# Effect of Astragalus Polysaccharides on Cancer Cells Studied by AFM

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***Abstract*—As a traditional Chinese medicine, astragalus and its products are used in cancer treatment aiming to reduce the side effects of chemotherapy. Cells are the most basic unit of living organisms, and AFM directly obtains information from living cells on the micro/nano scale. Therefore, the use of AFM to study the interaction between astragalus and cells is conducive to a full range of drug efficacy evaluation and provides a new way for drug development. In this paper, astragalus polysaccharides were extracted from astragalus, which were diluted into solutions of different concentrations. Combined with the MTT experiment, the effects of Astragalus polysaccharide on cancer cells and benign cells were studied by AFM.**

***Keywords—Astragalus polysaccharides, cancer cells, MTT, AFM***

## I. Introduction

According to the advantages of less damage to organs such as the liver and kidneys [1], prescriptions that can be adjusted at any time based on patient feedback [2-3], minor side effects [4-5] and less irritation to the stomach and intestines [6], traditional Chinese medicine attracts a lot of attention around the world [7-9]. In recent years, with the rapid increase in the number of cancer patients worldwide, scholars have considered applying some Chinese medicines with specificity and tumour treatment potential to cancer treatment [10-12]. These traditional Chinese medicines can be roughly divided into two categories based on their efficacy. One is to enhance human immunity, such as ginseng [13-15] and Ganoderma lucidum [16-17], and the other is effective against cancer, such as Scutellaria barbata [18-19], Lobelia chinensis [20-21] and Spina gleditsiae [22-23]. Astragalus is also among them because of its ability to replenish vitality, strengthen body protection, and eliminate toxins and pus from the body [24].

Most of the products of astragalus used in research are astragalus polysaccharides [25] and astragalus saponins [26]. Li et al. extracted astragalus polysaccharide in 2009 and applied it to a rat model of gastric cancer. The results showed that the spleen lymphocytes of rats proliferated significantly, proving that astragalus effectively regulated immune activity [27]. In 2019, Li found that astragalus polysaccharide had no obvious inhibitory effect on the proliferation of MCF-7 cells, but it activated macrophages to achieve the purpose of killing breast cancer cells [28]. Lin concluded in the case of 1409 subjects that the combination therapy of astragalus and chemotherapy reduced nausea and diarrhea and other chemotherapy-related reactions in the treatment of colon cancer, and effectively improved the tumour remission rate [29]. In 2021, Georgieva used astragalus saponins to act on Graffi tumour cells, and the results showed that astragalus saponins had anti-tumor cell proliferation effects [30]. However, these studies mainly focused on the application of astragalus and its extracts, but did not reflect the changes in cancer cells when the drug interacted with these cells. This is of great significance for the study of the pharmacology of astragalus and the development of derivative drugs.

In this work, the astragalus polysaccharide powder was extracted and concentrated from astragalus membranaceus, and after dilution, different concentrations of astragalus polysaccharide solution were obtained to study the effects of different concentrations of astragalus polysaccharide on cancer cells and healthy cells. Human immortalized liver cells HL-7702 and human liver cancer cells SMMC-7702 were cultured for 24 hours and then respectively co-cultured with different concentrations of astragalus polysaccharide solution for 24 hours and 48 hours. Representative drug concentrations were selected by MTT experiment. Afterwards, it was detected by atomic force microscope (AFM, NanoWizard® R3, JPK instruments) to obtain the characteristic values before and after the two kinds of cells were co-cultured, and to study the effect of incubation time and the concentration of astragalus polysaccharide on healthy cells and cancer cells.

## II. Method and Experiment

### A. Extraction of Astragalus Polysaccharides

Currently known active ingredients in astragalus are astragalus saponins and polysaccharides, and the extraction methods are different. The extraction of astragalus polysaccharides mainly included four steps, followed by

rough extraction, concentration, and precipitation of active ingredients and freeze-drying. The solvent used in the rough extraction was deionized (DI) water, and the solvent used in the final refinement was absolute ethanol. The finally obtained astragalus polysaccharide powder was light yellow-brown, which was sealed and stored in a refrigerator at -4ºC, and would be used in subsequent MTT experiments.

### *B. Cell Culturing*

In order to study the effect of astragalus polysaccharide in cancer cells and healthy cells, two kinds of cells derived from human liver were mainly used in this experiment, including human immortalized liver cell line HL-7702 and liver cancer cell line SMMC-7721. The two kinds of cells were incubated with Roswell Park Memorial Institute medium (RPMI, HyClone), appropriate concentration of Fetal Bovine Serum (FBS, ABW) and Penicillin-streptomycin solution (Solarbio), and the two cells were cultured in a constant temperature incubator at 37° C with a $CO_2$ concentration of 5%.

### *C. MTT*

Astragalus polysaccharide solutions with different concentration gradients were prepared. Based on the reduction reaction between living cells and MTT, the content of purple crystal formazan formed by living cells after co-incubation with different concentrations of astragalus polysaccharide solution was tested. The cell viability is calculated as

$$\text{Cell viability} = \nu_e/\nu_c \times 100\% \quad (1)$$

$\nu_e$ represents the average cell activity of a single sample in the experimental group, and $\nu_c$ represents the average cell activity of the control group. The higher the cell viability, the lower the drug toxicity.

### *D. AFM*

AFM uses the interaction force between the probe tip and the measured sample to determine the force feedback and convert it into data, which can directly obtain the physiological characteristics of living cells in a liquid environment. Astragalus polysaccharide solutions of several concentrations which showed special changes in the results of the former MTT experiment were selected and then incubated with the two kinds of cells for 24h and 48h, respectively. Finally the physical properties of the cells before and after the incubation were obtained.

## III. Results and Discussion

When astragalus is used as a medicinal material in traditional Chinese medicine prescriptions, it has the effects of curing weakness and eliminating edema. In addition, Astragalus can also be cooked with chicken as a medicinal diet. Therefore, it can be proved that the toxicity of astragalus itself is too little to ignore. After the effective ingredients are extracted, the toxicity is indeed enhanced. However, it still does not cause much damage to the cells incubated with FBS in pre-experiment, and even promotes cell proliferation conversely. Therefore, in the formal MTT experiment, the culture medium without FBS was used to eliminate the promotion effect of FBS on cell proliferation.

Figs. 1(a) and (b) show the cell viability of two kinds of cells respectively co-cultured with different concentration gradients of astragalus polysaccharide solution for 24 hours and 48 hours. In Fig. 1(a), when the concentration of astragalus polysaccharide solution is not higher than 1 mg/mL, the cell viability of benign cells HL-7702 does not change much, and the cell viability is all above 90%. The cell viability is even higher than 100% at several low concentrations. However, when the concentration of astragalus polysaccharide solution is higher than 1 mg/mL, the cell viability of HL-7702 cells is greatly affected, and there shows a significant decrease. At this time, the cancer cells SMMC-7721 has different results from healthy cells. When the concentration of astragalus polysaccharide solution is not higher than 1 mg/mL, the cell viability of SMMC-7721 cells nearly shows a linear decline, reaching the lowest point at the concentration of 1 mg/mL, which is about 10%. When the drug concentration is increased later, the cell activity is increased, but it isstill about 10%.

The result in Fig. 1(b) is quite different from that in (a). After 48 hours of incubation with different concentrations of astragalus polysaccharide solution, the cell viability of healthy cells HL-7702 is not higher than 70%. When the drug concentration is between 0.25 mg/mL and 1 mg/mL, the cell activity does not change much, and when the drug concentration is 4 mg/mL, the cell activity reaches the lowest level, which is about 20%. As for SMMC-7721 cells, when the concentration of astragalus polysaccharide is not higher than 0.5 mg/mL, the cell activity is decreased with the increase of the drug concentration. After that, as the concentration of the drug is increased, the cell activity is increased slightly and stabilized at about 40%.

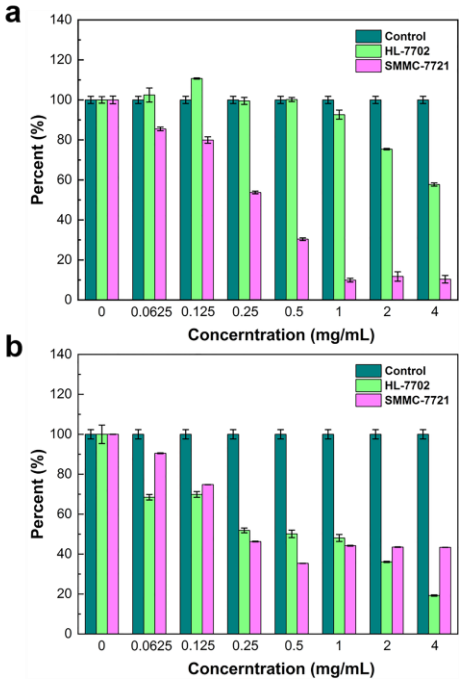


Fig. 1. The cell viability of the human immortalized hepatocytes HL-7702 and hepatoma cells SMMC-7721 culturing with multi-concentrations of astragalus polysaccharide solution after 24 h (a) and 48 h (b).

The results of the MTT experiment showed that in a short time period (24 hours) and at a low concentration (≤1 mg/mL), the astragalus polysaccharide solution had almost no negative effect on healthy cells, but within this concentration range, it inhibited the proliferation of cancer cells. After the co-incubation time was extended (48 hours), the astragalus polysaccharide solution had a relatively obvious proliferation inhibitory effect on healthy cells and

cancer cells. It could be seen that the concentration of astragalus polysaccharide solution had a greater impact on the proliferation of these two types of cells, so the drug concentrations (0.5 mg/mL, 1 mg/mL and 4 mg/mL) that had a greater impact on cell activity would be selected for subsequent AFM experiments.

After the selected three concentrations of astragalus polysaccharide solutions incubated with the cells for 24 hours and 48 hours, the results are respectively shown in Fig. 2 and Fig. 3.

Fig. 2(a) and (b) respectively show the morphologies of cancer cells, healthy cells and drugs after 24 hours of co-cultivation. It can be seen from the figure that the liver cancer cells are closer to round than the liver cells. In addition, when the drug concentration reaches 1 mg/mL, the structure of liver cancer cells becomes unstable. At this time, the growth of liver cancer cells is affected by astragalus polysaccharides, which is consistent with the conclusions in the MTT experiment. Fig. 2(c) shows the tendency of the adhesion of the two types of cells as the concentration of the astragalus polysaccharide solution increases. It shows that the adhesion of hepatocytes continued to decrease when the drug concentration is increased, and the magnitude of change is much larger than that of cancer cell adhesion. The results of previous experiments show that the cell adhesion between healthy cells and their homologous cancer cells is not much different. Therefore, it can be proved that the astragalus polysaccharide solution affects the adhesion of the cells, which in turn affects the growth state of the cells even the cell survival. This result is also reflected in the previous MTT experiment results. Fig. 2(d) shows the result of the influence of astragalus polysaccharide on the Young's modulus of the two cells. From the overall trend, the Young's modulus of liver cancer cells is decreased with the increase of drug concentration, but the change is not so much, while the hepatocytes has the opposite conclusion. Therefore, astragalus increases the hardness of liver cells and makes the liver cancer cells slightly softer.

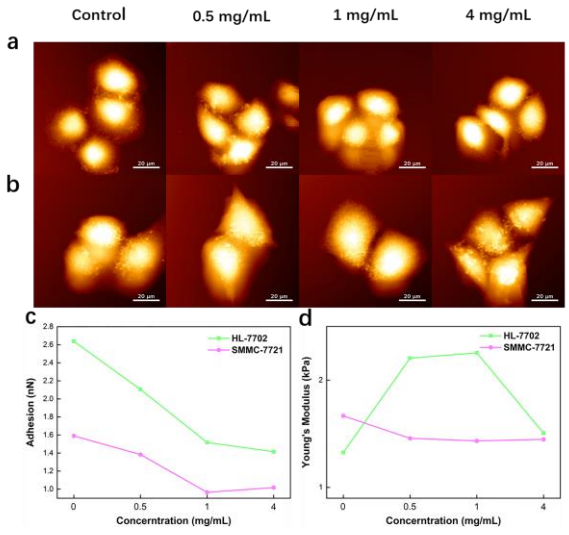


Fig. 2. The physical information of cancer cell SMMC-7721 and healthy cell HL-7702 obtained by AFM after incubation with different concentrations of astragalus polysaccharide solution for 24 hours. (a) The morphology of SMMC-7721 cells before and after incubation with polysaccharide solutions of different concentrations. (b) The morphologies of HL-7702 cells before and after incubation with polysaccharide solutions of different concentrations. (c) Changes in adhesion of two kinds of cells before and after co-incubation with different concentrations of astragalus polysaccharide solution respectively. (d) Changes of Young's modulus before and after two kinds of cells were incubated with the different concentrations of astragalus polysaccharide solution.

Fig. 3 is similar to Fig. 2, except that the co-incubation time has been increased to 48 hours. Fig. 3(b) shows that the cell morphology of hepatocyte HL-7702 can hardly be maintained when the drug concentration reached 4 mg/mL, which is close to lysis. In addition, the results of the adhesion of the two types of cells remained the same as that of 24 hour co-incubation. But the conclusion of Young's modulus is slightly different. When the concentration of astragalus polysaccharide is increased, the Young's modulus of the two types of cells is increased, which means that the two types of cells become harder under the action of astragalus polysaccharide.

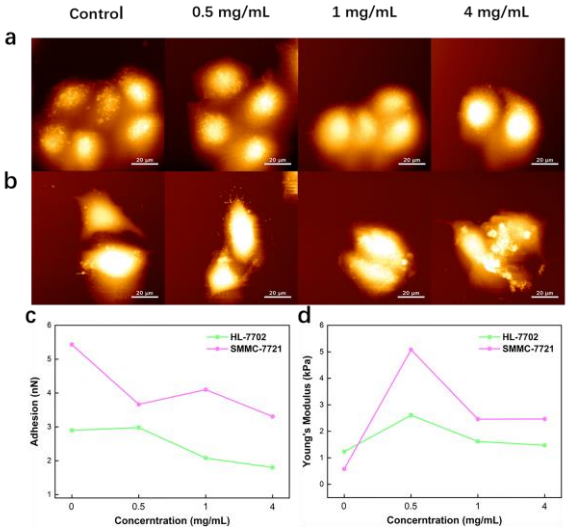


Fig. 3. The physical information of cancer cell SMMC-7721 and healthy cell HL-7702 obtained by AFM after incubation with the different concentrations of astragalus polysaccharide solution for 48 hours. (a) The morphologies of SMMC-7721 cells before and after incubation with the polysaccharide solutions of different concentrations. (b) The morphologies of HL-7702 cells before and after incubation with the polysaccharide solutions of different concentrations. (c) Changes in adhesion of two types of cells before and after co-incubation with the different concentrations of astragalus polysaccharide solution respectively. (d) Changes of Young's modulus before and after two types of cells are incubated with the different concentrations of Astragalus polysaccharide solution.

## IV. Conclusion

In this work, the effects of different concentrations of astragalus polysaccharide solution and different co-cultivation time on hepatocytes and its homologous cancer cells SMMC-7721 were studied. Through the MTT experiment and the use of AFM to obtain its morphology and characteristic physical information, it was found that when the concentration of astragalus polysaccharide solution was low and the incubation time was short, it promoted the proliferation of benign cells HL-7702 cells and inhibited proliferation of malignant cells SMMC-7721. When the concentration of astragalus polysaccharide and co-culturing time were increased, the astragalus polysaccharide solution reduced the adhesion of cells and changed the Young's modulus, thereby inhibiting the proliferation of the cells. The work in this article provides physical support for the pharmacology of astragalus polysaccharides.

## Acknowledgment

This work was supported by National Key R&D Program of China (No. 2017YFE0112100), EU H2020

Program (MNR4SCell No.734174), National Natural Science Foundation Program of China (No. 62175020), Jilin Provincial Science and Technology Program (Nos. 2020C022-1, 20190201287JC, 20190702002GH and 20200901011SF), Jilin Province Education Department Program (No. JJKH20210833KJ), and "111" Project of China (No. D17017).

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