Reviewer's report

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

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Reviewer: Susan Cohn

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Summary:
The authors treated 4 neuroblastoma cell lines with 5-Aza-dC with or without TSA and analyzed gene expression using whole-genome expression arrays. The data were combined with whole-genome DNA methylation arrays to identify genes that were epigenetically silenced in neuroblastoma. They report 8 genes that are methylated in neuroblastoma, and expression of 2 of the genes was lower in patients that have died from disease compared with patients with no evidence of disease. The authors conclude that “DNA methylation as biomarkers could potentially be used to classify NB tumors in the future and aid in patient stratification.”

Major revisions:
1. Methylation frequency varied amongst the cell lines examined, emphasizing the heterogeneity of these epigenetic changes. Did the authors find any differences between MYCN-amplified cell lines and those that lack MYCN amplification? Was there any phenotype difference between cell lines with a high frequency of methylation vs those that had fewer methylated genes?

2. The authors present their data regarding the clinical significance of gene methylation in an unconventional manner. It would be helpful to see Kaplan Meier curves of overall survival for patients with and without methylation of specific genes and/or combinations of genes. Also, it is unclear how many patients were evaluated. Further detail in the methods and a table showing the biologic characteristics of the patients (ie stage, age, 11q deletion, etc) should be included). This table should also include outcome data. Specific information regarding whether the cohort is representative is critical. If the cohort is not representative, the prognostic significance of the methylated genes can not be evaluated.

3. Mutivariable analyses should also be performed to determine the independent prognostic value of the methylated genes after correcting for risk group.

4. The authors should provide some information in the Discussion regarding what is known about the function of the genes that are epigenetically silenced and rationale regarding why silencing of these genes would be associated with worse outcome.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.