Phytoglycoprotein isolated from *Dioscorea batatas* Decne promotes intestinal epithelial wound healing

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**[ABSTRACT]** *Dioscorea batatas* Decne (DBD) has been used to heal various illnesses of the kidney and intestine as an herbal medicine in Asia. As a source of therapeutic agents, many glycoproteins have been isolated from mushrooms and plants, but the functional role of glycoprotein in intestinal epithelial wound healing has not been understood yet. In the present study, we investigated the wound healing potentials of the 30 kDa glycoprotein (DBD glycoprotein) isolated from DBD in human intestinal epithelial (INT-407) cells. We found that DBD glycoprotein (100 μg mL\(^{-1}\)) significantly increased the motility of INT-407 cells for 24 h by activating protein kinase C (PKC). DBD glycoprotein stimulated the activation of p38 mitogen-activated protein kinase (MAPK), which is responsible for the phosphorylation of NF-κB inhibitor α (IκBα). DBD glycoprotein increased the level of profilin-1 (PFN1), α-actinin and F-actin. DBD glycoprotein via activation of transcription factor, nuclear factor-kappa B (NF-κB) during its promotion of cell migration. Experimental mouse colitis was induced by adding dextran sulfate sodium (DSS) to the drinking water at a concentration of 4% (W/V) for 7 days. We figured out that administration of DBD glycoprotein (10 and 20 mg·kg\(^{-1}\)) lowers the levels of disease activity index and histological inflammation in DSS-treated ICR mice. In this regard, we suggest that DBD glycoprotein has ability to promote the F-actin-related migration signaling events via activation of PKC and NF-κB in intestinal epithelial cells and prevent inflammatory bowel disease.

**[KEY WORDS]** Cell migration; *Dioscorea batatas* Decne (DBD) glycoprotein; Intestinal epithelial cells; p38 MAPK; F-actin; Mouse colitis

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**Introduction**

Cell migration in intestinal epithelium is a dynamic biological event that is regulated by a complex microenvironmental network [1]. Intestinal epithelial migration is not only critical for intestinal homeostasis but also plays an essential role in wound healing, a process disrupted in inflammatory bowel diseases (IBDs) [2]. In response to injury, epithelial cells at the wound edge proliferate and migrate to cover denuded surfaces and re-establishing the critical barrier function [3]. Thus, rapid resealing of epithelial wounds is critical in maintaining intestinal mucosal homeostasis and protecting the host from hostile luminal environment. Migrating cells undergo a striking transition in cell shape that is orchestrated by the cytoskeletal reorganization-related factors, such as α-actinin, profilin-1 (PFN1), and filamentous (F)-actin [4]. There are also many factors that can regulate F-actin-related migration signaling events for cytoskeletal reorganization, such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and transcription factors.

Although there are many factors that can regulate the migration of intestinal epithelial cells, many studies have focused on the investigation of new functional substances which activate cytoskeletal reorganization factors [5, 6]. Therefore, one of the most interesting questions is center of the discovery and identification of safe new drugs that enhance wound healing process in the intestinal epithelium. In these contexts, many scientists have proposed that the increased dietary intake of herbal medicine and functional foods can promote the wound healing process in intestinal epithelium and have regulatory effects on cytoskeletal reorganization-related factors induced by F-actin-related migration signaling events [7].

*Dioscorea batatas* Decne (DBD), sweet soothing herb, is a perennial trailing rhizome plant widely distributed in Asia. DBD has traditionally been used for a food additive and herb-
al medicine as considered a potential functional food for the treatment of various illnesses of the kidney and intestine because of its nutritional fortification, improve anorexia, tonic, anti-diarrheal, antitussive, and expectorant effects. Recent studies have shown that mucilage of DBD extract contains pharmacological active agents harboring in vivo and in vitro antioxidant and anti-inflammatory properties \(^9\). In this context, we isolated a glycoprotein (30 kD) consisting of carbohydrate (61%) and protein (39%) components from DBD and reported that DBD glycoprotein has strong anti-oxidative activity and anti-inflammatory effects via the modulation of transcriptional activities in immune and epithelial cells \(^8,10\). Having shown that DBD glycoprotein has many biological functions, we speculated that DBD glycoprotein may be effective against intestinal diseases involving considerable impairments of wound healing which is a potential causative factor in IBD. In addition, there is no evidence of the functional role of glycoprotein in the promotion of cytoskeletal reorganization during intestinal epithelial wound healing. Therefore, we examined the wound healing potentials of the 30 kDa DBD glycoprotein isolated from DBD in human intestinal epithelial (INT-407) cells and in experimental mouse colitis.

**Materials and Methods**

**Chemicals**

Dulbecco’s modified Eagle’s medium (DMEM), phosphate-buffered saline (PBS) and fetal bovine serum (FBS) were purchased from GE Healthcare (Logan, UT, USA). The following antibodies were purchased: F-actin antibody (abcam, Cambridge, MA, USA); phospho-pan-PKC, pan-PKC, phospho-ERK, ERK, phospho-JNK, c-Jun N-terminal kinase (JNK), phospho-p38 MAPK, p38 MAPK, phospho-NF-κB, NF-κBp65, phospho-IκBα, IκBα, α-actinin, profilin-1 and β-actin antibodies (Santa Cruz Biotechnology, Paso Robles, CA, USA); Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse IgG antibodies (Gene Tex, Irvine, CA, USA). Bisindolylmaleimide I, SB203580 and Bay 11-7082 were purchased from MedChemExpress (Monmouth Junction, NJ, USA). The concentrations of all of the pharmacological inhibitors listed did not show any significant cytotoxic effects by themselves. All other reagents were of the highest purity commercially available and were used as received.

**Cells**

Human intestinal epithelial (INT-407) were purchased from American Type Culture Collection (Manassas, VA, USA). INT-407 cells were grown at 37 °C in 5% CO₂ in DMEM supplemented with 10% FBS and 100 U mL⁻¹ penicillin and 100 mg mL⁻¹ streptomycin, respectively. The cells were sub-cultured three times a week. These cell lines have previously been used to evaluate the physiological and pathophysiological function of intestinal epithelium \(^11\).

**Preparation of DBD glycoprotein**

* Dioscorea batatas* Decne (DBD) was purchased at Na-ju traditional market, located in the Chonnam province of South Korea, in October 2006 and authenticated by Prof. LIM Kye-Taek from Chonnam National University. DBD was cut into small pieces and soaked in ethanol (99%) at 4 °C for 5 months in a dark basement. After soaking, the solution was filtered through Whatman filter paper (No. 2), concentrated using a rotary evaporator (B465; Buchi, Flawil, Switzerland), and dried with a freeze dryer (SFD306; SamWon, Seoul, Korea) for 2 days. The dried powder (5.0 g) was dissolved in 10 mL distilled water and centrifuged at 7000 × g for 15 min at 4 °C to remove insoluble proteins. The supernatant was precipitated with 80% ammonium sulfate and centrifuged at 12 000 × g for 20 min at 4 °C. The pellet was redissolved in 5 mL distilled water and recentrifuged at 7000 × g for 20 min at 4 °C to remove remaining insoluble proteins. The supernatant was dialyzed with a dialysis membrane (Spectra/por, MWCO 6000–8000, Spectrum Medical Industries, Los Angeles, CA, USA) against 20 mmol L⁻¹ Tris-Cl (pH 7.4) at 4 °C overnight. After dialysis, the solution was further centrifuged at 3000 × g for 15 min at 25 °C using microcon concentrators (MWCO 10 000) to obtain protein with more than 10 kDa molecular weight according to the manufacturer’s protocol (Amicon Inc., MA, USA), and the supernatant was adsorbed to concanavalin A-sepharose 4B affinity chromatography (24–45 mm, confirmed by Sigma, C9017) and eluted with 0.5 mol L⁻¹ methyl α-D-glucopyranoside containing 0.5 mol L⁻¹ NaCl at pH 7.4. The eluted solution was further dried with a freeze dryer and stored at −70 °C. To confirm, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the sample of protein (50 mg mL⁻¹) containing 0.1% SDS using a 15% polyacrylamide minigel and a Mini-PROTEIN II electrophoresis cell (Bio-Rad, Hercules, CA, USA) at 110 V, 30 mA for 2.5 h. The DBD glycoprotein was confirmed by staining with Schiff’s reagent, a specific staining reagent for glycoprotein through a redox reaction \(^12\). The final amount of DBD glycoprotein was 1.6 mg (0.03%) from the dried powder (5.0 g) of initial DBD extract. After verification of high purity (approximately more than 95%) of glycoprotein, the analysis determined that DBD glycoprotein consists of carbohydrate content (61%) and protein content (39%), as described previously \(^13\).

**Experimental animals and ethics statements**

Male mice (ICR), 7-week-old were purchased from Dae-Han Lab (Animal Research Center Co., Ltd, DaeJeon, Korea). All procedures for the mouse colitis model were performed following the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The protocol of the animal study was approved by the Institutional Animal Care and Use Committee of Daegu Haany University (DHU-2020-001). Animals were maintained in a clean room at a temperature between 23 ± 2 °C with a 12 h light/dark cycle, lights (an illumination intensity of 150–300 lux) on 700 h and lights off 1900 h. The relative humidity was 55% ± 15% with air ventilation frequencies of 15–20 times per hour. All mice were fed a commercial diet and water ad libitum, and kept for at least 1 week before the experiments.
**Mouse colitis induced by DSS**

Experimental mice groups were divided into the five groups: mice given PBS (group 1, \( n = 5 \)); mice given 20 mg·kg\(^{-1} \) DBD glycoprotein (group 2, \( n = 5 \)); mice given 4% of dextran sulfate sodium (DSS) drinking water (group 3, \( n = 5 \)); mice given 10 mg·kg\(^{-1} \) DBD glycoprotein and 4% DSS drinking water (group 4, \( n = 5 \)); mice given 20 mg·kg\(^{-1} \) DBD glycoprotein and 4% DSS drinking water (group 5, \( n = 5 \)). To induce experimental colitis, 7-week-old male ICR mice performed an experiment by replacing normal drinking water with distilled water containing 4% DSS (\( W/V \); molecular weight 36 000–50 000; MP Biomedicals, Santa Ana, CA, USA). The body weight of each mouse was recorded on 0, 7, and 14 days after the start of the experiment, and the intake of food and drinking water was recorded every 2 days. At the end of the experiments, the distal colon was harvested and flushed with PBS. To confirm the effect that inhibits the colitis of DBD glycoprotein, the colon sample was embedded in optimum cutting temperature (O.C.T.) compound (Sakura Finetek, Torrance, CA, USA) for histological examinations by hematoxylin and eosin (H&E) staining and stored in a deep freezer at \(-70^\circ C\). Samples were then cut into 6 μm thick frozen sections. Disease activity index (DAI), characterized by the change of body weight loss, stool consistency, and blood in feces was scored daily from 0 to 4 for each mouse according to the method of Table 1, and then the averages of the sum of three scores for each mouse were calculated \(^{[14]}\).

**Western blot analysis**

Cells were harvested, washed twice with PBS, and lysed with buffer [20 mmol·L\(^{-1} \) Tris (pH 7.5), 1 mmol·L\(^{-1} \) EDTA, 1 mmol·L\(^{-1} \) EGTA, 1% Triton X-100, 1 mg·mL\(^{-1} \) aprotonin, and 1 mmol·L\(^{-1} \) phenylmethylsulfonyl fluoride (PMSF)] for 30 min on ice. Protein concentrations were determined by BCA Protein Assay kits (Pierce, Rockford, IL, USA). Equal amounts of protein (20 μg) were resolved by 8%–12% SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membranes. The membranes were washed with TBST solution [10 mmol·L\(^{-1} \) Tris-HCl (pH 7.6), 150 mmol·L\(^{-1} \) NaCl, and 0.05% Tween-20], blocked with 5% skim milk for 30 min and incubated with appropriate primary antibody at 4 °C for overnight. The membrane was then washed and incubated with a horseradish peroxidase-conjugated secondary antibody for 2 h. The bands were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) and detected by using the Bio-Rad ChemDoc™ XRS+ System (Bio-Rad, Hercules, CA, USA). The results of the western blot analysis were calculated in terms of relative intensity, using Scion imaging software (Scion Image Beta 4.02, Frederick, MD, USA).

**Scratch wound-healing assay**

INT-407 cells were cultured until 90% confluence in 6-well culture plates and scratched with pipetman diamond tip (Gilson, Middleton, WI, USA). The border of the denuded area was marked with a fine line immediately, and the cells were incubated with 100 μg·mL\(^{-1} \) of DBD glycoprotein in serum-free medium. PBS was treated for positive control. The cell migration was observed with an Olympus FluoView™ 300 confocal microscope (Tokyo, Japan) during incubation with a \( \times 100 \) objective. Results were expressed as a percent of control, determined as the average width of migrated cells in wells divided by the average width of migrating cells in control wells.

**Cell invasion assay**

*In vitro* cell invasion assay was performed in transwell permeable support with 8.0 μm pore size membrane coated with matrigel (Corning Incorporated Life Sciences, Lowell, MA) according to the manufacturer’s instructions. INT-407 cells suspensions (5 \( \times 10^4 \) cells/mL) were placed into the upper chamber in 0.2 mL of serum-free medium. The lower compartment was filled with 0.6 mL serum-free medium containing 100 μg·mL\(^{-1} \) of DBD glycoprotein. After incubation for 24 h, cells that had migrated to the lower surface of the filters were fixed in acetone for 5 min at room temperature and visualized with H&E staining method.

**General liver toxicity tests**

The serum tests for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed according to the manufacturer’s instructions (ALT/AST kit, Asanpharm, Dajeon, Korea) and measured by using a microplate reader at 505 nm (SPARK, Seestrasse, Männedorf, Switzerland).

**Statistical analysis**

Results are expressed as means ± standard errors (SE). All experiments were analyzed by ANOVA, followed in some cases by a comparison of treatment means with a control using the Bonferroni-Dunn test. Differences were considered statistically significant at \( P < 0.05 \).

**Results**

The effect of DBD glycoprotein on migration of INT-407 cells

To examine the role of DBD glycoprotein in the migration of intestinal epithelial cells, INT-407 cells were exposed to various concentrations (10–100 μg·mL\(^{-1} \)) of DBD glycoprotein for various times (0–24 h). The cell migration was determined by scratch wound-healing assay. As shown in Fig. 1A, the cell migration was increased by 19%, 28%, and 53% at 10, 50, and 100 μg·mL\(^{-1} \) of DBD glycoprotein for 24 h, respectively, compared to the control. The same results were visually confirmed with a microscope (Fig. 1B). In ad-

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**Table 1  Scoring of disease activity index (DAI)**

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight loss (%)</th>
<th>Stool consistency</th>
<th>Occult/gross bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>1–5</td>
<td>Loose stools</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>5–10</td>
<td>Loose stools</td>
<td>Hemoccult positive</td>
</tr>
<tr>
<td>3</td>
<td>10–20</td>
<td>Diarrhea</td>
<td>Hemoccult positive</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 20</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
</tbody>
</table>

Normal stools: well formed pellets; loose stools: pasty and semi-formed stools which do not stick to the anus; diarrhea: liquid stools that stick to the anus. The DAI value is combined the scores of weight loss, stool consistency, and bleeding \(^{[14]}\).
dition, we found that DBD glycoprotein significantly increases the number of cells that are migrated to the lower surface of the membrane coated with matrigel, compared to the control (Fig. 1C). These results suggest that DBD glycoprotein has the ability to promote the migration and invasion of intestinal epithelial cells.

**The effect of DBD glycoprotein on activation of protein kinase C (PKC)**

To know how DBD glycoprotein regulates intracellular signaling pathway, we next have evaluated the activation of protein kinase C (PKC) which is an important mediator for initiation of migration. The level of PKC phosphorylation was significantly augmented by 2.1 and 2.7-fold at 15 and 30 min after DBD glycoprotein treatment, respectively, compared to the control (Fig. 2A). To clarify the involvement of PKC in migrative signaling pathway occurred by DBD glycoprotein, cells were pre-treated with PKC inhibitor, Bisindolylmaleimide I for 30 min prior exposure to DBD glycoprotein for 24 h (Fig. 2B). Importantly, cell migration was abrogated by blockage of PKC in the cells treated with DBD glycoprotein. These data provide the important evidence that phosphorylation of PKC is required for the cell movement and DBD glycoprotein could promote the cell migration via the activation of PKC.

**DBD glycoprotein induces cell migration through phosphorylation of p38 MAPK**

Mitogen-activated protein kinases (MAPKs) are well-known mediator of cell migration at downstream of PKC. We next analyzed the functional role of DBD glycoprotein on the activation of MAPKs in INT-407 cells. The phosphorylation of p38 MAPK was significantly increased for 30–60 min by treatment with DBD glycoprotein, while the phosphorylation of JNK and ERK was not affected by the DBD glycoprotein (Fig. 3A). Interestingly, increased phosphorylation of p38 MAPK was significantly inhibited by PKC inhibitor, Bisindolylmaleimide I (Fig. 3B), indicating that the phosphorylation of p38 MAPK is regulated by the activation of PKC. Furthermore, pretreatment with the p38 MAPK inhibitor, SB203580 significantly blocked the cell migration induced by DBD glycoprotein (Fig. 3C). Taken together, the above results suggest that DBD glycoprotein is a functional substance that regulates activation of p38 MAPK in the promotion of migration process in intestinal epithelial cells.

**Regulatory effect of DBD glycoprotein on NF-κB activation**

We further examined the role of DBD glycoprotein in activation of nuclear factor-kappa B inhibitor α (IκBα) and nuclear factor-kappa B (NF-κB), that are important intermediates of PKC and MAPK. When the cells were incubated with...
DBD glycoprotein for 60 min, DBD glycoprotein significantly induced the phosphorylation of IκBα and NF-κB at 30 and 60 min (Figs. 4A and 4B). Especially the phosphorylation of NF-κB induced by DBD glycoprotein was significantly blocked by treatment with p38 MAPK inhibitor, SB203580 (Fig. 4C), suggesting that NF-κB activation is downstream event of p38 MAPK activation in the migration of intestinal epithelial cells induced by DBD glycoprotein. Importantly, pretreatment with NF-κB inhibitor, Bay 11-7082, significantly inhibited the DBD glycoprotein-induced migration of INT-407 cells (Fig. 4D), suggesting that activation of NF-κB is involved in the migration of intestinal epithelial cells induced by DBD glycoprotein. **DBD glycoprotein stimulates migration of INT-407 cells via cytoskeletal reorganization-related proteins**

We further determined the potential role of DBD glycoprotein in promoting cell migration mediated by dynamic regulation of cytoskeletal reorganization-related proteins. The levels of α-actinin and profilin-1 (PFN1), which are believed to be essential in the dynamic regulation of filamentous (F)-actin structure were significantly augmented by 2.2 and 1.7-fold at 6 h after treatment with 100 μg·mL−1 DBD glycoprotein, compared to the control. In addition, an increase in F-actin expression was observed after incubation with 100 μg·mL−1 of DBD glycoprotein for 6 h (Fig. 5A). Importantly, the expression of α-actinin, PFN1, and F-actin were significantly abrogated by NF-κB inhibitor, Bay 11-7082 (Fig. 5B), suggesting that the expression of cytoskeletal reorganization-related proteins was regulated by activation of NF-κB. These results indicate that DBD glycoprotein has ability to stimulate cytoskeletal reorganization by increasing expression of α-actinin, PFN1, and F-actin in the promotion of migration process in intestinal epithelial cells.

**DBD glycoprotein normalizes the level of disease activity index (DAI) and colonic length in mice treated with DSS**

To evaluate the functional role of DBD glycoprotein in colonic inflammation, ICR mice were divided into five groups according to the experimental design presented in Fig. 6A. 7-week-old ICR mice were given oral injection of DBD glycoprotein (10 and 20 mg·kg−1) for 7 days prior to co-treatment with 4% of dextran sulfate sodium (DSS) in drinking water for 7 days. As shown in Fig. 6B, treatment with DSS decreased the length of mouse colon by 4.1 cm, compared to the control. However, when the mice were treated with 20 mg·kg−1 of DBD glycoprotein in the presence of DSS, the length of mouse colon significantly recovered by 3.0 cm, compared to the DSS treatment alone. As shown in Fig. 6C, the disease activity index (DAI) characterized by diarrhea, occult blood, and body weight loss was quantified according to Table 1. Consequently, treatment with DSS resulted in reproducible colitis and showed a significant increase in values of DAI from day 3. The values of DAI increased by 9.15 on day 7 after treatment with DSS alone, compared to the control. However, when the mice were treated with 20 mg·kg−1 of DBD glycoprotein in the presence of DSS, the values of DAI significantly decreased by 0.1, 0.5, 1.9, 3.0, and 1.9 on...
day 3, 4, 5, 6, and 7. Histologic examination showed that epithelial ulceration and crypt damage from DSS-induced colitis were markedly restored in the mice treated with 20 mg·kg$^{-1}$ of DBD glycoprotein for 7 days (Fig. 6D). Moreover, we found that DBD glycoprotein has normalizing effect on the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as serum makers for early stage liver dysfunction that are increased by DSS treatment (Fig. 6E). We have further performed additional experiment to confirm the functional role of DBD glycoprotein in murine colitis. We found that DBD glycoprotein has inhibitory effect on the phosphorylation of p38 MAPK, IkBa, and NF-xB in DSS-induced colitis (Fig. 6F). In addition, DBD glycoprotein restored the expression of α-actinin, profilin-1, and F-actin that are decreased by DSS treatment (Fig. 6G).

**Discussion**

Our data demonstrate that 30 kDa phytoglycoprotein isolated from DBD induces cell migration by stimulating the F-actin-related migration signaling events through the PKC-
mediated p38 MAPK/NF-κB pathway and has ability to prevent mice colitis which disrupts the intestinal wound healing process. Recent studies have shown that phytochemicals in fruits and vegetables have a positive effect on the process of wound repair and that it can modulate the behavior of various functional cells in the gut, including their differentiation, proliferation, and migration processes [15]. Many functional glycoproteins have been isolated from various natural...
scales, like mushrooms, fungi, yeasts, algae, lichens, and plants due to the many biological activities including anti-oxidative, immunomodulatory, and proliferative functions based on their high structural variability and polarity. However, the underlying pharmacological mechanisms of these substances against the wound healing in intestinal epithelial cells have not been reported. In the present study, we are the first to show that INT-407 cells treated with DBD glycoprotein have the ability to stimulate cell migration by inducing the activation of PKC and p38 MAPK. Previously, it has been demonstrated that PKC and p38 MAPK implicated in the control of cell adhesion and migration. These results are supported by a previous study which showed that disruption of PKC and p38 MAPK results in impairment of wound healing. Moreover, phosphorylation of p38 MAPK is significantly modulated by PKC in the promoting of cell movement induced by DBD glycoprotein. Consequently, our results suggest that DBD glycoprotein has stimulating effect on wound healing process via activation of PKC and p38 MAPK in intestinal epithelial cells.

It has been shown that MAPKs exists downstream of PKC and regulates many related transcription factors, including nuclear factor-xB (NF-xB), in several cell types, including human intestinal epithelial cells. NF-xB is known as one of the most ubiquitous transcription factors that play critical roles in multiple physiological processes including cell migration, differentiation, and survival. Activation of NF-xB is a pivotal stimulator for migration. Inhibitor xB (IxBa) is an inhibitor of NF-xB, which is bound to NF-xB in the cytoplasm in unstimulated cells, the phosphorylated and degraded IxBa produces nucleus translocation of NF-xB and transcription expressions of NF-xB-related genes. Indeed, earlier studies have identified natural products such as green tea activate the MAPKs and NF-xB pathway in the regulating of many cellular responses. Our results in the present study further showed that the phosphorylation of NF-xB is mediated by p38 MAPK activation and the activation of NF-xB induced by DBD glycoprotein promotes the cell migration. Indeed, earlier work showed that p38 MAPK pathway can influence activation of NF-xB, at least partly, through the physical association. Based on these results, we suggest that DBD glycoprotein induces the NF-xB-dependent signaling pathways via activation of PKC and p38 MAPK, and promotes the migration of INT-407 cells.

Regarding the role of DBD glycoprotein in NF-xB activation, we also determined the potential role of DBD glycoprotein in the regulation of the cytoskeletal reorganization-related proteins α-actinin, profilin-1, and F-actin, which are critical requirements for cell migration. α-Actinin and profilin-1 are prominent F-actin associated proteins and play critical role in the regulation of cytoskeletal network and actin polymerization. Our results demonstrate that DBD glycopro-

Fig. 5 DBD glycoprotein stimulates migration of INT-407 cells via cytoskeletal reorganization-related proteins. (A) Time responses of DBD glycoprotein in the expression of α-actinin, profilin-1, and F-actin are shown (0–6 h). Data represent means ± SE, n = 3. *P < 0.05 vs control. (B) Cells pretreated with NF-κB inhibitor, Bay 11-7082 (10 μmol·L⁻¹) for 30 min and incubated with DBD glycoprotein for 6 h. The level of α-actinin, profilin-1, and F-actin expressions were determined by western blot. Data represent means ± SE, n = 3. *P < 0.05 vs DBD glycoprotein alone.
tein has ability to enhance cytoskeletal reorganization by regulating the level of α-actinin, profilin-1, and F-actin expressions via the activation of NF-κB in the promoting of cell migration. Therefore, we suggest that the signaling pathway induced by DBD glycoprotein is crucially linked to cytoskeletal reorganization process to promote the migration of intestinal epithelial cells.

Finally, we showed convincing in vivo proof that DBD...
glycoprotein has inhibitory effect of inflammatory bowel disease induced by DSS. The DSS is a polyanionic derivative of dextran, phagocytosed by gut macrophage that has ability to elicit the alternation of composition of intestinal flora and the permeation of luminal antigens into the mucosa, and thereby leading to IBD [28]. IBD is a chronic, relapsing and remitting inflammatory disorder of the bowel, consisting mainly of Crohn’s disease (CD) and Ulcerative colitis (UC). Although the exact etiology of IBD remains uncertain, many studies have reported that an impaired wound healing process is a major cause of IBD [29]. In response to injury, therefore, rapid recovering of denuded surfaces and gut barrier function is critical in maintaining intestinal mucosal homeostasis. In these regards, the inhibitory effects of DBD glycoprotein on the levels of DAI and histological inflammation induced by impaired wound healing process mean that DBD glycoprotein is a functional substance that improves wound repair and mucosal healing. Concerning the role of DBD glycoprotein on IBD process, Toll-like receptors (TLRs) signaling also has been shown to be abnormal in several intestinal inflammatory diseases. For example, it has reported that activation of TLR4 in intestinal epithelial cells leads to an inhibition of cell migration and proliferation as well as the induction of apoptosis [30]. This indicate that the potential interferences of DBD glycoprotein on activation of other targets that are related to the IBD process.

In conclusion, our results strongly suggest that DBD glycoprotein triggers PKC-dependent p38 MAPK phosphorylation to regulate NF-κB activation, and that this signaling pathway governs the cytoskeletal reorganization process to

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**Fig. 6** DBD glycoprotein normalizes the level of disease activity index (DAI) and colonic length in mice treated with DSS. (A) ICR mice (n = 5) were pretreated with either PBS or DBD glycoprotein (10 and 20 mg·kg$^{-1}$) for 7 days prior to co-treated with DSS for another 7 days. After 15 days, the changes of colonic length (B) and DAI level (C) were evaluated. Data represent means ± SE, n = 5. *P < 0.05 vs control; †P < 0.05 vs DSS alone. (D) Representative colonic tissues stained with H&E are shown, n = 5. Scale bars, 100 μm (magnification, × 100). (E) The effect of DBD glycoprotein on the serum levels of ALT and AST in mice treated with DSS is shown. Data represent means ± SE, n = 5. ‡P < 0.01 vs control; ‡‡P < 0.05 vs DSS alone. (F) The effect of DBD glycoprotein on the phosphorylation of p38 MAPK, IκBα, and NF-κB in mice treated with DSS is shown. Data represent means ± SE, n = 5. *P < 0.01 vs control; †P < 0.05 vs control; ††P < 0.01 vs DSS alone. (G) The effect of DBD glycoprotein on the expression of α-actinin, profilin-1, and F-actin in mice treated with DSS is shown. Data represent means ± SE, n = 5. *P < 0.05 vs control; †P < 0.05 vs control; ††P < 0.01 vs DSS alone.
promote the migration of intestinal epithelial cells. This functional bioactivity of DBD glycoprotein on cell migration is associated with wound healing effect on mice colitis induced by DSS. These results offer important insight into the potential for the development of therapeutic strategies and agents for various gastrointestinal diseases.

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