Reviewer's report

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

Version: 1 Date: 15 August 2011

Reviewer: Phillip Buckhaults

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Section 1. Major Compulsory Revisions

No major compulsory revisions are indicated.

Section 2. Minor Essential Revisions

1. The last sentence of the methods section of the abstract. “The conversion of estrone…… “. The authors may have intended “The conversion of estrone…..”

2. The entire manuscript can be made easier to follow and interpret by reporting the genomic locations of the gene, transcript start sites, specific cpgs assessed by methylation studies, HRM and CHIP primers, etc…. Please refer the reader to the genomic coordinates of the hg18 reference assembly, using the format that is utilized by the UCSC genome browser. For example,

(the numbers below are provided as examples only. The authors will have to replace these numbers with the correct ones)

Gene (long form ?) = chr17:37954758-37960750
Gene (short form?) = chr17:37,957,510-37,960,757
Transcription Start Site for Long form ??? = chr17:37,957,510
Promoter for Long form ???? = chr17:37957410-37957559
Transcription Start Site for Short form ???? = chr17:37,957,510
Promoter for Short form ???? = chr17:37958378-37958527
CpG Island = chr17:????????-????????
CpG 1 = chr17:??????
CpG 2= chr17:??????
CpG 3= chr17:??????
Etc…

3. Epigenomics data hosted on the UCSC genomics browser site indicates the strongest POL2 binding and DNA methylation in the neighborhood are here
(chr17:37,958,763-37,959,497) and here (chr17:37,953,464-37,954,756). Some readers may be interested in comparing the sites described in this manuscript with those characterized by the epigenomics projects. If the authors will provide hg18 genomic locations throughout the manuscript will make it easy for a reader to go to the UCSC browser and look up the exact locations and place the results into scientific context. Please comment on the location and relevance of the sites that were assessed in this study in comparison to the sites reported by encode.

Section 3. Discretionary Revisions

1. Figure 1, panel B shows three cancerous tissues have HSD17B1 protein levels that are 1 log lower than the median of the rest of the cancerous tissues, and the normal tissues. Are these the tissues identifiable on Figure 1, panel A, as having the lowest mRNA expression? Alternatively, are these three specimens significantly higher than background, or are they essentially zero? A one-sentence commentary by the authors on the correlation (or lack of) between mRNA and protein would be helpful.

2. Figure 1. The mRNA expression levels (Panel A) were all normalized to the geometric mean of a couple of control genes, however the protein levels (Panel B) are reported as absolute optical density obtained from scanning the film. The authors are encouraged to quantify a few randomly chosen background regions of the blot and normalize the protein optical densities to this background number. This would help the reader to appreciate if those three lowest spots on panel B are significantly different from background or if they are essentially zero. This point only comes to the attention of the reader because of the weakness of the intensity of some of the bands shown in the example blot, panel C.

3. Figure 3 shows the results of methylation at 31 cpg sites, obtained from sequencing 10 individual clones of bisulfite-converted PCR products. Were most clones either 5/10 or 10/10 methylated, with few clones having zero methylation? This appears to be the case, which raises the interesting possibility that samples with 50% methylation may have had allele-specific methylation. The authors took the trouble to painstakingly clone and sequence, providing an opportunity to see if the samples with 50% methylation had neighboring cpg sites methylated in cis or in trans. A similar question is relevant for the Aza treated HT29 cells presented in figure 6, panel A. Are the neighboring methylated cpgs in the untreated HT29 cells located on the same strand (and therefore show up in the same clones), or are they evenly assorted between strands (and therefore assort randomly between different clones)? Finally, If 50% methylation of adjacent cpgs in cis vs trans can be inferred from the high-resolution melt data presented in the supplemental figures, the authors are invited to comment on this and the as well.

4. Figure 7, Panel A shows that the rate of conversion of E1 to E2 is low in HT29 cells, and can be significantly increased by Aza C treatment. Panel B shows that this conversion rate is high in SW707 cells, and unaffected by AZA. The authors are encouraged to calculate the slopes of the four relevant lines (nM E2/ug protein per hr) for an easy comparison. The background rate of appearance of E2 in the absence of E1 substrate is precisely zero, and so the plots are lost in
the X axis. The authors are encouraged to set the X axis to intersect the Y axis at a point slightly below zero, so that the reader can easily visualize the rather impressive substrate-dependence of the assay.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.