Peer Review File

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Reviewer A

Comment: I found that the text was generally well-organized and readable. It is sufficiently informative to allow consideration for publication. I would add that the content is quite « dense » in that there are a lot of receptors and pathways that are being discussed. In my opinion it would be helpful to include a figure that illustrates the major pathways under discussion, and also highlights the novel pathways/ targets that have been identified by Tan and colleagues.

Response: We appreciated this suggestion very much. In the new version of the manuscript, we included a Transmission Electron Microscopy (**Figure 2**) illustrating and simulating the modifications in organelles, such as Golgi apparatus and lysosomes, as well as the basement membrane, in a case of human lung adenocarcinoma. We also composed a Figure 1 to illustrate de PIK pathway and related pathways. Thank you for the suggestion.

Reviewer B

This review paper focuses on the role of ZEB1 and PI4K2A in the epithelial-mesenchymal transition (EMT) pathway in non-small cell lung cancer (NSCLC). It examines how these factors contribute to metastasis and tumor progression. Although the paper touches upon the potential of targeting PI4K2A for treatment, it seems to be predominantly a summary of findings from Tan et al. and could benefit from a broader citation range and the provision of new insights, unique perspectives, or hypotheses.

Response: We totally agree with the Reviewer. In the new version of the manuscript, we addressed these points raised by the Reviewer. We appreciate the suggestion which improved considerably the scientific rigor of the manuscript. We also took the liberty to paraphrase the points raised by the Reviewer. Thank you.

Particularly, the following points could be considered for discussion:

Comment 1: On Metastasis Prevention and Cancer Cure: While preventing metastasis is essential for improving survival, fundamentally curing lung cancer seems more crucial. Does PI4K2A only play a role in metastasis, or is it involved in the primary development of lung cancer as well? It's essential to explore whether inhibiting PI4K2A is crucial only for metastasis or if it also has implications for the primary tumor.

Response: Thank you for the suggestion.

Page 3, line 93-109:

Regarding metastasis prevention and cancer cure, while preventing metastasis is essential for improving survival, essentially curing lung cancer should be more critical. Does PI4K2A only play a role in metastasis, or is it involved in the primary development of lung cancer as well? **Figure 1** illustrates the phosphatidylinositol 3-kinase (PI3K)–Akt pathway, involved in carcinogenesis. After activation by receptor tyrosine kinases or RAS, PI3K phosphorylates phosphatidylinositol 4,5-trisphosphate (PIP2) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3), which triggers Akt and 3-phosphoinositide-dependent protein kinase (PDK). Akt inhibits glycogen synthesis kinase 3 (GSK3) which stabilizes cyclin D1, inhibits p27, promoting cell-cycle progression. By inhibiting the Bcl2-antagonist of cell death, Akt enhances cell survival. Furthermore, Akt also controls protein synthesis and cell growth by phosphorylation of mammalian targets of rapamycin (mTOR), promoting translation of mRNA to synthesize protein for cell growth (16). Note that *PI4K2A* not only plays a role in metastasis but is also involved in the primary development of lung cancer as well (16). Therefore, inhibiting *PI4K2A* is crucial for metastasis and primary tumor control. As previously reported phosphatidylinositol 3-kinase inhibitor (LY294002) induces apoptosis of cancer cells *in vivo* and *in vitro* (17).

Comment 2: PI4K2A Expression Independent of NSCLC Driver Gene Mutations: Is the expression of PI4K2A independent of known NSCLC driver gene mutations such as KRAS, EGFR, ALK, BRAF, and RET? Understanding the relationship or independence of PI4K2A from these mutations can deepen our insights into NSCLC pathogenesis.

Response: Thank you for your comment. Please, see below the modified text as advised.

Page 4, lines 110-128:

Is the expression of *PI4K2A* independent of known NSCLC driver gene mutations such as

KRAS and EGFR? Dual inhibition of EGFR at protein and activity level via blocking of *PI4KIIα* have an anti-tumor effect and promote the handling of misfolded proteins to the lysosome, thus preserving malignant cell survival (15). Some preclinical models emphasize the potential for targeting *PI4KA* and *PI4KB* in cancer (18-20)). *PI4KA* generates the PI4P pool at the plasma membrane, which is phosphorylated into PIP2, and subsequently phosphorylated into the pro-development signal PIP3. In human cancer, the most frequently mutated genes HRAS, NRAS, and KRAS, activated the class IA PI3K p110α to generate PIP3 essential in Ras-driven tumorigenesis (21). Through the interaction between the PI4KA regulatory protein EFR3A and KRAS, an interruption of either PI4KA or EFR3A lead to decreased PI4P, PS, and KRAS levels at the cytoplasmic membrane, with a simultaneous decrease in oncogenic signaling and tumorigenesis (18). When mutant KRAS pancreatic cell cultures were treated with a combination of a G12C-specific Ras inhibitor (sotorasib) and 2-aminobenzothiazole, PI4KA inhibitor had a synergistic inhibitory effect on cancer cell growth. In experimental pancreatic tumors, PI4KA and EFR3A were upregulated compared to normal tissue (19). According to this result, we inferred that there is a beneficial, restricted therapeutic window of PI4KA inhibition in mutant KRAS-driven cancers when used in combination with either PI3K or KRAS inhibitors since careful analysis of toxicity will be essential.

Comment 3: Combination with Current Targeted Therapies: How should treatments targeting PI4K2A be utilized with existing molecular-targeted NSCLC therapy? The synergistic potential or interaction of PI4K2A inhibitors with current therapies needs exploration.

Response: Please, see the response to query 2. Thank you.

Comment 4: miR-200 Dependent EMT in Molecular Targeted Therapy Resistance: The role of miR-200 dependent EMT as a mechanism for resistance to molecular targeted therapy in NSCLC is well-known (Cancer Sci. 2020 Jul;111(7):2374-2384.Cancer Res. 2019 Apr 1;79(7):1658-1670.). Could inhibiting EMT enhance the effectiveness of these therapies?

Response: Thank you for your comment. Please, see below the modified text as advised.

Page 4, lines 129-139:

The next question is related to miR-200-dependent EMT in molecular targeted therapy resistance. Preclinical experiments with osimertinib-resistant lung cancer cells showed that EMT was

associated with decreased miR-200 and increased *ZEB1* expression. The pre-treatment of resistant clone cells with a histone deacetylase inhibitor helped minimize the resistance by reverting EMT (22). Another study in preclinical models with crizotinib-resistant lung cancer cells showed that EMT associated with decreased expression of miR-200c and increased expression of ZEB1 caused cross-resistance to new-generation ALK inhibitors alectinib, ceritinib, and lorlatinib. The pretreatment with the histone deacetylase inhibitor quisinostat inactivated this resistance by reverting EMT *in vitro* and *in vivo* (22). These studies indicate that inhibiting EMT enhance the effectiveness of targeted therapy.

Comment 5: AXL as an Initial Resistance Factor: AXL has been reported as an initial resistance factor in molecular targeted therapy (Nat Commun. 2020 Sep 14;11(1):4607). Could targeting the degradation of AXL overcome initial resistance to these therapies?

Response: Thank you for your comment. Please, see below the modified text as advised.

Page 5, lines 140-149

AXL has been reported as an initial resistance factor in molecular targeted therapy. A recent study showed that while AXL-low expressing EGFR mutated lung cancer (EGFRmut-LC) cells are more sensitive to osimertinib than AXL-high expressing EGFRmut-LC cells, a small population emerge osimertinib tolerance. In AXL-low-expressing EGFRmut-LC cell-derived xenograft and patient-derived xenograft models, transient insulin-like growth factor-1 receptor (IGF-1R) inhibition combined with continuous osimertinib treatment could eradicate tumors and prevent regrowth even after the cessation of osimertinib. These results indicate that optimal inhibition of tolerant signals combined with osimertinib may dramatically improve the outcome of EGFRmut-LC (23).

Comment 6. It would be better to add a summary figure.

Response: We totally agree with the Reviewer. In the new version of the Editorial we included two illustrative Figures.

These points aim to deepen the analysis in the review, proposing critical considerations for future research directions and therapeutic strategies in NSCLC.