Analysis of the Relationship between Scleritis and T Cell Activation in Patients with Hepatocellular Carcinoma Treated with PD-1 Carrelizumab

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In order to explore the function of inhibiting the immune effect, the relationship between programmed death receptor 1 (PD-1) carrelizumab in the treatment of hepatocellular carcinoma-induced scleritis and T cell activation is investigated. A total of 120 patients with primary liver cancer treated in the department of oncology of our hospital from July 2020 to January 2022 are selected and treated with carrelizumab. According to the occurrence of PD-1 carrelizumab treatment, the patients are divided into the scleritis group and nonscleritis group. IK_he levelsofTcells, PD-1, PD-L1 proteins, and serum inflammatory factors at different time points are compared. The experimental results show that the occurrence of scleritis after liver cancer treatment with PD-1 carrelizumab is closely associated with Treg cells, the percentage of Th17 cells, the expression of PD-1, PD-L1 proteins, and inflammatory factors. It is clearly evident that PD-1 carrelizumab can increase the risk of scleritis by affecting T cell activation.

1. Introduction

Primary liver cancer is one of the common malignant tumors in China, which is involved by many factors and plays a synergistic role. Among them, hepatitis B is the main factor inducing liver cancer. The number of people carrying the hepatitis virus is nearly 100 million in China, which is a high-risk group of primary liver cancer. Therefore, actively preventing the occurrence of liver cancer is an important issue that needs to be solved urgently in Chinese medicine [1]. Clinical surgical treatment requires a high liver function reserve, and it is easy to relapse after operation. Patients with liver cancer have poor sensitivity to radiotherapy, so radiofrequency ablation and chemoembolization can be used for local treatment. This treatment has a certain clinical effect, but it is only used in cases of small nodular lesions and a small number of lesions. The effect of comprehensive treatment of patients with advanced liver cancer is not obvious, which fails to meet clinical expectations [2]. Immune checkpoint inhibitors are new targets for clinical cancer therapy. It can inhibit the immune activity of checkpoints and activate the tumor immune response effect of T cells, thus playing a certain antitumor effect. Among them, PD-1 carlizhu as a new drug has made some achievements in clinical tumor treatment in recent years, with less adverse reactions. However, PD-1 carrelizumab treatment can cause certain toxic reactions, including the skin, eyes, and heart. Scleritis is one of the ocular toxic reactions after PD-1 carrelizumab treatment in patients with liver cancer, and its pathological features include inflammation of the scleral matrix layer caused by cell infiltration, collagen destruction, and vascular reconstruction. Scleritis is caused by mediated vasculitis and is related to immune system diseases [3]. Currently, there is no unified conclusion...
on the mechanism of interaction between programmed cell death-1 (PD-1) carrelizumab in the treatment of scleritis induced by primary liver cancer and T cell activation [4]. Therefore, this study further analyzed the relationship between scleritis induced by PD-1 carrelizumab treatment and T cell activation in primary liver cancer, providing a new target for subsequent clinical treatment of liver cancer.

The rest of the paper is organized as follows: Section 2 discusses related studies, and Section 3 presents the general information and experimental method. Section 4 describes the experimental results and analysis. The conclusions are provided in Section 5.

### 2. Related Work

As a transmembrane protein, PD-1 is widely expressed in T cells and B cells. The T cell receptor (TCR) and cytokines can activate PD-1 on T cells. PD-1 can bind to T cells and inhibit the crenation of its proximal myase during the activation of T cells, so as to inhibit the immune effect [5]. As a natural ligand of PD-1, PD-L1 can inhibit the immune-mediated killing process of cancer cells by transmitting antiapoptotic signals of cancer cells. Therefore, because of the high expression of PD-1, PD-L1 can cause the immune escape of cancer cells, thus aggravating the development of the disease [6]. Lyon et al. [7] indicate that the expression of PD-1 and PD-L1 proteins in both groups showed a significant downward trend, which may be due to the existence of two common antigens between tumor cells and scleral fibroblasts. One is that the muscle antigens of tumor antigens are homologous with different TCR targets and the other is specific with different TCR targets. PD-1 and PD-L1 protein expressions are decreased when PD-1 carrelizumab treatment is performed, and the drug may target the common antigen, resulting in scleral injury and scleritis.

Th17 cells are a kind of helper T cells that are different from Th1 and Th2 cells. Th17 cells can secrete pro-inflammatory factors such as IL-17, IL-6, and TNF-α to participate in the occurrence and development of various inflammatory diseases [8]. IL-17 secreted by Th17 cells can induce and promote macrophage synthesis, inflammatory factor secretion, and complement C3 synthesis [9]. Treg cells can inhibit the production of IL-13, IL-5, and other proinflammatory factors, thus inhibiting the increase of eosinophils. In addition, Treg cells can secrete IL-10, which is an anti-inflammatory factor. When the body's immunity is activated, it can inhibit the activation of relevant cells and the generation of proinflammatory cells in the process of immune response. IL-10 can effectively control inflammation by reducing the expression of reactivity, and inhibit the secretion and generation of IL-6 and other proinflammatory factors. Treg cells regulate the body's antigen immune response through this pathway [10, 11]. Further analysis of the changes of Treg cell population, IL-10, Th17 cell population, Th17/Treg, IL-6, TNF-α, and other indicators in the scleritis group indicates that the correlation between the activation of T cells and the expression of related inflammatory factors in scleritis after PD-1 carrelizumab treatment should be explored in-depth. The reason may be that ruili is resistant to PD-1 single treatment and can play an active role by blocking the immune signaling pathway targeted by PD-1/PD-L1, enhancing the immune system function, activating T cells, and improving inflammation. It should be noted that the immune function of the tumor microenvironment of selective may selectively restore immune deficiency induced by tumors, which can cause immune system imbalance and immune tolerance [12]. From the perspective of IFN-γ analysis, IFN-γ is the main cytokine secreted by immune Th1 and can be conductive to promote the differentiation of T cells and B cells and clearing human pathogens. Proliferation of CD8+T cells after overactivation of T cells expressing IFN-γ

### Table 1: Percentage difference between Treg cells and Th17 cells (x ± s).

| Group | Number of Treg cells (%) | Number of Th17 cells (%) | Th17/Treg |
|-------|--------------------------|--------------------------|-----------|
|       | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 |
| The nonscleral group (n = 105) | 4.72 ± 1.19 | 5.49 ± 0.81 | 1.59 ± 0.49 | 1.27 ± 0.39 | 2.26 ± 0.69 | 1.12 ± 0.49 |
| Scleritis group (n = 15) | 4.68 ± 1.18 | 6.38 ± 0.78 | 1.58 ± 0.51 | 1.03 ± 0.28 | 2.29 ± 0.71 | 0.56 ± 0.29 |
| t    | −0.122 | 4.896 | −0.074 | −2.296 | 0.157 | −4.310 |
| P    | 0.903 | <0.001 | 0.941 | 0.023 | 0.876 | <0.001 |

**Figure 1:** Percentage changes of Treg cells and Th17 cells.
will result in abnormal increase of IFN-c. Activated myeloid cells express TNF-α and IL-6 inflammatory substances and cxCL9/10 chemokines, which further induce peripheral T cells to infiltrate the intestine and cause inflammation [13].

3. General Information and Experimental Method

3.1. General Information. A total of 120 patients with primary liver cancer treated in the Department of Oncology, First Hospital of Hebei Medical University, from July 2020 to January 2022 are selected. According to the occurrence of PD-1 carrelizumab treatment, the patients are divided into the scleritis group (15 cases) and nonscleritis group (105 cases), respectively. The age ranged from 45 to 75 years, with an average of 60.41 ± 4.32 years, including 76 males and 44 females, 49 stage III and 71 stage IV. The inclusion criteria are as follows: (1) combined CT/MRI and tumor marker (AFP) detection of primary liver cancer confirmed clinically or by preoperative biopsy and pathology; (2) there is no indication for surgical resection, or the patient refuses surgery; (3) Child–Pugh A or B; (4) estimated survival ≥ 12 weeks. Besides, the exclusion criteria are as follows: (1) use of immunosuppressive drugs within 14 days before administration; (2) the presence or history of any active autoimmune disease; (3) patients with vitiligo and asthma; (4) AIDS, active hepatitis B, hepatitis C, and patients requiring antiviral treatment during the study; (5) severe infection occurred within 4 weeks before medication; (6) participated in any other drug clinical studies within 4 weeks prior to initial administration [14].

Table 2: Protein expression differences between PD-1 and PD-L1 (x ± s).

| Group                  | PD-1 | PD-L1 |
|------------------------|------|-------|
|                        | T1   | T2    | T1          | T2          |
| Scleritis group (n = 15)| 288.72 ± 21.40 | 225.49 ± 0.81* | 341.59 ± 40.59 | 271.27 ± 0.39* |
| Nonscleritis group (n = 105) | 290.68 ± 21.38 | 246.58 ± 0.78* | 341.63 ± 40.61 | 301.03 ± 0.28* |

Table 3: Expression differences of inflammatory factors (x ± s).

| Group                  | IL-10 (pg/mL) | IL-6 (pg/L) | TNF-α (ng/L) | IFN-γ (ng/L) |
|------------------------|---------------|-------------|--------------|--------------|
|                        | T1            | T2          | T1           | T2           | T1            | T2            | T1             | T2             |
| Scleritis group (n = 15)| 2.12 ± 0.91   | 3.52 ± 0.42* | 54.62 ± 7.19 | 42.29 ± 5.16* | 58.82 ± 6.31 | 45.31 ± 5.26* | 633.44 ± 91.02 | 1001.22 ± 215.34* |
| Nonscleritis group (n = 105) | 2.14 ± 0.89   | 4.55 ± 0.22* | 54.56 ± 7.18 | 22.28 ± 5.13* | 58.86 ± 6.28 | 21.28 ± 5.11* | 630.44 ± 88.21 | 1213.45 ± 294.82* |

Figure 2: Protein expression differences between PD-1 and PD-L1.
powder is added to seal for 2 h, a primary solvent is added placed in a 37°C environment. the bed is fully shaken and mixed, and incubated overnight. Clean with TBST for 10 min and repeat 3 times. The second antibody is added and the reaction is continued for 2 h at room temperature. Then, clean with TBST for 10 min and repeat the operation 3 times. Exposure is carried out in ECL chemiluminescence liquid obscura. Gel-pro32 software is used for gray analysis of the obtained results, and the protein expression of PD-1 and PD-L1 is calculated [18, 19].

3.3. Inflammatory Factors. 3 ml venous blood samples are taken and centrifuged at 4°C for 10 min at a speed of 1000 r/min and a radius of 20 cm at 3000 g. The levels of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), interleukin-6 (IL-6) in serum are measured by ELISA. IL-6 and interferon γ (IFN-γ) are tested in strict accordance with the kit instructions.

3.4. Statistical Methods. SPSS 26.0 software is used for statistical analysis [20, 21]. The mean ± standard deviation (x ± s) is used to represent the measurement data of the normal distribution. The T test and the x^2 test is used to compare the count data. P < 0.05 denotes that the difference is statistically significant.

4. Results and Analysis

4.1. Percentage Difference between Treg Cells and Th17 Cells. The number of Treg cells on T1~T2 in 120 patients showed an increasing trend, while the number of Th17 cells and Th17/Treg showed a decreasing trend. In addition, the number of Treg cells on T2 in the scleritis group was higher, and the number of Th17 cells and Th17/Treg were smaller. There are statistical differences in all indicators (P < 0.05), as shown in Table 1 and Figure 1. The symbol "∗" means that the value is compared with T1 and P < 0.05.

4.2. Protein Expression Differences between PD-1 and PD-L1. PD-1 and PD-L1 proteins showed a decreasing trend from T1 to T2, and PD-1 and PD-L1 protein levels are lower in the scleritis group at T2, with statistical differences in all indicators (P < 0.05), as shown in Table 2 and Figure 2.

4.3. Differences in Expression of Inflammatory Factors. The levels of IL-10 and IFN-γ increased from T1 to T2 and are lower in the scleritis group, while the levels of IL-6 and TNF-α decreased and are higher in the scleritis group, with statistical differences (P < 0.05), as shown in Table 3 and Figure 3.

5. Conclusion

In this study, the relationship between programmed death receptor 1 (PD-1) carrelizumab in the treatment of hepatocellular carcinoma-induced scleritis and T cell activation is investigated. The levels of T cells, PD-1, PD-L1 proteins, and
serum inflammatory factors at different time points are compared. The occurrence of scleritis after liver cancer treatment with PD-1 carrelizumab is closely associated with Treg cells, the percentage of Th17 cells, the expression of PD-1, PD-L1 proteins, and inflammatory factors. The results indicate that PD-1 carrelizumab can increase the risk of scleritis by affecting T cell activation. Also, there are still some deficiencies in this study, such as a small sample size and incomplete inclusion of immune indicators. A small sample size may lead to data bias in subsequent analysis, and there are many types of immune T cell indexes, which may be related to the occurrence of scleritis in patients with liver cancer after PD-1 carrelizumab treatment. In the future, we will expand the sample size and include more immune indicators in the follow-up research for more in-depth research.

Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

[1] A. M. Beyer, M. G. Bonini, and J. Moslehi, “Cancer therapy-induced cardiovascular toxicity: old/new problems and old drugs,” American Journal of Physiology - Heart and Circulatory Physiology, vol. 317, no. 1, pp. 164–167, 2019.

[2] L. Xia, Y. Liu, and Y. Wang, “PD-1/PD-L1 blockade therapy in advanced non-small-cell lung cancer: current status and future directions,” The Oncologist, vol. 24, no. S1, pp. S31–S41, 2019.

[3] A. Khunger, L. Battel, A. Wadhawan, A. More, A. Kapoor, and N. Agrawal, “New insights into mechanisms of immune checkpoint inhibitor-induced cardiovascular toxicity,” Current Oncology Reports, vol. 22, no. 7, 65 pages, 2020.

[4] National Health Commission of Prc, “Guidelines for the diagnosis and treatment of primary liver cancer (2022 edition),” Chin J Dis, vol. 35, no. 1, 2022.

[5] Y. Wu, L. Lin, and X. Liu, “Identification of PDL1-related biomarkers to select lung adenocarcinoma patients for PD1/PD-L1 inhibitors,” Disease Markers, vol. 2020, pp. 1–11, 2020.

[6] A. Inno, G. Metro, P. Bironzo et al., “Pathogenesis, clinical manifestations and management of immune checkpoint inhibitors toxicity,” Tumori, vol. 103, no. 5, pp. 405–421, 2017.

[7] A. R. Lyon, N. Yousaf, N. M. L. Battisti, J. Moslehi, and J. Larkin, “Immune checkpoint inhibitors and cardiovascular toxicity,” The Lancet Oncology, vol. 19, no. 9, pp. 447–458, 2018.

[8] M. Wang, Y. Zhao, and Q. Zhang, “Human mesenchymal stemcell-derived exosomes accelerate wound healing of mice eczema,” Journal of Dermatological Treatment, vol. 33, no. 3, pp. 1–5, 2020.

[9] A. L. Jones, D. Everett, and D. Y. M. Leung, “Staphylococcus aureus colonization is associated with increased peanut allergy sensitization in children with atopic dermatitis (AD),” The Journal of Allergy and Clinical Immunology, vol. 137, no. 2, pp. 183–191, 2016.

[10] M. Chen, H. Xu, and Q. Wang, “Changes and significance of IL-6, IL-10, IL-17 and HMGB1 in bronchoalveolar lavage fluid in children with severe pneumonia,” Chinese Journal of Emergency Medicine, vol. 37, no. 1, pp. 171–172, 2017.

[11] M. S. M. Tsang, P. C. Shaw, I. M. T. Chu et al., “High-throughput immunological analysis of dictamni cortex: implication in the quality control of herbal medicine,” Molecules, vol. 24, no. 16, pp. 2880–2933, 2019.

[12] R. Martin Huertas, C. Saavedra Serrano, C. Perna, A. Ferrer Gomez, and T. Alonso Gordoa, “Cardiac toxicity of immune-checkpoint inhibitors: A clinical case of nivolumab-induced myocarditis and review of the evidence and new challenges,” Cancer Management and Research, vol. 11, pp. 4541–4548, 2019.

[13] D. Y. Chen, W. K. Huang, V. Chien-Chia Wu et al., “Cardiovascular toxicity of immune checkpoint inhibitors in cancer patients: a review when cardiology meets immunoncology,” Journal of the Formosan Medical Association, vol. 119, no. 10, pp. 1461–1475, 2020.

[14] H. Y. Li, S. Dai, and W. Zhang, “Analysis of the relationship between peripheral blood T lymphocyte subsets, PD-1 and prognosis in patients with gastric cancer,” Modern Medical Journal, vol. 22, no. 7, pp. 231–237, 2018.

[15] W. Tang, S. Wan, Z. Yang, A. E. Teschendorff, and Q. Zou, “Tumor origin detection with tissue-specific miRNA and DNA methylation markers,” Bioinformatics, vol. 34, no. 3, pp. 398–406, 2018.

[16] J. Yan, Y. Yao, S. Yan, R. Gao, W. Lu, and W. He, “Chiral protein supraparticles for tumor suppression and synergistic immunotherapy: an enabling strategy for bioactive supramolecular chirality construction,” Nano Letters, vol. 20, no. 8, pp. 5844–5852, 2020.

[17] S. Sun, H. Liu, Y. Hu et al., “Selection and identification of a novel ssDNA aptamer targeting human skeletal muscle,” Bioactive Materials, vol. 20, pp. 166–178, 2023.

[18] W. F. Lai, “Non-conjugated polymers with intrinsic luminescence for drug delivery,” Journal of Drug Delivery Science and Technology, vol. 59, p. 101916, 2020.

[19] T. Aoki, M. Kudo, K. Ueshima et al., “Exploratory analysis of lenvatinib therapy in patients with unresectable hepatocellular carcinoma who have failed prior PD-1/PD-L1 checkpoint blockade,” Cancers, vol. 12, no. 10, 3048 pages, 2020.

[20] X. Liu, R. Lin, B. Zhao, R. Guan, T. Li, and R. Jin, “Correlation between oxidative stress and the NF-κB signaling pathway in the pulmonary tissues of obese asthmatic mice,” Molecular Medicine Reports, vol. 13, no. 2, pp. 1127–1134, 2016.

[21] J. J. Wang and D. E. Johnson, “An examination of discrepancies in multiple imputation procedures between SAS® and SPSS®,” The American Statistician, vol. 73, no. 1, pp. 80–88, 2019.