Author's response to reviews

Title: Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity

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Author's response to reviews: see over
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Dear Editor

*BMC Cancer*

**Title:** Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity

**Authors:** Zahid N. Rabbani, Mitchell S. Anscher, Rodney J. Folz, Emerald Archer, Hong Huang, Liguang Chen, Maria L. Golson, Thaddeus S. Samulski, Mark W. Dewhirst, Zeljko Vujaskovic

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Please find attached revised manuscript and revision details. We have revised the manuscript according to the reviewer’s report. Thank you for your consideration of this manuscript.

Sincerely yours,

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Major Compulsory Revisions

A) Page 10 last para, Fig 3 a and b

The authors state that the increase in BAL cell count is mainly due to an increase in macrophages. Comparison of the figures suggest that only about 10% of the increase can be accounted for by macrophages. The major influence by EC-SOD overexpression on BAL is thus decreases in other cell types. Which? Which is the effect of that on the radiation toxicity. This may change the whole interpretation of the paper.

- We are thankful for reviewer’s observation regarding to our this data presentation mistake.
  After thoroughly revising our manuscript, figures and data, we figure out the presentation mistake in scaling of Fig 3a. The Fig 3a (Total Leukocytes Count) is scaled at $10^6$, which is supposed to be $10^5$. After making correction for this error, the comparison of Fig3a and b and data, shows that macrophages, make major bulk of inflammatory cells. So the interpretation of the paper remains the same. We included the updated file of Figure 3a.

B) Page 11 2nd para), Fig 5: (2nd para which is now last para of page 11)

The authors show an increased activation of TGF-β1 over time, as % of whole TGF-β1. This cannot be interpreted in the absence of data for whole TGF-β1 levels in the lungs. Which are the absolute levels of active TGF-β1?

- The biological activity of TGFβ1 is primarily regulated by the conversion of latent TGFβ1 to active TGFβ1. Normal tissues contain significant quantities of latent TGFβ1 and activation of only a small fraction of this latent TGFβ1 generates maximal cellular responses. Active TGFβ1 increased significantly after irradiation concomitant with decreased latent TGFβ1 has already been demonstrated in previous studies. This rapid shift in activation of TGFβ1 mediates normal tissue toxicities. Radiation-induced activation of TGFβ1 may have profound implications for understanding tissue effects caused by radiation therapy.

- Radiation induced lung injury is believed to be a consequence, in part, of oxidative stress from radiolytic hydrolysis and the formation of reactive oxygen species that are important in the activation of latent TGFβ1. This active TGFβ1 influences inflammation and fibrotic disease process at the molecular/cellular levels and also increases the recruitment and activation of inflammatory cells into an area of injury Furthermore, active TGFβ1 induces macrophages to secrete inflammatory and fibrogenic cytokines, including TGFβ1 itself. This mechanism of autoinduction of TGFβ1 is important for maintaining the level of locally active TGFβ1 in injury and the wound healing process (ref # 13, 41 of manuscript).

Minor Essential Revisions

C) Abstract, material and Methods

It is meaningless to state that "immunohistochemistry and ELISA" were performed to determine lung injury. If included the sentence must be more specific as to which factors were analysed, not techniques used.

- Changes has been made as suggested by reviewer.

D) Abstract, results.

....whereas there was significant reduction in these parameters .. should be ...whereas these parameters were significantly less increased. (These parameters were in fact not reduced, but increased)

- Changes has been made as suggested by reviewer.

E) P4, last para

"RT-induced"....RT has not been defined anywhere in the paper.

- Corrected. After defining RT (radiation) in P3, 1st paragraph, we replaced radiation with RT in manuscript.

F) P9, 2nd para

Psmad 3 should be Psmad 2/3 (???)

- Corrected