Author's response to reviews

Title: Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data

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Response to Reviewer’s (ALFONSO DUENAS-GONZALEZ) comments

(1) Figure 2 which is excellent, gives an overview on the global results, stills is notorious the high frequency of methylation in normal tissues for FHIT and AR. For interested readers it will be used to add a table to show literature findings concerning methylation status for these genes in normal tissues.

Authors:
We would like to thank the reviewer for the recommendation. Since the AR (androgen receptor) gene is located on the X chromosome, females are expected to have one copy of the X chromosome inactivated by methylation. Both methylated and unmethylated alleles should be present in normal tissues of females. Therefore, the high frequencies of methylation in normal tissues for AR in our sets of samples are not unexpected. We did not exclude this gene from our study simply because we would like to use it to test our Pooled DNA approach. We proved that the Pooled DNA approach worked well for rapid identification of specific methylation in tumor tissues.

Concerning the FHIT gene, we did a literature search and found that many publications did not indicate clearly the numbers of normal samples used or analyzed in their studies. Majority of the studies used the same primer pairs as we used to detect the methylated and unmethylated FHIT sequences (Zöchbauer-Müller et al., 2001; Ref. No. 28 in the text). At any rate, the methylation status for FHIT gene in normal and tumor tissues of other human cancers reported in the last two year time have been found and listed in the newly added Supplementary Table 2 for those interested readers. Three studies reported the occurrence of high frequencies (ranging from 8.3% to 30%) of FHIT methylation in noncancerous tissues (Please see Supplementary Table 2 and Ref. Nos. 5 and 8 under the table) using the same primer pairs as used in our own study. This indicates that FHIT methylation is not necessarily tumor specific. The result in present study is at least in line with others.

(2) Secondly, the numbers in table 2 regarding patients who received primary radiation are different to what is stated in text. In this is not clear still the definition of radiosensitivity and radioresistance (it is residual vs no residual viable cells in biopsies?). If this is the case then is remarkable the high complete pathological responses rate (no viable cells) (70%).I Literature indicates that with RT it is around 45% and with RT-cisplatin 55%.

Authors:
For the first point, we are not able to find the difference between the numbers in Table 2 and in the text. As indicated in Table 2 (Footnote b), a total of 63 cases received primary radiotherapy and have the data of response to radiotherapy. 44 cases were radiosensitive and 19 cases were radioresistant.

For the second point, the definition of radiosensitivity and radioresistance are further clarified. A sentence “If there are no residual viable tumor cells in cervical biopsies after treatment, the tumor is defined as radiosensitive.” is added to the last paragraph of Page 11. For the last point, the reviewer is absolutely right. The present data have a higher complete pathological response rate than in the literature. This may be due to the small sample size being used in the present study. In another study by our group (Liu et al., 2004), we found that the response rate is about 48% (56/112). We have made a suggestion in the Conclusion Sections in the Abstract and at the end of the text that a larger sample size should be used to confirm the potential role of
methylated DNA marker in cancer management (Page 16).