Author's response to reviews

Title: DNA repair deficiency in peripheral blood lymphocytes of endometrial cancer patients with a family history of cancer

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Author's response to reviews: see over
Dear Editor,

We are pleased to resubmit the revised version of MS: 1843428373114847 Research article “DNA repair deficiency in peripheral blood lymphocytes of endometrial cancer patients with a family history of cancer” to BMC Cancer.

We would like to thank the reviewers for the comments and suggestions. Our responses follow (the reviewer’s comments are in italics).

Response to Referee #2 Comments

1) On page 6, Line 7: Typo. Write "genotoxic"
   Reply: The correction has been made.

2) On page 6, Line 8: Delete "according to recommendation Schmezer et al [20]" as it seems to misplaced in this sentence. It should be inserted at the end of the sentence behind "30min": "as recommended by Schmezer et al. [20]."
   Reply: The correction has been made.

3) On page 6, Line 16: Use a decimal point (0.8 V/cm)
   Reply: The correction has been made.

4) On page 6, Line 17: Please give source/manufacturer of the Comet Score programme
   Reply: The correction has been made.

Response to Referee #3 Comments

I still concern about the same issues I stated in my previous revision. I agree that the obtained results can be meaningful but I still have some doubts about it. The paper of Schmezer et al is confusing in some aspects. In a preliminary study with lymphocytes of 7 healthy volunteer they showed that 20 µg/ml bleomycin saturates the assay, as it is shown in figure 1A and in the text (“Treatment with bleomycin at concentrations of #20 µg/ml induced a dose dependent increase of DNA damage (Figure 1A), but higher concentrations let to no further increase”). I do not think bleomycin does not induce more damage at higher concentration so it seems the assay is saturated at that concentration and with a tail moment of about 30. Nevertheless later on in the paper, when they analyze the real samples using 20 µg/ml bleomycin, they obtained a tail moment between 5 and 60. (Please also note that they used phytohaemagglutinating stimulated cell in the real samples but it is not clear if the stimulate the cells in the preliminary study –according with the material and methods section it seems that they did not-, and this can be a reason to have different tail moments since it changes with different cell types.) Anyway in your case, when percentage of DNA in tail is used, the saturation level of the assay is clearer (that is one of the reasons why the tail moment is less used) and it is reached when treating the lymphocytes with 20µg/ml bleomycin.

Regarding the issue about the damage inflicted by bleomycin being different between healthy subjects and cancer patients I disagree with the authors since this observation is common. For instance, in the paper of Schmezer et al they showed that lymphocytes from patients with lung cancer were more sensitive to bleomycin than lymphocytes from control subject (see Figure 2 and text –p<0.001). When this observation is done it is difficult to analyse the removal of damage since the starting point is different in both groups (there are other approaches). In this case cells are not going to repair with the same efficiency very different amounts of damage.

I recognize that this work has a lot of work behind it and the samples taken are very valuable. As I mentioned in my previous revision the comet assay is saturated at about 70 or
80% DNA in tail. I would be happy to reconsider my decision if the authors showed me that the comet assay was not saturated in the conditions used in this paper (and you can reach 100% DNA in tail). To do so I suggest you to perform a dose response (twice) with different concentrations of bleomycin (including 20 µg/ml) until the assay is saturated.

Reply: We performed a concentration-response curve with bleomycin (5, 10, 15, 20, 25, 30, 35, 40, 50 µg/ml) and lymphocytes of healthy volunteer (see Figure). According to the obtained results the comet assay was saturated at about 95% DNA in tail (30 µg/ml bleomycin) in conditions we used. DNA damage induced by concentration of 20 µg/ml was about 80% DNA in tail and was in the linear part of the curve. So we suggest that chosen concentration in the paper of 20 µg/ml allowed to determine the real level of DNA damage.

We agree that there are other approaches to measure DNA repair activity that are possibly more accurate but method we used has commonly been applied by many other researches. Our data indicate that with starting levels of bleomycin-induced DNA damage that are not significantly different in healthy subjects and endometrial cancer patients, the repair capacity of the cells from healthy subjects was apparently greater, since at the end of the incubation the cells had returned to the background damage level, whereas in the case of the cancer patients, there was still substantial unrepaired damage - 25.4% tail DNA compared with background level of 9.3%.