Tropical Journal of Pharmaceutical Research May 2018; 17 (5): 767-771
ISSN: 1596-5996 (print); 1596-9827 (electronic)
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Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i5.3

Original Research Article

Role of IL-33 and ST2 signaling and inflammatory responses in non-small cell lung cancer

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Sent for review: 23 November 2017 *Revised accepted: 18 April 2018*

Abstract

Purpose: *To investigate the role of the interleukin (IL)-33 and ST2 pathway in non-small cell lung cancer (NSCLC), and to further explore the critical relationships among inflammation, immunity, and cancer.*
Methods: *From January 2014 to December 2015, paraffin-embedded sections of surgical specimens were obtained from 40 patients definitively diagnosed with NSCLC by pathological examination in Changzhou Wujin People's Hospital and Taicang Hospital of Traditional Chinese Medicine. Sections were further immunostained with antibodies directed against IL-33 and ST2 cardiac biomarker. Inflammatory reactions were determined by hematoxylin and eosin (H&E) staining. Paracancerous control sample tissues were also collected. In addition, 60 primary NSCLC patients without any complications were enrolled, and 60 healthy volunteers were enrolled at the same institutions. Serum samples of patients were collected, and protein expressions of IL-33, ST2, IL-4, and interferon (IFN)-γ were detected by enzyme-linked immunosorbent assay (ELISA) or western blot assay.*
Results: *The results indicate that IL-33, ST2 and IL-4 expressions in cancer tissues and blood were significantly increased when compared with control groups.*
Conclusion: *IL-33/ST2 in NSCLC microenvironment enhances T helper cell 2 (Th2) response, which may be beneficial for tumor growth.*

Keywords: *Interleukin, IL-33, ST2, IL-4, non-small cell lung cancer (NSCLC)*

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INTRODUCTION

Lung cancer is the leading cause of cancer deaths both in males and females throughout the world, including China. It is estimated that the incidence and mortality of lung cancer were approximately 7.33 and 6.10 % in China in 2015, respectively [1]. Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancers diagnosed at an advanced stage and comprises about 85 % of the cases. Efforts to improve the poor prognosis of patients with NSCLC depend on a better understanding of the biology of the cancer [2]. There are few reports regarding the molecular features of the tumor microenvironment in dynamic conditions, and links to inflammation, immunity, and cancer remain unclear. In the 19th century, Rudolf

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Virchow observed the presence of leukocytes within tumors, and suggested a possible link between inflammation and cancer [3,4]. Persistent *Helicobacter pylori* infection can cause gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. Hepatitis B virus (HBV) infections increase the risk of hepatocellular carcinoma (HCC), and *Schistosoma* or *Bacteroides* species infections have some links with bladder and colon cancer [5,6]. Aggarwal argued that about 20 % of cancers are associated with chronic infections and 30 % are linked to tobacco smoking and inhaled pollutants (such as silica and asbestos) [7]. Particulate materials and other irritants from tobacco can cause chronic obstructive pulmonary disease, a condition associated with higher lung cancer risk. Inhaled asbestos or silica particles also give rise to lung cancer, but have no obvious mutagenic effects. COX2 inhibitors, aspirin and anti-inflammatory steroids, can prevent colon cancer, breast cancer [8], and reduce prostate cancer risk, but only in individuals who carry a particular polymorphic allele which specifies high lymphotoxin production [9]. Therefore, tumor-promoting inflammation is just a single aspect of cancer biology.

In the process of immune surveillance, precancerous and malignant cells can express “altered self” and “non-self” antigens, and induce an immune response, which results in the destruction of transformed and malignant cells. However, an immune surveillance is not always successful, resulting in tumor cells escaping the immune surveillance and progressing to cancer. Ostrand-Rosenbergs proposed that the immune response is a double-edged sword in tumorigenesis [10]. T cells can exert both tumor-suppressive and tumor-promoting effects, as determined by their effector functions. Type 1 CD4+ T cells (Th1 cells) facilitate tissue destruction and tumor rejection by providing help to cytotoxic CD8+ T cells, while Type 2 CD4+ T cells T (Th2 cells) facilitate antibody production by B cells and promote tumor progression [11]. In the present study, we have shown that IL-33/ST2 in the NSCLC microenvironment enhanced the Th2 cell response, which might be beneficial for tumor growth.

EXPERIMENTAL

Subjects

From January 2014 to December 2015, paraffin-embedded sections from surgical specimens were obtained from 40 patients definitively diagnosed with NSCLC by pathological examinations. The sections were further immunostained with antibodies directed against IL-33 and ST2, and inflammatory reactions were determined by hematoxylin and eosin (H *&* E) staining. Paracancerous tissues were collected as control samples. In addition, 60 primary NSCLC patients without any complications were enrolled, and 60 healthy volunteers were enrolled as the control group. Blood samples were collected from the vein and the serum samples were separated. Protein expression levels of IL-33, ST2, IL-4 and IFN–γ were detected by enzyme-linked immunosorbent assay (ELISA) or western blot assays.

All the patients were required to read and sign an informed consent form voluntarily before enrollment. All the experimental protocols were carried out in accordance with the Declaration of Helsinki promulgated in 1964 as amended in 1996 [12]. Approval by Changzhou Wujin People's Hospital (no. CZ 2017-0121) was obtained.

H & E and immunohistochemistry staining

Samples were fixed in 4 % formaldehyde, embedded in paraffin wax, and processed for H&E and immunohistochemical analyses of IL - 33 and ST2. Noncancerous tissues at least 10 cm from the tumor were analyzed as control samples. Sections (5 μm thick) were cut and subsequently hydrated. Endogenous peroxidase activity was blocked with 3 % tris hydroxymethyl aminomethane-buffered saline for 30 min. Immunohistochemistry was performed using rabbit anti-human IL-33 antibody (Cloud-Clone Corp., Houston, TX, USA) and goat anti-human ST2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The negative control involved omission of the primary antibody.

Western blotting analysis

Lung cancer tissues were lysed in radioimmunoprecipitation assay (RIPA) buffer including protease and phosphatase inhibitors, and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS - PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with skimmed milk, incubated with primary antibodies (rabbit anti-human IL - 33 antibody and goat anti-human ST2 antibody), and horseradish peroxidase (HRP)-conjugated secondary antibody. β-actin was used as an internal control. Specific bands were visualized using an ECL - PLUS chemiluminescence system.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples from each individual were collected at the time of diagnosis, before chemotherapy, and after second chemotherapy. Sera were stored at -70 °C. The optical density (OD) values of IL-33, IL-4, and IFN-γ from the supernatants of each group were detected after being centrifuged, while ST2 was detected from centrifuged cells. These proteins were measured by a sandwich ELISA kit, according to the manufacturer's instructions. The concentration of each cytokine was obtained from a standard curve.

Statistical analysis

All data were analyzed using SPSS, version 19.0 software (SPSS, Chicago, IL, USA), and are shown as mean ± standard deviation (SD). Statistical differences between groups were computed by independent sample *t*-tests, with $p < 0.05$ considered statistically significant.

RESULTS

Histopathological features of lung cancer tissues

IL-33 - positive immunostaining was located in tumor cells (adenocarcinoma and squamous cell carcinoma), and adjacent normal tissues at least 10 cm from the tumor were used as normal lung tissues (Figure 1 A). In either adenocarcinoma (Figure 1 D) or squamous cell carcinoma (Figure 1 E) tissues, there were many inflammatory and immune cells, such as macrophages, neutrophils, dendritic cells, and T and B lymphocytes with surrounding stroma (consisting of fibroblasts, endothelial cells, pericytes, and mesenchymal cells). ST2 was immunostained positively at the areas of inflammation and infiltrating immune cells (Figure 1 F).

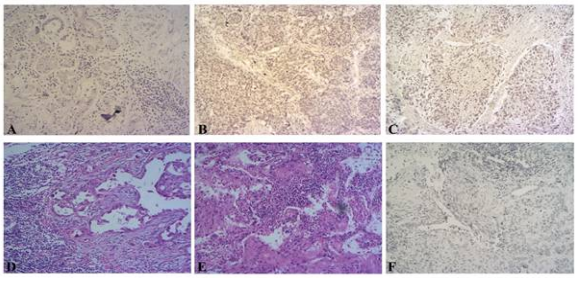

Figure 1: Hematoxylin-eosin and immunohistochemistry staining of lung tissues. A-F represented as the paracancer tissues (A), IL-33-positive cells of adenocarcinoma (B) squamous cell carcinoma (C), *HE* staining tissues of adenocarcinoma (D) squamous cell carcinoma (E), ST2 positive staining (F)

Expressions of proteins IL-33 and ST2 in NSCLC tissue

As can be seen in Figure 2, compared to normal lung tissues, the protein levels of ST2 and IL-33 in NSCLC tissues were significantly upregulated ($p < 0.05$; Figure 2). No other significant differences were observed between adenocarcinoma and squamous cell carcinoma tissues.

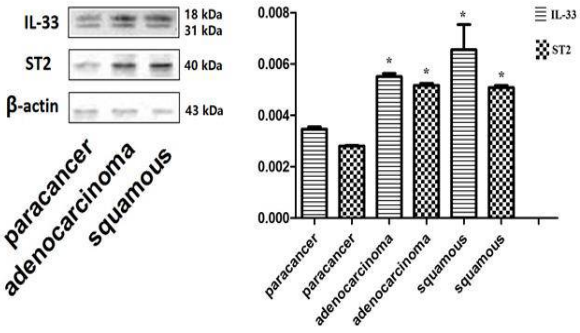

Figure 2: Results of the western blotting of IL – 33 and ST2. (squamous means squamous carcinoma cell). Data were expressed as Mean ± SD; * $p < 0.05$ vs paracancer groups (control group)

Serum levels of IL-33, ST2, IL-4, and IFN - γ

As shown in Figure 3, the serum levels of IL-33, ST2, and IL-4 of NSCLC patients were significantly higher than those in normal lung tissues ($p < 0.05$). In contrast, the IFN-γ levels in the serum of NSCLC patients remained lower than that of normal lung tissues, but showed no statistical differences ($p > 0.05$). There were no significant differences of IL-33, ST2, IL-4, and IFN-γ between patients with adenocarcinoma and patients with squamous carcinoma cell.

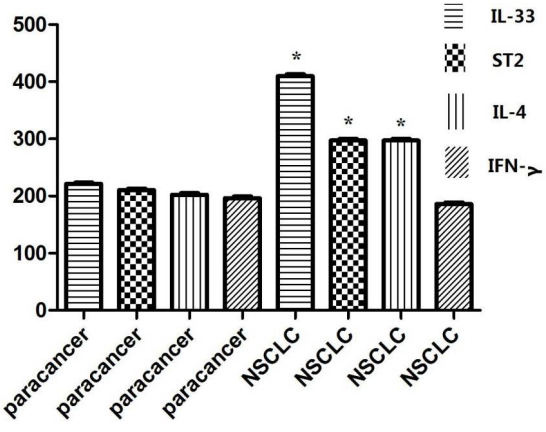

Figure 3: Blood levels of IL-33, ST2, IL-4, IFN-γ by using ELISA. Data were expressed as Mean ± SD; *$p < 0.05$ vs paracancer groups (control group)

DISCUSSION

IL-33 is a cytokine of IL-1 family, also called IL-1F11 according to systematic nomenclature. Produced as a pro – IL-33, the 31 kDa protein, IL-33 is cleaved to form a mature 18 kDa protein, which works as a cytokine through its IL-1 receptor family members [11,12]. ST2, the most prominent orphan IL-1 receptor (also designated T1, Fit-1, and DER4), belongs to Toll-like receptor-IL-1 receptor (TLR-IL-1R) superfamily [13-15]. Schmitz *et al* first identified the orphan receptor "ST2" (also called IL-1R4) as a receptor for IL - 33. Co-immunoprecipitation demonstrated that a combination of IL-33 and ST2 can ultimately lead to the activation of NF-κB and MAPK kinases involved in the control of cellular proliferation and apoptosis [11]. Xu *et al* have shown that *ST2* gene products are predominantly expressed in Th2 cells but not in Th1 cells, and have been recognized as stable markers of Th2 cells [16,17]. Recently, IL - 33/ST2 signaling has been studied in many fibrotic diseases such as scleroderma, progressive systemic sclerosis [18,19], and liver fibrosis in mice and humans [20].

This study was the first to determine whether IL - 33/ST2 signaling existed in NSCLC. IL -33 and ST2 were analyzed in NSCLC tissues by immunohistochemistry, with L - 33 - positive cells being tumor cells, while ST2-positive reactions were located in inflammatory cells. The greater levels of IL-4 and ST2, and the lower level of IFN-γ in NSCLC patients' sera indicated that there was a Th1/Th2 imbalance with a striking Th2 response in NSCLC. In breast cancer, the presence of tumor-infiltrating lymphocytes with high CD4+/CD8 + and Th2/Th1 ratios were indicative of poor prognoses [21]. Th2 CD4+ T cells stimulate mammary cancer progression and metastasis by activing tumor-associated macrophages (TAMs) to produce proangiogenic and pro-metastatic factors [22]. Dunn *et al* proposed that the presence of tumor-infiltrating lymphocytes was insufficient for curtailing tumor growth, and the cancer cells escaping the immune surveillance system were constantly edited and modulated by the host antitumor immune response [23]. Consistent with this hypothesis, it was speculated that tumor cells expressing and releasing IL - 33, as a proinflammatory mediator, recruited monocytes and changed the tumor dynamic microenvironment, which consisted of macrophages, neutrophils, and T lymphocytes. IL-3, as an immune modulator, in combination with ST2 of Th2 cells or other cells in the tumor microenvironment and NSCLC blood, can enhance the Th2 response in favor of tumor growth, thus tipping the balance of tumor-promoting vs. tumor-suppressive immunity.

CONCLUSION

The findings of the present investigation demonstrate that activated tumor environmental cells provide both anti- and protumorigenic signals, which may represent targets to be harnessed or attacked for therapeutic advantages. In addition, the data also indicate that tumor-promoting IL-33 provides a target preventive approach for NSCLC treatment.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. In addition, all authors read and approved the manuscript for publication. Jiao Xu conceived and designed this study; Hong Lv, Tiefeng Qiu, Xianwen Li and Yanjuan Zhou collected and analysed the data; Jiao Xu and Hong Lv wrote the manuscript. In addition, Hong Lv and Tiefeng Qiu contributed equally to this work.

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