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# Effects of Ginsenoside Rg3 on HLA-DR, HLA-ABC and Cellular Immune Function in Patients with Tumor Radiotherapy

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**XL and SZ contributed equally to this work as co-first author. LL, XW and YX participated in conceiving the design of the study and collecting and reviewing the data and coordination of project. XW and YL participated in doing literature review, collecting the data and analysis and in preparing the manuscript and helped in critical revision and finalizing the manuscript. All authors read, revised, and approved the final manuscript.**

**Key words**
**Ginsenoside Rg3, Human leukocyte DR antigen, Human leukocyte ABC antigen, Cellular immune function, Cancer radiotherapy patients**

## ABSTRACT

The objective of this study was to study the effect of Ginsenoside Rg3 on HLA-DR, HLA-ABC and cellular immune function in patients with tumor radiotherapy. From July 2017 to August 2018, 80 patients who were diagnosed as tumor and received radiotherapy were randomly divided into Ginsenoside Rg3 group and control group. The lymphocyte increment rate, the expression rate of cell membrane surface molecules (HLA-DR, HLA-ABC, CD3, CD4, CD8) were measured by flow cytometry. The positive expression rates of lymphocyte antibodies (IgG, IgM) and Th1, Th2 cells were measured by ELISA. Compared with the control group, different doses of ginsenoside monomer Rg3 can significantly promote the proliferation of lymphocytes induced by LPS and Rg3. The expression of HLA-DR and HLA-ABC in Rg3 group was significantly higher than that in the control group, and the effect of middle dose Rg3 was the strongest. Compared with the control group, the expression of CD3, CD4 positive cells and the ratio of $CD4^+$ / $CD8^+$ in different doses of Rg3 were significantly up-regulated, and the expression of CD8 positive cells was down regulated. Different doses of Rg3 can significantly up regulate the expression of IgG and IgM antibody, down regulate the positive expression rate of Th2, and the middle and high doses of Rg3 can significantly up regulate the positive expression rate of Th1. Ginsenoside Rg3 can up regulate the expression of HLA-DR and HLA-ABC in peripheral blood lymphocytes of patients with tumor radiotherapy, and enhance the cellular immune function, which is worth further promotion in clinical application, and provide reference value for adjuvant treatment of patients with tumor radiotherapy.

## INTRODUCTION

Tumor is a common disease that endangers human health, it is also an important reason for the death of patients (Chen and Liu, 2022). Today, many treatment methods, especially immunotherapy, are used to control cancer, and many targeted and fundamental researches are being conducted in this field (Pak *et al*., 2015). The idea that the immune system can detect tumors and control their

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growth dates back to 1983 (Yang, 2015), when McCarthy (2006) used live bacteria as immune system stimulators to treat cancer. Some scholars have shown that when the immune function of the body is low, the immune monitoring function of the body to tumor cells will also be weakened (Albers *et al*., 2013). The immune function of tumor patients is low, indicating poor prognosis of the disease.

Radiation therapy is one of the three major treatment methods of tumor, which can effectively inhibit and kill cancer cells, but it can also lead to the decline of immune function, bone marrow suppression, inflammation and other side effects, and even harm the life of patients. Therefore, it is of great significance to enhance the body resistance, prolong the survival period and improve the quality of life of patients by using drugs to improve immunity at the same time of radiotherapy. Ginsenoside Rg3 is a kind of tetracyclic triterpenoid panaxadiol saponin monomer extracted from ginseng. According to relevant experimental studies, Ginsenoside Rg3 has significant antitumor and immunity enhancing effects, and is one of the most active ginsenoside monomers (Zhang *et al*., 2013; Geng *et al*., 2002). Therefore, the purpose of this study was to study the effect of ginsenoside Rg3 on HLA-DR, HLA-ABC and cellular immune function in patients with tumor radiotherapy, so as to provide reliable basis and new treatment ideas for patients with tumor radiotherapy.

## MATERIALS AND METHODS

*General information*

From July 2017 to August 2018, 80 patients who were diagnosed as tumor and received radiotherapy were randomly divided into Ginsenoside Rg3 group and control group. There were 40 people in Rg3 group, 24 men and 16 women, aged 46-65 years, with an average age of (54.36 ± 10.02) years, including 12 cases of breast cancer, 18 cases of lung cancer, 6 cases of lymph cancer, 2 cases of colon cancer and 2 cases of nasopharyngeal carcinoma. The control group consisted of 40 patients, 25 males and 15 females, aged 45-64 years, with an average age of (55.44 ± 9.89) years, including 10 cases of breast cancer, 21 cases of lung cancer, 5 cases of lymphoid cancer, 3 cases of colon cancer and 1 case of nasopharyngeal carcinoma. There was no significant difference between the two groups in gender, age and tumor type (P>0.05). Patients in both groups were eligible to join the present study if they met the following inclusion criteria: the tumor was confirmed by operation and pathology, the patients received radiotherapy, the functions of heart, liver, kidney and other important organs were basically normal, with the permission of the Ethics Committee of the hospital, all patients were informed and agreed. Patients with distant metastasis of cancer cells and primary systemic immune system diseases were excluded from the study.

*Materials and reagents*

Constant temperature incubator (Beijing Ruite Instrument Equipment Co., Ltd., model: SLI-400), low-temperature high-speed centrifuge (Shanghai Precision Instrument Company, model: GL-2050MS), Flow cytometry (Beijing Pangu Innovation Biotechnology Co., Ltd., model: ACEA NovoCyte), Ginsenoside Rg3 (Dalian Fusheng Pharmaceutical Co., Ltd.), HLA-DR antibody (Shanghai Lianmai Bioengineering Co., Ltd.), HLA-ABC antibody (Shanghai Yubo Biotechnology Co., Ltd.), CD3 antibody (Beijing Tongli Haiyuan Biotechnology Co., Ltd.), CD4 antibody (Shanghai Fusheng Industrial Co., Ltd.), CD8 antibody (Shanghai Yansheng Industrial Co., Ltd.), IgG antibody (Wuhan Okbotai Biotechnology Co., Ltd.), IgM antibody (Biolab Biotechnology Co., Ltd.) were used in this study.

*Lymphocyte separation and purification*

Peripheral venous blood was drawn from the two groups 24 h after radiotherapy for routine anticoagulation, and the same amount of PBS solution was added for mixing, and then the same volume of lymphocyte separation solution was added respectively, 2500rpm, centrifugation for 10 min. Then RPMI1640 containing 10% FBS was added to the culture medium for re suspension and adherent cells were removed.

*Lymphocyte proliferation rate measurement*

Collect the above-mentioned lymphocytes, after corresponding treatment, suspend the lymphocytes again, adjust the cell density, lay the plates, and then put them into constant temperature incubator for 2 h, and add LPS (5 μg/ml) and concanavalin A (5 μg/ml) respectively for stimulation. Ginsenoside Rg3 group was added into the culture hole according to 5, 25 and 100 μg/ml respectively, while the control group was added into the culture hole with the same amount of DMSO, and the lymphocyte increment rate was measured after 72 h of incubation in incubator.

*Measurement of the molecular expression rate on the cell membrane surface*

For measurement of molecular expression rate on the cell membrane surface antibodies (HLA-DR, HLA-ABC, CD3, CD4, CD8) were added to the above lymphocytes, respectively, washed with PBS after reaction for a certain period of time. Then cell suspension was used to measure the molecular expression rate on the cell membrane surface.

**Table I. Lymphocyte proliferation in different mitogen induced tumor radiotherapy patients ($\bar{x}\pm s$).**

| Project | Control group (n = 40) | Rg3 group (n = 40) | | |
|---|---|---|---|---|
| | | 5μg/ml | 25μg/ml | 100μg/ml |
| LPS (5μg/ml) | 0.04±2.39 | 2.36±0.24* | 3.22±2.64* | 4.35±0.68* |
| ConA (5μg/ml) | 0.05±2.84 | 22.07±2.54* | 27.89±3.46* | 42.33±2.05* |

* $P<0.01$

**Table II. Effect of Ginsenoside Rg3 on for the study patients ($\bar{x}\pm s$).**

| Indicator | Control group (n = 40) | Rg3 group (n = 40) | | |
|---|---|---|---|---|
| | | 5μg/ml | 25μg/ml | 100μg/ml |
| HLA-DR (%) | 21.89±1.38 | 27.43±1.28 | 31.36±2.12 | 25.11±2.19 |
| HLA-ABC (%) | 26.43±1.41 | 27.09±1.76 | 32.36±1.48 | 33.37±1.09 |
| CD3+ (%) | 30.98±1.38 | 32.64±1.35 | 39.41±2.09 | 47.42±1.86 |
| CD4+ (%) | 27.26±2.09 | 36.82±1.94 | 42.16±1.33 | 46.44±2.13 |
| CD8+ (%) | 38.54±2.28 | 32.08±1.36 | 26.45±1.26 | 21.58±1.32 |
| CD4+/CD8+ | 0.74±0.03 | 1.08±0.19 | 1.27±0.13 | 1.44±0.06 |
| IgG (mg/ml) | 0.95±0.26 | 1.50±0.18 | 1.72±0.15 | 1.88±0.23 |
| IgM (mg/ml) | 0.56±0.13 | 2.88±0.26 | 4.01±0.27 | 5.80±0.49 |
| Th1+ (%) | 5.54±0.49 | 5.01±0.62 | 11.34±1.80 | 16.44±1.28 |
| Th2+ (%) | 3.17±0.47 | 1.47±0.19 | 1.26±0.14 | 0.81±0.24 |

*Determination of lymphocyte IgG, IgM antibody and cytokines*

According to the above-mentioned different doses of Rg3, they were added to the culture wells for regular culture, then washed and hung again. After taking corresponding measures, the expression levels of lymphocyte IgG, IgM antibody and Th1 cells (IL-2, IFN - γ positive lymphocytes), Th2 cells (IL-4, IL-10 positive cells) were detected by ELISA.

*Statistical analysis*

SPSS18.0 software was used for data statistics, t test was used for measurement data, expressed with ($\bar{x}\pm s$), $\chi^2$ test was used for counting data, expressed with %, and P<0.05 was used as the difference with statistical significance.

## RESULTS

The results of comparison of lymphocyte proliferation in different mitogen induced tumor radiotherapy patients showed that compared with the control group, different doses of ginsenoside monomer Rg3 can significantly promote the proliferation of lymphocytes induced by LPS and Rg3 ($P<0.05$) after 72 h of treatment of peripheral blood lymphocytes of tumor radiotherapy patients, and it is dose-dependent (Table I).

The results of ginsenoside monomer Rg3 effects on the expression of HLA-DR and HLA-ABC and lymphocyte subsets in peripheral blood lymphocytes of patients with tumor radiotherapy are shown in Table II. Compared with the control group, Rg3 could significantly increase the expression of HLA-DR and HLA-ABC (P<0.05), among which the low dose (5 μg/ml) and medium dose (25 μg/ml) of Rg3 could significantly increase the expression of HLA-DR (P<0.01), while the medium dose of Rg3 had the strongest effect on the expression of HLA-DR. The high dose (100 μg/ml) of Rg3 had lower and weaker effect on the expression of HLA-DR (P<0.05). Compared with the control group, the expression of HLA-ABC increased significantly (P<0.01) at medium and high doses of Rg3 (Table II). Compared with the control group, the expression of CD3, CD4 positive cells and the ratio of CD4+ / CD8+ in the low, medium and high doses of Rg3 were significantly up-regulated (P<0.05), and the expression of CD8 positive cells was down regulated (P<0.05) (Table II). Compared with the control group, low, medium and high doses of Rg3 can significantly up regulate the expression level of IgG

and IgM antibody ($P < 0.05$); compared with the control group, medium and high doses of Rg3 can significantly upregulate the Th1 positive expression rate ($P < 0.01$), while three doses of Rg3 can significantly downregulate the Th2 positive expression rate ($P < 0.01$) (Table II).

## DISCUSSION

Tumor is a genetic or non-genetic disease in which the cells change their genes under the action of carcinogenic factors, thus losing the regulation of normal growth, resulting in the formation of new organisms due to the abnormal proliferation of monoclonal. In clinic, the main methods of tumor treatment are surgery, radiotherapy and chemotherapy, among which radiotherapy refers to the treatment method of irradiating tumor with various energy rays to inhibit and kill cancer cells. Radiotherpy has the advantages of wide application, non-invasive and exact curative effect, but at the same time, it also destroys normal cells of surrounding tissues besides killing tumor cells. Radiation was also used to reduce the immune function of the body. Some animal experiments show that ginsenoside Rg3 can significantly improve the specific and non-specific immune function of mice (Zhang *et al*., 2004). Therefore, the effect of ginsenoside Rg3 on HLA-DR, HLA-ABC and cellular immune function in patients with tumor radiotherapy is of great significance, providing reference value for clinical patients with tumor radiotherapy.

In recent years, studies have shown that the strength of anti-tumor response of the body is related to the infiltrated lymphocytes in the tumor tissue, and with the increase of the number of infiltrates, the immune killing power of the body to the tumor is stronger, and the increase of the number of peripheral blood lymphocytes is of great significance to improve the immunity of the body (Zhang *et al*., 2013; Davies *et al*., 2005). After chemotherapy and radiotherapy, the absolute total number of peripheral lymphocytes in many patients with malignant tumors also decreased. At this time, in order to maintain the stability of the immune environment, the body started the self-stable proliferation of endogenous lymphocytes, so that the lymphocytes gradually returned to the normal physiological level. However, some studies have shown that the lymphocytopenia caused by radiotherapy will lead to the abnormal immune function of the body, which is not conducive to the induction of anti-tumor immune response. Ginsenoside Rg3 has the strongest antitumor activity among known ginsenosides, with the molecular formula of c42h72o13. He *et al*. (2015) showed that Rg3 can promote the proliferation of peripheral lymphocytes stimulated by different mitogens, thus improving the level of immune response. Combined with the results of this study, Rg3 at different doses can significantly promote the proliferation of lymphocytes induced by LPS and Rg3, and it is dose-dependent. The results showed that ginsenoside Rg3 could reverse the inhibition of lymphocyte proliferation induced by tumor chemotherapy, and with the increase of dose, the effect of ginsenoside Rg3 on lymphocyte proliferation was more significant. But at present, its mechanism still needs to be further explored.

The main function of human leukocyte antigen (HLA) is to present molecules for antigens, and then present the combined polypeptides to T cells to activate T cells. Studies have shown that HLA is closely related to the occurrence and development of tumor, treatment and prognosis, determines the histocompatibility of the body, and is closely related to the immune response and immune regulation of the body (Xu *et al*., 2012; Morandi *et al*., 2010). Many tumor patients have low expression of HLA, which makes T cell activation lack of messenger, cannot recognize foreign antigen, make tumor escape immune surveillance, and then reduce the level of immune response. The results of this experiment show that Rg3 can significantly increase the expression of HLA-DR and HLA-ABC, among which the middle dose Rg3 has the strongest effect on the expression of HLA-DR, and the middle and high dose Rg3 can also significantly increase the expression of HLA-ABC. The results showed that ginsenoside Rg3 could significantly improve the antigen presenting ability of HLA in patients with tumor radiotherapy, thus enhancing the immunity of the body.

T-lymphocyte is an important immune cell in the body, which can be divided into $CD3^+$, $CD4^+$, $CD8^+$ subsets. The expression level of subsets is an important index to observe the activity of T-lymphocyte. The number of $CD3^+$ directly reflects the immune activity and quantity of the body participating in the immune response. $CD4^+$ as a T-helper cell, its main function is to promote the secretion of B-lymphocyte, $CD8^+$ as a T-killer cell, and directly participate in the disease The ratio of $CD4^+$ / $CD8^+$ represents the state of immune function (Zhou *et al*., 2008). Studies have shown that radiotherapy can significantly reduce the expression of CD3 and the ratio of $CD4^+$/ $CD8^+$ (Zhang *et al*., 2004; Mullen *et al*., 2012). Combined with the results of this study, different doses of ginsenoside Rg3 can significantly up regulate the expression of CD3, CD4 positive cells and the ratio of $CD4^+$/ $CD8^+$. The results showed that Rg3 could improve the function of T-lymphocytes by regulating T-lymphocyte subsets, and ultimately improve the level of cellular immune response, so that the immunosuppressive effect of radiotherapy on the body was reduced.

IgG and IgM antibodies are immunoglobulins produced by B lymphocytes stimulated by antigens. When

IgG and IgM antibodies combine with corresponding antigens, they can mediate a variety of physiological effects of the body and participate in the immune response of the body (Begeman *et al*., 2017). Combined with the results of this study, different doses of ginsenoside Rg3 can significantly upregulate the expression level of IgG and IgM antibody, induce antigen antibody reaction, and enhance the immune activity in the body. Th1 and Th2 cells are two subtypes of helper T cells, which are closely related to the development of tumor. Some studies have shown that radiotherapy can down regulate Th1 expression and up regulate Th2 expression, resulting in the decrease of Th1/ Th2 ratio, resulting in the immunosuppression of the body, which is conducive to the escape of immune surveillance of tumor cells (Wei *et al*., 2002). The results show that Th1/ Th2 is a better index to observe the immune dynamic changes of tumor patients. Combined with the results of this study, Rg3 can significantly upregulate Th1 positive expression rate and downregulate Th2 expression, so as to increase Th1/ Th2 ratio and improve immunity. It is suggested that Rg3 can improve the immune response by regulating the expression of Th1/ Th2 in peripheral blood lymphocytes of patients with tumor radiotherapy.

## CONCLUSION

In conclusion, ginsenoside Rg3 can upregulate the expression of HLA-DR and HLA-ABC in peripheral blood lymphocytes of patients with tumor radiotherapy and enhance the cellular immune function, which can be used as potential immunoenhancer and provide reference value for clinical adjuvant treatment of patients with tumor radiotherapy.



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*IRB approval*

This study was carried out with the approval of Research Guidance Workshop Committee (Shanghai Pulmonary Hospital, Tongji University School of Medicine).

*Ethics statement*

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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