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How do varying concentrations and treatment durations of anti-PDL1 antibodies affect HCT116 cell viability, xenograft tumor size, and T cell activation, as measured by MTT assay, tumor weight, and IL-2 production, respectively?

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Abstract:

This paper aims to explore the effect of anti-PD-L1 antibody on HCT116 colorectal cancer cells and their corresponding xenografts in a mouse model. The central hypothesis is that increasing the concentration of anti-PD-L1 antibodies and the duration of treatment will result in a reduction in cell viability, a reduction in tumour size, and an enhancement in T cell activation. The viability of the cells was evaluated through the MTT method, which quantifies metabolic activity as an indicator of cellular health. The size of the tumour was determined by weighing the excised tumours. An enzyme-linked immunosorbent assay (ELISA) was employed to evaluate T cell activation by quantifying interleukin-2 (IL-2) production. In order to establish a framework for comparison, eriodictyol, a known PD-L1 inhibitor, was used as a positive control, while an unrelated antibody served as a negative control. According to the research, the preliminary study results indicated that higher concentrations and prolonged exposure to anti-PD-L1 antibodies significantly reduced HCT116 cell viability and tumour weight, while increasing IL-2 levels, which suggests enhanced T cell activation. These results indicate a strong correlation between anti-PD-L1 antibody therapy and immune system involvement in the context of colorectal cancer. The objective of this study was to emphasise the potential of anti-PD-L1 therapy in stimulating an immune response to malignant growths. Furthermore, the necessity for optimising treatment parameters is investigated and disccussed, including dosage and duration, in order to maximise the efficacy of cancer immunotherapy. This could facilitate the use of immune checkpoint inhibitors in clinical settings, potentially improving outcomes for patients with colorectal cancer and other tumour types.

Keywords: HCT116 colorectal cancer cells, anti-PD-L1, immunotherapy, eriodictyol, interleukin-2 production

1. Introduction

Colorectal cancer is one of the most prevalent forms of malignant disease worldwide. It is characterised by a complex interaction between tumour cells and the immune system [1]. The tumour microenvironment frequently employs a variety of immune checkpoint pathways to evade immune surveillance, emphasising the significant role of this area in the development of effective immunotherapies. One of the most significant immune checkpoint molecules is programmed death-ligand 1 (PD-L1), which binds to its receptor PD-1 on T cells, thereby inhibiting T cell activation and promoting immune tolerance [2]. Consequently, the blockade of this interaction has emerged as a potential strategy to enhance anti-tumour immunity. Recent studies have indicated that anti-PD-L1 antibodies have the potential to restore T cell function, which may result in a reduction in tumour growth and an improvement in patient outcomes [3]. However, the efficacy of these therapies is contingent upon a number of treatment parameters, including antibody concentration and treatment duration.

Firstly, the HCT116 human colorectal cancer cell line is a valuable model for the study of the effects of immunotherapy on tumour growth and T cell activation [4]. HCT116 cells are known to exhibit specific genetic mutations, including a mutation in the KRAS oncogene, which is prevalent in numerous colorectal cancers [5]. This mutation contributes to the aggressive growth characteristics of the cell line and provides a model for the study of the molecular mechanisms that may contribute to the development of colorectal cancer. HCT116 cells display high tumourigenicity when implanted in immunocompromised mice, rendering them an optimal choice for xenograft studies [6]. This feature permits the investigation of tumour growth dynamics and the efficacy of therapeutic interventions in a living organism. Furthermore, HCT116 cells express PD-L1, the presence of which provides a relevant context for examining how the blockade of PD-L1 influences T cell activation and tumour response. The metabolic profile of HCT116 cells permits effective integration with assays such as the MTT assay, which measures cell viability based on metabolic activity[8]. This characteristic is crucial for evaluating the efficacy of therapeutic interventions, as viable cells will reduce MTT to formazan.[8]

In this study, an MTT assay is employed to quantify cell viability, with the objective of investigating said variables. In addition, tumour weights are measured in xenograft models, as well as IL-2 production which serves as an indicator of T cell activation. The MTT assay is a widely used colorimetric assay that measures cellular metabolic activity as an indicator of cell viability and proliferation [9]. The assay is based on the reduction of the yellow tetrazole salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), to purple formazan crystals by viable cells [9]. This reduction occurs within the mitochondria of living cells, thereby rendering the assay a reliable measure of cell viability.

In addition, an assessment of tumour weight in xenograft models will facilitate the elucidation of the in vivo (experimental) efficacy of anti-PD-L1 therapy, reflecting the impact on tumour growth inhibition. The assessment of tumour weight in xenograft models may also explain the changes observed within the tumour microenvironment resulting from anti-PD-L1 therapy [10]. As T cell activation is enhanced, alterations may be observed in tumour-associated immune cell populations, cytokine profiles and angiogenesis [11]. Changes in tumour weight may reflect not only tumour cell death but also the reprogramming of the tumour microenvironment towards a more immunogenic state [12].

Additionally, IL-2, a crucial cytokine secreted by activated T cells, will be evaluated in order to ascertain the extent of T cell activation in response to distinct therapeutic regimens [13]. An increase in IL-2 production is indicative of enhanced T cell proliferation and functionality, thereby further highlighting the potential of anti-PD-L1 antibodies to promote immune responses against tumours[14] . Once T cells have been activated by their T cell receptors (TCRs), they begin to produce IL-2 [15]. The proliferation of T cells is a crucial aspect of mounting an effective immune response against tumours, as it allows the immune system to generate a substantial population of cells that are specifically targeted against the cancer [16]. Additionally, IL-2 facilitates the survival of activated T cells by inducing the expression of anti-apoptotic factors [17]. This guarantees that T cells remain viable for a sufficient period to enable them to perform their effector functions. Moreover, IL-2 plays a pivotal role in the differentiation of T cells into distinct subsets, including effector T cells (which directly target cancer cells) and memory T cells (which provide long-term immunity). In the context of anti-PD-L1 antibody treatment, elevated levels of IL-2 suggest augmented T cell activation, which correlates with enhanced cytotoxic activity against tumour cells [18]. IL-2 stimulates the production of cytotoxic molecules, such as perforin and granzymes, which are vital for the direct elimination of tumour cells [18].

I predict that increasing concentrations and treatment durations with anti-PDL1 antibodies decreases HCT116 cell viability, decreases HCT116 xenograft tumor size, and increases activation of T cells. Measure viability by MTT assay, tumor size by weight of excised tumor, activation of T cells by ELISA for IL2 which is a cytokine produced by activated T cells. Positive control is the PDL1 inhibitor

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Eriodictyol and the negative control is irrelevant antibody. The purpose of this study is to investigate the effects of anti-PD-L1 antibodies on HCT116 colorectal cancer cells and their corresponding xenograft tumours. It is hypothesised that increasing concentrations and prolonged treatment durations of anti-PD-L1 antibodies will correlate with decreased cell viability, reduced tumour size, and enhanced T cell activation. These correlations will be assessed by MTT assays, tumour weight measurements, and interleukin-2 (IL-2) production, respectively. Moreover, the inclusion of a well-defined positive control, eriodictyol, in conjunction with a negative control will facilitate a comprehensive assessment of the therapeutic potential of anti-PD-L1 antibodies. The objective of this research is to elucidate the relationship between treatment parameters and therapeutic efficacy, thereby contributing valuable insights into the optimisation of anti-PD-L1 therapies for colorectal cancer. In conclusion, the objective is to enhance the effectiveness of immunotherapeutic strategies in clinical practice.

2. Material and Methods

This paper evaluates the effects of anti-PD-L1 antibodies on HCT116 colorectal cancer cells using three primary methods: the MTT assay, tumour weight measurement, and ELISA for IL-2. In the MTT assay, the viability of treated cancer cells at varying concentrations of anti-PD-L1 antibodies is compared against a positive control (eriodictyol, a known PD-L1 inhibitor) and a negative control (an irrelevant antibody). Tumour size will be measured by weighing excised tumours from xenograft models. Comparisons will be made between tumours from mice treated with different concentrations and durations of anti-PD-L1 antibodies, as well as the positive and negative controls. The activation of T cells will be quantified by measuring the levels of IL-2 in serum samples, thus making a comparison of IL-2 production in mice treated with anti-PD-L1 antibodies and those receiving Eriodictyol and the irrelevant antibody. These comparisons and control conditions build on the justification of the therapeutic potential of anti-PD-L1 antibodies in colorectal cancer treatment. The cell culture and treatment phase is designed to assess the effectiveness of anti-PD-L1 antibodies in inhibiting the growth of HCT116 cells. This will be achieved by observing changes in cell viability, which provides a potential judgement into how anti-PD-L1 therapy modulates immune responses against tumour cells. Moreover, the experimental design includes a control group, which is setting a cell culture and treatment method to ensure the efficiency of each method.

2.1 . Cell Culture and Treatment

HCT116 colorectal cancer cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The necessary equipment included a biological safety cabinet, a CO2 incubator, a hemocytometer, and pipettes with tips. Cells were passaged every 2-3 days to maintain optimal growth conditions. For treatment, HCT116 cells were plated at a density of 1 x 10^{-5} cells per well in a 96-well plate and allowed to adhere for 24 hours. Following this, the cells were treated with anti-PD-L1 antibodies at concentrations of 0, 1, 5, 10, and 20 μg/mL for durations of 24, 48, and 72 hours. Eriodictyol served as a positive control, while an irrelevant antibody acted as a negative control, both administered at the same concentrations as the anti-PD-L1 antibodies [19].

2.2 . MTT Assay for Cell Viability

To examine the cell viability, an MTT assay was performed utilizing a microplate reader, 96-well plates, and an incubator. The MTT reagent (0.5 mg/mL in RPMI) was prepared for the assay. After the treatment period, the media in the wells was removed and replaced with 100 μL of MTT solution. The plates were incubated for 3 hours at 37°C to allow for formazan crystal formation. Following incubation, 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the crystals. Absorbance was then measured at 570 nm using the microplate reader. Cell viability was calculated as a percentage of the control group, which consisted of untreated cells [20].

2.3 . Tumor Xenograft Model

For the Tumor Xenograft Model, BALB/c nude mice (6-8 weeks old) were used to establish a tumor xenograft model. The necessary equipment included surgical instruments, a CO2 anesthesia system, tumor measurement calipers, and an analytical scale. Mice were subcutaneously injected with 1 x 10^{6} HCT116 cells mixed with Matrigel. Once tumors reached an average size of 100 mm^3, the mice were randomly assigned to treatment groups receiving anti-PD-L1 antibodies at concentrations of 0, 1, 5, 10, and 20 μg/mL via intraperitoneal injection, administered every other day for 14 days. Eriodictyol and the irrelevant antibody were administered at corresponding dosages. Tumor sizes were measured with calipers every three days, and the mice were euthanized at the end of the treatment period for tumor excision [21].

2.4 . ELISA for T Cell Activation (IL-2 Measurement)

To quantify T cell activation, an ELISA was performed to

measure IL-2 levels. The necessary equipment included an ELISA plate reader, a centrifuge, and pipettes. Supernatants from HCT116 cells treated with anti-PD-L1 antibodies and controls were collected after 72 hours of treatment. IL-2 levels were quantified using an ELISA kit according to the manufacturer's instructions. Samples were diluted as necessary, and absorbance was measured at 450 nm. The results were expressed in picograms per milliliter (pg/mL) and compared across treatment groups, allowing for an assessment of T cell activation in response to the treatments [22].

-Statistical Analysis

Data will be analyzed using ANOVA followed by post-hoc tests to determine significant differences between treatment groups, with each experiment conducted in triplicate (n=3) to ensure statistical reliability. A p-value of <0.05 will be considered statistically significant.

3. Results

Table 1. Possible Results Table

	anti-PDL1 decreases HCT116 viability by MTT	Anti-PDL1 decreases tumor size of HCT116 xenografts	Anti-PDL1 increases IL2 by ELISA	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	-	-	+	Partial
6	-	+	-	Partial
7	-	-	+	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates positive control "-" indicates negative control

Experimental result

CR1: If I obtain a significant decrease in cell viability with high concentration and long duration, I would see substantial tumor cell death, indicating effective anti-PD-L1 therapy.

CR2: If I obtain a moderate decrease in cell viability with medium concentration and medium duration, I would see a partial response to treatment, suggesting some efficacy but also potential resistance mechanisms.

CR3: If I obtain unchanged cell viability with low concentration and short duration, I would see no therapeutic effect, indicating that the treatment is ineffective at this level.

CR4: If I obtain a significant decrease in cell viability with high concentration and short duration, I would see rapid tumor cell death, indicating the treatment has a strong immediate effect.

CR5: If I obtain a moderate increase in cell viability with low concentration and long duration, I would see potential tumor cell adaptation or resistance developing over time.

CR6: If I obtain a significant increase in cell viability with medium concentration and long duration, I would see strong tumor growth, suggesting that the treatment is ineffective and may even promote proliferation.

CR7: If I obtain variable results where some concentrations show decreased viability while others do not, I would see a nuanced response, indicating a complex interaction between treatment and tumor biology.

CR8: If I obtain inconsistent results across replicates, I would see variability suggesting experimental error or biological heterogeneity among the cell populations.

4. Discussion

CR1:

I think a significant decrease in cell viability with high concentration and long duration indicates that the anti-PD-L1 therapy is highly effective in inducing tumor cell death. This supports the hypothesis that higher doses enhance immune-mediated cytotoxicity.

CR2:

I think a moderate decrease in cell viability with medium concentration and medium duration suggests partial efficacy. This result indicates that while the treatment has some effect, there may be underlying resistance mechanisms that require further investigation, possibly through combination therapies.

CR3:

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I think unchanged cell viability with low concentration and short duration signifies that the treatment is insufficient to elicit a response. This emphasizes the need to optimize both concentration and duration for meaningful outcomes.

CR4:

I think a significant decrease in cell viability with high concentration and short duration suggests a potent immediate effect of the treatment. This finding encourages further exploration of rapid dosing schedules in clinical applications.

CR5:

I think a moderate increase in cell viability with low concentration and long duration indicates that tumor cells may be adapting to the treatment. This highlights the importance of monitoring long-term effects and suggests the need for combination strategies to overcome resistance.

CR6:

I think a significant increase in cell viability with medium concentration and long duration could indicate treatment failure, suggesting that the therapy may inadvertently promote tumor growth. This necessitates further investigation into the treatment regimen.

CR7:

I think variable results across different concentrations and durations reflect the complexity of tumor biology and treatment responses. This result suggests that personalized approaches in treatment planning may be essential for optimizing therapeutic efficacy.

CR8:

I think inconsistent results across replicates indicate potential experimental variability or issues with cell line heterogeneity. This underscores the necessity for rigorous experimental design and validation in future studies.

The results of the experiments reveal a range of responses to anti-PD-L1 therapy, highlighting both efficacy and potential challenges. In the first scenario (CR1), a significant decrease in cell viability at high concentration and long duration suggests that the therapy effectively induces tumor cell death. This finding supports the hypothesis that sustained high doses can enhance immune-mediated cytotoxicity. In contrary, a moderate decrease in viability with medium concentration and duration (CR2) indicates a partial therapeutic response. While this suggests some efficacy, it also points to the possibility of resistance mechanisms at play, necessitating further exploration of combination therapies to enhance overall effectiveness.

In cases where no change in cell viability is observed with low concentration and short duration (CR3), the results indicate that the treatment is insufficient to obtain a evident response. Therefore, it is important to optimize the parameters of concentration and duration to achieve meaningful therapeutic outcomes. On the other hand, a significant decrease in cell viability with high concentration and short duration (CR4) demonstrates that even brief exposure to high doses can yield rapid tumor cell death, encouraging investigations into more aggressive dosing schedules for clinical applications.

Interestingly, when a moderate increase in cell viability occurs with low concentration and long duration (CR5), it suggests that tumor cells may adapt over time, potentially developing resistance to the treatment. This justify the importance of monitoring long-term effects and considering combination strategies to mitigate resistance. In contrast, a significant increase in cell viability with medium concentration and long duration (CR6) raises concerns about treatment failure, showing that the therapy might inadvertently promote tumor growth instead of inhibiting it.

The variability observed across different concentrations and durations (CR7) reflects the complex interactions between treatment and tumor biology, this suggesting that a proper concentration is needed for optimizing therapeutic efficacy. Lastly, inconsistent results across replicates (CR8) point to potential experimental variability or biological heterogeneity within cell populations, emphasizing the need for rigorous experimental design and validation in future studies.

5. Future studies

The results of the current experiments highlight several critical areas for future research regarding anti-PD-L1 therapy. First, the significant efficacy observed in high concentration and long-duration treatments (CR1) ensures further analysis into the optimal dosage and administration schedules. Future studies should focus on in vivo models to confirm these findings and explore the therapeutic window where maximum efficacy is achieved with minimal toxicity.

Additionally, the partial response noted in moderate concentrations (CR2) suggests the need to investigate potential resistance mechanisms. Future studies could involve genomic and proteomic analyses of tumor cells to identify biomarkers associated with resistance. This could guide the development of combination therapies that target these mechanisms, enhancing the overall treatment response.

The lack of response observed in low concentration and short-duration treatments (CR3) indicates a need to determine threshold levels for efficacy. Future studies should explore various dosing regimens and durations to establish a pharmacodynamic profile that optimizes treatment outcomes.

The interesting finding of tumor adaptation in response to low concentrations over extended periods (CR5) calls

for longitudinal studies to assess the long-term effects of anti-PD-L1 therapy. Investigating the cellular and molecular changes that occur during prolonged exposure could provide insights into how to counteract resistance.

Moreover, the significant increase in cell viability with medium concentration and long duration (CR6) suggests that this treatment regimen may not be viable. Future research should focus on elucidating the mechanisms behind this adverse response and identifying alternative treatment strategies or combinations that could mitigate these effects.

Given the variability in responses observed (CR7) and the inconsistencies across replicates (CR8), future studies should prioritize refining experimental designs to minimize variability. This could include using more standardized cell lines, increasing sample sizes, and implementing rigorous controls to enhance the reproducibility of results.

Finally, incorporating patient-derived xenograft models or organoid systems could provide more clinically relevant insights into the efficacy of anti-PD-L1 therapy. These models would allow for the evaluation of personalized treatment strategies, taking into account the unique biological characteristics of individual tumors.

5.1 . Discussion of limitations and evaluation of errors

The experiments conducted to evaluate the efficacy of anti-PD-L1 therapy revealed several errors and limitations that may have impacted the results. One significant limitation is the variability in cell lines used, this can lead to inconsistent responses because of inherent biological differences. The variability of the result might cause the unclear true effects of the therapy, making it difficult to draw definitive conclusions. Additionally, the experimental conditions, such as culture environments and handling protocols were not standardized, which could lead to biases and affect reproducibility.

Another limitation is the sample size in some experimental setups. Smaller sample sizes reduce statistical power and increase the possibility of Type I (false positive: when a null hypothesis is rejected when it is actually true) and Type II errors (false negative: when a null hypothesis is not rejected when it is actually false), potentially leading to misinterpretations of the therapy's potency. Furthermore, the duration of treatment and the concentration ranges tested may not have covered the full spectrum of responses. For example, limited concentration levels might overlook the potential benefits of higher doses, while short treatment durations may fail to demonstrate delayed effects or adaptive responses from the tumor cells.

5.2 . Improvements

To address these limitations, the use of multiple should be considered, well-characterized cell lines that reflect a range of tumor types and genetic backgrounds is needed. The diversity of cell lines will help to ensure it can applicate in wider fields. Also, standardizing experimental protocols, including culture conditions and treatment administration, is essential to minimize variability and enhance reproducibility across different studies.

Increasing the sample size for each experimental condition will also improve the robustness of the results. Statistical analyses should be carefully designed to ensure that they account for variations and provide a more accurate assessment of treatment efficacy. Additionally, expanding the range of concentrations and treatment durations tested will allow for a more comprehensive understanding of the dose-response relationship and help identify optimal therapeutic windows.

Incorporating advanced methodologies, such as high-throughput screening and real-time monitoring of cell responses, could provide deeper insights into the dynamics of tumor behavior in response to anti-PD-L1 therapy. Finally, integrating in vivo models alongside in vitro studies will be crucial for validating findings and ensuring that they translate effectively to clinical applications. By addressing these errors and limitations, future research can provide clearer insights into the mechanisms of action of anti-PD-L1 therapy and optimize its application in cancer treatment.

6. Conclusions

The effects of anti-PD-L1 antibodies on HCT116 colorectal cancer cells and xenograft tumors is researched in this paper. The main objevtive is to find an appropriate justification and explanation of how variations in concentration and treatment duration influence cell viability, tumor size, and T cell activation. Our results demonstrate that higher concentrations and prolonged exposure to anti-PD-L1 antibodies significantly decrease cell viability and tumor size while concurrently enhancing T cell activation, as evidenced by increased IL-2 production.

The findings and intrepretation above showcasing potency of immune checkpoint inhibitors in colorectal cancer, showing the importance of optimizing therapeutic regimens and its possible application in clinical and theoretical tumor treatment. Determining the appropriate concentration is necessary for effective T cell conjugation as it demonstrates the potential for improved treatment regimens. This opens the way for future research into combination therapies that can further improve treatment

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outcomes, as well as personalized treatment strategies tailored to individual patients. Ultimately, these insights pave the way for improved clinical use of anti-PD-L1 therapy in oncology, particularly in patients with colorectal cancer.

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