Author's response to reviews

**Title:** Cyclin A1 promoter hypermethylation in human papillomavirus-associated cervical cancer

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I have corrected the manuscript according to the reviewers suggestions as follow:

**Minor Essential Revisions of Shuchi Agrawal and Carsten Muller-Tidow**

There are several points that need to be revised:

1. The authors claim that cyclin A1 due to its inactivation by methylation acts as a tumor suppressor. However, the authors only demonstrate a correlation which does not establish any causative relationship. These sections need to be clarified.

Response: All sentences claimed cyclin A1 as a tumor suppressor gene has been deleted.

2. Figure 1: The authors refer to HeLa (K) and HeLa (S) cells that differ in cyclin A1 methylation. The authors need to describe the orifin of these cells and the potential reasons for varying levels of cyclin A1 methylation in more detail. It would be preferable to demonstrate that HeLa (K) and HeLa (S) cells indeed show a common genetic background.

Response: Sources of all cell lines were added in the method section.

3. Figure 2: It is unclear how many PCR cycles were performed for the MSP and the USP reactions.

Response: 30 cycles have been indicated in the method section.

4. Figure 2B needs more explanation. Was densitometry used to quantitate the PCR bands? Inter- and Intra-assay variability need to be explained in more detail? Do the error bars represent standard error or standard deviation? How many replicates were performed for individual experiments and how may independent experiments were performed?

Response: Intensity of bands were measured by phosphoimager and indicated in the method section. Error bars were SD and more than triplicate experiments were performed. Both were clarified in the paper.

5. The method used for methylation analysis is at best semiquantitative. It would have been preferable to use real-time PCR. At least, the authors should remove the statements about correlation and regression equation (page 9, first paragraph)

Response: Regression equation has been removed.

6. Figure 3: The normal tissue surrounding the tumor also shows cyclin A1 methylation but retain expression. This would either mean that methylation detection is sensitive (even when only the minority of cells is affected) or that expression and methylation do not always correlate with each other inversely. The authors should comment on this.
Response: The comment sentences regarding the issue has been improved by indicating a possibility of multistep carcinogenesis.

7) Several typos and spelling errors occur throughout the manuscript.

Response: The article has been checked by several readers. However, we failed to identify any numerous misspells that the reviewers noticed.

martin widschwendter

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Figure 4B: Indicate number of samples analyzed (although already shown in Table 2).

Response: the number of samples has been added in the legend.

Both reviewers
Needs some language corrections before being published Quality of written English:

The paper has been language edited by one experience scientist: Dr. Virote Sriuranpong, one native English speakers: Ms Petra Hirsch, two language professionals: Asia Science Editing and Mrs. Nita Suyarnsestakorn.