中文題目：藉由腫瘤免疫浸潤微陣列分析評估M2巨噬細胞對於Rituximab臨床抗藥性之影響

英文題目：Evaluation of the effect of M2 macrophages on clinical resistance of Rituximab by tumor immune-infiltration microarray analysis

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Background*:* Diffuse large B-cell lymphoma is the most common hematological cancer in adults and is a highly invasive blood tumor that can occur almost anywhere in the body. Although the etiology of DLBCL is still unclear, it is currently recognized to be mainly formed by abnormal B cell proliferation or malignant transformation of chronic lymphocytic leukemia (CLL). Rituximab is an FDA proved monoclonal antibody that antagonizes the B cell-specific antigen CD20. The complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) caused by Rituximab further trigger apoptosis in the target cells. Currently, Rituximab is combined with CHOP chemotherapy as the first-line treatment regimen for malignant lymphoma, which significantly improved the complete response rate in patients with DLBCL clinically. However, clinical resistance to rituximab is usually developed after 6 months of treatment with this regimen, so exploring factors that may contribute to rituximab resistance is urgent for improving the prognosis of patients with DLBCL. While the development of B-cell depends on numerous highly orchestrated interactions with the stromal and immune cells, the importance of studying the impact of the tumor microenvironment in NHL has been increasingly recognized. This study focused on identifying the impact of immune infiltration connecting resistance of rituximab in the tumor microenvironment, especially the M2 tumor associated macrophage (TAM).

Method*:* To analyze the effect of Rituximab on the immune infiltration of the tumor microenvironment in clinical DLBCL patients, we used the GSE10846 database, which contained 414 DLBCL patients with tumor microarray gene expression data from lymph nodes. Of these, 233 received treatment with rituximab plus CHOP and 181 received CHOP [1]. We used the CIBERSORT approach to assess the tumor-infiltrating cell population in each sample from DLBCL patient [2]. Corresponding follow-up time and survival status were collected at the same time to evaluate the correlation between the profiling of immune infiltration and prognosis. Moreover, the T cell accumulation, T cell exhaust and T regulatory scores were further evaluated by signatures of T cell dysfunction provided in TIDE to predict the patient's resistance to ICB therapy [3]. Finally, GSVA was used to assess the potential signaling pathways as a novel combination therapy to increase the susceptibility to rituximab [4].

Results: Firstly, Kaplan-Meier survival curve analysis was performed between groups with or

without rituximab treatment, showing that rituximab plus CHOP can significantly improve the prognosis of patients with DLBCL. To evaluate the tumor microenvironment immune-infiltration, CIBERSORT was utilized to present the detailed profiling of various immune cells. Results suggest that rituximab could reduce the proportion of naïve and memory B cell but have no impact on plasma B cell (low CD20 expression), indicating that the method can actually reflect the composition of the tumor microenvironment in silico. In addition, the usage of rituximab promotes the ratio of CD4+ memory T cell activation and reduces regulatory T in the tumor, which agree well with existing studies. However, the observations from the results of these data seem to indicate that rituximab have no impact on changing the proportion of macrophages. To assess the impact of M2 macrophages on the efficacy of rituximab, we defined M2 Z score greater than 1 for high M2 infiltration and others for low M2 infiltration. For comparison with M2, we define M1 Z score to be less than -1 for low M1 infiltration and others for high M1 infiltration. The results showed that rituximab had no significant effect on the prognosis of DLBCL patients with high M2-infiltrating, which is similar to the one with low M1 infiltration, indicating that rituximab may be affected when a higher proportion of M2 infiltration or a lower proportion of M1 infiltration existed in the DLBCL tumor microenvironment. To understand whether high M2 macrophage infiltration was associated with increased T regulatory cell and exhaust T cell, we collected T cell dysfunction signature from TIDE and analyzed the relevant scores, indicating that patients receiving rituximab had significantly higher M2 infiltrates. High T reg score and T exhaust score. It is further shown that high M2 infiltration in the tumor microenvironment may be highly resistant to treatment with the Immune checkpoint block. Finally, HALLMARK signature GSVA analysis of patients with DLBCL receiving rituximab showed that high M2 infiltrates were significantly associated with IL6 and IL2 signaling pathway activation compared with low M2 infiltration.

Conclusions: This study used a microarray database published ten years ago and employed the latest immune-infiltration analysis approach published recently to discover the inhibitory effect of M2 macrophages on the therapeutic effect of rituximab. Further analysis showed that activation of IL6-JAK-STAT pathway may be characteristic of high M2-infiltrating DLBCL tumors, so we anticipate that combination therapy with R-CHOP with IL6R antagonist or macrophage inhibitor may effectively inhibit clinical lymphoma recurrence.

References

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