Reviewer's report

Title: YKL-40 regulate Epithelial-Mesenchymal Transition and enhance migration/invasion in non-small cell lung cancer

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Reviewer: Siyuan Zhang

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In the recent manuscript submitted for publication, Malvin and colleagues attempted to show that YKL-40 affected invasion via EMT gene regulation, such as Twist, Snail, Slug, N-cadherin, Vimentin and E-cadherin and cancer cell migration in NSCLC cell lines. By using cell invasion and migration assays and western blotting the authors attempted to demonstrate the significance of YLK-40 as clinical target for non-small cell lung cancer. The major weaknesses are lacking in vivo functional assay, small sample size and unclear sampling methods. Quality of written English is not suitable for publication at the current form unless extensively edited. The following are some concerns that should be addressed prior to publication:

Major Compulsory Revisions:

1. The major concern is lacking in vivo functional validation of the role YKL-40 in metastasis. An in vivo model would help support authors claim to analyze the impact that the microenvironment has EMT compared to cell culture.

2. The statement “We believe that YKL-40 may serve as therapeutic targets for NSCLC patients in the future.” May be an overstatement if only “diagnosis, 39% to 91% of patients with metastatic disease had elevated serum levels of YKL-40.” This is a very large deviation and may not prove meaningful for clinical testing. This large deviation is evidenced in the authors own western blot data in which patient 10 has tumor tissue with equal protein levels of YLK-40 than subject 1 non-tumor tissue. Additionally while some studies have found that YLK-40 increases, it only does so for 24 hours and decreases thereafter leading to the elevated levels are only detectible for a short period of time as evidenced by Salvatore, V., Teti, G., Bolzani, S., Focaroli, S., Durante, S., Mazzotti, M. C., & Falconi, M. (2014). Simulating tumor microenvironment: changes in protein expression in an in vitro co-culture system. Cancer Cell International, 14(1), 40.

3. It has also been shown that YKL40 is known to be regulated by PI3K/AKT rather than YKL-40 may play a role in the regulation of PI3K/AKT/mTOR cascade as claimed in the article as evidenced by Jeet, V., Tevz, G., Lehman, M., Hollier, B., & Nelson, C. (2014). Elevated YKL40 is associated with advanced prostate cancer (PCa) and positively regulates invasion and migration of PCa cells. Endocr Relat Cancer, 21(5), 723-737. doi: 10.1530/erc-14-0267. It will be interested to whether modulation of YKL-40 impacts on cell proliferation.

5. Please justify the inclusion of the 5 cell lines measured in the invasion and
migration assays and why they were placed in the respective categories.

Minor Essential Revisions:

1. No mention is made that any of the 10 patient samples were screened for any of the inflammatory conditions such as “asthma and chronic obstructive pulmonary disease” which could confound results as these conditions increase YKL-40.

2. The statement “It is believed that inflammatory mediators, such as pro-inflammatory cytokines of …IL-6,” is not correct. IL-6 has been shown to have anti-inflammatory properties as recently described by Reilly, S. M., & Saltiel, A. R. (2014). Countering inflammatory signals in obesity. [News and Views]. Nat Immunol, 15(5), 410-411. doi: 10.1038/ni.2874

3. The statement “high expression of YKL-40 correlated with poor prognosis in NSCLC patients.” should be elaborated on. Did the patients die of lung cancer or another illness? This is not clear in from the article.

4. Consider changing “further YKL-40 knockdown of the same cell” to “YKL-40 knockdown”; adding “cells” after “tumor than in non-tumor.” line 169; “knockdowned-cell” to “knockdown cells” line 187; “after overexpression or knock down YLK-40 gene in NSCLC cell lines.” to “after YLK-40 overexpression or knock down in NSCLC cell lines.” Line 36-37; “gene was found” to “was” line 72; “in this study focus on YKL-40 correlated with” this study explored the relationship between YLK-40 and” line 75; “in a vertical slab gel unit” to “by vertical gel electrophoresis” line 136; delete “and instantly” line 139; “Following three times wash” to “After washing three times” line 143; “investigate” to “investigated” line 168; “in sustaining” to “sustained” line 220; delete “gene” or change to “genes” line 222; change “short survival in a number of poorly prognoses” to poor survival in” line 223;

5. Change “gene” to “genes” line 37, 136; “regulated” to “regulate” line 78; “level” to “levels” line 147; “significant” to “significance” line 165; “shows” to “show” line 235; “undergoes” to “undergo” line 240; “promote” to “promotes” line 252; “promote” to “promotes” line 202; “patient” to “patients” line 31; “is” to “was” line 149; “significant.” To “significance.” Line 165; “Result” to “Results” line 166; “effect” to “effects” line 185; “promote” to “promotes” line 202. “cell” to “cells” line 203; “finding” to “findings” line 250; “through” to “by” line 252; “one of” to “a” line 256-257; “Reference” to “References” line 275.

6. This sentence is a fragment “To investigate the YKL-40 expression level effect tumor migration and invasion in NSCLC.” Line 175

7. Clarify “shRNA to re-knockdown in CL1-1 YKL-40 overexpressed-cell.” line 188, “YKL-40 knockdown-reoverexpression CL1-5 was reversely processing EMT (Figure 4B).” line 216

8. A comma is needed after markers line 215, “Mesenchymal” shouldn’t be capitalized

9. The paragraph between lines 202 and 216 needs to be reworked due to poor use of the English language.
Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.