SciMedCentral

Journal of Cancer Biology & Research

Research Article

Expression and Significance of CD4, CD45 In Patients with Non-Small Cell Lung Cancer

Weiyu Wang[1,2*], Yang Yang[3*], Shengyao Ma[1,2], Huaijie Wang[2], Hanyue Li[1], Qingtao Yan[4], Kunning Yang, Zhigin Gao[1#], Mei-Hua Qu[1,2#]

[1]*Bioscience and Technology College, Weifang Medical University, Weifang 261053, China*
[2]*Translational Medical Center, Weifang Second People's Hospital, Weifang 261041, China*
[3]*School of Public Health, Qingdao University, Qingdao 266071, China*
[4]*Department of Pediatric Surgery, Weifang People's Hospital, Weifang 261041, China*
[#]*Corresponding authors and*Authors contributed equally*

***Corresponding authors**

Mei-Hua Qu, Bioscience and Technology College, Weifang Medical University, Weifang 261053, China; Translational Medical Center, Weifang Second People's Hospital, Weifang 261041, China, Tel: Tel: +86-536-8214176; E-mail:qumeihua2016@163.com

Zhiqin Gao, Bioscience and Technology College, Weifang Medical University, Weifang 261053, China, Tel: +86-536-8462066; E-mail: zhiqingao2013@163.com

Submitted: 22 December 2020

Accepted: 24 December 2020

Published: 26 December 2020

ISSN: 2373-9436

OPEN ACCESS

Abstract

Objective: To study the clinical significance of CD4 and CD45 expression in patients with non-small cell lung cancer.

Methods: Biopsies from 140 patients with non-small cell lung cancer in Weifang Second People's Hospital from January 2011 to December 2015 were collected and made into tissue microarray. The expression of CD4 and CD45 in tissue microarray was detected by immunohistochemistry. The overall survival (OS) of patients was followed up by telephone in August 2020. Graphpad primes 8 and BIM SPSS statistics 22 were used for statistical analysis.

Results: Immunohistochemical staining showed a significant positive correlation between the expression of CD4 and CD45 from the same patients (Person correlation=0.4, P<0.0001). Kaplan-Meier survival analysis showed that the OS of $CD4^{high}$ patients was higher than that of $CD4^{low}$ patients (log-rank=0.0456); the OS of $CD4^{high}CD45^{high}$ patients was higher than that of $CD4^{low}CD45^{low}$ patients (log-rank=0.0362). No significant difference was found in OS between $CD45^{high}$ and $CD45^{low}$ patients (log-rank = 0.5454).

Conclusion: There was a positive correlation between CD4 and CD45 in non-small cell lung cancer, and the expression of CD4 and CD45 in tumor tissue was positively correlated with OS.

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the deadliest malignant tumors in humans and the main cause of cancer deaths worldwide, accounting for 80–85% of lung cancers [1, 2]. Non-small cell lung cancer is divided into lung adenocarcinoma and lung squamous cell carcinoma by the histological phenotype [3].

The purpose of non-small cell lung cancer immunotherapy is to promote the activity of cytotoxic T lymphocytes in patients, help activate non-small cell lung cancer-specific cytotoxic T lymphocytes in lymphoid organs, and establish effective and durable anti-tumor immunity [4-6]. There are two main types of T cells: CD4+T cells and CD8+T cells [7]. Here we mainly study the clinical significance of CD4 and CD45 in non-small cell lung cancer. T cells with CD4+ can target and kill cancer cells through direct pathways (eliminating tumour cells through cytolytic mechanisms) and indirect pathways (regulating the tumor microenvironment) [8, 9]. CD4 + T cells not only have the function of enhancing the B cell response, but also have the function of enhancing the activity of cytotoxic T lymphocytes [10]. CD4+ T cells participate in different immune responses which are also known as helpers of the immune system and account for the largest proportion of lung cancer infiltrating lymphocytes (25.9%) [11, 12]. They kill target cells by secreting granzyme B and perforin, so they play an important role in anti-tumor immunity [13].

CD45 molecules are expressed on all white blood cells, called leukocyte common antigen (Leukocyte Common Antigen, LCA), widely present on the surface of white blood cells, and there is also a large amount of CD45 expression on the surface of T cells [14, 15]. CD45 is a key molecule of signal transduction on the cell membrane and has important significance in the development and maturation of lymphocytes, function regulation and signal transmission [15, 16].

This study was done by observing the expression of CD4 and CD45 in non-small cell lung cancer tissues and their relationship with prognostic survival. To further understand the role of tumor microenvironment in the treatment of lung cancer.

MATERIALS AND METHODS

Samples

Biopsies from 140 non-small cell lung cancer patients with medical records in Weifang Second People's Hospital from January 2011 to December 2015 were collected. The tissue microarray containing non-small cell lung cancer tissues was prepared with the assistance of Shanghai Outdo Biotech Co., Ltd. (Shanghai, China). The clinicopathological information of patients with tissue samples was obtained from hospital information system. The overall survival (OS) of patients was followed up by telephone in August 2020. This study was approved by the Ethics Committee of Weifang Medical University and Weifang Second People's Hospital. All analysis were carried out in accordance with the Declaration of Helsinki.

Tissue Microarray and Immunohistochemistry (IHC)

Confirmed, formalin-fixed, paraffin-embedded human non-small cell lung cancer tumor tissues array contained 140 samples of lung cancer from the 140 patients. All organizations come from the Sample Archives of the Pathology Department of the Weifang Second People's Hospital. IHC was performed with the tissue chip. The tissue chip slice was cut in 0.03um, baked in an oven at 60°C for 60 minutes. After dewaxing, hydrogen peroxide blocking, citric acid (BL604A, Biosharp) antigen retrieval and goat serum (SL038, Solarbio) blocking for 30mins at 37°C, the tissue chip was incubated with the CD4 rabbit monoclonal antibody (1:1000) (ab183685, Abcam) and CD45 rabbit polyclonal antibody (1:500) (ab10558, Abcam) overnight at 4 °C, respectively. After incubated with the HRP-coupled-goat-anti-rabbit secondary antibody (1:500) (abab6721) at room temperature for 2 h, the tissue chip was stained with DAB Chromogenic Kit (ZLI-9018, Beijing Zhongshan Golden Bridge) 5 mins at RT, and counterstained with Mayer' Hematoxylin solution (G1080, Solarbio). The slides were scanned by Pannoramic 250 Flash III (3DHistech Ltd., Budapest, Hungary).

Digital Image Analysis and IHC Scoring

The images captured by Pannoramic Viewer software (3DHistech Ltd., Budapest, Hungary) was analyzed using the DensitoQuant software module, and the immune-positive rate of the tissue was calculated (3DHistech Ltd., Budapest, Hungary). The DenstioQuant algorithm of QuantCenter is a stain-intensity measurement module enabling a user-controlled whole slide analysis on a pixel basis. As an initial step, the IHC signal was controlled and manually optimized to get reliable monochrome intensity values. Next, pixel intensity levels of the positive reaction were scaled and displayed in three levels: weak (yellow), moderate (orange), and strong (red). The background of negative cells was represented by the blue-stained nuclei and unstained pixels in white color. The module automatically calculated immuno-positive rate using individual pixel intensity levels and the total area of pixels. Ratios for negative, weak, moderate, and strong positives, as well as background pixels were generated. The immuno-positive rate representing specific immunolabeling for each slide was used for further evaluation. For statistical evaluation, the immuno-positive rate ≥35% was regarded as high expression, the immuno-positive rate 35% as low expression.

Statistics

IBM SPSS Statistics 22 software performed chi-square test to analyze the expression of CD4 and CD45 in non-small cell lung cancer tissues, and Graphpad Primes 8 performed Kaplan-meier to analyze the relationship between the expression of CD4, CD45 and the overall survival of patients with non-small cell lung cancer.

RESULTS

The expression of CD4 and CD45 in lung adenocarcinoma arrayed in tissue chip

CD4+ T cells can promote the proliferation and differentiation of B cells, T cells and other immune cells, and coordinate the interaction between immune cells. CD45 is a receptor-type protein tyrosine phosphatase, which plays an important role in T cell recepter signal transduction[17]. In order to detect the expression of CD4 and CD45 in non-small cell lung cancer tissue, lung cancer tissues microarray chip was made and performed immunohistochemical analysis. Seven of the 140 samples were excluded from the analysis for severe tissue damage, and the total 133 samples were analyzed in this experiment. As showed in Figure 1A, CD 4 or CD 45 positive rate high than 35% (case 1) was considered as high expression ($CD4^{high}$, $CD45^{high}$); positive rate less than 35% (case 2) was considered as low expression ($CD4^{low}$, $CD45^{low}$), respectively. Out of the 133 tissues, there were 51 samples $CD4^{high}$ expression, 82 samples with $CD4^{low}$ expression; and 53 samples with $CD45^{high}$ expression, and 80 samples with $CD45^{low}$ expression. Analyzed by SPSS software, 33 out of 133 samples (24.81%) are $CD4^{high}$ $CD45^{high}$, and 62 out of 133 samples (46.62%) are $CD4^{low}$ $CD45^{low}$ expression (Figure 1B). Analyzed by SPSS, person correlation=0.4, P<0.0001. The results showed that the expression of the two had a significant correlation, and he correlation was statistically significant (Figure 1B). In order to assess the correlation of CD4 and CD45, we conducted a T test analysis on the expression of CD4 and CD45 in 133 cancer tissues. The results showed that r=0.9061, P<0.0001. CD4 and CD45 in non-small cell lung cancer tissue is correlated.

The relationship between CD4 CD45 expression and prognosis

In order to analyze the relationship between the expression of CD4/CD45 and the prognostic survival of non-small cell lung cancer patients, we used tissue microarray to obtain the positive rate of gene expression in 140 non-small cell lung cancer patients (with 7 tissue damaged samples removed). Telephone follow-up to obtain the survival time of the included samples, the survival time is greater than 60 months according to 60 months statistics. Graphpad Primes 8 was used for drawing and statistical analysis. The results showed that the prognostic survival time of patients with $CD4^{high}$ was significantly greater than patients with $CD4^{low}$ (Log-rank P=0.0456) (Figure 2A). No significant difference was found in OS between $CD45^{high}$ and $CD45^{low}$ patients (log-rank = 0.5454) (Figure 2B). In order to detect the impact of CD4 and CD45 co-expression on the prognosis of patients with non-small cell lung cancer, we divided 133 samples into four groups: CD4+CD45+, CD4-CD45+, CD4+CD45-, CD4-CD45-. The CD4+CD45+ and CD4-CD45- groups were selected for Kaplan-

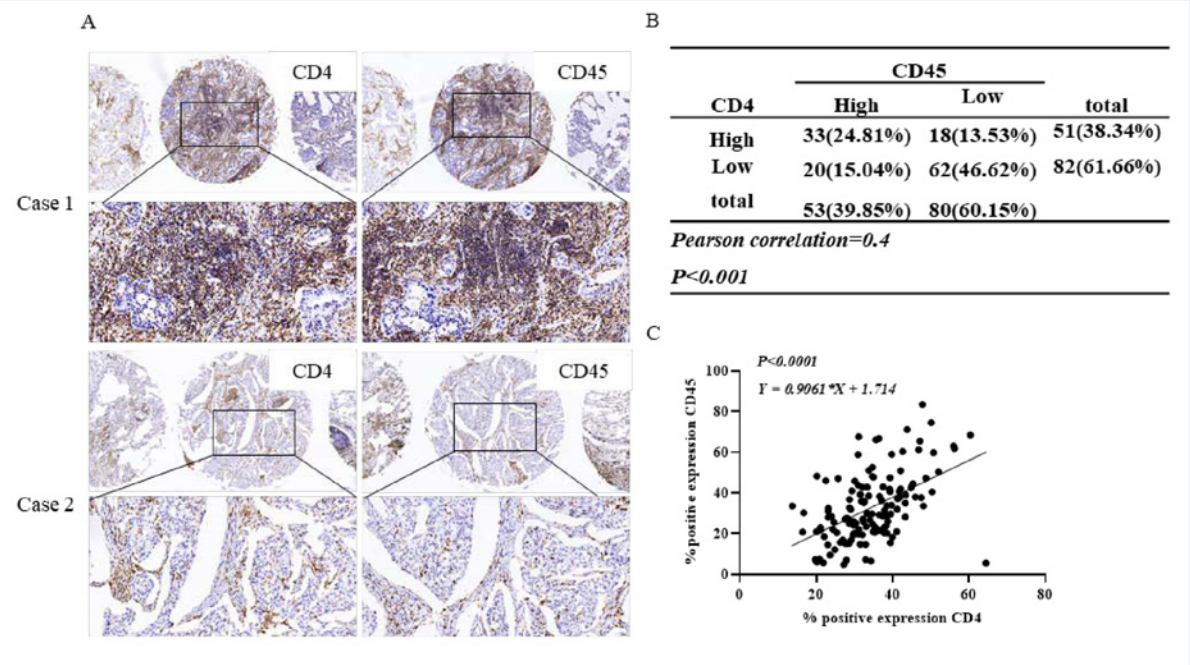

Figure 1 The expression of CD4 and CD45 in non, [A]: Immunohistochemical detection of the expression of CD4 and CD45 in the same field of the same sample. [B]: Person correlation analysis based on the expression of CD4 and CD45 in immunohistochemistry (Person correlation=0.4, P<0.0001). [C]: Linear correlation analysis and statistics based on the positive rate of CD4 and CD45 in immunohistochemistry (r=0.9061, P<0.0001).

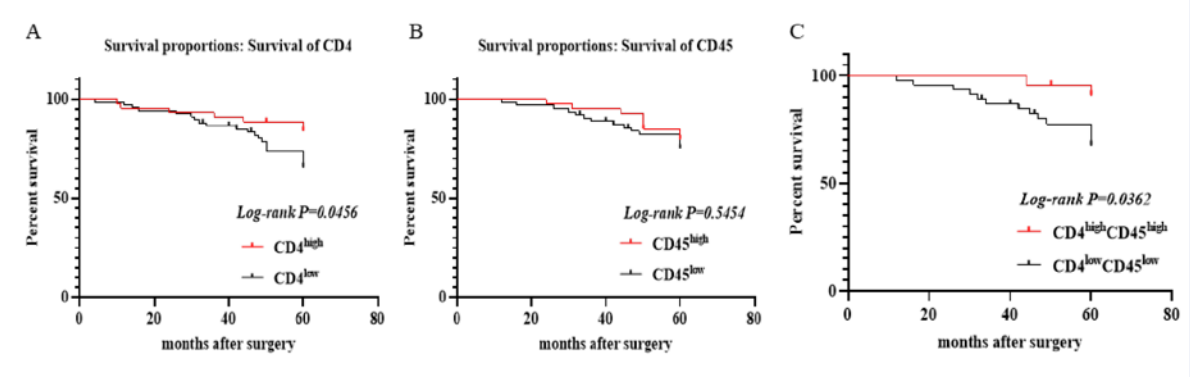

Figure 2 CD4 and CD45 co-expression and Kaplan-Meier survival analysis. [A]: CD4 expression and Kaplan-Meier survival analysis (Log-rank P=0.0456). [B]: CD45 expression and Kaplan-Meier survival analysis (Log-rank P=0.5454). [C]: CD4 and CD45 co-expression and Kaplan-Meier survival analysis (Log-rank P=0.0362).

Meier survival analysis. The results showed that the survival time of CD4+CD45+ was significantly higher than that of CD4-CD45- (Log-rank P=0.0362) (Figure 2C). Therefore, it can be determined that the increased expression of CD4 and CD45 can improve the prognostic survival of patients with non-small cell lung cancer.

DISCUSSIONS

Despite the recent decline in morbidity and mortality, lung cancer is still the world's second most common malignant tumor with the highest mortality rate[1]. Beginning in 2015, immunotherapy has been gradually applied to the clinic and achieved good therapeutic effects. Therefore, it is particularly important to further explore the mechanism of immunotherapy in the treatment of lung cancer.

CD4+ T cells are the main regulatory T cells[11]. Preliminary research in our laboratory found that the absolute count of CD4 in lung cancer patients was lower than the reference value, and there was a general decline. CD4 and CD45 are surface molecules on T cells. When activated by antigen stimulation, they will cause the active expression and secretion of a large number of cytokines, including IL-4, IL-5, IL-6, IL-10 and IL-13, accelerate immune regulation and improve immune resistance function[18, 19]. This study found that the expression of CD4 and CD45 in non-small cell lung cancer tissues has a significant correlation. Combined with the cell signaling effect of CD45, this related effect may be mediated by CD45[17].

The purpose of immunotherapy is to activate or inhibit

related immune genes, and to inhibit the growth of tumor cells or tumor tissues. This study found that the prognostic survival of CD4+CD45+ non-small cell lung cancer patients was significantly higher than that of CD4-CD45- non-small cell lung cancer patients. Therefore, we believe that under the condition of controlling excessive immune response, proper activation of CD4 and CD45 can effectively improve the prognosis and survival of patients.

The further determination of immune checkpoint markers and the inspection of immune checkpoints are the directions for further research and breakthroughs in basic scientific research and clinical examination. In the next work, the research team will further analyze and research the results of the lavage fluid and blood samples of respiratory patients with flow cytometry, and discover new immune checkpoints to better serve and clinically.

AUTHORS' CONTRIBUTIONS

The manuscript writing were instructed: MQ, ZG. Experimental operation, statistical analysis and wrote the manuscript: WW. All coauthors commented on the manuscript and agreed with the conclusions of the manuscript.

FUNDING

This study is supported by funds from National Natural Science Foundation of China (81871892 Meihua Qu, 82070856 Zhiqin Gao), Natural Science Foundation of Shandong Province (No. ZR2019BH036), the Science and technology plan of Shandong Health Committee (2019WS244-2019WS243), Science and Technology Development Project of Weifang (No. 2018YX058, 2020YQFK017, 2018YX027, WFWSJK-2020-004, WFWSJK-2020-077).

ACKNOWLEDGEMENTS

The author thanks the clinical doctors of Weifang Second People's Hospital.

REFERENCES

1. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA: A Cancer Journal for Clinicians 70: 7-30.
2. Kathleen, Madden, Mary, Kate, Kasler (2019) Immune Checkpoint Inhibitors in Lung Cancer and Melanoma. Semin Oncol Nurs 35: 150932.
3. Cui T, Srivastava AK, Han C, Yang L, Zhao R, et al. (2015) XPC inhibits NSCLC cell proliferation and migration by enhancing E-Cadherin expression. Oncotarget 6: 10060-10072.
4. Jannie B, Tomasz A, Nikolina BBA, Melief CJM, Wolfgang K (2018) CD4 + T cell help in cancer immunology and immunotherapy. Nat Rev Immunol 18: 635-647.
5. Leonetti A, Wever B, Mazzaschi G, Assaraf YG, Giovannetti E (2019) Molecular basis and rationale for combining immune checkpoint inhibitors with chemotherapy in non-small cell lung cancer. Drug Resist Updat 46: 100644.
6. Ajona D, Ortiz-Espinosa S, Moreno H, Lozano T, Pajares MJ, et al. (2017) A Combined PD-1/C5a Blockade Synergistically Protects Against Lung Cancer Growth and Metastasis. Cancer Discov 7: 694-703.
7. Koyalta D, Jenabian MA, Nadjiouroum N, Djouater B, Djemadji-Oudjeil N, et al. (2013) Single-platform, volumetric, CD45-assisted pan-leucogating flow cytometry for CD4 T lymphocytes monitoring of HIV infection according to the WHO recommendations for resource-constrained settings. BMC Research Notes 30: 6-169.
8. Melssen M, Slingluff CL (2017) Vaccines targeting helper T cells for cancer immunotherapy. Curr Opin Immunol 47: 85-92.
9. Kennedy R, Celis E (2010) Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunol Rev 222: 129-144.
10. Bevan MJ (2004) Helping the CD8(+) T-cell response. Nat rev Immunol 4: 595-602.
11. Stankovic B, Bjørhovde HAK, Skarshaug R, Aamodt H, Frafjord A, Müller E, et al. (2019) Immune Cell Composition in Human Non-small Cell Lung Cancer. Front Immunol 9: 3101.
12. Zander R, Schauder D, Xin G, Nguyen C, Cui W (2019) CD4+ T Cell Help Is Required for the Formation of a Cytolytic CD8+ T Cell Subset that Protects against Chronic Infection and Cancer. Immun 51: 1028-1042.
13. Arata T, Takashi S (2017) CD4 CTL, a Cytotoxic Subset of CD4+ T Cells, Their Differentiation and Function. Front Immunol 8:194.
14. Hermiston ML, Xu Z, Weiss A (2003) CD45: a critical regulator of signaling thresholds in immune cells. Annu Rev Immunol 21: 107-137.
15. Altin JG, Sloan EK (1997) The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol 75: 430-445.
16. Chang VT, Fernandes RA, Ganzinger KA, Lee SF, Siebold C, Mccoll J, et al. (2016) Initiation of T cell signaling by CD45 segregation at 'close contacts'. Nat Immunol 17: 574-582.
17. Koretzky GA, Picus J, Schultz T, Weiss A (1991) Tyrosine phosphatase CD45 is required for T-cell antigen receptor and CD2-mediated activation of a protein tyrosine kinase and interleukin 2 production. Proc Natl Acad Sci USA 88: 2037-2041.
18. Nobuhiko K, Tadakazu H, Susumu O, Hiroshi C, Taku K, et al. (2008) Unique CD14+ intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-γ axis. J Clin Invest 118: 2269-2280.
19. Bamias G, Jia LG, Cominelli F (2013) The tumor necrosis factor-like cytokine 1A/death receptor 3 cytokine system in intestinal inflammation. Curr Opin Gastroenterol 29: 597-602.

Cite this article

Wang W, Yang Y, Ma S, Wang H, Li H, et al. (2020) Expression and Significance of CD4, CD45 In Patients with Non-Small Cell Lung Cancer. J Cancer Biol Res 8(1): 1130.