Carbohydrate Polymers 81 (2010) 340–347

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Relationship of the inhibition of cell migration with the structure of ginseng pectic polysaccharides

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ARTICLE INFO

Article history:
Received 31 December 2009
Received in revised form 13 February 2010
Accepted 15 February 2010
Available online 4 March 2010

Keywords:
Cell migration
Cell adhesion
Cell spreading
Ginseng pectin
Homogalacturonan
Rhamnogalacturonan I

ABSTRACT

In this paper, ginseng pectin (WGPA) was fractionated and two new fractions were characterised. Nine fractions, including seven characterised in our previous paper, were tested for their effects on cell migration. The HG-domain rich pectins caused significant inhibition on cell migration. The order of the inhibitory effects is WGPA-1-HG < -2-HG < -3-HG < -4-HG. At 0.015 mg/ml, WGPA-3-HG significantly inhibited L-929 cell migration. WGPA-3-RG and -4-RG, containing both HG- and RG-I-domains, showed a slightly stronger inhibition than the HG-domain rich fractions. WGPA-N, WGPA-1-RG and -2-RG, containing no, trace or minor HG and RG-I domains, showed less effects on cell migration. The inhibitory effect of ginseng pectic polysaccharides was related to GalA content (HG domain) and Rha content (RG-I domain). The effect is probably mediated through decreasing cell adhesion and cell spreading.

1. Introduction

Panax ginseng C.A. Meyer (ginseng) is a plant medicine that has been used in Asian countries for a long time and has many pharmacological activities (Attele, Wu, & Yuan, 1999; Chang, Seo, Gyllenhaal, & Block, 2003; Choi, 2008). Pectin is an active component of ginseng that can inhibit gastric lesions (Kiyoyaha et al., 1994), inhibit adhesion of bacteria to host cells (Lee, Shim, Chung, Lim, & Kim, 2009; Lee et al., 2006), and protect animals from the lethal effects of ionising radiation (Kim et al., 2007; Song et al., 2003). In addition, it can reduce blood glucose levels in normal and hyperglycemic mice (Konno, Sugiyama, Kano, Takahashi, & Hikino, 1984; Suzuki & Hiking, 1989), inhibit tumor growth and metastasis (Kim, Kang, & Kim, 1990; Shin et al., 2004; Yun, Lee, Jo, & Jung, 1993), and modulate the immune system (Du, Jiang, Wu, Won, & Choung, 2008; Han et al., 2005). Ginseng pectin is mainly composed of arabinogalactan (AG), type I rhamnogalacturonan (RG-I), and homogalacturonan (HG) (Zhang et al., 2009). Although many studies on the bioactivities and structures of ginseng pectin have been performed, the mechanisms by which ginseng pectin exerts these effects are still not well understood, and the structure–activity relationship remains unclear.

Cell migration is a fundamental process in normal physiology as well as pathology. Many biological phenomena are related to cell migration, such as the movement during the differentiation of stem cells in normal tissue turnover (Min et al., 2008) and leukocyte infiltration into areas of injury in immune responses (Szabo, Ablin, & Singh, 2004). Cell migration contains four interdependent physical steps: protrusion, adhesion, contraction, and de-adhesion and retraction (Lauffenburger & Horwitz, 1996; Nobes & Hall, 1995; Vicente-manzanares, Webb, & Horwitz, 2005). The experimental results indicated that cell migration is influenced by a variety of extracellular molecules such as soluble growth factors and the extracellular matrix (ECM) components (Gupton & Waterman-storer, 2006; Ren, Kiosses, & Schwartz, 1999; Toole, 2002). Hyaluronan and proteoglycans are the main carbohydrate components of the ECM. Their functions in regulating cell migration have been demonstrated by a number of studies in recent years (Cattaruzza & Perris, 2005; Cohen, Kam, Addadi, & Geiger, 2006; Evanko, Tammi, Tammi, & Wight, 2007; Lin, Ren, Greiling, & Clark, 2005; Sugahara et al., 2003). It has been reported that a modified citrus pectin MCP blocked chemotaxis of human umbilical endothelial cells (Nangia-Makker et al., 2002). Another modified citrus pectin (GCS-100) blocks vascular endothelial growth-factor-induced migration of multiple myeloma cells (Chauhan et al., 2005). The pectic polysaccharides from citrus and swallow root have

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doi:10.1016/j.carbpol.2010.02.028

inhibitory effects on MDA-MB-231 and cancer buccal cell invasiveness *in vitro* (Sathisha, Jayaram, Nayaka, & Dharmesh, 2007).

Ginseng pectin, like most pectins from plants, has very complex structures, and is difficult to separate into homogenous fractions. This difficulty prohibits studying mechanisms of activity and structure–activity relationships. Recently, our research group has fractionated ginseng pectin (WGPA) into 2 homogenous fractions of arabinogalactans (WGPA-1-RG and -2-RG) and 4 homogenous HG-rich pectic polysaccharides (WGPA-1-HG, -2-HG, -3-HG and -4-HG), allowing studies of this kind (Zhang et al., 2009). Subsequently, AG, RG-I and HG domains in ginseng pectin were analysed (Yu et al., 2010). In this paper, we further fractionated ginseng pectin and collected two new fractions, WGPA-3-RG and WGPA-4-RG. The WGPA and all fractions from WGPA were then tested for their effects on cell migration in an attempt to determine whether the medicinal uses are supported by pharmacological effects.

2. Materials and methods

2.1. Materials

Sepharose CL-6B gel and mannan were purchased from Sigma. RPMI 1640 medium and fetal calf serum were obtained from Gibco. Crystal violet was purchased from Genview. Trypsin (E.C. 3.4.21.4) was obtained from Amresco. Penicillin/streptomycin antibiotics were obtained from PAA. All other reagents were of analytical grade or better.

2.2. Ginseng pectin preparation and fractionation

Ginseng pectin WGPA and its fractions WGPA-N, -1-RG, -2-RG, -1-HG, -2-HG, -3-HG, and -4-HG were prepared and characterised in our previous publication (Zhang et al., 2009). Briefly, ginseng roots were extracted with hot water and the polysaccharides were precipitated by ethanol. After deproteination using the Sevag method, the polysaccharides were fractionated by a combinatory procedure shown in Fig. 1. WGPA-N, -1-RG, -2-RG, 1-HG, -2-HG, -3-HG and -4-HG were characterised in our last publication. In the present paper, WGPA-3-RG and -4-RG were characterised by sugar determination and chromatography on Sepharose CL-6B.

2.3. Chromatography on Sepharose CL-6B

For analytical chromatography, each sample (in 0.15 M NaCl) was applied to a Sepharose CL-6B column (1.5 cm × 90 cm) and eluted with 0.15 M NaCl at a flow rate of 0.15 ml/min. The eluate was collected at 3 ml per tube and assayed for distribution of total sugar and uronic acids contents. WGPA-3-RG and -4-RG were prepared on a preparative Sepharose CL-6B column (3.0 cm × 90 cm) and eluted with 0.15 M NaCl at 0.5 ml/min. The eluate (10 ml per tube) was collected and assayed for total sugar and uronic acids. The appropriate fractions were combined, concentrated, dialysed against distilled water and lyophilised.

2.4. Determination of sugar composition

Sugar composition analysis was performed as described by Zhang et al. (2009). Each polysaccharide sample (2 mg) was hydrolysed with 2 M TFA at 120 °C for 1 h. The resulting monosaccharides were derivatised with 1-phenyl-3-methyl-5-pyrazolone (PMP) and subsequently analysed on a pre-calibrated DIKMA Inertsil ODS-3 column (4.6 mm × 150 mm) assembled on a Shimadzu HPLC system, eluting isocratically at 1 ml/min with 18% (v/v) acetonitrile 82% (v/v) in 0.1 M phosphate buffer, pH 7.0 and monitored by absorbance at 245 nm.

2.5. Cell culture

L-929 (mouse embryonic fibroblast), HT-1080 (human fibrosarcoma) and HeLa (human adenocarcinoma of cervix) cells were obtained from American Type Culture Collection. All cells were grown in RPMI 1640 medium supplemented with 10 mmol/l HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2.

2.6. Cell migration by scratching wound assays

Cells were plated at the density of 2.5×10^5 cells/ml in RPMI 1640 growth medium containing 10% fetal calf serum into 12-well cell culture plates (Costar) and incubated for 24 h. The culture medium was aspirated and the pretreatment medium (culture medium plus various carbohydrate samples) was added and incubated for 24 h. The cell monolayer was scratched with a horizontal and a vertical line crossing each other with a 200-μl pipette tip. The cross made for each well was routinely performed to allow subsequent measurements. The cells were washed twice with PBS and fresh pretreatment medium was added. Migration of cells into wounded areas was observed using an inverted microscope at 100× magnification and photographed. The width of the scratch was measured and referred to as W_{before}. The wounds in the culture were allowed to heal for 3 h at 37 °C and the width was measured again and referred to as W_{after}. Control cells were pretreated and

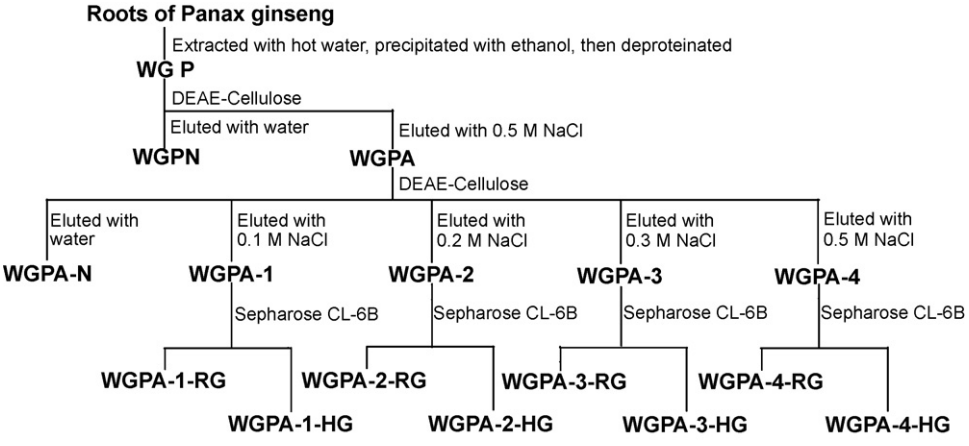

Fig. 1. Fractionation procedure of ginseng polysaccharides.

scratched similarly but pretreatment medium without carbohydrates was used.

Migrating distance was determined by W_{before} minus W_{after}. The relative migration, defined as the ratio of the migrating distance of carbohydrate-treated cells to that of control cells, was presented for each assay. The migration of the control was set as 100.

2.7. Cell migration by transwell assays

Transwell assays were performed using 24-well Transwell units with 8 μm pore size polycarbonate inserts (BD Biosciences). Cells grown on tissue culture plates were incubated for 24 h in pretreatment medium with or without 0.5 mg/ml of pectin as described in the scratching wound assays and collected with PBS containing 1 mM EDTA after a 30 min incubation at 37 °C. Cells were resuspended at a density of 5×10^4 in 200 μl pretreatment medium without serum and seeded into the upper chamber of the Transwell unit. The lower chamber was filled with complete pretreatment medium containing 10% fetal calf serum. Cells were allowed to migrate for 3 h at 37 °C in a CO_2 incubator. The cells on the upper side of the filter were removed using a cotton swab. The cells that migrated to the underside were fixed in 2.7% paraformaldehyde for 15 min at room temperature and stained in 0.5% crystal violet in 20% methanol 80% H_2O for 10 min. Subsequently, the filter was washed three times with distilled water to remove excess dye and cells solubilised with 1% SDS for 30 min. The resulting cell extract was transferred to a 96-well plate at 150 μl per well and read at 570 nm using a microplate reader. The absorbance determines the number of cells that migrated and is compared to untreated control.

2.8. Cell adhesion assays

L-929 cells grown on a 12-well tissue culture plate (Costar) were incubated for 24 h in pretreatment medium with or without 0.5 mg/ml polysaccharide samples. The medium was then changed to PBS and the plate was rotated at 250 rpm for 2 h on an orbital shaker. The cells released into the medium were collected into microcentrifuge tubes and pelleted by centrifugation. Both the released cells and the cells remaining on the plate were fixed with paraformaldehyde, stained with crystal violet, and measured at 570 nm as described above in the transwell migration assay. The percentage of cells remaining on the plate after rotation, representing relative cell adhesion strength, was calculated in each case.

2.9. Cell spreading assays

L-929 cells grown on tissue culture plates were incubated for 24 h in pretreatment medium with or without(as control) 0.5 mg/ml of pectin and then detached as described in the transwell assays. Collected cells were re-suspended in their respective pretreatment medium at the density of 2×10^5 cells/well. Cells were re-plated onto 12 mm diameter glass coverslips and adhered for 1, 2, and 3 h at 37 °C in a CO_2 incubator. Afterwards, the plate was gently rinsed three times with PBS by rotating at 100 rpm for 5 min each to remove the non-adherent cells. The remaining cells on the coverslips were fixed in 2.7% paraformaldehyde for 15 min at room temperature and viewed using a phase contrast microscope. Ten randomly chosen fields per condition with 120–500 cells each were photographed at 200× magnification. All images were analysed for cell morphologies that were classified as non-spreading and spreading cells.

2.10. Statistical analysis

The data were expressed as the means ± SD. The significance of differences between values was checked with one-way ANOVA. A level of $p < 0.001$ was accepted as statistical significance.

3. Results

3.1. Ginseng pectin preparation and fractionation

In our previous study, ginseng pectin WGPA was separated and sequentially fractionated into five fractions, WGPA-N and WGPA-1 to WGPA-4 on DEAE-Cellulose, as shown in Fig. 1 (Zhang et al., 2009). Neutral polysaccharide WGPN containing glucans and arabinogalactans had wide molecular weight distribution without further fractionation. Each of the acidic fractions WGPA-1 to WGPA-4 gave two main populations on Sepharose CL-6B column and eight fractions named WGPA-1-RG to -4-RG and WGPA-1-HG to -4-HG were obtained. WGPA-1-RG and 2-RG and WGPA-1-HG to -4-HG were characterised in our previous publication and all showed homogenous molecular weight distribution. In the present paper, we characterised WGPA-3-RG and WGPA-4-RG. WGPA-3-RG and WGPA-4-RG show wide molecular weight distribution (Fig. 2). Their sugar compositions were mainly GalA, Ara, Gal and Rha. Additionally, they contained small amounts of Glc, GlcA and Man (Table 1). Compared to WGPA-1-RG and -2-RG, WGPA-3-RG and -4-RG contained more GalA and Rha. The sugars GalA/Rha in WGPA-3-RG and -4-RG were 20.2/7.3 and 38.4/11.4, respectively, GalA/Rha of both fractions is larger than 1, implying that WGPA-3-RG and -4-RG probably contained both RG-I and HG domains. The sugar compositions and structural features of all obtained fractions are listed in Table 1.

3.2. Inhibitory effects of WGPA on cell migration

The effects of ginseng pectin WGPA on the migration of L-929, HT-1080 and HeLa cells were tested using both scratching and transwell assays. In scratching assays, quantitative analyses revealed that the migration of L-929 and HT-1080 cells was reduced by WGPA treatment, but the migration of HeLa cells was not affected (Fig. 3A). When L-929 and HT-1080 cells were treated with 0.50 mg/ml of WGPA, the relative migration was 30% and 56% of

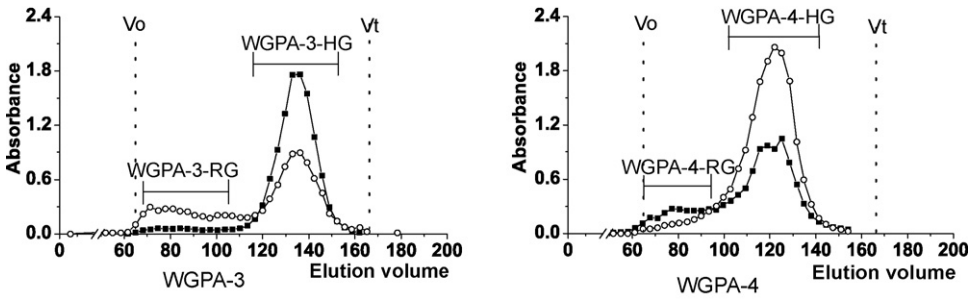

Fig. 2. Molecular weight distributions of WGPA-3 and WGPA-4 on Sepharose CL-6B, eluted with 0.15 M NaCl (total sugars, -■-; uronic acid, -○-).

Table 1
Composition and structure of ginseng pectic polysaccarides.

Fraction	Main sugar composition (%)						Structure
	GalA	Rha	Ara	Gal	Glc	GalA/Rha	
WGPA	44.2	2.5	15.5	18.0	18.5	–/–	AG, RG-I and HG
WGPA-N[a]	–	–	15.7	18.0	66.3	0.0/0.0	Glucan and AG
WGPA-1-RG[a]	1.8	0.2	34.0	56.2	3.5	1.8/0.2	AG with trace RG-I
WGPA-2-RG[a]	5.3	4.1	40.9	44.4	2.9	5.3/4.1	AG with minor RG-I
WGPA-3-RG	20.2	7.3	38.0	29.0	3.2	20.2/7.3	HG and RG-I
WGPA-4-RG	38.4	11.4	26.1	13.5	4.4	38.4/11.4	HG and RG-I
WGPA-1-HG[a]	62.4	3.6	7.1	15.2	7.6	62.4/3.6	HG with minor RG-I
WGPA-2-HG[a]	83.6	3.0	4.6	5.1	1.9	83.6/3.0	HG with minor RG-I
WGPA-3-HG[a]	90.9	1.5	2.2	3.5	1.3	90.9/1.5	HG with trace RG-I
WGPA-4-HG[a]	92.1	–	–	5.9	2.0	92.1/0.0	HG with little RG-I

[a] The fractions from our previous publication, Zhang et al. (2009).

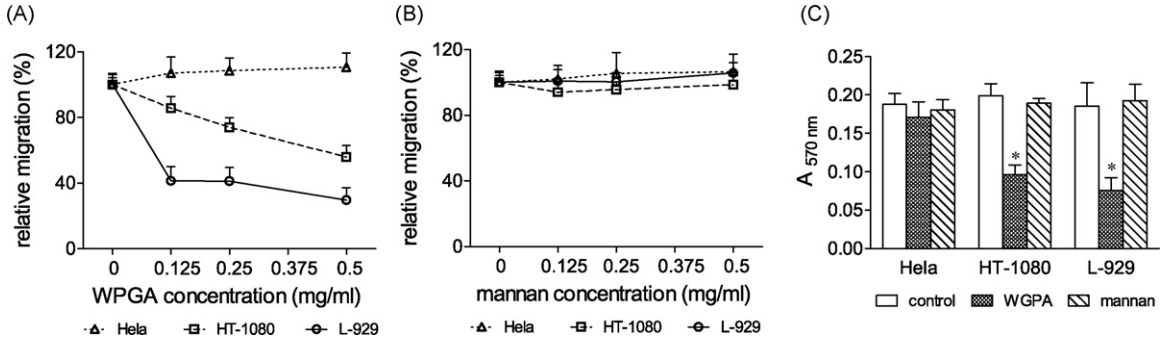

Fig. 3. The effect of WGPA on the cell migration. (A) The effect of WGPA on cell migration as measured by scratching assays; (B) the effect of mannan on cell migration as measured by scratching assays; (C) the effect of WGPA and mannan on cell migration as measured by transwell assays. The values are means ± SD from three experiments. $^{*}p < 0.001$.

control, respectively. In contrast, treatment with yeast mannan did not affect the migration of the three test cell lines (Fig. 3B). The higher concentrations of mannan slightly increased the migration. These data indicate that the inhibitory effect on cell migration was related to the structures of WGPA and the effect was different on the cell lines.

To further verify the above findings, transwell assays were also employed to check the migration effect of WGPA on L-929, HT-1080 and HeLa cells. The migration unit was assembled such that the upper chamber of the transwell unit contained pretreated cells in the pretreatment medium but lacked serum, while the lower chamber contained complete pretreatment medium. Thus, the serum (10%) in the lower chamber functioned as the chemoattractant in this assay. After incubation for 3 h, the number of cells that had migrated to the underside of the filter was determined by measuring the absorbance at 570 nm following crystal violet staining and solubilisation. As shown in Fig. 3C, the numbers of L-929 and HT-1080 cells migrating through the filter were reduced by 0.50 mg/ml of WGPA. The maximum reduction at 3 h was 59% and 52% for L-929 and HT-1080 cells, respectively. Mannan had no effect on the migration of any test cell line and the migration of HeLa cells was not affected by either WGPA or mannan. The results of cell migration in the transwell assay were consistent with the observations in scratching assay.

3.3. Ginseng pectin did not alter cell viability

Cell viability was determined using an MTT assay. Live cells can utilise MTT and produce formazan, which can be measured by absorbance at 570 nm after being solubilised. The assay results showed that cells pretreated with 0.5 mg/ml of WGPA for 24 h had similar absorbance to that of control cells. The absorbance was 1.93 ± 0.13 for the control and 1.97 ± 0.09 ($n = 3$) for WGPA. In addition, pretreated cells continued to proliferate. Even with pretreatment for 48 h, the absorbance was similar to control. These data indicate that ginseng pectin did not alter cell viability, at least under the conditions used in cell migration assay, implying that the effect of ginseng pectin on cell migration was not the consequence of cytotoxicity.

3.4. The effects of different ginseng pectic polysaccharides on cell migration

Ginseng pectin WGPA is a mixture of structurally different pectic polysaccharides. Therefore, the observed effect was from the contribution of all its components. To investigate the structural features of polysaccharides that caused the inhibitory effects on cell migration, we examined each fraction of WGPA for their activities on L-929 cell migration. Fig. 4 shows representative images of L-929 cell migration affected by WGPA-3-HG and WGPA-3-RG. The quantitative analysis results in Fig. 5 show that the fractions without (WGPA-N) and with low GalA (WGPA-1-RG and -2-RG) had little effect on L-929 cell migration. All HG-domain rich fractions (WGPA-1-HG, -2-HG, -3-HG and -4-HG) had significant effects. The lowest inhibition was caused by WGPA-N, -1-RG and -2-RG, followed by -1-HG, -2-HG, and -3-HG and -4-HG, increasing with the content of GalA. However, WGPA-3-RG and -4-RG had slightly stronger effects than -3-HG and -4-HG, with relative migrations at 0.5 mg/ml of about 46% and 40% of control, respectively, though they contained moderate GalA content. In comparing their structural features, it was noted that WGPA-3-RG and -4-RG contained both RG-I and HG domains, while WGPA-3-HG and -4-HG mainly contained the HG domain, implying that the RG-I domain might play an important role on the inhibition of cell migration. The inhibitory effects of all tested pectins on L-929 cells showed a dose-dependency. Further dose-effect relationship tests indicated that the reduction

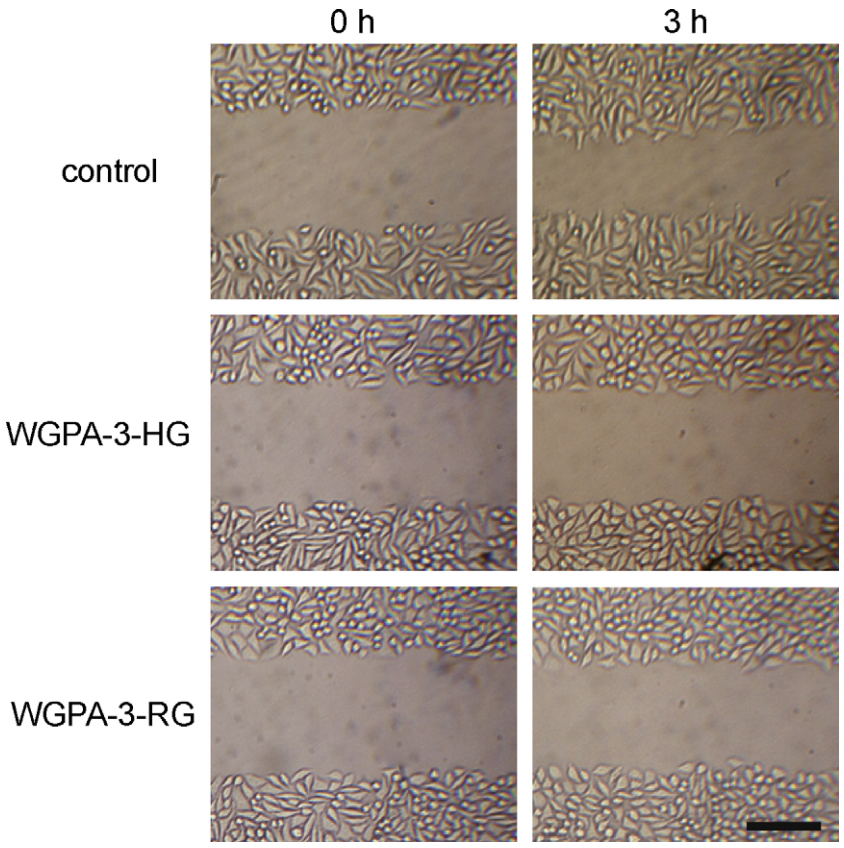

Fig. 4. Representative images of the inhibition of L-929 cell migration by ginseng pectic polysaccharides in a scratching assay. L-929 cells grown on a 12-well tissue culture plate were incubated for 24 h in medium without (control) or with 0.5 mg/ml of polysaccharides. Then, the cells were scratched and allowed to migrate for 3 h; the cells were photographed at 0 h and 3 h. Bar, 300 μm.

of L-929 cell migration by WGPA-3-HG was 25%, 33%, 34%, 45%, 47% and 50% at the concentrations of 0.015, 0.031, 0.062, 0.125, 0.25, and 0.5 mg/ml, respectively. The statistical analysis shows that WGPA-3-HG significantly inhibited L-929 cell migration from a concentration as low as 0.015 mg/ml ($p < 0.001$).

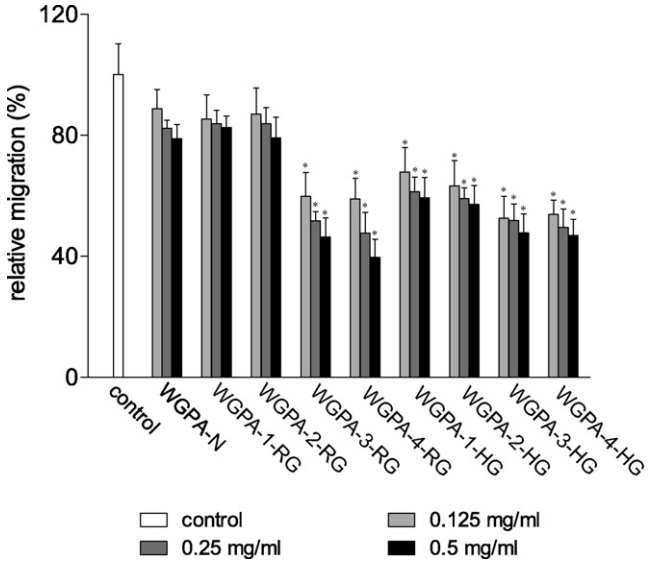

Fig. 5. The effects of different pectic ginseng polysaccharides on L-929 cell migration. Cells treated without (control) or with 0.125, 0.25 or 0.5 mg/ml of polysaccharides were tested in scratching assays. Values are means ± SD from three experiments, $^{*}p < 0.001$.

3.5. Effects of different pectic ginseng polysaccharides on cell adhesion to substratum

Cell adhesion plays a pivotal role in cell migration. It has been shown that moderate adhesion usually gives rise to fast migration, while both weak and strong adhesion lead to retarded migration (Ananthakrishnan & Ehrlicher, 2007). Therefore, we investigated the effects of ginseng pectins on L-929 cell adhesion, which might be related to the retardation of cell migration. To quantify the strength of cell adhesion, cells pretreated without (control) or with ginseng pectins were subjected to vigorous rotation on an orbital shaker. The speed of rotation was adjusted such that weakly adherent cells were released into the medium while firmly adherent cells remained on the plate. At the end of the rotation, both the cells in the medium and the cells on the plate were measured. Under the rotation of 250 rpm for 2 h at room temperature, 40% of control L-929 cells remained on the plate, and 39%, 25% and 25% of the cells treated by WGPA-1-RG, WGPA-3-RG and WGPA-3-HG remained, respectively (Fig. 6). These data indicate that control cells adhered more strongly on the substratum than the cells treated by WGPA-3-RG and WGPA-3-HG, but not WGPA-1-RG. The result that WGPA-3-RG and -3-HG decreased L-929 cell adhesion and WGPA-1-RG had no effect is similar to the observation that WGPA-3-RG and -3-HG inhibited L-929 cell migration but WGPA-1-RG had no effect on the migration. This implies that the decrease of L-929 cell adhesion might cause the inhibition of cell migration.

3.6. Effects of different pectic ginseng polysaccharides on L-929 cell spreading

As cell spreading is an important process during cell migration, we investigated the effects of pectins on L-929 cell spreading. After

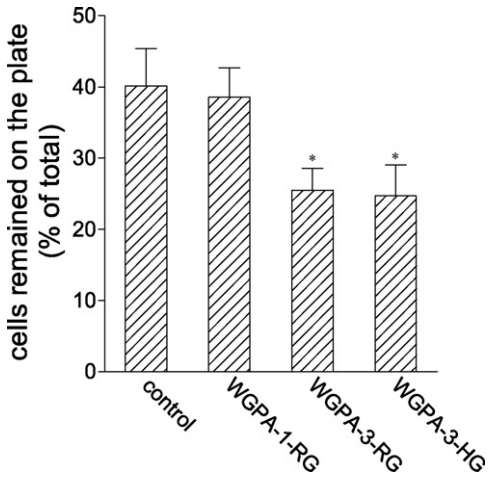

Fig. 6. Effects of different pectic ginseng polysaccharides on L-929 cell adhesion. L-929 cells pretreated with 0.5 mg/ml of polysaccharides for 24 h were washed with PBS and rotated at 250 rpm for 2 h. The cells remaining on the plate under each condition was plotted. This experiment was repeated three times. Values were means ± SD, $^{*}p < 0.001$.

pre-treatment with WGPA-1-RG, WGPA-3-RG or WGPA-3-HG, the cells were detached, re-plated on glass cover slips and incubated for 1, 2, and 3 h for cell adhesion and spreading. The adherent cells were fixed and examined using a phase contrast microscope, and the images representing the morphologies of the control and pectin-treated cells are shown in Fig. 7A. There were three typical morphologies under the microscope: non-spreading cells that were round with smooth rims, partially spread cells that were enlarged and flattened with small protrusions, and well-spread cells that exhibited polygon or fibroblastoid morphologies. Here, both partially spread and well-spread cells were categorised and counted as spreading cells. The percentage of spreading cells was calculated and plotted (Fig. 7B). As shown in Fig. 7A and B, control cells spread quickly, and spreading cells accounted for 37%, 59%, and 68% of total adherent cells after 1, 2, and 3 h of incubation, respectively. At these time points, spreading cells were 1.8%, 24% and 35% in the case of WGPA-3-RG, and 2.4%, 28%, and 39% in the case of WGPA-3-HG, indicating that WGPA-3-RG and WGPA-3-HG treatment significantly inhibited L-929 cell spreading. However, by WGPA-1-RG treatment, which had no significant inhibition of cell migration, there was no significant effect on cell spreading. These data indicate that WGPA-3-RG and -3-HG specifically impaired cell spreading, which might contribute to their inhibition on cell migration.

4. Discussion

The effects of some plant pectins on cell migration and related phenomenon have been reported in the literature (Chauhan et al., 2005; Nangia-Makker et al., 2002; Sathisha et al., 2007), but the structural features related to such effects have not yet been elucidated. In this paper, we tested structurally different polysaccharides from ginseng pectin for their effects on cell migration. The results showed that structurally different ginseng pectins caused significantly different effects. WGPA-1-HG through -4-HG, which are characterised to be HG-domain rich pectins, significantly inhibited L-929 cell migration in a dose-dependent manner. Among the HG-domain rich pectins, the inhibitory effects on cell migration increased with the content of GalA. WGPA-3HG and -4-HG (which contain the highest content of GalA) exhibited significant inhibition from 0.015 mg/ml and reached maximum inhibition (about 50%) at about 0.125 mg/ml. These results suggested that the HG domain might be an important functional element for the inhibition of cell migration.

The WGPA-1-RG and -2-RG fractions, characterised as AG polysaccharides containing little GalA, had no significant inhibitory effects on L-929 cell migration, which further supported the idea that the inhibitory effect on cell migration is related to the content of GalA. WGPA-3-RG and -4-RG contained moderate GalA content, 20.2% and 38.4%, respectively, a much lower amount than the HG-domain-rich fractions. However, they showed significant inhibitory effects, similar to that caused by the HG-domain-rich fractions. In comparing the structural features of tested fractions, it was noted that WGPA-3-RG and -4-RG contain both RG-I and HG-domains. This contrasts the AG fractions WGPA-1-RG and -2-RG, which lack the RG-I and HG domains, and the HG-rich fractions WGPA-1-HG through -4-HG, which contain the HG but not RG-I domains. Thus, we speculated that in addition to HG domain, the RG-I domain (representing the Rha-containing structure unit) might also be an important functional element for cell migration.

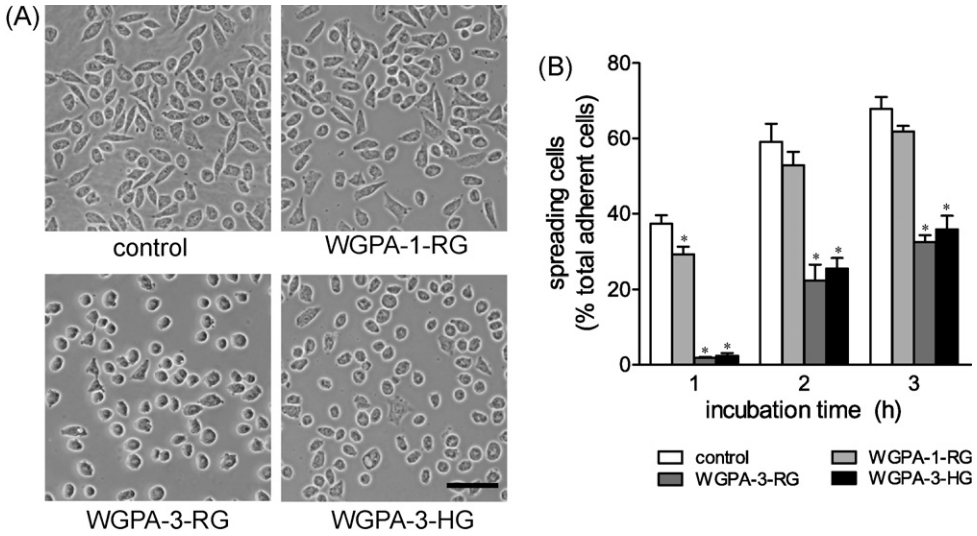

Fig. 7. Effects of pectic ginseng polysaccharides on L-929 cell spreading. Cells pretreated without (control) or with 0.5 mg/ml of each pectin sample were plated on glass coverslips and incubated for 1, 2 or 3 h. The adherent cells were measured by a phase contrast microscope. (A) Representative images of the control and pectin-treated cells after 2 h incubation. Bar, 100 μm. (B) Quantification of cell spreading. Values were means ± SD from three experiments, $^{*}p < 0.001$.

The L-929 cell viability was not changed under the conditions used in the cell migration assays, implying that the inhibition of cell migration was not caused by cytotoxic effects. Further evidence for a lack of cytotoxicity was the finding that ginseng pectin selectively inhibited the migration of different cells. Specifically, ginseng pectin inhibited L-929 and HT-1080 cell migration, but did not affect HeLa cells. Fibroblastic and epithelial cells express different cell adhesion molecules, possess different actin cytoskeletal organizations, and have different migration properties (Omelchenko et al., 2001). Ginseng pectin may target the migration machinery specific to fibroblastic cells. To investigate the mechanism of inhibition of cell migration, we tested the effects of the pectic polysaccharides from ginseng pectin on cell adhesion and cell spreading. WGPA-3-RG and -3-HG reduced L-929 cell adhesion about 15% as compared to control, while WGPA-1-RG had no effects. Combining the results that WGPA-3-RG and -3-HG inhibited about 50% of L-929 cell migration and WGPA-1-RG had no inhibition, we deduced that the effect on the adhesion might partially account for the reduction of migration. Other processes, such as cell spreading, might be involved in the effects on L-929 cell migration, too. Indeed, both WGPA-3-RG and -3-HG caused about 35% reduction of L-929 cell spreading compared to control. Thus, we deduced that the effect of ginseng pectin on both cell adhesion and spreading might contribute to the reduction of cell migration.

Many factors act on cell adhesion and cell spreading and these may ultimately lead to the inhibition of cell migration. The molecules that ginseng pectin binds to or associates with remain unknown. Galectin-3 is a widely expressed galactoside-binding lectin that participates in a variety of cellular processes both in normal physiological and pathological conditions (Glinsky & Raz, 2009; Gunning, Bongaerts, & Morris, 2009; Inohara & Raz, 1995; Sathisha et al., 2007). It has been reported that some pectins and pectin fragments could specifically bind to galectin-3. Therefore, it is reasonable to think that ginseng pectins might exert their effects on cell adhesion and spreading through binding to galectin-3. However, Gunning et al. (2009) recently reported that an HG-domain-rich citrus pectin showed no interact with galectin-3. Thus, it is unlikely that WGPA-1-HG to -4-HG fractions interact with galectin-3. However, WGPA-3-RG and WGPA-4-RG may affect cell migration via interaction with galectin-3 as they contain more galactose. Ongoing studies in our laboratory are focused on searching for the target molecules and detailed studies of the structure–activity relationships in order to elucidate the molecular mechanisms underlying the effects of ginseng pectin on cell migration.

In summary, ginseng pectin WGPA was fractionated and two new fractions, WGPA-3-RG and WGPA-4-RG, were characterised. Thus, including the fractions purified in previous work, nine fractions have been obtained from ginseng pectin. The effects of these fractions on cell migration were tested. The results showed that the inhibition was related to GalA content (HG domain) and Rha content (RG-I domain). The HG-rich pectins caused significant inhibition on L-929 cell migration. The inhibitory sequence was least in WGPA-1-HG, followed by -2-HG, -3-HG, and -4-HG. WGPA-3-RG and -4-RG containing both HG and RG-I domains, showed significant inhibition, too. WGPA-N, WGPA-1-RG and -2-RG containing little HG nor RG-I domains, showed less effects on cell migration. The mechanism of the inhibitory effect is probably due to decreasing cell adhesion and cell spreading.

Acknowledgements

This work was supported by National Natural Science Foundation of China (30670478, 30973857) and Natural Science Foundation of Jilin Province (20070710).

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