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Quantitation of trk-A mRNA by RT-PCR Identifies Biologically and Clinically Distinct Subsets of Neuroblastoma

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Higher expression of the *trk*-A gene encoding a tyrosine kinase nerve growth factor receptor correlates with a good prognosis in neuroblastoma. A method was developed for quantitative analysis of the copy number of *trk*-A mRNA using fluorescent RT-PCR. Co-amplification of *trk*-A cDNA with a known number of copies of a 162 bp deletion product was used as an external standard for quantitation of *trk*-A transcripts per ng of RNA. 116 primary neuroblastomas representing all clinical stages were analysed for *trk*-A mRNA copy number and the results were compared with *MYC*N status, histopathological classification (Shimada), and event-free survival (EFS).

trk-A	No. pts	*MYC*N amplification	Unfavourable histology	EFS
> 970 000	23	4%	13%	85%
36 900–970 000	70	4%	21%	65%
0–35 300	23	39%	75%	0%

Three prognostic groups with different *MYC*N and histopathological characteristics were distinguished by the analysis of *trk*-A mRNA copy number ($P < 0.001$). This study demonstrates quantitative RT-PCR can identify biologically and clinically distinct subsets of neuroblastoma.

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Genes Involved in Metastatic Dissemination and in Response to Chemotherapy in the Neuroblastoma IGR-N-91 Model

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A model of metastasis has been established to study genes involved in the response of neuroblastoma to chemotherapy. Briefly, when the *MYC*N amplified neuroblastoma IGR-N-91 cell line was subcutaneously xenografted into nude mice, malignant neuroblasts dissemination was observed in various tissues (blood, bone marrow and the myocardium) which had given rise to established neuroblastoma metastatic sublines, in culture [1]. Transcript levels of genes involved in multi-drug resistance phenotypes (MDR1, MRP and GST-pi) and in apoptosis (*MYC*N, p53 and bcl-2) were measured by Northern blotting. Cell sensitivity to doxorubicin and cisplatin were determined by MTT assays. Metastatic cells showed drug-resistance phenotypes and *MYC*N and p53 gene over-expression. Whereas expression of the *MDR1* gene was not detectable in parental IGR-N-91 cells, it was significant in neuroblastoma sublines derived from nude mouse tissues. As animals bearing neuroblastoma xenografts were not treated by chemotherapeutic agent capable of activating the *MDR1* gene, it is strongly suggested that MDR1 expression is a marker of invasiveness. In addition, results obtained with this model indicate that chemoresistance in metastatic neuroblastoma is multifactorial and involves not only detoxification genes but also genes implicated in apoptosis.

1. Ferrandis E, Da Silva J, Riou G, Bénard I. Coactivation of the MDRI and MYCN genes in human neuroblastoma cells during the metastatic process in the nude mouse. *Cancer Res* 1994, **54**(8), 2256–2261.