Peer Review Report

Review Report on Application of the FISH method and High-Density SNP Arrays to the assessment of genetic changes in neuroblastoma -research by one institute

Original Research, Acta Biochim. Pol.

Reviewer: Walentyna Balwierz
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EVALUATION

Q 1 **Please summarize the main findings of the study.**

The article presented to review has six pages of text, two table, nine good quality figures and forth up to date and relevant positions of references. The manuscript is prepared with great precision and the results are presented clearly. The references contain of current papers concerning the issue of the study. The article is written in very clear and logical fashion. The most references contain of current papers concerning the issue of the study. Very interesting and valuable results in Table No. 2, showing the tumor heterogeneity in NBL.
The best methods should be used for these tests and should be systematically verified. The authors conducted studies to detect NMYC amplification using fluorescence in situ hybridization (FISH), currently the most recommended method. They used tissue sections embedded in paraffin as well as impression smears from tumor specimens obtained during patient surgeries for this assessment. The authors rightly believe that fluorescence in situ hybridization of intraoperative tumor smears should be the gold standard used to investigate the presence of N-MYC gene amplification. Tissue sections embedded in paraffin should only be used for this purpose if impression smears cannot be obtained.
Because treatment failures also occur in patients without NMYC gene amplification in tumor cells, it is necessary to search for other prognostic genetic markers. Therefore, patients with neuroblastoma should also undergo investigations for other abnormalities, including chromosomal aberrations and molecular disorders. Hence, the authors rightly believe that it is very important for genetic test results to be based on both fluorescence in situ hybridization and molecular karyotyping using the CytoScan HD array.
The manuscript shows very important issue and in my opinion this paper qualifies for publication in after correction, see below. The prognosis of high-risk neuroblastoma is still not favourable that why each new additional way of diagnostic is very welcome.

Q 2 **Please highlight the limitations and strengths.**

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The main shortcomings of the work are associated with some interpretation of research results - see below.

Q 3 **Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns.**

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The main shortcomings of the work are associated with some interpretation of research results.

The main concerns are as follows:

Lines 49 and 50: The statement that the MYCN gene remains only in one copy at locus 2p24 during amplification is very misleading. Structural changes leading to amplification can take the form of so-called double minute chromosomes (dmins), which are additional extrachromosomal material, or the form of homogeneous staining regions (HSRs), which are homogeneous and duplicated material within the chromosome [Jeison, M., Ash, S., et al., 2010, 2p24 gain region harboring MYCN gene compared with MYCN amplified and nonamplified neuroblastoma: Biological and clinical characteristics., American Journal of Pathology 176 (6), 2616-2625 oraz Van Noesel, M.M., Versteeg, R., 2004, Pediatric neuroblastomas: Genetic and epigenetic “Danse Macabre”, Gene 325 (1-2), 1-15].

According to the literature, dmins are typical for cells derived from tumors, while HSRs are for cell lines, [Schwab, M., Westermann, F., et al., 2003, Neuroblastoma: Biology and molecular and chromosomal pathology., Lancet Oncology 4 (8), 472-480], but without classical karyotype analysis, we cannot say with 100% certainty what occurs in the patient.

Line 132:

The statement that more than 10 MYCN signals indicate amplification is insufficient, because according to the guidelines, the cutoff point was set at 10 signals (amplification ≥10; gain <10) only in the case of disomy of chromosome 2!!! If tumor cells are characterized by polysomy of chromosome 2, then, according to the guidelines, MYCN gene amplification is confirmed if the number of signals tested exceeds the number of control signals by at least 4-fold. (Ambros, P.F., Ambros, I.M., et al., 2009, International consensus for neuroblastoma molecular diagnostics: Report from the International Neuroblastoma Risk Group (INRG) Biology Committee., British Journal of Cancer 100 (9), 1471-1482).

Line 157: The number of probes marking duplications and deletions was provided, but the detection limit was not mentioned (the smallest CNV change reported, as was done for LOH).

Lines 185 and 187: Incorrect numbering of figures or their captions, with descriptions of 5 figures but 9 images inserted.

Line 185: The term "duplication" is reserved for 3 copies of a given chromosomal region. It would be better to use the term "multiplication" = copy amplification, as the authors stated that there are 10 copies, or directly use the term "amplification".

Lines 194 and 195: Whether the patient had ruled out germinal origin of duplication 22q11.21 and ruled out suspicion of Turner syndrome in mosaic, is important especially since it is a female patient?

The minor revisions are as follows:

Line 137: the statement that the analysis started from patient No. 60 is unclear. It would be much better to specify the total number of patients examined using the SNP microarray technique.

Lines 185 and 186: I suggest including the chromosomal locus in parentheses for the MYCN and ALK genes, so that they can be easily located on the figure, as these genes are not displayed in magnification on the graphic.

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Check List

Q 4 **Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List)**

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Q 5 **Is the English language of sufficient quality?**

Yes.

Q 6 **Is the quality of the figures and tables satisfactory?**

Yes.

Q 7 **Does the reference list cover the relevant literature adequately and in an unbiased manner?**

Yes.

Q 8 **Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)**

Not Applicable.

Q 9 **Are the methods sufficiently documented to allow replication studies?**

Yes.

Q 10 **Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication)**

Not Applicable.

Q 11 **Does the study adhere to ethical standards including ethics committee approval and consent procedure?**

Yes.

Q 12 Have standard biosecurity and institutional safety procedures been adhered to?

Yes.

QUALITY ASSESSMENT

Q 13	Originality	■ ■ ■ □ □
Q 14	Rigor	■ ■ ■ ■ □
Q 15	Significance to the field	■ ■ ■ □ □
Q 16	Interest to general audience	■ ■ ■ ■ □
Q 17	Quality of the writing	■ ■ ■ ■ □
Q 18	Overall quality of the study	■ ■ ■ ■ □