TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

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Review Comments:

Comment 1: What was the relationship between cGAS-STING expression and tumour grade? did authors look into this issue and exclude tumour grade as a potential interfering factor?

Reply 1: We appreciate the comment from the reviewer. However, to explore the tumor grade in tumor tissue, tumor blocks or at least core needle aspirations have to be available. Unfortunately, patients in this study were evaluated by fine needle aspirations. Furthermore, tumor grading is not part of the standard diagnostic work-up in lung cancer as it not used in the clinic. Decisions on treatment are based on the TNM staging as well the clinical performance of the patient. Therefore, due to the nature of this study, we do not have this information.

Changes in the text: The following has been added to the manuscript in the discussion section:

Page 17, line 403
Since the tumor tissue examined in this study was obtained by fine-needle aspirations, we did not have information on infiltrating immune cells nor tumor grade.

Comment 2: Did authors investigate the relationship between the cGAS-STING pathway expression and the density, type and distribution of immune cells in the tumour tissue?

Reply 2: To explore the tumor density, type and distribution of immune cells in tumor tissue, tumor blocks or at least core needle aspirations have to be available. Unfortunately, patients in this study were evaluated by fine needle aspirations. Therefore, due to the nature of this study, we do not have this information.

Changes in the text: The following has been added to the manuscript in the discussion section:

Page 17, line 403

Since the tumor tissue examined in this study was obtained by fine-needle aspirations, we did not have information on infiltrating immune cells nor tumor grade.

Comment 3: The authors should speculate by which mechanisms tumour cells in high stage lung cancer supress cGAS-STING expression?

Reply 3: We agree with the reviewer that this is important to discuss. In our dataset, we do not see a significant decrease in STING and cGAS expression in high stage lung cancer, although there is a tendency. Xia et al. 2016(1) showed that cGAS and STING expression was decreased in late stage melanoma due to epigenetic silencing and not tumor mutations. Since, we do see decreased cGAS expression in PBMCs it could be speculated whether this is epigenetically regulated or due to inherent genetic variation such as SNPs in the MB21D1/CGAS gene. Such a genetic variation could potentially influence the ability of immune cells to suppress cancer progression.

Changes to the text: The following has been added to the discussion section
Page 17, line 412

The decrease in CGAS expression from non-cancer to cancer and from localized disease to late stage disease may be due to some not yet identified inherent genetic variation or due to epigenetic regulation of cGAS expression as previously described for tumor cells(1).

Comment 4: I am not sure whether the sample size is large enough to draw any statistically strong conclusions? Given that there is already another study with some contradictory results, these findings have to recapitulate by other investigators.

Reply 4: It would be very beneficial to our conclusions to extend the investigations to a larger cohort. We do unfortunately not have access to such a cohort and especially RNA samples from patients with metastatic disease are scarce. As mentioned in the discussion this study did not stratify for tumor stage and hence is not directly comparable. We hope that our study can contribute to the discussion of the role of cGAS/STING in cancer and lead to further investigations.

Comment 5: The retrospective nature of the study is also a big drawback.

Reply 5: We agree that there are some limitations when performing a retrospective study. We do however also think that this study gives important insight to a potential stage dependent role for cGAS/STING as well of the potential for PBMC gene expression as a biomarker.

Comment 6: I think the authors should clearly describe the inclusion/exclusion criteria for the recruitment of patients rather than simply reference to another publication of their own!

Reply 6:

The inclusion and exclusion criteria for recruitment of patients for the original study as well as for this specific substudy have been clarified.

Changes to the text:The following has been added to the manuscript:

Page 9, line 225

Patients were selected from a cohort of patients suspected of lung cancer and referred to the Department of Pulmonary Medicine, Aarhus University Hospital, Denmark between April 2011 and June 2015. For the original cohort the inclusion criteria were 1) age ≥ 18 years, 2) the patient had to sign a written informed consent. The only exclusion criterion was the presence of a current cancer. A total of 1921 patients were included. At time of inclusion a blood sample was collected. If the diagnostic work-up led to a fine needle aspiration, a small part of the diagnostic biopsy was obtained and further processed as described below in the RNA purification section. The cohort has been utilized for studies on the epidermal growth factor system, exosome analyses and comorbidity evaluations(2–7).

References

1. Xia T, Konno H, Barber GN. Recurrent loss of STING signaling in melanoma correlates with susceptibility to viral oncolysis. Cancer Res. 2016 Nov 15;76(22):6747–59.