Spirulina polysaccharide induces the metabolic shifts and gut microbiota change of lung cancer in mice

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A polysaccharide obtained from Spirulina (PSP) and its effect on lung cancer in mice was investigated. Our results indicate that the tumor volume and weight of the lung cancer-bearing mice treated with PSP decreased significantly. Metabolite analysis showed that 27 differential accumulated metabolites (DAMs) changed significantly, in which 24 DAMs increased while 3 DAMs decreased. KEGG enrichment results showed that these differential metabolites were enriched significantly in the high-affinity IgE receptor (FcεRI) signaling pathway and arachidonic acid metabolism. In addition, PSP modulated gut microbiota of the lung cancer-bearing mice. PSP increased the abundance of Lactobacillus, Allobaculum, Alloprevotella, and Olsenella, decreasing Bacteroides and Acinetobacter. The results might be related to suppressing lung cancer. Based on our study, we hypothesized that PSP inhibited lung cancer through FcεRI signaling pathway and arachidonic acid metabolism and regulated the balance of gut microbiota. Nevertheless, the relationship between these two pathways and gut microbiota needs further study.

1. Introduction

Cancer has been a public health concern worldwide, and although the death rate from cancer has continued to decline since 1991 (Siegel et al., 2019), the annual number of cancer cases remains large. In terms of mortality, cancer deaths remain a severe problem worldwide (8.97 million deaths), only after ischemic heart disease (Bray et al., 2018). Therefore, the cancer problem still needs to be solved. Lung cancer is one of the deadliest cancers (Siegel et al., 2017). According to the WHO Global Cancer Observatory (GLOBOCAN), 2018 Registry, lung cancer cases are the largest in the Cancer epidemic, with about 2 million cases (Mattiuzzi and Lippi, 2019). Although recent treatment advances have significant clinical impact in patients with lung cancer, but the side effect still existed, some effect is not satisfactory (Mohindra and Patel, 2022). It is still vital to find new drugs and new treatment strategies for lung cancer.

Polysaccharides are large molecular compounds composed of multiple monosaccharide molecules and usually possess a complex structure. Due to their unique structure, polysaccharides usually have superior pharmacological applications, such as immunoregulatory, anti-tumor, anti-virus, antioxidation, and hypoglycemic activity (Yu et al., 2018), even the application for microencapsulation of probiotics (Liu et al., 2020), and therefore they play an important role in nature (Chen and Huang, 2018; Liu et al., 2020). In recent years, seaweed polysaccharides have been receiving widespread attention owing to their special biological activities, including anti-tumor activities, which have...
attracted wide attention in biochemistry and medicine (Florez et al., 2017; Zhao et al., 2020). Among them, the biological activities of Spirulina polysaccharides were particularly outstanding. Based on the research, PSP has anti-virus functions, enhancing immunity (Wu et al., 2016), anti-oxidation, anti-tumor, lowering blood sugar, and microbial-modulating activities (Matloub et al., 2013; Finamore et al., 2017). It was found that the water-soluble polysaccharide of Spirulina had anti-Hepatitis C virus, antioxidant, cytotoxic, and lipidemic activities (Matloub et al., 2013). Spirulina complex polysaccharide (CPS) has been researched and it contributed to down-regulating angiogenesis through Toll-like receptor 4 signaling to inhibit glioma growth (Kawanishi et al., 2013). A study aimed to determine a protective effect on dopaminergic neurons of the polysaccharide extracted from Spirulina platensis, and the results revealed that they had a protective effect on the damage of dopaminergic neurons, and the antioxidant properties of polysaccharides might be the basis of its neuroprotective effect (Zhang et al., 2015). Moreover, research also pointed that the polysaccharide extracted from Spirulina platensis evokes antitumor activity in gastric cancer cells via modulation of galectin-3 and exhibited cyto/DNA protection (Upfin et al., 2022). Otherwise, another research preliminarily talked that the polysaccharide from Spirulina platensis could remarkably inhibit the growth of A549 lung cancer cells but without the clear details of biological mechanism (Cai et al., 2022). Obviously, although there have been many studies on Spirulina polysaccharides, there was still a lack of research on Spirulina polysaccharides treating lung cancer, especially the potential mechanism. Therefore, it is important to deeply study Spirulina polysaccharides treating lung cancer.

To explore the effect of Spirulina polysaccharides on lung cancer, we extracted a Spirulina polysaccharide named PSP. The Lewis lung cancer-bearing mice were treated with PSP, and some apparent indicators such as tumor weight and tumor volume were measured. Furthermore, metabolic analysis and 16S rRNA sequencing was carried out in this research.

2. Materials and methods

2.1. Materials

Dried Spirulina powder (purchased from Foshan Lanqiang Biotechnology Co., Ltd.) was stored in the Guangdong Provincial Key Laboratory of Food Quality and Safety, South China Agricultural University (Guangzhou, China). Lewis lung cancer (LLC) cells and C57BL/6 female mice were purchased from the Guangdong Medical University (Guangzhou, China). Fetal bovine serum (FBS) was supplied by Gibco Life Technologies (Grand Island, NY, USA). Other mentioned chemical materials in this study were purchased from Guangzhou Chemical Co. LTD (Guangzhou, China). All the reagents used were of analytical grade.

2.2. Preparation of PSP

PSP was extracted by the method of the reports with some slight modifications (Song et al., 2019; Han et al., 2021). The dried Spirulina powder (100 g) was added to 2000 mL of water twice at 90 °C for 2 h. Then the filtrate was concentrated to a quarter of the original volume by rotary evaporation at 52 °C. After centrifugation, the supernatant was mixed with triply distilled ethanol for overnight precipitation to get the precipitate. The precipitate was dissolved in distilled water and then deproteinated by Sevag reagent (CHCl3: n-C6H12OH = 4:1, v/v). The protein-free polysaccharide solution was dialyzed in distilled water for three days. The cut-off molecular weight of the dialysis bag is 3000 Da. Finally, PSP was obtained after lyophilizing the dialysate. The total sugar content, protein content, relative molecular weight, mono-saccharide composition, and functional groups of PSP were measured (Hao et al., 2019; Song et al., 2020). These results are shown in Tables S1–S2 and Fig. S1.

2.3. Animal experiment

5 × 10^5 LLC cells were resuspended in a free FBS medium and injected subcutaneously in a 100 μL volume into the right flank of 8-week-old C57BL/6 female mice. After the tumor grew to about approximately 60 mm^3, the mice were divided into two groups (five mice per group). The vehicle group was treated with vehicle and the PSP group was treated with PSP (200 mg/kg) via intrastragastric administration daily for 15 days. The mice’s body weights were recorded every day, and the signs of toxicity were monitored. The tumors of the mice were measured with a digital caliper every three days. The tumor volumes were calculated by the following formula:

\[ \text{volume (mm}^3) = \text{length (mm)} \times \text{width (mm)}^2/2 \]

2.4. Metabolic analysis

The metabolic analysis is carried out according to the reported methods (Xia et al., 2021). In short, the cell sample stored at −80 °C refrigerator was thawed on ice. Each sample was taken 50 mg and added to 1 mL icy methanol/water (70%, v/v) to homogenize and vortexed for 3 min. The sample was centrifuged (4 °C, 12000 rpm, 15 min), then collected and desiccated in a centrifuge. Then, 200 μL aliquots of supernatant were transferred for LC-MS/MS analysis. The samples of quality control (QC) were performed by mixing 10 μL of every single sample, then researching the other samples to control the reproducibility of instrumental research. And, an ACQUITY UPLC BEH column (2.1 mm × 100 mm, 1.7 μm, waters, Ireland) was used to analyze the samples for separation. 25 mM ammonium hydroxide and 25 mM ammonium acetate in water (A) and acetonitrile (B) were performed as the mobile phase in both electrospray ionization (ESI) positive patterns (5500V) and negative patterns (−4500V).

2.5. Microbial analysis

Based on 16S rRNA sequencing, microbial analysis was carried out according to the reported methods (Abd-Elaziz et al., 2020). DNA was extracted based on the instructions of the Omega Kit e.z.n.a TM Mag-Bind SOIL DNA Kit (http://omegabiotek.com/store/product/soil-dna-kit/). Before the experiment, DNA integrity was measured through an agarose gel. Primers were used to amplify the v3-v4 region of the microbial 16S ribosomal gene. The primers were 341F (5′-CCTAC GGGNGGCWGGCAG-3′) and 805R (5′-GACTACHVGGGTATCCTAAT CC-3′). Two rounds of PCR amplification were conducted. The first round of amplification was carried out under the following conditions: 94 °C for 3 min, 5 cycles at 94 °C for 30 s, 45 °C for 20 s, 65 °C for 30 s, and then 20 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s and finally extending at 72 °C for 5 min. After amplification, PCR primers from the first round were introduced into the Illumina Bridge PCR compatible primers for the second round of amplification. Reaction conditions of the second amplification were as follows: 94 °C for 3 min, 5 cycles at 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s and finally extending at 72 °C for 5 min. After amplification, the PCR products were detected using agarose electrophoresis to purify and recover the DNA. The recovered DNA was quantified accurately by the Qubit3.0 DNA detection kit and mixed in an equal amount of 1:1 to facilitate the sequencing.

2.6. Statistical analysis

Data were shown as mean average ± standard deviations (SD) (n = 3). Data in all the bioassays were evaluated by Student’s t-test at a univariate level, p-value<0.05 or p-value<0.01 were respectively regarded as significant differences or extremely significant differences. **p < 0.01, ***p < 0.001 as compared to control sample. The online Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.
0.001), which was statistically significant (Fig. 1B). In addition, there were also significant differences in tumor weight between the two groups (p < 0.01) (Fig. 1C). According to the result, we speculated that PSP had an inhibitory effect on lung cancer.

3. Results and discussion

3.1. PSP on tumor volume and tumor weight in lung cancer-bearing mice

We first established a lung cancer mouse model to demonstrate whether PSP inhibited tumor growth. As shown in Fig. 1A, tumors in the Spirulina polysaccharide (SPI) group were significantly smaller than in the Vehicle group. Within 15 days, tumor volume increased gradually in the Vehicle group but not in the SPI group. On day 15, there was a significant difference in tumor volume between the two groups (p < 0.001), which was statistically significant (Fig. 1B). In addition, there were also significant differences in tumor weight between the two groups (p < 0.01) (Fig. 1C). According to the result, we speculated that PSP had an inhibitory effect on lung cancer.

3.2. Metabolomics analysis

To explore changes in tumor metabolites, we performed metabolomics analyses on the tumors of treated and control mice. A total of 27 DAMs were screened, among which 24 DAMs were up-regulated while 3 DAMs were down-regulated. To see the differences in metabolites more intuitively, we drew a differential metabolite volcano plot (Fig. 2A). 27 DAMs were grouped into different categories. As shown in Fig. 2B, these DAMs were mainly oxidized lipid and organic acid and its derivatives.

KEGG enrichment results were shown in Fig. 2C. Among the enriched pathways, Fructose and mannose metabolism, FcεRI signaling pathway, and arachidonic acid metabolism are highly enriched with the p-value under 0.05. In Fructose and mannose metabolism, L-fucose and L-rhamnose were significantly increased. Since both L-fucose and L-rhamnose are the main components of PSP, it was reasonable to believe that their significant upregulation of them was due to the intake of PSP. The other two significantly enriched pathways may be related to the anticancer activity of PSP. Consequently, there is a strong relationship between the two pathways.

FcεRI signaling pathway is closely related to immunity. IgE binds to the IgE receptor is the FcεRI and a member of the antigen receptor superfamily and is generally expressed on eosinophils and mast cells. It plays a vital role in connecting pathogen or antigen-specific IgE and cellular immune effector function (Sherings et al., 2018). FcεRI, which activates eosinophils or mast cells to release and secrete mediators such as histamine, heparin, proteinoids, and metabolites of arachidonic acid, cytokines, and so on (Olivera et al., 2018). After being activated, eosinophils or mast cells play their biological roles mainly through releasing and secreting mediators. When eosinophils or mast cells are activated, mediators released into the extracellular environment participate in the inflammatory response, significantly impacting the body (Lu et al., 2011). Arachidonic acid metabolism was a downstream pathway of the FcεRI signaling pathway. In this pathway, arachidonic acid does not exist free in cells but is esterified in the cell membrane as a phospholipid. When stimulated, the arachidonic acid is dissociated by phospholipase and converted into active metabolites under the action of various arachidonic acid metabolic enzymes, such as Leukotrienes (LTs) (Hanna and Hafez, 2018). LTs are a class of highly bioactive arachidonic acid derivatives. They can directly or indirectly promote innate immune response by affecting leukocyte effector function (Nicolete et al., 2008), stimulating the production of other inflammatory mediators (Liu and Yokomizo, 2015), inducing phagocytosis (Zhang et al., 2017), and activating antimicrobial mechanisms (Yokomizo et al., 2018). In this study, the FcεRI signaling pathway and arachidonic acid metabolism both enriched significantly. Leukotriene D4 (LTD4) and leukotriene E4 (LTE4) were significantly up-regulated in the FcεRI signaling pathway, while leukotriene B4 (LTB4), LTD4, and LTE4 were significantly up-regulated in arachidonic acid metabolism. LTD4 is a strong pro-inflammatory factor, which can increase vascular permeability, induce the release of lysosomal enzymes, promote the production of reactive oxygen species and enhance the expression of TNF-α, IL-1, IL-6, IL-2 (Laye et al., 2018). LTD4 and LTE4 can enhance the posterior venules’ plasma penetration and mucus secretion in the inflammatory process and regulate the Th2 cell-dependent pneumonia response (Austen, 2007).

Based on the metabolomics analysis results, it can be speculated that B lymphocytes transform into plasma cells and produce IgE in response to PSP stimulation. IgE then binds to FcεRI on eosinophils or mast cells to activate eosinophils or mast cells, thereby activating the arachidonic acid pathway. Arachidonic acid is free from the cell membrane. Then, 5-lipoxygenase (5-LOX) catalyzed the transformation of arachidonic acid into Leukotriene A4 (LTA4) with the assistance of 5-LOX activated proteins. Due to the instability of LTA4, LTD4 was catalyzed by epoxide hydrolase (LTA4H) to produce LTB4 or combined with glutathione to transform into LTC4. LTC4 and LTD4 are subsequently transported extracellularly and lysed into LTD4 and LTE4 (Tian et al., 2020). Therefore, LTD4, LTE4, and LTD4 are significantly increased. As critical inflammatory mediators, LTD4, LTE4, and LTD4 participate in inflammatory responses by stimulating mucus secretion (Otaka et al., 2019), increasing vascular permeability (Papadaki et al., 2013), and causing smooth muscle contraction (Bonvini et al., 2020), thus influencing tumor development (Fig. 3).

3.3. PSP on gut microbiota in lung cancer-bearing mice

A total of 6136 operational taxonomic units (OTUs) were detected at a similar level of 97% in the sequencing. There are 2996 OTUs overlaps between the Vehicle group and SPI group (Fig. 4A). In this study, we reflect the abundance and diversity of microbial communities through a single-sample diversity analysis (Alpha diversity). According to the sequencing results (Fig. 4B), compared with the Vehicle group, all the indexes, including Shannon, Simpson, Chao, and ACE, decreased in the SPI group. Although the Alpha index between the two groups showed no
Fig. 2. (A) Volcanic plot. (B) Proportion diagram of DAMs classification. (C) KEGG classification map. (D) KEGG enrichment map.

Fig. 3. Diagram of the potential pathways of PSP against lung cancer. Syk: Spleen tyrosine kinase; LAT: Linker for activation of T cells; Grb2: Growth factor receptor-bound protein 2; Sos: Son of sevenless; Ras: GTPase HRas; Raf-1: RAF proto-oncogene serine; MEK: Mitogen-activated protein kinase kinase 1; ERK: Mitogen-activated protein kinase 1/3; cPLA2: Cytosolic phospholipase A2; FLAP: Arachidonate 5-lipoxygenase-activating protein.
could increase the abundance of some beneficial bacteria according to the changes in the genera level community, PSP treatment nant phyla showed changes in abundance. Compared with the Vehicle group, the abundance of Bacteroidetes and Firmicutes increased while Bacteroidetes and Proteobacteria decreased (Fig. 4E). According to the data in Fig. 4F, the gut microbiota, Firmicutes/Bacteroidetes ratio (F/B) was increased under the PSP treatment. Firmicutes and Bacteroidetes are the two main phyla of gut microbiota, and the F/B value is mainly related to maintaining the balance of gut microbiota (Grigor’eva, 2021). A high F/B value can lead to obesity (Abenavoli et al., 2019), while a low F/B value leads to inflammatory bowel disease (Shen et al., 2018). In our study, the PSP treatment increased the F/B value and made the F/B value more toward 1. These results suggested that PSP could contribute to regulating the balance of gut microbiota to maintain intestinal homeostasis.

As shown in Fig. 4G, the abundance of Turicibacter in Firmicutes increased (p = 0.016), while the abundance of Desulfovibrio increased (p = 0.058) in Proteobacteria decreased. The abundance of Bifidobacterium (p = 0.059) in Actinobacteria and Alloprevotella (p = 0.097) in Bacteroidetes increased. According to the classification comparison results, we selected the classification of dominant species and drew a circular tree diagram combining the species abundance information (Fig. 4H). According to the changes in the genera level community, PSP treatment could increase the abundance of some beneficial bacteria such as Allobaculum, Lactobacillus, Alloprevotella, and Olsenella. Meanwhile, it reduced the abundance of some conditional pathogenic bacteria, such as Bacteroides and Acinetobacter (Qin, 2019). Therefore, we speculated that PSP exerted its activity by changing the abundance and composition of beneficial bacteria and pathogenic bacteria in the gut microbiota.

We obtained critical gut microbes through LEfSe analysis. The results were shown below (Fig. 4I–J). Desulfovibrionaceae, Deltaproteobacteria, and Desulfovibrionales in the Vehicle group showed selective enrichment. In contrast, Bifidobacteriaceae, Bifidobacterium, and Turicibacter increased in the SPI group. As an essential probiotic in the human body, Bifidobacterium plays a vital role in human health. By metabolizing organic acids and other antibacterial active substances, Bifidobacterium inhibits the proliferation or infection of harmful bacteria and pathogenic bacteria to change the physical and chemical characteristics of the intestine and finally achieves the goal of inhibiting the production of carcinogens (Feng et al., 2019). Some Turicibacter bacteria were associated with inflammation in the host (Kollermayer et al., 2011). The Desulfuribrio family, a kind of sulfate-reducing bacteria, can produce H₂S (Peek et al., 2019), which may be associated with cancer such as the colorectal cancer (Guo et al., 2016). Excessive H₂S is harmful to the colon epithelium’s energy metabolism and DNA integrity (Beaumont et al., 2016). Deltaproteobacteria is one of the classes of Proteobacteria and may be related to the occurrence of the inflammatory intestinal disorder (Wang et al., 2009). LEfSe analysis results led us to speculate that the increase in the abundance of Bifidobacterium and the decrease of some Proteobacteria might be related to the inhibition of lung cancer by PSP.
4. Conclusion

In this study, we first studied the apparent effects of PSP on tumors in LLC tumor-bearing mice. Then we elucidated the effects of PSP on tumor metabolism and gut microbiota. According to the results, we could see that the tumor volume decreased significantly after PSP treatment. Based on the metabolomic analysis, the LTB₄, LTD₄, and LTE₄ showed a significant increase, and they were both involved in the FceRI signaling pathway and arachidonic acid metabolism pathway, which were the two significantly enriched pathways in KEGG pathway enrichment. 16S rRNA sequencing results showed an increase in some beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, *Allobaculum*, and *Alloprevotella*. In contrast, some conditional pathogenic bacteria, such as *Bacteroides* and *Acinetobacter*, were down-regulated, suggesting that PSP might maintain intestinal homeostasis by altering the abundance of probiotics and harmful bacteria in the gut microbiota to achieve anti-tumor goals. This study provides a basic investigation of new potential molecular mechanisms of PSP on lung cancer, including the tumor metabolism and gut microbiota, our results may contribute to a deeper development of PSP and the research of biological treatment on lung cancer. However, further research is still required to obtain a deeper understanding of this research.

Ethics statements

This study includes animal experiments. The animal experiments involved were conducted through the Animal Ethics Committee of Guangdong Medical University (No.110324210104725223, animal experiments were performed in compliance with institutional animal care guidelines and according to committee-approved protocols).

CRediT authorship contribution statement

**Yingfang Lu:** Methodology, Writing – original draft. **Bo Peng:** Resources, Writing – original draft. **Yuqi Lin:** Writing – original draft, Data curation. **Qianmin Lin:** Data curation. **Xuewei Xia:** Data curation. **Saiyi Zhong:** Writing – review & editing, Supervision. **Lianxiang Luo:** Conceptualization, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.08.010.
