Reviewer's report

Title: Association of RASSF1A and CASP8 promoter methylation in neuroblastoma

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Reviewer: Yoshitaka Sekido

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General

Lazcoz P. et al.: Association of RASSF1A and CASP8 promoter methylation in neuroblastoma. The authors analyzed the loss of heterozygosity status of 3p21 and hypermethylation status of the RASSF1A, NORE1A, BLU, and CASP8 genes in 41 neuroblastic tumors. Twelve cell lines were also studied for the methylation status and expression levels of these genes. They found a statistically significant association between hypermethylation of RASFF1A and CASP8.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. One of the most critical points of this paper is that the methylation status of each gene is not correlated with its mRNA expression except for RASSF1A. From this standpoint, the authors’ conclusion is very ambiguous if hypermethylation of the three genes, BLU1, NORE, and CASP8, is important as an epigenetic alteration in the development of neuroblastoma.

2. The authors showed that there was just a statistical association between the hypermethylation status of RASSF1A and CASP8 in 12 cell lines. However, there seems to be a very weak biological relationship between two gene products, and epigenetic modifications of these genes seem more likely to be independent, and probably due to coincidental events. Therefore, the title of this paper should be rephrased more appropriately. For example: Frequent promoter hypermethylation of RASSF1A and CASP8 in neuroblastoma.

3. The authors should provide the data on MYCN amplification, 1p deletion and the aggressive histological pattern of the tumor by making a new table or incorporating the data in Table 3. If the data are not given in detail, the authors should delete their statement about this from the Abstract.

4. The author’s conclusion, LOH and MSI at 3p21 may be responsible for tumor formation in a small number of neuroblastic tumors, seems unclear. Although the low frequency of LOH at 3p may indicate that a candidate tumor suppressor gene of neuroblastic tumors is not located in this region, MSI at chromosome 3p does not seem to indicate any clear scientific meaning. Since MSI is not usually limited to a specific chromosomal region, which usually implies a phenotype over a whole genome, this conclusion should be rephrased.

5. In Table 3, the term (not determined), for LOH/MSI data is quite confusing. Does it mean non-informative?

6. There seems to be a discrepancy between Table 3 and Figure 1 about the results of D3S3522. Although the data of #48 look like no LOH/MSI, and the one of #77 shows a deletion of one band among three, the results are just described and, respectively, in Table 3. The authors should carefully check the data again.

7. In Figure 2B, C, and D, the authors should give the patient’s number for each lane to be compared with the data in Table 3.

8. The authors should indicate the definition of hypermethylation and hemimethylation.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
How did the authors measure the 75 ng of cDNA that was synthesized from RNA with reverse-transcriptase?

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.