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Research Article

Ginseng berry polysaccharides on inflammation-associated colon cancer: inhibiting T-cell differentiation, promoting apoptosis, and enhancing the effects of 5-fluorouracil

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ABSTRACT

Background: Ginseng is a commonly used herbal medicine in treating various medical conditions. Chronic gut inflammation is a recognized factor for the development of colorectal cancer (CRC). In this project, Asian ginseng berry polysaccharide preparations were used to assess their effects on CRC and related immune regulation mechanisms.

Methods: Ginseng berry polysaccharide extract (GBPE) and purified ginseng berry polysaccharide portion (GBPP) were used to evaluate their activities on human HCT-116 and HT-29 CRC cell proliferation. Interleukin-8 secretion analysis was performed on HT-29 cells. Naive CD4 cell isolation and T-helper cell differentiation were performed and determined using flow cytometry for Th1 and Treg in addition to cell cycle and apoptotic investigation.

Results: GBPE and GBPP significantly inhibited interleukin-8 secretion and cancer cell proliferation, inhibited $CD4^+IFN\text{-}\gamma^+$ cell (Th1) differentiation, and decreased $CD4^+FoxP3^+$ cell (Treg) differentiation. Compared to the GBPE, GBPP showed more potent antiinflammatory activities on the malignant cells. This is consistent with the observation that GBPP can also inhibit Th1-cell differentiation better, suggesting that it has an important role in antiinflammation, whereas Treg cells hinder the body's immune response against malignancies. Supported by cell cycle and apoptosis data, GBPE and GBPP, at various degrees, remarkably enhanced the anticancer activities of 5-fluorouracil.

Conclusion: Data from this project suggested that Asian ginseng berry potentially has clinical utility in managing enteric inflammation and suppressing CRC through immunomodulation mechanisms.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is the most commonly used herbal medicine worldwide [1,2]. In Oriental countries, the root of Asian ginseng has been used for centuries as a panacea to dismiss various medical conditions and improve general well-being [1,3]. A case–control study showed that the ginseng intakers had a remarkably decreased risk for cancer compared with nonintakers

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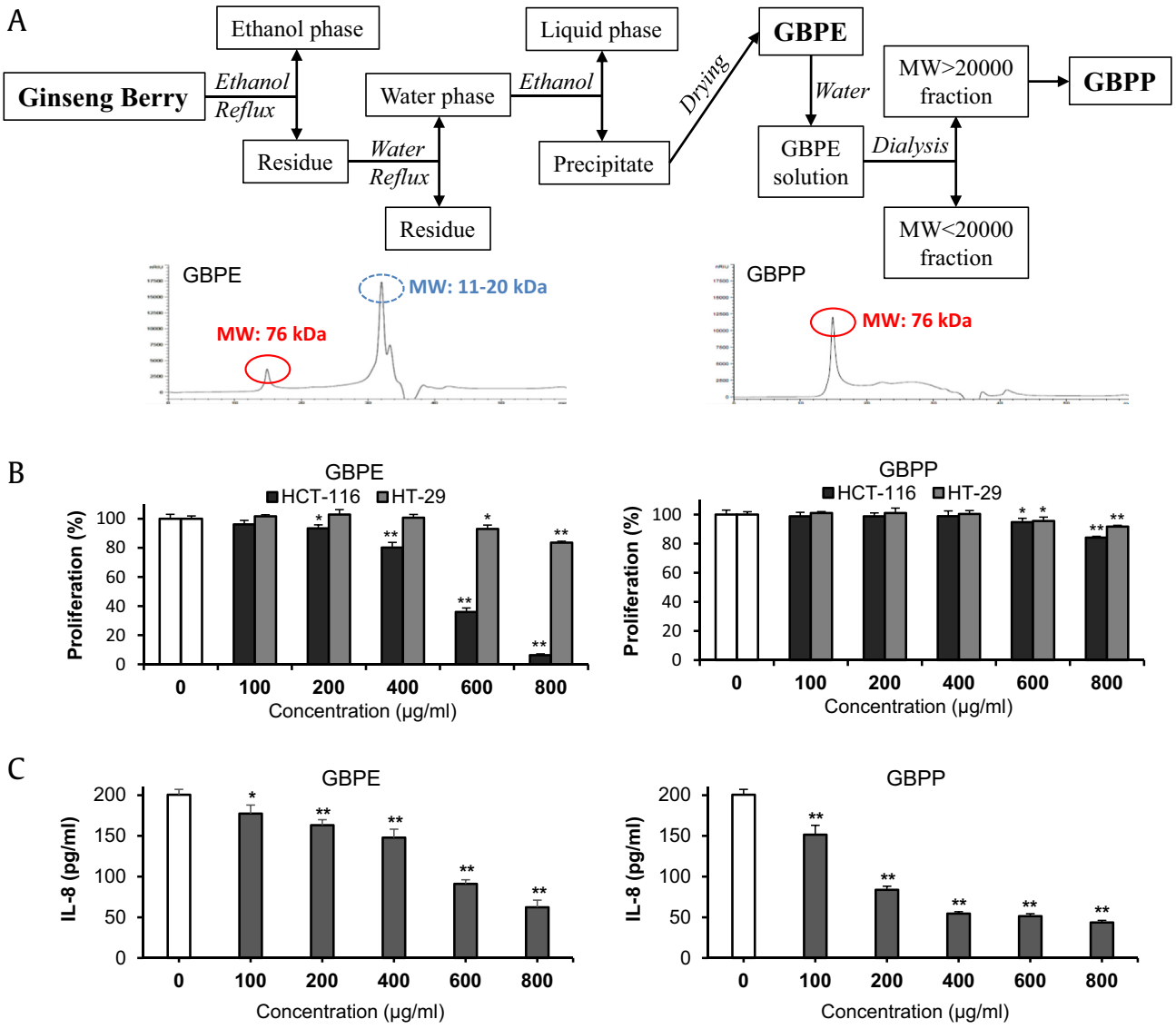

Fig. 1. Preparation of ginseng berry polysaccharide extract (GBPE) and ginseng berry polysaccharide portion (GBPP) and evaluation of their antiproliferative and antiinflammatory effects on human colorectal cancer cells. (A) Preparation flow chart (upper) for GBPE and GBPP and their high-performance size-exclusion chromatograms (lower). (B) Effects of GBPE and GBPP on colon cancer cell proliferation. HCT-116 and HT-29 cells were exposed to GBPE and GBPP for 48 h, and cell proliferation was determined by the MTS assay. (C) Effects of GBPE and GBPP on inflammatory cytokine IL-8 secretion in HT-29 cells. The basal level of IL-8 secretion from HT-29 cells was very low (<20 pg/ml). LPS-induced IL-8 secretion was inhibited by both berry polysaccharides. Results are expressed as the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle control; student *t* test. IL-8, interleukin-8; LPS, lipopolysaccharide; MW, molecular weight; SD, standard deviation.

[4–6]. Our group previously reported the preventive effects of ginseng on colorectal cancer (CRC) in different animal models [7–10].

Inflammatory bowel disease (IBD) is a group of inflammatory conditions in the large and small intestine. Chronic inflammation has been recognized as a risk factor for CRC [11,12]. Interleukin-8 (IL-8) is a proinflammatory cytokine, which plays a crucial role in the pathogenesis of IBD, and links to multiple aspects of the inflammatory response [13]. To prevent colon carcinogenesis and malignant progression, targeting inflammatory pathways has been shown to be effective [14]. Published data suggests that ginseng suppresses colitis by reducing enteric inflammation, and its inflammatory inhibitory property may play an important role in CRC chemoprevention [9,15].

One of the major types of IBD is Crohn's disease. Previous studies have shown that in the pathogenesis of this disease, it is pivotal for the dysregulation of the local immune system [16,17]. In this medical condition, the local immune response appears to consist of predominantly T-helper 1 cells or Th1 cells, and the Th1-mediated immunopathology plays a critical role in the induction and perpetuation of intestinal inflammation [18,19]. Furthermore, regulatory T cells or Treg cells are a component of the immune system that suppresses immune responses of other cells [20,21], and Treg cells obstruct the body's immune response against malignancies [20,22]. Natural products have been widely used by patients with cancer to boost their immune systems [14,23]. To study ginseng's effects on CRC, it is important to evaluate the action of this botanical on Th1 and Treg cells.

Many botanical polysaccharides have been identified as potential gastroprotective agents. These compounds could also prevent CRC formation and suppress tumor growth through modulation of the immune responses [24–26]. Compared to the frequently used root of Asian ginseng, investigations on ginseng berry are considerably limited. With regard to oncology studies, published reports have shown that triterpenoid glycosides of the berry inhibited human cancer cell growth and that these compounds could enhance

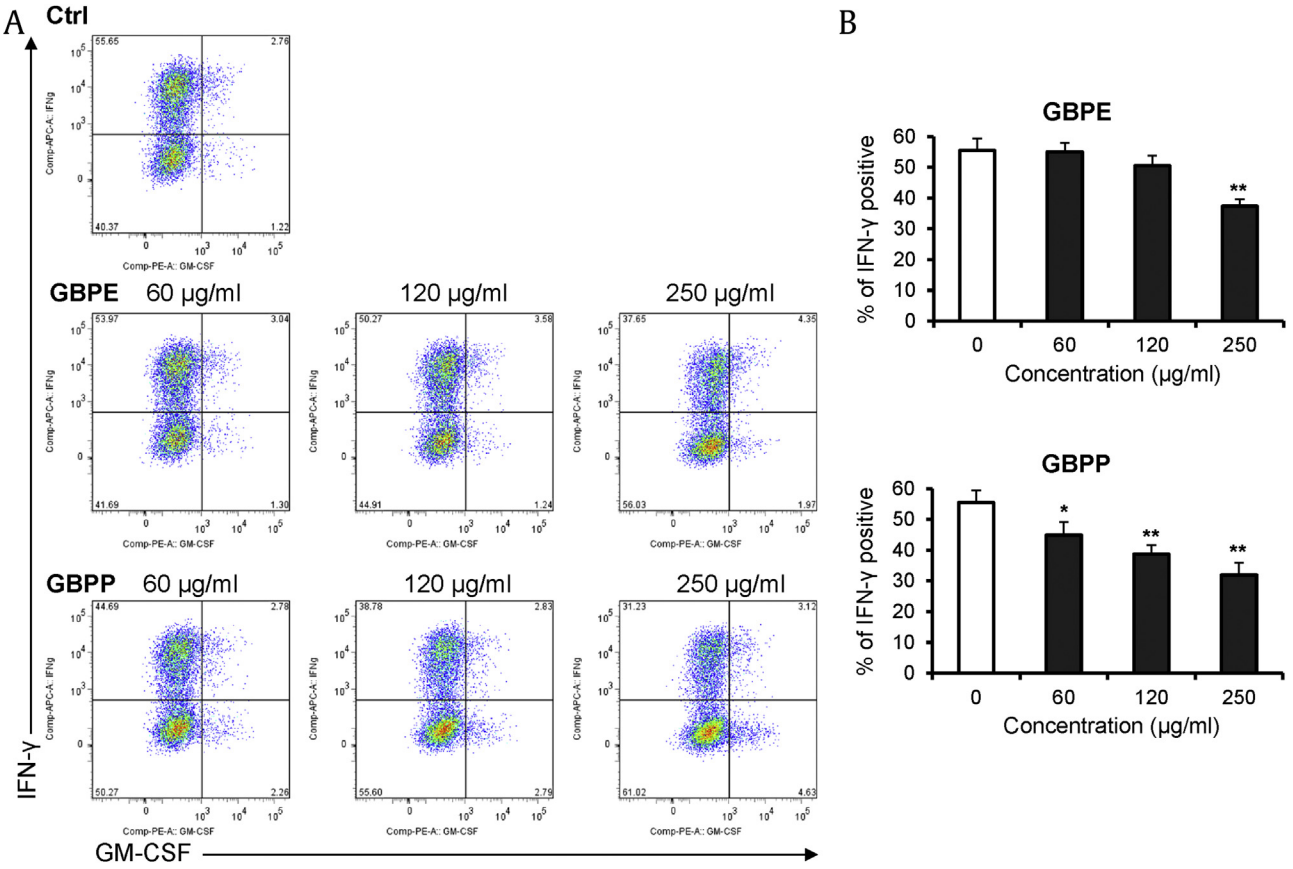

Fig. 2. GBPE and GBPP inhibited T-helper 1 cell differentiation. (A) Naive CD4+ T cells isolated from spleens and draining lymph nodes from WT B6 mice were stimulated under Th1-polarizing condition in the presence or absence of 60, 120, and 250 μg/mL for 3 days. Cells were then restimulated with PMA and ionomycin for 4 h, followed by an intracellular cytokine staining and flow cytometry. (B) Concentration-related decrease in Th1 frequency was observed after both ginseng berry polysaccharide treatment. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control; student t test.
GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion; IFN-γ, interferon-γ; WT, wild-type; GM-CSF, granulocyte-macrophage colony-stimulating factor.

the chemopreventive activities of 5-fluorouracil (5-FU) on cancer cells [27,28]. We recently observed the anti-CRC effects of ginseng berry polysaccharides on human colon cancer cell lines [29]. However, the mechanism of action of the berry polysaccharides on gut inflammation–medicated CRC has not been explored.

In this study, two preparations of Asian ginseng berry polysaccharides, GBPE and GBPP, were used to assess antiproliferation and antiinflammation effects. The adaptive immune response of these polysaccharide compounds on differentiations in Th1 and Treg cells was subsequently evaluated. Cancer cell cycle and apoptosis analyses were also investigated. Finally, whether these ginseng berry polysaccharides enhanced the antiproliferative actions of 5-FU was quantified.

2. Methods and materials

2.1. Study materials

Crude ginseng berry polysaccharide extract (GBPE) and purified ginseng berry polysaccharide portion (GBPP) were obtained from the AmorePacific Corp. (Seoul, South Korea). In brief, Asian ginseng berries were extracted with 90% ethanol. Then, the remaining residues were extracted with water. We precipitated the crude berry polysaccharides GBPE from the supernatant with ethanol and then lyophilized. The GBPP was prepared by dialyzing GBPE solution with a molecular weight cutoff at 20,000. GBPE and GBPP were analyzed with a Young-Lin 9500 HPLC (YL Instruments, Gyeonggi, South Korea). Separation was carried out in a 200 GL Superdex column (GE Healthcare, Anaheim, CA, USA) (Fig. 1A). Sigma-Aldrich Inc. provided 5-FU.

2.2. CRC cells and proliferation assay

HCT-116 and HT-29 human colon cancer cells were cultured in McCoy's 5A medium at 37°C (5% CO_2, 10% fetal bovine serum). The cancer cells were seeded in 96-well plates, and drug treatment time was 48 hr. At the end of the treatment, MTS assay, a colorimetric method for quantification of viable cells, was used to test cell proliferation.

2.3. IL-8 secretion assay

Using 24-well plates, HT-29 cells were cultured for 48 h. Fresh medium containing lipopolysaccharide (LPS, 100 ng/ml) was added, which served as a control. Different concentrations of GBPE or GBPP and LPS added in the medium were set as the experimental group. After incubating for 6 hr, the secreted IL-8 in the medium was determined by enzyme-linked immunosorbent assay.

2.4. Naive CD4 cell isolation and differentiation of T-helper cells

The spleens of 4- to 6-week-old naive C57BL/6 mice were used to prepare single-cell suspensions. Using biotinylated primary antibodies and streptavidin-coated secondary magnetic particles,

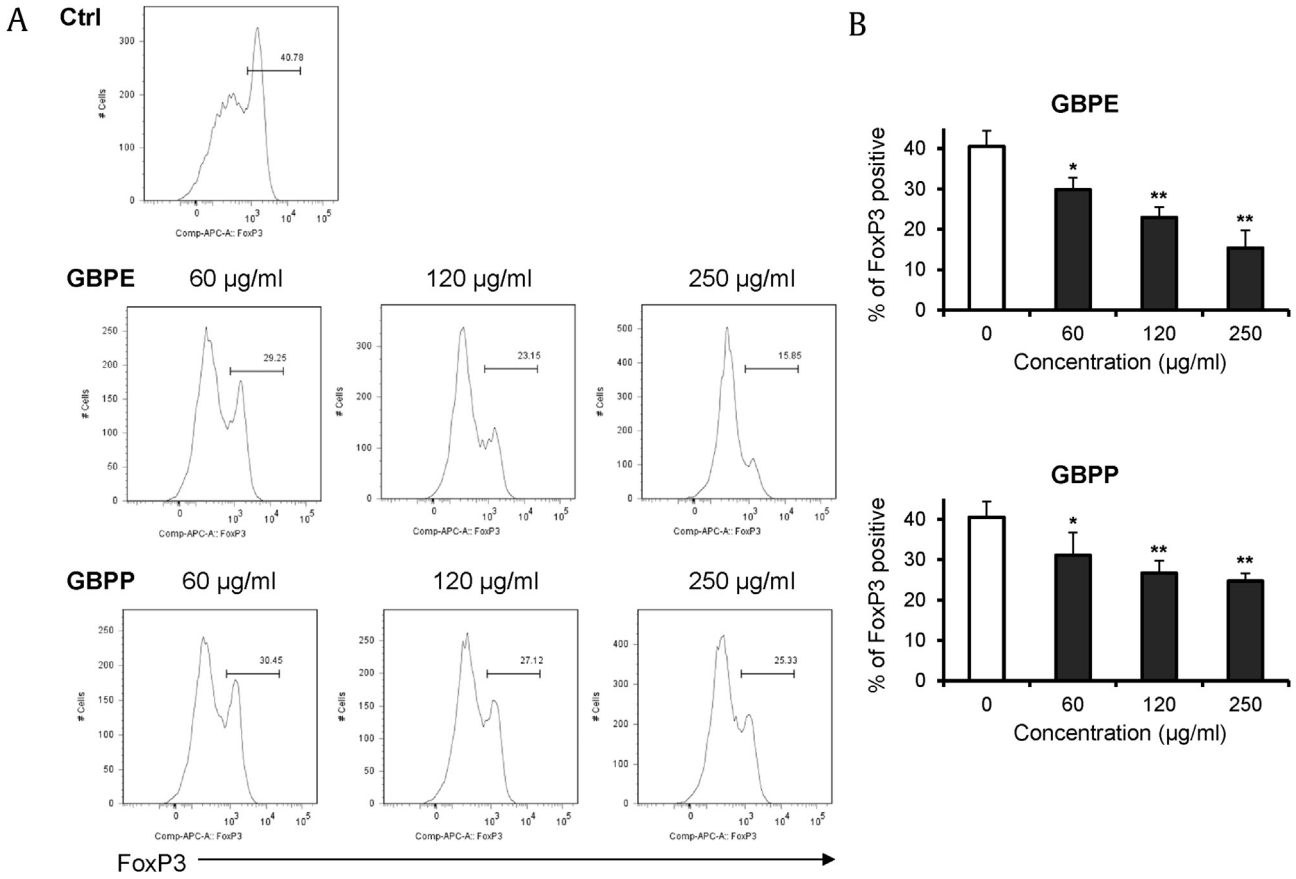

Fig. 3. GBPE and GBPP negatively regulate Treg generation. (A) A Treg generation assay was performed *in vitro* in the presence of GBPE or GBPP or medium alone (control). Naive CD4+ T cells extracted from WT B6 mice by magnetic microbeads were cultured with anti-CD3/CD28 antibodies, TGF-β, and increasing concentrations of GBPE and GBPP (both at 60, 120, and 250 μg/mL) or control. After 3 days of culture, the percentage of $CD4^+FoxP3^+$ cells was evaluated (Tregs). (B) Both GBPE and GBPP reduced Treg generation. $^*p < 0.05$ and $^{**}p < 0.01$ versus control; student *t* test.
GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion; TGF-β, transforming growth factor-β; WT, wild-type; APC-A, allophycocyanine-autofluorescence.

pooled splenocytes were depleted of $CD11b^+$, $CD8\alpha^+$, and $CD19^+$ cells. The enriched cells were sorted on the flow cytometer for naïve CD4 cells.

Naive CD4 T cells precoated with anti-CD3 and anti-CD28 were cultured in RPMI medium containing polarizing cytokines. The cytokines were anti-mIL-4, anti-mIFN-γ, hTGF-β1, and mIL-2 for Treg; anti-mIL-4 and mIL-12 for Th1; and anti-mIL-4, mIL-6, anti-mIFN-γ, and hTGF-β1 for Th17. Th0 cells were considered to be stimulated in "neutral" conditions (anti-mIFN-γ, anti-mIL-4, but without cytokines). At the start of cell culture, testing compounds were added. After 3 days, the differentiated cells were harvested and restimulated with ionomycin and phorbol myristate acetate (PMA).

2.5. Flow cytometry for Th1- and Treg-cell determination

After fixing, permeabilizing, and intracellularly staining with fluorochrome-conjugated anti-mGM-CSF, anti-mIFN-γ, and anti-FoxP3, harvested cells were analyzed using a BD FACSCanto II cytometer (BD Biosciences, San Jose, CA, USA). Data were analyzed using FlowJo software (Tree Star, Oten, Switzerland).

2.6. Cancer cell cycle analysis

After treatment with GBPE or GBPP for 48 h, cells were harvested. After fixing and permeabilizing, cells were stained with propidium iodide (PI) and RNase and then analyzed using BD LSR II flow cytometer (BD Biosciences, San Jose, CA, USA). At least 20,000 cells were counted for each assay.

2.7. Apoptotic analysis

After treatment with GBPE or GBPP for 48 h, cells, including floating cells, were harvested. Cells were stained with annexin V-fluorescein isothiocyanate (FITC) and PI and then analyzed using BD LSR II flow cytometer. At least 20,000 cells were counted for each assay.

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD) (n = 3). A student *t* test and a one-way analysis of variance with Tukey's *post hoc* test were used to test the significance of the differences between treatment and control groups. The statistical significance was set at $p < 0.05$.

3. Results

3.1. GBPE and GBPP inhibited CRC proliferation and IL-8 secretion

The molecular weight profile of the crude polysaccharides GBPE was analyzed using size-exclusion HPLC. As shown in the chromatogram in Fig. 1A, the two peaks were well separated. The molecular weight for the first peak is 76 kDa, and that for the second

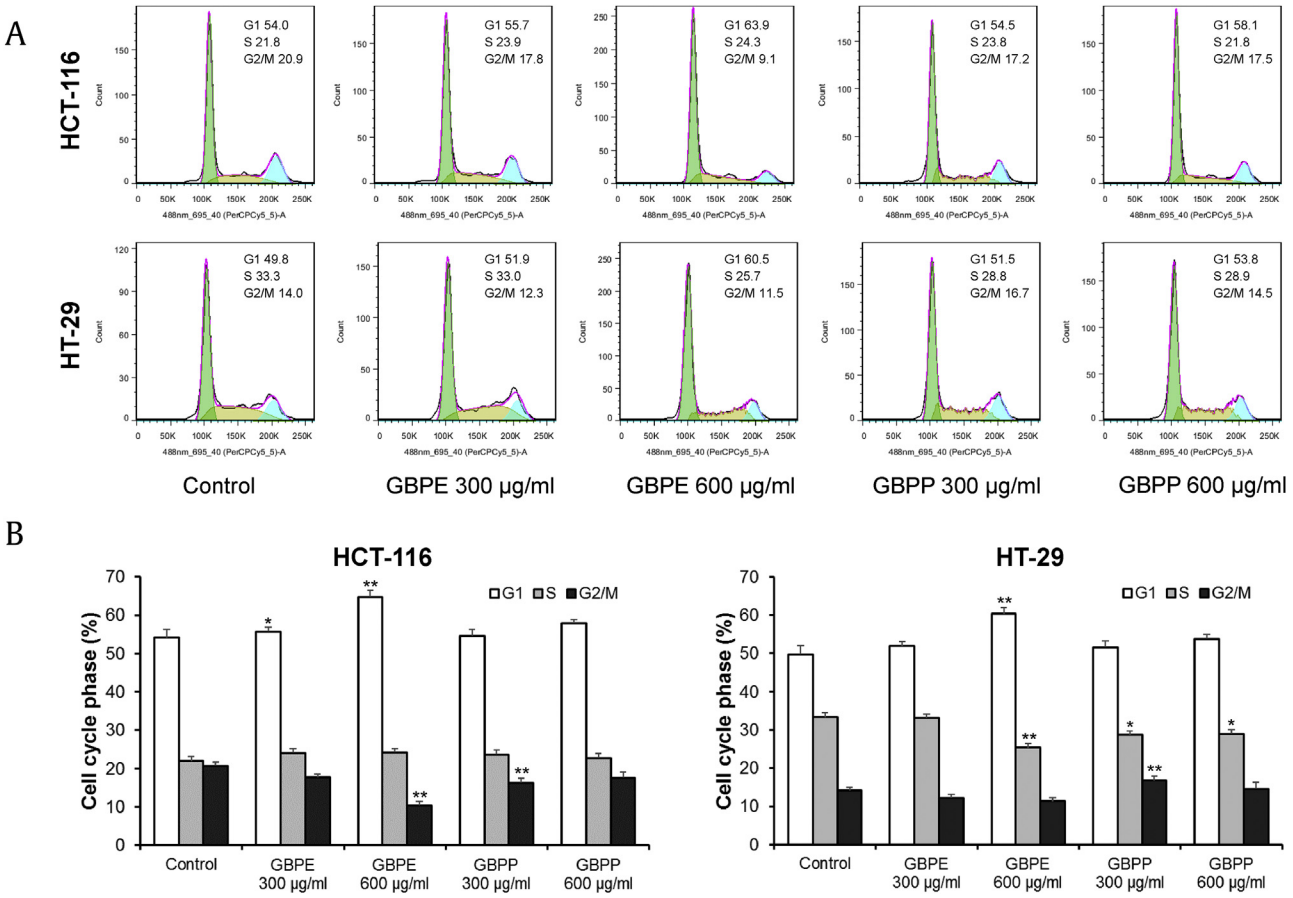

Fig. 4. Cell cycle analysis of HCT-116 and HT-29 human colorectal cancer cells using by cytometry after staining with propidium/RNase. Cells were treated with GBPE or GBPP at the concentrations of 300 and 600 μg/mL for 48 h. (A) Representative histograms of the DNA content in each experiment group. (B) Percentage of each cell cycle phase with various treatments or control. $^{*}p < 0.05$, $^{**}p < 0.01$ verses control; student *t* test.
GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion.

peak is 11–20 kDa. To remove the lower molecular weight fraction, dialysis process was performed with a molecular weight cutoff at 20 kDa. The procedures for preparing both crude GBPE and purified GBPP are shown in Fig. 1A.

HCT-116 and HT-29 cells were exposed to GBPE and GBPP at different concentrations. As shown in Fig. 1(B), using the MTS assay, the effects of GBPE and GBPP on colon cancer cell proliferation were determined. Results indicated that GBPE had higher cancer growth inhibition effects on HCT-116 than on HT-29 cells. Previous research has reported that IL-8 recruited and activated neutrophils, which contribute to the gut mucosal damage [30]. In this study, the antiinflammatory effects of GBPE and GBPP on LPS-induced IL-8 secretion in HT-29 cells were evaluated. Both GBPE and GBPP significantly inhibited this secretion (both $p < 0.01$). Fig. 1C also shows that the purified polysaccharide, GBPP, has stronger antiinflammatory effects than GBPE.

3.2. GBPE and GBPP inhibited $CD4^{+}IFN\text{-}\gamma^{+}$ cell (Th1) differentiation

Fig. 2 shows the role of GBPE and GBPP on adaptive immune responses. It has shown that inhibiting Th1-cell differentiation from naive $CD4^{+}$ T cells helps to alleviate inflammatory diseases. To investigate the effects of GBPE and GBPP on the differentiation of Th1, isolated $CD4^{+}$ T cells were cultured under Th1-polarizing conditions. As shown in Fig. 1, in the untreated control, approximately 55.4% of $CD4^{+}$ T cells were IFN-γ^{+}. Treatment with 250 μg/mL of GBPE inhibited Th1 differentiation by 37.4% ($p < 0.01$). In addition, treatment with 60, 120, and 250 μg/mL of GBPP inhibited Th1 differentiation down to 44.9%, 38.6%, and 31.9% ($p < 0.05$, $p < 0.01$), respectively (Fig. 1B). These data suggest that both ginseng polysaccharides showed concentration-dependent negative regulation potential in Th1 differentiation, whereas GBPP showed more potent activities.

3.3. GBPE and GBPP decreased $CD4^{+}FoxP3^{+}$ cell (Treg) differentiation

To investigate whether conversion from $CD4^{+}CD25^{-}$ cells to $CD4^{+}FoxP3^{+}$ cells was affected by GBPE or GBPP, a Treg differentiation assay was performed. For the control group, $CD4^{+}FoxP3^{+}$ cells accounted for 40.5%. When 60, 120, and 250 μg/mL of GBPP was added, the percentages for $CD4^{+}FoxP3^{+}$ cells were decreased to 31.1%, 26.7%, and 24.7%, whereas the same concentrations of GBPE decreased Treg frequency to 29.8%, 22.9%, and 15.4%, respectively (Fig. 3). For the Treg frequency, we observed a concentration-dependent decrease when there was treatment with both ginseng polysaccharides, and a stronger inhibitory effect was observed with GBPE treatment.

3.4. Cell cycle and apoptosis investigation

After staining with propidium/RNase M, flow cytometry was used to assay the cell cycle. Cells were treated with GBPE or GBPP at 300 and 600 μg/mL for 48 h. For the cell line HCT-116, both the

berry polysaccharides significantly induced cell cycle arrest at G2/M phase. For HT-29 cells, these compounds promoted cell cycle arrest in both the G1 and G2/M phases (Fig. 4).

After staining with annexin V-FITC/PI, apoptotic cells were assayed by flow cytometry. Cells were treated with GBPE or GBPP at 400 and 800 μg/mL for 48 h. Our data shows that GBPE or GBPP exhibited significant apoptotic induction effects ($p < 0.01$, $p < 0.05$). In comparison with GBPP, GBPE induced more apoptotic cells in both early- and late-stage apoptosis (Fig. 5) ($p < 0.01$).

3.5. *Effects of GBPE and GBPP on antiproliferative activities of 5-FU*

Fig. 6 shows that 5-FU and GBPE significantly inhibited HCT-116 cell growth. Cell proliferation for 5, 10, and 20 μM of 5-FU was 91.4%, 75.9%, and 64.0%, respectively. Proliferation for 200 μM of GBPE was 95.3%. When GBPE (200 μM) was combined with three concentrations of 5-FU (5, 10, and 20 μM), cell proliferations were decreased to 59.9%, 42.6%, and 34.7%, respectively (all $p < 0.01$ vs. 5-FU only). The combination of 5-FU with 400 μM of GBPE further decreased cancer cell proliferation, suggesting that GBPE significantly enhanced the effects of 5-FU. The effects, however, were not obviously enhanced when GBPP was combined with 5-FU.

For cell line HT-29, higher concentrations of 5-FU were used because this cell line is more resistant to 5-FU treatment than HCT-116 cells. As shown in Fig. 7, 5-FU and GBPE significantly inhibited the cell proliferation. When GBPE was combined with 5-FU, the effects of 5-FU were significantly enhanced ($p < 0.01$). When GBPP was combined with 5-FU, the effects of 5-FU were somewhat enhanced.

4. Discussion

Chronic gut inflammation is recognized as a risk factor for CRC initiation and development. To prevent colon cancer initiation and progression, targeting inflammatory pathways has been shown to be effective [9,12,14]. Nonsteroidal antiinflammatory drugs can reduce CRC tumorigenesis. However, owing to its long-term risks [11,31], it is critical to explore alternative strategies with botanicals against malignancies [32,33].

In this study, the effects of GBPE and GBPP on colon cancer cell proliferation were investigated. Compared to GBPP, we observed that the crude polysaccharides GBPE possessed greater anticancer effects. In addition to its major constituent polysaccharides (with molecular weight of 11–20 kDa), crude GBPE also contains small molecules such as trace ginsenosides, polyphenols, and other unidentified small-molecule substances [25,34]. These small-molecule substances likely possess antiproliferative potential.

IL-8 plays an important role in the pathogenesis of IBD, and the production of IL-8 is restricted to areas with histological signs of inflammatory activity and mucosal destruction. Targeting the IL-8 expression likely has a therapeutic benefit [35]. However, in our IL-8 secretion observation, we observed that the purified

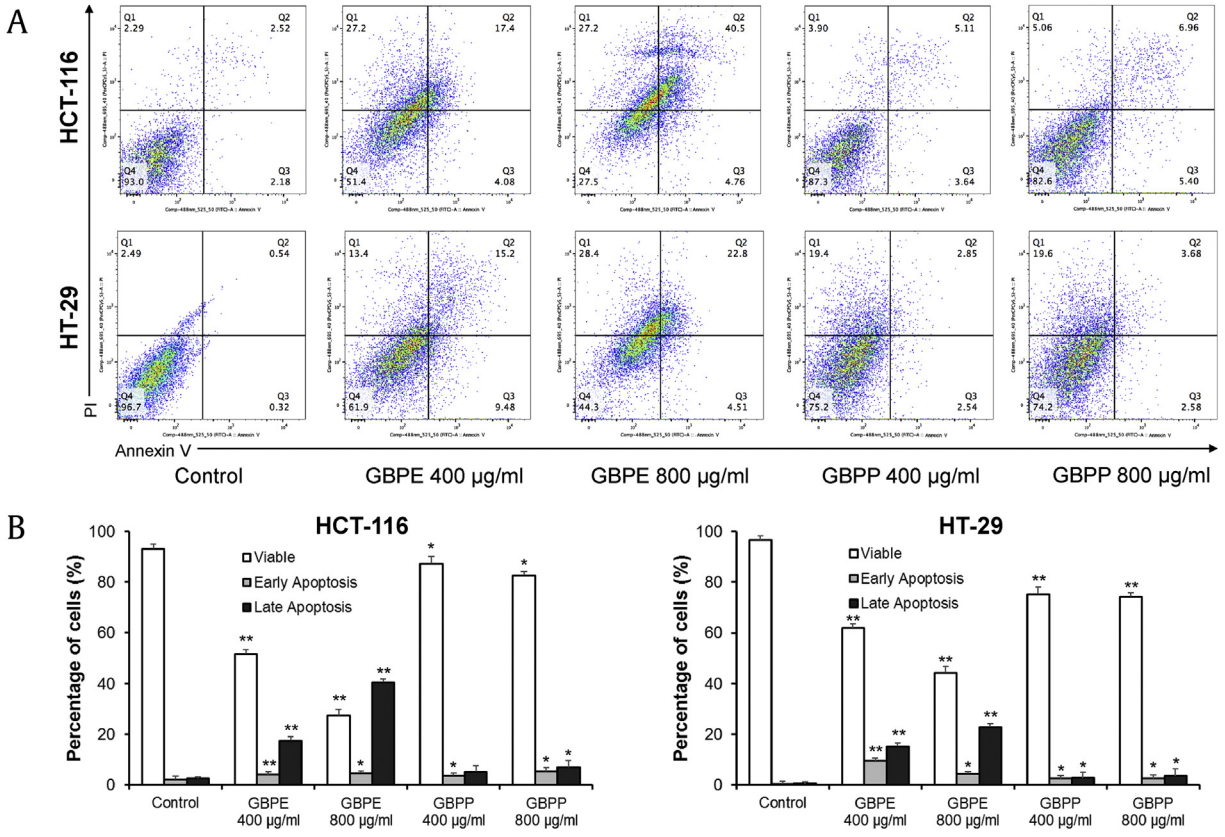

Fig. 5. Apoptosis assay of HCT-116 and HT-29 cells by flow cytometry after staining with FITC-annexin V/propidium iodide. Cells were treated with GBPE or GBPP at the concentrations of 400 and 800 μg/mL for 48 h. (A) Representative scatter plots of PI (y-axis) verses annexin V (x-axis). (B) Percentage of viable, early apoptotic and late apoptotic cells. $^{*}p < 0.05$, $^{**}p < 0.01$ verses control; student t test.
GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion; PI, propidium iodide.

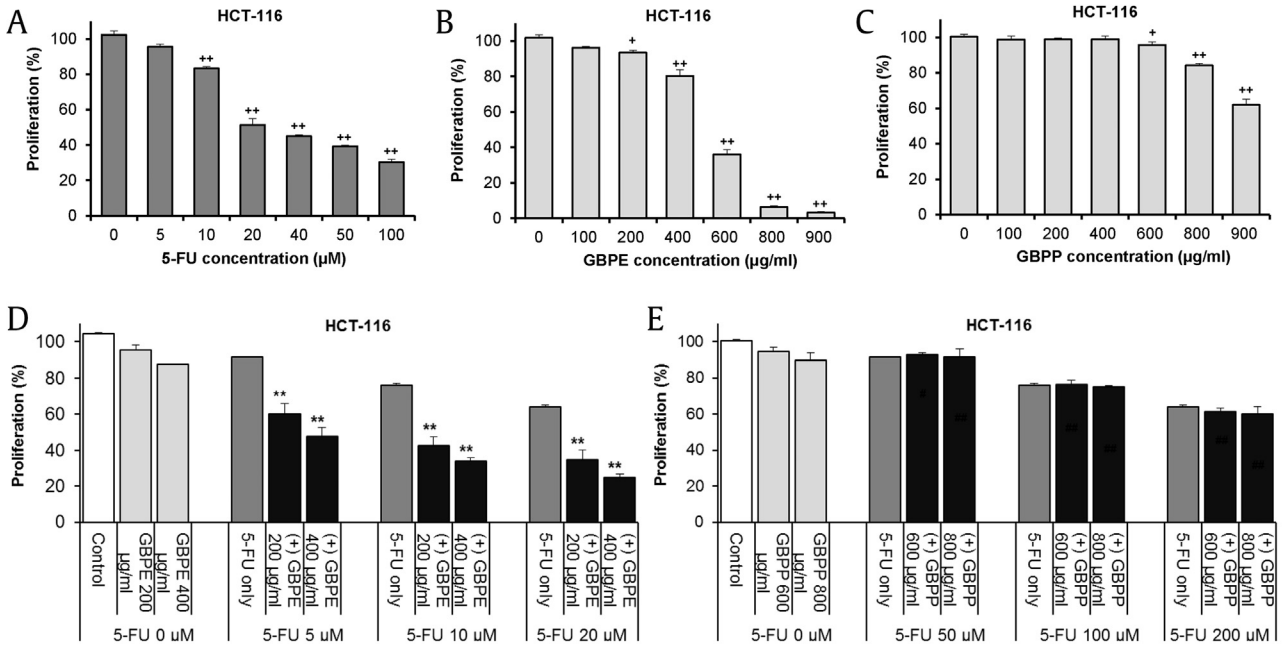

Fig. 6. Antiproliferative effects of (A) 5-FU, (B) GBPE, and (C) GBPP on HCT-116 colorectal cancer cells after 48 h of treatment. Effects of (D) 5-FU and GBPE or (E) 5-FU and GBPP on HCT-116 cell proliferation after 48 h of treatment. + $p < 0.05$; ++ $p < 0.01$ verses control; student *t* test. *$p < 0.05$, **$p < 0.01$ verses 5-FU group; one-way ANOVA with Tukey's *post hoc* test. 5-FU, 5-fluorouracil; ANOVA, analysis of variance; GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion.

polysaccharides GBPP exhibited stronger antiinflammatory effects than crude GBPE. Our results are consistent with those of previous reports state that ginsenosides, polyphenols, and some other ginseng small molecules possess potent antiproliferative activities, whereas botanical polysaccharides are believed to be more effective as immunomodulating agents [24–26]. Detailed phytochemical analysis should be conducted in the future to identify individual small-molecule substances in GBPE and their specific antiproliferative effects on cancer cells.

As a principal type of IBD, Crohn's disease is characterized by discontinuous and transmural inflammation. Previous studies have shown that for the pathogenesis of the disease, the dysregulation of the local immune system plays an important role [16,36]. It has been well documented that there is an increased risk for CRC in patients with IBD, especially in patients with Crohn's disease.

In Crohn's disease, the immune response tends to be predominantly production of Th1 cells due to the local release of proinflammatory cytokines [36,37]. Studies that suggest that

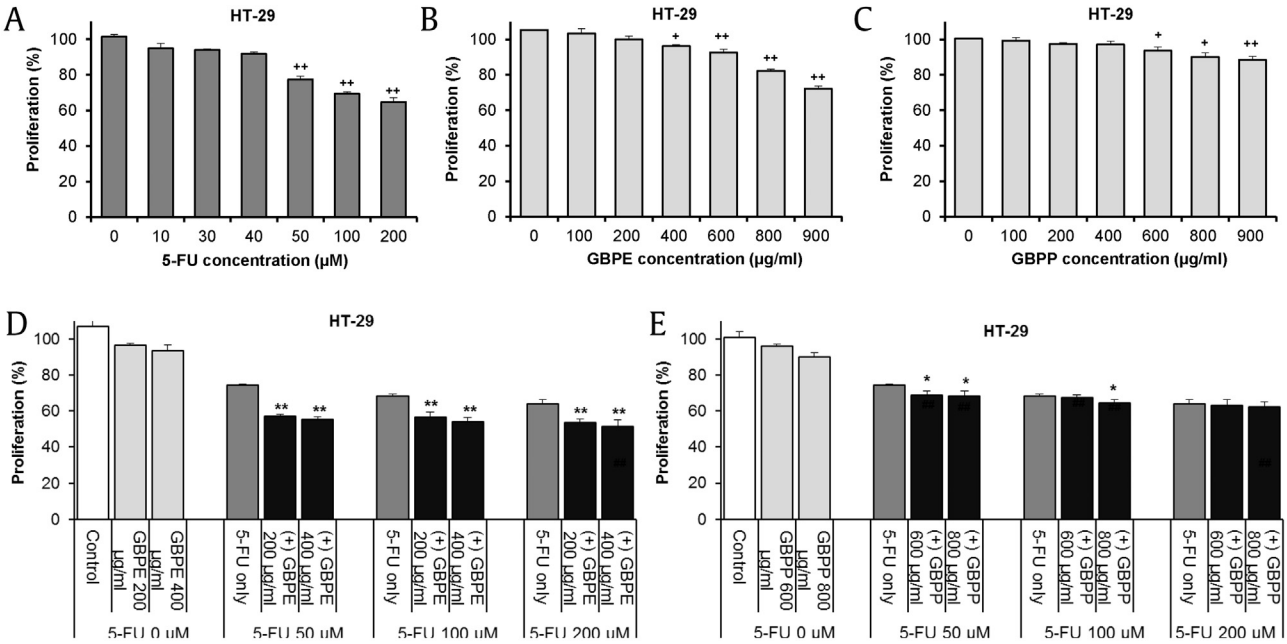

Fig. 7. Antiproliferative effects of (A) 5-FU, (B) GBPE, and (C) GBPP on HT-29 colorectal cancer cells after 48 h of treatment. Effects of (D) 5-FU and GBPE or (E) 5-FU and GBPP on HT-29 cell proliferation after 48 h of treatment. + $p < 0.05$; ++ $p < 0.01$ verses control; student *t* test. *$p < 0.05$, **$p < 0.01$ verses 5-FU group; one-way ANOVA with Tukey's *post hoc* test. 5-FU, 5-fluorouracil; ANOVA, analysis of variance; GBPE, ginseng berry polysaccharide extract; GBPP, ginseng berry polysaccharide portion.

neutralizing antibodies against these proinflammatory cytokines will prevent gut inflammatory injuries lend further support to a Th1-predominant immunopathology in Crohn's disease [38,39]. Antiinflammatory and immunosuppressive medications can thus be used to treat IBD, including Crohn's disease, and CRC chemoprevention can also be attained.

To explore the role of ginseng berry polysaccharides on the adaptive immune response, we investigated the effects of these polysaccharide compounds on T cells, a type of lymphocyte that plays a central role in cell-medicated immunity. Our data indicated that the berry polysaccharides significantly inhibited Th1-cell differentiation. Compared to GBPE, GBPP is obviously better in the inhibition. This is consistent with previous reports that compare different constituents of herbal medicines and has shown that polysaccharides possess significantly better actions for increasing immunological activities against various medical conditions [40,41]. It appears that the inhibition of Th1-cell differentiation is at least partially responsible for the berry polysaccharides' antiinflammatory effects that we previously observed.

To date, limited information is available regarding the relationship of Th1 differentiation and CRC development. It appears that there should be a connection between the attenuation of Th1 differentiation and risk reduction of IBD-linked CRC because inflammation is a recognized factor for the development of CRC. In a recent study, however, based on a transcriptomic data set of human CRC samples, it was found that compared to healthy controls, CRCs displayed increased ratios of Th1 to naive T-cell genes. Thus, suppressing Th1-cell differentiation may have clinical significance for the prevention and treatment of CRC [42]. Further studies are needed to characterize the relationship between Th1 immune response and CRC.

In this study, we also observed that GBPE and GBPP inhibited Treg-cell differentiation. By mediating immune homeostasis, Treg cells promote the maintenance of peripheral tolerance [22,43]. By shaping the tumor microenvironment, Tregs are involved critically to prevent the optimal function of effector cells. In addition, new therapeutic strategies have been explored by using Treg-suppressive functions [22]. Evidence has indicated that depleting populations of Treg cells contribute to the tumor inhibitory potential for several anticancer agents [44]. High levels of Treg cells in the tumor microenvironment are associated with poor prognosis in many cancers [22,43]. Therefore, downregulation of Treg-cell differentiation could contribute to ginseng berry polysaccharides' anticancer potential [21]. We observed that ginseng berry polysaccharides significantly reduced Treg-cell differentiation.

To further explore mechanisms of cancer chemopreventive effects of ginseng berry polysaccharides, we investigated their cell cycle and apoptotic induction. Data showed that both polysaccharide treatments increased G1 cell proportions at certain concentrations in HCT-116 and HT-29 cells. Our data also showed that these polysaccharides exhibited significant cancer apoptotic effects. Compared to GBPP, GBPE induced more apoptotic cells in both early- and late-stage apoptosis.

The two cell lines used in this study varied in p53 expression. HCT-116 is a p53 wild type, whereas HT-29 cells contain a p53 mutation. Cancer cells with p53 mutations are resistant to many chemotherapeutic agents [41,42]. We observed that both GBPE and GBPP had greater antiproliferation and apoptotic induction abilities in the p53 wild-type cell line (HCT-116) than in the p53 mutation cell line (HT-29), suggesting that p53 may play a role in ginseng polysaccharides' anticancer potential.

A commonly used cancer therapeutic compound for CRC is 5-FU. This drug, however, has strong adverse effects, especially at high doses. Thus, decreasing the dose of 5-FU, while maintaining or enhancing its effect by combining with botanicals, is considered to be a new strategy. Compared with the control, GBPE significantly enhanced 5-FU antiproliferation effects. Our data suggest that combining GBPE could reduce the dose of 5-FU and could increase the antiproliferation activity on CRC significantly, therefore further decreasing the dose-related toxicity of 5-FU. As GBPP had limited cancer cell antiproliferation effects, it did not effectively increase the action of 5-FU on both colon cancer cells.

In summary, we report that by comparing the two Asian ginseng berry polysaccharide preparations, GBPE and GBPP, it was found that GBPE possessed much stronger antiproliferation effects on CRC cells. Cell cycle and apoptosis data provided the mechanisms of action for the observed anticancer effects of the berry polysaccharides. On the other hand, we observed that compared to the GBPE, GBPP showed much better antiinflammatory activities on the malignant cells. This is consistent with the observation that GBPP also has better inhibition on Th1-cell differentiation. This suggests that GBPP has a role in depression immune activities, while Treg cells hinder the body's immune response against malignancies. In addition, the ginseng berry polysaccharides can enhance the antiproliferative actions of 5-FU, suggesting that there is potential clinical utility of Asian ginseng berry in colon cancer.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2018.12.010.

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