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Short Communication

Expression of the Apoptosis-Suppressing Protein *bcl-2*, in Neuroblastoma Is Associated with Unfavorable Histology and N-*myc* Amplification

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Survival rate in neuroblastoma, a tumor of postganglionic sympathetic neuroblasts, correlates with disease stage, tumor histology, and N-myc gene amplification. N-myc amplification is associated with rapid tumor progression and poor survival, but is not present in all cases of poor prognosis neuroblastoma. Moreover, overexpression of N-myc is not sufficient to cause cellular transformation. These data suggest that other genetic factors are important for neuroblastoma development. We investigated the expression of the, bcl-2 proto-oncogene in untreated cases of neuroblastoma. bcl-2 is a novel proto-oncogene that promotes cell growth by inhibiting programmed cell death (apoptosis), a form of cellular demise common during normal neurogenesis. Immunocytochemical localization using a monoclonal anti-bcl-2 antibody revealed that 16 of 40 patient specimens stained positive for bcl-2. bcl-2 was strongly associated with unfavorable histology (P = 0.002) and N-myc gene amplification (P = 0.002) and marginally associated with poor stage disease (P = 0.06). A logistic regression model evaluating the simultaneous association of stage, histology, and N-myc revealed that bcl-2 was most associated with unfavorable histology and N-myc gene amplification. These results support the notion that bcl-2 may play an important role in the genesis or progression of malignant neuroblastoma. (Am J Pathol 1993, 143:1543–1550)

Neuroblastoma is a malignant neoplasm of childhood that arises in the adrenal medulla or sympathetic ganglia. It is one of the most common malignancies of childhood and disease survival rates correlate with the clinical stage of disease, specific histologic features,[1] and the number of copies of the N-*myc* oncogene found in tumor cells.[2] N-*myc* gene amplification, which correlates with prognosis, is not sufficient to cause complete cellular transformation and does not occur in every case of malignant neuroblastoma.[3,4] A number of chromosomal abnormalities have been described that are frequently associated with neuroblastoma but, to date, the genetic mechanism responsible for the genesis of neuroblastoma is unknown.[5]

A fundamental feature of carcinogenesis is that abnormal expression of normal cellular genes termed protooncogenes can lead to transformation.[6] Typically, the products of proto-oncogenes act to cause increased cellular proliferation[7] or increased tissue invasive characteristics.[8] Recently, a new class of proto-oncogenes has been defined, which contributes to malignancy by inhibiting programmed cell death (PCD).[9] *bcl-2* is the first member of this class of oncogenes, and originally was identified at the breakpoint site of the t(14;18) (q32;p21) chromosomal translocation seen in follicular B cell lymphoma.[10] Deregulated *bcl-2* expression contributes to the genesis of follicular lymphoma by inhibiting PCD without affecting cell proliferation.[11–13]

In both normal tissue development and malignant cell growth, the maintenance of cell number reflects a balance between cell proliferation and cell death.

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PCD is a normal process that eliminates specific cells during development and balances the effects of proliferation in regenerating tissue types.[14] During neurogenesis, PCD eliminates 20 to 80% of the cells formed,[15] effectively removing defective cells and creating morphologically permissive environments for axonal growth.[16,17] PCD also plays a significant role in determining the survival of cultured neural cells. For example, sympathetic ganglia cells undergo PCD after withdrawal of neurotrophic factors.[18,19]

In neural tissue, *bcl-2* is expressed in a developmentally regulated manner.[19] It is also expressed in cell lines derived from neural tumors, including some neuroblastomas.[21] Among positive neuroblastoma cell lines, *bcl-2* expression appears to be greatest in cells that exhibit a less differentiated neuronal phenotype.[21] Importantly, *bcl-2* prevents neural cell death induced by nerve growth factor (NGF) withdrawal from cultured sympathetic neurons.[22] These observations prompted us to hypothesize that deregulated PCD possibly due to overexpression of *bcl-2* contributes to the genesis or progression of neuroblastoma. Our observations demonstrate that *bcl-2* expression correlates with features associated with aggressive neuroblastoma including unfavorable histology and N-*myc* amplification, and to a lesser extent with poor stage disease.

Materials and Methods

Tumor Specimens, Histology, and N-myc Amplification

Tumors specimens were obtained from patients referred to the University of Michigan Hospitals or tissue samples submitted to the Children's Human Tissue Network (Columbus, OH). In total, 40 patient samples were analyzed. Tumor specimens were staged according to the Evans staging system:[23] 1) stage I tumors were confined to the organ or structure of origin; 2) stage II tumors extended beyond the structure of origin but did not cross the midline; 3) stage III tumors extended beyond the midline with possible bilateral regional lymph node involvement; 4) stage IV tumors were large primaries with distant disease in multiple sites including bone, bone marrow, organs, soft tissues, or distant lymph nodes; and 5) stage IV-S tumors were small primary tumors with remote tumor in liver, skin, or bone marrow in infants under 1 year. The tumor specimens represented all stages of disease from nonmetastatic stage I to metastatic stage IV disease. Five samples belonged to patients with stage IV-S disease. Tumor specimens were analyzed before treatment except in six specimens that were obtained after chemotherapy. In two patients, pre- and postchemotherapy specimens were analyzed. All patient samples were coded and reviewed blindly by one pathologist (K.P.H.) and histology was determined according to the Shimada classification. This classification system includes assessment of patient age in addition to histological features of tumor specimens.[24] Analysis of N-*myc* DNA amplification was performed by the Children's Cancer Group Neuroblastoma Reference Laboratory (Los Angeles, CA). N-*myc* amplification (>3 copies) was determined by Southern blot analysis using previously described methods.[25] In five specimens, N-*myc* expression was assessed by immunocytochemical staining of N-*myc* protein in frozen sections.[25] Three specimens were N-*myc* negative by staining, which correlates with a single copy of N-*myc*. Two specimens showed 3+ staining with a heterogeneous pattern, which correlates with genomic amplification of >10 copies.[26]

Immunoperoxidase Staining

Formalin-fixed tissue was sectioned, deparafinized in xylene and ethanol, and fixed in methanol and acetic acid. Duplicate, sequential tissue sections from the same block reviewed for hematoxylin and eosin were incubated with a mouse monoclonal antibody specific for the human *bcl-2* gene product[27] (Dako Corporation, Carpinteria, CA). Antigen-antibody complexes were detected with an avidin-biotin-peroxidase technique (Vectastain Kit, Vector Laboratories Inc., Burlingame, CA). Peroxidase activity was detected with chromogen 3-amino-9 ethylcarbazole, which resulted in a red reaction product. This reaction was followed by a 1% hematoxylin counterstain. Samples were scored positive if the tumor cells exhibited a red staining product in the absence of staining with the control monoclonal antibody. Anti-CD-34 (anti-human progenitor cell antigen HPCA-1, Becton Dickinson Co., Mountain View, CA) monoclonal antibody, which recognizes only endothelial cells in peripheral tissues, was used as a control for the staining technique. To determine the percentage of *bcl-2*$^+$ cells in each tumor specimen, cell counts were taken from two random high power fields and expressed in relation to the total number of cells counted.

Western Analysis

In three patients fresh tissue was available for Western analysis. Tissue fragments were suspended and boiled in lysis buffer containing 0.1 M NaCl, 0.01 M Tris, 0.001 M EDTA, 1 mg/ml aprotinin, and 100 mg/ml phenylmethylsulfonyl fluoride. After sonification, samples were centrifuged at 10,000 *g* for 10 minutes at 23 C and supernatants were resolved on a 12.5% Sodium dodecyl sulfate polyacrylamide gel. Electrophoresed products were transferred to nitrocellulose membrane (Schleicher and Schuell, Keene, NH), blocked in 3% nonfat dried milk, and incubated with the anti-*bcl*-2 antibody for 2 hours at 23 C. Binding was detected with biotinylated rabbit anti-mouse IgG (Jackson Immunoresearch, West Grove, PA), followed by incubation with horseradish-conjugated streptavidin (Zymed Labs. Inc., San Francisco, CA). Blots were developed with 0.4 mg/ml diazobenzidine in 0.001% H_2O_2 and enhanced with 0.03% nickel chloride. Controls for this experiment were lysates from a mouse cell line transfected with control plasmid or a human *bcl*-2 expression vector.[12]

Statistical Analysis

Pearson's χ^2 test for differences in proportions was used to assess the significance of the association of *bcl*-2 staining (positive vs negative) with histology (favorable vs unfavorable), N-*myc* amplification (nonamplified vs amplified >3 copies), stage of disease (I, II, IV-S vs III, IV), and age in months (0 to 11 vs 12 to 23 vs 24 or more) on samples from untreated patients. The strength of the association was expressed as an odds ratio and exact significance levels are calculated.[28] In addition, the linear association between the three age groups and *bcl*-2 expression was assessed by the Cochran-Armitage test for linear trend using an exact significance level. A two-sample *t*-test was used to test the significance of the difference in age in months between those who expressed *bcl*-2 and those who did not. The Kruskal-Wallis two-sample rank sum test was used to test whether patients with tumors that expressed *bcl*-2 had a greater number of unfavorable prognostic factors than patients whose tumors did not express *bcl*-2. Logistic regression was used to assess the simultaneous association of stage, histology, and N-*myc* amplification with *bcl*-2 expression. Age was not included in the logistic model because it was not significantly associated with *bcl*-2 expression. The χ^2 statistic assessing the fit of the model with two independent variables compared with the intercept-only model and the Hosmer-Lemeshow-Cornfield goodness of fit statistic were calculated as previously described.[29]

Results

Expression of bcl-*2 Protein in Untreated Cases of Neuroblastoma*

Forty tumor specimens from 38 patients with neuroblastoma were assessed for *bcl*-2 expression by immunoperoxidase staining (Table 1). Sixteen (42%) patients had favorable stage disease (stage I, II, and IV-S) and 22 (58%) patients had poor stage disease (stage III, IV). Histology determined according to Shimada classification[24] revealed an equivalent number of tumor specimens with favorable and unfavorable histology. Eleven (28%) tumor specimens contained amplified N-*myc* DNA. Six tumor specimens were from patients who had received prior chemotherapy and in two of these prechemotherapy specimens were available for analysis.

Immunoperoxidase staining using a monoclonal anti-*bcl*-2 antibody revealed 16 of 40 (40%) specimens stained positive for *bcl*-2 (Table 1). Five of six postchemotherapy specimens stained positive for *bcl*-2. In the two patients where pre- and postchemotherapy specimens were evaluated, one was *bcl*-2^- pretherapy and one was *bcl*-2^+ (Table 1). After chemotherapy treatment, tumor specimens from both patients were *bcl*-2^+. In 9 of 11 prechemotherapy specimens, *bcl*-2 immunoreactive cells were small and small to medium in size with staining localized to the cytoplasm, the known localization of *bcl*-2 (Figure 1A and B).[30] Three of 11 prechemotherapy specimens showed elements of ganglioneuroblastoma differentiation and a heterogeneous staining pattern with small, medium, and large cells staining positive for *bcl*-2 (Figure 1C). These three specimens also displayed focal areas of ganglioneuroma that were negative for *bcl*-2. All of the tumor specimens expressing *bcl*-2 showed a focal staining pattern with nests of immunoreactive cells (Figure 1). The percentage of *bcl*-2^+ cells varied from specimen to specimen and ranged from 20 to 80%. Nuclear staining was not seen in any tumor specimen analyzed. *bcl*-2 immunoreactive cells did not show evidence of mitotic activity or karyorhexis.

In three patients, tumor tissue was available for both immunocytochemical and Western analysis. All three patients were positive for *bcl*-2 by immunoperoxidase staining and the 26-kd *bcl*-2 product was identified in each by Western analysis (Figure 2).

Table 1. bcl-*2 Staining of Neuroblastoma Tumors*

Patient	Age	Tumor specimen no.	Stage	Shimada class	N-*myc* copy no.	*bcl-2* staining
1	19m	01	I	F	1	–
2	22m	02	I	F	1	–
3	Newborn	03	I	F	1†	–
4	16m	04	II	UF	1	+
5	13m	05	II	UF	1	–
6	9m	06	II	F	1	–
7	7m	07	II	UF	1	+
8	7y 8m	08	II	F	1	–
9	5y 3m	09	II	F	1	–
10	14m	10	II	F	1	–
11	2m	11	II	UF	1	–
12	18m	12	II	F	1†	–
13	23m	13	III	UF	1	+
14	3y 2m	14	III	F	100	+
15	30m	15	III	UF	250	+
16	24m	16	III	UF	1	–
17	10m	17	III	F	1	–
18	27m	18–	IV	UF	>10†	+
	—	19–	IV*	UF	>10†	+
19	2y 1m	20–	IV	UF	1	+
20	6y 7m	21	IV	F	1	–
21	27m	22	IV*	UF	1	–
22	20m	23	IV	UF	1	–
23	16m	24	IV	UF	74	+
24	27m	25	IV	F	1†	–
25	32m	26	IV*	UF	15	+
26	13m	27	IV	UF	136	+
27	7y	28	IV	UF	1	–
28	13y	29	IV	UF	1	–
29	19m	30	IV	F	1	–
30	3y 4m	31	IV*	UF	1	+
31	25m	32	IV	F	25	–
32	22m	33	IV	UF	100	+
33	24m	34	IV	UF	6	–
34	14m	35	IV	UF	>10	+
35	2m	36	IVS*	F	1	+
36	2m	37	IVS	F	1	–
	—	38	IVS*	F	1	+
37	3m	39	IVS	F	1	–
38	1m	40	IVS	F	1	–

—Indicates specimen tested by Western blot.
* Postchemotherapy sample.
† N-*myc* determined by immunostaining of N-*myc* protein on fresh tissue sample.

bcl-*2 Expression Correlates with Features of Poor Prognosis Neuroblastoma*

The correlation between *bcl-2* expression and prognostic features of neuroblastoma was assessed in tumor specimens from untreated patients. There was no statistically significant difference in age ($P = 0.27$) between patients whose tumors express *bcl-2* (mean 32 months, SD 38 months) and those whose tumors did not (mean 22 months, SD 10 months). Nor was there a significant linear trend between *bcl-2* expression and age when patients are categorized into three age groups, less than 12 months, 12 to 23 months, 24 months or older: slightly more than 10% of those in the youngest age group, nearly half of those in the middle age group, and slightly less than a third of those in the oldest age group had tumors that expressed *bcl-2* ($P = 0.69$). The tumor specimens that stained positive for *bcl-2* were most often from patients whose tumors exhibited features associated with poor prognosis neuroblastoma (Table 2). *bcl-2* expression was strongly associated with unfavorable histology (odds ratio 18.4, $P = 0.002$) and N-*myc* amplification (odds ratio 22.9, $P = 0.002$); there was a marginally significant association between *bcl-2* expression and poor stage disease (stage III/IV, odds ratio 5.9, $P = 0.06$). Less than 20% of the patients in the favorable category for stage, histology, or N-*myc* expressed *bcl-2*, whereas half or more of the patients in the unfavorable category for these prognostic features had tumors that expressed *bcl-2*. *bcl-2* expression by immunoperoxide staining also correlated with the number of unfavorable prognostic features found in the tumor specimens (Table 3). As the number of unfavorable features increased, the proportion of patients expressing *bcl-2* also

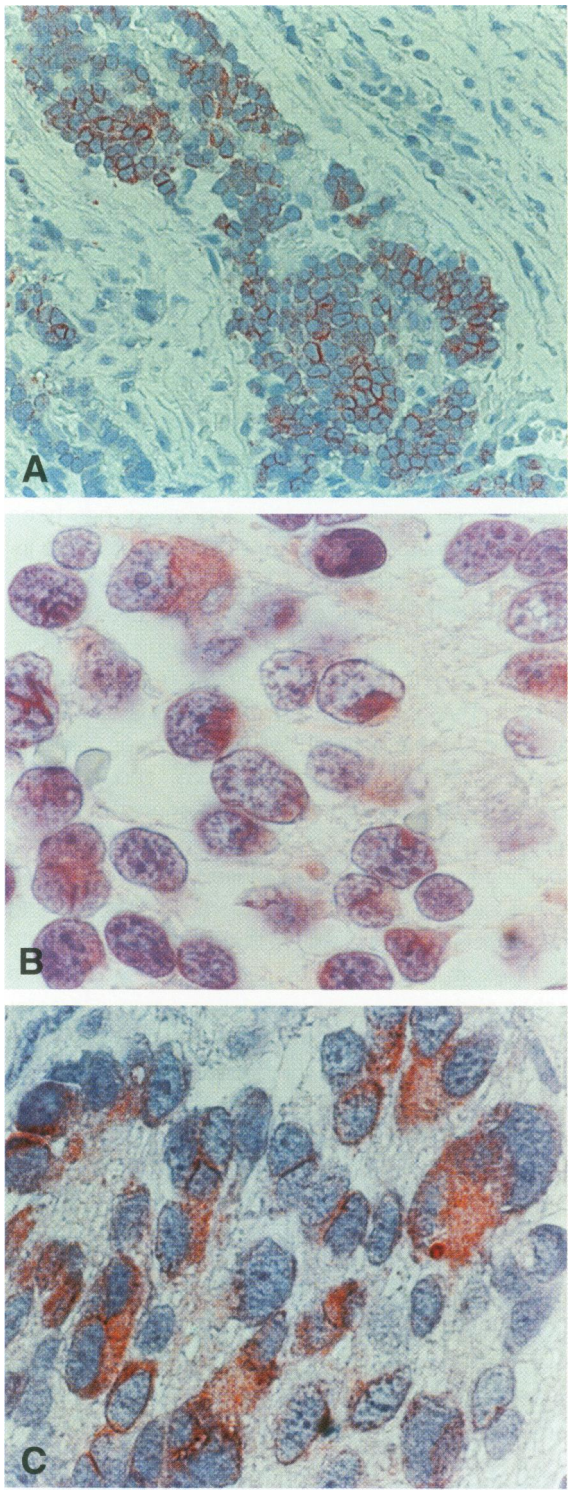

Figure 1. *Immunoperoxidase staining of neuroblastoma tumor specimens. Tumor specimens were assessed for* bcl-*2 staining using a mouse monoclonal anti-*bcl-*2 antibody in an immunoperoxidase reaction.* A *(×450) and* B *(×1000) show the characteristic staining pattern of* bcl-2^+ *tumor specimens. The staining was localized to the cytoplasm and was seen in small to medium sized cells. Three specimens showed a heterogeneous staining pattern with small, medium, and large cells staining positive for* bcl-*2, a representative specimen is depicted in* C *(×1000).*

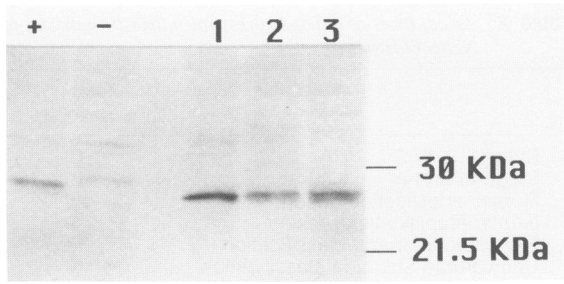

Figure 2. *Western blot of protein extracts from neuroblastoma tumors reacting with anti-*bcl-*2 monoclonal antibody. Fresh tumor specimens from patient 18 (tumor specimen 18 prechemotherapy, lane 1 and tumor specimen 19 postchemotherapy, lane 2) and patient 19 (tumor specimen 20, lane 3) were assayed for* bcl-*2 expression by Western blot analysis. The first two lanes represent positive and negative controls for this experiment. The first lane contains lysate from a mouse cell line transfected with a human* bcl-*2 expression vector.[12] The second lane represents lysate from the same cell line transfected with a control plasmid. All three specimens revealed* bcl-*2 immunoreactive protein migrating at the expected molecular weight of 26 kd.*

increased. There were 11 patients in the favorable category for all three prognostic categories and none of them had tumors that expressed *bcl*-2. This is in marked contrast to patients with one or more unfavorable prognostic features. Twenty-five percent of patients with one unfavorable feature expressed *bcl*-2. 37.5% of patients with two unfavorable features expressed *bcl*-2 and 85.7% of those in the unfavorable category for all three poor prognostic features had tumors that expressed *bcl*-2. This association was highly significant (Kruskal-Wallis test, $P = 0.0005$).

bcl-*2 Expression is Most Associated with Histology and N-*myc *Amplification in Neuroblastoma*

To evaluate the simultaneous association of stage, histology, and N-*myc* amplification with *bcl*-2 expression, a logistic model was fit to the pretreatment specimens. When all three of the prognostic variables are analyzed, the independent association of stage with *bcl*-2 expression was statistically insignificant ($P = 0.73$) and stage was removed from the model. The logistic model for *bcl*-2 expression with histology and N-*myc* as independent variables fit the data well (model $\chi^2 = 19.5$, 2 df, $P = 0.0001$; Hosmer-Lemeshow goodness of fit test, $\chi^2 = 0.40$, 4, df, $P = 0.82$). The model confirmed the results of the univariate analysis showing that *bcl*-2 expression correlates with both histology and N-*myc* amplification.

Table 2. *Association of* bcl-*2 Expression with Clinical Stage, N-*myc *Amplification and Histology in Untreated Patients with Neuroblastoma*

	Total no. patients	*bcl-2*$^+$ (absolute no.)	*bcl-2*$^+$ (%)	Odds ratio	*P*
Stage I, II, IV-S	15	2	13.3		
Stage III and IV	19	9	47.4	5.9	0.06
N-*myc* amplification absent	25	4	16.0		
N-*myc* Amplification present	9	7	77.8	22.9	0.002
Favorable Shimada classification	17	1	5.9		
Unfavorable Shimada classification	17	10	58.8	18.4	0.002

Pearson's χ^2 test was used to assess the significance of the association of *bcl-2* staining with stage of disease (I, II, IV-S vs III, IV), histology (favorable Shimada classification vs unfavorable Shimada classification), and N-*myc* amplification (>3 copies) (present vs absent) in untreated neuroblastoma tumor specimens.

Table 3. *Association of* bcl-*2 Expression with Number of Unfavorable Prognostic Factors in Untreated Neuroblastoma Tumors*

Number of unfavorable prognostic factors*	Number of *bcl-2*$^+$ tumors		
	N	Frequency	%
0	11	0	0.0
1	8	2	25.0
2	8	3	37.5
3	7	6	85.7

* Unfavorable prognostic features included stage III/IV disease, unfavorable Shimada classification, and N-*myc* DNA amplification. Only tumor samples from untreated patients were used for analysis.

The association of N-*myc* with histology and stage was also investigated. In this cohort of patients, N-*myc* was more associated with stage and less strongly associated with histology than was the expression of *bcl-2*. The odds ratio for N-*myc* and histology was 5.3 ($P = 0.12$), which was less than one-fifth the magnitude of the odds ratio for *bcl-2* and histology (odds ratio 22.9, $P = 0.002$) (Table 4). On the other hand, all of the patients with N-*myc* amplification had disease at unfavorable stage, resulting in an infinite odds ratio ($P = 0.002$), whereas the odds ratio for *bcl-2* and stage was marginally significant (odds ratio 5.9, $P = 0.06$).

Discussion

Neuroblastoma is a childhood malignancy in which the cancer cells are derived from postganglionic sympathetic neuroblasts. Despite aggressive chemotherapy regimens, most patients with poor prognosis neuroblastoma succumb to their disease. Several disease factors correlate with prognosis, however, no uniform genetic mechanism has been defined that is operative in this disease. We have investigated whether *bcl-2*, a novel protooncogene that inhibits PCD, is expressed in primary cases of neuroblastoma. *bcl-2* was assessed by immunocytochemical evaluation of untreated neuroblastoma tumors. These studies show that *bcl-2* is expressed in tumors from patients with poor stage disease (stage III/IV), unfavorable histology, and in tumor specimens that contain amplified copies of N-*myc* DNA. Logistic regression analysis assessing the simultaneous association of these factors with *bcl-2* expression revealed that unfavorable histology and N-*myc* amplification were strongly associated with *bcl-2* expression. When a tumor specimen exhibited either of these two characteristics it had a greater than 30% chance of expressing *bcl-2*. When the patients' tumor exhibited both unfavorable histology and N-*myc* amplification there was a 90% probability that the tumor was also *bcl-2*$^+$.

The results of this study indicate that *bcl-2* is a new marker for assessing patients with neuroblastoma and that its expression correlates with features associated with poor prognosis neuroblastoma: unfavorable histology and N-*myc* DNA amplification. Interestingly, the expression of *bcl-2* correlates only marginally with stage of disease. It is possible that *bcl-2* will provide a unique prognostic marker that could identify low stage patients who will develop

Table 4. *Association of N-*myc *Gene Amplification with Histology and Stage of Disease in Untreated Neuroblastoma Tumors*

	N-*myc* amplification (n= 9)	N-*myc* nonamplification (n = 25)	Odds ratio	*P*
Stage III or IV disease	9 (100%)	10 (40%)	*	0.002
Unfavorable Shimada classification	7 (77.8%)	10 (40%)	5.3	0.12

* Odds ratio is infinite because all patients with N-*myc* gene amplification had stage III or IV disease.

recurrent or progressive disease. Future studies will need to determine this possibility as well as the importance of *bcl*-2 in predicting response to therapy and disease-free survival.

Analysis of N-*myc* amplification in these specimens revealed a strong association between N-*myc* amplification and stage of disease ($P = 0.002$) (stronger than the association of *bcl*-2 with disease stage). Our data is comparable to other work indicating a high correlation between N-*myc* and advanced stages of disease.[31] The association of N-*myc* amplification and unfavorable histology was significant but not as strong as the association between *bcl*-2 and unfavorable histology. These results strongly suggest that *bcl*-2 and N-*myc* are independent prognostic factors that may define unique subsets among poor prognosis patients.

The mechanism by which *bcl*-2 contributes to the prognosis of neuroblastoma is unknown. Based on gene transfer experiments[11,13] and transgenic mouse models,[32,33] *bcl*-2 functions to inhibit cell death rather than the rate of cell proliferation. Thus, it is likely that the inappropriate expression of *bcl*-2 in neuroblastoma enables the cells to bypass normal control mechanisms for cell death. This would then leave cells open to further genetic alterations such as N-*myc* gene amplification or other oncogenic activation events that may be required for a full malignant phenotype. The observed *bcl*-2 expression in neuroblastoma cannot be attributed to known chromosomal rearrangements in neuroblastoma.[5] Furthermore, DNA analysis of neuroblastoma cell lines overexpressing *bcl*-2 have failed to detect any rearrangement of the *bcl*-2 gene by Southern analysis.[21] Thus, *bcl*-2 expression may be the consequence of an as yet unidentified cytogenetic event, be secondary to the deregulated expression of a growth factor such as NGF, or be related solely to the stage of differentiation of tumor cells. This latter possibility is unlikely because *bcl*-2 immunoreactive cells included undifferentiated small and small to medium sized cells but also large cells that displayed ganglioneuroblastoma differentiation. Furthermore, tumors that stained negative for *bcl*-2 included specimens with small undifferentiated cells arguing that factors other than the stage of differentiation are important for *bcl*-2 expression.

It is interesting to speculate on the association between *bcl*-2 expression and N-*myc* amplification. It is unclear whether this association is direct or indirect. One possibility is that the deregulated expression of *bcl*-2 is the result of N-*myc* amplification. Although possible, this seems unlikely because in our studies 20% of the *bcl*-2-expressing tumors were not N-*myc* amplified. In addition, neuroblastoma cell lines that express high levels of *bcl*-2 frequently do not contain DNA amplification of N-*myc*.[21] What is more likely is that the expression of *bcl*-2 and N-*myc* are independent events, but their separate biological functions cooperate in the genesis of neuroblastoma tumors. This possibility for oncogene cooperation is often observed during oncogenesis[34] and is supported by findings that deregulated *bcl*-2 expression augments the clonogenicity and latency of tumor induction in c-*myc*-transformed human B lymphoblastoid cell lines.[35] Additionally, *bcl*-2 has been shown to provide a mechanism for inhibiting PCD induced by c-*myc*.[35,36] Although the function of N-*myc* is not well understood, neuroblastoma cells with amplified N-*myc* may display a proliferative advantage and *bcl*-2 provides a survival signal by inhibiting PCD. These functions, although separate, could cooperate to maintain the progression and survival of neuroblastoma tumors. Regardless of the mechanism, determination of *bcl*-2 expression in neuroblastoma will probably provide important prognostic information for the treatment stratification of patients.

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