Author’s response to reviews

Title: Microarray analysis of DNA damage repair gene expression profiles in cervical cancer cells radioresistant to 252Cf neutron and X-rays

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Version: 3 Date: 29 August 2009

Author’s response to reviews: see over
Dear editors:

Thank you for your letter on August 9, 2009 regarding our manuscript entitled “Microarray analysis of DNA damage repair gene expression profiles in cervical cancer cells radioresistant to $^{252}$Cf neutron and X-rays” (Ms: 1254505758272283) that was submitted to your journal for publication. Comments and suggestions provided by the two reviewers have been very helpful to improve our manuscript quality and our study. We have carefully addressed the issues raised by the reviewers, point by point, as highlighted in the revised manuscript. We believe their comments and suggestions have significantly improved the quality of manuscript and made it publishable.

This manuscript has been reedited by some commercial editing service. Thanks again for your reconsideration of our manuscript for publication in your journal. We look forward to your favorable decision.

With best wishes,

Qing Yi

Comments from reviewer 1:

Question 1: Current finding in the manuscript is adequate but could be significantly enhanced if the authors further examine the cross-resistance patterns of these two cell lines with respect to DNA damaging and non-DNA targeting chemotherapeutic agents to better characterize these cells.

Answer: This question is very important to our further research and actually such experiments are undergoing in our lab currently. The radioresistant cell sub-lines, HeLaNR and HeLaXR, are very important models, we plan to apply them to investigate the resistance to chemoradiotherapy in cervical cancer and your suggestion is one of the important components. Though the relationship of chemoresistance and radioresistance of tumor cells isn’t definite, we are interested in the project with hope of some innovative findings, and we will show the data in our future paper.

Question 2: The author should also point out the differences between neutron- and x-irradiation and describe the rationale for the studies in the manuscript, including the reasons for generating the two resistant cell lines.

Answer: It is very important to distinguish the differences between these two subcategories of irradiation. We therefore have put these points into background section. Generally, neutron-ray belongs to a high-LET line and X-ray belongs to a low-LET line. Theoretically, high LET radiation, as provided by neutron rays, has several advantages over low LET radiation: (a) more damage to hypoxic cells; (b)
decreased repair of IR-induced damage; and (c) effectiveness at all stages of the cell cycle. Radioresistance is the most common reason for therapeutic failure of cervical cancer. Despite the improved efficacy of $^{252}$Cf neutron rays in the therapy of cervical cancer, tumors recurrence has been reported. Furthermore, the mechanism causing radioresistance to neutron- and X-irradiation is not well elucidated. So we establish two radioresistant cell strains that might serve as new models for radioresistance investigation. The ultimate goal of this study is to provide some information regarding the genes which are responsible for intrinsic radioresistance.

Question 3: The differential gene expression patterns observed between HeLaNR and HeLaXR cells should be further examined and the authors should discuss in greater detail the significance of these differences with respect to the differential clinical response in patients to these treatment modalities, and their implications.

Answer: Yes. We have added some contents in “Discussion” section. For the two radioresistant sub-lines, the similar overall trend in gene-expression changes indicated that long-term exposure to $^{252}$Cf neutron and X-rays had resulted in a similar induction of genes involved in DNA damage signaling pathways. The number of genes that underwent homologous recombination and nonhomologous end-joining was higher in HeLaXR cells (8 and 3, respectively) than in HeLaNR cells (2 and 1, respectively). Furthermore, the number of cell cycle arrest and mismatch repair genes expressed was higher in HeLaXR cells(7 and 3, respectively) than in HeLaNR cells (3 and 1, respectively) whereas the number of base excision repair genes expressed in the two sub-lines was almost the same (4 in HeLaXR cells and 5 in HeLaNR cells). Thus, genes encoding homologous recombination, nonhomologous end-joining, mismatch repair, and cell cycle arrest functions were more highly expressed in cells resistant to X-rays than in cells resistant to 252Cf neutron rays. Our hospital treats cervical cancer patients with neutron-ray intracavitary brachytherapy combined with X-ray external radiation and the 5-year survival rate is 85.2%. According to the results provided by this study, we examined some critical gene products, including p53, BTG2 and GADD45α, in groups with different radiosensitivity. We found that there are differential expression of GADD45α and p53 between sensitive and resistant group. However, this is a new project that failed to include sufficient patients. We are concentrating on the sample collection and trying to get the reliable data for publication. It is our belief that this future study will be helpful for developing therapeutic target for cervical caner.

Comments from reviewer 2:

Question 1: Review of the previous reports of radiosensitivity (cervical cancer) I think it is desirable to review the previous reports concerning the differential expression profiles of radiosensitive and radioresistant genes.
Answer: Yes. Your advice can improve our manuscript. We have reviewed previous reports and added some reviews in “Discussion” section. Gene expression profiling by DNA microarray has been applied to classify disease stages and predict treatment response to radiotherapy in cervical cancer. In these previous studies, sets of genes associated with pathways such as apoptosis (e.g. bax, bcl-2), DNA damage repair (e.g., Ku80, GADD45, XRCC5), cell adhesion (e.g., ICAM-3), angiogenesis (e.g., HIF-1α), and tumor cell invasion (e.g., CTSL, CTSB) were identified. Especially, Klopp et al detected 12 cervical cancer patients with microarray and found that GADD45α was upregulated by radiation in NED (no evidence of disease) patients (1.13) and downregulated (0.96) in recurrent-disease patients (p = 0.36). Based on the previous reports and our academic interest, we focus on the differential expression in DNA repair related genes in the present study. We found that the expression of GADD45α is downregulated in radioresistant sublines HeLaNR and HeLaXR. Thus, low-level expression of GADD45α may in part explain the radioresistance of HeLaNR and HeLaXR cells.

Question 2: Fig.1
I think it is desirable to add the standard deviation from three independent experiments in each point.

Answer: Yes. We have added the standard deviation in Fig.1 with Microsoft Excel replace of Graphpad Prism 4.0 software.