Author’s response to reviews

Title: Gli promotes tumor progression through regulating epithelial-mesenchymal transition in non–small-cell lung cancer

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Author’s response to reviews:

Dear Prof. Vipin Zamvar:

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We appreciate the editor and reviewers greatly for their positive and constructive comments and suggestions on our manuscript entitled "Gli promotes tumor progression through regulating EMT in non–small-cell lung cancer" (JCTS-D-19-00282).

We have studied reviewer’s comments carefully and have made relevant revisions which were marked in red in the paper. We have tried our best to improve our manuscript according to the comments. Please find the revised version in the attachment, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for the comments on our paper. Looking forward to hearing from you.

Thank you very much!

Yours sincerely,

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List of Responses

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled "Gli promotes tumor progression through regulating EMT in non–small-cell lung cancer" (JCTS-D-19-00282). Those comments are all valuable and very helpful for improving our paper. We have studied the comments carefully and have made relevant revisions. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewers’ comments are as follows:

Responds to the reviewer’s comments:

Reviewer #1

1. Response to comment: For the in vivo analysis, the authors describe the percentage of positiveness for each marker without exploring the possible correlation between variables analyzed. For example, the authors indicated that 61.1% (22/36) of tumor samples add Gli1 overexpression over normal counterpart, while 77.8% (28/36) had a similar trend for NCAD. Is there a positive correlation between NCAD and Gli expression in these samples? the same for the other markers analyzed. Adding this information will allow a more stringent correlation between gli expression and EMT markers.

Response: It is really true as reviewer commented. We have added relevant analysis involving correlation as “Subsequent correlation analyses between Gli1 and EMT or AKT pathway markers showed positive correlation as 0.7774 (p < 0.001) of Gli1 and N-cadherin, 0.6701 (p < 0.001) of Gli1 and Vimentin, 0.7237 (p < 0.001) of Gli1 and p-AKT, respectively.” and marked red in the text.

2. Response to comment: The authors use siRNA against Gli1 and 2 showing changes in the EMT markers profile in two different NSCLC cell lines. A recent work by Manzotti et al (Clinical Cancer Research) showed that H1299 cell line has a more mesenchymal-like phenotype as compared with H1975 used in this manuscript. This cell line, being gone further in the EMT process, may hence represent a more reliable cell model to test Gli effect on EMT. The authors may also want to discuss this work in their discussion.
Response: We really appreciated the reviewer’s remind. We have added relative discussion as “Considering cell lines used in the current study, some reports have suggested that H1299 cell line might be a more suitable tool in EMT research. However, H1975, which was selected in the current study, has also been widely used in many other studies.” in the Discussion section and marked red.

3. Response to comment: The authors posted background information in the introduction section to introduce the rationale of their work. However, some contents are suggested to be placed in the discussion section for making the first part of your article succinct.

Response: Considering the Reviewer’s suggestion, we have moved some contents in the Background section to the discussion section as “Because of the advanced lung cancer presented at the time of diagnosis, few if any available interventional options for effective therapies were left [3]. Therefore, tumor invasion and metastasis constitute critical steps in the pathogeneses of lung cancer, the understanding of which may provide novel molecular insight in tumor progressive and metastatic mechanisms and ultimately yield novel molecular-targeted therapeutic strategies [4].”, “While recognized as critical factors in biological development, SHH signal pathway has rapidly underwent intense study in oncology [8]. Significantly, interaction between tumor cells and the tumor microenvironment was mediated by Hh signaling, which might result in proliferation and metastasis in multiple tumor types [9]. In other words, the SHH pathway is implicated in several types of cancers, such as basal cell carcinomas, medulloblastomas, gliomas, sarcomas, and pancreatic carcinomas. Although many cancers activate the pathway either by activation of smoothened or inactivation of patched, some tumors involve in SHH signaling by increasing the expression levels of GLI, such as rhabdomyosarcoma, osteosarcoma, glioma, breast cancer, pericytoma, prostate cancer, and Ewing sarcoma family of tumors. Therefore, GLI would be a potential beneficial therapeutic target for a wide spectrum of cancer patients.

EMT-inducing transcription factors, such as Twist1, Snail1, ZEB and Six1, have been proved to be associated with metastasis by experiments involving loss-of-function and gain-of-function about EMT [16]. For example, E-cadherin exhibits the ability of promoting cell adhesion and preventing tumor invasion and metastasis [17]. Moreover, Twist1 has been proved the role of enhancing metastasis in many types of cancers [18]. These data demonstrated that induction of EMT may induce tumor metastatic progression in vivo. In response to EMT-inducing pathway, the expression level of epithelial cell markers is down-regulated in epithelial cells. By contrast, the mesenchymal markers are up regulated. Pivotal functional processes, such as cell proliferation, migration and invasion, could be regulated by EMT-inducing transcription factors [17]. Moreover, EMT-inducing transcription factors are downregulated post embryogenesis, but re-expressed when carcinogenesis, which would result in increased tumor initiation and enhanced metastasis. In tumor cells, Twist1 and Snail1 could repress E-Cadherin and upregulate mesenchymal genes [4].”, “During carcinogenesis, progression and metastasis, EMT act together with other key signaling pathways, such as Wnt, SHH, and so on [10]. Till now, there is no direct evidence linking SHH networks to lung cancer in the aspect of EMT. Furthermore, Shh signaling has been found to be essential for lung cancer onset and progression [11]. Consistently, Twist1 is intimately associated with Hh/GLI signaling pathway during normal and disease-related
procedures [19]. Recently, Twist1 and Snail1 were proved to take part in the Hh signaling in tumor-initiating cells [20]. EMT transcription factors Twist1, Snail1 and Six1 would influence carcinoma cells by inducing EMT characteristics and aggressive properties [21]. EMT transcription factors activate GLI by employing different mechanisms, including Hh ligand depended signaling [22].” and marked red.

4. Response to comment: I would suggest using "epithelial-mesenchymal transition" instead of the abbreviation "EMT" directly in the title.

Response: As Reviewer suggested, we have changed the title as “Gli promotes tumor progression through regulating epithelial-mesenchymal transition in non–small-cell lung cancer” and marked red.

5. Response to comment: It would be better to mention GANT61 as the selective inhibitor of Gli transcription in the Hedgehog pathway in the first place.

Response: As the Reviewer suggested, we have added relative information as “GANT61, the selective inhibitor of Gli transcription in the Hedgehog pathway, was commercially purchased (Selleckchem, Munich, Germany).” and marked red.

6. Response to comment: Forty references were cited at the end of the discussion, however, only 30 of them were listed in the manuscript. Please make sure all references are cited in a proper manner.

Response: We have carefully reviewed all references in the main text and in the reference section to make sure all references are cited in a proper manner.

Many thanks to you and your good comments.

Reviewer #3

1. Response to comment: While mentioning the vendos for consumable or equipment, the company name and place needs to be provided. For example, page number 8 line number 48 [Mammalian Protein Extraction Reagent (Thermo) and Complete Protease Inhibitor Cocktails (Roche, Lewes, UK)]. Need to do for all vendors throughout the manuscript.

Response: It is really true as reviewer commented. We have added relevant information as “(Selleckchem, Munich, Germany)”, “(Thermo, San Jose, CA)”, “(Promega, Madison, WI)” and marked red.
2. Response to comment: Reference number 8, 23 is not in the required format.

Response: We have carefully reviewed all references in the main text and in the reference section to make sure all references are cited in a proper manner.

Many thanks to you and your excellent comments.

Reviewer #4

1. Response to comment: In Figure 5B, I believe the legend of Y axis is not percent of wound. Maybe that is fold change compared with 0h.

Response: It is really true as reviewer commented. We have changed the legend of Y axis to be “fold change compared with 0h” in Figure 5B.

2. Response to comment: In Figure 6 and 7, it should indicate magnification and areas where the authors used to count invaded cells.

Response: We have added relevant information as “Representative images captured by a light microscope are shown (100x), average of 5 picture fields at 100x total magnification.” and marked red.

Many thanks to your helpful comments.

Reviewer #5

1. Response to comment: In Result section, authors found that Gli was upregulated and associated with AKT and EMT pathway markers in NSCLC tissue samples. Here, they just showed figure 1 about western blotting. I wondered whether any statistical analysis evidence to prove it and show us.

Response: It is really true as reviewer commented. We have added relevant analysis involving correlation as “Subsequent correlation analyses between Gli1 and EMT or AKT pathway markers showed positive correlation as 0.7774 (p < 0.001) of Gli1 and N-cadherin, 0.6701 (p < 0.001) of Gli1 and Vimentin, 0.7237 (p < 0.001) of Gli1 and p-AKT, respectively.” and marked red.
2. Response to comment: The expression of Gli was proved to be associated with EMT markers expression in 36 matched NSCLC and normal patient tissue samples. Besides, Gli inhibition and siRNA knockdown reduces EMT, cell viability in vitro. Is there any possibility that the expression of Gli associates with the lymph node metastasis of these 36 NSCLC patients?

Response: In the current study, we are focusing the effect of Gli in tumor progression, instead of metastases. Therefore, only matched NSCLC and normal patient tissue samples were analyzed to avoid potential confusion. However, we are conducting another study involving metastasis. We are planning to report the information in the near future.

3. Response to comment: This article presented so many figures where there are some figures should be combined. Figure 3 and 4, Figure 5 and 6, Figure 7 and 8 should be presented in one big figure, respectively.

Response: Considering the Reviewer’s suggestion, we have combined Figure 3 and 4, Figure 5 and 6, Figure 7 and 8. Relative modifications in the figure and figure legends were conducted and marked red in the main text.

Many thanks to your good comments.

Reviewer #6: Many thanks to your excellent comments.

We tried our best to improve the manuscript and made significant revisions in the manuscript. These changes will not influence the content and framework of the paper. And here we listed the changes and marked in red in revised paper.

We appreciate the Editors and Reviewers’ work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.