**Author's response to reviews**

**Title:** Hyperoxia retards growth and induces apoptosis and loss of glands and blood vessels in DMBA-induced rat mammary tumors

**Authors:**

- Anette Raa (anette.raa@biomed.uib.no)
- Christine Stansberg (christine.stansberg@helse-bergen.no)
- Vidar M Steen (vidar.martin.steen@helse-bergen.no)
- Rolf Bjerkvig (rolf.bjerkvig@biomed.uib.no)
- Rolf K Reed (rolf.reed@biomed.uib.no)
- Linda EB Stuhr (linda.stuhr@biomed.uib.no)

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**Author's response to reviews:** see over
To the Editor
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Dear Iratxe Puebla

We would like to thank the reviewers for their constructive comments on our manuscript 6244373861151655. We have now addressed their concerns in a revised version of the manuscript as specified in the enclose letter. We now hope that the article is acceptable for publication in BMC. Thank you very much for your consideration regarding this work.

Yours sincerely

Associate Professor Linda Elin Birkhaug Stuhr
RESPONSES:

Referee 1:

Table 2 did not include the treatment group with 5-FU as indicated by the referee. However, this group was only used to show whether hyperoxia had a more pronounced effect on tumor growth than 5-FU (a commonly used chemotherapeutic drug). The morphological effects of 5-FU on tumors have been well documented and was therefore not the scope of this study. Consequently, we have chosen not to implement any changes in the manuscript regarding this aspect.

Referee 2:

The “holes” in the tumor tissue found in the present study was suggested as a possible decrease in glandular components in the tumor itself and not in normal glandular tissue surrounding the tumor. These DMBA tumors are round and only slightly bound to the tissue beneath by blood vessels. At present we have not found any studies in the literature indicating that hyperoxia influences normal gland structures. Although interesting, we feel that this issue could be addressed in a study focusing on the physiological aspects of hyperoxia on gland structures. This can be followed up in future studies.

Referee 3:

As the referee pointed out the present study builds on a previous published article by us. The 2 bar represent a “proof of principle” study indicating that hyperoxia may exert a cytotoxic action on tumor tissue in vivo. The present study was initiated to see if 1 bar and 1.5 bar would have similar effects The aim was therefore to find the least pressure gradient that gave a therapeutic effect. If the responses seen at lower pressures were the same as observed by 2 bar we would have excluded 2 bar in future studies. Hopefully this aspect is more clearly outlined in the introduction (p.4, last paragraph).

For your interest: The 2 bar exposures had no effect on rat morbidity. The rats were fine through the experiments. As a consequence of the present results, we have initiated a follow-up study on 2 bar trying to address further the mechanisms behind the hyperoxic induced reduction in mamma tumor growth.

As pointed out by the referee, we agree that it would be interesting to know if RNA expression of identified genes is increased above baseline two weeks after treatment, and if these changes correlated with increase in tumor size. However, to address this rather complicated issue a completely new study is warranted that is not directly related to the present work. We plan, however, to address this issue in future studies.

Our laboratory has tried through Western blot to give an answer to whether VEGF and HIF-1 α was down-regulated or not after hyperoxic treatment. Unfortunately our technician has tried for months without being able to have a reliable blot and we therefore decides to finish.
As mentioned by the referee, lungs are the most sensitive organ to hyperoxia. The effect on lungs as other tissue depends on concentration and length of exposure. We do not believe that normobaric hyperoxia and 1.5 bar oxygen given intermittently for 90 min will significantly affect on the lungs. In the literature numerous studies indicating oxygen damage to the lungs, but this occur after >95% oxygen continuous exposure for 24-72 hours or at > 2 bar for a longer period of time period than the 90 min used in the present study. This aspect has hopefully now been more clearly describes in the discussion of the manuscript (p.16, 1 paragraph). Additionally, hyperbaric oxygen therapy (1-2 bar oxygen) is recommended for use in humans for treating decompression sickness, selected problem wounds and so forth (review by Gill & Bell 2004) without causing any adverse effects on lungs.

The fig 2 shows tumor histology not lung histology as the referee have stated. We could of course show 1.5 bar morphology also but since 1 and 1.5 bar showed identical responses we chose to show just the one (inserted in the results p. 13, line5-6). The samples are always taken day 11 (after hyperoxic treatment), as now stated in the figure legends (and not during as it was written previously)(Fig 2, p. 25).

We deleted Fig 4 (from p.25) as recommended by the referee.