Pergamon

European Journal of Cancer, Vol. 33, No. 12, pp. 1911–1916, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00284-0

Original Paper

Evidence that the *MYCN* Oncogene Regulates *MRP* Gene Expression in Neuroblastoma

M.D. Norris,[1] S.B. Bordow,[1] P.S. Haber,[2] G.M. Marshall,[1] M. Kavallaris,[1] J. Madafiglio,[1] S.L. Cohn,[3] H. Salwen,[3] M.L. Schmidt,[4] D.R. Hipfner,[5] S.P.C. Cole,[5] R.G. Deeley[5] and M. Haber[1]

[1]Children's Leukaemia and Cancer Research Centre, Sydney Children's Hospital, High Street, Randwick, Sydney, N.S.W. 2031; [2]Department of Gastroenterology, Prince of Wales Hospital, Sydney, Australia; [3]Department of Pediatrics, Northwestern University, Chicago, Illinois; [4]Department of Pediatrics, University of Illinois, Chicago, Illinois, U.S.A.; and [5]Cancer Research Laboratories, Queens University, Kingston, Ontario, Canada

We have recently shown that expression of the multidrug resistance-associated protein (*MRP*) gene is a powerful prognostic indicator in childhood neuroblastoma and have suggested that the *MYCN* oncogene may regulate *MRP* gene expression. To address this hypothesis, we have examined the relationship between *MYCN* and *MRP* gene expression in neuroblastoma tumours and cell lines. *MYCN* and *MRP* gene expression were highly correlated in 60 primary untreated tumours both with ($P = 0.01$) and without *MYCN* gene amplification ($P < 0.0001$). Like *MRP*, high *MYCN* gene expression was significantly associated with reduced survival, both in the overall study population and in older children without *MYCN* gene amplification (relative hazards = 13.33 and 19.61, respectively). Inhibition of *MYCN*, through the introduction of *MYCN* antisense RNA constructs into human neuroblastoma cells *in vitro*, resulted in decreased *MRP* gene expression, determined both by RNA–PCR and Western analysis. The data are consistent with *MYCN* influencing neuroblastoma outcome by regulating *MRP* gene expression. © **1997 Elsevier Science Ltd.**

Key words: multidrug resistance, multidrug resistance-associated protein (*MRP*) gene, neuroblastoma, *MYCN* oncogene, polymerase chain reaction (PCR), antisense RNA
Eur J Cancer, Vol. 33, No. 12, pp. 1911–1916, 1997

INTRODUCTION

AMPLIFICATION OF the *MYCN* oncogene is one of the most powerful indicators of poor outcome in childhood neuroblastoma [1, 2]. *MYCN* is involved in growth and differentiation of neuroblastoma cells [3, 4] and clearly contributes to the malignant phenotype of this disease [5]. Nevertheless, its precise role in neuroblastoma pathogenesis remains to be elucidated. *MYCN* encodes a nuclear phosphoprotein which appears to act as a transcriptional regulator. However, the specific target genes regulated by *MYCN*, either in this or other malignancies, are still undefined.

The risk of treatment failure in patients whose tumours display *MYCN* gene amplification is high [1] and is largely attributable to simultaneous resistance to a diverse range of structurally and functionally unrelated drugs. The association of *MYCN* amplification with clinical multidrug resistance raises the possibility that *MYCN* may be involved in the regulation of critical drug-resistance genes. Amongst genes known to mediate a multidrug-resistance phenotype *in vitro*, the best characterised are the *MDR*1 gene, encoding P-glycoprotein [6], and the multidrug resistance-associated protein (*MRP*) gene [7]. We have recently shown that *MRP* gene expression is significantly higher in tumours with *MYCN* gene amplification [8, 9] and that expression of the *MRP* gene is a powerful predictor of outcome in primary untreated neuroblastoma [9]. Moreover, we have previously demonstrated coordinated downregulation of the *MYCN* and *MRP* genes following retinoic acid-induced differentiation of neuroblastoma cell lines [8]. As a result of these findings, we have

Correspondence to M. Haber.

hypothesised that the *MYC*N oncogene regulates expression of *MRP*, thereby modulating the response of neuroblastoma to cytotoxic drugs.

To address this hypothesis, we analysed *MYC*N expression in 60 primary untreated neuroblastoma tumours whose *MRP* expression and *MYC*N gene copy number had previously been determined and related *MYC*N expression both to *MRP* expression and clinical outcome. We also utilised neuroblastoma cells transfected with *MYC*N antisense RNA constructs to investigate the effect of *MYC*N down regulation on *MRP* expression. Collectively, the data provide evidence consistent with the hypothesis that *MYC*N influences neuroblastoma outcome by regulating *MRP* gene expression.

MATERIALS AND METHODS

Patients and tumour specimens

Sixty primary untreated neuroblastoma samples, obtained either from the Neuroblastoma Tumor Bank of the Pediatric Oncology Group, U.S.A. or from the Sydney Children's Hospital (formerly the Prince of Wales Children's Hospital), Sydney, Australia, have been described previously [9]. All tumours had previously been subjected to Southern blot analysis to determine the number of copies of the *MYC*N oncogene per haploid genome [1, 10] and tumours were classified as having *MYC*N amplification where more than three *MYC*N copies were present. In addition, the level of *MRP* gene expression had been determined in each tumour using RNA–PCR analysis [9]. Outcome measures studied were survival, defined as time from diagnosis to death and event-free survival, defined as time from diagnosis to the first major event (relapse, failure to achieve remission or death).

Cell lines

NBL-S [11] is a *MYC*N non-amplified human neuroblastoma cell line established in one of our laboratories (S.L.C.). The NBL-S-derived *MYC*N antisense transfectant cell lines NBAS-4 and NBAS-5 and their vector-only transfectant control, NBV-1, were developed in the same laboratory and have been described previously [12]. Briefly, the antisense cell lines were derived by cotransfecting into the NBL-S cells a $pCMV_2$ vector containing a 1.2 kb *MYC*N cDNA insert in the antisense orientation together with the pSV2neo plasmid, followed by isolation of neomycin-resistant clones. The BE(2)-C human neuroblastoma cell line with *MYC*N gene amplification, and the drug-resistant MCF7/VP human breast cancer cell line [13] with *MRP* gene amplification, were generously supplied by Dr J. Biedler (Memorial Sloan-Kettering Cancer Center, New York, U.S.A.) and by Dr E. Schneider (Wadsworth Center for Laboratories and Research, Albany, New York, U.S.A.), respectively. All lines were maintained as monolayer cultures at 37°C in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum and were free of mycoplasma.

Analysis of MYC*N gene expression by the polymerase chain reaction (RNA–PCR)*

The synthesis of complementary DNA (cDNA) and the competitive polymerase chain reaction (RNA–PCR) assay we employed have been described previously [8, 9]. Aliquots of cDNA corresponding to 50 ng of RNA were amplified for 30 cycles and the *MYC*N target gene sequence was co-amplified with a control gene sequence (β_2-microglobulin), using gene-specific oligonucleotide primers, described elsewhere [8]. Following triplicate PCR analyses and polyacrylamide gel electrophoresis of PCR products, the level of expression of *MYC*N in each tumour was determined by densitometric scanning of photographic negatives and was expressed relative to the level of control β_2-microglobulin gene expression.

Western analysis

For MRP analysis using monoclonal antibody (MAb) QCRL-1 [14], protein from cell membranes was prepared as described previously [15]. For analysis of *MYC*N using MAb NCMII 100 (Santa Cruz Biotechnology, Inc. Santa Cruz, California, U.S.A.), whole cell lysates were prepared as follows. Cells were suspended in RIPA buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) and incubated on ice (30 min). Following homogenisation and a further incubation on ice, remaining whole cells, nuclei and debris were removed by centrifugation (15 000*g*; 20 min; 4°C). Protein samples (10 μg) diluted in 2X Laemmli buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, pH 6.8) were electrophoresed on standard 7% or 10% SDS–polyacrylamide gels, prior to electrophoretic transfer on to nitrocellulose membranes. Membranes were blocked in 5% skimmed milk in TBS (0.02 M Tris-HCl, 0.15 M NaCl, pH 7.6) containing 0.1% Tween for 1 h at room temperature before washing three times in the same solution and incubation with primary antibody (MAb QCRL-1 at 1:250 dilution and MAb NCMII 100 at 1:1000 dilution) in 0.5% skimmed milk/TBS at room temperature for 1 h. Membranes were washed again as previously, incubated with secondary antibody (antimouse Ig antibody–horseradish peroxidase conjugate; Amersham, Arlington Heights, Illinois, U.S.A.) in 0.5% skimmed milk/TBS at room temperature for 1 h, and washed again prior to detection using the ECL Chemiluminescence Western Blotting detection kit (Amersham, Arlington Heights, Illinois, U.S.A.).

Statistical analysis

The relationship between levels of *MYC*N and *MRP* gene expression in the neuroblastoma tumours was analysed by linear regression, whereby the PCR ratio for *MRP* expression was related to that of the *MYC*N gene for each sample. For the survival analyses, the *MYC*N PCR ratio of each individual tumour was categorised as 'low' or 'high' by dichotomising around the median *MYC*N PCR ratio obtained from all 60 tumour specimens [9]. Survival analyses involving *MRP* gene expression were performed as described previously [9]. Survival analyses were performed according to the method of Kaplan and Meier, and comparisons of outcome between subgroups were performed by the log-rank test for univariate comparisons, using two-tailed tests. Relative hazards were determined using the Cox proportional hazards regression model. Survival probabilities and relative hazards are given with 95% confidence intervals. Differences between groups of tumours or cell lines in terms of their *MRP* or *MYC*N PCR ratios were assessed by Student's *t*-test, using two-sided *P* values.

RESULTS

MYC*N and* MRP *gene expression in primary neuroblastoma*

The relationship between *MYC*N and *MRP* gene expression was initially analysed in the overall study population of 60 primary neuroblastoma specimens (Table 1). Consistent with our previous findings [8], linear regression analysis

Table 1. *Correlation between* MYC*N and* MRP *gene expression in primary untreated neuroblastoma*

Study population	*n*	Correlation coefficient (*R*)	*P* value
All patients	60	0.74	< 0.0001
Single-copy *MYC*N	47	0.78	< 0.0001
Amplified *MYC*N	13	0.67	0.01
Age > 1 year	31	0.85	< 0.0001
Age < 1 year	29	0.49	0.007

revealed a highly significant correlation between expression of these two genes ($R = 0.74$). However, since we have previously shown that the highest levels of *MRP* expression are present in tumours displaying *MYC*N gene amplification [8, 9], it was important to determine whether a similarly close relationship between *MYC*N and *MRP* gene expression would be observed in the subset of tumours without *MYC*N amplification. Linear regression analysis revealed that in these tumours, the relationship between *MRP* and *MYC*N gene expression was even stronger ($R = 0.78$) than that which had been observed in the overall study population (Figure 1 and Table 1) despite the smaller sample size. *MYC*N and *MRP* gene expression were also significantly correlated in the subset of tumours having multiple copies of the *MYC*N oncogene (Table 1) and the slopes of the regression lines in the analyses performed on the non-amplified and amplified tumours were almost identical (0.531 and 0.565, respectively). When the overall study population was subdivided according to different criteria, strong correlations between *MRP* and *MYC*N gene expression were observed in each subgroup analysed. Thus, expression of these two genes was highly correlated both in children aged greater than 1 year at diagnosis, who tend to have poor outcome in this disease, and also in infants, who tend to have a good prognosis (Table 1).

MYC*N gene expression and outcome*

Cumulative survival according to expression of the *MYC*N gene is shown in Figure 2. High *MYC*N expression was strongly associated with reduced survival. For the overall study population, the 5-year cumulative survival rates of the groups with high- and low-level *MYC*N expression were 61% (95% confidence interval, 43–79%) and 97% (95% confidence interval, 90–100%), respectively. Event-free survival was also closely associated with *MYC*N gene expression, the 5-year event-free survival rates of the groups expressing high and low levels of *MYC*N being 54% (95% confidence interval, 35–73%) and 90% (95% confidence interval, 79–100%), respectively. The relative hazards associated with high *MYC*N gene expression were 13.33 and 5.13 for survival and event-free survival, respectively (Table 2).

The prognostic value of *MYC*N gene expression was also determined in the subset of 47 patients who lacked amplification of the *MYC*N oncogene. Amongst these patients, the 5-year cumulative survival rates of the groups with high- and low-level *MYC*N expression were 76% (95% confidence interval, 55–97%) and 97% (95% confidence interval, 90–100%), respectively. Although high levels of *MYC*N expression in this subset of patients tended to be associated with reduced rates of survival and event-free survival (relative hazards = 7.69 and 3.44, respectively), these trends did not achieve statistical significance (Table 2). To determine why *MYC*N gene expression was not a powerful prognostic factor in patients without *MYC*N gene amplification, the pattern of *MYC*N expression in these patients was further investigated. In view of our previous finding that age was the only significant prognostic factor for neuroblastoma independent of *MRP* gene expression [9], the level of *MYC*N gene expression in *MYC*N unamplified tumours from infants (less than 1 year at diagnosis) was compared with that from older children. *MYC*N gene expression was significantly higher in the tumours of infants (mean ± SE *MYC*N PCR ratio = 0.705 ± 0.054) compared to older (mean *MYC*N PCR ratio = 0.364 ± 0.072) children ($P < 0.0005$), despite the fact that infants with neuroblastoma generally have a good prognosis. Consistent with this result, survival analysis revealed that *MYC*N expression was not predictive of outcome in infants ($P > 0.05$ for all analyses). When infants were excluded from the analysis, high *MYC*N gene expression was strongly associated with reduced rates of survival and event-free survival, both in children whose tumours lacked *MYC*N amplification, and in the overall study population (Table 2). As anticipated from the close relationship observed between *MYC*N and *MRP* gene expression (Table 1), high expression of the *MRP* gene was also strongly predictive of poor outcome in children

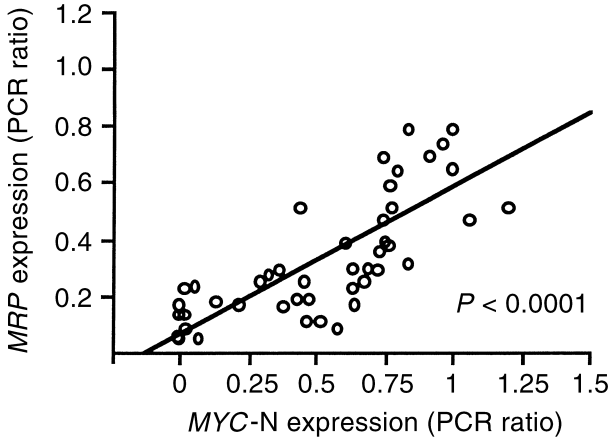

Figure 1. Correlation between expression of the *MYC*N and *MRP* genes in 47 primary untreated neuroblastoma tumours lacking amplification of the *MYC*N oncogene. Linear regression analysis indicated a highly significant relationship.

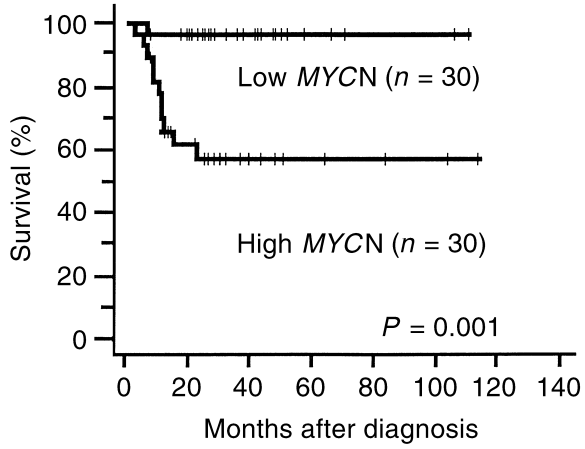

Figure 2. Cumulative survival in 60 neuroblastoma patients according to expression of the *MYC*N oncogene. Tick marks indicate the length of follow-up of individual patients.

Table 2. Prognostic value of MYC*N gene expression in primary untreated neuroblastoma*

Study population	*n*	Survival		Event-free survival	
		Relative hazard (95% CI*)	*P* value	Relative hazard (95% CI)	*P* value
All patients	60	13.33 (1.71–100.0)	0.013	5.13 (1.46–18.2)	0.011
Single-copy *MYCN*	47	7.69 (0.86–71.43)	0.068	3.44 (0.82–14.2)	0.091
Age > 1 year	31	14.93 (1.87–125.0)	0.011	17.54 (2.22–142.9)	0.007
Single-copy *MYCN*; Age > 1 year	21	19.61 (2.0–200.00)	0.011	19.61 (2.02–200.0)	0.010

*CI denotes confidence interval.

aged greater than 1 year at diagnosis, both in the overall study population (relative hazards for survival and event-free survival, 7.4 (95% confidence interval, 1.56–34.48%) and 8.7 (95% confidence interval, 1.87–40.0%), respectively), and in children whose tumours lacked *MYCN* gene amplification (relative hazards for survival and event-free survival, 19.61 and 19.61, respectively) ($P \leq 0.01$ for all analyses).

For all analyses, high *MYCN* expression remained a significant predictor of poor outcome when *MYCN* values were dichotomised around the mean PCR ratio for the 60 tumours rather than the median ratio [9]. Moreover, analysis by the Cox proportional hazards regression model showed that, in common with *MRP* expression, the risk of an adverse event increased in proportion to increasing levels of *MYCN* expression, with a relative hazard of 2.76 (95% confidence interval, 1.26–6.0%) associated with each unit increase in the PCR ratio for the *MYCN* gene.

MRP *expression in* MYC*N antisense transfectants*

Western blot analysis was initially used to confirm that introduction of *MYCN* antisense RNA constructs into the NBL-S cell line had led to downregulation of *MYCN* protein expression in the NBAS-4 and NBAS-5 cell lines, by comparison with the NBL-S parent cell line and the vector-only control, NBV-1 (Figure 3(b), *MYCN*). Consistent with previous results [12], *MYCN* protein was reduced by more than 50% in each of the antisense clones. *MRP* gene expression in each of the four cell lines was first determined by RNA–PCR. In both NBAS-4 and NBAS-5, *MRP* gene expression was lower than in the parent or vector-only control cell lines (Figure 3a). Triplicate analyses indicated that the mean PCR ratio in each of the antisense clones was significantly lower than in either the parent or vector-only control ($P < 0.001$ for each comparison). To determine whether MRP downregulation in the *MYCN* antisense cell lines had occurred at the protein level, as well as the RNA level, Western blot analysis was performed (Figure 3b, MRP). Consistent with the RNA–PCR analysis, the level of *MRP* in both the NBAS-4 and NBAS-5 cell lines was markedly reduced by comparison with either the NBL-S or NBV-1 cell lines.

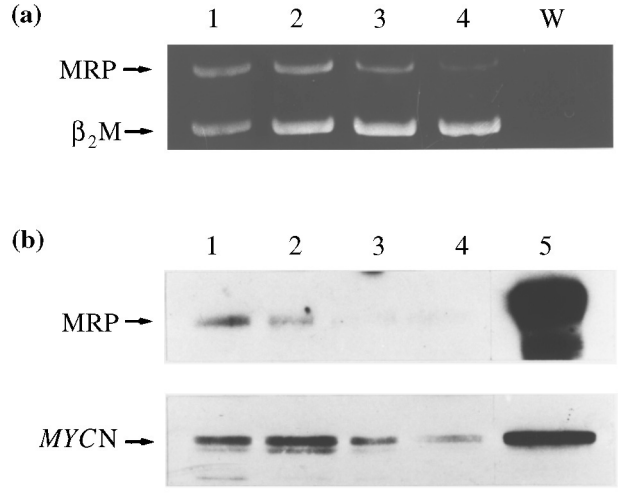

Figure 3. Downregulation of *MRP* expression in *MYCN* antisense transfectant cell lines. (a) Competitive RNA–PCR, co-amplifying *MRP* and β_2-microglobulin; (b) immunoblot analysis of *MRP* and *MYCN* proteins. Lane 1, NBL-S; Lane 2, NBV-1; Lane 3, NBAS-4; Lane 4, NBAS-5. W: Water control. Lane 5, MRP, MCF7/VP and *MYCN*, BE(2)-C.

DISCUSSION

The *MYCN* oncogene belongs to a family of proto-oncogenes whose members appear to be critically involved in the aetiology of a number of malignancies [16]. Available evidence indicates that these genes function as transcriptional regulators, with *MYCN* apparently playing a central role in the regulation of normal neural crest cell differentiation [17, 18]. However, despite extensive study, the number of *MYC*-activated genes identified to date has proven to be disappointingly small [16]. For *MYCN* in particular, the critical target genes regulated by this proto-oncogene either in normal or neoplastic cells are still unknown. Our own results [8, 9] have demonstrated a strong correlation between *MYCN* amplification and high levels of *MRP* expression, a concomitant downregulation of these genes following retinoic acid-induced differentiation and that expression of the *MRP* gene appears to account for the established association between *MYCN* gene amplification and reduced survival. These results have led us to hypothesise that *MYCN* influences neuroblastoma outcome by regulating *MRP* gene expression. The results of the present study provide further support for this hypothesis. Thus, even in the absence of *MYCN* gene amplification, *MYCN* and *MRP* gene expression were closely correlated in primary neuroblastoma tumours, and *MYCN* expression, like *MRP*, was a significant predictor of outcome. Moreover, selective inhibition *in vitro* of *MYCN* expression through the use of *MYCN* antisense RNA resulted in the downregulation of *MRP* RNA and protein in two independent transfectant clones. The data are consistent with the hypothesis that *MRP* gene expression is regulated by the *MYCN* oncogene.

The consensus DNA-binding sequence of MYC oncoproteins is a characteristic six-nucleotide E-box motif [19]. The promoter sequence of the *MRP* gene [20] contains three such motifs and we are currently investigating the possibility that *MYCN* mediates *MRP* gene expression via these motifs. Preliminary data from our laboratories indicates that the

activity of an *MRP* promoter-luciferase vector construct which contains the E-box motifs, following transfection into neuroblastoma cell lines, correlates with the level of endogenous *MYC*N present in these cells. It is also possible, however, that the putative regulatory interaction between *MYC*N and the *MRP* gene occurs at site(s) on the *MRP* gene distinct from the promoter region. Thus, for two genes, α-prothymosin [21] and ornithine decarboxylase [22], known to be targets for C-*MYC* activation, E-box elements present in the first intron of each of these genes have been shown to be the sites of *myc* activation.

While *MYC*N gene amplification is undoubtedly one of the most powerful prognostic indicators for neuroblastoma, studies of the prognostic significance of *MYC*N gene expression in this disease have yielded conflicting results [23–27]. Thus, Nisen and associates [25] concluded that expression of *MYC*N did not appear to correlate with prognosis. Nakagawara and associates, however, found *MYC*N expression to be prognostic of outcome in two separate studies [26, 27], although the predictive power of *MYC*N RNA expression was not as great as *MYC*N gene amplification [27]. This result could be attributed to the frequent presence of high levels of *MYC*N expression in tumours from infants. The present study has yielded results consistent with those of Nakagawara and associates [26, 27], in that high levels of *MYC*N expression predicted poor outcome in the overall study population as well as in older children, but not in infants. This result is not surprising given that we [9] and others [28, 29] have shown that age at diagnosis is itself a powerful prognostic marker, such that children under the age of 1 year are likely to have good outcomes despite the presence of otherwise adverse indicators. The present study has further demonstrated that in older children, *MYC*N expression, like *MRP*, is a powerful prognostic indicator and can predict for outcome even in the absence of *MYC*N gene amplification.

A large body of evidence indicates that the *MYC*N oncogene is crucial to the growth and development of neuroblastoma. While the primary role of the *MYC*N protein in this malignancy may well be to provide a block to cellular differentiation, it is known that advanced stage neuroblastomas with poorly differentiated histology and *MYC*N amplification also tend to be most refractory to drug treatment. Given the established role of *MRP* as a drug-resistance gene, the present results provide evidence linking *MYC*N to the chemoresistant phenotype of neuroblastoma and are consistent with the hypothesis that *MYC*N influences cytotoxic drug response in this malignancy by regulating expression of the *MRP* gene. We are currently addressing this by examining the response of our *MYC*N antisense transfectant cell clones to a number of cytotoxic drugs. These studies, together with ongoing investigations of the interactions between *MYC*N and the *MRP* gene promoter, may lead to increased understanding of the role of the *MYC*N oncogene in this refractory malignancy.

1. Seeger RC, Brodeur GM, Sather H, *et al.* Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985, **313**, 1111–1116.
2. Brodeur GM, Azar C, Brother M, *et al.* Neuroblastoma: effect of genetic factors on prognosis and treatment. *Cancer* 1992, **70**, 1685–1694.
3. Schwab M, Varmus HE, Bishop JM. Human N-*myc* gene contributes to neoplastic transformation of mammalian cells in culture. *Nature* 1985, **316**, 160–162.
4. Thiele CJ, Reynolds CP, Israel MA. Decreased expression of N-*myc* precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* 1985, **313**, 404–406.
5. Schwab M. Molecular cytogenetics of human neuroblastoma. *Biochim Biophys Acta* 1992, **1114**, 43–50.
6. Roninson IB, ed. *Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells.* Plenum, New York, 1991.
7. Cole SPC, Bhardwaj G, Gerlach JH, *et al.* Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992, **258**, 1650–1654.
8. Bordow SB, Haber M, Madafiglio J, *et al.* Expression of the multidrug resistance-associated protein (*MRP*) gene correlates with amplification and overexpression of the N-*myc* oncogene in childhood neuroblastoma. *Cancer Res* 1994, **54**, 5036–5040.
9. Norris MD, Bordow SB, Marshall GM, *et al.* Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. *N Engl J Med* 1996, **334**, 231–238.
10. Telford DJ, Kavallaris M, White L, *et al.* Association of N-*myc* amplification with neuroblastoma: The Australian and New Zealand experience. *J Paediatr Child Health* 1992, **28**, 58–63.
11. Cohn SL, Salwen H, Quasney MW, *et al.* Prolonged N-*myc* protein half-life in a neuroblastoma cell line lacking N-*myc* amplification. *Oncogene* 1990, **5**, 1821–1827.
12. Schmidt ML, Salwen HR, Manohar CF, Ikegaki N, Cohn SL. The biological effects of antisense N-*myc* expression in human neuroblastoma. *Cell Growth Differ* 1994, **5**, 171–178.
13. Schneider E, Horton JK, Yang C-H, Nakagawa M, Cowan KH. Multidrug resistance-associated protein gene overexpression and reduced drug sensitivity of topoisomerase II in a human breast carcinoma MCF7 cell line selected for etoposide resistance. *Cancer Res* 1994, **54**, 152–158.
14. Hipfner DR, Gauldie SD, Deeley RG, Cole SPC. Detection of the M_r 190,000 multidrug resistance protein, MRP, with monoclonal antibodies. *Cancer Res* 1994, **54**, 5788–5792.
15. Haber M, Norris MD, Kavallaris M, *et al.* Atypical multidrug resistance in a therapy-induced drug-resistant human leukemia cell line (LALW-2): resistance to *Vinca* alkaloids independent of P-glycoprotein. *Cancer Res* 1989, **49**, 5281–5287.
16. Ryan KM, Birnie GD. *Myc* oncogenes: the enigmatic family. *Biochem J* 1996, **314**, 713–721.
17. Zimmerman KA, Yancopoulos GD, Collum RG, *et al.* Differential expression of myc family genes during murine development. *Nature* 1986, **319**, 780–783.
18. Grady EF, Schwab M, Rosenau M. Expression of N-*myc* and c-*src* during the development of fetal human brain. *Cancer Res* 1987, **47**, 2931–2936.
19. Blackwell TK, Huang J, Ma A, *et al.* Binding of myc proteins to canonical and noncanonical DNA sequences. *Mol Cell Biol* 1993, **13**, 5216–5224.
20. Zhu Q, Center MS. Cloning and sequence analysis of the promoter region of the *MRP* gene of HL60 cells isolated for resistance to adriamycin. *Cancer Res* 1994, **54**, 4488–4492.
21. Gaubatz S, Meichle A, Eilers M. An E-box element localized in the first intron mediates regulation of the prothymosin alpha gene by c-myc. *Mol Cell Biol* 1994, **14**, 3853–3862.
22. Bello-Fernandez C, Packham G, Cleveland JL. The ornithine decarboxylase gene is a transcriptional target of c-*myc*. *Proc Natl Acad Sci USA* 1993, **90**, 7804–7808.
23. Slavc I, Ellenbogen R, Jung W-H, *et al. myc* gene amplification and expression in primary human neuroblastoma. *Cancer Res* 1990, **50**, 1459–1463.
24. Grady-Leopardi EF, Schwab M, Ablin AR, Rosenau W. Detection of N-*myc* oncogene expression in human neuroblastoma by *in situ* hybridization and blot analysis: relationship to clinical outcome. *Cancer Res* 1986, **46**, 3196–3199.
25. Nisen PD, Wabwe PG, Rich MA, *et al.* N-*myc* oncogene RNA expression in neuroblastoma. *J Natl Cancer Inst* 1988, **80**, 1633–1637.
26. Nakagawara A, Kadomatsu K, Sato S, *et al.* Inverse correlation between expression of multidrug resistance gene and N-*myc* oncogene in human neuroblastomas. *Cancer Res* 1990, **50**, 3043–3047.
27. Nakagawara A, Arima-Nakagawara M, Scavarda NJ, *et al.* Association between high levels of expression of the TRK gene and favorable outcome in human neuroblastoma. *N Engl J Med* 1993, **328**, 847–854.

28. Joshi VV, Cantor AB, Altshuler G, *et al.* Age-linked prognostic categorization based on a new histologic grading system of neuroblastomas. *Cancer* 1992, **69**, 2197–2211.
29. Evans AE, D'Angio G, Properet K, Anderson J, Hann HL. Prognostic factors in neuroblastoma. *Cancer* 1987, **59**, 1853–1859.

Acknowledgements—The authors thank the POG Neuroblastoma Subcommittee for reviewing and approving the present research project, and for providing neuroblastoma tumour samples for analysis. This work was supported by the Children's Leukaemia and Cancer Foundation Inc. (Australia) and by grants to MDN, MH and GMM from the National Health and Medical Research Council, Australia and from the New South Wales State Cancer Council, Australia. MLS was supported by the Schweppe Foundation, Chicago, Illinois and the Children's Cancer Research Fund, Los Angeles, California. S. Bordow is the recipient of an Australian Postgraduate Research Award.