in each group by using total protein extraction kit (#2140; Millipore, USA), after measuring the concentration of isolated total protein by using BCA protein quantification kit (#GV357366; Yiji), they were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), subsequently, transferred onto polyvinylidene difluoride (PVDF) membrane and blocked with 5% skim milk, then incubated with primary antibodies against Bcl-2 (1:1000, #ER1802-97; HUAIBIO, Hangzhou, China), anti-Bax (1:1000, #ER0907; HUAIBIO), anti-caspase-3 (1:1000, #ET1602-39; HUAIBIO), anti-PTEN (1:1000, #RT1519; HUAIBIO), anti-P13K (1:1000, #bs-0128R; Bioss, Beijing, China), anti-p-P13K (1:1000, #bs-5587R; Bioss), anti-Akt (1:1000, #bs-2056R; Bioss), anti-p-Akt (1:1000, #bs-5188R; Bioss), and anti-GAPDH (1:1000, #ER1901-65; HUAIBIO) overnight. The next day, the PVDF membrane was treated with secondary antibody (1:1000, #HA1024; HUAIBIO) and visualized by using SuperBrite™ ECL Western Blot Substrate/Detection Kit (#BIV-K823-200; BioVision, USA). The gray values of bands were analyzed by using ImageJ used to software.

2.13. Cell Count Kit-8 (CCK-8). Cell Count Kit-8 (CCK-8, #BA00208; Bioss) was used to detect cell viability of FRTL-5 and Htori-3 in each group according to the manufacturer's instructions. Then, the absorbance value was detected at a wavelength of 450 nm by using microplate reader.

2.14. Flow Cytometry. Flow cytometry was employed to measure cell cycle distribution and cell apoptosis rate. In order to measured cell cycle distribution, FRTL-5 and Htori-3 were treated with 50 mg/mL propidium iodide (PI) and RNase at 37°C for 30 min and then analyzed by flow cytometry. In order to measure cell apoptosis rate, FRTL-5 and Htori-3 were treated with 10 μL Annexin-V–Fluorescein Isothiocyanate (FITC) and PI at 4°C for 30 min in the dark, and analyzed by flow cytometry.

2.15. Dual Luciferase Reporter Genes Gene Assay. Dual luciferase reporter gene assay kit (#GV357366; Yiji) was used to verify relationship between miR-511-3p and PTEN. In brief, the 3’-Untranslated Regions (UTR) of PTEN wild type (WT) or mutant type (MUT) was cloned into the pGLO luciferase vector and transfected them with or without miR-511-3p mimics into 293T by using Invitrogen™ Lipofectamine™ 2000 Transfection Reagent. After 48 h, the luciferase activity was detected by using dual luciferase reporter assay system.

2.16. Statistical Analysis. Data were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed by using a Student’s t-test between two groups and one-way analysis of variance (ANOVA) among groups with GraphPad Prism 7.0 software. P values < 0.05 were considered statistically significant.
3. Results

3.1. Effect of SHB Prescription on Rat in Goiter Model. To verify the function of SHB prescription in goiter rat, first, the goiter model rat was established by using PTU, after PTU applied for 28 d, rats showed rough fur, loose skin, and slow reaction, and as Table 1 showed, the body weight of rat in the goiter group is less than the normal group. Then, we treated goiter rat with light, moderate, and high concentration of SHB prescription, and the LST treatment was the positive medicinal control group, after treatment for 28 d, their body weight increased compared with goiter rat without any treatment (Table 1); besides, the rats showed smooth fur, tight skin, and faster reaction. Besides, both SHB prescription and LST treatment reduced the thyroid coefficient (P < 0.05) (Table 2 and Figure 1(a)) and improved thyroid function (P < 0.05; Figure 1(b)). Moreover, HE staining results also indicated that high concentration of SHB prescription promoted morphology of thyroid tissue return to normal as LST done (Figure 1(c)). These results suggested that SHB prescription ameliorated goiter in concentration-dependent manner.

3.2. Regulation of SHB Prescription on Expression of miRNAs in Goiter Rat. In order to further explore the underlying mechanism of therapeutic effect of SHB prescription on goiter, we employed miRNA microarrays to detect the relative miRNA expression levels in thyroid tissue of rats in the above experimental groups. From this analysis, there were many differentials expressed miRNAs in the normal, goiter, LST, and SHB prescription groups (Figure 2(a)). We identified the top 10 miRNAs whose expression was upregulated in the goiter group compared with normal and decreased with LST and SHB prescription treatment, besides there were also the top 10 miRNAs whose expression was downregulated in the goiter group, and increased after LST and SHB prescription treatment (Table 3 and Figure 2(b)). Finally, miR-511-3p was chose for further experiments. As a first step in confirming the validity of the array data, we performed RT-qPCR on miR-511-3p, as shown in Figure 2(c) (P < 0.05), these it was overexpressed in the goiter group compared with the normal group, while its expression decreased after LST or SHB prescription treatment, and this was statistically significant, consistent with the microarray experiments. Therefore, we speculated that one of the ways that SHB prescription might improve goiter was through down-regulation of miR-511-3p.

3.3. SHB Prescription Effected Proliferation and Apoptosis of Thyroid Cells through miR-511-3p. miRNAs participate in the regulation of cell physiological process and can be regulated by TCM, based on the miRNA microarrays results, miR-511-3p mimics was transfected into thyroid cell lines FRTL-5 and Htori-3, after treated with SHB prescription in different concentration, cell cycle, viability, and apoptosis ability were measured. First, RT-qPCR results showed that compared with the normal and the blank group, expression of miR-511-3p significantly increased in miR-511-3p mimics group, while it decreased with the increase of SHB prescription concentration (P < 0.05; Figure 3(a)). Moreover, flow cytometry, CCK-8, and western blotting results indicated that the overexpression of miR-511-3p accelerated cell cycle, promoted proliferation, and inhibited apoptosis of FRTL-5 and Htori-3, but SHB prescription reversed it in concentration-dependent manner (P < 0.05; Figures 3(b)–3(e)). Hence, SHB prescription suppressed proliferation and promoted apoptosis of thyroid cells as observations in more cells in G1 phase and less cells in G2/M phase through downregulated expression of miR-511-3p.

3.4. Relationship between miR-511-3p, PTEN, and PI3K/Akt Pathway. The target genes of miR-511-3p have predicted by using bioinformatics. PTEN aroused our great interest, and the binding sites are shown in Figure 4(a). In order to confirm the relationship between miR-511-3p and PTEN, a dual luciferase reporter gene assay was carried out, and the results revealed that the luciferase activity of PTEN-WT was markedly reduced in miR-511-3p mimics (P < 0.05), but the luciferase activity of PTEN-MUT had no significant different between NC and miR-511-3p mimics group (P > 0.05; Figure 4(b)). Besides, western blotting indicated that the overexpression of miR-511-3p significantly reduced the expression of PTEN protein (P < 0.05; Figure 4(c)), in addition, both overexpression of miR-511-3p and knockdown of PTEN activated PI3K/Akt pathway, while overexpression of PTEN inhibited PI3K/Akt pathway and relaxed the activation of PI3K/Akt pathway caused by overexpression of miR-511-3p (P < 0.05; Figure 4(d)). These results indicated that miR-511-3p targeted PTEN and regulated PI3K/Akt pathway in thyroid cells.

3.5. SHB Prescription Effected Proliferation and Apoptosis of Thyroid Cells through Regulated miR-511-3p/PTEN/PI3K/Akt Pathway. Aimed to clarify whether SHB prescription regulated proliferation and apoptosis of thyroid cells through miR-511-3p/PTEN/PI3K/Akt pathway, we first activated PI3K/Akt pathway by using 740 Y–P and then treated with SHB prescription, transfected miR-511-3p inhibitor or pcDNA-PTEN into FRTL-5 and Htori-3 and then measured the expression of protein related to PI3K/Akt pathway (P < 0.05; Figure 5(a)) and apoptosis (P < 0.05; Figure 5(e)) by western blotting, detected cell viability by CCK-8 (P < 0.05; Figure 5(c)), and measured cell cycle (P < 0.05; Figure 5(b)) and apoptosis rate (P < 0.05; Figure 5(d)) by flow cytometry. The results showed that 740 Y–P activated PI3K/Akt pathway in thyroid cells, enhanced cell viability ability, and suppressed cell apoptosis through increased cell in G2/M phase. However, treatment of SHB prescription, inhibition of miR-511-3p, or overexpression of PTEN reverted these functions. These results indicated that SHB prescription inhibited proliferation and promoted apoptosis of thyroid cells through regulated miR-511-3p/PTEN/PI3K/Akt pathway.
4. Discussion

In this study, we found that rat in goiter model could be cured by SHB prescription, and then we explored the molecular mechanism via which SHB prescription promotes apoptosis of thyroid cells. We confirmed that SHB prescription induced downregulation of miR-511-3p and promoted apoptosis of thyroid cells. We suggested that SHB prescription effectively inhibited proliferation and promoted apoptosis of thyroid cells by arresting the cell cycle at G_1 phase. As a disease with thyroid enlargement, abnormal proliferation and apoptosis of thyroid cell exist in goiter. Thus, promoting apoptosis and inhibiting proliferation of thyroid cells are an effective method for the treatment of goiter. Therefore, our work further demonstrated that SHB prescription was an ideal technique for the treatment of goiter.

TCMs have been widely used against a broad spectrum of biological activities, and a kind of the underlying mechanism is the regulation of miRNA. Such as downregulation of miR-294 by Baicalin was its key mechanism of action in decreasing embryonic stem cells proliferation [27]. In our study, the overexpression of miR-511-3p in thyroid cells significantly stimulated cell proliferation and restrained cell apoptosis, while SHB prescription reversed it through downregulated expression of miR-511-3p in concentration-dependent manners. Although there was some study reported miR-511-3p involved in regulation of inflammation or progression of cancer [28], it is seldom reported miR-511-3p play a role in thyroid disease. Our study has demonstrated that miR-511-3p promoted proliferation, effected cell cycle, and inhibited apoptosis of thyroid cells, it might a prospective marker of goiter, while the newest research indicated that miR-511-3p is involved in cell cycle, proliferation, and metastasis through regulated AKT3 [29].

Table 1: Weight changes of rats (\(\bar{x} \pm s, n = 8, \text{g}\)).

| Treatment | Concentration | Goiter 1 d | Goiter 14 d | Goiter 28 d | Treatment 28 d |
|-----------|---------------|------------|------------|------------|----------------|
| Normal    | —             | 188.58 ± 13.5 | 231.67 ± 21.5 | 248.67 ± 27.4 | 297.75 ± 48.4 |
| Blank     | —             | 200.75 ± 22.7 | 229.67 ± 30.7 | 224.67 ± 30.0* | 270.42 ± 38.9# |
| LST       | Light         | 201.56 ± 13.7 | 224.33 ± 24.5 | 235.83 ± 27.6* | 287.00 ± 48.7# |
| Goiter    | SHB prescription | Light | 200.75 ± 16.3 | 236.17 ± 34.4 | 227.25 ± 36.8* | 276.17 ± 57.4# |
|           | Moderate      | 200.42 ± 15.9 | 235.33 ± 26.4 | 228.83 ± 27.2* | 277.00 ± 41.5# |
|           | High          | 200.75 ± 19.5 | 230.17 ± 16.8 | 228.33 ± 16.7* | 280.42 ± 40.4# |

* \(P < 0.05\) vs. normal group; *\(P < 0.05\) vs. Goiter 28 d group.

Table 2: The weight of thyroid and body of rat after treatment (\(\bar{x} \pm s, n = 8\)).

| Treatment | Concentration | Body weight (g) | Thyroid weight (g) | Thyroid coefficient (%) |
|-----------|---------------|-----------------|-------------------|------------------------|
| Normal    | —             | 300.88 ± 49.6   | 0.0496 ± 0.00596  | 0.0169 ± 0.00362       |
| Blank     | —             | 281.25 ± 44.2   | 0.0848 ± 0.01694  | 0.0306 ± 0.00631**     |
| LST       | Light         | 287.00 ± 48.79  | 0.0592 ± 0.0087   | 0.0207 ± 0.00187***### |
| Goiter    | SHB prescription | Light | 258.25 ± 45.86   | 0.0681 ± 0.01069 | 0.0268 ± 0.00409**ΔΔ |
|           | Moderate      | 269.75 ± 41.55  | 0.0495 ± 0.00751  | 0.0184 ± 0.00183###Δ   |
|           | High          | 280.25 ± 46.63  | 0.0522 ± 0.00864  | 0.0192 ± 0.00472#      |

* \(P < 0.05\) vs. normal group; **\(P < 0.001\) vs. normal group; ###\(P < 0.01\) vs. blank group; #Δ\(P < 0.001\) vs. blank group; #Δ\(P < 0.05\) vs. Goiter + LST group; ΔΔ\(P < 0.01\) vs. Goiter + LST group.
Figure 1: Continued.
miRNAs take part in the regulation of cell physiological processes through binding its target genes. In our study, miR-511-3p targeting and downregulated the expression of phosphatase and tensin homolog deleted on chromosome ten (PTEN), which is a phosphatase, regulated cell cycle, migration, growth, DNA repair, and survival signaling [30]. It has been found that PTEN plays an important role in thyroid function and disease, and the loss of PTEN resulted in a
Table 3: Selected significantly different miRNAs in SHB prescription treatment compared to other groups.

| miRNA        | Regulation | Fold change | P value |
|--------------|------------|-------------|---------|
| miR-378      | Down       | −2.45       | 0.003   |
| miR-30a-3p   | Down       | −0.58       | 0.014   |
| miR-511-3p   | Down       | −0.25       | 0.008   |
| miR-223-3p   | Down       | −0.35       | 0.021   |
| miR-760      | Down       | −0.63       | 0.023   |
| miR-6412     | Down       | −0.31       | 0.025   |
| miR-322-3p   | Down       | −0.34       | 0.028   |
| miR-582      | Down       | −0.76       | 0.022   |
| miR-345      | Down       | −0.97       | 0.039   |
| miR-130a-5p  | Down       | −1.48       | 0.004   |
| Mir-1246-3p  | Up         | 1.11        | 0.016   |
| miR-2478     | Up         | 2.05        | 0.023   |
| Let-7a-5p    | Up         | 0.27        | 0.039   |
| miR-129-5p   | Up         | 1.51        | 0.005   |
| miR-350-3p   | Up         | 0.74        | 0.014   |
| miR-125a     | Up         | 1.12        | 0.014   |
| miR-690      | Up         | 2.70        | 0.014   |
| miR-217-5p   | Up         | 1.92        | 0.020   |
| miR-409-3p   | Up         | 0.73        | 0.021   |
| miR-433-3p   | Up         | 1.10        | 0.023   |

Figure 3: Continued.
Figure 3: SHB prescription effected proliferation and apoptosis of thyroid cells through miR-511-3p. (a) Expression of miR-511-3p measured by RT-qPCT. (b) Cell cycle distribution of FRTL-5 and Htori-3 measured by flow cytometry. (c) Cell viability rate of FRTL-5 and Htori-3 measured by CCK-8. (d) Cell apoptosis rate of FRTL-5 and Htori-3 measured by flow cytometry. (e) Expression of apoptosis-related proteins Bcl-2, Bax, and caspase-3 measured by western blotting.

Figure 4: Continued.
Figure 4: Relationship between miR-511-3p, PTEN, and PI3K/Akt pathway. (a) Binding sites between miR-511-3p and PTEN. (b) Relationship between miR-511-3p and PTEN verified by dual luciferase reporter gene assay. **P < 0.01 vs. NC group. (c) Expression of PTEN protein measured by western blotting. **P < 0.01 vs. NC group. (d) Expression of protein in PI3K/Akt pathway. *P < 0.05 vs. NC group; **P < 0.01 vs. NC group; ##P < 0.01 vs. miR-511-3p mimics group; ΔΔP < 0.01 vs. OE-PTEN group.

Figure 5: Continued.
significant increase in the thyrocyte proliferative index [31]. PTEN serves as a negative regulator of phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway, which is an important regulator of many cellular events, including apoptosis, proliferation, and motility [32]. Our results fund that the activation of PI3K/Akt signaling pathway enhanced proliferation and inhibited apoptosis of thyroid cells, while SHB prescription treatment, miR-511-3p inhibition, or PTEN overexpression reversed these effects. Interestingly, PTEN is a tumor suppressor gene mutated in many human cancers [33], and the loss of PTEN function leads to activation of PI3K/Akt signaling pathway and is strongly associated with progression of cancer [34]. Therefore, our result might suggest that SHB prescription not only help to treat goiter but also may have the effect of preventing thyroid cancer.

In summary, our results showed that were found to cure rat in goiter model and downregulated miR-511-3p in thyroid tissues and cells in concentration-dependent

Figure 5: SHB prescription effected proliferation and apoptosis of thyroid cells through regulated miR-511-3p/PTEN/PI3K/Akt pathway. (a) Protein expression of PTEN measured by western blotting. (b) Cell cycle distribution of FRTL-5 and Htori-3 measured by flow cytometry. (c) Cell viability rate of FRTL-5 and Htori-3 measured by CCK-8. (d) Cell apoptosis rate of FRTL-5 and Htori-3 measured by flow cytometry. (e) Expression of apoptosis-related proteins Bcl-2, Bax, and caspase-3 measured by western blotting.
manner. Besides, there was a target relationship between miR-511-3p and PTEN in thyroid cells, and PTEN negatively regulated the activation of PI3K/Akt signaling pathway. Furthermore, the inhibition of apoptosis in thyroid cells caused by overexpression of miR-511-3p or activation of PI3K/Akt signaling pathway was reversed by treatment of SHB prescription, inhibition of miR-511-3p, or over-expression of PTEN. Therefore, our results indicated that SHB prescription promoted apoptosis of thyroid through decreased miR-511-3p and regulated PTEN/PI3K/Akt pathway.

The greatest regret of this study is that not enough and complete data of SHB prescription treated goiter patient were collected. Besides, the function of miR-511-3p and PTEN/PI3K/Akt pathway in the treatment of SHB prescription in goiter rat are performing now. Importantly, our study confirmed that SHB prescription not only help to treat goiter but also may prevent thyroid cancer; thus, research on SHB is warranted.

5. Conclusion

This study demonstrated that SHB prescription relieved goiter through inhibited proliferation and promoted apoptosis of thyroid cells via downregulated miR-511-3p and PTEN/PI3K/Akt pathway.

Abbreviations

SHB: Sageretia hamosa brongn
PTU: Propylthiouracil
T3: Triiodothyronine
T4: Free thyroxine
FT3: Free triiodothyronine
FT4: Free thyroxine
TSH: Thyroid-stimulating hormone
TCM: Traditional Chinese medicine
miRNA: MicroRNA
LST: Levothyroxin sodium tablets
HE: Hematoxylin-eosin
RT: Reverse transcription
qPCR: chain reaction
CCK-8: Cell Count Kit-8
FITC: Fluorescein isothiocyanate
UTR: Untranslated regions
WT: Wild type
MUT: Mutant type
PTEN: Phosphatase and tensin homolog deleted on chromosome ten
PI3K: Phosphatidylinositol-3-kinase
SBD: Scutellaria barbata D. Don
HDW: Hedyaotis diffusa willd.

Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Yang-lin Deng and Su Chen contributed equally to this work.

Acknowledgments

This work was supported in part by the National Natural Science Foundation of China (Grant no. 41964007).

References

[1] J.-L. Wémeau, “Prise en charge thérapeutique des goitres,” La Presse Médicale, vol. 40, no. 12, pp. 1147–1154, 2011.
[2] L. J. Holland and S. W. J. Lamberts, “Somatostatin receptors and disease: role of receptor subtypes,” Baillière’s Clinical Endocrinology and Metabolism, vol. 10, no. 1, pp. 163–176, 1996.
[3] P. Bel Lassen, A. Kyrill, M. Lytrivi, and B. Corvillain, “Graves’ disease, multinodular goiter and subclinical hyperthyroidism,” Annales d’Endocrinologie, vol. 80, no. 4, pp. 240–249, 2019.
[4] G. W. Randolph, J. J. Shin, H. C. Grillo et al., “The surgical management of goiter: part II. surgical treatment and results,” The Laryngoscope, vol. 121, no. 1, pp. 68–76, 2011.
[5] H. Luo, M. Lù, X. Pei, and Z. Xia, “Chinese herbal medicine for subacute thyroiditis: a systematic review of randomized controlled trials,” Journal of Traditional Chinese Medicine, vol. 34, no. 3, pp. 243–253, 2014.
[6] L. Liu, M. Heinrich, S. Myers, and S. A. Dworjanyn, “Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: a phytochemical and pharmacological review,” Journal of Ethnopharmacology, vol. 142, no. 3, pp. 591–619, 2012.
[7] M. L. Yang and B. Lu, “Treatment of goiter with traditional Chinese medicine regimen xing Qi Hua ying Tang: a clinical study on 72 patients with multinodular and diffuse goiter,” Journal of Alternative and Complementary Medicine (New York, N.Y.), vol. 24, no. 1, pp. 374–377, 2018.
[8] Y. Zhang, Y. Li, X. Mao et al., “Thyroid hormone synthesis: a potential target of a Chinese herbal formula Haizao Yuhu Decoction acting on iiodine-deficient goiter,” Oncotarget, vol. 7, no. 32, pp. 51699–51712, 2016.
[9] D. Liu, F. Chen, X. Yu et al., “Do different species of sargassum in Haizao Yuhu decoction cause different effects in a rat goiter model?” Evidence-Based Complementary and Alternative Medicine, vol. 2019, Article ID 5645620, 19 pages, 2019.
[10] T. K. Hyun, S. C. Song, C.-K. Song, and J.-S. Kim, “Nutritional and nutraceutical characteristics of Sageretia theezans fruit,” Journal of Food and Drug Analysis, vol. 23, no. 4, pp. 742–749, 2015.
[11] J. M. Riesco, J. A. Juanes, J. Carretero et al., “Cell proliferation and apoptosis of thyroid follicular cells are involved in the involution of experimental non-tumoral hyperplastic goiter,” Anatomy and Embryology, vol. 198, no. 6, pp. 439–450, 1998.
[12] H. Undeutsch, C. Löf, P. Pakarinen, M. Poutanen, and J. Kero, “Thyrocye-specific Dicer1 deficiency alters thyroid follicular organization and prevents goiter development,” Endocrinology, vol. 156, no. 4, pp. 1590–1601, 2015.
[13] Y. Han, L. Gao, N. Sun et al., “Effects of Kang-Jia-Wan, a Chinese medicinal herb official, on apoptosis induction in goiter of rats,” *Journal of Ethnopharmacology*, vol. 122, no. 3, pp. 533–540, 2009.

[14] Y. Liu, M. Wang, Y. Luo et al., “MiRNA-target network analysis identifies potential biomarkers for traditional Chinese medicine (TCM) syndrome development evaluation in hepatitis B caused liver cirrhosis,” *Scientific Reports*, vol. 7, no. 1, p. 11054, 2017.

[15] Y.-P. Wang, X.-Q. Fu, C.-L. Yin et al., “A traditional Chinese medicine formula inhibits tumor growth in mice and regulates the miR-34b/c-Met/β-catenin pathway,” *Journal of Ethnopharmacology*, vol. 260, Article ID 113065, 2020.

[16] C. S. Fuziwara and E. T. Kimura, “MicroRNAs in thyroid development, function and tumorigenesis,” *Molecular and Cellular Endocrinology*, vol. 456, pp. 44–50, 2017.

[17] H. Yu, W. Zhang, C. Shen et al., “Liver dysfunction induced by Levothyroxine Sodium Tablets (Euthyrox) in a hypothyroid patient with Hashimoto's thyroiditis: case report and literature review,” *Endocrine Journal*, vol. 66, no. 9, pp. 769–775, 2019.

[18] A. F. Hlaihel and M. Z. H. Al-Khairalla, “Levothyroxine-induced liver injury followed by complete recovery upon cessation of the drug: a case report,” *Journal of Medical Case Reports*, vol. 13, no. 1, p. 311, 2019.

[19] S.-K. Chung, Y.-C. Kim, Y. Takaya, K. Terashima, and M. Niwa, “Novel flavonol glycoside, 7-O-methyl mearnsitrin, from Sageretia theezans and its antioxidant effect,” *Journal of Agricultural and Food Chemistry*, vol. 52, no. 15, pp. 4664–4668, 2004.

[20] S.-K. Chung, C.-Y. O. Chen, and J. B. Blumberg, “Flavonoid-rich fraction from Sageretia theezans leaves scavenges reactive oxygen radical species and increases the resistance of low-density lipoprotein to oxidation,” *Journal of Medicinal Food*, vol. 12, no. 6, pp. 1310–1315, 2009.

[21] L. Wang, W. Chen, M. Li, F. Zhang, K. Chen, and W. Chen, “A review of the ethnopharmacology, phytochemistry, pharmacology, and quality control of Scutellaria barbata D. Don,” *Journal of Ethnopharmacology*, vol. 254, Article ID 112260, 2020.

[22] Y. Wang, B. Li, and X. Zhang, “Expression of Concern: Scutellaria barbata D. Don (SBD) protects oxygen glucose deprivation/reperfusion-induced injuries of PC12 cells by up-regulating Nrf2,” *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 48, no. 1, p. 715, 2020.

[23] X. Xu, F. Chen, L. Zhang et al., “Exploring the mechanisms of anti-ovarian cancer of Hedyotis diffusa Willd and Scutellaria barbata D. Don through focal adhesion pathway,” *Journal of Ethnopharmacology*, vol. 279, Article ID 114343, 2021.

[24] X. Han, X. Zhang, Q. Wang, L. Wang, and S. Yu, “Antitumor potential of Hedyotis diffusa Willd: a systematic review of bioactive constituents and underlying molecular mechanisms,” *Biomedicine & Pharmacotherapy*, vol. 130, Article ID 113065, 2020.

[25] J. Wazir, R. Ullah, P. Khongorzuul et al., “The effectiveness of Hedyotis diffusa Willd extract in a mouse model of experimental autoimmune prostatitis,” *Andrologia*, vol. 53, no. 1, Article ID e13913, 2021.

[26] C. Y. Wang, T. C. Wang, W. M. Liang et al., “Effect of Chinese herbal medicine therapy on overall and cancer related mortality in patients with advanced nasopharyngeal carcinoma in Taiwan,” *Frontiers in Pharmacology*, vol. 11, Article ID 607413, 2020.

[27] J. Wang, J. Masika, J. Zhou et al., “Traditional Chinese medicine baicalin suppresses mesenchymal stem cell (mESCs) proliferation through inhibition of miR-294 expression,” *Cellular Physiology and Biochemistry*, vol. 35, no. 5, pp. 1868–1876, 2015.

[28] D. C. Do, J. Mu, X. Ke et al., “mir-511-3p protects against cockroach allergen-induced lung inflammation by antagonizing CCL2,” *JCI Insight*, vol. 4, Article ID e126832, 2019.

[29] J. Ma, L. Zhang, A. Shang et al., “LINC02163 promotes colorectal cancer progression via mir-511-3p/AKT3 axis,” *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 48, no. 1, pp. 961–968, 2020.

[30] B. D. Hopkins, C. Hodakoski, D. Barrows, S. M. Mense, and R. E. Parsons, “PTEN function: the long and the short of it,” *Trends in Biochemical Sciences*, vol. 39, no. 4, pp. 183–190, 2014.

[31] N. Yeager, A. Klein-Szanto, S. Kimura, and A. Di Cristofano, “Pten loss in the mouse thyroid causes goiter and follicular adenomas: insights into thyroid function and Cowden disease pathogenesis,” *Cancer Research*, vol. 67, no. 3, pp. 959–966, 2007.

[32] T. Jamaspishvili, D. M. Berman, A. E. Ross et al., “Clinical implications of PTEN loss in prostate cancer,” *Nature Reviews Urology*, vol. 15, no. 4, pp. 222–234, 2018.

[33] C.-Y. Chen, J. Chen, L. He, and B. L. Stiles, “PTEN: tumor suppressor and metabolic regulator,” *Frontiers in Endocrinology*, vol. 9, p. 338, 2018.

[34] V. Álvarez-Garcia, Y. Tawil, H. M. Wise, and N. R. Leslie, “Mechanisms of PTEN loss in cancer: it's all about diversity,” *Seminars in Cancer Biology*, vol. 59, pp. 66–79, 2019.