Peer Review File

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Review Comments

Reviewer A

This is a comprehensive review on the role that chronic inflammation induced by asbestos plays in development of malignant mesothelioma, with special emphasis on high mobility group box1 (HMGB1), which is released by mesothelial cells after exposure to asbestos. Also, several ways to inhibiting HMGB1 activity are described in the manuscript.

GENERAL COMMENTS:

- The role that HMGB1 might play as a biomarker to detect asbestos exposure and mesothelioma development at early stages is described in detail in the manuscript, and -together with the possibility to inhibit HMGB1 activities using several compounds- is particularly promising.
- Also, the role that combination of BAP1 germline with other tumor suppressor gene mutations can play in mesothelioma is described.

SPECIFIC COMMENTS:

Comment 1: ROLE OF "FRUSTRATED PHAGOCYTOSIS"? After reading this manuscript and other published works, it appears that the concept that incomplete phagocytosis of long asbestos fibers leading to leakage of cells contents by partial rupture of the cell membrane is currently outdated. According to references #2 and #3 in the present manuscript, the old hypothesis on the relevancy of the mechanical interference of asbestos fibers with cell division and subsequent carcinogenicity has been ruled out. Instead, it seems that fiber chemical structure itself plays an important role in inducing DNA damage.

Reply 1:

We appreciate reviewer's comments.

We have clarified the concept of "frustrated phagocytosis". Moreover, we have reviewed the previous hypothesis of asbestos-led carcinogenesis via mechanical interference and explained in detail the updated model of iron-rich ferruginous body after asbestos exposure, specifically free radical-mediated DNA damage via Fenton reaction.

Changes in text:

The relevant text has been modified: (lines 14-17, page 7), (lines 2-9, page 7), and (line 22, page 7- line 4, page 8), respectively.

Comment 2: Although the present review makes clear that chronic inflammation linked to HMGB1 is critical to development of mesothelioma, a specific comment on this particular aspect would be welcome in the manuscript.

Reply 2:

We are grateful for the reviewer's comment.

According to your suggestion, we discussed the importance of chronic inflammation in asbestos-induced carcinogenesis.

Changes in text:

Please find the updated text at: lines 6 - 9, page 7.

Comment 3: Also, a comment on the role of macrophage ferroptosis and asbestos exposure would be welcome.

Reply 3:

We thank the reviewer for the suggestion.

The mechanism of macrophage ferroptosis after asbestos exposure and its role in generation of ROS and subsequently DNA damage is commented in the text.

Changes in text:

Updated text can be found at lines 2 -12, page 8.

Reviewer B

In this review, Zolondick and colleagues discuss the mechanisms of asbestos-induced mesothelioma carcinogenesis focusing on chronic inflammation and pro-survival autophagy. This is generally a nicely written review that points toward a novel clinically interesting avenue, namely targeting the HMGB1. This soluble factor, which is released from mesothelial cells upon exposure to asbestos, triggers the pro-inflammatory and pro-survival response and thus might promote malignant transformation.

To complement the discussion, I suggest adding the following:

Comment 1: a paragraph on the crosstalk between asbestos-induced ROS production, TGFbeta and EMT, which appears to be important in malignant transformation (as reported e.g. by Turini et al. Int J Mol Sci 2019).

Reply 1:

We would like to thank the reviewer for the favorable comments.

We added a discussion in details now the role of asbestos-induced ROS and intracellular signaling pathways, like TGF-β, HMGB1 and TNF-α, in EMT process of human mesothelial (HM) cells transformation. Asbestos-exposed HM upregulate these pathways, which accelerate mesothelioma development.

Changes in text:

Please find the new paragraph at lines 14-22, page 8.

Comment 2: reference to the report in Cancer Research 2002, showing that AP-1 component, Fra-1, plays important role in asbestos-induced mesothelial cells transformation. This is worth mentioning given that AP-1 is an important pro-inflammatory regulator.

Reply 2:

We appreciate the reviewer's comment.

We have better outlined now the role of transcription factor AP-1 as an important mediator of asbestos toxicity and mesothelioma cell transformation.

Changes in text:

For the updated text and the references please see line 22, page 8 – line 1, page 9.

Reviewer C

The objective of this manuscript is to review the mechanisms of asbestos carcinogenesis, including chronic inflammation and autophagy-mediated cell survival and possible innovative therapeutic targets to reduce inflammation and chemoresistance to prevent or delay mesothelioma development.

This manuscript is well written and it is molecularly informative and valuable to mesothelioma study. I would recommend resubmission after making a minor revision.

Minor comments:

Comment 1: Line 98: In the sentence: "HMGB1 is a prototypical damaged-associated molecular pattern (DAMP) that, in the presence of asbestos fibers, initiates the inflammatory process" should be modified to "HMGB1 is a prototypical damaged-associated molecular pattern (DAMP) that initiates the inflammatory process". Indeed extracellular HMGB1 does not mandatorily needs asbestos fibers to sustain inflammation.

Reply 1:

We thank the reviewer for the favorable comment. We have modified the sentence accordingly.

Changes in text:

Updated text can be found at lines 11 – 14, page 5.

Comment 2: Line 225: discuss in this context that asbestos can generate DNA double strand breaks (dsb) (Both, et al 1994 Int J Cancer 59, 538-542; Pietruska, et al 2010 Environmental health perspectives 118, 1707-1713) and that dsb have been shown to lead to loss of nuclear HMGB1 (White et al, 2015 DOI: 10.1038/ncomms7790).

Reply 2:

We appreciate your comment. We have updated the text to address how asbestos can cause double stranded breaks, and how double stranded breaks can lead to the loss of nuclear HMGB1.

Changes in text:

Updated text can be found in the discussion subsection "Autophagy as a mechanism for pre-cancer cell survival in response to chronic inflammation in mesothelioma", lines 18-20, page 12.

Comment 3: Sections referring to inhibition of HMGB1: discuss also heparan sulfate octadecasaccharide, which has been recently shown able to neutralize the proinflammatory activity of HMGB1 (Arnold et al., 2020, Sci. Transl. Med. 12, eaav8075).

Reply 3:

We thank the reviewer for the suggestion.

We have included a sentence stating how heparan sulfate octadecasaccharide can neutralize the proinflammatory activity of HMGB1.

Changes in text:

Updated text can be found in the discussion subsection "Possible therapeutic approaches to reduce chronic inflammation and cancer cell survival in mesothelioma", lines 19-23, page 14.