Reviewer's report

Title: Verification of genes differentially expressed in neuroblastoma tumours; a study of potential tumour suppressor genes.

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Reviewer: Barbara Weber

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Thorell and colleagues present a comparison of transcript abundance between neuroblastomas of favorable and unfavorable outcome. This study attempts to identify tumor suppressor genes by recognizing those genes that are downregulated in neuroblastomas with poor prognosis.

Major Compulsory Revisions

The authors suggest that the primary objective for this study is to identify potential tumor suppressor genes. Alterations of many bona-fide tumor suppressor genes, such as CDKN2A and APC, are well documented as early events in some tumors. Thus, the identification of potential tumor suppressor genes by querying those which are downregulated in neuroblastomas of less favorable prognosis (a diagnosis based largely on stage) is flawed. This is further highlighted by the association of CCND1 (a candidate oncogene) expression with tumors of a favorable diagnosis.

Microarray based studies of DNA copy number alterations in neuroblastomas indicate a degree of heterogeneity even those tumors of the same clinical subtype (e.g. MYCN+, 1q-). More recent analyses of neuroblastomas suggest that the same is true at the transcript level. The approach taken by this analysis (due to small sample sizes in the microarray based stages) requires all tumors in the unfavorable group to harbor an alteration to a candidate tumor suppressor. This is not a likely scenario given these previous results.

The three step approach used to identify and validate those genes downregulated in neuroblastomas of a less favorable prognosis is also problematic. First, the method of selection of candidate genes for PCR-based follow up analysis needs improvement. While microarrays serve as a benchmark technique for transcriptome analysis and are a logical starting point for a study such as this, the use of a total of 6 tumors for the initial query is not reasonable. It is likely that with such a small sample size a much larger number of genes could emerge as potential candidates at this stage. The authors indicate that this was based on significance, but don’t define this term. Published microarray data sets may help make this important stage more data rich (and thus, more inclusive).

As the authors point out, neuroblastoma cytogenetics are very well documented. The authors make little attempt to map genes at each stage of gene analysis to
common regions of loss in neuroblastomas (1p, 11q, etc....). This would be especially important to the selection stage as many tumor suppressor genes are mapped to regions of LOH (and homozygous deletions) in a subset of tumors. In other words, genes mapping to these regions of known loss may be a viable stage one selection criteria.

Minor Essential Revisions

1. The presentation of the gene analysis from the microarray-based selection step though qPCR validation is confusing. A venn diagram and a much more detailed flow chart may improve this.
2. The presentation of results appears incomplete and should be expanded. Specifically the results of the gene validation stages need more detail.
3. Some parts of this manuscript are casually presented and require revision. e.g. Page 14 paragraph 2 “Preliminary data from our lab”

Discretionary Revisions
1. Figure 3 is not valuable as the high degree of concordance between qPCR and microarray results is not noteworthy.
2. Table 4 is not worth presenting as the negative results it presents are fully described in the text.

Level of interest: An article of limited interest

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