Reviewer's report

Title: Identification of Epigenetically Regulated Genes that Predict Patient Outcome in Neuroblastoma

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Reviewer: Rani E George

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Identification of Epigenetically Regulated Genes that Predict Patient Outcome in Neuroblastoma

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Comments to the Authors

This is an interesting study aimed at identifying epigenetic genes that regulate outcome in neuroblastoma. Four neuroblastoma cell lines were treated with a demethylating agent and a histone deacetylase inhibitor and whole genome expression arrays were analyzed to identify genes activated by such treatment. Data from whole genome methylation arrays were then combined with the expression data to identify candidates silenced in neuroblastoma lines due to methylation. Increased of some of these genes was verified by qRT-PCR. Data from the cell lines were compared with methylation arrays from tumor samples. Eight genes were selected as being methylated in neuroblastoma of which 2 (or 3) showed differential methylation. Low expression of these genes was associated with a poor outcome. Although, the concept for the study is likely to yield important observations, the paper would be enhanced by the addition of more information regarding methylation and survival analyses.

Major Comments

1. How many genes were demethylated and overexpressed in the cell lines, compared to tumor samples? What is number of overlapping genes within the methylation analysis and the expression analysis? It is unclear how many tumor samples were analyzed for methylated and underexpressed genes. Did the methylation status correlate with expression analysis in the tumor samples? In how many tumor samples the candidate gene expression was verified by qRT-PCR?

2. The authors state that expression of certain methylated genes was lower in patients that died from the disease; here more details should be provided, for example, number of patients in each group, the P value etc. in the abstract.

3. Similarly when referring to favorable outcome in subsets with normal expression of the 3 candidate genes – the authors refer to “low” methylation, - this is not clear – how is low versus high methylation defined?

4. Details regarding the survival analysis would be beneficial, for example how
many patients were in each group? What is the overall and disease/progression free survival rate?

5. Please refer to previous publications referring to methylated genes in neuroblastoma for example; what was the status of caspase-8 in this data set?

6. In the conclusion the authors make the statement that “as a prognostic tool, the methylation of most of the genes presented here is just as good or better as the known prognostic risk factors, when it comes to predicting accurate outcome in this set of tumors.” This appears to be an overstatement of the results. Was methylation status independently prognostic in a multivariate analysis? In the different risk groups, what is the effect of methylation in patients over 18 months with stage 4 disease and without tumor MYCN amplification?

7. Figure 1B: Please show methylation specific PCR data for the 3 most significant candidate genes, KRT19, PRKCDDBP, SCNN1A.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests