Xiaoyan Decoction's Intervention on WISP2/CCN5 gene Knockdown Breast Cancer Transplanted Tumor Growth

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Research Article

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Abstract

Xiaoyan decoction, as a classical traditional Chinese medicine (TCM) formula, has been widely used for decades of years due to its safety and efficiency in tumor therapy. CCN5, also known as WISP-2(WNT-1 induced secreted protein 2) factor, plays an important role in inducing tumor cell apoptosis and inhibiting tumor proliferation. This study elucidated the mechanisms of inhibiting the *WISP2/CCN5* gene knockdown nude mice tumor growth by Xiaoyan decoction. MCF-7 cells transfected by s*h-WISP2/CCN5* were transplanted into nude mice and *WISP2/CCN5* gene knockdown nude mice model was established. Xiaoyan decoction was orally administered to *WISP2/CCN5* gene knockdown nude mice so as to intervene the growth of nude mice tumor model. As a result, the tumor volume of Xiaoyan decoction intervention group was significantly smaller,compared with *WISP2/CCN5* gene knockdown group($P = 0.032$). Besides, our study demonstrated that Xiaoyan Decoction was effective for raising the mRNA and protein levels of *WISP2/CCN5* and *p27Kip1*, reducing the *Skp2* mRNA and protein levels. Quantitative RT-PCR and western blotting were used to evaluated the mRNA and protein levels of *WISP2/CCN5, Skp2 and p27Kip1*. Increased mRNA and protein levels of *WISP2/CCN5* and *p27Kip1*, decreased *Skp2* mRNA and protein levels under the intervention by Xiaoyan decoction was found out in nude nice model. Overall, these results suggested that Xiaoyan decoction was an effective drug in inhibiting the growth of *WISP2/CCN5* gene knockdown nude mice tumor, and *WISP2/CCN5* might act as a potential anti-breast cancer target of Xiaoyan decoction.

Introduction

Breast cancer is one of the leading causes of cancer-related death in women worldwide. Exploring the molecular mechanism of the breast cancer occurrence is of great significance for the treatment of breast cancer[1–2]. Although extensive knowledge about the pathogenesis of breast cancer has been reported, the treatment of breast cancer still remains a challenge. Routine treatments, including Chemotherapy, endocrinotherapy and target therapy, are costly and side effect is significant.

Tradition Chinese medicine (TCM) has been widely applied in the therapy of breast cancer in China for thousands of years. Recently, in favor of the safety and the advantage of TCM, it has drawn more and more attentions in breast cancer treatment. Among lots of effective prescriptions, Xiaoyan decoction, which is composed of Astragalus mongholicus Bunge, radix pseudostellariae, radix curcumae, Curcuma longa L., Prunella vulgaris L, raw oyster shell, Hedyotis diffusa, is one of the most frequently applied prescriptions for breast cancer treatment. Those traditional Chinese medical materials are joined in Xiaoyan decoction and play integral cooperative role together in resolving hard lump, enhancing immunity and removing stasis.The prescription is based on removal of pathogenic and stasis, strengthening the body immunity and fighting against cancer[3]. TCM pharmacology centers on the wholeness and systematic interaction between components, targets and diseases. Although curative effect of Xiaoyan decoction has been significant, mechanistic hypotheses need to be explored. To resolve the question, the current study reveals the pharmacological mechanisms of Xiaoyan decoction by combining TCM pharmacology with in vitro experiments.

Previous studies have shown that Xiaoyan decoction suppresses the proliferation and infiltration of malignant tumor cells through [4–5]. It is supposed in traditional Chinese medicine that: hypoimmunity is one cause of cancer origin. Pathogen and blood stasis are two key factors of cancer occurrence[6]. Hence, strengthening immunity should be combined with eliminating pathogen and dissolving blood stasis. However, removal of pathogenic factors and stasis depends on strengthening immunity.

CCN5, also known as WISP-2(WNT-1 induced secreted protein 2) factor, has an critical role in inducing tumor cell apoptosis as well as restraining tumor cells multiplication[7–8]. Moreover, *WISP2/CCN5* compresses breast cancer cells proliferation through the Skp2/p27Kip1 pathway[9].

The present study was carried out in nude mice to assess the effectiveness of Xiaoyan decoction on breast cancer. Moreover, comparisons between the target genes of Xiaoyan decoction and breast cancer-related genes were conducted to reveal potential targets of Xiaoyan decoction for the treatment of breast cancer. Finally, the effects of Xiaoyan decoction on

WISP2/CCN5, Skp2 and p27Kip1 were investigated by qRT-PCR and Western blotting. Remarkably, this research was beneficial for elucidating the pharmacological mechanisms of Xiaoyan decoction.

Materials And Methods

Preparation of Xiaoyan Decoction

Xiaoyan decoction was composed of Astragalus mongholicus Bunge(30g), radix pseudostellariae(15g), radix curcumae(10g), Curcuma longa L.(15g), Prunella vulgaris L.(10g), raw oyster shell(15g), Hedyotis diffusa(10g), which were decocted by Pharmaceutical department of First Teaching Hospital of Tianjin Chinese Medicine and accredited by Dr. Xiaojiang Li. The voucher specimen(No.1806011 for Astragalus mongholicus Bunge, No.18091702 for radix pseudostellariae, No. 18051102 for radix curcumae, No.18041918 for Curcuma longa L., No.for 1803024017 Prunella vulgaris L., No.191001 for raw oyster shell, No.180501 for Hedyotis diffusa.) was deposited.

To prepare this decoction, a total of 105 g of mixed Xiaoyan decoction crude herbs were immersed in 1050 ml of distilled water for 40 minutes and then boiled and decocted for 30 minutes to yield a final concentration of 30mg/ml. Raw materials were stored at room temperature. Xiaoyan decoction preparations were stored at 4℃for the next experiment.

Establishment of WISP2/CCN5 gene knockdown MCF-7 cell line

MCF-7 cell line was purchased from KGI biotechnology co.LTD, Jiang Su, China and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin solution (Gibco, USA) in a incubator at 37°C and a humidified 5% CO_2 atmosphere. MCF-7 cell line were prepared using 0.05% trypsin solution (Invitrogen, USA) and seeded in 96 well tissue culture plates.

The lentivirus plasmid with a short hairpin RNA targeting human *WISP2/CCN5* gene, sh-*WISP2/CCN5*, was purchased from He Sheng Gene Biotechnology Co.Ltd, Beijing, China. The sequences of *sh-WISP2/CCN5* were designed according to targeting human *WISP2/CCN5* gene and it was anchored with lentivirus plasmid so as to knock down *WISP2/CCN5 gene*.

The sequence *sh-WISP2/CCN5* as follows:

5'GCCTACACACACAGCCTATATCGAAATATAGGCTGTGTGTGTFTAGTCC3';

Another short hairpin RNA sequence was designed and used as one noneffective sequence banding with null vector or negative vector, sequence as follows:

5'CCTAAGGTTAAGTCGCCCTCGCCGAAGCGAGGGCGACTTAACCTTAGG 3'

MCF-7 cell line was incubated in transfection solution consisting of *sh-WISP2/CCN5* and 5μg/ml polybrene (transfection fortifier) with purpose of establishing cell line with *WISP2/CCN5* gene knockdown. Eight hours later, DMEM culture medium took the place of transfection solution. The *sh-WISP2/CCN5* cell line, null vector cell line and control cell line were cultured and ogarithmic growth phase cells were digested and resuspended in PBS.

Establishment of WISP2/CCN5 gene knockdown nude mice tumor

Sixty specific pathogen-free nude mice(female, 4–6 weeks old, 19 ± 1g) were purchased from Si Bei Fu Biotechnology Co., Ltd., Beijing, China, and maintained in the Institute of Radiation Medicine Chinese Academy of Medical Sciences, Tianjin, China. All procedures were approved by the Ethics Committee of Tianjin University of TCM, China and conducted under the authority of the Project Licence(TCM-LAEC2019043). All mice were housed under a 12h/12h light/dark cycle and constant temperature (20 ± 2°C) and humidity (30 ± 10%) with food and water available ad labium. After 3 days of acclimatization,the nude mice were allocated randomly into four groups with 15 nude mice each group and each group were treated differently.

Throughout the trial, all mice were weighed each week, and the drug doses were adjusted accordingly.The cell density was adjusted to 2×10^6 cells/ml. Afterwards, 0.2ml of 2×10^6 cells/ml, including *sh-WISP2/CCN5*-MCF-7 cell line, null vector cell line and control cell line were injected into the nude mice in right nipple region subcutaneously. The injection was executed at 9 a.m. everyday. Tumor volume was measured by a caliper, and tumor volume was determined with the standard formula: $W^2\times L/2$. In the formula, L is the longest diameter and W is the shortest diameter[10]. When the tumor volume was up to $2mm^3$, it was considered as success of tumor model and started to execute gavage of Xiaoyan decoction .

Xiaoyan Decoction Intervention on tumor

Dosage of Xiaoyan decoction were determined based on the conversions from clinical adult dosages.The dosage of Xiaoyan decoction for adult is 140g (the total raw materials)/day, equivalently, the dosage for mice is 30g/kg/day, calculated by the formula that transforms the dosage of human into that of mouse according to the respective body surface areas in accordance with the Chinese Medicine Pharmacology Research Technology. Xiaoyan Decoction were given to nude mice by gavage administration with a concentration of 1.5g/ml and 30g drug per kilogram of nude mice weight, twice a day,one at 9 a.m. and another at 3 p.m. and last for two weeks.

Excarnation of Tumor

Approximately two weeks later, tumor-bearing mice were sacrificed. One hour before surgery, we administered analgesia to the mice by offering them with 3% pentobarbital sodium 30mg/kg by intraperitoneal injection[11]. The dosage of pentobarbital sodium was recorded. Nude mice were fixed in the dorsal position on the surgery board, skin and subcutaneous soft tissue around nipple were incised, and the tumors were extracted and reserved respectively in liquid nitrogen for quantitative RT-PCR and western blotting.

Quantitative Reverse-Transcription Polymerase Chain Reaction.

Quantitative RT-PCR was used to detect mRNA level of WISP2/CCN5,Skp2 and p27Kip1. RNA prep Pure Tissue Kit, Fast King cDNA First-strand synthesis Kit and SYBR Green PCR Kit were purchased from Tiangen Biochemical Technology (Beijing) Co., LTD, China. Total RNA was extracted from the nude mice tumor and the RNA concentration was determined. The amount of RNA in the gDNA reaction system was calculated. Then, gDNA removal reaction was carried out as follows: 5×gDNA Buffer 2μl, Total RNA, RNase-free ddH_2O supplement the reaction system to 10μl, reaction conditions: 42℃, 3minutes, placed on ice.

Reverse transcription reaction was performed to reverse RNA into cDNA. The reaction system was as follows: 10×King RT Buffer 2μl, Fast King RT Enzyme Mix 1μl, FQ-RT Primer Mix 2μl, RNase – Free ddH_2O complement reaction system to 10μl, and mixed with gDNA removal reaction system to form 20μl reaction system.

The PCR reaction system was as follows: SYBR Premix Ex Taq II (Tli RNaseH Plus) (2×) 12.5μl, Primer F (10μM) 1μl, Primer R (10μM) 1μl, cDNA 2μl, dH_2O 8.5μl, reaction system up to 25μl.

β-actin was selected as internal reference. Primer sequences of *WISP2/CCN5, Skp2* and *p27Kip1* are as follows:

Gene	Primer sequence
WISP2/CCN5	Forward 5'CTGGGCTGATGGAAGATGGT3'
	Reverse 5'TGTGTGTGTAGGCAGGGAGTG3'
Skp2	Forward 5'ATGGACCAACCATTGGCTGAA3'
	Reverse 5'ACACTGAGACAGTATGCCGTGGAG3'
p27Kip1	Forward 5'CAAATGCCGGTTCTGTGGAG3'
	Reverse 5' TCCATTCCATGAAGTCAGCGATA3'

Western blotting

Western blotting was used to detect protein level of WISP2/CCN5,Skp2 and p27Kip1.Western blotting related kits were purchased from Nanjing KGI Upgrade Technology Co., LTD,China.

After incubating MCF-7 for 48h with Xiaoyan Decoction, the cells were dissociated by standard trypsinization procedure (0.25%Trypin + 0.02%EDTA), and the cell concentration was evaluated. 1×10^7 of cells were lysed by 1ml Phenylmethanesulfonyl fluoride, and lysate solution was transferred to 1.5ml centrifuge tube, and lasted for 20–30 minutes. After centrifugation at 12000r/min and 4℃ for 15minutes, the supernatant was taken and the protein concentration was measured. The protein concentration was adjusted to 2μg/μl and stored at -80℃. Quantitative protein was heated up to 100℃ for 5 minutes, After gel electrophoresis analysis, 15% SDS-PAGE electrophoresis were turn into PVDF membrane and continuous for 90 minutes, with 1 ml primary antibody (dilution ratio: WISP2/CCN5 1:1000, Skp2 1:500, p27Kip1 1:150) at 4 ℃ overnight, with 1ml second antibody(dilution ratio:1:500) labeled by horseradish peroxidase, incubated at room temperature for 2 hours, rinsed, and luminous fluid image developed, finally imaged. *β*-actin was used as internal control.

Statistical methods

The statistical analysis was performed using the Graph Pad Prism 4 (Graph Pad Software, Inc, La Jolla, CA, USA) and $PASS^{15.0}$ software. Results are shown as mean ± S.D. Means were calculated and compared among or within groups using analysis of variance and a two-sided Student's t-test. *P* value of < 0.05 was considered as statistical significance.

Results

MCF-7 cells was transfected with sh-WISP2/CCN5 labeled by fluorescent.

Figure 1A showed the growth status of MCF-7 cell before *sh-WISP2/CCN5* was transfected, while figure 1B showed the MCF-7 cells transfected with negative vector and figure1C displayed that MCF-7 cells transfected with *sh-WISP2/CCN5* labeled by fluorescent. Compared with those of negative vector group, MCF-7 cells of *sh-WISP2/CCN5 group* got more stronger proliferation without *WISP2/CCN5* suppression.

Effect of Xiaoyan decoction intervention on the growth of WISP2/CCN5 gene knockdown nude mice tumor.

As shown in figure 2, compared with the control group and negative vectors group, the nude mice tumor volume in the *WISP2/CCN5* gene knockdown group was significantly increased ($P<0.043$), suggesting that *WISP2/CCN5* gene knockdown led to the remarkable growth of nude mice breast tumor and *WISP2/CCN5* displayed an prohibitive impact on the growth of breast cancer.

In addition, compared with tumor volume of *WISP2/CCN5* gene knockdown group, that of Xiaoyan decoction intervention group was significantly inhibited ($P<0.032$). These results indicated that Xiaoyan decoction had inhibitory effect on the growth of *WISP2/CCN5* gene knockdown transplanted tumors.

Finally, there was no significant difference in the tumor volume between the negative vector group and the control group ($P>0.05$), suggesting that the negative vectors had no intervention effect on the growth of the transplanted tumor.

Xiaoyan decoction raised the levels of WISP2/CCN5 mRNA and protein.

Compared with the negative vector group and the control group, *WISP2/CCN5 mRNA* of *WISP2/CCN5* gene knockdown group decreased ($P<0.026$ and $P<0.032$), at the same tim, while there was a significant increase in Xiaoyan decoction intervention group than that in *WISP2/CCN5* gene knockdown group (Figure 3, $P<0.023$). These results manifested that *WISP2/CCN5 mRNA* level displayed a remarkable rise in nude mice tumor with *WISP2/CCN5* gene knockdown under the interference of Xiaoyan decoction. No significant difference was observed between negative vector group and control group in *WISP2/CCN5* mRNA level(Figure 3, $P>0.05$).

Meanwhile, figure 4 displayed WISP2/CCN5 protein electrophoretic band in western blotting of four groups, including no.1 negative vector group and no.2 control group, no.3 Xiaoyan decoction intervention group and no.4 *WISP2/CCN5* gene knockdown group. WISP2/CCN5 protein electrophoretic band displayed partly deficiency .

Meanwhile, figure 5 showed that lower protein level of WISP2/CCN5 was observed in *WISP2/CCN5* gene knockdown group than those in negative vector group (P<0.021) and control group(P<0.025). Moreover, increased protein level of WISP2/CCN5 has been detected in Xiaoyan decoction intervention group than that in *WISP2/CCN5* gene knockdown group(P<0.035).

Xiaoyan decoction reduced the levels of Skp2 mRNA and protein

Compared to the negative vector group and the control group, *Skp2* mRNA level increased in *WISP2/CCN5* gene knockdown group (Figure 3, P<0.021, P<0.027). Meanwhile, compared with the negative vector group and the control group, *Skp2* protein level also rose up in *WISP2/CCN5* gene knockdown group (Figure 5, P<0.031, P<0.028). Those results indicated that *WISP2/CCN5* gene knockdown had been associated with *Skp2* increase of mRNA and protein levels.

Furthermore, compared to *WISP2/CCN5* gene knockdown group, *Skp2* mRNA level decreased in Xiaoyan intervention group(Figure 3, P<0.016). Simultaneously, lower *Skp2* protein level has been found out in Xiaoyan intervention group(Figure 5, P<0.023). The results indicated that Xiaoyan decoction promoted decrease of *Skp2* mRNA and protein levels on the condition that *WISP2/CCN5* gene knockdown. Skp2 electrophoretic bands in four groups were showed in figure 4.

Xiaoyan Decoction up-regulated the levels of p27Kip1 mRNA and protein.

The results of quantitative RT-PCR and Western blotting displayed a fall of the *p27Kip1* mRNA in the *WISP2/CCN5* gene knockdown group, which was lower than those of the negative vector group and the control group (Figure 3, P<0.039, P<0.041). In addition, a decrease of p27Kip1 protein in the *WISP2/CCN5* gene knockdown group has been observed compared with those of the negative vector group and the control group (Figure 5, P<0.017, P<0.019). It was supposed from these results that there was some relationship between *WISP2/CCN5* gene knockdown and decrease levels of p27Kip1 mRNA and protein.

While compared to *WISP2/CCN5* gene knockdown group, the *p27Kip1*mRNA level increased in Xiaoyan intervention group (Figure 3, P<0.028). Furthermore, p27Kip1 protein level was elevated in Xiaoyan decoction intervention group(Figure 5, P<0.013). These results implied that intervention of Xiaoyan decoction had some effect on increased p27Kip1 protein level. P27Kip1 electrophoretic bands in four groups were showed in figure 4.

All of the detailed data could be found out in Table 1-3.

Table 1 Effect of Xiaoyan Decoction on tumor volume of *WISP2/CCN5* Gene Knockdown Transplantation (mean±SD,mm)

Days	numbers	*WISP2/CCN5* gene knockdown group	Xiaoyan Decoction intervene group	negative vector group	control group
1	15	5.54±0.25	5.37±0.22	2.12±0.51	2.22±0.13
2	15	6.47±0.12	4.94±0.13	3.67±0.36	3.31±0.22
3	15	6.82±0.36	4.82±0.11	3.24±0.15	3.35±0.36
4	15	6.87±0.43	4.73±0.26	4.17±0.25	4.46±0.32
5	15	7.83±0.54	4.71±0.17	4.03±0.35	4.5±0.19
6	15	8.23±0.33	4.69±0.32	4.87±0.18	4.61±0.13
7	15	11.26±0.24	4.53±0.15	5.13±0.27	5.65±0.16
8	15	11.89±0.31	4.52±0.42	6.08±0.34	5.68±0.24
9	15	12.04±0.13	4.48±0.23	6.87±0.35	5.73±0.45
10	15	12.58±0.11	4.41±0.32	6.93±0.16	6.35±0.33
11	15	13.1±0.37	4.39±0.41	7.06±0.14	6.94±0.27
12	15	13.65±0.26	4.37±0.14	7.45±0.28	7.37±0.29
13	15	13.93±0.12	4.32±0.21	7.81±0.34	7.56±0.36
14	15	14.21±0.18	4.27±0.45	8.26±0.29	7.74±0.21

Table 2 Effect of Xiaoyan Decoction intervention on mRNA level of proliferation-related factors of *WISP2/CCN5* gene knockdown transplanted tumor (mean±SD,mm)

group	numbers	*WISP2/CCN5*	Skp2	P27Kip1
WISP2/CCN5 gene knockdown group	15	0.64±0.018	1.85±0.023	0.83±0.020
Xiaoyan decoction intervention group	15	1.48±0.014	0.26±0.032	1.59±0.016
negative vector group	15	1.22±0.013	0.59±0.012	1.13±0.011
control group	15	1.14±0.016	0.67±0.015	1.23±0.024

Table 3 Effect of Xiaoyan Decoction intervention on protein level of proliferation-related factors of *WISP2/CCN5* gene knockdown transplanted tumor (mean±SD,mm)

group	numbers	WISP2/CCN5	Skp2	p27Kip1
*WISP2/CCN5*gene knockdown group	15	0.51±0.017	2.05±0.024	0.36±0.028
Xiaoyan decoction intervention group	15	0.98±0.019	1.07±0.022	1.24±0.024
negative vector group	15	1.11±0.013	1.23±0.013	1.27±0.025
control group	15	1.24±0.015	1.15±0.021	1.21±0.031

Discussion

A lot of randomized controlled trials have proven that Xiaoyan decoction was an effective therapeutic medicine for malignant tumors[12–14]. Although Xiaoyan decoction is a traditional Chinese formula, but it has developed into varieties of dosage forms according to modern pharmacological research on the theory basis of enhancing immunity and killing cancer cells, dissolving pathogens and get rid of bacteria, narrowing lump and relieving mass.

Some of researchers have confirmed that Xiaoyan decoction distinctly suppressed the proliferation of lung adenocarcinoma cells and improved the life quality and prolonged the survival period of patients[15–16]. Combined with chemotherapy, it can play a synergistic effect of relieving toxic effect and improving curative effect in lung adenocarcinoma patients[17]. Especially, Xiaoyan decoction improved the bone marrow hematopoietic micro-environment and promote bone marrow hematopoiesis[18]. Another clinical research indicated that Xiaoyan decoction significantly reduced the side effects of chemotherapy in non-small cell lung cancer patients and increased patient weight and immunity [19].

Quantitative RT-PCR and Western blotting displayed that *WISP2/CCN5* mRNA and protein levels increased under the intervention of Xiaoyan decoction, moreover, affecting the expression of Skp2 and p27Kip1 in mRNA and protein expression levels. WISP2/CCN5, as a tumor suppressor gene, is also known as WNT-1 induced secreted protein 2 *(WISP2)*, which was closely related to the WNT pathway[20], for instance, *WISP2*, as a regulatory factor in the WNT pathway, plays an important role in inhibiting the progression of gastric cancer[21]. Before further studies, some scholars haven't found that Xiaoyan decoction up-regulated the expression of a series of apoptosis-related proteins by mediating WNT pathway related regulatory factors, and played a key role in inhibiting the proliferation and invasion of lung cancer cells[22]. WISP2/CCN5 inhibits the proliferation and invasion of esophageal carcinoma cells[23], further emphasizing the close relationship between *WISP2/CCN5* gene and tumor growth. Studies on the correlation between WISP2/CCN5 expression and hormone receptors in breast cancer suggest that the expression of *WISP2/CCN5* gene can be used as a monitoring gene for early breast cancer[24].

Furthermore, Skp2 and p27Kip1, as two cell cycle regulators closely related to *WISP2/CCN5* gene, are related to the occurrence and progression of breast cancer. Skp2 (S-phase kinase associated protein 2) acts as a promoter of cell cycle through ubiquitination and degradation of a variety of cell cycle suppressors[25–26], is associated with the regulation of cell proliferation and the occurrence of tumor[27–28]. The present studies have found that *WISP2/CCN5* restrains the proliferation of TNBC (triple negative breast cancer) by affecting Skp2 or p27Kip1 expression[29–30], however, the adjustment of estrogen receptor plays an important role during cell proliferation. MCF-7 cell line, as a hormone receptor-positive cell line, was used to explore the relationship of *WISP2/CCN5, Skp2* and *p27Kip1* in the present research. Therefore, the association between ER and WISP2/CCN5 would be the next research program.

Conclusions

Xiaoyan decoction influence the expression of *WISP2/CCN5* through the control of *Skp2* and *p27Kip1*, and inhibits the proliferation of transplanted breast tumor with *WISP2/CCN5* gene knockdown.

Abbreviations

WNT-1 induced secreted protein 2 WISP2

Declarations

Ethics approval and consent to participate

All procedures were approved by the Ethics Committee of Tianjin University of TCM, China and conducted under the authority of the Project license (TCM-LAEC2019043).

Consent for publication

All co-authors gave consent for publication.

Availability of data and materials

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

Competing interests

The co-authors declared no conflicts of interest.

Author's contributions

The work presented here was carried out in collaboration among all co-authors. Yan Lv conceived of the study, worked on associated data collection and their interpretation, and drafted the manuscript. Xiao Jiang Li participated in the design and coordination of the study. Chang Zhang and Shan Gao carried out the RT-PCR studies and western blotting experiment. Xu Zheng and Yan Yan Han performed the cell line culture and the statistical analyses. Chong Li collected the references about manuscripts. Qiang Geng performed the MTT assay. All authors read and approved the final manuscript.

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Figures

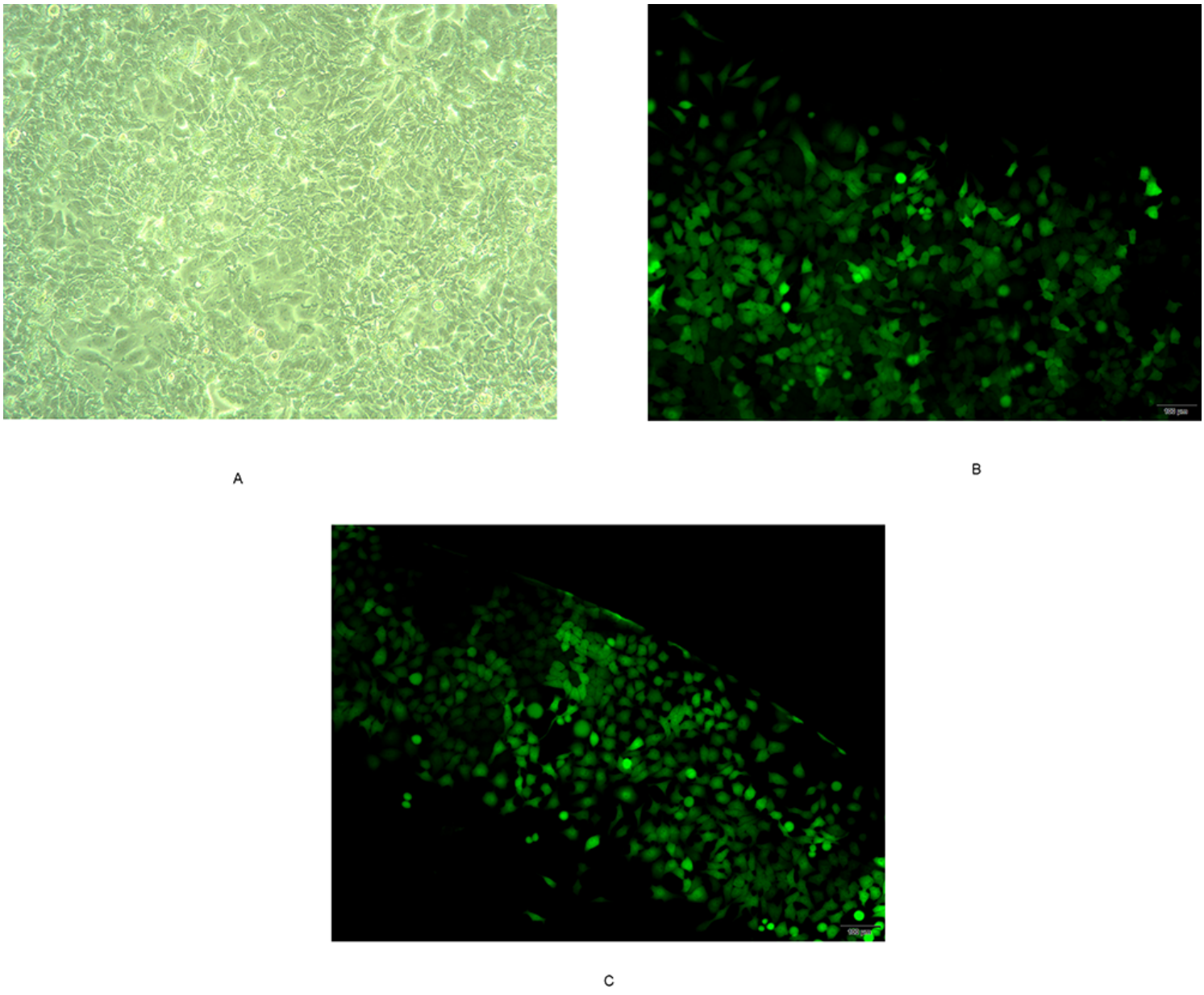

Figure 1

Legend not included with this version

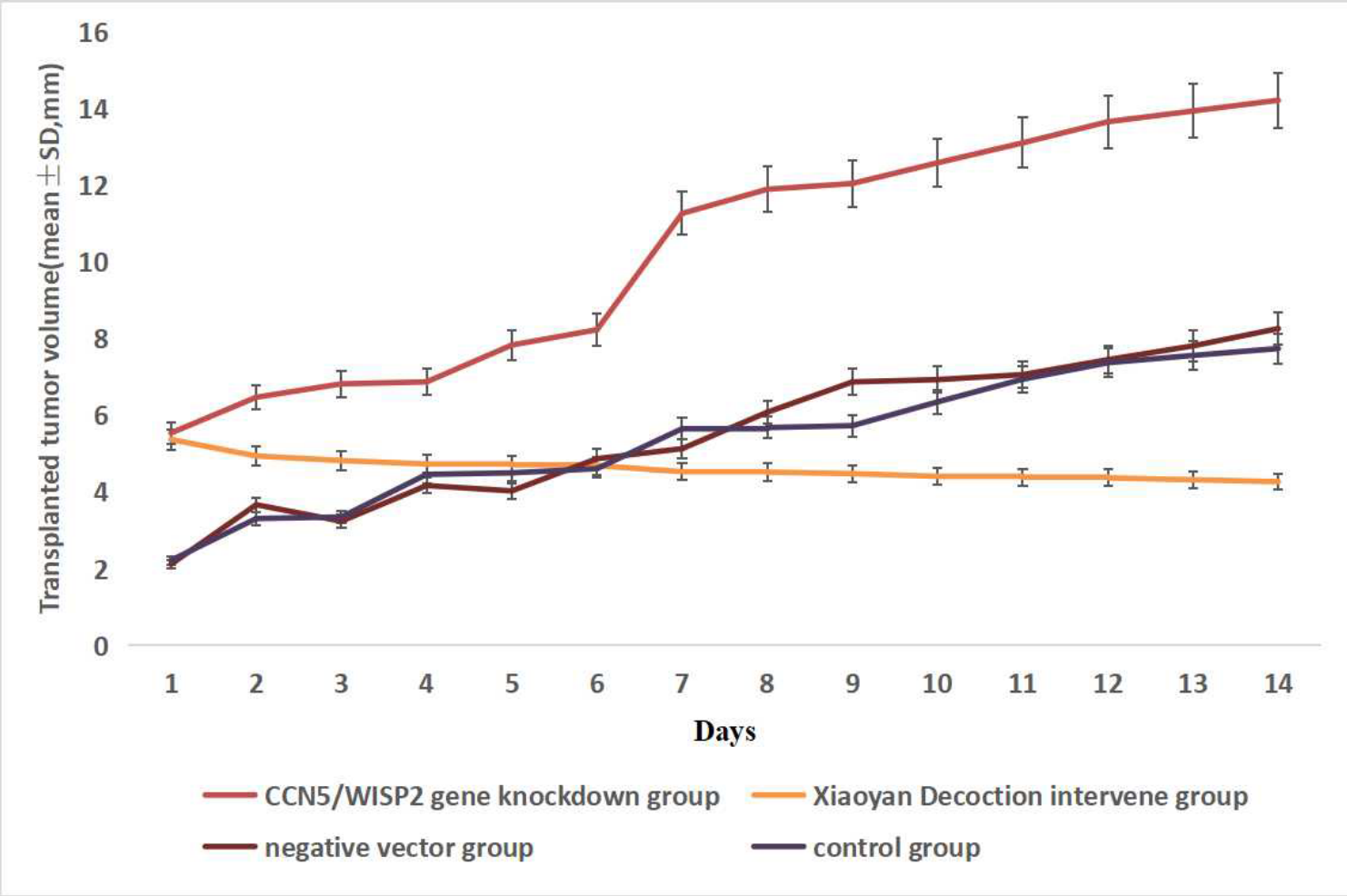

Figure 2

Xiaoyan decoction intervention on the growth of WISP2/CCN5 gene knockdown nude mice tumor

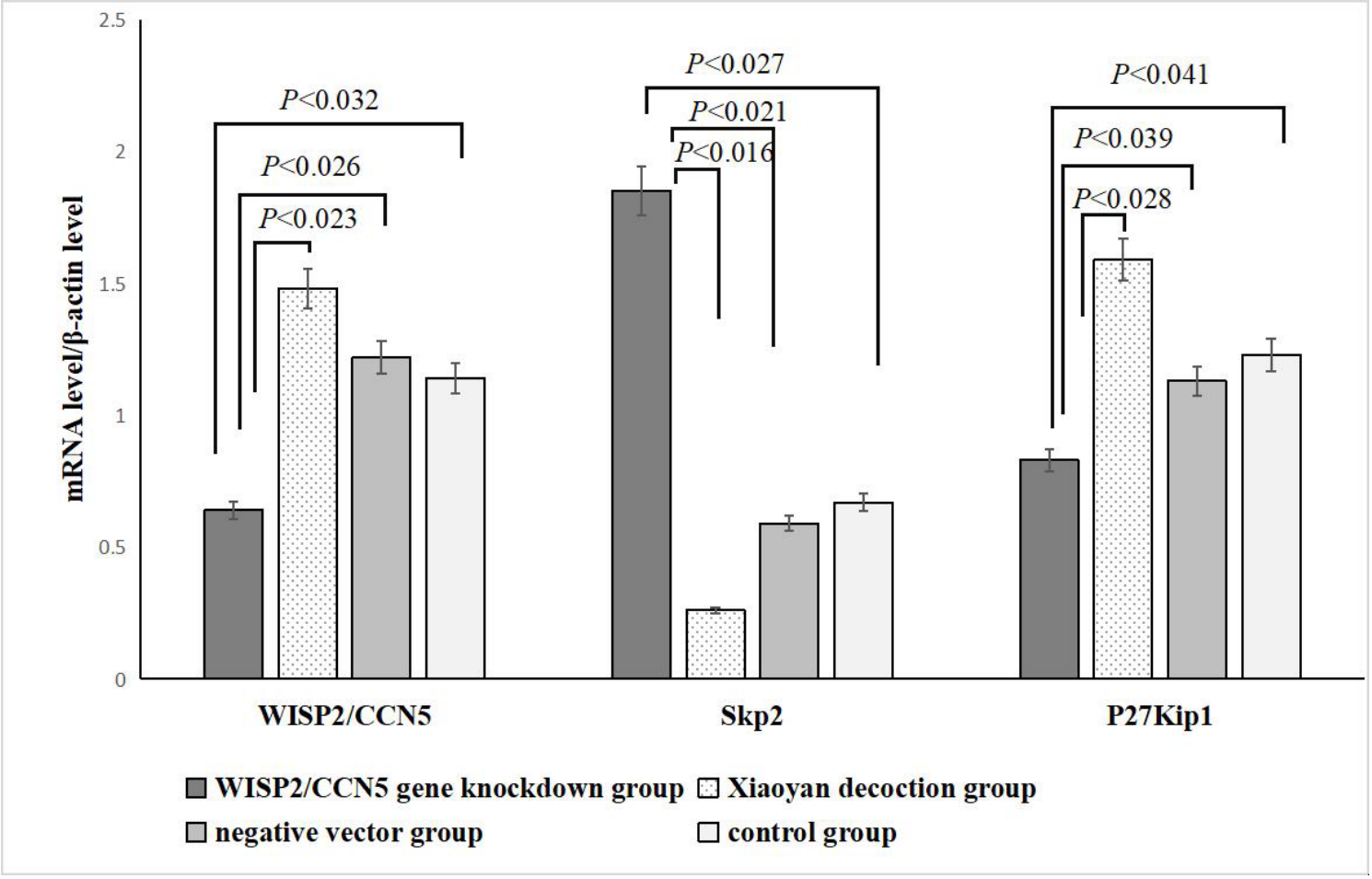

Figure 3

Xiaoyan decoction raised the levels of WISP2/CCN5 mRNA and protein.

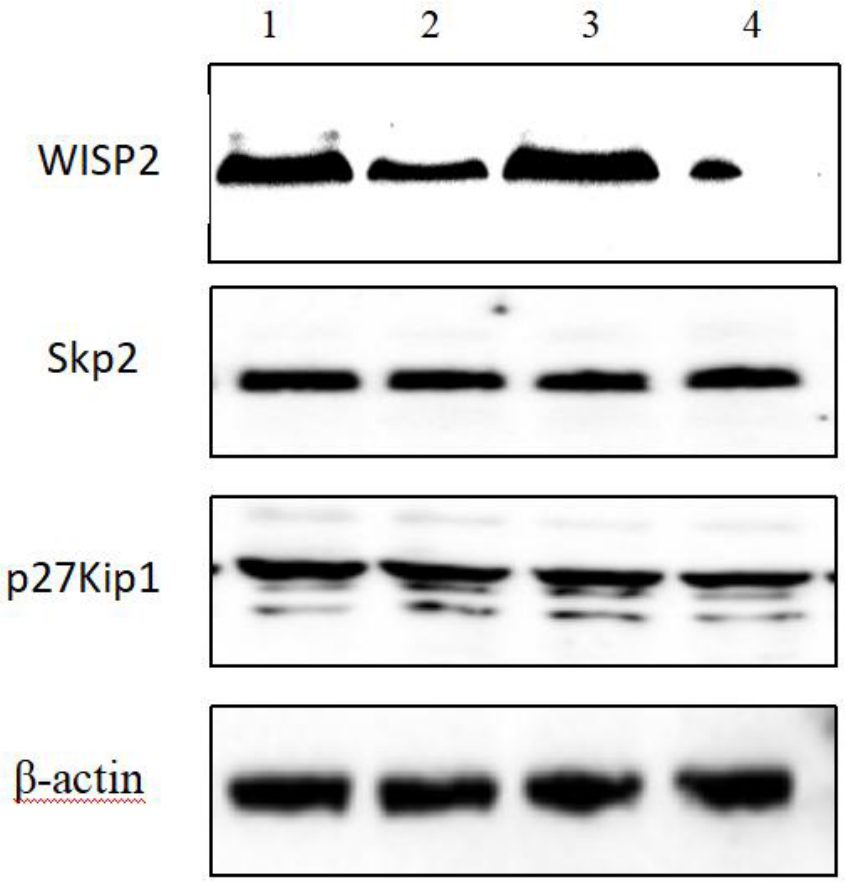

Figure 4

western blotting electrophoretic band of four groups. No.1 negative vector group and No.2 control group, No.3 Xiaoyan decoction intervention group and No.4 WISP2/CCN5 gene knockdown group

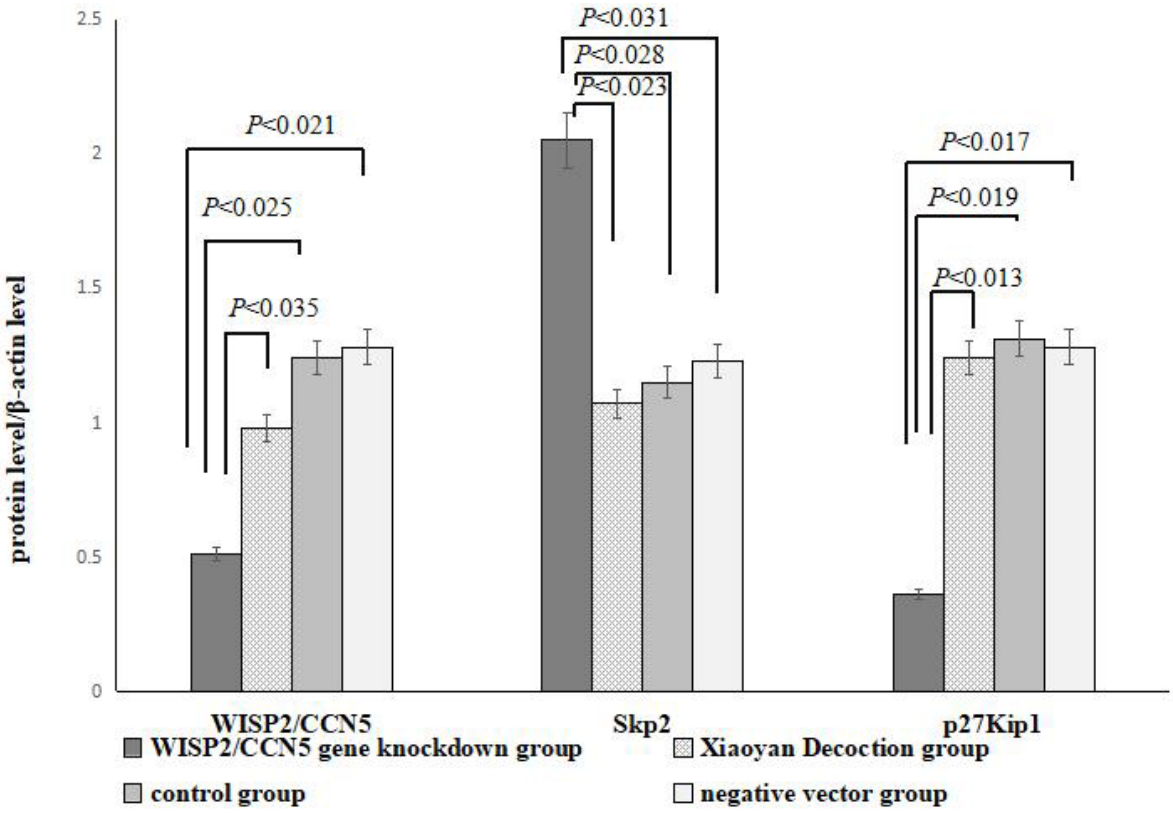

Figure 5

Xiaoyan decoction raised the levels of WISP2/CCN5 mRNA and protein.