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Combining Anti-IL-8, Tasquinimod Treatment, and Blockage of Pyruvate Dehydrogenase in Breast Cancer Immunotherapy

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Abstract. Using immunotherapy to treat breast cancer, one of the most common cancers that affects women and the second most common cancer among women in the United States, has achieved many successes. However, how to solve the problem of cancer resistance to therapies caused by myeloid-derived suppressor cells and immunosuppressive tumor microenvironment remains a major challenge. To improve the performance of immunotherapy, we chose to explore the three putative therapeutic approaches including anti IL 8 antibody injection, PDH inhibition, and the use of tasquinimod, then combining them to observe anti-tumor effects in a murine model. We anticipate the greatest decrease of tumor volume in triple combination, concluding the feasibility of our method on treating breast cancer.

Keywords: Breast Cancer, Myeloid-derived suppressor cells, Anti-IL-8, Tasquinimod, PDH inhibition, Immunotherapy

1. Introduction

Breast cancer is an uncontrolled growth of epithelial cells lining ducts and lobules of breast. It begins as an in-situ carcinoma, over time, invading basement membrane. It is the most common cancer in female population. Approximately 23.8 percent of female who got cancer have been diagnosed as breast cancer. The age-Standardized Rate (World) per 100 000 for both sexes is 46.8, top one in all cancer [1]. When alveolar cells undergo division, there is a chance of genetic mutation, which can lead to breast cancer. Several risk alleles are strongly associated with the development of breast cancer, including mutations in breast cancer gene 1 (BRCA-1) and breast cancer gene 2 (BRCA-2) and tumor protein p53 (TP53) [2,3]. The physiological of these genes is to control cell division cell division, and to induce apoptosis if cells divide uncontrollably. If there are mutations on those genes, epithelial cells begin to grow and replicate out of control, causing tumor. Several additional factor can contribute to breast cancer development including alcohol consumption, absence of excess body fatness (postmenopausal), aging, family history of breast cancer, exposure to

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diethylstilbestrol (DES). Common treatments include surgery (partial mastectomy and total mastectomy), radiation therapy, chemotherapy, and hormonal therapy [4].

Cancer cells deploy multiple mechanisms to avoid detection by the immune system. Those cells express less surface antigens, by downregulating the major histocompatibility class I (MHC I, which could be detected by CD8 T cells) antigen presentation machinery, to avoid elimination by the immune system [5]. Also, cancer cells can inhibit T cell function by expressing immune checkpoint molecules such as lymphocyte-activation gene 3 [6]. Moreover, those cells employ myeloid-derived suppressor cells (MDSCs) to establish immunosuppressive tumor microenvironment (TME) [7,8].

Possible immunotherapy approaches had been already proposed or used in clinical trials. A prime example is to target immune checkpoint inhibitors [8], enabling cancer cells to become detected by T cells. Also, T-cell transfer therapy is also a possible approach which patient's T cells will be collected and chimeric antigen receptor (CAR) T cells will be grown. Those T cells will be infused back into patients, so more T cells will be available in patient's body ("T-cell Transfer Therapy").

However, cancer immunotherapy still needs further improvements. Current immunomodulators have limited accumulation at tumor sites [8], which limit the effect of immunotherapies. Inevitably, cancer cells will develop resistance to immunotherapy, so more immunotherapy methods should be developed.

In the following section, we will summarize 3 primary research articles that our group selected as foundation for this project.

2. Literature review

2.1. Tasquinimod modulates suppressive myeloid cells and enhances cancer immunotherapies in murine models

Tasquinimod is an antitumor agent for castration-resistant prostate cancer. Inflammatory protein S100A9 would influence function and survival of tumor-suppressive myeloid cells.

The team used two mouse models: "tumor vaccine for prostate cancer", SurVaxM; and TTS, which is a melanoma "tumor-targeted superantigen".

During experiment, the teams performed splenocytes and tumor suspension, cell staining and flow cytometry, IFN-γ induction assay, granzyme B induction assay, T-cell suppression, splenocyte-mediated and CD8 T cell-mediated cytotoxicity assay, antigen-specific tetramer binding assay, immunofluorescence staining of tumor sections, immunohistochemistry staining, PCR, nitric oxide synthase activity assay, and statistical analysis of difference in tumor weight using nonparametric Mann-Whitney U test.

Tasquinimod inhibit MDSCs, and tumor-associated macrophages of polarized phenotype of myeloid cells. Moreover, CD8(+) T cells which are tumor-specific increased in both circulation and in tumors. Last but not least, this antitumor agent promotes T-cell effector functions.

Results from this study suggest that target suppressive myeloid cells by tasquinimod pharmacologically would lead to therapeutic benefits, and clinical testing of this antitumor agent should be performed.

2.2. Anti-IL-8 antibody activates myeloid cells and potentiates the anti-tumor activity of anti-PD-1 antibody in the humanized pancreatic cancer murine model

To solve the limited response that pancreatic ductal adenocarcinoma (PDAC) to single-agent immune checkpoint inhibitor therapy, Li et al used a humanized PDAC NOD SCID

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IL2RgammaNULL (NSG) mouse model reconstituted with human T cells and $CD14^+/CD16^+$ myeloid cells to test an anti-IL-8 and anti-PD-1 combination treatment.

The team found out that, anti-IL-8 + antiPD-1 significantly enhanced antitumor activity compare to monotherapy, the efficiency was depended on myeloid cells, particularly $CD16^+$ granulocytes. Also, aIL-8 increased intratumoral granulocytic myeloid cells and activated innate immune/type I cytokine pathways (shown via FACS/snRNA-seq).

This research supports clinical testing and offers ideas into myeloid cell re-education for immunotherapy [9].

2.3. LAL deficiency induced myeloid-derived suppressor cells as targets and biomarkers for lung cancer

MDSCs promote tumor immune evasion in non-small cell lung cancer (NSCLC), but their metabolic regulation and biomarker potential are unclear.

The team utilized Lysosomal Acid Lipase-deficient (LAL-D) mouse models, NSCLC patient samples, and healthy controls in their experiment. Their techniques include scRNA sequencing, flow cytometry, western blot, qPCR, metabolomics, and bioinformatics.

The main results are: MDSCs in NSCLC exhibited metabolic reprogramming, with altered key enzymes (PDH, G6PD) in glycolysis/TCA cycles, linked to tumor progression in LAL-D models; significant differences in MDSC subset proportions and metabolic enzyme expression distinguished NSCLC patients from healthy individuals, correlated with PD-L1; immune checkpoint inhibitor treatment altered MDSC proportions and metabolic enzyme expression; patient responses were associated with specific metabolic biomarkers.

This study is significant, since it identifies crucial metabolic alterations in NSCLC MDSCs, highlighting specific enzymes and MDSC features as promising diagnostic/prognostic biomarkers and therapeutic targets for immunotherapy [10].

3. Hypothesis

We hypothesized that combining anti-IL-8, tasquinimod treatment, and blockage of pyruvate dehydrogenase, would reduce breast cancer myeloid-derived suppressor cells survival. This would lead to decreased establishment of TME. Hence, make cancer immunotherapy more effective.

4. Experimental outline

4.1. Mouse model

We will use NSG humanized mice as our primary animal model because NSG mice only has innate immune cells and lack functional T, B, and NK cells, making the mice suitable recipients for implanting human cells and tissues [11]. We chose NSG mice model because we aim to simulate the tumor microenvironment of human breast cancer [12]. Then, in order to humanize the mouse model, we will add human $CD34^+$ hematopoietic stem cells (HSC) to provide mice with adaptive immune cells. Next, since we target to repress myeloid-derived suppressor cells, which comes from the human myeloid cell lineage, we will reconstitute NSG-SGM3 mice with human myeloid cells by intravenously injecting HSC through the tail vein. Finally, implant NSG mouse with human breast cancer cell line to create the breast cancer tumor environment. The cell lines we propose to use are MDA-MB-231 cells, a model of late-stage triple-negative breast cancer known for high IL-8 production. High IL-8 production can strongly recruit MDSCs into the microenvironment to

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suppress anti-tumor immune response. In this study, recruiting MDSCs is necessary because we aim to evaluate the impact of anti-IL-8, PDH inhibition, and tasquinimod on MDSCs suppressive activity. Therefore, humanizing mice model injected with MDA-MB-231 provides us with a suitable model to investigate the effect of multiple treatment. As shown in figure 1, we plan to implant the human breast cancer cell line into the NSG mouse model, and apply different combinations of tasquinimod, anti-IL 8 antibody, PDH inhibitor to the experimented mice.

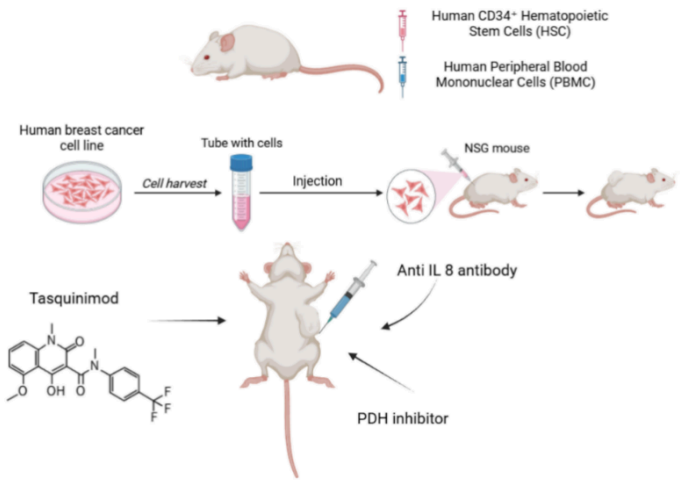

Figure 1. Experiment design mechanism diagram

4.2. Treatment groups

Each group will contain 7 to 10 mice.

1) Vehicle control
2) AntiIL8 antibody alone
3) Tasquinimod alone
4) PDH inhibitor alone
5) AntiIL8 + Tasquinimod
6) AntiIL8 + PDH inhibitor
7) Tasquinimod + PDH inhibitor
8) Triple combination (antiIL8 + Tasquinimod + PDH inhibition)

Dependent variables

During the experiment, our measures will include measurements on tumor progression as well as the dynamics of immune response. For measurements on tumor progression, we will measure tumor size of each model, using the formula $Tumor\ Volume = \frac{Length * Width^2}{2}$.

In addition, for measurements on the dynamics of immune response, MDSC dynamics will be measured through fluid cytometry, which is used to evaluate the immune suppression is inhibited by each type of treatment. $CD8^+$ T cell activity will be measured through intracellular cytokine staining quantifying IFNg, TNFa, granzyme B and perforin secretion. This variable is measured to test mainly anti-IL-8 and tasquinimod's ability to increase $CD8^+$ T cell effector functions by reducing the immunosuppression effect generated by MDSC [13]. PDH activity is mainly used to measure the effect of PDH inhibition by using western blot. Moreover, we will measure cytokine activity via ELISA because cytokine activity can be influenced by all three treatments. While anti-IL-8

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treatment can directly lower IL cytokine activity, tasquinimod and PDH inhibition can otherwise change cytokine activity through altering metabolic signaling [14].

Finally, we will use two-way ANOVA with Fisher's LSD test [7]. Specifically, P value will be used to measure the probability that the observed data is occurred by random chances. Specifically, a p-value less than 0.05 will indicate that the results has statistically significance.

5. Anticipated result

We anticipated our study to yield four results, which are as follows. Firstly, Tasquinimod will reduce the MDSCs suppressive function. Secondly, $CD8^+$ T cell activity will be enhanced. Thirdly, Inhibit PDH will cause MDSC immune suppression and reduction in tumor size. Finally, the triple combination treatment may yield the best suppression in tumor growth.

6. Potential caveats

A mismatch between the MHC haplotype of MDA-MB-231 and the engrafted human immune system increases the risks of graft-versus-host disease (GVHD), which may cause death in experimental mice.

Moreover, though we use NSG-SGM3 mice that are engineered to express stem cell factor to enhance HSC proliferation, insufficient myeloid cell engraftment may still occur when the HSCs in NSG-SGM3 mice fail to grow or produce new myeloid cells.

7. Alternative approach

The alternative human breast cancer cell lines include MCF-7 and SUM-159PT. Alternative cell lines can generate diverse tumor microenvironment, IL secretion, and different MDSCs recruitment ability, which is helpful for evaluating the treatment efficacy in a broader tumor environment.

To mitigate insufficient myeloid cell engraftment, we will treat the mice with a second stem cell transplanting. Alternatively, we will use supportive therapy including injecting growth factors and mesenchymal stem cells to stimulate the production.

If encounter GVHD, we will use corticosteroids as an initially treatment to reduce inflammation and suppress immune response. Furthermore, we will use ruxolitinib or extracorporeal photopheresis (ECP) to treat mice that develop steroid refractory GVHD.

8. Discussion

Tasquinimod could inhibits T cell–suppressing factors, induces antigen specific $CD8^+$T cells, and binds to S100A9, an inflammatory protein that affects the accumulation of immunosuppressive myeloid cells, inhibiting its downstream signaling, affecting myeloid cells. By reducing the ability of immunosuppressive myeloid cells to support tumor growth simultaneously, tasquinimod reduced MDSCs suppressive function, become one of the factors that reduce tumor size in our study.

Myeloid cells exert an immune-enhancing effect by promoting

CD8+ T cell infiltration, while aIL-8 enhances the infiltration of granulocytic myeloid cells. IL-8 blockade counteracts the immunosuppressive effect of granulocytic myeloid cells, thus lead to reducing tumor size.

LAL-D promotes the expansion of MDSCs derived from hematopoietic progenitors in the bone marrow, increases endothelial permeability, and facilitates their infiltration into various organs. These cells inhibit T-cell proliferation and impair T-cell function, while also promoting tumor

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growth and invasion. Inhibition of PDH, a central enzyme connecting glycolysis and the TCA cycle, by using CPI-613 or PDH siRNA, significantly reduced the abundance of $Ly6G^{+}$ cells in Lal^{-+} backgrounds and diminished their reactive oxygen species (ROS) generation. Furthermore, this intervention counteracted their immunosuppressive effects on T cells and hinder their promotive influence on tumor cell proliferation and growth.

Our anticipated observations would also be in line with findings of previous published studies [7]. Anti IL 8 antibody alone group, Tasquinimod alone group, and PDH inhibitor alone group all show some amount of reduce on tumor size, while triple combination will show the most significant reduction. This led us to our conclusion: Our results demonstrate that combining anti-IL-8, tasquinimod treatment, and blockage of pyruvate dehydrogenase will effectively reduce myeloid-derived suppressor cells' survival. This is a feasible method for cancer immunotherapy.

Our research proved the possibility of combining PDH inhibitor Tasquinimod and Anti-IL-8 antibody on enhancing performance of immunotherapy. However, to see weather combining these treatments will have any side effect or how these treatments interact to affect real human instead of mice still need further research. The huge difference between human and mice cause the requirement of adjusting dosage, the exactly amount should be developed.

The practical application is constrained, though enhancing tumor treatment, financial cost of combining all three treatments making real life application impractical, unless better way of reducing cost could developed. Our proposal is the first to combine those treatments and apply them to treat breast cancer specifically. We hope our proposal could provide an inspiration for future study, that by combining treatment groups that functions similarly there is a chance of enhancing overall performance. In addition, we think our research could be used to treat more cancer type other than breast cancer.

9. Conclusion

Our results demonstrate that combining anti-IL-8, tasquinimod treatment, and blockage of pyruvate dehydrogenase will effectively reduce myeloid-derived suppressor cells' survival. This might be a potentially attractive method for cancer immunotherapy.

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Yingfei Wang, Weizhi Song, Kaihan Zheng and Zejia Li, contributed equally to this work and should be considered co-first authors.

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