Peer Review File

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Reviewer A

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer deaths. In the manuscript "An inter-correlation among chemokine (C-X-C motif) ligand (CXCL) 1, CXCL2 and CXCL8, and their diversified potential as biomarkers for tumor features and survival profiles in non-small-cell lung cancer patients", authors explored the interaction among chemokine (C-X-C motif) ligand (CXCL) 1/2/8 expressions, and their associations with clinicopathologic features and survival profiles in non-small-cell lung cancer (NSCLC) patients.

Couple questions are required to be answered before it will be accepted.

(1) There was a similar report (Anticancer Res. 2019 Dec;39(12):6645-6652) about CXCL1, CXCL2, and CXCL8 as biomarkers for gastric cancer in the PubMed. What is the novel idea in the PubMed? Please elaborate in the introduction.

Reply 1: Thank you for your comments. This previous report [Anticancer Res. 2019 Dec;39(12):6645-6652] explores the clinicopathological significance of CXCR2 ligands (including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8) in gastric cancer patients, and reveals that among the CXCR2 ligands, CXCL7 and CXCL1 server as important roles in the malignant progression of gastric cancer by regulating CXCR2 signaling. Whereas little is known about the clinical implication of CXCL1, 2, 8 in NSCLC patients. Hence, to solve this problem, we conducted this study with aim to explore the interaction among CXCL1, 2, 8 expressions, and their associations with clinical characteristics and prognosis in NSCLC patients. As your suggestion, we have added the relevant detailed explanations in the **Introduction Section**. The corrected parts were highlighted in red color. Thanks again for your suggestions.

Changes in the text: Page 5, line 72-88.

(2) Chemokines were the key point of the research. Please supplement the roles of CXCL1, 2 and 8 in lung cancer in the introduction. How about the association between CXCL1, 2, 8 and microenvironment of lung cancer? Please supplement the illustration in the introduction.

Reply 2: Thank you for your suggestion. As your suggestion, we have supplemented the roles of CXCL1, 2 and 8 in lung cancer in the **Introduction Section** as follows: "Regarding lung cancer, several previous studies have shown that CXCL1, CXCL2 and CXCL8 participant in the microenvironment of lung cancer through participating in multiple mechanisms (including recruitment of tumor-associated neutrophils, involving in anlotinib resistance, or affecting tumor cell proliferation and

angiogenesis).". The corrected parts were highlighted in red color. Thank again for your suggestion.

Changes in the text: Page 5, line 72-76.

(3) The figure 1 was not clearly enough. Please replace it with a new. And scale bar and more magnification of images were also needed to be added in the figure 1.

Reply 3: Thank you for your comments. As your suggestion, we have replaced Figure 1, and added the scale bar and more magnification of images in **Figure 1 (revised)**. Thanks again for your suggestion.

Changes in the text: Figure 1 (revised)

(4) Since there were CXCL1, 2, 8 antibodies and NSCLC tissues, it is more convincing to test the expressions of CXCL1, 2, 8 by Western blot.

Reply 4: Thank you for your comments. In this study, we used the formalin-fixed and paraffin-embedded tumor tissue specimens obtained from the storeroom of pathology department, but not fresh tissues. Besides, if the expressions of CXCL1, 2, 8 were to be detected by Western blot, it was not easy to distinguish whether CXCL1, 2, 8 were expressed in tumor cells or in other cells. Instead, the expressions of CXCL1, 2, 8 could be shown in different cells by IHC assay. Therefore, we selected IHC to present the expressions of CXCL1, 2, 8 in this study.

Changes in the text: None

(5) Please supplement the measure method of CEA in the methods.

Reply 5: Thank you for your comments. As your suggestion, we have added the measure method of CEA in the **Methods Section** as follows: "Preoperative carcinoembryonic antigen (CEA) level in the serum of patients was also collected, which was determined by electrochemiluminescence immunoassay (ECLIA) using RocheElecsys601 automatic immune analyzer (Roche diagnostics, Mannheim, Germany) and corresponding test kits.". The corrected parts were highlighted in red color. Thanks again for your suggestion.

Changes in the text: Page 6, line 109-113.

(6) It is necessary to validate the effect of CXCL1, 2, 8 on NSCLC cell by in vitro and in vivo experiments.

Reply 6: Thank you for your comments. In this study, we aimed to explore the interaction among CXCL1, 2, 8 expressions, and their associations with clinical characteristics as well as prognosis in NSCLC patients, but not the effect of CXCL1, 2, 8 on NSCLC cells, thus, in vitro and in vivo experiments were not the main point in

this study. Considering your good suggestion, we have added the relevant information in the **Limitation Parts** of **Discussion Section** as follows: "Furthermore, although the clinical implication of CXCL1, CXCL2 and CXCL8 in NSCLC patients was explored, their detailed mechanism underlying NSCLC pathology was not investigated in this study, further in vitro and in vivo experiments were needed". The corrected parts were highlighted in red color. Thanks again for your suggestion.

Changes in the text: Page 15, line 304-309.

(7) The expressions of CXCL1, 2, 8 were upregulated in half of NSCLC tissues, and downregulated in half of NSCLC tissues in the research. It is hard to understand them could be as predictive factors for NSCLC. Please supplement in the discussion.

Reply 7: Thank you for your comments. (1) For the common cancer biomarkers, their prognostic values are usually not correlated with their expressions (high or low) in tumor. For instance, CEA and CA199 are common blood indicators in lung cancer patients, whose expressions are dysregulated in only half of the lung cancer patients, while for the rest of patients, their expressions are normal. However, CEA and CA199 are still commonly used prognostic markers in lung cancer. Hence, the expressions (high or low) of biomarkers in tumor tissues are not correlated with their values as prognostic factors. Actually, the original intention of this study was to detect expressions of CXCL 1, 2, 8, and explore their associations with clinical features in NSCLC patients. Since most patients completed follow-up, we then further explored the prognostic values of CXCL 1, 2, 8. The prognostic values of CXCL 1, 2, 8 in NSCLC might be explained by their mechanisms in tumor microenvironment.

(2) There are several assessment criteria for the expressions of CXCL 1, 2, 8 (IHC score<3 or IHC score≤3 or IHC score<1 or IHC score≤1 have been used to distinguish low expression), and we used one of them (based on the density and concentration of CXCL1, 2, 8 positive cells to calculate the IHC scores, and the IHC score>3 was defined as high expression, while the IHC score≤3 was defined as low expression). If we use IHC score=1 as threshold, we will have a high positive rate of CXCL1, 2, 8 high expression. However, considering the statistical power, we used IHC score=3 as threshold because there is not much difference between the CXCL1, 2, 8 high expression and low expression proportion.

Changes in the text: None

(8) Chemokines play an important role in microenvironment of lung cancer. So it is suggested to test the association between CXCL1, 2, 8 and inflammatory factors and lymphocytes.

Reply 8: Thank you for your comments. (1) In this study, the association between CXCL1, 2, 8 and inflammatory factors was not the main purpose. If we explore the

association between CXCL1, 2, 8 and inflammatory factors, archived tissue specimens were needed to detect several inflammatory factors by additional IHC assays. Owning to the limited funding, no budget was left for the additional assays. However, considering your good suggestion, we have added the relevant information in the **Limitation Part** of **Discussion Section**.

(2) Regarding lymphocytes, we reviewed lymphocytes data from the medicine records, then explored the correlation of CXCL1, 2, 8 with lymphocyte count, and we found no correlation of CXCL1 (P=0.963), CXCL2 (P=0.209), CXCL8 (P=0.434) with lymphocyte count in NSCLC patients. The possible explanation might be that lymphocytes counts in blood sample reflect the whole body conditions, thus, their associations with tumor CXCL1, 2, 8 might be weak. In addition, we have added the relevant information in the **Results Section** and **Supplementary Table 1 (revised)**. The corrected parts were highlighted in red color. Thanks again for your suggestions.

Changes in the text: Page 10, line 183-188; Page 15, line 304-309;

(9) Please supplement the action mechanisms of CXCL1, 2, 8 in the microenvironment of NSCLC in the discussion.

Reply 9: Thank you for your comments. As your suggestion, we have added the action mechanisms of CXCL1, 2, 8 in the microenvironment of NSCLC in the **Discussion Section** as follows: “In lung cancer, CXCL1 has been reported to interact with miR141-CXCR2 pathway regulates migration of regulatory T cells (Treg) into malignant pleural effusion (MPE). CXCL2 is discovered to obviously offset anlotinib-induced cell migration inhibition and promote invasion of anlotinib-treated cells in NSCLC, indicated that CXCL2 is involved in the resistance in anlotinib resistant NSCLC cells. CXCL8/Interleukin-8 analogue CXCL8(3-72) K11R/G31P interacts with CXCR1/2 to surpress tumor cell proliferation and inhibit angiogenesis, thereby restricting lung cancer growth.”. The corrected parts were highlighted in red color. Thanks again for your suggestion.

Changes in the text: Page 12, line 230-237.

Reviewer B

Great paper, hold a great perspective for the future.

Reply: Thank you for your comments. Thanks again for your energy and time.