Research Article

Dendrobium officinale Polysaccharides Inhibit CDCA-Induced Gastric Intestinal Metaplasia through Activating NRF2/HO-1 and Modulating HNF4α/CDX2 Signaling Pathway

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1. Introduction

Gastric adenocarcinoma (GAC) is one of the most common digestive tract cancers in China, and its morbidity and mortality rates exceeded 10 and 13%, respectively, in 2018 [1]. Gastric intestinal metaplasia (GIM) is related to pre-malignant conditions in which areas of human stomach epithelium express mixed gastric and intestinal features [2]. Substantial epidemiologic evidence has indicated that GIM is the result of the comprehensive effects of multiple environmental factors, such as Helicobacter pylori (Hp) infection, bile reflux, aging, race, and lifestyle [3, 4]. Many epidemiologic studies established a close relationship between long-term bile reflux and GIM risk [4], and bile reflux was considered to be an independent risk factor for the development of precancerous gastric lesions and gastric cancer (GC) [5]. Although the antibiotics can eliminate Helicobacter pylori in clinical treatment, such as amoxicillin or clarithromycin, many of them cause undesirable subsequent health complications, including hypersensitivity, arrhythmias, hematopoietic disorders [6, 7], and gynecomastia [8]. Therefore, the IM stage can be an important broad time window to block the progression from gastritis to GC. Currently, there is no effective progress in the treatment or prevention of IM [9]. Importantly, some natural compounds and small molecules that could be used for the treatment have been discovered.

Bile reflux, such as chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA)-induced GIM model in vivo and in vitro, has been widely applied [10, 11]. Nuclear factor erythroid-related factor 2 (NRF2), a central transcriptional regulatory factor, can be activated by oxidative stress to achieve self-protection and increase antioxidase expression [12]. Upregulated NRF2/HO-1 shows an important...
protective function in various inflammatory diseases [13]. After chenodeoxycholic acid (CDCA) treatment, the activity of various transcription factors in gastric epithelial GES-1 cells was changed, and the result showed that NRF2 was increased [14]. Several studies showed that the plenty of polysaccharides from herbal medicines induce NRF2-mediated antioxidant/detoxifying enzymes to improve antioxidant capacity and reduce oxidative stress in cancer, diabetes, and Alzheimer’s and inflammation disease [15–18].

Caudal type homeobox 2 (CDX2) is an essential intestinal marker in the development and maintenance of intestinal tissue in adult mammals [19]. Hepatocyte nuclear factor 4 alpha (HNF4α) in the gastric epithelium and subsequent progression of IM contribute significantly to the expression of bile acid-induced columnar genes via regulating KLF4 and CDX2 [20]. Trefoil family factor 2 (TFF2) is a mucin-associated peptide expressed in gastrointestinal epithelial cells where it serves to maintain mucosal integrity and promote epithelial repair [21]. Meanwhile, TFF2-promoted epithelial restitution does not require cyclooxygenase activation [22]. Indomethacin and aspirin upregulated TFF2 by the activation of PPAR gamma to reduce gastric mucosal injury since TFF peptides play an important role in gastric mucosal protection [23, 24]. Therefore, TFF2 will enhance the gastric mucosal protection, and HNF4α/CDX2 will directly reduce damage from CDCA.

Alternative and complementary therapies have brought favorite results in precancerous lesions of gastric cancer (PLGC) which is based on traditional Chinese medicine (TCM) theory [25]. Dendrobium officinale Kimura et Migo (called Tie Pi Shi Hu in Chinese, usually used as stems) is listed in the pharmacopoeia of the People’s Republic of China which belongs to the orchid genera. It has been reported to have anticancer, anti-inflammation, and immunomodulatory properties [26–29]. Dendrobium officinale (DO), its dried leaves, and flowers have been permitted for use as food resources in Guizhou, Zhejiang, Yunnan, and Fujian provinces [30]. Modern research confirmed that DO had protected gastric mucosal injury, modulated enteric dysbacteriosis, improved gut barrier, and promoted di-stasis [31–33]. We found that DOP from stems reduced intestinal metaplasia and made the atypical hyperplasia be kept at the moderate or mild degree in a N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced PLGC rat model [34, 35]. However, it is unclear whether DOP has an inhibitory effect on bile reflux-induced GIM in vitro.

Based on the above facts, we hypothesized that DOP inhibits the intestinal metaplasia process on the bile acid-induced gastric intestinal metaplasia model by activating NRF2/HO-1 and regulating the HNF4α/CDX2 signal pathway. Therefore, we could better confirm and understand the effect and the mechanism of Dendrobium officinale polysaccharides on precancerous lesions of gastric cancer.

2. Materials and Methods

2.1. Chemicals and Reagents. DOP was isolated and purified from D. officinale, its purity was more than 80%, and the molecular weight was detected by high-performance gel permeation chromatography (HPGPC) [35]. Generally speaking, 50 g water extraction was dissolved in 2.5 L water two times and then mixed with 95% alcohol at a ratio of 1 : 5. After 24 hours, the mixture was collected by centrifuging at 4000 rpm for 15 min, then deproteinized by savage reagent, and dried to form crude DOP. Crude DOP is dissolved in water at a concentration of 0.1 g/mL as the mother liquor, and then it is diluted to the final concentration (800 μg/mL, 400 μg/mL, and so on). Human gastric epithelial GES-1 cell lines were purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS) and 1640 medium were purchased from Solarbio (Shanghai, China), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric ion-reducing antioxidant power (FRAP) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Cell counting kit-8 (CCK-8) assay kit was received from MCE (New Jersey, USA), total protein extraction kit (Beyotime, Shanghai, China), SYBR Premix Ex Taq II (Takara, Japan), Lowry protein assay, and nuclear protein extraction kit (Thermo Fisher Scientific, MA, USA). HNF4a, HO-1, and TFF2 antibodies were purchased from Abcam (Cambridge, MA, USA). CDX2 and β-actin antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA) and SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Pierce Biotechnology, Rockford, USA). Secondary antibodies were purchased from Beijing Zhongshan Biotechnology Company (Beijing, China).

2.2. Antioxidant Activities of Dendrobium officinale Polysaccharides by DPPH Assay. The DPPH assay is the most popular method for measuring antioxidant activity because they are reliable and economical [36]. First, DOP was diluted 10-fold, and 45 μL of the diluted sample was added to a 96-well plate. Then, 100 μL of 0.2 mM DPPH solution (in ethanol) was added, mixed well, and placed in the dark for 30 min at room temperature. The absorbance A1 at 516 nm was measured by a spectrophotometer, and 45 μL of anhydrous ethanol was used to replace the sample as blank absorbance A0. 0.05 μg/mL ascorbic acid was selected as the positive control.

The unit of the DPPH-scavenging rate is recorded as “%”. DPPH-scavenging rate (%) = (A0 − A1)/A0 × 100 where A0 is the absorbance of the blank sample and A1 is the absorbance of the DOP. All experiments were performed at least 3 times.

2.3. Antioxidant Activities of Dendrobium officinale Polysaccharides by FRAP Assay. The ferric reducing/antioxidant power (FRAP) assay was used to determine the total antioxidant capacity [37]. FRAP assay was carried out according to the kit instructions [38]. 180 μL of FRAP working solution was added to the 96-well plate, and 5 μL of sample (400 μg/mL) or ultrapure water was added to the working solution and mixed. The absorbance was recorded at 593 nm by a spectrophotometer after incubation at 37°C for 3 min. The standard curve was determined with FeSO4 as the standard product. The results were expressed as Trolox equivalents. The unit of the total antioxidant capacity of the
sample is expressed in “mM FeSO4 equivalent.” All experiments were performed at least 3 times.

2.4. Establishment of CDCA-Induced Intestinal Metaplasia Model on GES-1 Cells by Western Blot (WB). According to [39], GES-1 cells were seeded in 60 mm dishes at a density of $8 \times 10^4$ cells/well, were cultured in an environment of 37°C and 5% CO$_2$ for 24 hours to allow cells to adhere, and then were serum-deprived for 12 hours prior to treatment with 50, 100, and 200 μm CDCA. After being treated with CDCA for 24 hours, harvested cells were lysed with RIPA cell lysis buffer supplemented with 1% PMSF to extract total protein. Protein concentration was evaluated by Lowry protein assay [40]. The protein sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes by the wet blotting method. Membranes were blocked with 5% dried skimmed milk powder in Tris-buffered saline Tween-20 (TBST). Membranes were incubated with primary antibodies to CDX2 (1 : 1000) and β-actin (1 : 1000). After washing in TBST, secondary antibodies (1 : 10000) conjugated with horseradish peroxidase (HRP) were incubated on the membranes for 1 hour. ECL, the enhanced chemiluminescence detection reagent, was used to visualize the protein bands after the incubation. All experiments were performed at least 3 times.

2.5. Establishment of CDCA-Induced Intestinal Metaplasia by RT-PCR (Reverse Transcription-Polymerase Chain Reaction). These genes CDX2, MUC2, HNF4α, KLF4, VILLIN1, SOX2, and TFF2 were key intestinal metaplasia genes. After choosing the appropriate concentration to establish CDCA-induced intestinal metaplasia, the expression of key intestinal metaplasia genes was investigated by RT-PCR. The cell was collected, and its total RNA was extracted by the TestaTm Super Total RNA Extraction Kit. The integrity of the RNA sample was measured by the Agilent Bioanalyzer 2100 system. 1 μg of total RNA was used to synthesize first-stand cDNA by the PrimeScript RT reagent kit with gDNA Eraser following the manufacturer’s instructions. Primers and templates were mixed with TB Green Premix Ex Taq II. The primers are listed in Table 1. RT-PCR was conducted using the SYBR method on a CFX96™ Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The 2$^{-\Delta\DeltaCT}$ method was used to quantify the relative mRNA expression levels [41]. All experiments were performed at least 3 times.

2.6. The Inhibitory Effect of DOP at Different Concentrations on Normal GES-1 Cell and CDCA-Induced Intestinal Metaplasia Model [42]. A 96-well plate at a density of $4 \times 10^4$ cells/well was tested for the inhibitory effect of DOP at final concentrations of 800, 400, 200, 100, and 50 μg/mL on normal GES-1 cells. In the CDCA-induced intestinal metaplasia model, DOP were at final concentrations of 400, 200, 100, and 50 μg/mL, which combined with 100 and 200 μm CDCA. The control group was cultured in a medium supplemented with 10% FBS. The model groups were induced by 100 and 200 μm CDCA. After twenty-four hours, 10 μL of CCK-8 solutions was added to 100 μL medium and incubated for 4 hours according to the CCK-8 kit. Absorbance was measured at 450 nm after shaking and in the dark for 5 min. The cell survival rate was calculated as follows: cell survival rate (%) = (OD value in sample group/OD value in normal group) × 100%. All experiments were performed at least 3 times.

2.7. Effect of DOP on CDCA-Induced Intestinal Metaplasia by RT-PCR. GES-1 cells were seeded in 60 mm dishes for 24 hours to adhere and then were serum-deprived 12 hours later, and 100 μm CDCA was used to induce intestinal metaplasia. High, medium, and low concentrations of DOP (400, 200, and 100 μg/mL) were added lasting for 24 hours. The cells were collected for RT-PCR. The procedure of RT-PCR was the same as above. The genes were several intestinal markers (CDX2, HNF4a, KLF4, MUC2, VILLIN1, and TFF2), NFR2, HO-1, and NQO-1. All experiments were performed at least 3 times.

2.8. Nuclear Translocation of NRF2 on CDCA-Induced Intestinal Metaplasia Treated with DOP. The nuclear translocation of NRF2 was assessed by WB. Nuclear and cytoplasmic proteins were extracted using the Nuclear Protein Extraction Kit. Histone H3 (1 : 1000) and β-actin (1 : 1000) were used as internal reference antibodies and subsequently incubated with the horseradish peroxidase-conjugated goat antirabbit secondary antibody (dilution 1 : 5000) for 1 h at room temperature. Protein bands were then detected using enhanced chemiluminescence Western blot detection reagents (Thermo Fisher Scientific, MA, USA). All experiments were performed at least 3 times.

2.9. Effect of DOP on CDCA-Induced Intestinal Metaplasia by WB. The method of collecting and dealing with the cell was the same as above, the primary antibodies to HNF4α (1 : 1000), HO-1 (1 : 2000), NQO-1 (1 : 1000), and TFF2 (1 : 2000) at 4°C for 12 hours. β-Actin (1 : 1000) was used as an internal reference antibody after all experiments were performed at least 3 times.

2.10. Statistical Analysis. Data were analyzed using SPSS version 16.0 software (IBM, Chicago, IL, USA). Data were presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences between groups. P value <0.05 was considered to be statistically significant.

3. Results

3.1. Antioxidant Activities of Dendrobium officinale Polysaccharides by DPPH and FRAP Assay. DPPH value is expressed as ascorbic acid (GAE) having an equivalent antiradical capacity. The clearance rate of 50, 100, 200, 400, and 800 μg/mL DOP was from 18.5% to 50.8%. For FRAP
Table 1: RT-PCR primer information.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sense (5′ – 3′)</th>
<th>Antisense (5′ – 3′)</th>
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<tr>
<td>NRF2</td>
<td>TCAGGGACGGAAAGTAGTGA</td>
<td>CCACCTGTTTCTCTGAGATGT</td>
</tr>
<tr>
<td>HO-1</td>
<td>AAACCTTGAAGGGGGCGAAG</td>
<td>GACAGTGCCACATTAGGTT</td>
</tr>
<tr>
<td>NQO-1</td>
<td>AAGAGCCCTGATGCACTGG</td>
<td>CTTCACTTTACTCTGTGAGCC</td>
</tr>
<tr>
<td>CDX2</td>
<td>GCTATAATGCGAGGGGACACC</td>
<td>CACAGCACCACACCCACAG</td>
</tr>
<tr>
<td>MUC2</td>
<td>AGTCCATCTCTGACCTCACC</td>
<td>GTGGTACGCTGCTCTTCC</td>
</tr>
<tr>
<td>KLF4</td>
<td>AAGATTTCCACTTCAAGGCAA</td>
<td>GGCGAATTTCCTACGAC</td>
</tr>
<tr>
<td>Villin1</td>
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<td>TGAGTTGCTGGTTCATGAC</td>
</tr>
<tr>
<td>TFF2</td>
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<td>CAGTCTTCACAGACATCG</td>
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<tr>
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<td>AGGCATACCTGTTGTCATG</td>
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<td>SOX2</td>
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<td>CGCCGCCGATGATTGTTAT</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GAAAACATCCTCAACTCCATC</td>
<td>CGAGGCGAGGAGGCGCC</td>
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3.5. Effect of DOP on CDCA-Induced Intestinal Metaplasia by RT-PCR. The mRNA expression of CDX2, HNF4α, KLF4, MUC2, and VILLIN1 was significantly increased, and TFF2 was decreased by stimulation of CDCA in the model group by RT-PCR. The mRNA expression of NRF2, HO-1, and NQO-1 was obviously increased. After DOP treatment, the mRNA expression of CDX2, HNF4α, and VILLIN1 was found to be significantly decreased. Although there was a trend of lower KLF4α mRNA expression treated with H-DOP and M-DOP compared with the model group, they did not have statistical significance. MUC2 has not been affected. Interestingly, the mRNA expression of NRF2 and HO-1 was further found to be increased by DOP, but NQO-1 was decreased. TFF2 mRNA was downregulated in CDCA-treated GSE-1 cells and was upregulated by DOP in the cell model. Collectively, these results suggest that DOP increases NRF2, HO-1, and TFF2 expression but decreases CDX2, NQO-1, and HNF4α expression (Figure 5).
was remarkably decreased. The protein expression of HO-1 and TFF2 was increased by three concentrations of DOP \( (p < 0.05; p < 0.01) \). The protein expression of NQO-1 was downregulated by DOP \( (p < 0.05) \) (Figure 7).

### 4. Discussion

Dendrobium plants are famous traditional medicines and are mainly distributed in South Asia and Oceania. There are 1547 accepted Dendrobium species worldwide, more than 80 Dendrobium species have been found in China with a large annual production [44]. DO has a strong effect on nourishing the stomach Yin, playing an important role in treating gastrointestinal diseases, and modern pharmacology research has shown that DO protects against gastrointestinal injury and aids in digestion [45, 46]. GIM is considered an important precursor to gastric cancer. In this study, DOP inhibited CDCA-induced gastric intestinal metaplasia in the cell model, and furthermore, it improved the NRF2/HO-1 and HNF4α/CDX2 signal pathway, which was a further extension of our preliminary work in vitro. Our previous studies had proved that the DOP prevented MNNG-induced PLGC along with subsequent liver and kidney damage, and the mechanism is associated with the reduction of 8-OHdG levels and the activation of the NRF2 pathway and its related antioxidant enzymes HO-1 and NQO-1 [35].

For DOP, many studies highlighted advances in antioxidant properties from in vivo and in vitro by decreasing free radicals, enhancing the antioxidant system, inhibiting nuclear factor-kappa B, and downregulating inflammatory response [47–49]. In this study, we evaluated the antioxidant activities of *Dendrobium officinale* polysaccharides by DPPH and FRAP assay. The result showed that the clearance rate of 50, 100, 200, 400, and 800 μg/mL DOP was from 18.5% to 50.8%. Xing et al. found when the concentrations of DOP
were 0.5–5.0 mg/mL, the DPPH radical-scavenging rates were between 40 and 50% [50], which was consistent with our experimental results.

NRF2 is a key transcription factor regulating antioxidant enzymes and, therefore, protecting against antioxidant stress and anti-inflammation [51–53]. After CDCA treatment, the activity of various transcription factors in GES-1 cells was changed, and the result showed that NFE2 was increased [14]. HO-1 as a downstream gene of NRF2 has a cytoprotective effect in normal tissues via restraining oxidative dysregulation, inappropriate immune response, and related disorders, especially in cancer carcinogenesis [52, 54]. Much data report and in vivo studies revealed the increased expression of the HO-1 enzyme in tumors compared with the surrounding normal tissues [55]. Our previous research demonstrated the ability of DOP to induce NRF2 upregulation and to increase HO-1 in the PLGC rat model [35]. In this study, we also found that CDCA enables NRF2 to translocate into the nucleus and upregulate redox-regulated genes HO-1. After treatment with DOP, it could promote the entry of NRF2 into the nucleus and upregulate the protein expression of NRF2, especially the high concentration of DOP. At the same time, the protein expression of NRF2 in the cytoplasm was decreased by CDCA and increased by high and medium doses of DOP. Meanwhile, the HO-1 was upregulated after being treated with DOP on the CDCA-induced intestinal metaplasia model. These data indicated that DOP had the potential capacity to reduce the damage from intestinal metaplasia through the NRF2/HO-1 pathway in vitro. The data have similarities with published reports that D. nobile extract protects retinal pigment epithelial cells from UV and oxidative stress damage via NRF2/HO-1 and MAPK pathways [56]. The bile acid-induced gastric intestinal metaplasia model was widely used [57, 58], especially chenodeoxycholic acid (CDCA), which could increase caudal type homeobox 2 (CDX2) and induce following intestine-specific markers in GES-1 cells [39]. CDX2 is an essential intestinal marker in the development and maintenance of intestinal tissue in adult mammals [19]. HNF4α, one of the TFs (Transcription factors), activates ectopic CDX2 expression through a shadow 3′enhancer [59]. HNF4α, in gastric epithelium and subsequent progression of
Figure 5: The inhibition effect of DOP on CDCA-induced intestinal metaplasia model by RT-PCR. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. The relative level was calculated with target genes/β-actin. Compared with the control group: *p < 0.05, **p < 0.01, and ***p < 0.001. Compared with the CDCA model group: #p < 0.05, ##p < 0.01, and ###p < 0.001. n = 3 and repeated three times.

Figure 6: The protein expression of NRF2 on CDCA-induced intestinal metaplasia treated with DOP in the nucleus and cytoplasm. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. (a, b) NRF2 expression in the nucleus; (a, c) NRF2 expression in cytoplasm. Compared with the CDCA model group: #p < 0.05 and ##p < 0.01. n = 3 and repeated three times.
IM, contributes significantly to the expression of bile acid-induced columnar genes via regulating KLF4 and CDX2 [20]. TGR5-ERK1/2-HNF4α axis plays an important role in the bile acid-induced gastric intestinal metaplasia model [20, 60]. In our data, CDCA could increase CDX2 and HNF4α mRNA as well as protein expression, while DOP could decrease their expression. We demonstrate that DOP could inhibit CDCA-induced gastric intestinal metaplasia by downregulating the HNF4α/CDX-2 signal pathway. A relation between HO-1 and HNF4α has been previously proposed for both to share a common microRNA, and HNF4α regulates miR-377 leading to upregulation of HO-1 [61]. This will be a potential pathway for further investigation.

TFFs play a major role within the gastrointestinal mucosal barrier. It has been demonstrated to promote cell migratory and antiapoptotic activities so as to mediate mucosal repair in epithelial cell culture models [62]. TFF2 is illustrated with a homeostatic pattern during inflammatory processes, which results in an anti-inflammatory effect that would be conducive to create the necessary microenvironment for tissue repair [21]. TFF2 drives epithelial repair by activating CXCR4 in gastric organoids [63]. In the results, DOP could upregulate the mRNA and protein expression of

![Western blot analysis of HNF4α, CDX2, TFF2, HO-1, and NQO-1 protein expression.](image-url)

**Figure 7:** Western blot analysis of HNF4α, CDX2, TFF2, HO-1, and NQO-1 protein expression. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. (a) The western blot bands and (b) the relative level of HNF4α, CDX2, TFF2, HO-1, and NQO-1. The relative level was calculated with target proteins/β-actin reference protein. Compared with the control group: *p < 0.05, **p < 0.01, and ***p < 0.001. Compared with the CDCA model group: #p < 0.05, ##p < 0.01, and ###p < 0.001. n = 3 and repeated three times.
TFF2, indicating that DOP generates an anti-inflammatory effect which will help to defend against mucosal injury.

5. Conclusion

DOP inhibits the intestinal metaplasia process on the bile acid-induced gastric intestinal metaplasia model by activating NRF2/HO-1 and regulating the HNF4α/CDX2 signal pathway. These findings provide an experimental basis for further application in gastric precancerous lesions.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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