Author’s response to reviews

Title: Decreased expression of 17beta-hydroxysteroid dehydrogenase type 1 is associated with DNA hypermethylation in colorectal cancer located in the proximal colon

Authors:

Agnieszka A Rawłuszko (arovluszko@ump.edu.pl)
Karolina Horbacka (karolinahorbacka@hotmail.com)
Piotr Krokowicz (chiroq@szpital.strusia.poznan.pl)
Paweł P Jagodzinski (pjagodzi@am.poznan.pl)

Version: 2 Date: 19 October 2011

Author’s response to reviews: see over
Poznań, October 19, 2011

Christina Chap

Executive Editor

BMC Cancer, BioMed Central

Dear Doctor Chap,

Thank you very much for the careful inspection of our manuscript (1170757703573795) “Decreased expression of 17beta-hydroxysteroid dehydrogenase type 1 is associated with DNA hypermethylation in colorectal cancer located in the proximal colon”, which we submitted to BMC Cancer, and for the criticism of the two reviewers. We corrected the manuscript strictly according to the reviewers’ comments.

In reply to the comments of reviewer Jarom Heijmans,

- Since the short isoform of mRNA is translated and most accurately reflects total HSD17B1 mRNA, we focused on data only for this short transcript and its promoter epigenetic studies (page 7, lines 73, 85, 88, 89; page 11, line 168; page 12, lines 202, 207, 210; page 14, lines 249, 251-252; page 15, line 264, 266, 271, 273; page 17, line 316; page 18, lines 347-348; page 21, line 403; page 22, line 443; page 29, line 586; page 30, line 626; page 31, line 647; page 32, lines 662-664; Table 1; Figure 4; Figure 6B; Additional file 2).
• According to the reviewer’s suggestion, we reconsidered using the 2-way ANOVA test. However, consultation with an experienced statistician indicated that our hard data are not modeled by a Gaussian distribution; hence the 2-way ANOVA test could not be applied. Statistic analysis of differences within subgroups (male cancerous, male histopathologically unchanged, female cancerous, female histopathologically unchanged etc.) indicated that the most suitable test is the nonparametric U-Mann-Whitney test. Moreover, only a few data subgroups had normal distribution and we evaluated them by unpaired t-test. Additional information about the statistical analysis was given in the manuscript on page 12, lines 191, 194-195.

• Per the reviewer’s suggestion, we cited (position [33]) and shortly discussed (page 16, lines 298-301) the study performed by the Women’s Health Initiative.

• According to the reviewer’s suggestion, the immunohistochemical analysis was discussed independently with two experienced pathologists; they confirmed correctly chosen healthy tissue and specificity of antibody binding. Moreover, the negative control (slides incubated only with secondary antibody) did not reveal unspecific staining within the analyzed tissue. Information about the negative control was stated on page 9, lines 119-121.

• Per the reviewer’s suggestion, we performed the western blot with cancerous and histopathologically unchanged material from the proximal colon in one run. The old Figure 1C was replaced with a new one.

• According to the reviewer’s suggestion, the sentence “we observed slightly demethylation” was changed ”we observed slight demethylation” (page 15, line 272-273)
• According to the reviewer’s suggestion, the sentence “Moreover, the cohort’s studies indicated that” was replaced by “Moreover, the cohort studies indicated that” (page 16, lines 308-309).

In reply to the comments of reviewer Philip Buckhaults,

• According to the reviewer’s suggestion, the sentence in the abstract “The conversion of estrone” was changed to “The conversion of estrone” (page 2, line 18)

• Per the reviewer’s suggestion, the genomic locations of the gene, transcript start sites, specific CpG assessed by methylation studies, HRM and ChIP primers were stated in UCSC format (page 9, lines 129, 132; page 18, lines 346, 354; page 30, line 616; page 31, line 652; Additional file 1; Additional file 2).

• Per the reviewer’s suggestion, information about the epigenomics data presented on the UCSC genomics browser was extended and discussed (page 18, line 353-356).

DISCRETIONARY REVISIONS

• Information about the correlation between the mRNA and protein was stated on page 12, lines 192-194 and lines 205-207.

• Previous determinations of protein optical densities were conducted with elimination of the background signal. Moreover, the levels of HSD17B1 protein were normalized to the level of a control protein, β-actin. Hence, in Figure 1 panel B, the observed lowest band densities were different from the surrounding background. Additionally, to make Figure 1B more clear, we changed the description of the graph presenting protein levels “log [optical density of HSD17B1 protein]” to “log [optical density of HSD17B1 protein normalized to β-actin]”.
• We observed the same pattern of methylation within 10 individual clones of each patient as well as in 5-dAzaC treated HT29 and SW707 cells (page 13, lines 231-232; page 15, line 267). To make Figures 3 and 6A (bisulfite sequencing results) more clear we extended the figure legends (page 30, lines 621-624; page 31, lines 657-658).

• We also changed the intersection of the X and Y axis in graphs in Figure 7 in agreement to the reviewer’s comment. According the reviewer’s suggestion, we added the slope values in Figure 7 (Figure 7; page 32, lines 676-677).

In reply to the editorial request,

• According to the editorial request the paper was copy-edited by a native English speaking person.

We do hope that these corrections are sufficient, and in this form the paper will be suitable for publication in *BMC Cancer*.

With kind regards.

Yours sincerely,

Paweł P Jagodzinski Ph.D.