Author’s response to reviews

Title: DNA HYPERMETHYLATION ASSOCIATED WITH UPREGULATED GENE EXPRESSION IN PROSTATE CANCER DEMONSTRATES THE DIVERSITY OF EPIGENETIC REGULATION

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Author’s response to reviews:

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Dr. Jun Yao
BMC Genomics

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Subject: Revision and resubmission of manuscript MGNM-D-19-00142

Dear Dr. Yao,

Thank you for the opportunity to revise our manuscript entitled “DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation” by Ieva Rauluseviciute, Finn Drabløs and Morten Beck Rye. We appreciated your comments and suggestions on revising the figures and other aspects of the manuscript. Below we describe the changes made to the manuscript point-by-point according to your recommendations.

1. Visualization of gene expression vs methylation, for top genes from each category.
Taking into account the first comment, we visualized gene expression and DNA methylation changes for the Top 150 genes from each regulation pattern group. The changes are displayed as fold changes for the methylation data. The expression data is displayed as scaled from -1 to 1 fold changes for the expression data since they are based on ranks from a meta-analysis. The top 150 genes from each group are visualized in different colors.

2. Figure S1 mainly marked probe locations, ideally this should let the readers appreciate the level of differential methylation between sample groups and the distribution of differential methylated probes plus the direction of gene expression changes.

We used your suggestion to improve Figure S1 for the gene GSC by adding a chart with methylation fold change values for each methylation probe visualized. We updated Figure 5 (Figure 6 in the revised manuscript) similarly for the methylation probes associated with gene TLX1.

3. Figure 4, rather than using absolute basepair distances the authors should consider using normalized relative distances to TSS, exon ½, and the whole gene structure, for example, are UPUP probes more residing at 3’-end and UPDOWN at promoter exon1/2?

We have made a great effort to respond to your third comment by performing an analysis of how significantly hypermethylated and non-differentially methylated probes from UPUP-only and UPDOWN-only groups are located in different gene regions. We overlapped methylation sites with gene 3’UTR, 5’UTR, coding and non-coding exons, introns, first exon, first coding exon and first intron for both groups. We analysed the differences between UPUP-only and UPDOWN-only probes, and also took into account the differences between significantly hypermethylated and non-differentially methylated probes in each of the gene region categories. We did not observe any large changes between the UPDOWN and UPUP groups. The results have been summarized in Table 2.

We discussed your suggestion of using normalized relative distances to TSSs in Figure 4 (Figure 5 in the revised manuscript). However, we decided to keep use distances in basepairs, because we think basepair distances better reflects the biological effect of a methylation site on the transcription of the gene.

4. Do UPUP genes also have UPDOWN probes and vice versa? If yes, can we tell which part is more dominant and the counterpart may be just sidekicks and by-products?

When analysing the initial 27K methylation probes, we observed that genes were consistently classified in the UPUP and UPDOWN groups, and less than 2% of genes with multiple probes in each group are associated with both hypermethylated and hypomethylated probes. When later expanding the probe sets to 450K, we only selected those genes that were associated with hypermethylated and non-differentially methylated probes, resulting in two refined groups of
genes we called UPUP-only and UPDOWN-only. Thus none of the UPUP-only and UPDOWN-only genes are associated with any hypomethylated probes.

We appreciate your time for editing our manuscript and hope that this revised version is suitable for publication in BMC Medical Genomics.

Sincerely,

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