Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 256 (2007) 56–63
CANCER Letters
www.elsevier.com/locate/canlet

Concomitant *DDX1* and *MYCN* gain in neuroblastoma

Raffaella Defferrari [a,b], Gian Paolo Tonini [b,*], Massimo Conte [c], Filippo Papio [d], Angela Rita Sementa [e], Alexander Valent [f], Francesca Schena [g], Patrizia Perri [a,b], Katia Mazzocco [a,b]

[a] *Laboratory of Neuroblastoma Research, Italian Neuroblastoma Foundation, National Institute for Cancer Research (IST), Largo R. Benzi 10, 16132 Genoa, Italy*
[b] *Translational Paediatric Oncology, National Institute for Cancer Research (IST), Largo R. Benzi 10, 16132 Genoa, Italy*
[c] *Hematology and Oncology Department, G. Gaslini Children's Hospital, Largo Gerolamo Gaslini 5, 16147 Genoa, Italy*
[d] *Epidemiology and Biostatistics Section, Scientific Directorate, G. Gaslini Children's Hospital, Largo Gerolamo Gaslini 5, 16147 Genoa, Italy*
[e] *Department of Histopathology, G. Gaslini Children's Hospital, Largo Gerolamo Gaslini 5, 16147 Genoa, Italy*
[f] *Laboratoire de Genomique Cellulaire des Cancers, Institut Gustave Roussy, Unite Mixte de Recherche 8125, Centre National de la Recherche Scientifique, rue Camille Desmoulins 39, 94805 Villejuif Cedex, France*
[g] *Immunology and Rheumatic Diseases Laboratory, Pediatrics II, G. Gaslini Children's Hospital, Largo Gerolamo Gaslini 5, 16147 Genoa, Italy*

Received 5 April 2006; received in revised form 11 May 2007; accepted 18 May 2007

Abstract

DDX1, a gene mapping to the 2p24 region, has been observed to be co-amplified with *MYCN* in neuroblastoma. Co-amplification of the *DDX1* gene is a consequence of the short physical distance between the two genes. Recently, it has been found that neuroblastoma cells can show a low increase in *MYCN* gene copy number, defined as *MYCN* gain. We studied 13 neuroblastomas with *MYCN* gain to evaluate the status of the *DDX1* gene. We investigated *DDX1*/*MYCN* gain by double-colour FISH on interphase nuclei. All cases showed concomitant low extra copy number of *DDX1* and *MYCN*. Heterogeneous distribution of nuclei displaying *DDX1*/*MYCN* gain was observed in almost all tumours, suggesting a clonal evolution of cells with *DDX1*/*MYCN* gain. This is the first report that shows *DDX1* co-gained with *MYCN* in neuroblastoma and indicates that *DDX1* over-representation is closely associated with an increase in *MYCN* copy number in neuroblastoma cells. Since *DDX1* has already been found co-amplified with *MYCN*, *DDX1* gain seems to be a further rearrangement due to the physical proximity of the two genes. Moreover, all patients with *DDX1*/*MYCN* gain show a good overall survival but a high frequency of adverse events.

Keywords: Neuroblastoma; *MYCN*; *DDX1*; Gain; FISH

1. Introduction

Neuroblastoma (NB) is the most common extra cranial solid tumour in children below the age of 5

* Corresponding author. Tel.: +39 010 5737381/487/490/430; fax: +39 010 5737487.
E-mail address: gianpaolo.tonini@istge.it (G.P. Tonini).

0304-3835/$ - see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2007.05.010

years. This tumour shows a great clinical and genetic heterogeneity [1]. Extra copies of the *MYCN* oncogene, which maps to the chromosome 2p24.3 region [2,3], have been detected in 20–30% of NB [4–6]. An increase in *MYCN* copy number ranging between 4 and over 100 (per haploid genome) is defined as *MYCN* amplification [2,7–9] whereas an increase from one to 4, relative to the number of chromosome 2, has been described as *MYCN* gain [9]. *MYCN* gain has recently been observed in about 6–8% of neuroblastomas [10–12]. *MYCN* amplification is associated with more aggressive tumours and is a strong marker of poor prognosis [4,13,14] whereas the meaning of *MYCN* gain remains to be clarified. *MYCN* amplification is frequently associated to *MYCN* over-expression [15] although the role of increased *MYCN* expression is still controversial [16]. The size of *MYCN* amplicon ranges from 100 to 1.500 kb [17] supporting the hypothesis that additional genes mapping in that region can be involved in the amplification process contributing to tumour growth.

In a recent paper, Weber et al. [18] demonstrated that more than one gene located at 2p24–25 can be co-amplified with *MYCN* in human neuroblastoma and that the frequency of gene amplification directly correlates to the physical distance from *MYCN*. *DDX1* (DEAD/H-BOX1) is one of the *MYCN* co-amplified genes; it maps to 2p24.3, 340 kb distal from *MYCN* and belongs to a family of genes that encode DEAD box proteins, putative ATP-dependent RNA helicases [19–21]. It has been shown to act as an oncogene but its biological function is still unexplained [22]. *DDX1* gene has been observed co-amplified with *MYCN* in human neuroblastoma cell lines and in 50–70% of *MYCN* amplified NB primary tumours [23–28]. *DDX1* amplification correlates with increased mRNA expression in primary tumours [24,18] as well as in cell lines [23,17]. The prognostic significance of *MYCN*/*DDX1* co-amplification is still controversial [18,29,30].

Since *DDX1* and *MYCN* are closely located and are co-amplified in neuroblastoma, we investigated the status of the *DDX1* gene in tumours with *MYCN* gain from patients with neuroblastoma.

Here, we report the first evidence of *DDX1* and *MYCN* co-gain in neuroblastoma cells of primary tumours.

2. Materials and methods

From January 2003 to June 2005, 313 primary neuroblastomas from patients enrolled in the Italian Neuroblastoma Registry (INBR) were studied by fluorescence in situ hybridisation (FISH) to evaluate *MYCN* gene status. Informed consent for biological studies was obtained before collection of the samples. Fifty cases (16%) showed *MYCN* amplification, whereas 30 (9.6%) had *MYCN* gain. Given the poor availability of tissue from primary or metastatic tumours at the time of onset of the disease, we studied the status of *DDX1* in 13 cases out of the 30 showing *MYCN* gain. Expression analysis of *MYCN* and *DDX1* was carried out for five cases available for RNA extraction. Clinical data have been collected to evaluate overall survival (OS) and event free survival (EFS).

2.1. Tumour histology

Tumours were classified according to the pathologic classification of the International Neuroblastoma Pathology Committee (INPC) [31]. In 11 cases tissue was available from primary tumours whereas in two cases material was obtained from metastatic sites (lymph node). All but one tumour were classified as neuroblastoma, Schwannian-stroma poor, poorly differentiated. One was classified as ganglioneuroblastoma, nodular classical with a poorly differentiated neuroblastomatous nodule. Tumour cell content of the samples utilised to study *MYCN* and *DDX1* status was determined histologically on the specular half of each sample.

2.2. FISH analysis

Double-colour FISH was performed on interphase nuclei using Chromosome 2p24 (*MYCN*)/Alphasatellite 2 Cocktail Probe (Qbiogene, France) and Chromosome 2 α-Satellite (D2Z) (Oncor Appligene, Illkirch, France) for *MYCN* gene copy number detection and BAC (RP11-422A6) for *DDX1* gene copy number detection. Commercial probes were handled according to the manufacturer's recommendations. The *DDX1* probe was labelled by Biotin-16-dUTP, and FISH was carried out as previously described [32]. For simultaneous detection of *MYCN* and *DDX1* genes, cosmid pNB101 and BAC (RP11-422A6) were directly labelled with Cy™3-dUTP and FluorX™ dCTP (Amersham Pharmacia, UK), respectively. For each tumour and probe combination we surveyed at least 200 nuclei. *MYCN* gain has been also confirmed by the use of N-MYC/LAF cocktail probe (2p24-2q11) (Qbiogene, France) (data not shown). As recommended by the European Neuroblastoma Quality Assessment (ENQUA) group, *MYCN* amplification was defined as a 4-fold or greater increase in *MYCN* signals in relation to the number of chromosome 2, whereas a copy increase up to 4-fold was defined as *MYCN* gain [33].

2.3. RT-PCR

The expression analysis was performed on five available NB samples. Total RNA was isolated from NB samples and from two cell lines (*MYCN* amplified SK-N-BE