HIGH-RESOLUTION OF COMPARATIVE GENOMIC HYBRIDIZATION IMPROVES DETECTION OF CHROMOSOMAL ABERRATIONS WITH PROGNOSTIC SIGNIFICANCE IN NEUROBLASTOMA

INTRODUCTION

Neuroblastoma (NB) is a genetically very heterogeneous pediatric malignant tumor. The clinical course of NB varies markedly. Therefore molecular and cytogenetic markers are studied as strong predictors of clinical outcome, to amend clinical staging and aid in treatment planning. This malignancy is characterized by a broad spectrum of clinical behavior. Low-, intermediate-, and high-risk groups have been defined based upon expected outcome following conventional therapy using both clinical and biological criteria. The criteria currently used to assign risk-group are as follows: Clinical stage, MYCN status, Shimada histology and DNA ploidy. Recently, other cytogenetic changes as 1p, 3p, 11q deletions and 17q rearrangements may also have prognostic value. There are a number of molecular genetic features known which are of prognostic importance in neuroblastoma - expression of different molecular markers as neurotrophin tyrosin kinase receptors (TRKA, B and C), tyrosine hydroxylase, neuroendocrine protein gene product 9.5 (PGP9.5), telomerase reverse transcriptase (hTERT) and vascular endothelial growth factor-A (VEGF-A). Some of them are already candidates stratifying markers. A global view of cytogenetic imbalances in NB patients can be detected by CGH. With recently developed high resolution (HR-CGH) method, we can find aberrations of < 10 Mb.

AIMS

In this study we report about the application of FISH and CGH/HR-CGH method for the detection of chromosomal imbalances in NB patients. In our work, FISH technique and CGH analyses were applied to 49 neuroblastomas of both low and high stage disease and the data were reviewed and correlated with clinical characteristics, including survival analysis.

MATERIAL AND METHODS

Interphase Fluorescence In Situ Hybridization (I-FISH)

dual colour I-FISH on fixed tumour touch imprints or bone marrow smears:
- N-myc gene status: Abbott-Vysis LSI N-myc/CEP – 2 DNA Probe
- upper and lower fluorescence ratio thresholds: 1.15 and 0.85
- total genomic reference DNA labelled with SpectrumRed dUTP
- total genomic test DNA labelled with SpectrumGreen dUTP

Comparative Genomic Hybridisation (CGH)

- total genomic test DNA labelled with SpectrumGreen dUTP (CGH Nick Translation Kit, Abbott-Vysis Inc., Downers Grove, USA)
- total genomic reference DNA labelled with SpectrumRed dUTP (CGH Nick Translation Kit, Abbott-Vysis Inc., Downers Grove, USA)
- upper and lower fluorescence ratio thresholds: 1.15 and 0.85

High-Resolution Comparative Genomic Hybridization (HR-CGH)

- total genomic test DNA labelled with SpectrumGreen dUTP (CGH Nick Translation Kit, Abbott-Vysis Inc., Downers Grove, USA)
- total genomic reference DNA labelled with SpectrumRed dUTP (CGH Nick Translation Kit, Abbott-Vysis Inc., Downers Grove, USA)
- 99.5% dynamic standard reference interval (based on an average of 17 normal CGH analyses) compared to 99.5% confidence interval of the mean ratio profile of the test sample

Digital Image Analysis

- Fluorescence Microscope Olympus BX-61
- CCD Camera COHU 4910
- software: LUCIA G 4.82 – KARYO/FISH/CGH/CGH-Advanced Statistics (Laboratory Imaging Ltd., Prague, Czech Republic)

RESULTS

Event-free survival of 49 patients with neuroblastoma according to risk status.

Comparison of CGH and HR-CGH profiles of the same patient showing advantages of usage of HR-CGH technique.

CONCLUSIONS

The results from our study confirmed different CGH profiles in the three major clinicogenetic subgroups. Using conventional CGH the highest incidence of genetic imbalances was observed on chromosomes 1p, 2p, 3p, 11q and 17q. In addition, by means of HR-CGH we were able to detect clones with whole or partially chromosome losses or gains occurring with low frequency. Our results have illustrated the power of CGH/HR-CGH as a sensitive method for the detection of all clinically important genetic alterations in neuroblastoma with good correlation to other relevant methods, e.g. FISH.

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