Dynamic alterations of myeloid-derived suppressor cells and CD68+CD163+M2-like macrophages of non-small cell lung cancer patients in radiotherapy

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Research

Keywords: Myeloid-derived suppressor cells, M2-like macrophages, non-small cell lung cancer, radiotherapy

Posted Date: June 17th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-35586/v1>

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Abstract

Background

Recent studies showed that myeloid-derived suppressor cells (MDSCs) and M2-like macrophages are involved in the treatment of non-small cell lung cancer (NSCLC) as immunosuppressive cells; however, the changes of MDSCs and M2-like macrophages, and their prognostic value are rarely unknown in NSCLC patients during radiotherapy. In this article, we aim to explore dynamic alteration of the circulating MDSCs and M2-like macrophages, to examine their relationship, and to evaluate their prognostic value for NSCLC patients in radiotherapy.

Method:

Peripheral blood mononuclear cells from healthy controls and NSCLC patients in radiotherapy were isolated to examine the circulating MDSCs and M2-like macrophages. The peripheral MDSCs defined as CD11b⁺CD33⁺HLA-DR⁻ and M2-like Macrophages signified as CD68⁺CD163⁺ were determined by flow cytometry. 40 plasma inflammatory cytokines were measured by multiplex ELISA.

Results

Compared with health controls, the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients were significantly elevated, and were distinctly higher in NSCLC patients in radiotherapy than in pre-radiotherapy. Moreover, MDSCs correlated positively with CD68⁺CD163⁺M2-like macrophages in NSCLC patients in radiotherapy and post-radiotherapy. Interestingly, the alterations of the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages in RT were unrelated with radiotherapy area. During radiotherapy, the proportions of MDSCs were clearly increased in adenocarcinoma patients but not in squamous carcinoma patients, while the proportions of CD68⁺CD163⁺M2-like macrophages were markedly elevated in squamous carcinoma patients but not in adenocarcinoma patients. In addition, the percentages of MDSCs and CD68⁺CD163⁺ M2-like macrophages were also increased in NSCLC patients reached PR in radiotherapy compared to NSCLC patients reached SD and PD. IL-1ra and MIP-1β have a positive relation with MDSCs or M2 macrophages in NSCLC patients in radiotherapy, and Eotaxin was correlated with CD68⁺CD163⁺M2-like macrophages but not MDSCs in NSCLC patients after radiotherapy.

Conclusions

The dynamic alterations of MDSCs and M2-like macrophages, as well as their connection with plasm inflammation cytokines, could provide potentially prognostic and sensitive markers for NSCLC patients in radiotherapy.

Trial Registration: Jinshan Hospital of Fudan University, IEC-2020-S01. Registered 22 May 2020.

Background

Lung cancer is the major cause of cancer-related death among males in both more and less developed countries [1], and has surpassed breast cancer as the leading cause of cancer death among females in more developed countries [2]. Non-small cell lung cancer (NSCLC) as the most common type of lung cancer accounts for approximately 85% of all cases [3, 4]. Many NSCLC patients have already developed to the advanced stage when they are diagnosed, and then they have little chance to accept surgical treatment. Local radiotherapy is a traditional approach to treating terminal NSCLC [5], which can retard the deterioration of primary or secondary lesion and relieve pain by activating the body immune system [6, 7]. However, the correlation between local radiotherapy and the variation of the immune system of cancer patients has been rarely reported. Therefore, it is crucial to carry out further studies on their potential relations and mechanisms further, especially the effects of Immunosuppressive cells on cancer patients' prognosis in radiotherapy (RT).

Myeloid-derived suppressor cells (MDSCs), a subset of bone marrow-derived immature myeloid cells and one of the major factors negatively regulating immune responses [8], have been confirmed to suppress the activation of T cells [9] and the differentiation of dendritic cells (DCs) [10]. According to the recent research findings, tumor-secreted and host-secreted factors, many of which are pro-inflammatory molecules, can induce the accumulation of MDSCs [11]. Increased MDSCs have been implicated in the pathogenesis of cancer, which could also serve as a prognostic marker for treatment responses in cancer [12, 13]. Macrophages, a group of terminally differentiated myeloid cells derived from monocytes in peripheral blood, are also crucial drivers to promoting tumor inflammation [14]. M2 macrophages that are predominantly characterized as CD68⁺ (pan macrophages marker) and CD163⁺ (M2 specific marker) could dampen the immune responses and limit inflammation reactions. Ionizing radiation (IR) promotes the release of danger signals and chemokines that recruit inflammatory cells into the tumor microenvironment, including antigen-presenting cells that activate cytotoxic T cell function [15]. However, whether the alterations of MDSCs and M2 macrophages of NSCLC patients paly a potential role in improving sensitivities and outcomes in radiotherapy, has not yet been investigated in NSCLC patients.

The aim of this study is to analyze the dynamic alterations of circulating MDSCs and M2-like Macrophages of NSCLC patients in radiotherapy, to examine their relationships, and to evaluate the potential connections of plasm cytokines, thereby providing a novel insight as well as prognostic factors for NSCLC patients in radiotherapy.

Methods

Patients and Controls

From June 2018 to April 2019, Peripheral blood was obtained from Sixteen NSCLC patients in pre-radiotherapy (pre-RT), radiotherapy (RT), and post-radiotherapy (post-RT). The total radiation dose was 40 Gy with 20 times of radiation. According to eighth edition of the TNM Classification of Lung Cancer [16], all NSCLC patients (over 44 years old) were diagnosed with inoperable, locally advanced (Stage III) or metastatic (Stage IV) disease. The evaluation of target lesions in RECIST criteria[17] was: complete

response (CR) —the disappearance of all target lesions; partial response (PR) —at least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter; progressive disease (PD) —at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions; stable disease (SD) —neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started. For controls, blood samples were collected from 8 healthy volunteers (4 males and 4 females; age 56 ± 7 years). All patients provided a written informed consent and the study was approved by the ethics committee of Jinshan Hospital affiliated to Fudan University.

Isolation of peripheral blood mononuclear cells

EDTA-anticoagulated venous blood samples were freshly collected from NSCLC patients. According to the Manufacturer's manual and density-gradient centrifugation, Peripheral blood mononuclear cells (PBMCs) were isolated by using human lymphocytes separation medium (Haoyang biological products technology, Tianjin, China). The detached PBMCs were resuspended in cell freezing medium (10% DMSO in fetal bovine serum) and then stored at -80°C .

Flow Cytometry Analysis

MDSCs were defined as $\text{CD11b}^+\text{CD33}^+\text{HLA-DR}^-$. Single cell suspensions were surfacely stained with APC-conjugated anti-human CD11b (ICRF44; BD Biosciences, San Diego, CA), FITC-conjugated anti-human CD33 (HIM3-4; BD Biosciences), and PerCP-Cy5.5-conjugated anti-human HLA-DR⁺ (G46-6; BD Biosciences). M2-like macrophages were intracellularly stained with CD68 (Y1/82A; BD Biosciences) and CD163 (GHI/61; BD Biosciences). All samples were prepared according to the manufactures' instructions and were analyzed by using a flow cytometry (BD Biosciences).

Plasma inflammation cytokine array

Cytokine profiles were measured by Qu antibody Human Inflammation Array 3 (Ray Biotech, Norcross, GA) that detected 40 inflammation-associated cytokines simultaneously by using plasma samples (8 health controls and 16 NSCLC patients) cryopreserved at -80°C . The experiment was performed according to the manufacture's protocol.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism version 7.0 (GraphPad Institute Inc, USA) and Microsoft Office Excel 2016 (Microsoft, USA). One-Way ANOVA and Tukey's test were applied to continuous variables between groups. Statistical significance ($P < 0.05$) was determined by Two-tailed Student's non-paired t test in two independent groups. The correlation between MDSCs and M2-like macrophages was accessed by Pearson's correlation coefficient.

Results

Clinical characteristics of NSCLC patients

In the present study, the curative effects of 6 patients reached PR, and the curative effects of eight patients reached SD, while that of two patients reached PD after palliative radiotherapy. There was a distant metastasis (Stage IV) in twelve patients from sixteen NSCLC patients which included ten adenocarcinoma and six squamous carcinoma patients. Seven lung cancer patients received radiation to the lungs, five to the brain, and four to the bone. Clinical characteristics of NSCLC patients were presented in **Table 1**. The median age of eight health controls (4 females and 4 males) was 54.5 (ranging from 49 to 62).

The percentages of peripheral MDSCs and CD68⁺CD163⁺M2-like macrophages were significantly elevated in NSCLC patients

The percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages were analyzed by flow cytometry in healthy control (HC) and NSCLC patients (Fig. 1A, B). We found that the percentages of peripheral MDSCs and CD68⁺CD163⁺M2-like macrophages were significantly elevated in NSCLC patients in pre-RT and RT compared to HC group, while the proportions of MDSCs and CD68⁺CD163⁺M2-like macrophages were decreased in NSCLC patients in post-RT compared to in RT. Otherwise, there were obviously more peripheral MDSCs and CD68⁺CD163⁺M2-like macrophages in NSCLC patients in RT than in pre-RT (Fig. 1C, D).

MDSCs were correlated positively with CD68⁺CD163⁺M2-like macrophages in NSCLC patients

In HC, we found that MDSCs were correlated negatively with CD68⁺CD163⁺M2-like macrophages, but their correlation was not significant (Fig. 2A). Interestingly, the correlation between MDSCs and CD68⁺CD163⁺M2-like macrophages was not significant in NSCLC patients in pre-RT ($r = 0.072$, $p = 0.791$) (Fig. 2B), but peripheral MDSCs were positively correlated with CD68⁺CD163⁺M2-like macrophages in NSCLC patients in RT ($r = 0.5548$, $p = 0.0257$) and post-RT ($r = 0.51$, $p = 0.0435$) (Fig. 2C, D). These data indicated that ionizing radiation (IR) could cause the expansion of peripheric MDSCs and CD68⁺CD163⁺M2-like macrophages in NSCLC patients.

The value of MDSCs and CD68⁺CD163⁺M2-like macrophages in NSCLC patients with various clinical characteristics

To further investigate the value of MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in pre-RT, RT, and post-RT, we reviewed and classified the clinical data of these patients. In this study, we found that MDSCs as well as CD68⁺CD163⁺M2-like macrophages of NSCLC patients in RT and post-RT were elevated in NSCLC patients without metastasis but were not significantly increased in NSCLC patients with metastasis. Furthermore, there were higher percentages of MDSCs of NSCLC patients with

metastasis in pre-RT than NSCLC patients without metastasis in pre-RT (Fig. 3A, B). The selection of radiotherapy area could affect the response to radiotherapy. However, in this study, the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages did not show clear difference in NSCLC patients with lung, brain and bone radiation. Hence, we speculated that the alteration of the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in RT were unrelated with radiotherapy area (Fig. 3C, D). After radiotherapy (post-RT), the percentages of MDSCs in NSCLC patients with brain radiation were significantly less than NSCLC patients with lung and bone radiation, suggesting that MDSCs could be a more sensitive marker in NSCLC patients with brain radiation. We analyzed the percentages of MDSCs and CD68 + CD163 + M2-like macrophages in non-small cell lung squamous carcinoma and lung adenocarcinoma patients, finding that in radiotherapy, the proportions of MDSCs were markedly increased in non-small cell lung adenocarcinoma patients but not in non-small cell lung squamous carcinoma patients; however, the proportions of CD68⁺CD163⁺M2-like macrophages were markedly elevated in non-small cell lung squamous carcinoma patients but not in non-small cell lung adenocarcinoma patients (Fig. 3E, F).

The increasing MDSCs and CD68⁺CD163⁺M2-like macrophages improved the prognosis of NSCLC patients

After radiotherapy, the curative effects of NSCLC patients reached PR, SD and PD. We found that as for the NSCLC patients who reached PR, the frequencies of MDSCs and CD68⁺CD163⁺M2-like macrophages in RT were more than in pre-RT and post-RT. Furthermore, the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients reached PR in RT were higher than NSCLC patients reached SD or PD in RT. (Fig. 4A, B). By the correlation analysis between MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in pre-RT, RT, and post-RT at every curative effect group, we found that these two kinds of peripheral cells of PR group in RT had an obvious relation, demonstrating that radiotherapy could simultaneously increase their percentages in peripheral blood (Fig. 4C, D). This part of data illustrated that MDSCs and CD68⁺CD163⁺M2-like macrophages also act as a prognostic marker for treatment responses in NSCLC patients.

The crossed correlation analysis of CRP and WBCs with MDSCs and CD68⁺CD163⁺M2-like macrophages in NSCLC patients

Through retrospectively consulting the clinical data of NSCLC patients, we found that C-reaction protein (CRP) of NSCLC patient were increased in RT but decreased in post-RT compared with NSCLC patients in pre-RT (Fig. 5A). White blood cells (WBCs) of NSCLC patients were slightly decreased in post-RT (Fig. 5B). CRP was correlated positively with CD68⁺CD163⁺M2-like macrophages of NSCLC patients in RT ($r = 0.7571$, $p = 0.0044$), but did not have a linear relation with MDSCs of NSCLC patients in pre-RT, RT, and post-RT (Fig. 5C, D). As for WBCs, our study indicated that they were correlated negatively with MDSCs of NSCLC patients in post-RT ($r = -0.5$, $p = 0.0572$), but were irrelevant with CD68⁺CD163⁺M2-like macrophages of NSCLC patients in pre-RT, RT, and post-RT (Fig. 5E, F).

The plasma cytokines were examined by multiplex ELISA in HC and NSCLC patients

Measuring 40 inflammation-associated cytokines of NSCLC patients and HC, we found that 8 kinds of cytokines (BLC, Eotaxin, I-309, MCP-1, MIP-1 β , RANTES, TNF RI, and TNF RII) were significantly elevated in NSCLC patients compared to HC. Especially, MIP-1 β as well as Eotaxin was markedly decreased in NSCLC patients in pre-RT compared with HC group, but was significantly elevated after radiotherapy (Fig. 6A). 17 kinds of plasma cytokines (G-CSF, GM-CSF, ICAM-1, IFN- γ , IL-1 α , IL-2, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF- α , and TNF- β) were obviously decreased in NSCLC patients compared with HC (Fig. 6B). However, 15 kinds of plasma cytokines (Eotaxin-2, IL-1 β , IL-1ra, IL-6, IL-6R, IL-11, IL-12p40, IL-16, MCSF, MIG, MIP-1 α , MIP-1d, PDGF-BB, TIMP-1, and TIMP-2) hardly changed in NSCLC patients compared with HC (Fig. 6C).

Plasma cytokines were correlated with MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in RT

Ionizing radiation increased the percentages of MDSCs and M2-like macrophages in NSCLC patients. However, the correlation between MDSCs as well as CD68⁺CD163⁺M2-like and plasma cytokines had barely been illuminated in NSCLC patient in ionizing radiation. In this study, we found that MDSCs were positively correlated with IL-1ra ($r = 0.59$, $p = 0.0161$), MIP-1 β ($r = 0.4939$, $p = 0.0516$), TIMP-2 ($r = 0.5575$, $p = 0.0248$), and TNF RI ($r = 0.504$, $p = 0.0465$) in NSCLC patients of IR-middle group but were negatively related with IL-11 ($r = -0.4055$, $p = 0.1192$) (Fig. 7A). CD68⁺CD163⁺M2-like macrophages were positively related with IL-1ra ($r = 0.6527$, $p = 0.0061$), MIP-1 β ($r = 0.5577$, $p = 0.0248$), and IL-6 ($r = 0.5448$, $p = 0.0291$) in NSCLC patients of IR-middle group but were negatively related with TIMP-1 ($r = -0.4545$, $p = 0.0769$) and IL-12p40 ($r = -0.5128$, $p = 0.0422$) (Fig. 7B).

Plasma cytokines were correlated with MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in post-RT

In this part, we analyzed the relation of plasma cytokines with MDSCs, finding that MDSCs were negatively related with MIP-1d ($r = -0.6431$, $p = 0.0072$) in NSCLC patients in post-RT (Fig. 8A). CD68⁺CD163⁺M2-like macrophages were positively correlated with Eotaxin (CCL11) ($r = 0.7316$, $p = 0.0013$) (Fig. 8B) in NSCLC patients in post-RT but were negatively related with TMP-2 ($r = -0.4808$, $p = 0.0594$) (Fig. 8C) and TNF RII ($r = -0.4525$, $p = 0.0785$) (Fig. 8D).

Discussion

NSCLC is the leading pathology type of lung cancer [18]. Ionizing radiation therapy is a traditional method of treating advanced NSCLC patients [19]. As we all know, ionizing radiation (IR) is often regarded as an element of danger [20]. However, danger responses on the cellular and molecular level are often beneficial to the induction of anti-tumor immunity and amelioration of inflammation [21]. In the current study, through examining the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages, their

relationships, their further changes in ionizing radiation therapy, and the cytokine profiles of NSCLC patients in radiotherapy, we provided novel insights into the role of myeloid-derived immune modulator cells in NSCLC patients in radiotherapy.

Myeloid-derived suppressor cells (MDSCs) are critical in regulating immune responses by suppressing antigen presenting cells (APCs) and T cells [22]. In mice, MDSCs are usually defined as either CD11b⁺GR-1⁺ or CD11b⁺GR-1⁻ [23]. The surface markers of cells defining human MDSCs have not yet to be confirmed [24, 25], partly because no unified markers are currently available for these cells. However, these cells typically express the common myeloid markers, CD33 and CD11b, but lack markers of mature myeloid cells, such as HLA-DR[26]. Therefore, MDSCs are defined as CD33⁺CD11b⁺HLA-DR⁻ in NSCLC patients in this present study. Macrophages, key regulators of inhibition and resolution of inflammation [27], are a large group of immune cells, which can generally be divided into two subpopulations according to the specific immune responses involved, namely M1 and M2 types [28, 29]. M2 macrophages that are usually identified based on the expression patterns of a set of diverse markers, display anti-inflammatory properties and are implicated in debris scavenging and tissue repair [29, 30]. CD163, a scavenger receptor expressed on cells of the monocyte lineage [31], was stained intracellularly in the present study to show the potential monocyte-to-M2 macrophages polarization. This cell population was defined as M2-like macrophages because only monocytes but not activated macrophages exist in the peripheral blood.

MDSCs expansion are generally linked to inflammatory processes [32]. Recent studies showed that MDSCs could expand under pathological conditions including multiple sclerosis [33], 1 diabetes [34], rheumatoid arthritis [35], systemic lupus erythematosus [36], and autoimmune hepatitis[37]. Our study found that MDSCs accumulated markedly in NSCLC patients in RT, suggesting that MDSCs regulated the inflammation responses caused by ionizing radiation therapy, and could be beneficial to improving the curative effects for advanced NSCLC patients. In addition, M2-like macrophages as anti-inflammation ingredient were correlated positively to MDSCs in NSCLC patients in RT, which could further suppress the inflammation responses caused by ionizing radiation therapy. Usually, M2 or alternatively activated macrophages are activated by IL-4, IL-10, IL-13 [38] and glucocorticoid hormones, express high levels of IL-10 and low levels of IL-12 [39], and facilitate tumor progression [40]. Interestingly, plasm cytokines assay in our study showed the expression of IL-4, IL10, and IL-13 was significantly decreased in NSCLC patients compared to HC, whereas M2-like macrophages were obviously elevated. These evidences manifested that CD68⁺CD163⁺M2-like macrophages in peripheral blood were different from the alternatively activated M2 macrophages in issue or tumor microenvironment. Besides, although recent researches showed that the increasing MDSCs and M2 macrophages were markers for poor prognosis in cancer[41] due to promoting invasiveness [42] or tumor progression[43], MDSCs and CD68⁺CD163⁺M2-like macrophages in the present study only were provisionally elevated in NSCLC patients in RT. The frequencies of MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients in post-RT returned to the level of NSCLC patients in pre-RT, which could illustrate that the percentages of MDSCs and CD68⁺CD163⁺M2 macrophages were transiently increased in NSCLC patients in RT due to the inflammation reaction caused by ionizing radiation, and CD68⁺CD163⁺M2 macrophages could be

different from M2 or alternatively activated macrophages. However, whether the elevated MDSCs and CD68⁺CD163⁺M2-like macrophages promote tumor metastasis still needs a longer follow-up visit in NSCLC patients in radiotherapy, and also deserves further investigations. In this study, we found that the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages did not show clear difference in NSCLC patients with lung, brain and bone radiation, indicating that the alterations of the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages in RT were unrelated with radiotherapy area. In addition, we found that in radiotherapy, the proportions of MDSCs were markedly increased in non-small cell lung adenocarcinoma patients but not in non-small cell lung squamous carcinoma patients; however, the proportions of CD68⁺CD163⁺M2-like macrophages were markedly elevated in non-small cell lung squamous carcinoma patients but not in non-small cell lung adenocarcinoma patients. Hence, we predicted that in radiotherapy, alterations of the percentages of MDSCs are more sensitive in lung adenocarcinoma patients, while CD68⁺CD163⁺M2-like macrophages are more specific for lung squamous carcinoma patients. We also found that there were much more MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients without metastasis in RT and post-RT than in pre-RT. Moreover, the incremental range of NSCLC patients without metastasis was markedly wider than that in NSCLC patients with metastasis. Compared to NSCLC patients with metastasis, the percentages of MDSCs and M2-like was also decreased in NSCLC patients without metastasis in pre-RT. There were also more MSDCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients of PR group in RT than these patients of SD or PD group in RT. All data suggested that the elevated MDSCs and CD68⁺CD163⁺M2-like macrophages could provide a sensitive marker for NSCLC patients in radiotherapy.

C-reaction protein (CRP) is a pentameric protein synthesized by the liver, whose level rises in response to inflammation [44]. CRP has been determined as a prognostic factor in nasopharyngeal carcinoma [45] and colon cancer [46]. In the present study, we found that the levels of CRP were decrease in NSCLC patients in pre-RT and post-RT than these patients in RT, and the levels CRP of NSCLC patients in RT was positively correlated with CD68⁺CD163⁺M2-like macrophages. The increasing levels of CRP further indicated that ionizing radiation therapy induced the inflammation responses, which could be a referentially prognostic marker for NSCLC patients in radiotherapy. We also found that WBCs were hardly affected by ionizing radiation, but WBCs were negatively correlated with MDSCs in NSCLC patients in post-RT. Therefore, it is interesting to deeply investigate the relationship between WBCs and MDSCs in NSCLC patients after radiotherapy.

Eotaxin (CCL11) has been shown to promote the chemotaxis of eosinophils cells by binding to chemokine receptor 3 [47], but its effects on CD68⁺CD163⁺M2-like macrophages recruitment remain unknown. Our data indicated that Eotaxin was positively correlated with CD68⁺CD163⁺M2-like macrophages in NSCLC patients in post-RT, suggesting that the upregulated Eotaxin could emerge as a potential marker for the recruitment of CD68⁺CD163⁺M2-like macrophages in NSCLC patients after radiotherapy. MIP-1 β (CCL4), RANTES and the human cytokine I-309 are all monocyte and lymphocyte chemoattractant [48]. Our results showed that MIP-1 β was obviously elevated in NSCLC patients in post-RT compared with these patients in pre-RT, and there was more expression of RANTES and I-309 in

NSCLC patients in post-RT than HC group. In the present study, MDSCs and CD68⁺CD163⁺M2-like macrophages of PBMCs were detected by flow cytometry and were visibly raised in NSCLC patients. We found that MDSCs as well as CD68⁺CD163⁺M2-like macrophages were also correlated positively with MIP-1 β in NSCLC patients in RT. This data indicated that MIP-1 β could also recruit MDSCs and CD68⁺CD163⁺M2-like macrophages of NSCLC patients during radiotherapy, thereby playing a vital role in evaluating the inflammation environment of radiation therapy. In addition, we detected that the interleukin-1 receptor antagonist (IL-1ra) had a positive relationship with MDSCs and CD68⁺CD163⁺M2-like macrophages, but tissue inhibitor of metalloproteinases-2 (TIMP-2) and tumor necrosis factor receptor-I (TNF RI) were only correlated with MDSCs in NSCLC patients in RT. CD68⁺CD163⁺M2-like macrophages were negatively correlated with IL-12p40 but had a positive linear relation with IL-6. Some studies found that IL-1ra was a competitive inhibitor of IL-1(α/β) [49] and was neuroprotective in the mice that had a stroke [50]. IL-1ra could also suppress the growth of esophageal cancer cells by blocking IL-1 α [51]. In this article, IL-1ra could play a crucial role because it was clearly related with MDSCs and CD68⁺CD163⁺M2-like macrophages in NSCLC patients in RT, which deserved deep investigations in the following experiments.

In ionizing radiation treatment, the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages were clearly elevated in NSCLC patients. We speculated that the increase of MDSCs and CD68⁺CD163⁺M2-like macrophages could be a reactive mechanism that suppressed the inflammation response caused by ionizing radiation. However, we still need to build an animal model and increase the number of NSCLC patients for further investigations, and if necessary, we will also explore the molecular mechanism in detail.

Conclusion

In conclusion, compared to HC, the percentages of MDSCs and CD68⁺CD163⁺M2-like macrophages were significantly increased in NSCLC patients in RT, suggesting that radiotherapy upregulated the expression of peripheral MDSCs and CD68⁺CD163⁺M2-like macrophages. The increase of circulating MDSCs and CD68⁺CD163⁺M2-like macrophages was found to be correlated with partial responses, and MDSCs were positively correlated with CD68⁺CD163⁺M2-like macrophages in NSCLC patients in RT, indicating that it could provide more sensitive biomarkers, and predict a better prognosis of NSCLC patients in radiotherapy.

Abbreviations

NSCLC: non-small cell lung cancer; MDSCs: myeloid-derived suppressor cells; DCs: dendritic cells; pre-RT: pre-radiotherapy; RT: radiotherapy; post-RT: post-radiotherapy; CR: complete response; PR: partial response; PD: progressive disease; SD: stable disease; HC: healthy control; IR: ionizing radiation; CRP: C-reaction protein; WBCs: White blood cells; APCs: antigen presenting cells; IL-1ra: interleukin-1 receptor antagonist; TIMP-2: tissue inhibitor of metalloproteinases-2; TNF RI: tumor necrosis factor receptor-I.

Declarations

Acknowledgments

Not applicable.

Author contribution

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, visualization, writing—original draft preparation, writing—review and editing, Minghe LV; Writing—review and editing, funding acquisition, Xibing Zhuang; Methodology, Shali Shao; investigation, Xuan Li; Supervision, funding acquisition, Yunfeng Cheng; Supervision, Duoqiao Wu; Supervision, Xiangdong Wang; Conceptualization, funding acquisition, project administration, funding acquisition, writing—review and editing, Tiankui Qiao. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Shanghai Health Commission (Grant Number: ZK2019B30) and the Jinshan Science and Technology Commission (Grant Number: 2018-3-5).

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript.

Ethics approval

All experiments involving human were performed with the permission and under the strict guidance of Ethic Committee of Jinshan Hospital affiliated to Fudan University (Shanghai, China). Written informed consent was obtained from all patients and data was analyzed anonymously.

Consent for publication

All authors have given their consent for the publication of this article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 Clinical Characteristic of NSCLC patients

Characteristic	All of patients	
	No.	%
Gender		
Male	6	37.5
Female	10	62.5
Ages, years median (range)	72 (44, 83)	-
Histology		
Adenocarcinoma	11	69
Squamous	5	31
Stage		
III a/b	4	25
IV	12	75
Radiotherapy area		
Lung	7	43.75
Brain	5	31.25
Bone	4	25
Response to radiotherapy		
PR	6	37.5
SD	8	50
PD	2	12.5

Figures

Figure 1

Peripheral MDSCs and CD68⁺CD163⁺M2-like macrophages in HC and NSCLC patients. (A) Representative dot plots and peak plots of CD11b⁺CD33⁺HLA-DR⁻ MDSCs in HC and NSCLC patients. (B) Representative dot plots of CD68⁺CD163⁺M2-like macrophages in HC and NSCLC patients. (C) The percentages of peripheral MDSCs were significantly elevated in NSCLC patients. (D) The percentages of peripheral CD68⁺CD163⁺M2-like macrophages were distinctly increased in NSCLC patients. *p<0.05,

p<0.01, *p < 0.001, compared to HC in two-tailed Student's non-paired t test. #p<0.05. The bars denote mean values $\pm$ SEM.

Figure 2

The correlation between peripheral MDSCs and CD68+CD163+M2-like macrophages in HC and NSCLC patients. (A) The relationship between peripheral MDSCs and CD68+CD163+M2-like macrophages in HC. The correlation between MDSCs and CD68+CD163+M2-like macrophages in NSCLC patients in pre-RT (B), RT (C), and post-RT (D). Data was analyzed by Pearson's correlation test.

Figure 3

The percentages of MDSCs and CD68+CD163+M2-like macrophages of NSCLC patients in various clinical characteristics. (A) The proportions of MDSCs in NSCLC patients with or without metastasis. (B) The percentages of CD68+CD163+M2-like macrophages in NSCLC patients with or without metastasis. (C) The percentages of MDSCs in NSCLC patients with different radiotherapy area. (D) The percentages of CD68+CD163+M2-like macrophages in NSCLC patients with different radiotherapy area. (E) The proportions of MDSCs in NSCLC patients with diverse pathologic types. (F) The proportions of CD68+CD163+M2-like macrophages in NSCLC patients with diverse pathologic types. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to HC group in two-tailed Student's non-paired t test. # $p < 0.05$. The bars denote mean values $\pm$ SEM.

Figure 4

The percentages of MDSCs and CD68+CD163+M2-like macrophages of NSCLC patients in PR, SD, and PD group. (A) The percentages of MDSCs of PR, SD, and PD group in pre-RT, RT, and post-RT. (B) The frequencies of CD68+CD163+M2-like macrophages of PR, SD, and PD group in pre-RT, RT, and post-RT. (C) The correlation between MDSCs and CD68+CD163+M2-like macrophages of NSCLC patients of PR group in pre-RT, RT, and post-RT. (D) The relationship between MDSCs and CD68+CD163+M2-like macrophages of NSCLC patients of SD group in pre-RT, RT, and post-RT. * $p<0.05$, ** $p<0.01$, compared to HC group in two-tailed Student's non-paired t test. Data was analyzed by Pearson's correlation test. The bars denote mean values $\pm$ SEM.

Figure 5

The crossed correlation analysis of CRP and WBCs with MDSCs and CD68+CD163+ M2-like macrophages in NSCLC patients. (A) The bar graph of CRP concentration in NSCLC patients in pre-RT, RT, and post-RT. (B) The histogram of WBCs concentration in NSCLC patients in pre-RT, RT, and post-RT. (C) The correlation between CRP and MDSCs of NSCLC patients in pre-RT, RT, and post-RT. (D) The correlation between CRP and CD68+CD163+ M2-like macrophages of NSCLC patients in pre-RT, RT, and post-RT. (E)

The correlation between WBCs and MDSCs of NSCLC patients in pre-RT, RT, and post-RT. (F) The correlation between WBCs and CD68+CD163+M2-like macrophages of NSCLC patients in pre-RT, RT, and post-RT. Data was analyzed by Pearson's correlation test.

Figure 6

The expression of plasma cytokines in HC and NSCLC patients. (A) 8 kinds of the plasma cytokines were significantly increased in NSCLC patients. (B) 17 kinds of the plasma cytokines were significantly

decreased in NSCLC patients. (C) The changes of 15 kinds of the plasma cytokines were not significant (NS) in NSCLC patients compared to HC. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, compared to HC group. # $p < 0.05$, ## $p < 0.01$. The bars denote mean values $\pm$ SEM.

Figure 7

The correlation between plasma cytokines and MDSCs as well as CD68+CD163+M2-like macrophages of NSCLC patients in RT. (A) MDSCs were correlated with IL-1ra, MIP-1 β , TIMP-2, IL-11 and TNF RI in NSCLC patients in RT. (B) CD68+CD163+M2-like macrophages were correlated with IL-1ra, MIP-1 β , TIMP-1, IL-6 and IL-12p40 in NSCLC patients in RT. Data was analyzed by Pearson's correlation test.

Figure 8

The correlation between the plasma cytokines and MDSCs as well as CD68+CD163+M2-like macrophages in NSCLC patients in post-RT. (A) The correlation between MDSCs and IL-1d of NSCLC patients in post-RT. (B, C and D) CD68+CD163+M2-like macrophages were correlated with Eotaxin, TIMP-2, and TNF RII of NSCLC patients in post-RT. Data was analyzed by Pearson's correlation test.