Peer Review File

<mark>Reviewer A</mark>

Non-small cell lung cancer (NSCLC) accounts for 80-85% of lung cancer, which is the leading cause of cancer-related deaths worldwide. α3β1 integrin is a promising cancer biomarker and drug target in NSCLC. In the manuscript "High integrin α3 expression is associated with poor prognosis in patients with non-small cell lung cancer", authors determined the expression pattern and prognosis of INTA3 expression by immunohistochemistry (IHC) using commercially available antibodies on archived NSCLC tumors.

Couple questions are required to be answered before accepted.

Q1. There are many reports, such as Frontiers in oncology (PMID: 32117712), Cancer science (PMID: 23786209) and British journal of cancer (PMID: 23652300), about integrin α 3 and tumor invasion, metastasis, poor prognosis in other cancer. Please point out the novelty of this study or the difference between this study and published papers in the introduction.

Response: We appreciate the Reviewer pointed out this important question. Almost all previous studies were done on preclinical models and the clinical study was limited by lack of suitable antibodies for integrins. We have revised the following sentences, emphasizing the novelty of our current study. We have also added these three references suggested by the Reviewers in the Introduction as below.

Ref 13, Cancer science (PMID: 23786209); Ref 14, Frontiers in oncology (PMID: 32117712); and Ref 15, British journal of cancer (PMID: 23652300).

"Overexpression of α3β1 integrin has been detected in multiple tumor types and is associated with tumorigenesis, invasion, metastasis, as well as resistance to cancer treatment in several cancer types, including NSCLC (4, 12) , breast cancer (13), cervical cancer (14), glioma (15), and other cancer type metastasis to the lung (16, 17). Almost all these studies were done on preclinical models. Due to the poor sensitivity and specificity of early commercially available antibodies, limited study has been reported on the clinical significance of ITGA3 expression

using archival formalin fixed, paraffin embedded (FFPE) patient samples (14). Furthermore, there are conflicting data on the prognosis of ITGA3 expression in NSCLC (12, 18)."

Q2. Since authors have previously showed that α3β1 integrin is a novel cancer biomarker and drug target in non-small cell lung cancer (NSCLC), regardless of histology and tumor genotype. There are also many reports on the relationship between α 3 β 1 integrin and non-small cell lung cancer (Journal of hematology & oncology, PMID: 31182116; Cancer science, PMID: 14965364). What is the novel idea in the paper? Please illustrate detailed in the introduction.

Response: Again, we appreciate the Reviewer pointed out this important question. Our and others' previous studies ((Journal of hematology & oncology, PMID: 31182116; Cancer science, PMID: 14965364) mainly used in vitro and in vivo human cancer models. FFPE specimens are generally used in the clinical pathology laboratory as the fixing processing steps preserve the morphology of tumor and normal cells and the samples could be stored in stable condition for many years. However, the fixing processing steps usually change the configuration and number of antigens on tumor cells due to protein degradation. The antibody or peptide ligand LXY30 binds to live or frozen tumor cells but not tumor cells but not FFPE tumor specimens. The novelty of the current study is to establish an IHC assay and to determine the expression pattern and prognosis of INTA3 expression using archived NSCLC tumors. In addition to the addition in Q1, we also have a paragraph on the novelty and translational potential of our study in the Discussion:

"Our study has several translational potentials. First, …. The INTA3 IHC can be used to identify candidate NSCLC patients using archived FFEP tissue specimens for targeted therapy although the caution should be excised to select the primary antibody for INTA3."

Q3. What is the advantage of integrin α3 as a biomarker for NSCLC, compared to other biomarkers?

Response: As we stated at the beginning of Discussion: "α3β1 integrin is a promising biomarker for lung cancer detection and a potential drug target, being one of the most commonly expressed integrin subtypes found on tumor cells mediating metastasis and treatment resistance (6). Using a novel peptide ligand LXY30 that has higher affinity and longer half-life than natural integrin (4, 16), we recently showed that INTA3 was expressed in about 90% of live tumor cells and exosomes isolated from patients with metastatic NSCLC (4)." The result of current study also supports our effort in targeting INTA3 using LXY30 in patients with live NSCLC. I hope the Reviewers could agree this explains why we focus on

integrin α3 as a novel biomarker for NSCLC. Although there has been signifivant treatment advances in NSCLC in the past few decades, the prognosis for patients with metastatic NSCLC remains poor.

Q4. Scale bar is needed to be added in the Figure 2A. In the Figure 2B, the horizontal ordinate was partly covered.

Response: We have added a scar bar in Fig 2.

Q5. It is suggested to increase the results of in vitro study to confirm the relationship between integrin α 3 and malignant biological behavior of lung cancer cells.

Response: We agree with the Reviewers that there have been many reports on the relationship between α 3 β 1 integrin and NSCLC and other cancer types as we cited in the Introduction. In our previous study (Journal of hematology & oncology, PMID: 31182116), we have shown LXY30 alone or in combination with another integrin ligand LXW64 could modulate the activity of EGFR, the first molecular target in NSCLC. The objective of the current study was to establish an IHC assay and determine the expression pattern and prognosis of INTA3 expression on archived FFPE specimens. In our future study on targeting α3β1 integrin in NSCLC, we will further delineate the relationship between integrin α 3 and malignant biological behavior of lung cancer cells.

Q6. It is suggested that EMT, vascularization, invasion and migration should be detected in patients. It may provide the correlation between integrin α 3 and EMT, vascularization, invasion and migration.

Response: We appreciate the Reviewer pointed out this important issue. We have analyzed the TCGA databases and found that ITGA3 interacted with many key genes regulating epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and LUSC. We summarized this data in a new **Figure 7**. We also added this point to the Abstract.

Q7. In figure 3, it is suggested that Figure 3B, C, D should be made in the same style as figure 3A, that is, the number of cases should be supplemented below the horizontal ordinate, which is not only conducive to the consistency of picture results, but also to the readers' understanding of the survival of cases.

Response: We have revised the figures and use the X-axis of 216 months.

Q8. It is recommended to provide pictures of immunohistochemical staining results of integrin α 3 in histology subgroups of NSCLC.

Response: We have added the representative INTA3 IHC stains on LUAC and LUSC in **Figure 4D**. On page 11, we added "Representative INTA3 IHC stains on LUAC and LUSC are showed (**Figure 4D**)." to reflex this addition.

Q9. Figure 6A and B indicated that INTA3 shared many interactive genes mediating cell adhesion and motility in both LUSC and LUAD. However, the gene names in these two network maps are not clear, so it is recommended to present them in a table form, so it will be clearer.

Response: We have added a new Table 6 to summarize these genes and their key biological functions.

Q10. Since integrin α 3 expression is related to poor prognosis of lung cancer patients, it is suggested to increase the possible mechanism of integrin α 3 affecting the prognosis of patients, such as the relationship between integrin α 3 and EMT, angiogenesis, invasion and metastasis in the discussion.

Response: We appreciate the Reviewer's suggestion and have added the analysis of ITGA3 significantly interacted with many key genes regulating epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and LUSC in Figure 7 in the Result section. We also added the following sentences in the Discussion:

"Analysis of TCGA databases showing that ITGA3 significantly interacted with many key genes regulating cell adhesion, motility, epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and LUSC (**Table 6** and **Figure 7**). Further study is warranted to delineate the the mechanism by which ITGA3 may mediate these malignant processes, and to develop therapeutic strategies for NSCLC patients."