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Effect of Ganoderma Lucidum on Glycosylation Pathways and Immune Response Modulation in Colorectal Cancer Cells

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Abstract: Since glycosylation plays a key role in cancer progression and immune evasion in colorectal cancer (CRC), the study explores whether *Ganoderma lucidum* (GL) can modulate glycosylation pathways to inhibit tumor growth and strengthen anti-tumor immunity. The research will also investigate whether GL alters the glycosylation of immune checkpoint proteins, such as PD-L1, in HCT116 colorectal cancer cells, enhancing natural killer (NK) cell cytotoxicity and promoting the secretion of pro-inflammatory cytokines like TNF-alpha. Moreover, the study will investigate GL's potential as a complementary therapy for CRC, particularly in cases resistant to conventional treatments. The results will provide critical insights into Ganoderma lucidum's potential as a complementary therapy for colorectal cancer, particularly in enhancing immune responses. This research will contribute to the broader understanding of traditional medicines in cancer treatment and pave the way for future therapeutic developments.

Keywords: colorectal cancer, ganoderma lucidum, glycosylation pathways, immune checkpoint proteins.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, affecting over one million people each year. It is the third most common cancer and the second leading cause of cancer-related deaths. CRC develops from cells lining the colon or rectum and typically progresses from benign polyps to malignant tumors over many years. Various factors contribute to the development of CRC, including genetic mutations, environmental influences, dietary habits, and inflammatory conditions of the gastrointestinal tract. Despite advances in screening techniques like colonoscopy, which have improved early detection, many cases of CRC are still diagnosed at advanced stages, resulting in poor outcomes. Traditional treatments for CRC include surgery, chemotherapy, and radiotherapy, but these often come with significant side effects, and resistance to chemotherapy remains a major challenge, especially in advanced cases.

Given the limitations of conventional therapies for CRC, there is increasing interest in exploring alternative treatments rooted in traditional Chinese medicine. This research investigates the potential of *Ganoderma lucidum* (GL), a medicinal mushroom used in traditional Chinese medicine, to enhance anti-tumor immunity in CRC. The study aims to understand whether GL can influence glycosylation

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pathways—specific protein modifications that play a vital role in cancer progression and immune evasion.

The research will explore how GL might alter these pathways to inhibit tumor growth and strengthen the immune response. It will examine the effects of different concentrations and durations of GL treatment on the glycosylation of key immune checkpoint proteins in colorectal cancer cells. By targeting these modifications, GL could enhance the activity of natural killer cells, improving their ability to identify and eliminate cancer cells. The study will also assess whether GL can influence the secretion of pro-inflammatory cytokines, such as TNF-alpha, which are important in tumor suppression. Through these investigations, the research aims to uncover GL's potential as a complementary therapy to improve existing CRC treatments and as a new approach to managing the disease, especially in patients who do not respond well to conventional therapies.

Previous research on CRC has mainly focused on understanding the genetic and molecular mechanisms that drive the disease. Scientists have identified crucial genetic mutations and signaling pathways, such as APC, KRAS, and TP53, which play significant roles in the onset and progression of CRC. This understanding has led to the development of targeted therapies aimed at inhibiting these specific molecules. In parallel, the field of immunotherapy has made strides in using immune checkpoint inhibitors to boost the body’s natural defenses against cancer. Although these treatments have been successful in certain cancers, their effectiveness in CRC has been inconsistent, leading researchers to explore the potential of combination therapies.

In Chinese medicine, *Ganoderma lucidum* has been studied for its potential anti-cancer effects. Preclinical studies have shown that GL can prevent the development of CRC in animal models and reduce tumor size. Research has also highlighted the importance of glycosylation pathways in CRC, as abnormal glycosylation patterns are associated with tumor growth and immune evasion. Therefore, the study aims to determine whether *Ganoderma lucidum* can enhance anti-tumor immunity in colorectal cancer by influencing these glycosylation pathways.

I predict that increasing concentrations and treatment durations with Ganoderma lucidum extract will inhibit the glycosylation of key immune checkpoint proteins PD-L1 in HCT116 colorectal cancer cells, thereby enhancing NK cell-mediated cytotoxicity and promoting the secretion of cytokines such as TNF-alpha. This effect will be evaluated through western blot analysis of PD-L1 glycosylated proteins and flow cytometry to assess NK cell activity and ELISA to measure TNF-alpha cytokine levels. Positive control is Andrographolide, negative control is pbs/DMSO.

2. Materials and methods

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2.1. Cell culture

HCT116 colorectal cancer cells will be obtained from ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM) boosted with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/mL penicillin-streptomycin. The cells will be maintained in an incubator set at 37°C with 5% CO2. For each experiment, cells will be seeded at a density of 1×10^6 cells per well in 6-well plates and allowed to adhere for 24 hours before treatment.

2.2. Ganoderma lucidum (GL) treatment

Ganoderma lucidum extract will be prepared in DMSO, and the stock solution will be watered down in DMEM to create final concentrations of 10, 25, 50, and 100 μg/mL. Cells will be treated with these

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concentrations for durations of 24, 48, and 72 hours. Andrographolide (10 μM) will be used as a positive control, and cells treated with PBS containing 0.1% DMSO will serve as the negative control.

2.3. Western blot for PD-L1 glycosylation

After the treatment periods, cells will be lysed in RIPA buffer containing protease inhibitors. Protein concentrations will be quantified using a bicinchoninic acid (BCA) assay. Equal amounts of protein will be loaded onto SDS-PAGE gels for separation, followed by transfer to PVDF membranes. The membranes will be incubated with primary antibodies specific to glycosylated PD-L1 and a secondary antibody conjugated to HRP. Bands will be detected using enhanced chemiluminescence (ECL) and analyzed along with image software to assess glycosylation shifts compared to controls.

2.4. NK cell cytotoxicity assay by FACS

HCT116 colorectal cancer cells treated with *Ganoderma lucidum* (GL) will be co-cultured with natural killer (NK) cells at an effector-to-target ratio of 5:1. NK cells will be pre-activated with recombinant IL-2 before co-culture to enhance their cytotoxic activity. During co-culture, GFP (Green Fluorescent Protein)-labeled HCT116 cells will be used to allow easy identification and quantification of the cancer cells. Flow cytometry (FACS) will be employed to measure the extent of NK cell-mediated killing of HCT116 cells by detecting the reduction in GFP-labeled cancer cells. The cytotoxic effect of NK cells on GL-treated cancer cells will be compared to that of untreated cells (negative control) and cells treated with the positive control (Andrographolide) to evaluate the impact of *Ganoderma lucidum* on NK cell activity.

2.5. ELISA for TNF-alpha secretion

The supernatants from GL-treated HCT116 cells will be collected at 24, 48, and 72 hours, and TNF-alpha levels will be quantified using an enzyme-linked immunosorbent assay (ELISA). Absorbance will be measured at 450 nm using a microplate reader, and TNF-alpha concentrations will be calculated using a standard curve.

2.6. Statistical analysis

All experiments will be performed in triplicate, and data will be presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) will be used to determine statistical significance.

3. Results

Table 1: The combination of possible results

Combination Result # (CR#)	GL decreases glycosylation of PDL1 by western blot mass shift	GL increases NK killing of HCT116 by FACS of GFP labeled HCT116	GL increases TNFa by ELISA	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	-	-	+	Partial
6	-	+	-	Partial
7	-	-	+	Partial
8	-	-	-	Fully Contradicts

Note: "+" represents an increase or decrease relative to the positive control group. For PD-L1 glycosylation, a "+" means a decrease in glycosylation. For NK cell killing and TNF-alpha secretion, a "+" means an increase in activity or secretion. "-" represents no significant change compared to the negative control group.

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The table below outlines eight possible combinations of results (CR1 to CR8) for the three experiments involving glycosylation of PD-L1, NK cell cytotoxicity, and TNF-alpha secretion.

3.1. Description of each combination

Combination of possible results 1 (CR1): In this result, *Ganoderma lucidum* (GL) reduces the glycosylation of PD-L1, enhances the killing of HCT116 cells by NK cells, and increases TNF-alpha secretion. The western blot would show a mass shift in PD-L1 due to decreased glycosylation, and FACS analysis would indicate higher NK cell activity. The ELISA results would show increased levels of TNF-alpha in the supernatants. This outcome fully supports the hypothesis that GL enhances anti-tumor immunity by modulating glycosylation pathways and promoting immune responses.

Combination of Possible results 2 (CR2): In this case, GL reduces PD-L1 glycosylation and increases NK cell activity, as observed in the western blot and FACS results. However, TNF-alpha secretion remains unchanged compared to the control, as shown by the ELISA. This result partially supports the hypothesis by demonstrating that GL affects glycosylation and NK cell cytotoxicity but does not enhance cytokine secretion.

Combination of Possible results 3 (CR3): For result 3, GL decreases the glycosylation of PD-L1 and increases TNF-alpha secretion, as demonstrated by the western blot and ELISA results, but does not enhance NK cell cytotoxicity. The FACS analysis would show no significant increase in NK cell activity. This outcome partially supports the hypothesis by indicating that GL impacts glycosylation and cytokine production but does not improve NK cell-mediated killing.

Combination of Possible results 4 (CR4): In this result, GL does not affect the glycosylation of PD-L1, as shown by the western blot, but enhances NK cell cytotoxicity and increases TNF-alpha secretion. The FACS and ELISA results would demonstrate these changes. This outcome partially supports the hypothesis by showing that GL enhances immune activity through NK cells and cytokine production, despite not altering glycosylation.

Combination of Possible results 5 (CR5): Results 5, GL does not affect PD-L1 glycosylation or NK cell cytotoxicity, as indicated by the western blot and FACS results. However, the ELISA would show an increase in TNF-alpha secretion. This outcome partially supports the hypothesis by suggesting that GL's primary effect is on cytokine secretion rather than on glycosylation or NK cell activity.

Combination of Possible results 6 (CR6): In this case, GL does not impact PD-L1 glycosylation or TNF-alpha secretion, but FACS analysis shows increased NK cell activity. This outcome partially supports the hypothesis, suggesting that GL primarily enhances NK cell-mediated cytotoxicity without influencing glycosylation or cytokine production.

Combination of Possible results 7 (CR7): In this case, GL does not affect PD-L1 glycosylation or NK cell activity, but the ELISA shows increased TNF-alpha secretion. This result partially supports the hypothesis by indicating that GL's effect is limited to cytokine secretion without influencing glycosylation or NK cell cytotoxicity.

Combination of Possible results 8 (CR8): In this scenario, GL does not affect PD-L1 glycosylation, NK cell activity, or TNF-alpha secretion. The western blot, FACS, and ELISA results would all show no significant differences compared to the control. This outcome fully contradicts the hypothesis, suggesting that GL does not affect these immune pathways.

3.2. Possible results for treatment duration and concentration

The effects of treatment duration and concentration of Ganoderma lucidum (GL) can vary based on the immune mechanisms being targeted. At higher concentrations with longer durations, you might observe the strongest outcomes, such as reduced PD-L1 glycosylation, increased NK cell cytotoxicity,

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and elevated TNF-alpha secretion, which supports the hypothesis (like in Possible Result 1). In contrast, high concentration but shorter duration might yield partial effects, such as either a reduction in glycosylation or enhanced NK cell activity, but not both, due to insufficient time for full immune activation (like Possible Results 2 or 3). At lower concentrations with long durations, you might see gradual, mild increases in NK cell activity or cytokine secretion, indicating that GL could still have an effect over time, but it might not be enough to impact tumor immunity (like in Possible Result 4). However, low concentration and short duration treatments may lead to no observable changes in PD-L1 glycosylation, NK cell cytotoxicity, or TNF-alpha secretion, as the dose and exposure time may be insufficient to activate immune pathways (like in Possible Result 8). Moderate concentration and intermediate duration could lead to balanced effects, activating both NK cells and cytokine production without excessive immune modulation, showing an optimal approach (like in Possible Result 4 or 6).

4. Conclusion

In conclusion, this study evaluates the potential of *Ganoderma lucidum* (GL) to modulate immune mechanisms in colorectal cancer (CRC), specifically focusing on PD-L1 glycosylation, NK cell cytotoxicity, and TNF-alpha secretion. The possible results highlight various scenarios for how GL may influence these pathways. Result 1 suggests GL decreases PD-L1 glycosylation, increases NK cell activity, and enhances TNF-alpha secretion, indicating comprehensive immune modulation and supporting GL's role as a multi-target therapy. Result 2 shows GL decreases PD-L1 glycosylation and increases NK cell activity without enhancing TNF-alpha secretion, implying it reduces immune evasion and improves cytotoxicity but may require combination with cytokine therapies for a broader effect. Result 3 suggests GL decreases PD-L1 glycosylation and increases TNF-alpha secretion without boosting NK cell activity, highlighting its role in immune checkpoint regulation and inflammation rather than direct cytotoxicity. Result 4 shows no changes in glycosylation but an increase in NK cell activity and TNF-alpha secretion, suggesting GL activates immune responses through cytotoxicity and inflammation rather than modulating immune evasion.

The study also considers Result 5, where GL increases TNF-alpha secretion alone, suggesting its primary role as a cytokine modulator that could benefit from combination therapies targeting immune evasion and cytotoxicity. Result 6 highlights GL enhancing NK cell activity without changes in glycosylation or TNF-alpha secretion, pointing to its potential for selective cytotoxic activation. Result 7 shows GL promotes TNF-alpha secretion without impacting glycosylation or NK cell activity, positioning it as an immune adjuvant. Finally, Result 8 indicates no significant changes in any immune pathway, prompting a reassessment of GL's dosing, preparation, or testing in different cancer models.

The findings emphasize the critical importance of optimizing GL's concentration and treatment duration. Higher doses and extended treatment periods are associated with stronger effects but risk overstimulation or toxicity, necessitating careful balancing to maximize efficacy while minimizing side effects. Overall, this study will clarify whether GL can inhibit tumor growth and enhance anti-tumor immunity, particularly for CRC patients unresponsive to conventional therapies. Positive results could position GL as a complementary therapy, integrating traditional medicine into modern cancer treatment. If effects are limited or absent, further exploration of combination therapies and alternative approaches would be essential. This research ultimately contributes to the broader understanding of how traditional medicines can advance contemporary oncology.

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