PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Validation of EGFL6 expression as a prognostic marker in lung adenocarcinoma patients in Taiwan: a retrospective study
AUTHORS	Chang, Chun-Chi; Sung, Wen-Wei; Hsu, Hui-Ting; Yeh, Chung-Min; Lee, Chien-Hsun; Chen, Ya-Ling; Liu, Ta-Chih; Yeh, Kun-Tu

VERSION 1 – REVIEW

REVIEWER	Anil Sood MD Anderson Cancer Center Houston, TX USA
REVIEW RETURNED	16-Jan-2018

GENERAL COMMENTS	The authors examined EGFL6 expression in non-small cell lung cancer. This is an important topic but the following details may add to the paper: • Page 7. Additional methodological details would be important to present. How clean and specific is the antibody that was used? How was the specificity tested/proven? What positive and negative controls were used? Apparently three pathologists examined the samples – what was the inter-observer variation? What exactly was the scoring approach used? • Since EGFL6 can be produced by tumor and stromal populations, which compartment exactly was scored? Were both stromal and tumor staining accounted for? Which was more dominant? Was EGFL6 specifically in endothelial cells evaluated? • There are several grammatical and typographical errors that should be corrected

REVIEWER	Masaki Tomita Department of Thoracic and Breast Surgery, Faculty of Medicine, University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki, 889-1692, Japan
REVIEW RETURNED	18-Jan-2018

GENERAL COMMENTS	The authors reported high EGFL6 expression may serve as a marker for poor prognosis of non-small cell lung cancer, especially in younger patients. This is a well written and useful contribution, which I think is entirely suitable for publication in 'BMJ open'. However I have some comments. Comments (1)The present series consist of stage I-IV NSCLC. Although all patients received surgical treatment, this heterogeneous group might

	receive various therapies. Unfortunately, there are no information about treatment. How many patients received chemotherapy, radiotherapy and best supportive care ? Treatment modality might affect the patients' survival. However, treatment modality was not investigated in uni- and multi-variate analyses. If the treatments were too heterogenous to investigate, this should add in the limitation of this study. (2)There are no data about histology of NSCLC. In general. patients with adenocarcinoma histology had favorable prognosis than others. Furthermore, there is a possibility that expression of EGFL6 might be different between adenocarcinoma and others.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Anil Sood
Institution and Country: MD Anderson Cancer Center, Houston, TX, USA

The authors examined EGFL6 expression in non-small cell lung cancer. This is an important topic but the following details may add to the paper:
• Page 7. Additional methodological details would be important to present. How clean and specific is the antibody that was used? How was the specificity tested/proven? What positive and negative controls were used? Apparently three pathologists examined the samples – what was the inter-observer variation? What exactly was the scoring approach used?
Answer:
Thank you for this suggestion. In our study, Anti-EGFL6 antibody (Abcam, ab140079) was used. The tested applications include western blot and immunohistochemistry staining. The western blot showed dominant signal in around 61 kDa. Liver tissue was reported to have EGFL6 expression and served as positive control. IHC assay with a primary antibody in tandem with a specimen that is not exposed to the primary antibody served as negative control. We provide detail catalog information of the antibody in the revision which was missed in previous manuscript. As to the scoring variation. A final agreement was obtained for each score by having all three evaluators view the specimens simultaneously through a multi-headed microscope. We did not actually investigate the inter-observer variation. However, bios might happen while the clinical outcome of patients was known during scoring. Therefore, in our study, the pathologists were blinded to the prognostic data of the study. Thank you.

• Since EGFL6 can be produced by tumor and stromal populations, which compartment exactly was scored? Were both stromal and tumor staining accounted for? Which was more dominant? Was EGFL6 specifically in endothelial cells evaluated?
Answer:
In this study, EGFL6 expression in the tumor part was scored. Stromal expression was not dominant and was ignored. We did not analyze endothelial expression of EGFL6 in current study. Thank you.

• There are several grammatical and typographical errors that should be corrected
Answer:
Thank you for this suggestion. We sent this manuscript to the professional copyediting agency (Scribendi Inc.) for English improvement. The changes are marked in the tracked manuscript.

Reviewer: 2
Reviewer Name: Masaki Tomita

Institution and Country: Department of Thoracic and Breast Surgery,Faculty of Medicine, University of Miyazaki,
Kihara 5200, Kiyotake, Miyazaki, 889-1692, Japan

The authors reported high EGFL6 expression may serve as a marker for poor prognosis of non-small cell lung cancer, especially in younger patients.
This is a well written and useful contribution, which I think is entirely suitable for publication in 'BMJ open'.
However I have some comments.
Comments
(1)The present series consist of stage I-IV NSCLC. Although all patients received surgical treatment, this heterogeneous group might receive various therapies. Unfortunately, there are no information about treatment. How many patients received chemotherapy, radiotherapy and best supportive care ? Treatment modality might affect the patients' survival. However, treatment modality was not investigated in uni- and multi-variate analyses.
If the treatments were too heterogenous to investigate, this should add in the limitation of this study.
Answer:
Thank you for this comment. We are sorry that we cannot provide more detail information as mentioned in the comment since all the patients were de-linked previously. We added this limitation to the “strengths and limitations section” and discussion section. Thank you.

(2)There are no data about histology of NSCLC. In general. patients with adenocarcinoma histology had favorable prognosis than others. Furthermore, there is a possibility that expression of EGFL6 might be different between adenocarcinoma and others.
Answer:
Thank you for this suggestion about this important issue. In this study, all patients were diagnosed as lung adenocarcinoma. We revised the terms of NSCLC to adenocarcinoma. The changes are marked in the tracked manuscript. Thank you.