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Research paper

Expression of PD-1 on peripheral blood Treg cells is related to the diagnosis, prognosis and treatment of T cell non-Hodgkin lymphoma

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ABSTRACT

Purpose: The aim of study was to explore the PD-1 expression on Treg cells and its association with T-NHL.
Methods: 137 patients newly diagnosed with T-NHL and 115 healthy controls were enrolled. The expression level of PD-1 was measured by flow cytometry at the time of diagnose and 3–8 course of treatment.
Results: Median fluorescence intensity (MFI) of PD-1 on Treg cells in T-NHL patients was significantly higher than that in healthy controls ($P < 0.001$). MFI of PD-1 in medium/high-risk T-NHL patients were higher than that in low-risk patients ($P < 0.05$). After treatment with Chidamide combined with chemotherapy, MFI of PD-1 significantly decreased ($P < 0.05$). In patients with high PD-1 expression (percentage > 19.6% and MFI > 580), EFS was significantly lower than patients with low PD-1 expression (percentage < 19.6% and MFI < 580).
Conclusions: The PD-1expression on peripheral blood Treg cells of T-NHL patients is related to the diagnosis, prognosis and treatment of disease.

1. Introduction

T-NHL is a common malignant lymphoma in Asian, accounting for 10–15% of non-Hodgkin's lymphoma. What's more, the proportion of T-NHL in China can be as high as 15–20% [1,2]. The first-line therapy of T-NHL is traditional chemotherapy, with a total effective rate of 60–70% in most patients, but the 5-year survival rate is only about 30% [3]. So it is meaningful that more studies should focus on the pathogenesis and tumor immunity of T-NHL in order to improve the prognosis of patients.

T lymphocyte is an important component of immune system and play a critical role in tumor immunity. Regulatory T lymphocytes (Treg) can inhibit the function of B lymphocytes, $CD4^+$ Th cells, cytotoxic T lymphocytes and antigen presenting cells, and finally influenced the function of immune system [4]. A number of studies [5,6] have showed that the proportion of Treg cells in peripheral blood of lymphoma significantly increased, and was associated with tumor burden and prognosis. They thought that activation of Treg in peripheral blood may indirectly inhibit tumor immune environment, promoting the occurrence or development of tumor.

In addition, as an inhibitory costimulatory factor, PD-1 is of great significance for the function of T lymphocyte and tumor immunity [7,8]. In tumor microenvironment the PD-1 ligand, PD-L1, is highly expressed in tumor cells, and once combined with PD-1 expressed on T lymphocyte, the function of T lymphocyte will be altered to promote tumor immune escape. In lymphoma, the expression of PD-1 is significantly correlated with progression and prognosis of disease. Matthew [9] and his colleagues tried to detect the expression of PD-1 in 70 cases of DLBCL by immunohistochemical staining and explore their correlation with prognosis. The results showed that PD-1 was an independent prognostic factor in patients, and the LDH of patients with high PD-1 expression was lower than that of patients with low PD-1 expression. More than that, with the deepening of research in lymphoma, PD-1 inhibitor was gradually applied to the treatment of several lymphomas, such as Hodgkin's lymphoma, follicular lymphoma and refractory and relapsed large B cell lymphoma [10–12]. However, in T-cell lymphoma the study about PD-1 is very few, and its expression and clinical significance on specific subtypes of T lymphocytes, for example Treg cells, are not clear. Therefore, in this study, we detected the expression of PD-1 on peripheral blood Treg cells in T-NHL patients, and

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explored its relationship with diagnosis, prognosis and treatment of disease.

2. Patients and methods

2.1. Patients

This study included a total of 137 patients diagnosed with T-NHL in Peking Union Medical College Hospital from September 2015 to April 2018. Inclusion Criteria: ①pathologically confirmed and untreated; ②integrated clinical information. Exclusion criteria: ①cytomegalovirus (CMV) and Epstein–Barr virus (EBV) infection; ②previous medical history of indolent lymphoma and other primary malignant tumors; ③complication of autoimmune diseases; ④verification of pathologic diagnosis is inconsistent with the former. All the patients were confirmed based on WHO classification criteria (2008) [13]. The therapy regimens of patients include CHOP-like regimens (cyclophosphamide + doxorubicin + vincristine + prednisone), GDP-ML regimen, and Chidamide combined with conventional chemotherapy regimens. 115 cases of healthy people in PUMCH physical examination center were randomly selected in healthy control group.

Peripheral blood samples was collected from T-NHL patients and healthy controls once diagnosed. And the samples were collected again from T-NHL patients after 3–8 cycles of therapy. The study had been approved by the Ethics Committee and both patients and healthy controls had signed informed consent forms.

2.2. Flow cytometry

100 μl peripheral blood of patients or healthy controls were isolated and incubated with CD4, CD25 and CD279 (PD-1) antibodies for 25 min without light, then perforate and wash with Foxp3 transcription factor staining buffer according to instruction. And then incubated with Foxp3 antibody 25 min without light, wash twice in Foxp3 staining buffer and finally fixed with 1% formalin solution. CD4 (Clone RPA-T4; FITC), CD25 (Clone M-A251; PE-Cy™7) and CD279 (PD-1) (Clone MIH4; PE) were purchased from BD Biosciences, Foxp3 (Clone 236A/E7; APC) and Foxp3 Transcription Factor Staining Buffer Kit were purchased from eBiosciences™ (Invitrogen™). Samples were detected by FACS Canto II cytometer (BD, USA) and the data were analyzed by FACS Diva software for PD-1 expression on Treg cells. Firstly lymphocytes were gated out by forward and side corners, and then Treg cells were $CD4^+CD25^+Foxp3^+$ cells, which were finally used for PD-1 expression (presented as percentage and mean fluorescence intensity [MFI], showed in Fig. 1). The threshold of PD-1 percentage and MFI in the prognosis analysis was determined by the ROC curve, where the point of the maximal Youden exponent was used as the threshold.

2.3. Follow up

Patients diagnosed with T-NHL were followed up by telephone and hematology clinic. A total of 96 patients were monitored until June 9, 2017, with 11 (3–21) months of the median follow-up time. The event-free survival time (EFS) refers to the time from diagnosis of the disease to occurrence of any events (for example disease progression, death, etc.); total survival time (OS) refers to the time from diagnosis of the disease to the patient's death.

2.4. Statistical methods

SPSS 16.0 software was used for statistical analysis. The qualitative data were presented as the number and percentage of cases. The normal distribution measured data were present as the mean ± standard deviation. In addition, the non-normal distribution measured data were present as the median (range interquartile), and two groups of independent data were analyzed by the nonparametric Mann-Whitney *U* test, while the two groups of correlated data were analyzed by nonparametric Wilcoxon test and multiple groups of independent data were analyzed by nonparametric Kruskal-Wallis H test. Kaplan-Meier method was used to plot the survival curve. $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Clinical data

The median age of 137 patients with newly diagnosed T-NHL was 45 years (18–78 years), and the male: female ratio was 77:60. According to the international prognostic index (IPI) and NK/T cell lymphoma prognostic index (NKPI) score, 32.8% of patients were at low risk, while patients with medium/high risk accounted for 67.2%. Based on Ann Anbor staging, the stage I ~ II: stage III ~ IV was 23:114. On the basis of subtypes of newly diagnosed T-NHL patients, 26.3% of patients were extranodal NK/T cell lymphoma patients, followed by peripheral T-cell lymphoma (not otherwise specified), Angioimmunoblastic T cell lymphoma, ALK-negative anaplastic large

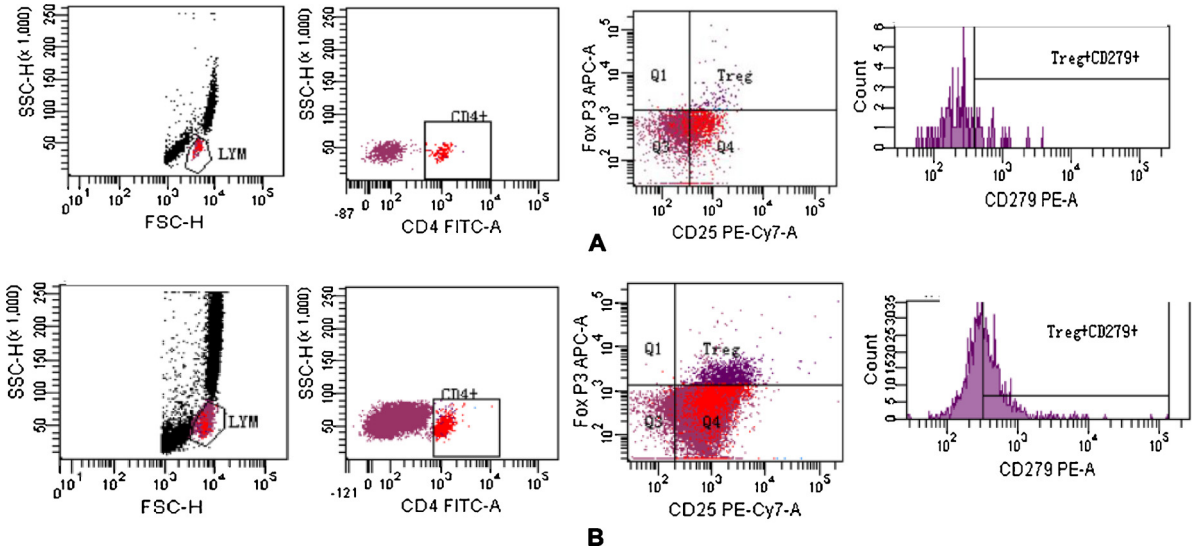

Fig. 1. Expression of PD-1 on the surface of Treg cells in healthy controls (A) and T-NHL patients (B).