Weipixiao ameliorates gastric precancerous lesions in a rat's model by regulating GSK3β and C-myc

Zeng Jinhao, Pan Huafeng, Guo Jing, Gong Daoyin, Cai Tiantian, Chen Xiaodong, Zhang Yi, You Fengming, Chen Longhui, Zhao Ziming, Liang Chao

Abstract

OBJECTIVE: To investigate the mechanism underlying the action of Weipixiao (WPX) in a rat's model with ameliorating gastric precancerous lesions (GPL).

METHODS: HPLC analysis was performed to identify the chemical constituents of WPX preparation. Sprague-Dawley rats were randomly assigned into control group, model group, vitacoenzyme group, high-dose WPX group (H-WPX), medium-dose WPX group (M-WPX) and low-dose WPX group (L-WPX). After modeling, the treated rats were administrated WPX or vitacoenzyme intragastrically for consecutive 10 weeks. Gene and protein expressions of GSK3β, C-myc, Cylin E were evaluated by quantitative real-time transcription-polymerase chain reaction (RT-qPCR) and immunohistochemistry, respectively.

RESULTS: WPX could efficiently attenuate the pathological alterations of "non-progressive GPL" in rats. As expected, mRNA and protein levels of C-myc and Cylin E were up-regulated in model rats, while GSK3β expression down-regulated (P < 0.01). WPX treatment, especially at low dose, could significantly down-regulate the mRNA as well as protein levels of C-myc, and could lead to remarkable up-regulation of mRNA and protein levels of GSK3β in GPL rats (P < 0.05). However, no significant changes were observed in WPX-treated rats.

CONCLUSION: Our findings suggested that WPX-mediated attenuation of GPL pathological alterations might be due to its regulatory effect on the expressions of GSK3β and C-myc, and on the dysregulation of Wnt/GSK3β pathway.

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Keywords: Gastric precancerous lesions; GSK3β; C-myc; Cylin E; Weipixiao

INTRODUCTION

Gastric cancer (GC) is one of the major malignant tumors detrimental to human health, and it is a major cause of cancer-related mortality. An estimated 951 600 new gastric cancer cases and 723 100 deaths occurred worldwide in 2008, accounting for 6.8% of the total cases and 8.8% of total deaths. There is a high prevalence of gastric cancer in China, one of the major...
determinants for the high prevalence may be credited to a high incidence of gastric precancerous lesions (GPL).\textsuperscript{2,3} GPL are generally defined as gastric intestinal metaplasia (GIM) and gastric epithelium dysplasia (GED), and GPL may serve as the crucial transitional lesions from normal gastric mucosa to gastric cancer. Therefore, suppression or reversion of GPL may serve to lower the incidence of GC.

It is well known that Wnt signaling pathway plays a key role in several developmental processes in embryonic state and also in the regulation of cell differentiation, proliferation, and apoptosis. Wnt signaling pathway comprises many genes that have been shown to be part of the signaling cascade, and their up-regulation or down-regulation could directly lead to the dysregulation of Wnt signaling pathway and thus lead to the occurrence and progression of numerous malignant tumors, including gastric cancer.\textsuperscript{7} GSK\textsubscript{3β}, C-myc and Cylin E are categorized as important components of the canonical Wnt/GSK\textsubscript{3β} pathway. The aberrant expression of these Wnt-related genes and thus the dysregulation of Wnt/GSK\textsubscript{3β} pathway could initiate gastric carcinogenesis, not only by promoting cellular proliferation, but also by virtue of its opposing role in engendering programmed cell death. However, the differential expressions of these genes/proteins in GPL are not fully understood.

Weipixiao (WPX), a traditional Chinese herbal formula consisting of Huangqi (\textit{Astragalus Membranaceus Bunge}), Taizishen (\textit{Pseudostellaria Heterophylla Pax}), Baishu (\textit{Rhizoma Atractylodis Macrocephalae}), Eshu (\textit{Curcuma phaeocaulis Val}), Danshen (\textit{Salvia Miltiorrhiza Bunge}) and Baihuasheshecao (\textit{Hedyotis Diffusa Willd}), can fortify the spleen, dissipate blood stasis and exert detoxification. It exhibits favorable clinical efficiency in preventing and treating GPL.\textsuperscript{3} Our previous studies revealed that WPX could suppress and reverse GPL by attenuating cellular proliferation, increasing apoptosis, and by inhibiting inflammatory reactions.

In present study, we used high performance liquid chromatography (HPLC) analysis to screen potential constituents of WPX separation. Condition optimization of fingerprint: JADE-PAK ODS-AQ column (250 \(\times\) 4.6 mm, 5 mm) and Inertsil ODS-SP column (4.6 \(\times\) 150 mm, 5 mm) were used, with acetonitrile 0.1% phosphoric acid solution and acetonitrile 0.4% phosphoric acid solution as the mobile phase respectively, under full wavelength detection. The following chromatographic analysis conditions were determined: the separation was determined on Inertsil ODS-SP column (4.6 \(\times\) 150 mm, 5 mm) with a mobile phase of acetonitrile (solvent A) 0.4% phosphoric acid solution (solvent B). For HPLC analysis, a 10 mL sample was injected into the column and eluted at a flow rate of 1.0 mL/min under room temperature. The detective wavelength was 203 nm.

**Methods**

**Ethical statement**

The study protocol and the handling of animals were in accordance with the international guidelines.

**Animals**

Seventy 7-week old male Sprague-Dawley (SD) rats, weighing 140-170 g, were provided by Experimental Animal Center, Sun Yat-sen University (certificate No. 0111909). The experiments were conducted in Guangdong Provincial Institute of Traditional Chinese Medicine [specific-pathogen-free (SPF) laboratory animal facility: No. SYXK (Guangdong) 2010-0059].

**Drugs and reagents**

WPX contains the following herbs: Huangqi (\textit{Astragalus Membranaceus Bunge}) 30 g, Taizishen (\textit{Pseudostellaria Heterophylla Pax}) 15 g, Baishu (\textit{Rhizoma Atractylodis Macrocephalae}) 15 g, Eshu (\textit{Curcuma phaeocaulis Val}) 10 g, Danshen (\textit{Salvia Miltiorrhiza Bunge}) 10 g, and Baihuasheshecao (\textit{Hedyotis Diffusa Willd}) 30 g. The herbs were provided by the First Affiliated Hospital of Guangzhou University of Chinese Medicine. The herbs were decocted in water twice, and then the filtrates were mixed and concentrated into a mixture of 1.5g/mL. All processes were performed by the Department of Pharmaceutical Preparation in Guangdong Provincial Institute of Traditional Chinese Medicine.

**High performance liquid chromatography (HPLC) analysis**

HPLC analysis was performed to identify the chemical constituents of WPX separation. Condition optimization of fingerprint: JADE-PAK ODS-AQ column (250 \(\times\) 4.6 mm, 5 mm) and Inertsil ODS-SP column (4.6 \(\times\) 150 mm, 5 mm) were used, with acetonitrile 0.1% phosphoric acid solution and acetonitrile 0.4% phosphoric acid solution as the mobile phase respectively, under full wavelength detection. The following chromatographic analysis conditions were determined: the separation was determined on Inertsil ODS-SP column (4.6 \(\times\) 150 mm, 5 mm) with a mobile phase of acetonitrile (solvent A) 0.4% phosphoric acid solution (solvent B). For HPLC analysis, a 10 mL sample was injected into the column and eluted at a flow rate of 1.0 mL/min under room temperature. The detective wavelength was 203 nm.

**Grouping, modeling and treatment**

Using random number table method, animals were randomly divided into 6 experimental groups: control group \((n = 10)\), model group \((n = 15)\), vitacoenzyme group \((n = 13, 0.2 \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\), high-dose WPX group \((H-WPX) (n = 13, 15 \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\), medium-dose WPX group \((M-WPX) (n = 13, 7.5 \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) and low-dose WPX group \((L-WPX) (n = 13, 3.75 \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\). Based on the literature,\textsuperscript{7} the composite method was adopted to modeling the GPL rats. Briefly, All the rats, except for 10 control rats, were allowed to drink MNNG solu-
tion (200 µg/mL) ad libitum, and underwent hunger-satiety shift every other day, and during the satiety day the rats were free to ranitidine diet. At the end of 9th week, 2 random rats in the model group were sacrificed and examined for gastric precancerous lesions. At the beginning of 10th week, the treated rats were administered WPX or vitacoenzyme by gastrogavage for 10 consecutive weeks, while the control rats and the model rats were given distilled water by gastrogavage (1 mL/100 g body weight) daily.

**Protein expression of GSK3β, C-myc and cyclin E by immunohistochemical method**

Paraffin-embedded gastric tissues were sliced into 3 µm sections. The sections were used for routine histopathological examination by hematoxylin and eosin (HE) staining and high-iron diamine-alcian blue-periodic acid Schiff (HID-AB-PAS) staining, and for protein expressions of GSK3β, C-myc and Cyclin E by EnVision immunohistochemical method. Five visual fields were randomly selected from each slice under light microscope (100 ×), and then images were acquired and quantitatively analyzed by Image Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD). Protein expression levels of GSK3β, C-myc and Cyclin E were all evaluated by scores of three indexes: mean of average optical density (AOD), sum of per area, object/total (PA), and mean of integrated optical density (IOD). Protein expression levels were considered to be up-regulation or down-regulation when any of the three indexes was increased or decreased.

**Gene expressions of GSK3β, C-myc and cyclin E by qRT-PCR**

The mRNA levels of GSK3β, C-myc and Cyclin E were determined by quantitative real-time reverse transcription-polymerase chain reaction (RT-qPCR) method via IQ™ 5 real-time PCR detection system (Bio-Rad, USA). The primers were designed according to the sequences of GSK3β (GenBank accession no. NM_032080), C-myc (GenBank accession No. AY679730), Cyclin E (GenBank accession No. NM_001100821), and 18S (GenBank accession No. M11188) from Genbank database. All primers were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd., China (Table 1).

### Table 1: Primer Sequences of GSK3β, C-myc and Cyclin E

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequences of primers</th>
<th>PCR products length (bp)</th>
<th>Anneal temperature (℃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>F (5’-3’): ACCGGTACCACATCC R (5’-3’): CAGACTTGCCCTCCCA</td>
<td>162</td>
<td>51</td>
</tr>
<tr>
<td>GSK3β</td>
<td>F (5’-3’): ACCAGCTGCCCTTCTC R (5’-3’): GTCTCCAGATTTAGATCTT</td>
<td>147</td>
<td>51</td>
</tr>
<tr>
<td>C-myc</td>
<td>F (5’-3’): ATCCGTGGCCTTCAGA R (5’-3’): AAGTCCAAATTGCCTGCA</td>
<td>187</td>
<td>50</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>F (5’-3’): GAACCTGATGATGATGAAAG R (5’-3’): CCACCTGAAAACTGAG</td>
<td>276</td>
<td>47</td>
</tr>
</tbody>
</table>

Notes: PCR reaction conditions were as follows: 94 ℃ for 5 min; 45 cycles of 94 ℃ for 30 s, anneal at respective temperature for 30 s, and 72 ℃ for 30 s, followed by 72 ℃ for 7 min elongation. Melting curve analyses were performed on the amplified PCR products. The relative transcript amount of the target genes was normalized to that of 18S by using 2^{-ΔΔCt} method.

### Statistical analysis

All data were presented as mean ± standard deviation (x ± s). Multiple group comparison was analyzed statistically by one-way analysis of variance (ANOVA), the comparison between two groups was performed with SNK method for the homogeneous variances, while the variances were heterogeneous, Dunnett’s T3 method should be adopted. All analysis was performed using IBM SPSS 19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY, USA). A P value < 0.05 was considered significant.

### RESULTS

#### Chemical constituents of WPX

Figure 1 shows the HPLC chromatograms of WPX test sample (A) and reference sample (B). The retention times of the major chemical constituents were 20.5 min (Calycosin-7-glucoside), 34.8 min (ginsenoside-Rg1), 48.3 min (ginsenoside-Rb1), 49.5 min (astragaloside N), 59.0 min (actractylenolide III), 71.7 min (actractylenolide II), and 81.7 min (actractylenolide I).

#### Histopathological changes of the gastric mucosa by HID-AB-PAS staining

In control rats, neutral mucusin present in normal gastric epithelium were stained red (Figure 2A). In model rats, sialomucins present in small intestinal-type metaplasia (S-IM) were stained blue, and sulfomucins expressed in colonic-type metaplasia (C-IM) were stained brown (Figure 2B), suggesting that diffuse lesions of both S-IM and C-IM were found in model rats. In treated rats, the scope of IM lesion was reduced slightly in vitacoenzyme-treated rats (Figure 2C). Comparative, the scope of IM lesion was reduced remarkably in all WPX-treated rats (Figure 2D-F).

#### Histopathological changes of the gastric mucosa by H-E staining

In control rats, normal structure/morphology of glands and epithelial cells was observed, and inflammatory infiltration in gastric epithelium was absent or minimal.
Changes of C-myc mRNA expression

In this study, level of C-myc mRNA expression in model group was significantly higher than that in control group ($P < 0.01$). Compared with model group, levels of C-myc mRNA expression in all WPX groups were significantly decreased ($P < 0.01$). Compared with vitacoenzyme, H-WPX and M-WPX groups, level of C-myc mRNA expression in L-WPX group was significantly decreased ($P < 0.01$ or $P < 0.05$). The data suggest that WPX might have no inhibitory effect on Cyclin E mRNA over-expression in GPL rats (Figure 5).

Changes of Cyclin E mRNA expression

In this study, level of Cyclin E mRNA expression in model group was significantly higher than that in control group ($P < 0.05$), whereas no significant differences were seen among model, vitacoenzyme, and all WPX groups ($P > 0.05$). The result suggest that WPX might have no inhibitory effect on Cyclin E mRNA expression in GPL rats (Figure 6).

Immunohistochemical analysis of GSK3β protein expression

The protective effect of GSK3β on Wnt-associated gastric carcinogenesis and progression was well established. In the present study, scores of GSK3β protein expression (AOD, PA and IOD) were significantly de-
creased as compared to that of control group ($P < 0.01$). Compared with model, vitacoenzyme and H-WPX groups, scores of GSK3β protein expression (PA or IOD) in L-WPX group were significantly decreased ($P < 0.01$ or $P < 0.05$). Therefore, it shows that L-WPX could markedly promote GSK3β protein expression in GPL rats (Table 2 and Figure 7).

**Immunohistochemical analysis of C-myc protein expression**

A contribution of C-myc in gastric carcinogenesis has been implied from previous studies. In this study, scores of C-myc protein expression (AOD, PA and IOD) in L-WPX group were significantly decreased ($P < 0.01$). Compared with model group, scores of C-myc protein expression in H-WPX (PA and IOD), M-WPX (IOD), and L-WPX (PA and IOD) were significantly decreased ($P < 0.01$ or $P < 0.05$). In addition, score of C-myc protein expression (PA) in L-WPX group was significantly decreased as compared to that of vitacoenzyme group ($P < 0.05$). The results revealed that WPX, especially at low dose, could markedly inhibit C-myc protein expression in GPL rats (Table 3 and Figure 8).

**Immunohistochemical analysis of cyclin E protein expression**

Aberrant expression of Cyclin E disrupts the cell cycle and may trigger cancerization of epithelial cells. In this study, scores of Cyclin E protein expression (AOD, PA and IOD) in model group were significantly higher than that of control group ($P < 0.01$ or $P < 0.05$), whereas no significant differences in all indexes were shown among model, vitacoenzyme, and all WPX groups and model group: given with distilled water (1 mL/100 g body weight) daily; vitacoenzyme group: administered with vitacoenzyme (0.2 $g \cdot kg^{-1} \cdot d^{-1}$); H-WPX group: treated with high dose of WPX (15 $g \cdot kg^{-1} \cdot d^{-1}$); M-WPX group: treated with medium dose of WPX (7.5 $g \cdot kg^{-1} \cdot d^{-1}$); L-WPX group: treated with low dose of WPX (3.75 $g \cdot kg^{-1} \cdot d^{-1}$). WPX: Weipixiao; HID-AB-PAS: high-iron diamine-alcian blue-periodic acid Schiff.
Control group and model group: given with distilled water (1 mL/100 g body weight) daily; vitacoenzyme group: administered with vitacoenzyme (0.2 g · kg⁻¹ · d⁻¹); H-WPX group: treated with high dose of WPX (15 g · kg⁻¹ · d⁻¹); M-WPX group: treated with medium dose of WPX (7.5 g · kg⁻¹ · d⁻¹); L-WPX group: treated with low dose of WPX (3.75 g · kg⁻¹ · d⁻¹). WPX: Weipixiao. *P < 0.01, compared with control group; †P < 0.01, compared with model group; ‡P < 0.01, compared with vitacoenzyme group; §P < 0.05, compared with H-WPX group; ||P < 0.01, compared with M-WPX group.

**DISCUSSION**

Wnt signaling pathway has a key role in normal embryonic development and wound repair but is largely quiescent in normal physiologic state apart from in pathological state, such as carcinogenesis. Different intrinsic and extrinsic factors, such as chronic inflammation, and Helicobacter pylori (Hp) infection, may be closely correlated to the abnormal expression of Wnt-related genes and the dysregulation of Wnt signaling pathway. Thus, inappropriate activation of Wnt signaling pathway via dysfunction in core pathway components, such as GSK3β. C-myc and Cyclin E, is firmly established as the critical factor in the development of gastric cancer.

GSK3β is evaluated as a central molecule necessary for the induction of β-catenin degradation and thus the inhibition of Wnt signaling pathway activation. In addition, GSK3β is a key tumor-suppressive gene and is also crucial to the pathogenesis of gastric cancer. A study reported that inhibition of GSK3β could stimulate COX-2 over-expression in gastric cancer cells, which might be involved in the underlying mechanisms of gastric cancerous progression. Thus, the deficiency or down-regulation of GSK3β may contribute to the dysregulation of Wnt signaling in gastric cancer.

Here, we demonstrated that mRNA and protein levels of GSK3β were significantly decreased in precancerous tissues when compared with those of normal tissues.

C-myc is an important regulator of a wide array of cellular processes, including cell cycle, cell growth, differentiation, apoptosis and transformation, and even angiogenesis. Furthermore, C-myc is also identified as a downstream target gene mediated by Wnt/GSK3β pathway. Recent studies revealed that C-myc amplification/over-expression was frequently found in specimens from patients with gastric cancer and is positively correlated with advanced tumor stage, lymph node metastasis as well as poor survival time. The critical role of...
Cyclin E expression may be regulated by Wnt signaling pathway.\(^{19}\) Several studies revealed that Cyclin E over-expression was observed in most cases of gastric cancer, and it was significantly associated with intestinal Lauren classification, size of the tumor (larger than 5 cm), advanced stages, and lymphatic and vascular invasion.\(^{20}\) In this study, dysfunction of C-myc and Cyclin E is not only one of the hallmarks in gastric cancer, but in gastric precancerous lesions as well, as proved by our RT-qPCR and immunohistochromy analysis. In this study, up-regulated gene and protein expressions of C-myc and Cyclin E, and down-regulated gene and protein expressions of GSK3β were found in gastric mucosa from GPL rats. These results demonstrated that the abnormal expressions of the Wnt-related genes/proteins and thus the dysregulation of Wnt/GSK3β pathway may be prominent in oncogenic transformation of gastric epithelium, strongly suggesting that the states of abnormality of cellular differentiation, increased cell proliferation and decreased cell apoptosis may be present in precancerous gastric mucosa. In addition, as the lesions progressed gradually from atrophic gastritis, to intestinal metaplasia, and to dysplasia (the so-called Correa pathway),\(^ {21} \) an increased level of C-myc gene/protein expression and a decreased level of GSK3β gene/protein expression were observed in GPL rats. Thus, down-regulated expression of GSK3β may induce gastric tumorigenesis, in combination with up-regulated expression of C-myc.

In accordance with the Traditional Chinese Medicine theory, GPL is conceptually referred to as “gastric stuffiness”, of which the major manifestations were abdominal distension, stomach duct pain or gastric upset, stomach reflux or vomiting, hiccup, diarrhea or constipation, poor appetite, and weight loss. The principal pathogenesis of GPL may include the following three aspects:\(^ {22} \) spleen-stomach weakness, blood stasis in the stomach collateral vessels, and pathogenic toxins dormant in the body. So the herbal preparation WPX is developed to fortify the spleen and invigorate the stomach collateral vessels, and pathogenic toxins dormant in the body. Thus, WPX shows obvious effects at relieving the clinical symp-

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AOD</th>
<th>PA</th>
<th>IOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>0.000±0.000</td>
<td>0.000±0.000</td>
<td>0.000±0.000</td>
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<tr>
<td>Model</td>
<td>9</td>
<td>0.117±0.040</td>
<td>0.173±0.067</td>
<td>40.778±11.712</td>
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<td>Vitacoezyme</td>
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<td>0.104±0.042</td>
<td>0.138±0.049</td>
<td>32.535±13.859</td>
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<td>H-WPX</td>
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<td>0.080±0.022</td>
<td>0.085±0.022</td>
<td>19.635±8.368</td>
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<tr>
<td>M-WPX</td>
<td>9</td>
<td>0.094±0.016</td>
<td>0.094±0.026</td>
<td>23.550±7.660</td>
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<tr>
<td>L-WPX</td>
<td>9</td>
<td>0.077±0.012</td>
<td>0.072±0.017</td>
<td>17.675±5.656</td>
</tr>
</tbody>
</table>

Notes: control group and model group: given with distilled water (1 mL/100 g body weight) daily; vitacoezyme group: administered with vitacoezyme (0.2 g·kg\(^{-1}\)·d\(^{-1}\)); H-WPX group: treated with high dose of WPX (15 g·kg\(^{-1}\)·d\(^{-1}\)); M-WPX group: treated with medium dose of WPX (7.5 g·kg\(^{-1}\)·d\(^{-1}\)); L-WPX group: treated with low dose of WPX (3.75 g·kg\(^{-1}\)·d\(^{-1}\)). WPX: Weipixiao. \(^{P}<0.01\), compared with control group; \(^{P}<0.05\), compared with model group; \(^{P}<0.05\), compared with vitacoezyme group.
WPX treatment at low dose may be more ideal for long-term intervention of GPL. Additionally, in the present study, L-WPX was shown to be more effective in inhibiting the gene/protein expression level of C-myc, promoting the gene/protein expression level of GSK3β, in contrast with those of H-WPX and M-WPX. However, our previous studies revealed that H-WPX is more superior to M-WPX and L-WPX in blocking gastric precancerous lesions, suppressing cell proliferation and promoting apoptosis. We speculate that the inconsistency might be due to the different treatment duration (10 weeks in the present study, 4 weeks in the previous studies). The results suggest that WPX, at low dose, may be more ideal for long-term intervention of GPL when compared with that at high and medium doses. We will explore and validate the correlations among treatment dosage, intervention duration and therapeutic effect in WPX-treated GPL in further studies. However, WPX showed no regulatory effect on Cyclin E protein and gene expression, which might be attributed to the limitation of sample number, or to the fact that Cyclin E may not be the potential target of WPX. In this study, however, some insufficiencies still existed. For example, limitation of sample number, and intermolecular interactions among GSK3β, C-myc and Cyclin E were not investigated. These limitations should be considered in future studies.

In conclusion, our findings suggested that WPX has regulatory effect on the expressions of GSK3β and C-myc, and on the dysregulation of Wnt/GSK3β pathway, thereby inhibiting cell hyper-proliferation in GPL.

Table 4 Scores of Cyclin E protein expression in gastric epithelium in various groups (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AOD</th>
<th>PA</th>
<th>IOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>0.027±0.029</td>
<td>0.004±0.005</td>
<td>3.827±6.508</td>
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<tr>
<td>Model</td>
<td>9</td>
<td>0.140±0.035</td>
<td>0.152±0.101</td>
<td>33.008±14.826</td>
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<td>Vitacoenzyme</td>
<td>9</td>
<td>0.121±0.045</td>
<td>0.128±0.059</td>
<td>29.410±9.639</td>
</tr>
<tr>
<td>H-WPX</td>
<td>9</td>
<td>0.118±0.033</td>
<td>0.083±0.043</td>
<td>22.088±8.988</td>
</tr>
<tr>
<td>M-WPX</td>
<td>9</td>
<td>0.112±0.032</td>
<td>0.085±0.042</td>
<td>25.191±10.256</td>
</tr>
<tr>
<td>L-WPX</td>
<td>9</td>
<td>0.101±0.043</td>
<td>0.066±0.025</td>
<td>19.308±11.463</td>
</tr>
</tbody>
</table>

Notes: control group and model group: given with distilled water (1 mL/100 g body weight) daily; vitacoenzyme group: administered with vitacoenzyme (0.2 g·kg⁻¹·d⁻¹); H-WPX group: treated with high dose of WPX (15 g·kg⁻¹·d⁻¹); M-WPX group: treated with medium dose of WPX (7.5 g·kg⁻¹·d⁻¹); L-WPX group: treated with low dose of WPX (3.75 g·kg⁻¹·d⁻¹). WPX: Weipixiao. *p < 0.01, **p < 0.05, compared with control group.
A: control group, GSK3β was mainly expressed in the cytoplasm, and GSK3β positive cells were distributed diffusely in gastric epithelium. B: model group, GSK3β was sparsely expressed in normal gastric epithelium. C: vitacoezyme group; D: H-WPX group, treated with high dose of WPX (15 g·kg⁻¹·d⁻¹) E: M-WPX group, treated with medium dose of WPX (7.5 g·kg⁻¹·d⁻¹); F: L-WPX group, treated with low dose of WPX (3.75 g·kg⁻¹·d⁻¹), WPX: Weipixiao. In the treated groups, the number of Cyclin E positive cells was relatively fewer than that of model group, of which the change among the groups showed no significant difference.

### REFERENCES