Shanxian granule-containing serum mediated inhibition of hepatic oval cell EMT and malignant transformation via the Wnt/β-catenin signaling pathway

mengyang qu¹, yanfang pan¹, yangqian yang¹, xinmao yang¹, yan fang¹, xiaoping ying¹, meiqian zhang¹, and jing wei¹

¹Affiliation not available

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Abstract

Precancerous lesions of the liver are the intermediate stage in the development of liver cancer from cirrhosis. Our previous studies indicated that Shanxian granules (SXG) can alleviate abnormal proliferation of liver cells and the formation of cirrhosis. To explore the mechanism of SXG-containing serum in reversing epithelial-mesenchymal transition (EMT) and malignant transformation of hepatic oval cells (HOC). The malignant transformation and EMT of rat HOC cell line WB-F344 were induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Subsequently, WB-F344 cells treated with MNNG were treated with with three doses of SXG to observe its inhibitory effect. The Wnt signaling pathway was blocked by adding Wnt activator CP21R7 on the basis of SXG high dose group to observe whether the inhibitory effect was regulated by Wnt/β-catenin signaling pathway. SXG-containing serum inhibited MNNG-stimulated proliferation, migration and invasion of WB-F344 cells in a time- and dose-dependent manner. In MNNG-stimulated WB-F344 cells, SXG-containing serum regulated the expression of tumor-related indicators and EMT-related proteins. The results showed that SXG-containing serum reversed WB-F344 EMT and malignant transformation through Wnt/β-catenin signaling pathway, which provides a scientific basis for the clinical application of SXG.
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Abstract
Hepatocellular carcinoma (HCC) is a malignant tumor with late detection and poor prognosis. Epithelial-mesenchymal transition (EMT) of hepatic oval cells (HOC) is a key step in malignant transformation of precancerous lesions. Shanxian granules (SXG) has the effect of inhibiting abnormal proliferation of liver cells and the formation of cirrhosis. To explore the mechanism of SXG-containing serum in reversing EMT and malignant transformation of HOC. The malignant transformation and EMT of rat HOC cell line WB-F344 were induced by N-methyl-N'-nitro-N-nitroso (MNNG). Subsequently, WB-F344 cells treated with MNNG were treated with SXG low, medium and high dose groups to observe its inhibitory effect. The Wnt signaling pathway was blocked by adding Wnt activator CP21R7 on the basis of SXG high dose group to observe whether the inhibitory effect was regulated by Wnt/β-catenin signaling pathway. The proliferation ability of cells was detected by MTT, the migration ability of cells was detected by Wound healing assay, and the expression levels of EMT-related proteins E-cadherin, N-cadherin, Vimentin, cleaved Caspase-3, P53 and CD34 and the expression levels of Wnt/β-catenin signaling pathway related marker proteins wnt3a, β-catenin and C-myc were detected by Western Blot. SXG-containing serum inhibited MNNG-stimulated proliferation, migration and invasion of WB-F344 cells in a time- and dose-dependent manner. In MNNG-stimulated WB-F344 cells, SXG-containing serum increased the expression of tumor-related indicators and EMT-related proteins E-cadherin, cleaved Caspase-3, and P53 proteins, and decreased the expression of N-cadherin, Vimentin, CD34 proteins. SXG-containing serum also inhibited the expression of wnt3a, β-catenin and C-myc. In addition, after CP21R7 activated Wnt/β-catenin signaling pathway, compared with SXG high dose group, the expression of E-cadherin, cleaved Caspase-3 and P53 protein decreased and other indicators increased significantly. The results showed that SXG-containing serum reversed WB-F344 epithelial-mesenchymal transition and malignant transformation through Wnt/β-catenin signaling pathway. It is suggested that it has potential clinical application value in the prevention of hepatocellular carcinoma.

KEY WORDS
Shanxian granule-containing serum, epithelial mesenchymal transition, malignant transformation, precancerous lesions of liver, hepatic oval cells, wnt/β-catenin signaling pathway

1 INTRODUCTION
Hepatocellular carcinoma (HCC) is the main pathological type of primary liver cancer with a high degree of malignancy and a dangerous prognosis. According to the latest global cancer burden data, hepatocellular carcinoma is one of the sixth most common malignant tumors in the world, and its fatality rate ranks third among tumor diseases in the world. The pathogenesis of HCC is mainly related to various factors such as viral hepatitis, alcoholic liver disease, fatty liver disease, and liver cirrhosis. The liver cancer microenvironment, that is, the surrounding tissue of tumor, including a complex mixture of extracellular matrix and non-HCC cells, may contribute to the occurrence and progression of liver cancer. The study found that HCC is unique in that it has a well-defined background of chronic liver damage. In the process of malignant transformation of liver cirrhosis-liver cancer, it will experience a relatively long period, that is, the period of precancerous lesions. The precancerous lesion of the liver is the intermediate stage from benign lesion to canceration, closely related to HCC, and may develop into liver cancer if treatment is not timely.

Recent studies have found that the cytological origin of HCC is the abnormal differentiation of undifferentiated stem cells or oval cells in the liver. Hepatic oval cells (HOC) is an endogenous stem cell in the
liver, which has the ability of self-proliferation, self-renewal and multi-directional differentiation. It can differentiate into mature bile duct epithelial cells and hepatocytes to repair liver regeneration, but under the influence of carcinogenic factors, it can also develop into precancerous cells. This stage is “Precancerous changes” stage. In a specific environment, atypical hyperplasia occurs and has the characteristics of cancer cells in its morphological properties, ultrastructure, enzymology, surface markers, etc., and further develops into nodular lesions, and eventually develops into HCC.

Studies have shown that epithelial-mesenchymal transition (EMT) is an important process in tumor progression and metastasis, and plays an important role in liver precancerous lesions. EMT and malignant transition of HOC play an important role in the occurrence and development of hepatocellular carcinoma. Under the action of different microenvironments, the differentiation direction of HOC is different, and the adverse microenvironment will cause the balance between the EMT of HOC and the transformation of mesenchymal epithelium to be broken, which makes it increase the susceptibility to carcinogens, the normal cell cycle is inhibited, accelerating the malignant transformation of HOC, thereby developing into hepatoma cells. It is confirmed that Wnt/β-catenin signaling pathway regulates the occurrence and development of various cancers including liver cancer. It has been found that Wnt/β-catenin signaling pathway inhibits EMT and malignant transformation in hepatic oval cells. The Wnt/β-catenin signaling pathway stabilizes β-catenin by inhibiting GSK3β, making it translocate to the nucleus to bind to the transcription factor TCF/LEF, and promote the expression of genes related to EMT and malignant transformation.

Shanxian granules (SXG) is a compound granule of Traditional Chinese Medicine (TCM) that is composed of American ginseng, turtle shell, turtle shell, Agrimony, Curcuma, Yuanhu, Hawthorn, Polyporus, Coix Seed. TCM believes that SXG can inhibit the abnormal proliferation of liver cells and the formation of liver cirrhosis. However, it is not clear whether SXG through Wnt/β-catenin signaling pathway inhibits EMT and malignant transformation of hepatic oval cells, thereby delaying or blocking the occurrence and development of precancerous lesions of liver cancer. The molecular pharmacological mechanism of SXG in protecting liver needs to be clarified. Therefore, in the present study, serum pharmacological methods were used to explore the effects of SXG on HOCs and the molecular changes that are related to the Wnt/β-catenin signaling pathway.

2 | MATERIALS AND METHODS

2.1 | Reagents

The rat HOC cell line WB-F344 was purchased from Wuhan Boster Biological Engineering Co., Ltd. SXG was purchased from Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine. DMEM and FBS from Beijing Soleibao Biotechnology Co., Ltd. N-methyl-N’-nitro-N-nitrosamine (MNNG) was purchased from Sigma-Aldrich (Merck KGaA). CP21R7 was purchased from Selleck. MTT was purchased from Thermo Fisher Scientific, Inc. Crystal violet staining solution was purchased from Sigma, USA. Rabbit anti-rat primary antibodies targeted against Beta-Actin(cat.no.BM3873), E-cadherin(cat.no.BM4166), N-cadherin(cat.no.MA01577), Vimentin(cat.no.PB9359), P53(cat.no.BM4309), CD34(cat.no.BM4082) and horseradish peroxidase-labeled goat anti-rabbit secondary antibodies were purchased from Wuhan Boster Biotechnology Co., Ltd. cleaved Caspase-3(cat.no.ab214430), Wnt3a(cat.no.ab219412), β-catenin(cat.no.ab2302), C-myc(cat.no.ab32072) purchased from Abcam, USA. BCA protein quantitative test kit was purchased from Beijing Boaosen Biotechnology Co., Ltd. The enhanced RIPA cracking solution was purchased from Wuhan Boster Biotechnology Co., Ltd. Ultrasensitive ECL chemiluminescence substrate and SDS-PAGE gel preparation kit were purchased from Wuhan Boster Biotechnology Co., Ltd. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.

2.2 | Preparation of SXG-containing drug serum

A total of 20 male Sprague-Dawley rats weighing 180±10g were adapted to the new experimental environment for 5 days and received humanized care based on the Guide for the Care and Use of Laboratory Animals
of Institutional Animal Care and Use Committee of Shaanxi Provincial Hospital of Traditional Chinese Medicine. The housing conditions were as follows: temperature 20-25°C, relative humidity 40-70%, 12-hour light-dark cycle, fixed water supply system, feeding 3 times a day. SXG was formulated into a 4.06 g/ml stock solution with 0.9% NaCl. The rats were intragastrically administered at a dose of 1 ml/100g twice a day for 7 days. On the 7th day, the rats were fasted for 12 hours before administration, and 1 hour after administration, the rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital. After successful anesthesia, 2-5 ml blood was collected from each rat by puncturing the abdominal aorta, placed at room temperature for 0.5-1 h, then centrifuged at 3000 r/min for 15 min, the supernatant was aspirated, sterilized in a water bath at 56°C for 30 min, and filtered with a microporous filter. The bacteria were removed by microporous filter (0.22 µm), repackaged, and stored at -80°C.

2.3 | Cell culture and SXG administration

The NCI-HOC cell line WB-F344 was cultured in DMEM high-glucose medium containing 10% fetal bovine serum at 37°C and 5% carbon dioxide. Change the culture medium every 2-3 days. The cells were divided into control group, model group, SXG-containing serum high-dose group (SXG-H), SXG-containing serum medium-dose group (SXG-M), SXG-containing serum low-dose group (SXG-L) and Wnt Activator CP21R7 group (SXG + CP21R7). In addition to the control group, the other groups were treated with 3μg/ml MNNG for 24h to induce a malignant transformation model, and then continuously stimulated with 7×10^-7 mol/L H_2O_2 for 12h to induce EMT formation. The cells in the control group were treated with the same amount of DMEM. Then, the original SXG-containing drug serum was applied to the SXG-H group, half of the concentration of the original SXG-containing drug serum was applied to the SXG-M group and a quarter of the concentration of the original SXG-containing drug serum was applied to the SXG-L group; FBS was used to prepare the half and one-quarter concentrations of the drug-containing serum. The cells in the control group and the model group were treated with DMEM. The dosage of each group was 20% of the culture medium. The cells were harvested after 24 h and 48 h of SXG treatment for subsequent experiments.

2.4 | MTT assay for cell proliferation

The proliferative ability of WB-F344 cells was detected by means of MTT assay. Cells were seeded in 96-well plates (2000 cells per well). After treatment with SXG-containing serum for 24h, 48h and 72h, respectively, and the 96-well plate was taken out. Add 10ul of MTT (Sigma) to each well and incubate in a cell incubator for 4 h. After the incubation, the medium was discarded, 150 ul DMSO was added to each well, and the crystal was shaken at a low speed on a shaker for 10 min to fully dissolve the crystal. Measure the absorbance (OD value) of each well at 490 nm of the microplate reader. According to the OD values measured at different time points, the experimental results were statistically analyzed.

2.5 | Wound Healing Assays

Cells were seeded in 6-well plates (1×10^5 cells/well) and grown to approximately 80% confluence, then wounded with 200 µL pipette tip at approximately 1.5 mm intervals and washed twice to remove detached cells and debris. After treatment with SXG-containing serum for 0h, 24h, and 48h, cell images and scratches were taken using a phase-contrast Olympus microscope. Throughout experiments, the same visual field was used. The gap lengths were measured by Image-Pro Plus software.

2.6 | Transwell invasion assay

Transwell assay (Costar, USA) was used to determine cell invasion capacities of WB-F344 cells. Cells were seeded at a density of 1×10^6 cells/well in 12-well plates. After treatment with SXG-containing serum for 24 h and 48 h, the cells in 500 mL serum-free medium were seeded onto the upper chamber, coated with growth factor-reduced Matrigel, and DMEM medium containing 10% FBS was added into the lower chamber as a chemoattractant. After further incubation, the cells on the surface of the membrane were removed, the invading cells were fixed with 70% ethanol and stained with 0.1% crystal violet. Cells were counted in five different fields using light microscopy at 200 magnification. The data were expressed as the mean of cells in
five fields based on three independent experiments.

2.7 | Western blotting assay

The cells were washed twice with pre-cooled PBS and lysed in RIPA lysate for 30 min. The supernatant was collected by centrifugation at 12000 r/min for 10 min at 4 degC as a total cell protein extract. Protein concentration was detected by BCA protein analysis kit. Protein samples were separated on 10% SDS-PAGE and then transferred onto polyvinylidene fluoride membranes. The membrane was incubated with 5% milk in TBST at room temperature for 1 h. Cells were incubated overnight with primary antibodies (E-cadherin 1 : 20000, N-cadherin 1 : 1000, Vimentin 1 : 1000, cleaved Caspase-3 1 : 1000, p53 1 : 1000, CD34 1 : 2000, Wnt3a 1 : 1000, β-catenin 1 : 4000, C-myc 1 : 1000, beta-actin 1 : 5000) at 4°C and washed three times with TBST. The membrane was further incubated with HRP-labeled goat anti-rabbit IgG secondary antibody (1 : 5000) at room temperature for 1 h. Display protein bands by enhanced chemiluminescence (ECL). The image gray value was calculated by ImageJ software, and the internal reference was used as the control. The ratio of the gray value of the target protein to the internal reference indicates the relative expression level of the protein.

2.8 | Statistical analysis

All statistical analyses were performed using SPSS 22.0 and graphically demonstrated using GraphPad Prism7 software (GraphPad Software, Inc.). All data were expressed as mean±standard deviation. The experiment was repeated three times. The difference between the two groups was analyzed by t-test, and the comparison between the two groups was evaluated by one-way analysis of variance (ANOVA). P<0.05 was considered statistically significant.

3 | RESULTS

3.1 | SXG-containing serum inhibited the proliferation of MNNG-stimulated WB-F344 cells.

MTT assay was used to detect the proliferation activity of WB-F344 cells in control group, model group, SXG-L group, SXG-M group, SXG-H group and Wnt Activator CP21R7 group after 24h, 48h and 72h respectively. As shown in (Figure 1), compared with the control group, the cell proliferation activity of the model group increased (P<0.05). However, the proliferation activity of WB-F344 cells decreased significantly in SXG groups compared with the model group(P<0.01), especially in SXG-H group(P<0.01). Moreover, with the increase of action time and dose, the cell proliferation activity gradually decreased. Compared with the SXG-H group, the cell proliferation activity of CP21R7 group was significantly increased (P<0.01). These results indicate that SXG-containing serum can inhibit the malignant proliferation of WB-F344 cells in a time-and dose-dependent manner.
FIGURE 1 The effect of SXG-containing serum on the proliferation activity of MNNG-stimulated WB-F344 cells. Data are expressed as mean±standard deviation. Compared with the control group, *P<0.05; compared with the model group, #P<0.05, ##P<0.01; compared with SXG-H group.

3.2 | SXG-containing serum inhibited the migration of MNNG-stimulated WB-F344 cells.

Wound healing assays was used to detect the changes of cell migration ability of WB-F344 cells in different groups after 24 h and 48 h, respectively. Compared with the control group, the cell migration ability was enhanced in the model group (P<0.05). Compared with the model group, the migration ability of WB-F344 cells was weakened in SXG groups (P<0.05). Moreover, with the increase of time and dose, the cell migration ability gradually weakened. The cell migration ability of CP21R7 group was enhanced in SXG-H group compared with the SXG-H group (P<0.01, Figure 2A,B). These results indicate that SXG-containing serum can inhibit the migration ability of WB-F344 cells in a time-and dose-dependent manner.

FIGURE 2 The effect of SXG-containing serum on the migration ability of MNNG-stimulated WB-F344 cells. (A) Wound healing assays of WB-F344 cells in 24h and 48h in different dosages of SXG. (B) The histograms summarize the effects of the different dosages of SXG on migration ability of WB-F344 cells in 24h and 48h. Data are expressed as mean±standard deviation. Compared with the Control group, *P<0.05; compared with the model group, #P<0.05, ##P<0.01; compared with SXG-H group.

3.3 | SXG-containing serum inhibited the invasion of MNNG-stimulated WB-F344 cells.

Invasion assay was used to detect the changes of invasion ability of WB-F344 cells. Compared with the control group, the number of transmembrane cells in the model group was significantly increased (P<0.05). Compared with the model group, the number of transmembrane cells in the SXG-L, SXG-M, SXG-H groups decreased significantly, especially in the SXG-H groups (P<0.05). The number of transmembrane cells decreased gradually with the increase of action time and the dose of SXG-containing serum. Compared with the SXG-H group, the number of transmembrane cells in the CP21R7 group increased significantly (P<0.01, Figure 3A,B). These results indicate that SXG-containing serum can inhibit the invasion ability of WB-F344 cells in a time-and dose-dependent manner.

FIGURE 3 The effect of SXG-containing serum on the invasion ability of MNNG-stimulated WB-F344
cells. (A) Invasion assay of WB-F344 cells in 24h and 48h in different dosages of SXG. (B) The histograms summarize the effects of the different dosages of SXG on invasion of WB-F344 cells in 24h and 48h. Data are expressed as mean±standard deviation. Compared with the Control group, *P<0.05 ; compared with the model group, #P<0.05, ##P<0.01 ; compared with SXG-H group,

3.4 | SXG-containing serum reversed the expression of tumor-related indexes and EMT-related proteins in MNNG-stimulated WB-F344 cells

As shown in (Figure 4A,B), compared with the normal group, the level of cleaved Caspase-3 and p53 protein were significantly decreased, the expression of CD34 increased in the model group (P<0.01). Compared with the model group, the expression levels of cleaved Caspase-3 and p53 protein in the SXG-L group, SXG-M group, SXG-H groups were significantly increased(P<0.05), and the expression level of CD34 protein was significantly decreased(P<0.05). Compared with SXG-H group, the expression levels of cleaved Caspase-3 and p53 protein in CP21R7 group were significantly decreased(P<0.01), and the expression level of CD34 protein was significantly increased(P<0.01). These results indicate that SXG-containing serum has a regulatory effect on malignant transformation-related proteins of MNNG-stimulated WB-F344 cells in a dose-dependent manner. Then, EMT related proteins were detected. As shown in (Figure 4C,D), compared with the control group, the expression level of E-cadherin protein decreased, and the expression levels of N-cadherin and Vimentin protein increased in the model group(P<0.01). Compared with the model group, the expression levels of E-cadherin protein in SXG-L group, SXG-M group, SXG-H group were significantly increased(P<0.05), and the expression levels of N-cadherin and Vimentin protein were significantly decreased(P<0.05). Compared with SXG-H group, the expression of E-cadherin protein in CP21R7 group was significantly decreased, and the expression of N-cadherin and Vimentin protein was significantly increased(P<0.01). These results indicate that SXG-containing serum has a regulatory effect on EMT related proteins in MNNG-stimulated WB-F344 cells in a dose-dependent manner.
FIGURE 4 Effects of SXG-containing serum on tumor-related indexes and EMT-related proteins of MNNG-stimulated WB-F344 cells after 24 h and 48 h. (A) The expression of tumor-related protein in WB-F344 cells of different groups after 24 h. (B) The effect of SXG-containing serum on tumor-related proteins of WB-F344 cells after 48 h. (C) The effect of SXG-containing serum on EMT related proteins in WB-F344 cells after 24 h. (D) The effect of SXG-containing serum on EMT related proteins in WB-F344 cells after 48 h. Data are expressed as mean±standard deviation. Compared with the Control group,*P<0.05,**P<0.01; compared with the model group,#P<0.05,##P<0.01; compared with SXG-H group.

3.5 | ΣΞΓ-ζονταινινγ σερυμ ινηβιτεδ τηε ατσιατιον ου Ωντ/β-καιτενιν σιγαλινγ πατηωαψ-ρελατεδ προτεινς ιν ΜΝΝΓ-στιμυλατεδ ΩΒ-Φ334 χελς

Ιν όρδερ το δετες ωηετερ ΣΞΓ-ζονταινινγ σερυμ μεερες τηε μαλιγναντ τρανσφορματιον ου ΩΒ-Φ344 χελς ανδ ωηετερ EMT πλαψις αντρελατορφ ρολε τηρουν τη Ωντ/β-καιτενιν signaling pathway, we added Wnt pathway activator CP21R7 to block the signaling pathway on the basis of the SXG-H. It was found that, compared with the SXG-H group, the proliferation, migration, invasion and malignant transformation of cells and the expression of EMT-related proteins were significantly changed after adding activators(P<0.01). MTT results showed that, the cell proliferation activity of Wnt activator group was significantly increased (P<0.01). The results of Wound healing assays and Transwell experiments showed that compared with the SXG-H group, the cell migration ability of Wnt activator group was enhanced (P<0.01), and the number of transmembrane cells was significantly increased (P<0.01). Western Blot results showed that the expression levels of malignant transformation and EMT-related proteins E-cadherin, cleaved Caspase-3 and p53 in Wnt activator group were significantly decreased, and the expression levels of N-cadherin, Vimentin and CD34 were significantly increased (P<0.01).
Further, Western Blot was used to detect the expression of Wnt/β-catenin signaling pathway-related proteins in WB-F344 cells treated with control group, model group, SXG-L group, SXG-M group, SXG-H group and Wnt activator CP21R7 group for 24 h and 48 h, respectively. As shown in (Figure 5A,B), compared with the control group, the expression levels of wnt3a, β-catenin and C-myc protein in the model group increased (P<0.01). Compared with the model group, the expression levels of wnt3a, β-catenin and C-myc proteins in the SXG-L group, SXG-M group, SXG-H group were significantly decreased (P<0.01). Compared with SXG-H group, the protein expression levels of wnt3a, β-catenin and C-myc in CP21R7 group were significantly increased (P<0.01). These results indicate that SXG-containing serum reversed WB-F344 malignant transformation and EMT through the Wnt/β-catenin signaling pathway.

**FIGURE 5** Effects of SXG-containing serum on Wnt/β-catenin signaling pathway related proteins after 24h and 48h. (A) The effect of SXG-containing serum on Wnt/β-catenin signaling pathway related proteins after 24h. (B) The effect of SXG-containing serum on Wnt/β-catenin signaling pathway related proteins after 48h. Data are expressed as mean±standard deviation. Compared with the Control group, *P<0.05; compared with the model group,#P<0.05,##P<0.01; compared with SXG-H group.

4 | DISCUSSION

The occurrence of liver cancer is a multi-stage process. The formation of liver cancer usually undergoes hepatitis—hepatic fibrosis—liver cirrhosis—hepatic precancerous lesions—the development of liver cancer. The precancerous lesions of liver cirrhosis to liver cancer is the intermediate stage of transition, but also a prelude to liver cancer, and hepatocellular carcinoma is closely related. Precancerous lesions of liver cancer refers to the liver can make the possibility of malignant benign liver lesions, if timely and effective prevention and treatment of precancerous lesions, inhibit its malignant transformation can reduce the occurrence of liver cancer. The main pathological features of precancerous lesions are abnormal differentiation and proliferation of HOC. As mentioned earlier, when liver cells are severely damaged, HOC with the ability to proliferate and differentiate is activated and proliferates in large quantities and further develops into atypical hyperplasia under long-term adverse environmental stimulation, further developing into cancer cells and eventually evolving into liver cancer. Rat HOC line WB-F344 cells have been widely used to establish models of liver precancerous lesions. MNNG is an experimental carcinogen. By stimulating WB-F344 cells, MNNG replicated some of the conditions that occurred during the transformation of HOC to HCC cells.

Studies have confirmed that the abnormal activity of Wnt/β-catenin signaling pathway is one of the important causes of the occurrence and development of liver cancer, which is related to the progression and metastasis.
of liver cancer.\textsuperscript{22} It has been found that Wnt/β-catenin signaling pathway is involved in the regulation of EMT and malignant transformation of WB-F344 cells. Wnt3a is an important member of the Wnt signaling pathway and plays a unique role in tumorigenesis and metastasis. It is highly expressed in hepatoma cells.\textsuperscript{23} β-catenin is a key molecule in EMT and malignant transformation of liver cancer cells, and is highly expressed in liver cancer cells.\textsuperscript{24} In the presence of Wnt ligands, Wnt ligands bind to the curled homologues and co-receptors on the cell membrane, causing the aggregation of scattered proteins, activating this pathway, GSK-3 inactivation, cytoplasmic β-catenin cannot be phosphorylated by GSK-3β, β-catenin gradually accumulates and enters the nucleus, interacts with the nuclear transcription factor T lymphocyte factor / lymphoid enhancer factor (TCF / LEF), regulates the expression of target genes such as c-myc, thereby regulating the malignant transformation of HOC and EMT\textsuperscript{25,26} further leading to the formation of liver cancer.

Chinese medicine compound granule SXG is composed of American ginseng, tortoise shell, turtle shell, agrimony, zedoary turmeric, yuanhu, raw hawthorn, polyporus, coix seed and other traditional Chinese medicines. The compatibility of the whole prescription is rigorous, and the combination of various medicines has the effects of promoting blood circulation and removing blood stasis, benefiting qi and nourishing yin, softening and eliminating disease, and strengthening the body resistance and anti-cancer. It is commonly used in the treatment of liver cirrhosis and liver cancer, and the curative effect is positive. This study further explored its mechanism and potential role.

In this study, different concentrations of SXG had different degrees of inhibition on EMT and malignant transformation of WB-F344 cells. Compared with the normal group, the cell proliferation activity and cell migration ability of the model group were enhanced, the number of transmembrane cells was significantly increased, the expression levels of E-cadherin, cleaved Caspase-3 and P53 protein were significantly decreased, and the expression levels of N-cadherin, Vimentin and CD34 protein were significantly increased. After treatment, compared with the model group, the proliferation activity and migration ability of cells in the SXG-L, SXG-M and SXG-G groups were significantly weakened, the number of transmembrane cells was significantly reduced, the expression levels of E-cadherin, cleaved Caspase-3 and P53 protein were significantly increased, and the expression levels of N-cadherin, Vimentin and CD34 protein were significantly decreased. In order to explore whether SXG-containing serum can reverse the malignant transformation and EMT of WB-F344 cells through Wnt/β-catenin signaling pathway, the Wnt pathway activator CP21R7 was added to the SXG-H to block the signaling pathway. The results showed that compared with the SXG-H, the proliferation, migration and invasion ability of cells were significantly enhanced after the addition of activator, and the expression levels of malignant transformation and EMT-related proteins E-cadherin, cleaved Caspase-3 and p53 were significantly decreased. The expression levels of N-cadherin, Vimentin and CD34 proteins were significantly increased. The Wnt/β-catenin signaling pathway-related proteins wnt3a, β-catenin, and C-myc were further detected by Western Blot. The results showed that compared with the control group, the expression levels of wnt3a, β-catenin, and C-myc proteins in the model group increased. Compared with the model group, the expression levels of wnt3a, β-catenin, and C-myc proteins in the SXG group decreased, compared with the SXG-H group. The expression levels of wnt3a, β-catenin and C-myc protein in CP21R7 group were significantly increased. These results suggest that SXG-containing serum reverses WB-F344 epithelial-mesenchymal transition and malignant transformation through the Wnt/β-catenin signaling pathway.

5 | CONCLUSION

In conclusion, the present study showed that SXG-containing serum inhibited WB-F344 epithelial-mesenchymal transition and malignant transformation through the Wnt/β-catenin signaling pathway. These findings lay a foundation for studying the mechanism of Wnt/β-catenin pathway drugs against precancerous lesions and provide a new treatment for patients with liver disease. However, due to the uncertainty of serum components containing drugs, the specific role of monomer composition cannot be determined, which may be the focus of further research.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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