

The Primary Antitumor Activities Study of a Novel Peach Gum Oligosaccharide

Jinfeng Deng^a, Yanhong Ran^{b,*}

Department of Bioengineering, College of Life Science, Jinan University, Guangdong, China

^a531699461@qq.com, ^btranyh@jnu.edu.cn

*Corresponding author: tranyh@jnu.edu.cn

Keywords: peach gum oligosaccharide (PGOP), anti-tumor, galectin-3, probiotics

Abstract: The peach gum oligosaccharide (PGOP) we reported is a novel bio-degradation product of peach gum. Because of the bone structure is galactan, PGOP is expected has prebiotic and antitumor activity. This study investigates the activity of PGOP to inhibit the tumor cell proliferation and the tumor cell agglutination. The result showed that when the concentration of PGOP above 25 µg/ ml, the proliferation of Hela cell would be inhibited, and when above 50 µg/ ml, the viability of Hela cell would be inhibited either. PGOP can inhibit the cell agglutination mediated by galectin-3, and has a better inhibition than lactose. Finally, PGOP plays the role of prebiotic to promote the growth of probiotics, assists in anti-tumor.

1. Introduction

As a kind of natural polysaccharide gum from Rosaceae, peach gum can be degraded to produce peach gum oligosaccharide (PGOP). PGOP mainly consists of arabinose, galactose, xylose and rhamnose. Its structure is similar to arabinogalactan, which has been reported to inhibit tumor proliferation [1]. At present, many polysaccharides from natural plants show antitumor activity, such as castanea mollissima polysaccharides [2], pumpkin polysaccharide [3], mushrooms [4], ect. Galectin-3 is a kind of galectin family protein with unique structure, which plays a role in the invasion and metastasis of tumor cells [5]. The C-terminal of galectin-3 has a glycosyl recognition domain and can specifically bind to polysaccharides containing β-galactoside structure [5], like PGOP. In order to investigate whether PGOP has the antitumor activity, this paper studies the effect of PGOP on tumor cell proliferation and the tumor cell agglutination.

2. Method

Effect of PGOP on tumor cell viability and proliferation: The Hela cell line was cultured in RPMI 1640 medium supplemented with 10% (v/v) FCS, 100units/mL of penicillin, and 100 µg/mL of streptomycin in a humidified 5 % CO₂ incubator at 37°C. After treating by different concentration of PGOP in 24h or 72h, the cell survival rate and proliferation were analyzed by MTT assay

Effect of PGOP on cell agglutination mediated by galectin-3: Using alsever solution to protect

the collected fresh chicken blood, wash the blood by 0.15 M NaCl several times, resuspend the blood by 0.02M PBS. The prepared red blood cells would be treated by different concentration of galectin-3 or PGOP. The agglutination of red blood cell was observed by microscope.

The effect of different concentrations of PGOP on the growth of different bacterium: Different weights of sugar were added to the culture medium to culture *Bifidobacterium*, *Bacillus subtilis* and *Enterococcus* 24h, compare the number of bacteria in the experimental group and the control group.

3. Results

3.1 PGOP inhibit tumor cell viability and proliferation

To investigate the effect of PGOP on tumor cell, we add different concentrations of PGOP to the cultured Hela cell, the results are shown in Figure 1. At 48h and 72h, the Cell survival rate of HeLa cells was decrease by the increase of PGOP concentration. When the concentration of PGOP is between 5 $\mu\text{g/ml}$ to 25 $\mu\text{g/ml}$, the inhibitory effect on Hela cell haven't significant difference. When the concentration of PGOP is above 50 $\mu\text{g/ml}$, the inhibitory effect will be enhanced with the increase of experimental time. High concentration PGOP can reduce the viability of Hela cell, however, low concentration of PGOP such 25 $\mu\text{g/ml}$ can also inhibit the proliferation of Hela cell. As shown in the Figure 2, compare with the control group, the increased number of Hela cell treated by PGOP was obviously less and this inhibition is dose-dependent. In general PGOP has inhibitory activity on tumor cell viability and proliferation.

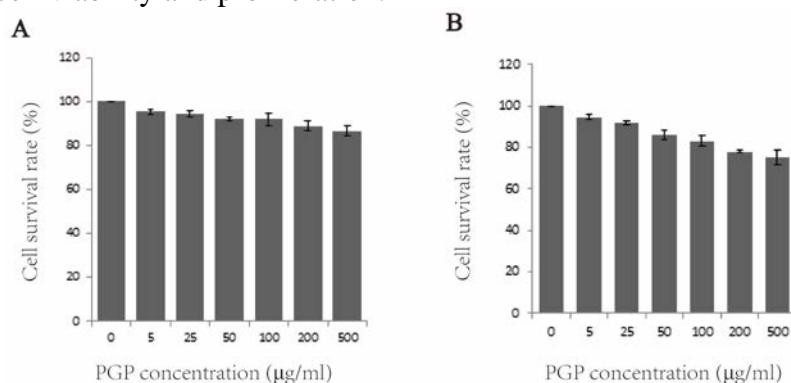


Figure 1. The effect of different concentrations of PGOP on the Cell viability. A: Hela cell treated with PGOP in 48h. B: Hela cell treated with PGOP in 72h.

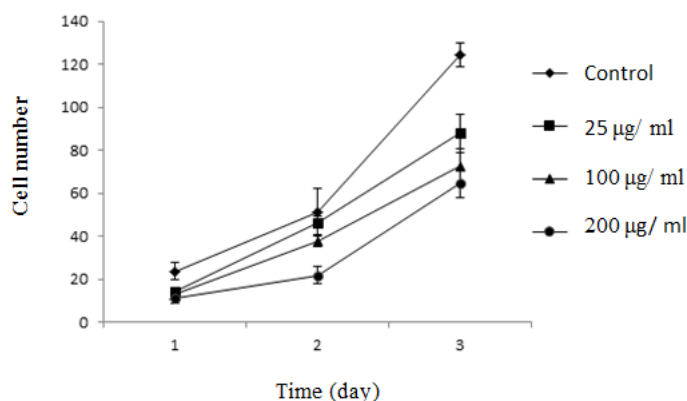
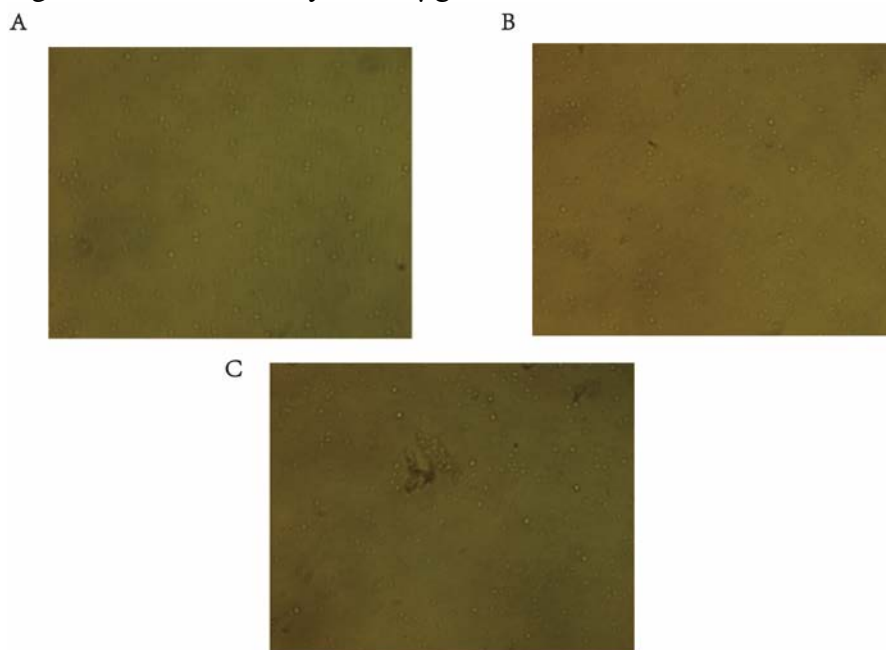


Figure 2. The effect of different concentrations of PGOP on Hela cell proliferation.

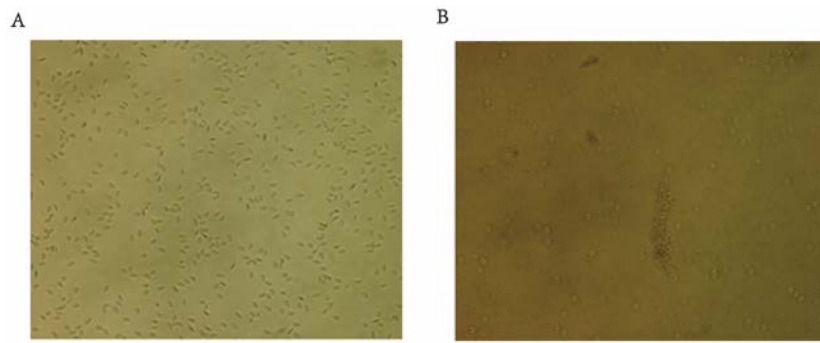
3.2 PGOP inhibit cell agglutination mediated by galectin-3

It has been reported that galectin-3 can mediate the adhesion and migration of tumor cells [6]. As the natural receptor of galectin-3, PGOP can competitively bind to the galectin-3 against the cell surface receptor, and then inhibit the cell aggregation mediated by galectin-3. Figure 3 shows the effect of different concentrations of Galectin-3 on Hela cell agglutination. When the treatment concentrations of Galectin-3 reaches 100 $\mu\text{g}/\text{ml}$, the Hela cell agglutinated. So the effective concentration of galectin-3 in this study is 100 $\mu\text{g}/\text{ml}$.



*Figure 3. The effect of different concentrations of Galectin-3 on Hela cell agglutination.
A: cell treated with 25 $\mu\text{g}/\text{ml}$ Galectin-3. B: cell treated with 50 $\mu\text{g}/\text{ml}$ Galectin-3.
C: cell treated with 100 $\mu\text{g}/\text{ml}$ Galectin-3*

As shown in the Figure 4, compared with the blank control, the red blood cell treated with galectin-3 the cell distribution was more intensive, which indicated that galectin-3 can also mediate agglutination of red blood cell. Through the β -galactoside structure, lactose can combine with galectin-3 and block the contact between galectin-3 and the cell surface receptor, this principle can also be applied to PGOP. Figure 5 is a comparison of the ability of PGOP and lactose to inhibit galectin-3 mediated cell agglutination. After lactose treatment, the cells still aggregated until the lactose concentration rise to 75 $\mu\text{g}/\text{ml}$ (Figure 5 A, B, C). Therefore, the minimum inhibitory concentration (MIC) of lactose is 75 $\mu\text{g}/\text{ml}$. However, even though only 25 $\mu\text{g}/\text{ml}$ PGOP treat the red blood cell, they do not aggregate, which suggests the MIC of lactose is 25 $\mu\text{g}/\text{ml}$. Generally, PGOP is better than lactose in inhibiting cell agglutination mediated by galectin-3.



*Figure 4. The effect of Galectin-3 on red blood cell agglutination.
A: blank control. B: cell treated with Galectin-3*

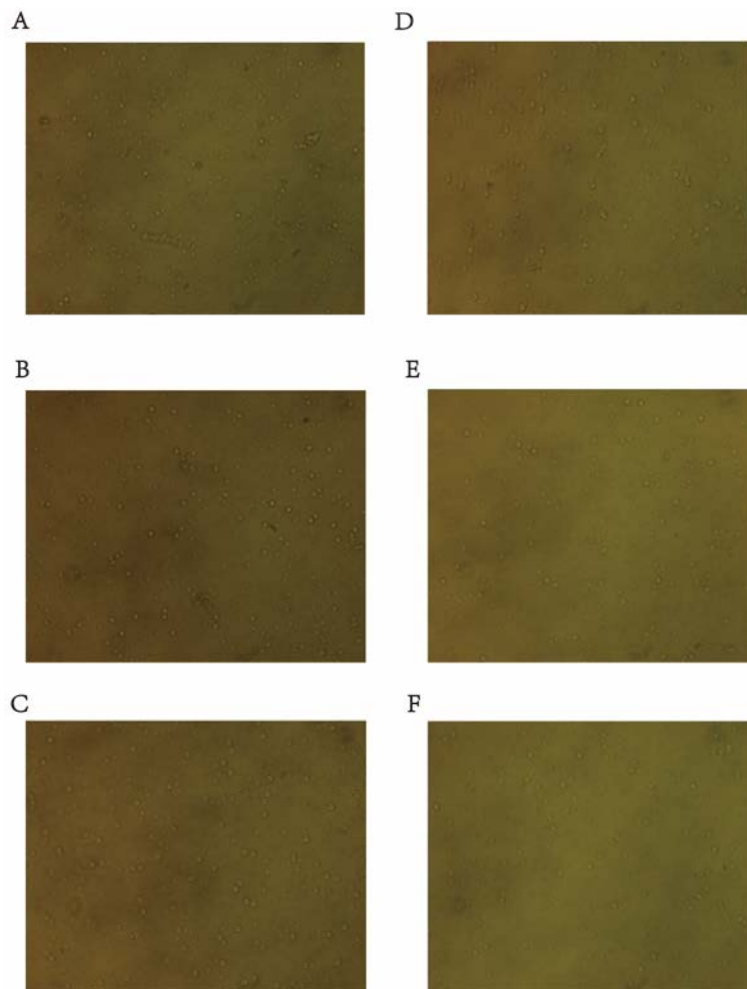


Figure 5. The effect of different concentrations of lactose and PGOP on the red blood cell agglutination inhibition.

*A: cell treated with 25µg/ml lactose. B: cell treated with 50µg/ml lactose.
C: cell treated with 75µg/ml lactose. D: cell treated with 25µg/ml PGOP.
E: cell treated with 50µg/ml PGOP. F: cell treated with 75µg/ml PGOP*

3.3 PGOP benefits to regulate intestinal flora

Many natural polysaccharides can act as prebiotics to promote the growth of probiotics and inhibit the propagation of harmful bacteria [7]. As is known to all, intestinal probiotics can regulate the immune function of the system and reduce tumorigenesis. In order to study whether PGOP can promote the growth of probiotics and regulate the balance of intestinal flora, *enterococcus*, *Bifidobacterium*, and *Bacillus subtilis* was cultured with different concentrations of PGOP. As shown in the Figure 6, PGOP inhibit the growth of *Enterococcus*, promote the growth of bifidobacteria, and when the concentrations of PGOP is more than 1000mg/ml, it can gradually promote the growth of *Bacillus subtilis*. This data indicate that PGOP inhibit the *Enterococcus* and is beneficial to *Bifidobacterium* and *Bacillus subtilis*, which can positively regulate the intestinal flora.

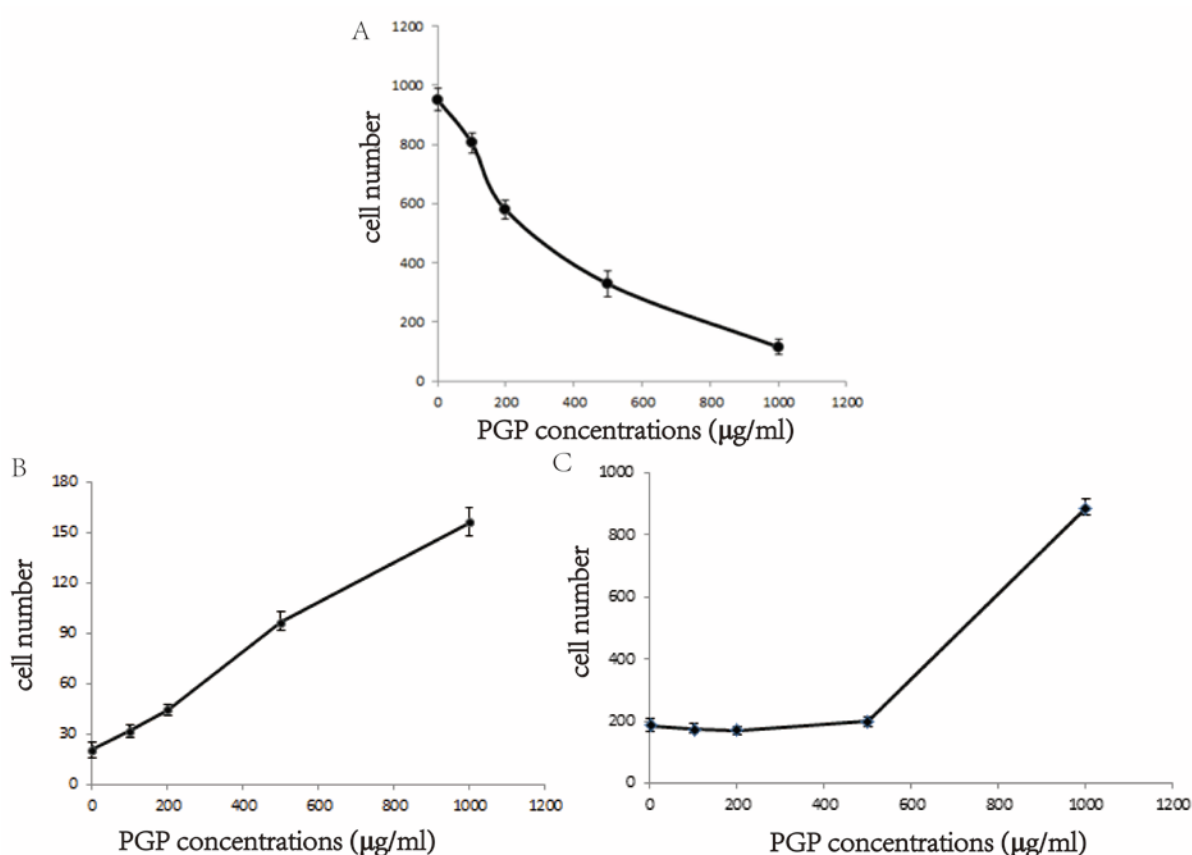


Figure 6. The effect of different concentrations of PGOP on the growth of different bacterium. A: *enterococcus*. B: *Bifidobacterium* C: *Bacillus subtilis*.

4. Conclusion

In this study, PGOP shows its inhibitory effect on the growth and proliferation of tumor cells. Though competitively bind to the galectin-3 against the cell surface receptor, PGOP can inhibit the cell agglutination mediated by galectin-3, and has a better inhibition than lactose. Besides, PGOP inhibit the harmful bacteria *Enterococcus* and is beneficial to *Bifidobacterium* and *Bacillus subtilis*, which are against to the colon cancer [8]. In summary, as a kind of polysaccharide, PGOP has a great anti-tumor activity and great development potential

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