Oncogene (1995) 10, 1417–1422

Co-amplification of *MYCN* and a DEAD box gene (*DDX1*) in primary neuroblastoma

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DEAD box proteins are putative RNA helicases that have been implicated in cellular processes involving alteration of RNA secondary structure, such as translation initiation and splicing. These proteins share eight conserved amino acid motifs, including Asp(D)-Glu-(E)-Ala(A)-Asp(D) which is part of a more extended motif. Recently, we have shown that the novel *DDX1* gene containing a DEAD box motif maps to the same chromosome band as *MYCN* at 2p24 and is co-amplified with *MYCN* in retinoblastoma cell lines. Here, we show that the *DDX1* gene is co-amplified with the *MYCN* gene in 2 of three neuroblastoma cell lines and that DDX1 RNA levels correlate with *DDX1* gene copy number. Since amplification of *MYCN* is an indicator of poor prognosis in neuroblastoma, it was of interest to determine whether co-amplification with *DDX1* occurred in clinical samples of neuroblastoma and whether such a finding carried any additional prognostic significance. We determined the gene copy number of *DDX1* in 32 neuroblastoma patient samples (representative of all stages): 13 were *MYCN* amplified and 19 had normal copy numbers of the *MYCN* gene. Of the 13 neuroblastomas that were *MYCN* amplified, seven were also *DDX1* amplified. Of the 19 that were not *MYCN* amplified, none were *DDX1* amplified. This is the first example of a gene that is co-amplified with *MYCN* at a high frequency in neuroblastoma. While there was a trend towards a worse clinical outcome with co-amplification, the numbers were too small to reach significance.

Keywords: gene amplification; neuroblastoma; MYCN; RNA helicase; prognosis

Introduction

Gene amplification is associated with aggressive neoplasms and may be an important mechanism contributing to the growth advantage of a tumor cell and its subsequent clonal expansion (Alitalo and Schwab, 1986; Schwab and Amler, 1990). The process of gene amplification usually involves co-amplification of extensive regions of DNA that share a common origin but which become rearranged with subsequent amplification events (Cowell *et al.*, 1982; Zehnbauer *et al.*, 1988; Akiyama and Nishi, 1991; Amler and Schwab, 1992; Schneider *et al.*, 1992). In human malignancies, gene amplification is more commonly seen in solid tumors. The most thoroughly studied amplified gene in human cancer is the *MYCN* gene in neuroblastoma; an increase in copy number of *MYCN* in this tumor is associated with advanced disease stage and poor prognosis (Brodeur *et al.*, 1984; Seeger *et al.*, 1985; Brodeur and Seeger, 1986).

MYCN is part of the family of *MYC* genes (including *MYCL, MYCC*) with similar structure, sequence homology in coding regions, and similar protein products (Alt *et al.*, 1986; DePinho *et al.*, 1987). All three *MYC* genes have been found to be amplified in a variety of different neoplasms. The *MYC* proteins are found in the nucleus where each can form a complex with another nuclear protein called MAX. The MYC-MAX heterodimers can bind to a specific DNA sequence (CACGTG) are believed to function as transcriptional factors (Blackwood and Eisenman, 1991; Cole, 1991). Recent evidence suggests that MYC proteins may cause increased expression of cyclins and translation initiation factors in fibroblasts, which may lead to their transformation (Jansen-Durr *et al.*, 1993; Rosenwald *et al.*, 1993). Down regulation of cell surface proteins by MYC proteins has also been implicated in altering cell growth and invasiveness (Luscher and Eisenman, 1990). The mechanism by which amplified *MYC* is involved in tumorigenesis is unknown, but cooperation with other oncogenes has been suggested (pim-1 in T-cell lymphomas) (van Lohuizen *et al.*, 1989).

Multiple copies of the *MYCN* gene, which normally maps to chromosome 2p23-24, have been shown to occur in the form of double minute chromosomes or as a single homogeneously staining region randomly incorporated into a chromosome in the form of tandem repeats (Schwab *et al.*, 1983, 1984). Recently, another gene, *DDX1*, has been shown to be co-amplified with *MYCN* in two retinoblastoma cell lines (Godbout and Squire, 1993). *DDX1* is a member of the DEAD box gene family (>30 identified so far in various species) which have eight highly conserved amino acid motifs, one of which is the DEAD (Asp-Glu-Ala-Asp) motif. *DDX1* has been mapped to the chromosomal region 2p24, the same chromosomal band as *MYCN* (Godbout and Squire, 1993). Proteins with the DEAD motif are putative RNA helicases that have been implicated in diverse cellular functions such as translation initiation, ribosomal assembly, RNA splicing, spermatogenesis, embryogenesis, and cell growth and division (Schmid & Linder, 1992).

The finding of co-amplification of these genes in cell lines is intriguing given the presumed function of both

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Received 23 September 1994; revised 28 December 1994; accepted 29 December 1994

in gene expression, one at the transcriptional level and the other, post-transcriptional. In this study, we examine the frequency of *DDX1* gene amplification in neuroblastoma cell lines and in patient samples of neuroblastoma tumors. We correlate *DDX1* gene amplification in neuroblastoma tumors with *MYCN* gene amplification and other prognostic factors in relation to clinical outcome.

Results

Amplification and expression of DDX1 and MYCN in neuroblastoma cell lines

Genomic DNA was extracted from the five neuroblastoma cell lines, IMR32, SK-N-SH, SK-N-MC, BE(2)-C and KAN, and processed for Southern blot analysis. The filter was sequentially hybridized with the DDX1 (Figure 1a–top) and MYCN (Figure 1a–bottom) probes. *DDX1* gene amplification was observed in IMR32 (~30X) and BE(2)-C (~60X) while *MYCN* amplification was observed in IMR32 (~30X), BE(2)-C (~85X) and KAN (~175X). The levels of *MYCN* amplification in these three cell lines is similar to that reported by others (Kohl *et al*., 1984; Montgomery and Melera, 1988; Schneider *et al*., 1991). Neither *DDX1* nor *MYCN* was amplified in SK-N-SH and SK-N-MC.

To determine whether amplification of the *DDX1* gene leads to elevated levels of DDX1 transcription, poly(A)$^+$RNA was extracted from the five neuroblastoma cell lines, run on a denaturing agarose gel and transferred to nitrocellulose filter. The filter was sequentially hybridized with the DDX1 (Figure 1b–top) and MYCN (Figure 1b–bottom) probes. Elevated DDX1 RNA levels were observed in the two *DDX1*-amplified cell lines, IMR32 (~20X) and BE(2)-C (~50X). As expected, MYCN RNA was also overexpressed in the three cell lines with *MYCN* gene amplification, IMR32, BE(2)-C and KAN. Because no MYCN RNA could be detected in the non-amplified cell lines, it was not possible to determine the fold increase in MYCN RNA levels in these three cell lines. However, there was a general correlation with gene copy number and RNA levels in IMR32 and KAN. MYCN RNA levels in the BE(2)-C cell line was lower than expected based on *MYCN* gene copy number.

Amplification of DDX1 and MYCN in neuroblastoma tumors and clinical outcomes

The 32 cases of neuroblastoma selected for this analysis included three stage 1, seven stage 2 (three stage 2A and four stage 2B), two stage 3, 17 stage 4 and three stage 4S tumors. The clinical and laboratory results are tabulated in Table 1. The *MYCN* gene was amplified in 13 tumors, including two from stage 2B, nine from stage 4 and two from stage 4S. Of these 13 tumors, seven also had amplified copies of the *DDX1* gene (six from stage 4 and one from stage 4S). All the tumors that had normal copy numbers of the *MYCN* gene were also not amplified for the *DDX1* gene.

The copy number of the amplified genes in the 13 neuroblastoma tumors described above was measured by densitometric scanning (Tables 1 and 2). The two

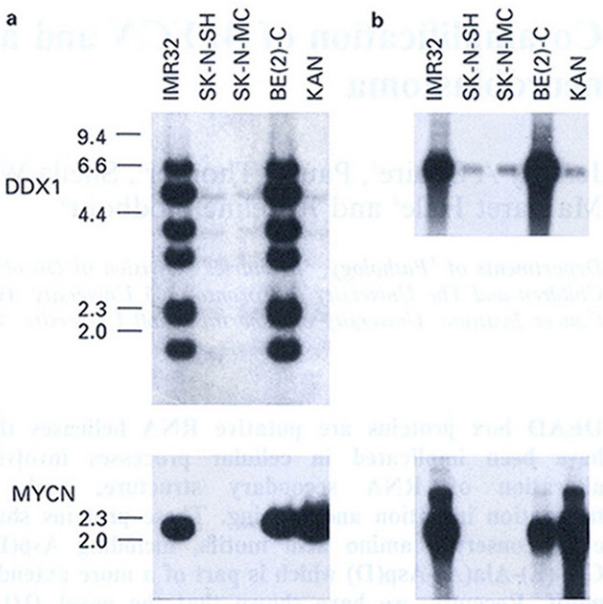

Figure 1 Southern and Northern blot analyses of DDX1 and MYCN in neuroblastoma cell lines. (**a**) Ten μg of genomic DNA from each of five neuroblastoma cell lines (IMR32, SK-N-SH, SK-N-MC, BE(2)-C and KAN) were digested with EcoRI, processed for Southern blotting and hybridized with ^{32}P-labelled DDX1 (top) and MYCN (bottom) probes. Markers (in kb) are indicated on the left. (**b**) Two μg of poly(A)$^+$RNA from IMR32, SK-N-SH, SK-N-MC, BE(2)-C and KAN, respectively, were loaded in each lane and hybridized with the DDX1 (top) and MYCN (bottom) probes. The DDX1 transcript (~3 kb) migrated at a slightly slower rate than the MYCN transcript (3.1 kb)

stage 2B neuroblastomas which had normal copies of *DDX1* had 4–12 and 20–40 copies of the *MYCN* gene, respectively. Of the nine stage 4 tumors, three were only amplified for *MYCN* (two had 5–10 copies of the *MYCN* gene while the third had 50–100 copies). Of the remaining six tumors co-amplified for *MYCN* and *DDX1*, there was a general correlation between the copy number of both genes. One tumor had 5–10 and 8–13 copies of the *DDX1* and *MYCN* genes, respectively; four had >20 copies of both genes while the sixth tumor had >50 copies of *MYCN* and <20 copies of *DDX1*. The only stage 4S tumor that was co-amplified for *DDX1* and *MYCN* had <20 copies of both genes.

The clinical outcomes of the 13 patients whose tumors showed amplification of *MYCN* were compared (Table 2). Of the six patients with *MYCN* amplified but not *DDX1* amplified, three were dead, all stage 4. The two stage 2B patients with only *MYCN* amplified were alive and disease-free at 35 and 53 months, respectively, and the one stage 4S patient was alive with disease at 62 months. Of the seven patients with both genes amplified, four were dead, all from stage 4. Two patients with stage 4 disease were alive and disease-free at 20 and 43 months, respectively. The first tumor had 30–50 copies of *DDX1* and >100 copies of *MYCN* and the other had 8–13 copies of *DDX1* and 5–10 copies of *MYCN*. The third surviving patient had stage 4S disease and was alive and disease-free at 32 months. This patient's tumor had 5–10 copies of *MYCN* and 10–20 copies of *DDX1*. Of the eight stage 4 patients with tumors that were neither *MYCN* nor *DDX1* amplified, one was free of disease at 46 months,

Table 1 Clinical and laboratory data for the 32 patients with neuroblastoma

Patient	*Age at diagnosis*	*Stage (INSS)*	*Histology (Shimada)*	*DNA index*	*MYCN copy no.*	*DDX1 copy no.*	*Status (as of 12/94)*	*Treatment*
1	1 yr 3 mo	1	Fav	1.4	No Amp	No Amp	Alive after 31 mo Complete response	S
2	1 yr	1	Fav	1.0	No Amp	No Amp	Alive after 29 mo Complete response	S
3	2 yr	1	Unfav	1.5	No Amp	No Amp	Alive after 28 mo Complete response	S
4	8 mo	2A	Unfav	1.8	No Amp	No Amp	Alive after 31 mo Complete response	S
5	7 mo	2A	Fav	1.3	No Amp	No Amp	Alive after 39 mo Complete response	S
6	6 mo	2A	Fav	1.4	No Amp	No Amp	Alive after 33 mo Very good partial response	S
7	7 mo	2B	Fav	1.4	No Amp	No Amp	Alive after 28 mo Complete response	S
8	3 yr 9 mo	2B	Unfav	ND	No Amp	No Amp	Relapsed after 23 mo Alive with disease after 70 mo	C > C + R > S
9	3 yr 3 mo	2B	Unfav	1.8	4-12	No Amp	Relapsed after 11 mo Alive with disease after 35 mo	S > C + R
10	1 yr 4 mo	2B	Unfav	1.5	20-40	No Amp	Alive after 53 mo Present status unknown	S > C
11	10 mo	3	Fav	ND	No Amp	No Amp	Alive after 57 mo Complete response	C > S
12	6 yr 2 mo	3	Unfav	1.4	No Amp	No Amp	Relapsed after 55 mo Dead after 116 mo	C + R > C > C + R > R > BMT
13	4 yr 7 mo	4	Unfav	ND	No Amp	No Amp	Dead after 12 mo (from complications)	C > ABMT
14	2 mo	4	Unfav	ND	No Amp	No Amp	Alive after 46 mo Complete response	C
15	4 yr 5 mo	4	Unfav	1.1	No Amp	No Amp	Relapsed after 16 mo Dead after 38 mo	C > S > R > C + R > ABMT
16	1 yr 3 mo	4	Fav	ND	No Amp	No Amp	Very good partial response Dead after 17 mo	C > S + R > ABMT
17	5 yr 9 mo	4	Fav	ND	No Amp	No Amp	Dead after 10 mo	C > S > R
18	2 yr 7 mo	4	Unfav	ND	No Amp	No Amp	Relapsed after 19 mo Alive with disease after 43 mo	C > S + R > ABMT
19	4 yr 5 mo	4	Unfav	1.6	No Amp	No Amp	Relapsed after 20 mo Dead after 22 mo	C > S > ABMT
20	1 yr 8 mo	4	Unfav	ND	No Amp	No Amp	Relapsed after 18 mo Dead after 23 mo	C > ABMT
21	1 yr 3 mo	4	Fav	ND	5-10	No Amp	Relapsed after 8 mo Dead after 9 mo	C > R
22	2 yr 2 mo	4	Unfav	1.0	5-10	No Amp	Relapsed after 23 mo Dead after 24 mo	C > R > ABMT
23	1 yr 5 mo	4	Unfav	1.3	5-10	8-13	Alive after 43 mo Complete response	C > R
24	13 yr 1 mo	4	Unfav	ND	50-100	No Amp	Relapsed after 7 mo Dead after 8 mo	C
25	1 yr 6 mo	4	Unfav	1.0	50-100	10-20	Relapsed after 16 mo Dead after 17 mo	C > S
26	9 yr 9 mo	4	Unfav	ND	> 100	30-50	Relapsed after 30 mo Dead after 39 mo	C > S
27	4 yr 7 mo	4	Unfav	ND	> 100	30-50	Alive after 20 mo Complete response	S > C > S > ABMT
28	1 yr 8 mo	4	Unfav	ND	> 100	80-110	No response Dead after 28 mo	C + R
29	Birth	4	Unfav	ND	> 100	40-60	Dead after 6 mo	C + R > ABMT > C + R
30	3 mo	4S	Unfav	ND	No Amp	No Amp	Alive after 27 mo Complete response	C > S
31	10 mo	4S	Unfav	0.5	5-10	10-20	Alive after 32 mo Very good partial response	S > C > ABMT
32	3 mo	4S	Unfav	1.0	25-50	No Amp	Relapsed after 14 mo Alive after 62 mo Complete response	C + R > R

S = surgery; C = chemotherapy; R = radiotherapy; ABMT = autologous bone marrow transplant; ND = not done; > in Treatment column = prior to

one was alive with disease at 43 months and six were dead. The one stage 4S patient with neither *MYCN* nor *DDX1* amplified was disease free at 27 months.

Statistical analysis showed no correlation between any of the parameters tested (age, stage, histology, DNA index, *MYCN* copy number, *DDX1* copy number). When relapse-free survival was analysed, only age, stage and DNA index appeared significant. When overall survival was analysed, only age and stage appeared significant. *DDX1* amplification carried no significant correlation with survivial or known prognostic indicators. Of note, *MYCN* gene amplification could also not be shown to be significant in terms of survival.

Table 2 Patient stage, copy numbers and survival data of *MYCN* and *DDX1* amplified neuroblastomas

Patient	*Stage*	*DDX1 copy number*	*MYCN copy number*	*Status*
9	2B	No Amp	4-12	A
10	2B	No Amp	20-40	A
21	4	No Amp	5-10	D
22	4	No Amp	5-10	D
23	4	8-13	5-10	A
24	4	No Amp	50-100	D
25	4	10-20	50-100	D
26	4	30-50	>100	D
27	4	30-50	>100	A
28	4	80-100	>100	D
29	4	40-60	>100	D
31	4S	10-20	5-10	A
32	4S	No Amp	25-50	A

Discussion

Gene amplification is rare in human tumors. It is therefore intriguing that 40% of all neuroblastoma tumors are amplified for the oncogene *MYCN* and that the presence of >10 copies of this gene is associated with an unfavorable outcome (Seeger *et al.*, 1985). This is one of the clearest examples of a clinically relevant mutation of a dominant oncogene in a human cancer and yet very little is understood concerning the basic biology of this mutation (Schwab and Amler, 1990). The proteins encoded by the *MYC* family are involved in the control of cell proliferation and differentiation, and aberrant expression of MYC proteins is believed to be central to the malignant process (Luscher and Eisenman, 1990).

Although increased copies of the *MYCN* gene correlate with a poor prognosis, about 60% of stage 4 (i.e. aggressive) neuroblastomas lack this change, implying that other factors are important in determining a poor outcome. We were therefore especially interested in determining whether any of these non *MYCN*-amplified tumors were amplified for the DEAD box gene, *DDX1*. We found, however, that of the 19 tumors with normal copies of the *MYCN* gene (including eight stage 4 patients), none were amplified for *DDX1*.

We did find that seven of 13 neuroblastoma tumors amplified for *MYCN* were also amplified for *DDX1* and this only occurred in stage 4 and 4S. These results raised the question as to whether amplification of *DDX1* might be of significance in the clinical outcome of this tumor, or more specifically, might co-amplification of both genes identify a subgroup of neuroblastoma patients with a less favorable response to treatment? In this study, none of the correlations of *DDX1* copy number with other known prognostic markers or survival reached statistical significance. Overall, three of six (50%) of patients with tumors amplified for *MYCN* only had died, whereas four of seven (57%) of patients with tumors amplified for both *MYCN* and *DDX1* had died. This lack of correlation may be due to the small number of patients studied since established prognostic indicators such as DNA index and *MYCN* copy number also failed to reach statistical significance. A larger study needs to be carried out to establish whether *DDX1* serves as an independent prognosticator of survival or whether a unique subcategory of neuroblastoma tumors can be defined based on *MYCN* and *DDX1* co-amplification.

With rare exceptions, MYCN overexpression is achieved by increasing the gene copy number per cell rather than by up-regulating basal expression of MYCN RNA or protein (Seeger *et al.*, 1985; Cohn *et al.*, 1991). This suggests that there may be a biological preference for the amplification process perhaps as the result of co-amplifying additional DNA sequences. Since the amplified units (amplicons) range from hundreds to thousands of kilobase pairs in size, it is logical to postulate that other genes may be co-amplified with *MYCN* (Stark and Wahl, 1984). However, co-amplification of *MYCN* with other genes has rarely been described in neuroblastoma (Schwab and Amler, 1990). One exception is the ornithine decarboxylase (*ODC1*) gene which maps to chromosome 2p24-25 and which has been shown to be co-amplified with *MYCN* in one of six primary neuroblastomas (Tonin *et al.*, 1989). Two other genes located on band 2p24-25, (encoding the M2 subunit of ribonucleotide reductase and a 55 000-Daltons protein), were not co-amplified with *MYCN*. Since a number of investigators have found that rearrangements are common in the neuroblastoma amplicons (Shiloh *et al.*, 1986; Akiyama and Nishi, 1991; Amler and Schwab, 1992), one may postulate that unless there is selection for a particular gene product, it is unlikely that the gene will be amplified. Based on Southern blot analyses, *DDX1* is a relatively large gene (>30 kilobase pairs), there are no gross rearrangements of this gene in amplified cells and all the bands (from the amplified tumors) that hybridize to DDX1 cDNA are amplified at similar levels. Furthermore, although it was not possible to study *DDX1* gene expression in the tumor samples, there was a correlation between *DDX1* gene copy number and DDX1 RNA levels in neuroblastoma cell lines. These data suggest that *DDX1* amplification may not be incidental to *MYCN* amplification but may be of relevance to tumor growth. Co-amplification of two or more genes has been described in other tumors: eg., co-amplification of *ERBB2* and *ERBBA* in mammary carcinomas (van de Vijver *et al.*, 1987) and co-amplification of multiple genes from 12q13-14 in sarcomas, the two most common being the cyclin dependent kinase, *CDK4*, and *SAS*, postulated to have a role in signal transduction and growth control (Forus *et al.*, 1993; Khatib *et al.*, 1993; Reifenberger *et al.*, 1994). In the case of mammary carcinomas, van de Vijver *et al.* (1987) concluded that amplification of *ERBBA* was fortuitous because this gene was not over-expressed in the tumors.

At the present time, we can only postulate as to the function of DDX1. Comparison of the predicted amino acid sequence of DDX1 with that of other DEAD box proteins implicated in translation initiation (such as elF4A1) or RNA splicing (such as SPP81) reveals a similar level of homology (Godbout and Squire, 1993). Furthermore, we have recently reported significant homology between a 128 amino acid region of DDX1 (located between conserved motifs la and lb) and a 144 amino acid subfragment of the heterogeneous nuclear ribonucleoprotein U (hnRNP U) (Godbout *et al.*, 1994). HnRNP U participates in the processing of hnRNA to mRNA and has RNA binding activity

(Kiledjian and Dreyfuss, 1992). Additional study of DDX1 is required to determine first of all whether it does have RNA binding and RNA helicase activity and second, to determine the biochemical pathways in which it is implicated. A provocative hypothesis is that the DDX1 protein could directly affect MYCN mRNA processing or stability or the rate of translation of the MYCN protein. Formation of an RNA-RNA duplex at the 5′ end of MYCN mRNA has previously been reported and is believed to have a role in the modulation of RNA processing (Krystal *et al.*, 1990). Alternatively, MYCN could play a role in the regulation of DDX1 RNA levels through its transcriptional activator function.

In conclusion, we report co-amplification of the *MYCN* and *DDX1* genes in a subset of stage 4 neuroblastoma tumors. We suggest that there may be interaction between the MYCN and DDX1 gene products. Larger studies are needed to determine whether co-amplification or over-expression of these two genes may be common in a subcategory of tumors with a less favorable response to treatment.

Materials and methods

Case material

DNA extracted from 32 neuroblastoma tumors was obtained from the Department of Pathology, The Hospital for Sick Children, where it had been stored at −70°C. Each tumor sample had previously been digested with proteinase K and the DNA extracted with phenol and chloroform and precipitated with ethanol. The DNA was digested with EcoRI (Boehringer Mannheim, Germany) and the DNA concentration measured using a spectrophotometer. DNA from fresh human tonsil and a human neuroblastoma cell line (IMR32) known to contain 50 copies of *MYCN* (Kohl *et al.*, 1984), both digested with EcoRI, were used as negative and positive controls, respectively.

Tissue sections from all tumors were reviewed by a pediatric pathologist (PT) to confirm the diagnosis of neuroblastoma and to assign a category according to the Shimada classification (Shimada *et al.*, 1984). DNA index (ploidy) as assessed by flow cytometry was recorded when available. The charts of all patients were reviewed by a pediatric oncologist (SW) and age, stage according to INSS (Brodeur *et al.*, 1988), relapse-free survival and overall survival were determined for each patient.

For statistical analysis, all parameters except stage were divided into 'favorable' and 'unfavorable' categories. Unfavorable categories include: age >12 months, unfavorable Shimada classification, diploid or tetraploid DNA index (Look *et al.*, 1991), *MYCN* gene-amplified and *DDX1* gene-amplified. Possible correlations between age at presentation, stage, histology, DNA index, *MYCN* gene amplification and *DDX1* gene amplification were tested by multivariate analysis of variance. For relapse-free survival and overall survival, Cox regression analysis was performed, and each parameter was tested by log-rank test on a Kaplan-Meier plot.

Probe preparation

MYCN levels were assessed using a 1.0 kb EcoRI–BamHI human MYCN cDNA insert from pNB-1 (Schwab *et al.*, 1983). This probe is specific for exon 2 of *MYCN*. The DDX1 probe was a 1.6 kb EcoRI insert from clone 1042 (Godbout and Squire, 1993). The human Jκ gene which is located at chromosome 2p14 and which represents the joining region of the immunoglobulin kappa light chain genes served as the control for DNA loading. The probe insert size is 1.8 kb and hybridizes to a 9.4 kb germline band in EcoRI-digested human DNA. The Jκ and DDX1 probes were labelled by nick translation while the MYCN probe was labelled using the random primer method (Amersham Corp., Arlington Heights, IL).

Southern and Northern blot analyses of neuroblastoma cell lines

The IMR32, SK-N-SH, SK-N-MC neuroblastoma cell lines were obtained from the American Type Culture Collection. The KAN and BE(2)-C cell lines were obtained from Dr Walter Dixon (University of Alberta, Canada). The origins of the five cell lines are described in Biedler *et al.* (1983). Genomic DNA extraction was according to Maniatis *et al.* (1989). Ten μg of each DNA was digested with EcoRI, run on a 1% gel and transferred to nitrocellulose filter. The filter was hybridized with the DDX1 probe, stripped using 0.1 N NaOH in 2×SSC, and re-hybridized with the MYCN probe. Poly(A)$^+$RNA was extracted from the five neuroblastoma cell lines using the hot phenol method and oligo(dT)-cellulose chromatography as previously described (Godbout *et al.*, 1992). Two μg of each RNA preparation was electrophoresed in a 6% formaldehyde–1.4% agarose gel in MOPS buffer (Godbout *et al.*, 1992). The RNA was transferred to filter and sequentially hybridized with the DDX1 and MYCN probes. Quantitation was by densitometric scanning of autoradiograms at different exposures using an LKB Ultroscan XL laser densitometer and the Gel-Scan XL V2.1 program (Pharmacia, Sweden).

Southern blot analysis of tumor samples

Serial dilutions of all restriction endonuclease-digested DNA samples were size-fractionated on 0.8%. TRIS-borate/EDTA agarose gels and transferred to nylon membranes (Boehringer Mannheim, Germany). Following prehybridization with Hybrisol (Oncor Inc., Gaithersburg, MD), the membranes were sequentially hybridized with the MYCN, Jκ and DDX1 probes. The membranes were washed to a final stringency of 0.2× SSC at 60°C for 15 min and exposed for 24 to 48 h at −70°C to Kodak XAR-5 film (Rochester, N.Y.) using intensifying screens.

The MYCN, Jκ and DDX1 hybridization signals on the autoradiograms were quantitated by densitometric scanning using a Molecular Dynamics computing densitometer model 300AV (Molecular Dynamics Corp., CA). The MYCN signal of each sample was normalized by comparison with the Jκ signal in each sample lane and then compared with the DDX1 signal.

Acknowledgements
We would like to thank Mr Blair Gerrie for excellent technical assistance with the project. This work was supported by grants from the National Cancer Institute of Canada with funds from the Terry Fox Marathon of Hope (RG) and the Canadian Cancer Society (JS).

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