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Research Paper

Effects of *Ganoderma lucidum* polysaccharides on IEC-6 cell proliferation, migration and morphology of differentiation benefiting intestinal epithelium healing *in vitro*

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Abstract

Objectives Restoration of epithelial continuity in the intestinal surface after extensive destruction is important since intestinal epithelial cells stand as a boundary between the body's internal and external environment. Polysaccharides from *Ganoderma lucidum* (Gl-PS) may benefit intestinal epithelial wound healing in different aspects, which awaits clarification. To identify potential effects, a non-transformed small-intestinal epithelial cell line, IEC-6 cells, was used.

Methods Effects on epithelial cell proliferation, migration, morphology of differentiation and transforming growth factor beta (TGF-β) protein expression, as well as the cellular ornithine decarboxylase (ODC) mRNA and c-Myc mRNA expression, were assessed, respectively, by MTT assay, wound model *in vitro*, observation under a microscope after hematoxylin and eosin staining, enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction assays.

Key findings It was shown that Gl-PS stimulated IEC-6 cell proliferation and migration significantly in a dose-dependent manner; 10 μg/ml Gl-PS improved the morphology of differentiation in IEC-6 cells. Inefficacy in expression of TGF-β in IEC-6 cells indicated a possible TGF-β independent action of Gl-PS. However, Gl-PS increased ODC mRNA and c-Myc mRNA expression in a dose-dependent manner, indicating, at least partially possible involvement of *ODC* and *c-Myc* gene expression in improvement of intestinal wound healing.

Conclusions These results suggest the potential usefulness of Gl-PS to cure intestinal disorders characterized by injury and ineffective repair of the intestinal mucosa.

Keywords c-Myc; *Ganoderma lucidum* polysaccharides; IEC-6 cell line; intestinal epithelium healing; ODC

Introduction

The intestinal epithelium acts as a gateway that allows absorption of dietary nutrients yet restricts uncontrolled entry of luminal antigens. This selective barrier function is critical for maintaining mucosal immune homoeostasis, as demonstrated in pathologic circumstances (e.g. inflammatory bowel disease, infectious diarrhoea, radiogenic colitis and celiac disease) associated with loss of epithelial integrity. Observations over the past several years have demonstrated the ability of the gastrointestinal tract to restore the continuity of the surface epithelium after extensive destruction.[1] Basically, three different phases can be identified. First, epithelial cells adjacent to, or just beneath, the injured surface migrate into the wound to cover the denuded area. This process has been termed epithelial restitution. Secondly, epithelial cell proliferation takes place to replenish the decreased cell pool, and then maturation and differentiation of the epithelial cells finally enable the epithelium to maintain its functional activity.[2] Therefore, improvement of migration, proliferation and differentiation in epithelial cells, theoretically, can contribute to the restoration of the epithelial integrity after destruction.

Some substances, such as ornithine decarboxylase (ODC), c-Myc and transforming growth factor-β (TGF-β), are closely associated with the restoration of the epithelial integrity after destruction.[3–5] The polyamines, including spermidine, spermine and their

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precursor putrescine, have been widely investigated for nearly three decades.[6] Polyamines are found necessary for normal intestinal mucosal growth. Decreasing cellular polyamines inhibits cell proliferation and disrupts epithelial integrity.[7] A great deal of evidence has shown the importance of polyamines in gastrointestinal mucosal growth.[8] However, intracellular polyamine levels are highly dependent on the activity of ODC, the initial rate-determining enzyme in polyamine biosynthesis.[9] The *ODC* gene is a physiological transcriptional target of c-Myc in association with induction of cell proliferation and transformation, but not with induction of apoptosis.[10] The c-Myc is a transcription factor involved in the regulation of cell proliferation, differentiation and apoptosis. The c-*Myc* gene expression can be stimulated by polyamines, which play a role in the stimulation of cell proliferation in intestinal crypt cells (IEC-6 line).[4,11,12] TGF-β is a multifunctional cytokine widely distributed in tissues. It is produced by many cell types including the gastrointestinal epithelium.[13] TGF-β has been shown to inhibit cell proliferation, to stimulate epithelial migration and to increase the production of extracellular matrix, all processes which promote restitution.[14,15]

Obviously, effects on the ODC and c-Myc as well as TGF-β are important to maintain the continuity of the surface epithelium and rapidly restore it after destruction. Many natural products are known to possess multiple bioactivities.[16–21] We suppose the polysaccharides from *Ganoderma lucidum* (Gl-PS), which are natural products with multiple actions, may possess the activity to improve the restoration of the continuity of the surface epithelium after destruction.

Ganoderma lucidum (Fr.) Krast (Ganodermataceae) is a well-known medicinal mushroom used in China for more than centuries to promote longevity and improve vigor without considerable adverse effects.[22] Gl-PS is the critical component of *G. lucidum* with biological activity. As a natural product, Gl-PS has been widely reported to be effective in inhibiting tumour growth mainly by improving host immune function,[23] preventing oxidative damage,[24] protecting liver and reducing serum glucose levels, along with possessing no toxicity,[25,26] modifying biological response and potentiating immune effectiveness,[27] and antagonizing the suppression of lymphocytes induced by culture supernatants of melanoma cells.[28] The promoting effects of Gl-PS on B16F10 cells to activate lymphocytes have been reported as well.[29] However, little attention has been paid to the effect of Gl-PS in modulating gastrointestinal mucosal function. In this study, we made use of the intestinal epithelial cell line IEC-6 to evaluate the role of Gl-PS on epithelial cell proliferation and restitution in an established in-vitro wounding model and also examined its effects on the morphology of differentiation. Furthermore, the roles of TGF-β, c-Myc and ODC in the Gl-PS-mediated intestinal epithelial cell restoration were investigated as well.

Materials and Methods

Materials

Intestinal epithelial cell line IEC-6 was derived from rat jejunum crypt epithelium[30] and obtained from the cells bank of Peking Union Medical College (Beijing, China) and routinely maintained in the presence of Dulbecco's modified Eagle medium (DMEM) containing 5% inactivated fetal calf serum (FCS), insulin 10 μg/ml, sodium bicarbonate 2.8 g/l, penicillin G sodium 100 U/ml and streptomycin sulfate 100 μg/ml. Recombinant human transforming growth factor beta (TGFβ1) ELISA kit was from Boster Company (Wuhan, China). 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Co (St. Louis, MO, USA). A microplate-reader (Model 550, Bio-Rad, Hercules, CA, USA) and IX71 Inverted fluorescence microscope (Olympus, Tokyo, Japan) were used.

Preparation of the *Ganoderma lucidum* polysaccharides

The *G. lucidum* polysaccharides (Gl-PS), a glycopeptide, hazel-coloured water-soluble powder, was kindly provided by Fuzhou Institute of Green Valley Bio-Pharm Technology.[31] As we previously described,[28] the Gl-PS was isolated from a boiling water extract of fruit bodies of *Ganoderma lucidum*, followed by ethanol precipitation, dialysis and protein depletion using the Sevag method. The Sevag method used in this study was as follows: polysaccharides were dissolved in distilled water, mixed with 0.2 volumes of trichlormethane and 0.04 volumes of n-butyl alcohol, shaken for 30 min, followed by centrifugation and then the precipitate was removed. This procedure was repeatedly performed and discontinued when precipitate was no longer found at the interface between the water and trichlormethane. The monosaccharides in the polysaccharides were determined by gas chromatography. The amino-acid residues contained in the peptides were determined by an Amino Acid Automatic Analyzer.

The Gl-PS was dissolved in serum-free DMEM medium (Gibco BRL, Gaithersburg, MD, USA), then filtered through a 0.22-μm filter and stored at 4°C. It was further diluted to indicated concentrations before each assay.

Determination of cell proliferation

IEC-6 cells (3×10^4) were seeded into 96-well plates (Costar, Pleasanton, CA, USA) in the presence of DMEM containing 5% FCS. Cultures were then supplemented with different concentrations of Gl-PS. After 44 h incubation at 37°C and 5% CO_2, a colorimetric MTT assay was performed, as described previously.[32] Tetrazolium salt thiazolyl blue was added to each well (20 μl) at a concentration of 5 mg/ml and incubation was continued for 4 h. The supernatants were discarded and cells were lysed with dimethyl sulfoxide (DMSO). Metabolization of MTT directly correlating with the cell number was quantitated by measuring the absorbance at 570 nm (reference wavelength 450 nm) using a microplate-reader.

Monolayer wounding and measurement of epithelial cell restitution

IEC-6 cell restitution assays were performed in a modified version of a previously described technique.[2,33] IEC-6 cells were plated in six-well polystyrene plate (Costar, USA) to reach confluence in normal growth media. A denuded epithelial 'cross-centre' wound was created in a standardized fashion by scraping the IEC-6 monolayers with a 200 μl pipette tip. After the scrape, cells were washed twice with D-Hanks to remove any residual cell debris, and the wounded

monolayers were then cultured for 42 h in 2%FCS DMEM in the presence or absence of Gl-PS. Wound areas were viewed under the microscope at various times after scrape-wounding and photographed with an Olympus IX71 microscope. The denuded wound area (ROI, region of interest) was quantified with LEIKA QWIN software (Germany). Two wound areas per well were analysed and each group were triplicates, in at least three independent experiments that were performed. Restitution was calculated as migration ratio (i.e. ((ROI0h-ROI42h)/ROI0h) × 100%). Data were expressed as mean value ± SD (standard deviation), representing at least three independent experiments.

Determination of transforming growth factor-β levels in IEC-6 cell supernatants

IEC-6 cell supernatants were collected using the technique previously described.[34] IEC-6 cells were plated in six-well culture dishes at a density of 2.5×10^5 cells/dish. Twenty-four hours later, media supplemented with different concentrations of Gl-PS were added. At confluence, monolayers were wounded with a 200 µl pipette tip as previously described.[6,8] Cultures were washed twice with D-Hanks. Fresh media supplemented with Gl-PS were added and cell supernatants were collected 24 h later. Secretion of TGF-β in the supernatants was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol.

Assessment of the cell morphology of differentiation

Sterilized coverslips were placed on six-well plates on which there was a drop of DMEM to make the coverslip attach to plate basal firmly. IEC-6 cells were plated at a density of 5×10^5 cells (2 ml/well) and 24 h later, media supplemented with or without 10 µg/ml Gl-PS were added. Forty-eight hours later, coverslips were removed and stained with hematoxylin and eosin (H&E). The cells were observed under a microscope for differentiation.

Determination of ornithine decarboxylase and c-Myc mRNA expression by reverse transcription-polymerase chain reaction analysis

IEC-6 cells treated with or without Gl-PS were harvested 12 h later and total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The concentration of RNA was quantified spectrophotometrically. The cDNA was synthesized according to the Advantage RT-for-PCR Kit protocol of Promega (San Luis Obispo, CA, USA). Diluted samples from these reactions were then used as templates for polymerase chain reaction (PCR).

The mRNA expression of ODC and c-Myc in IEC-6 cells was performed by reverse transcription-polymerase chain reaction (RT-PCR) using Access RT-PCR system (Takara, Ohtsu, Shiga, Japan) according to the manufacture's procedures. Commercial primers to rat glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) (sense 5′-GCC AAG GTC ATC CAT GAC AAC-3′ and antisense 5′-GTC CAC CAC CCT GTT GCT GTA- 3′), rat ODC (sense 5′-TGC TTG ACA TTG GTG GTG-3′ and antisense 5′-TTC TCA TCT GGC TTG GGT-3′) and rat c-Myc (sense 5′- GCT CGC CCA AAT CCT GTA-3′ and antisense 5′-ACC CTG CCA CTG TCC AAC-3′), provided by Shanghai Sangon (Beijing, China), were used to generate products of 498 bp, 355 bp and 385 bp, respectively. PCR reactions included 0.4 µM of each primer, 0.2 mM deoxy-ribonucleoside triphosphate (dNTPs), 1 × PCR-buffer with 15 mM $MgCl_2$ and 2U/µl Taq polymerase (Takara, Japan). The PCR cycling protocol was as follows: 45 s at 94°C, 45 s at 60°C (GAPDH)/57°C (ODC)/59°C (c-Myc) and 2 min at 72°C. This protocol was carried out for 24 cycles (GAPDH)/26 (ODC)/26 cycles (c-Myc) and included an initial 5-min denaturation at 94°C and a final 10-min extension at 72°C. The performed cycles of PCR were chosen to ensure that amplification of all specific cDNA products were exponential after all PCR procedures. Products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide and visualized by UV trans-illumination. The detected DNA was compared with a molecular weight routine (marker) consisting of a 100, 200, 500, 1000, 2000 bp ladder to confirm PCR product size. By using Alpha EaseFC V4.0.0 software (Alpha Innotech Corp), PCR products were quantified by densitometric scanning. ODC and c-Myc expression were normalized relative to the steady-state expression of GAPDH used as internal control, respectively.

Statistical analysis

Data were analysed using one-way analysis of variance followed by least- significant difference (LSD). $P < 0.05$ was considered statistically significant.

Results

Chemical constituents of *Ganoderma lucidum* polysaccharides

The Gl-PS was a glycopeptide with molecular weight of 584 900, and the ratio of polysaccharides to peptides was 93.61 : 6.49%. According to the gas chromatography data, the polysaccharides consisted of D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose and D-glucose with molar ratio of 0.793 : 0.964 : 2.944 : 0.167 : 0.389 : 7.94 and linked together by β-glycosidic linkages. According to the Amino Acid Automatic Analyzer, the 16 kinds of amino acid contained in the peptides were Asp, Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Leu, Phe, Lys, His, Arg, Pro, with a mass ratio of 9.49 : 5.32 : 4.88 : 8.10 : 4.71 : 4.63 : 1.82 : 3.70 : 7.44: 0.54 : 2.56 : 3.05 : 2.22 : 1.23 : 3.47 : 1.76. The prepared *G*l-PS was identified by the wave spectrum.

Effect of *Ganoderma lucidum* polysaccharides on the proliferation of IEC-6 cells

Cells were incubated with different concentrations of Gl-PS (0.1, 1, 10 µg/ml) for 48 h. Gl-PS significantly promoted the proliferation of IEC-6 cells in a dose-dependent manner compared with untreated controls (Figure 1). A maximal promotion of proliferation (35%) effect in IEC-6 cells was observed with a Gl-PS concentration of 10 µg/ml.

Ganoderma lucidum polysaccharides promoted IEC-6 cell restitution

Gl-PS caused a dose-dependent promotion of intestinal epithelial cell restitution in a well-established wounding model

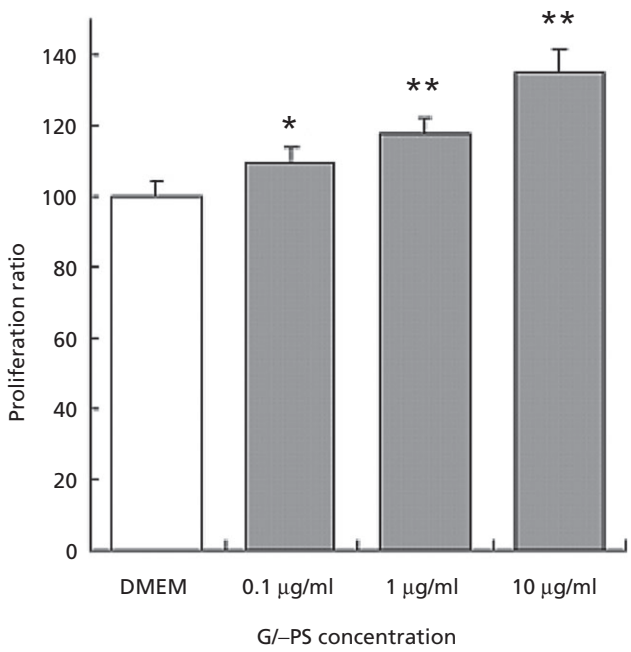


Figure 1 *Ganoderma lucidum* polysaccharides stimulated IEC-6 cell proliferation in a dose-dependent manner. $n = 6$, mean ± SD; $^{*}P < 0.05$ vs DMEM, $^{**}P < 0.01$ vs DMEM.

Table 1 Effect of *Ganoderma lucidum* polysaccharides on transforming growth factor-β level in IEC-6 cell culture supernatant

Group	TGF-β (pg/ml)
DMEM	9.602 ± 0.001
G*l*-PS 5 μg/ml	9.603 ± 0.001
G*l*-PS 10 μg/ml	9.605 ± 0.001
G*l*-PS 20 μg/ml	9.607 ± 0.002

G*l*-PS, *Ganoderma lucidum* polysaccharides; TGF-β, transforming growth factor-β. IEC-6 cells were grown in six-well plates and maintained in media supplemented with 0, 5, 10 or 20 μg/ml G*l*-PS to confluence. IEC-6 monolayers were wounded as described in Materials and Methods. G*l*-PS-supplemented media was replaced following wounding in cultures. Cultures were maintained for 24 h at which time conditioned media was collected and processed as described in Materials and Methods. Values represent means ± SEM from three independent experiments performed in duplicate.

in confluent monolayers of IEC-6 cells (Figure 2). Maximal promotion of restitution (25%) in IEC-6 cells was observed with a *Gl-PS* concentration of 10 μg/ml.

Effects of *Ganoderma lucidum* polysaccharides on transforming growth factor-β level in IEC-6 cell culture supernatants

Recent evidence has supported a central role for TGF-β in the process of intestinal epithelial restitution.[35,36] Therefore, the production of TGF-β was measured to determine any possible involvement of this cytokine in Gl-PS modulation of restitution (Table 1). No significant effect was observed on the production of TGF-β by Gl-PS treatment.

Effect of *Ganoderma lucidum* polysaccharides on IEC-6 cell morphology of differentiation

Cell morphology can reflect the cell status and function. As shown in Figure 3, under microscope after H&E staining, when treated with 10 μg/ml Gl-PS, IEC-6 cells showed a little more typical epithelial form, seemed to arrange more in trabs, even in gland-like forms such as glandular lumen-like or glandular-tube-like, in some parts, suggesting that Gl-PS seems to promote differentiation in IEC-6 cells.

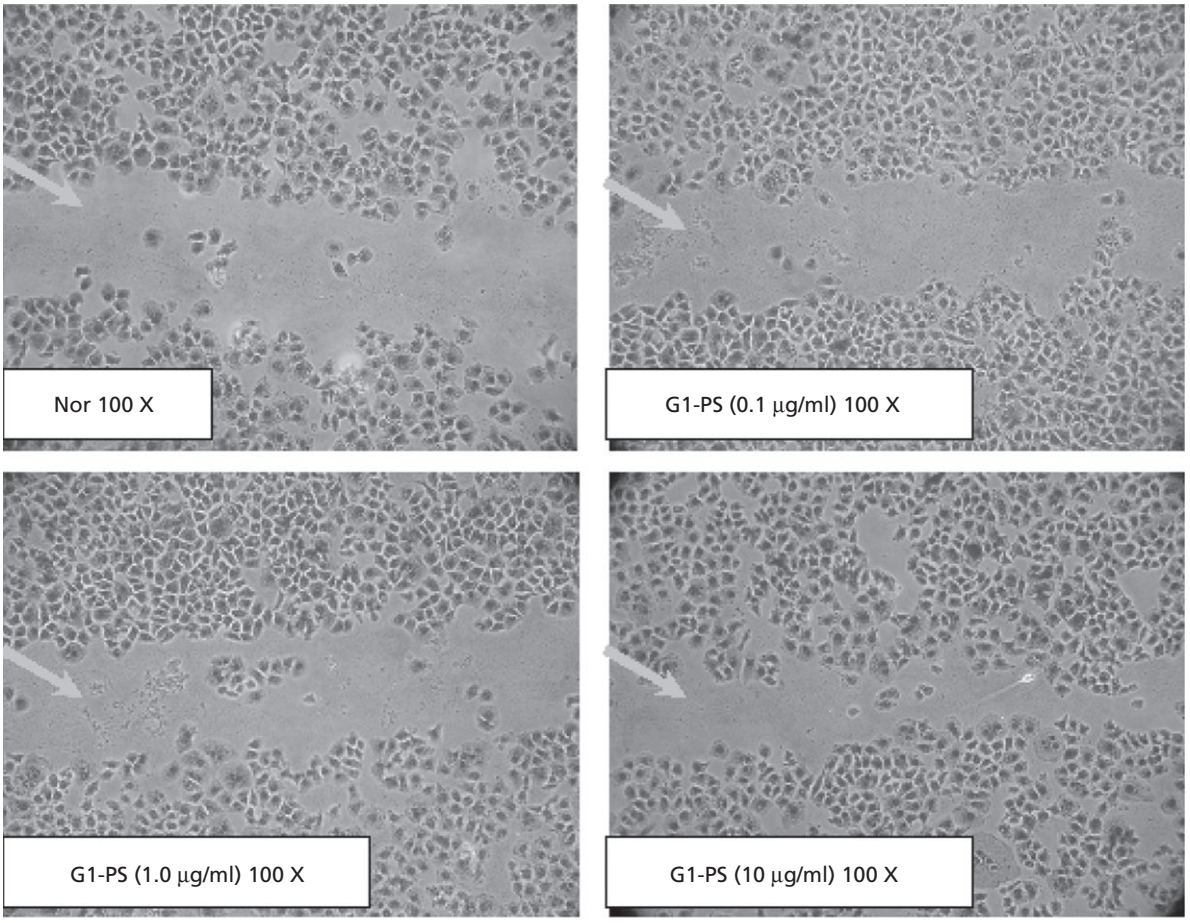
Ganoderma lucidum polysaccharides modulated mRNA expression of ornithine decarboxylase and c-Myc by IEC-6 cells

Semiquantitative RT-PCR was used to determine the effect of Gl-PS on ODC and c-Myc mRNA expression by IEC-6 cells after 12 h. Figures 4 and 5 show that mRNA of ODC and c-Myc was expressed in IEC-6 cells. It also showed that ODC and c-Myc mRNA expression by IEC-6 cells in experimental groups to which Gl-PS (10 and 20 μg/ml) was added were higher than that in normal control groups.

Discussion

Intestinal epithelial cells have traditionally been regarded as passive cells primarily responsible for maintaining the integrity of the intestinal barrier. However, it is now widely appreciated that they are also important regulators of natural and acquired immunity.[37] The intestinal epithelial cell stands as the boundary between the body's internal and external environment. As such, its importance in regulation of the intestinal immune system is paramount.[38] The effects of Gl-PS in inhibiting tumour growth and promoting cellular immunity and humoral immunity[23] have been extensively investigated and it is believed that the anti-tumour effect of Gl-PS is primarily mediated by improvement of immune function. However, there are few data about the effects of Gl-PS on intestinal mucosa, which prompted us to examine whether Gl-PS has direct effects on proliferation, restitution and morphology of differentiation in intestinal epithelial cells. Since it is still difficult to keep primary human intestinal epithelial cells in long-term culture and to simultaneously maintain the capacity of the intestinal epithelium to rapidly proliferate, resembling the high cell turnover rate found *in vivo*, we chose the IEC-6 model cell line. Previous studies had demonstrated the similarity between the non-transformed jejunal crypt-derived IEC-6 cell line and normal rat small intestinal crypt epithelial cells, including growth characteristics, morphology and extracellular matrix synthesis as well as the presence of cell-specific plasma membrane antigens.[30,39,40] The cell line

Figure 2 The effect of *Ganoderma lucidum* polysaccharides (Gl-PS) on IEC-6 cell wound restitution. IEC-6 cells were scrape-wounded and exposed to 0, 0.1, 1 or 10 μg/ml Gl-PS as described in Materials and Methods. Wounds were photographed immediately after addition of *Ganoderma lucidum* polysaccharides (Gl-PS) post-wounding and at 42 h. $n = 3$, mean ± SD; $^{**}P < 0.01$ vs DMEM.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G1-PS (0.1 μg/ml) 100 X
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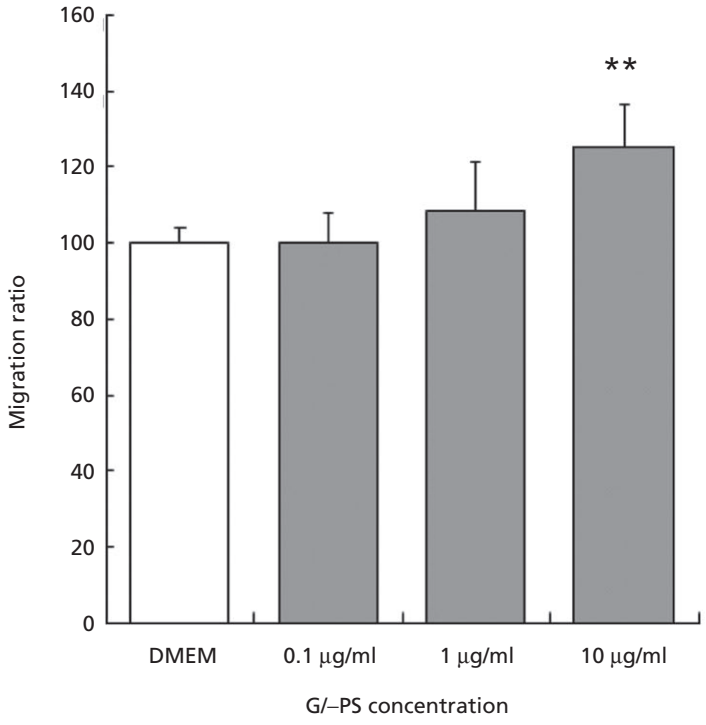
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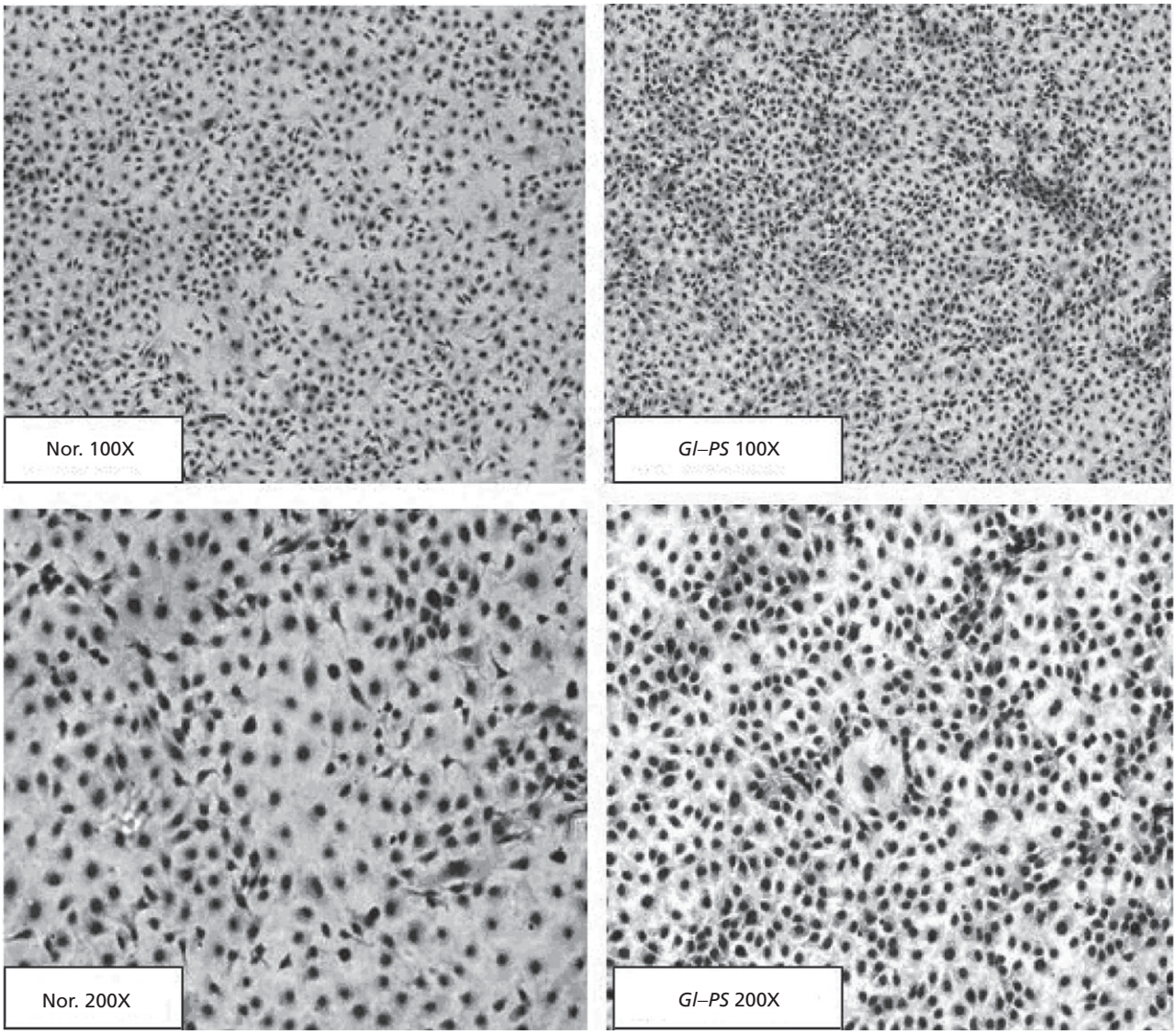

Figure 3 Effects of *Ganoderma lucidum* polysaccharides (Gl-PS) on IEC-6 cell morphology. IEC-6 cells were cultured with or without 10 μg/ml G*l*-PS. Forty-eight hours later the cells were stained with hematoxylin and eosin and observed under a microscope. A little more typical epithelial form, more trabs-like arrangement, even in gland-like forms such as glandular lumen-like or glandular tube-like, in some parts, was observed in the *Gl*-PS-treated IEC-6 cells, suggesting that *Gl*-PS seems to promote differentiation on IEC-6 cells.

keeps the non-differentiated characteristics of small intestinal epithelial stem cells and can be differentiated well under suitable conditions.

The initial mechanism contributing to rapid resealing of epithelial defects after mucosal injury is migration of viable epithelial cells from the wound margin into the denuded area, a process not requiring cell proliferation.[41] The data presented in this study revealed that Gl-PS at a concentration of 10 μg/ml augments the migration of intestinal epithelial cells in an in-vitro model that mimics the early cell division-independent stages of epithelial restitution. When Gl-PS was given immediately after wounding, it dose-dependently increased intestinal epithelial cell migration in a statistically significant manner. Recent advances in oncological study have indicated that increase in cancer cell migration is closely related to the phenomenon of epithelial-mesenchymal transition (EMT), which correlates with the metastatic potential of cancer cells. But Gl-PS with anti-tumour activity[23] (which conflicts with the metastatic potential) increases intestinal epithelial cell migration in physiological conditions, different from oncological conditions.

Although the exact mechanism of restitution has not been elucidated, the cytokine TGF-β has been shown to play an important role in the stimulation of cell migration after wounding. Some data revealed that the intestinal epithelial cells restitution process can be stimulated through TGF-β-dependent pathway.[42,43] However, in others have reported that intestinal epithelial cells restitution is independent of TGF-β.[2] In our study, Gl-PS had no effect on TGF-β expression in IEC-6 cells after wounding, which indicates that Gl-PS stimulates restitution possibly through a TGF-β-independent pathway.

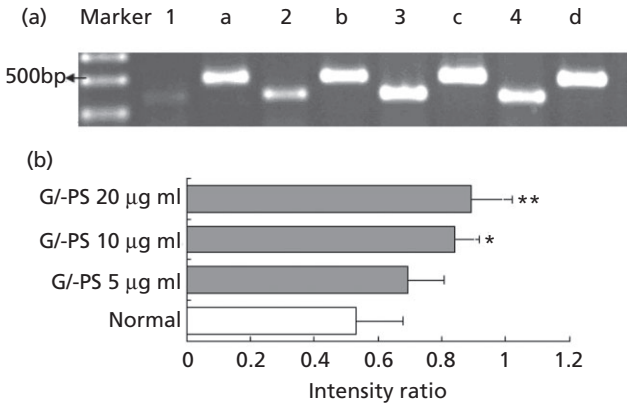

Figure 4 The mRNA expression of ornithine decarboxylase (ODC) and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) in IEC-6 cells assessed by reverse transcription-polymerase chain reaction (RT-PCR). (a) 1–4: mRNA expression of ODC in IEC-6 cells treated with 0, 5, 10 and 20 μg/ml Gl-PS. a–d: mRNA expression of GAPDH in IEC-6 cells treated with 0, 5, 10 and 20 μg/ml *Ganoderma lucidum* polysaccharides (Gl-PS). (b) PCR products were quantified by densitometric scanning and ODC expression was normalized relative to the steady-state expression of GAPDH used as internal control (intensity ratio: ODC to GAPDH). Values represent means ± SEM from three independent experiments. $^{*}P < 0.05$ vs normal, $^{**}P < 0.01$ vs normal.

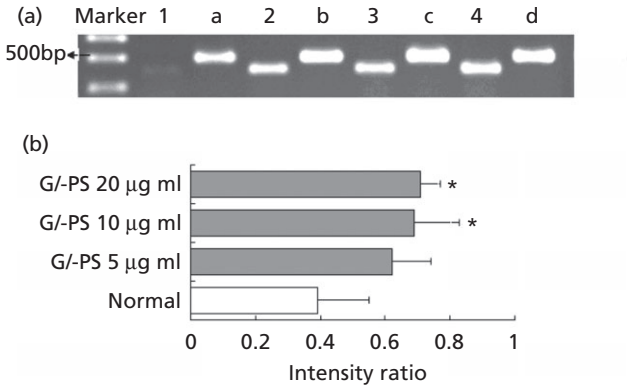

Figure 5 The mRNA expression of c-Myc and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) in IEC-6 cells by reverse transcription-polymerase chain reaction (RT-PCR). (a) 1–4: mRNA expression of c-Myc in IEC-6 cells treated with 0, 5, 10 and 20 μg/ml *Ganoderma lucidum* polysaccharides (Gl-PS). a–d: mRNA expression of GAPDH in IEC-6 cells treated with 0, 5, 10 and 20 μg/ml Gl-PS. (b) PCR products were quantified by the same methods as ODC. $n = 3$, $^{*}P < 0.05$ vs normal.

Epithelial cell proliferation, another essential mechanism for resealing of mucosal wounds in the intestine, was substantially promoted by Gl-PS. This effect was dose-dependent and maximal at a concentration of 10 μg/ml. Besides, epithelial cell differentiation and maturation is the third phase of gastrointestinal tract to be resealed under repair. Morphological examination revealed the possibility that Gl-PS prompts intestinal cell differentiation, although this awaits confirmation by electron microscopy or immunocytochemistry with reasonable molecular markers.

The polyamines are a group of ubiquitously distributed organic cations, which are intimately involved in regulation of growth of the gastrointestinal mucosa.[44] Ornithine decarboxylase (ODC), a pyridoxal phosphate-dependent enzyme, is the first rate-limiting enzyme for the biosynthesis of polyamines. Theoretically, an increase in ODC may lead to an increase in polyamines, although this awaits confirmation, and the extent of the ODC mRNA expression was correlated with the cell proliferation. Treatment of cells of gastrointestinal origin with difluoromethylornithine, a suicide substrate inhibitor of ODC that induces depletion of intracellular polyamines, inhibits proliferation.[45,46]

In addition, induction of the *ODC* gene may play a role in the signalling pathways of several oncogenes. Transformation by activated *ras, v-src* and *myc* appears to be tightly coupled to *ODC* gene expression and polyamine accumulation[47–49] and *ODC* is a transcriptional target of c-Myc.[50] c-Myc has a central and necessary role in the proliferation of normal cells. Following mitogenic stimulation of quiescent cells, c-Myc is rapidly induced and remains elevated, suggesting that it is required for continuous cell growth.[51] Transcription factor *c-Myc* and *ODC* genes may be induced by Gl-PS, while overexpression of these genes may lead to transformation of cells or activation of oncogenes, such as *ras* or *v-src*. Therefore, the oncogenic potential of Gl-PS is of concern. However, it has been shown that Gl-PS possesses anti-tumour activity,[23] which conflicts with its potential oncogenic activity, therefore, withstands the former concern.

In this study, an increase in *c-Myc* and *ODC* gene expression was observed after 12 h exposure to G*l*-PS. It is coincident to the effect of stimulating cell proliferation, indicating the possibility of involvement of promoted *c-Myc* and *ODC* gene expression by Gl-PS, at least partially, in the stimulation of intestinal epithelial cell proliferation by Gl-PS. However, it is reported that *c-Myc* and *ODC* expression were down-regulated during cell differentiation and/or at entry into quiescence.[52,53] Therefore, the effect of Gl-PS in the stimulation of IEC-6 cells differentiation and its mechanism should be further investigated.

Conclusions

It is important to restore continuity in surface epithelium after extensive destruction because intestinal epithelial cell stands as boundary between body's internal and external environment. In this study, it was demonstrated that G*l*-PS accelerate wound repair by stimulation of cell proliferation, migration and possibly differentiation in intestinal epithelial cells. The improvement of migration is independent of the TGF-β pathway. The ODC and c-Myc transcription were increased by Gl-PS implying the possibility of Gl-PS acting through the *c-myc* gene, which activates ODC expression and subsequently stimulates cell proliferation. These results suggest the possible use of Gl-PS for the treatment of intestinal mucosal healing.

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