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Expression of *Apo-3* and *Apo-3L* in primitive neuroectodermal tumours of the central and peripheral nervous system

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Abstract

Deregulation of apoptosis has been implicated in the pathogenesis, spontaneous regression and treatment resistance of neuroblastoma. A newly recognised member of the tumour necrosis factor (TNF)-family of death receptors known as *Apo-3* has been mapped to human chromosome 1p36.3, a region commonly deleted in aggressive neuroblastoma. Based on its localisation and function, *Apo-3* is a candidate for the putative neuroblastoma tumour suppressor gene. Therefore we analysed mRNA expression of the Apo-3 receptor/ligand (*Apo-3*/*Apo-3L*) system in a representative panel of 18 neuroblastoma cell lines, 41 primary neuroblastoma and 13 ganglioneuromas/ganglioneuroblastomas by semi-quantitative RT-PCR. We compared the level of expression with the well-established prognostic factors age, stage, histology, *MYCN*-amplification and *TrkA* expression, as well as outcome. For comparison, we studied *Apo-3*/*Apo-3L* expression in 27 central nervous system (CNS) primitive neuroectodermal tumours/medulloblastomas (PNET/medulloblastoma) and in six normal brain samples. Neuroblastoma cell lines with 1p deletion and *MYCN*-amplification expressed significantly lower levels of *Apo-3* ($P=0.009$ and $P=0.03$, respectively) compared with neuroblastoma cell lines without 1p deletion or *MYCN*-amplification. The mean expression level of *Apo-3L* was significantly higher in ganglioneuromas/ganglioneuroblastomas compared with neuroblastomas ($P=0.001$) and in normal brain compared with PNET/medulloblastoma ($P<0.0001$). Expression of *Apo-3L* was significantly associated with survival in neuroblastomas ($P<0.049$) and in PNET/medulloblastomas ($P=0.01$). Expression of *Apo-3* was significantly associated with survival in PNET/medulloblastomas ($P=0.03$). Thus, the *Apo-3* receptor/ligand system might be involved in the regulation of apoptosis in neuroblastomas and PNET.

Keywords: Neuroblastoma; Apo-3/DR3; Apo-3 ligand; Apoptosis

1. Introduction

Apoptosis, or programmed cell death, plays a critical role in normal development and tissue homeostasis of multicellular organisms. Deregulation of apoptosis has been implicated in autoimmune diseases, neurogenerative disorders and cancer. Some of the well-known regulators of apoptosis are cytokines of the tumour necrosis factor (TNF) ligand family, such as Fas ligand and TNF, which induce apoptosis by activation of their corresponding receptors. Members of the TNF receptor (TNFR) family are type I membrane molecules that contain a structurally homologous cytoplasmic 'death domain', which activates the cell's apoptotic machinary through interaction with adaptor proteins and initiates a series of caspase-dependent events that lead to cell death.

Recently, a new member of the TNF family known as Apo-3/DR3/Wsl-1/TRAMP/LARD (hereafter referred to as Apo-3) was identified [1]. Apo-3 is a transmembrane protein of approximately 47 kDa that has similarity to other members of the TNFR family in its extracellular, cysteine-rich domains. In addition, *Apo-3* resembles TNFR1 and CD95 in that it contains a cytoplasmic death domain. The *Apo-3* gene maps to human chromosome 1p36.3, and *Apo-3* mRNA was detected in several human tissues including spleen, thymus, peripheral blood lymphocytes, small intestine and colon [1]. Alternative pre-mRNA splicing of *Apo-3* generates several

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isoforms including one membrane anchored and several soluble molecules [2].

The ligand for Apo-3, Apo-3L, has also been recently discovered [1]. The extracellular sequence of *Apo-3L* shows the greatest similarity to that of TNF. *Apo-3L* has been mapped to human chromosome 17p13, and *Apo-3L* mRNA has been detected in many human tissues. Activation of Apo-3 by soluble *Apo-3L* has been shown to induce nuclear factor κB (NF-κB) activation and apoptosis in human cell lines [1].

Neuroblastoma is the most common extracranial malignant solid tumour of childhood and arises from the sympathetic nervous system. Apoptotic pathways and their disruption may not only be involved in tumour formation and spontaneous regression of neuroblastoma [3], but defective apoptotic programmes may also confer resistance to therapy [4,5]. Advanced neuroblastomas with *MYCN*-amplification have a particularly poor prognosis and almost always have loss of heterozygosity (LOH) of chromosome 1p36.2-3 [6,7]. Deletion and/or alteration of death receptors in tumour cells may contribute significantly to the proliferative effects of oncogenes like *MYC,* possibly by preventing the cellular suicide that might otherwise be triggered by environmental or genotoxic damage [8–12]. Tumour cells unable to undergo normal apoptosis will accumulate genomic mutations that might further enhance their transformed nature [9,11].

Based on both its function and localisation to 1p36, *Apo-3* is a candidate for the putative neuroblastoma tumour suppressor gene, and so it is of particular interest to analyse its expression in human neuroblastoma. We analysed the expression of *Apo-3* and *Apo-3L* in a panel of 41 primary neuroblastomas, 13 ganglioneuromas/ganglioneuroblastomas, 27 central nervous system (CNS) primitive neuroectodermal tumours/medulloblastomas (PNET/medulloblastoma) and six normal human brain samples by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). We compared the level of expression with well-established prognostic factors such as age, stage, histology, *MYCN*-amplification and *TrkA* expression and with outcome. Finally, we examined mRNA expression of *Apo-3* and *Apo-3L*, as well as the protein expression of Apo-3 in 18 neuroblastoma cell lines.

2. Patients and methods

2.1. Neuroblastoma cell lines and tumour samples

All cell lines were obtained from the Children's Hospital of Philadelphia (CHOP) cell line bank and grown at 37 °C and 5% CO in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum, L-glutamine, penicillin and streptomycin. 1p deletion was determined in neuroblastoma cell lines by loss of heterozygosity (LOH) analyses as previously described in Ref. [6]. We studied tumour specimens from 41 children with neuroblastoma who had been diagnosed in the United States and Canada from 1990 to 1997. 39 patients were identified at the Children's Hospital of Philadelphia, PA, USA and 2 patients were identified by the Quebec Screening Study, Canada. The selection of tumours for study was based solely on the availability of sufficient amount of tumour tissue from which to prepare mRNA for the RT-PCR analysis. All diagnoses were confirmed by histological assessment of the tumour specimen obtained at surgery. Tumour staging was based on the criteria of the International Neuroblastoma Staging System (INSS) [13]. From the neuroblastoma tumour specimens, 19 patients (46%) had a favourable stage (stage I, $n=6$; stage II, $n=10$ and stage IVs, $n=3$) and 22 patients (54%) were diagnosed with advanced stages of disease (stage III, $n=9$ and stage IV, $n=13$). The ages of patients at diagnosis ranged from 1 day to 211 months, with a median of 22.2 months. The overall survival rate of the patients was 83.8%, with a median follow-up time of 52.2 months (range 6–118 months). *MYCN* gene copy number of cell lines and primary tumours was determined by southern blot analysis as previously described in Ref. [14]. *MYCN* amplification was detected in 7 of 41 tumours (6 stage IV neuroblastoma, 1 stage III neuroblastoma), 34 tumours had a single copy of the *MYCN* proto-oncogene. Tumour tissue was classified histologically according to the criteria described by Shimada in Ref. [15]. Twenty-two tumours had a favourable histology and 19 had an unfavourable histology. In addition to these 41 tumours, we included six ganglioneuromas and seven ganglioneuroblastomas in the comparison of gene expression with tumour histology of stored human tissue for these studies was reviewed and approved by the Institutional Review Board of the Children's Hospital of Philadelphia.

2.2. PNET/medulloblastoma tumour samples and near normal brain

We studied PNET/medulloblastoma samples from 27 children diagnosed at the Children's Hospital of Philadelphia between 1988 and 1998. The selection of tumours for this study was based on the availability of sufficient amounts of tumour tissue from which to prepare mRNA for the RT-PCR analysis. All diagnoses were confirmed by histological assessment of a tumour specimen obtained at surgery by one neuropathologist. The median age at diagnosis for all patients was 6.7 years (range 0.3–14.8 years). The median follow-up period for the 16 patients who remain alive and progression-free at the time of this report was 66 months (range 15–105 months). Evidence of leptomeningeal metastasis (M1-3) was present in 8 patients (30%),

whereas 19 patients had M0 (70%). 21 patients had $\geqslant 90\%$ surgical resection, whereas resection of the tumour was $\geqslant 50\%$, but $<90\%$ in 6 patients. Postoperative therapy included radiation and/or chemotherapy according to previously described protocols in Ref. [16,17]. Human brain samples included fetal cerebellum (Clontech, Palo Alto, CA, USA), normal cerebellum of a 4-year-old glioma patient, temporal cortex from a 4-year-old epilepsy-surgery patient, temporal cortex from a 14-year-old epilepsy-surgery patient, occipital cortex from a 19-year-old epilepsy-surgery patient and adult whole brain (Clontech, Palo Alto, CA, USA).

2.3. RNA extraction and first-strand cDNA synthesis

Total RNA was extracted from primary tumour samples using the Qiagen RNeasy Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. One μg of total RNA was reverse transcribed using the SuperScript Preamplification System (Gibco BRL/Life Technologies). Reactions were carried out in a total volume of 20 μl containing 150 ng random hexamers (Gibco BRL), 0.5 mM deoxynucleotide triphosphates (dNTPs) (Gibco BRL), 10 mM dithiothreitol, 200 U SuperScriptII Reverse Transcriptase (Gibco BRL) in the reaction buffer 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$. Initially, the total RNA was denatured at 70 °C for 10 min and immediately chilled on ice. First-strand cDNAs were obtained after 10 min at 23 °C and 50 min at 42 °C. The reaction was terminated at 70 °C for 15 min. RNAse H (2 U, Gibco) was added to each RT reaction followed by incubation at 37 °C for 20 min.

2.4. Semi-quantitative RT-PCR

Semiquantitative RT-PCR was performed according to a previously published protocol in Ref. [18]. PCR was carried out in a final volume of 10 μl containing 0.5 U Taq Gold Polymerase, 200 μM dNTPs, 0.4 μM of each primer, in a buffer consisting of 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 2.0 mM $MgCl_2$ and 1 μl of the RT product (reverse transcribed total RNA). Biotinylated PCR primers for *TrkA* (primer sequences: ACC ATG CTG CCC ATT CGC TG (sense) and GAG GGC AGG CCC CAG TAT TC (antisense)), and glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) (primer sequences: CAT CAA GAA GGT GGT GAA GC (sense) and GAG CTT GAC AAA GTG GTC GT (antisense) have been described previously in Refs. [18,19]. Biotinylated PCR primers for *Apo-3* (membrane-anchored isoform, primer sequences: CAG CCA ATG TGT CAG CAG TT (sense) and TGG CGG TAT GTG TAG GTC AG (antisense)) and *Apo-3L* (primer sequences: TCG CAG AAG TGC ACC TAA AG (sense) and AGC CTT CCC CTC ATC AAA GT (antisense) were designed according to published gene sequences (Genbank # AB051850 and AF055872, respectively). All samples were overlaid with mineral oil. Amplification was performed on a PTC-100 Programmable Thermal Controller (MJ Research). The samples were denatured initially at 95 °C for 12 min, followed by 20 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s. The final cycle was followed by a 5 min extension step at 72 °C. The housekeeping gene *GAPD* was coamplified as an internal standard control as previously described in Ref. [18].

2.5. Analysis of amplified products

Each PCR-sample (10 μl + 2 μl Ficoll Dye Reagent) was analysed in parallel with a biotinylated molecular weight marker (Amersham, Arlington Heights, IL, USA) on a non-denaturing 6% polyacrylamide gel. DNA was transferred to a nylon membrane (Hybond N+, Amersham) and immobilised by ultraviolet (UV)-crosslinking. Detection of biotin-labelled DNA was performed according to the 'Southern-Light-Protocol' (Tropix, Bedford, MA, USA). Quantification of RNA transcript expression was performed by densitometric analysis on X-ray films using NIH 1.55 Image software. A modification of the *GAPD* primers (biotinylated: non-biotinylated at a ratio of 1:49) allowed accurate quantification within the linear range of X-ray detection of both the target transcript and *GAPD* [18]. The expression of the target transcript was normalised by taking the ratio between the densitometric unit of the transcript and that of the internal control, *GAPD* [18].

2.6. Western blot analysis

Cells were rapidly lysed in 800 μl/dish Nonidet P-40 (NP-40) lysis buffer (1% NP-40, 20 mM Tris pH 8.0, 137 mM NaCl, 0.5 mM ethylene diamine tetra acetic acid (EDTA), 10% glycerol, 1 mM phenylmethylsulphonyl fluoride, 0.15 U/ml aprotinin, 20 μM leupeptin, 1 mM sodium vanadate). Samples normalised for total protein content were separated by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS/PAGE), electroblotted onto nitrocellulose and immunostained. Anti-DR3 rabbit polyclonal antibody (Santa Cruz Biotechnology) was used according to the manufacturer's instructions. Detection of immunocomplexes was conducted using an enhanced chemiluminescence (ECL) system (Amersham Corp., Arlington Heights, IL, USA).

2.7. Statistical analysis

A two sample *t*-test was used to examine possible associations between clinical stages and the expression

of the genes of interest. The Pearson correlation coefficient (r) and P value for each gene pair examined were calculated. Cox regression models were used to explore associations between target gene expression and survival, as well as other prognostic variables such as age, stage and *MYCN* amplification. Overall survival data were determined by Kaplan–Meier analysis, and differences between survival curves were calculated using the log-rank test. Statistical analysis was performed using the STATA version 6.0 (State Corp., College Station, TX, USA).

3. Results

3.1. Expression of Apo-3/Apo-3L in the neuroblastoma cell lines and tumours

We used semiquantitative RT-PCR analysis to examine the expression of the *Apo-3L*/receptor system in the neuroblastoma cell lines (Fig. 1, Table 1). From the 18 neuroblastoma cell lines examined, 15 expressed various levels of *Apo-3L* and 18 cell lines expressed various levels of Apo-3. Interestingly, we detected significantly lower levels of the Apo-3 receptor in the neuroblastoma cell lines with a deletion of chromosome 1p in comparison to cell lines without a 1p deletion ($P=0.009$). Significantly lower levels of *Apo-3* were also detected in *MYCN*-amplified versus *MYCN* single copy cell lines ($P=0.03$). There was no significant correlation of *Apo-3L* with 1p deletion or *MYCN* status. None of the target genes demonstrated any correlation with the level of *TrkA* expression. We confirmed the mRNA expression pattern of *Apo-3* at the protein level by western blotting analysis with a polyclonal Apo-3/DR3 antibody (data not shown).

We found transcripts of *Apo-3L* in 40 out of 41 primary neuroblastoma tumours and in all 13 ganglioneuromas/ganglioneuroblastomas. Transcripts of *Apo-3* were detected in 28 out of 41 neuroblastomas tumours and in 10 out of 13 ganglioneuromas/ganglioneuroblastomas. High-level expression (defined as expression > median expression level) of *Apo-3L* was detected in 39% of neuroblastomas and 85% of ganglioneuromas/ganglioneuroblastomas. *Apo-3* was expressed at high levels in 54% of neuroblastomas and 46% of the ganglioneuromas/ganglioneuroblastomas.

3.2. Correlation of Apo-3/Apo-3L expression with prognostic factors and survival in neuroblastomas

We analysed the relationship between tumour stage, tumour histology, the patient's age, *MYCN*-amplification, *TrkA* expression and the expression of the Apo-3 receptor and ligand. The mean expression level of *Apo-3L* was significantly higher in ganglioneuromas/ganglioneuroblastomas versus neuroblastomas ($P=0.001$). The tendency of *MYCN*-amplified neuroblastoma tumours to express lower levels of *Apo-3* only approached significance ($P=0.09$). There was no significant correlation of tumour histology, age of the patients or *TrkA* expression with the expression levels of the Apo-3 receptor or ligand.

Cox regression analysis including all 54 tumours revealed that children with neuroblastoma/ganglioneuromas/ganglioneuroblastomas expressing low levels of *Apo-3L* had a significantly greater risk of death than children whose tumours had a high *Apo-3L* expression (hazard ratio 0.08, 95% CI: 0.012–0.56, $P=0.01$). In the subset of neuroblastoma patients (41 tumours) only, the influence of *Apo-3L* expression levels on survival just reached significance ($P=0.049$).

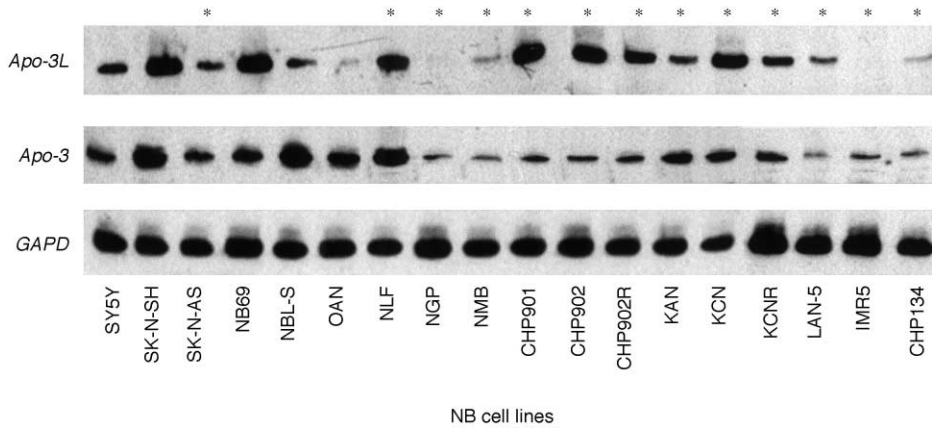

Fig. 1. Reverse transcriptase-polymerase chain reaction (RT-PCR) of *Apo-3* receptor and ligand expression (*Apo-3L*) in 18 neuroblastoma cell lines. Representative example of semiquantitative RT-PCR showing expression levels of *Apo-3* and *Apo-3L* in 18 neuroblastoma cell lines. The first six cell lines from the left are the *MYCN* single copy lines, the other 12 cell lines are those with amplified *MYCN*. The cell lines marked with an asterix demonstrate 1p deletion. Expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) was used as internal control.

Table 1
Expression levels of *TrkA*, *Apo-3* and *Apo-3L* in 18 neuroblastoma cell lines in comparison to their *MYCN*- and 1 p-status

Cell line	*MYCN*	*TrkA*	1pdel	*Apo-3L*	*Apo-3*
SY5Y	Single copy	0.38	No	0.23	0.43
SK-N-SH	Single copy	0.62	No	0.66	0.83
SK-N-AS	Single copy	0.17	Yes	0.09	0.22
NB69	Single copy	0.22	No	0.52	0.28
NBL-S	Single copy	0.37	No	0.1	0.72
LAN6/OAN	Single copy	0.08	No	0	0.45
NLF	Amplified	0.29	Yes	0.27	0.71
NGP	Amplified	0.35	Yes	0	0.09
NMB	Amplified	0	Yes	0.05	0.08
CHP901	Amplified	0.14	Yes	0.52	0.17
CHP902	Amplified	0.09	Yes	0.6	0.14
CHP902R	Amplified	0	Yes	0.41	0.25
KAN	Amplified	0	Yes	0.08	0.54
KCN	Amplified	0.09	Yes	0.56	0.39
KCNR	Amplified	0.09	Yes	0.23	0.33
LAN5	Amplified	0	Yes	0.05	0.05
IMR5	Amplified	0.59	Yes	0	0.07
CHP134	Amplified	0.15	Yes	0.01	0.07

The 5-year cumulative-survival was 90% for patients with tumours expressing high levels of *Apo-3L* and 74% for patients with tumours expressing low levels of *Apo-3L* (defined as $<$median expression level, data not shown). However, analysis of the Kaplan–Meier curve with a log-rank test only approached significance ($P=0.07$).

3.3. *Expression of* Apo-3/Apo-3L *in primary PNETs/medulloblastoma and correlation with prognostic factors and survival*

We detected transcripts of *Apo-3L* in 26 out of 27 PNETS. Transcripts of *Apo-3* were found in 22 out of 27 PNETs.

Expression of *Apo-3L* was significantly lower in PNETs compared with the six samples of human brain ($P<0.0001$). Expression of *Apo-3L* was significantly higher in patients <3 years ($P=0.03$), whereas expression of the *Apo-3* was significantly higher in patients $\geqslant 3$ years ($P=0.008$). There was no significant correlation of gender, metastatic stage or extent of surgical resection with the expression levels of the Apo-3 receptors and ligand.

Cox regression analysis revealed that children with PNET expressing high levels of *Apo-3L* had a significantly lower risk of death than children whose tumours had a low *Apo-3L* expression (hazard ratio 0.02, 95% CI: 0.0012–0.48, $P=0.01$). The 5-year cumulative survival was 92% (95% CI: 57–99) for patients with tumours expressing high levels of *Apo-3L* and 64% (95% CI: 28–78) for patients with tumours expressing low levels of *Apo-3L* ($P=0.046$) (Fig. 2a). Children with tumours expressing high mRNA levels of *Apo-3* had a greater chance of survival than children with low levels of *Apo-3* (hazard ratio $=0.006$; 95% CI: 0.00009–0.637, $P=0.03$). The 5-year cumulative survival was 100% for patients with high levels of *Apo-3* and 57% (95% CI: 28–78) for patients with low levels of *Apo-3* ($P=0.014$) (Fig. 2b).

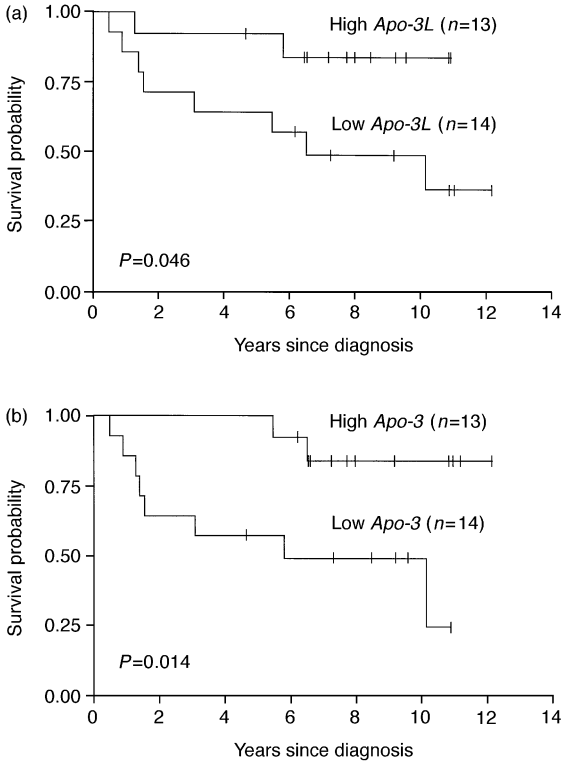

Fig. 2. Cumulative survival curves of PNET/medulloblastoma patients, according to expression of Apo-3 ligand (*Apo-3L*) (a) and Apo-3 receptor (*Apo-3*) (b). The Kaplan–Meier curves show the probability of overall survival in terms of the level of expression of *Apo-3L* (a) and *Apo-3* (b). High expression of *Apo-3L*/*Apo-3* was defined as expression $>$ median expression level and low expression of *Apo-3L*/*Apo-3* was defined as $\leqslant$ median expression level. The survival curve was analysed by the log-rank test.

We analysed the effect on survival of the expression of Apo-3 receptor and ligand compared with the effect of the patient's age, metastatic stage and extent of surgery. On the basis of this univariate analysis, only *Apo-3* and *Apo-3L* expression were significant prognostic factors.

4. Discussion

The human *Apo-3* gene encodes a tumour-necrosis factor-related receptor that is capable of inducing both NF-κB activation and apoptosis when overexpressed in mammalian cells or activated by its ligand *Apo-3L*. It has been reported previously that the human *Apo-3* gene region is duplicated at chromosome 1p36.2-3 and that hemizygous deletions and/or translocations involving the *Apo-3* gene occur relatively frequently in *MYCN* amplified neuroblastoma cell lines [20]. To further explore the biological significance of the Apo-3 receptor/ligand system in paediatric neuronal tumours,

we performed the first systematic analysis of expression of *Apo-3* and *Apo-3L* in neuroblastoma cell lines as well as neuroblastoma and PNET tumour samples.

Our data demonstrate for the first time differential expression of *Apo-3* and/or *Apo-3L* in neuroblastoma and PNET/medulloblastoma patients with different survival probabilities. We found that high levels of *Apo-3L* expression correlated with a favourable outcome for patients with neuroblastoma and PNET and that high levels of *Apo-3* correlated with a favourable outcome in PNET. A variety of clinical and biological variables have been proposed as prognostic factors for neuroblastoma and PNET. *MYCN* amplification, deletion of chromosome 1p and *TrkA* expression have independent prognostic significance for neuroblastomas that equals or exceeds any of the clinical prognostic factors for these tumours [14,21]. Single-institution and collaborative group studies have identified clinical factors such as metastatic stage, patient's age, tumour location, and extent of tumour resection as characteristics that have independent prognostic significance for PNET/medulloblastoma survival outcomes [22–25]. These factors are currently used to distinguish children with a high risk of PNET/medulloblastoma recurrence (e.g. leptomeningeal metastasis, age <3 years, or large postoperative residual tumour) from those with a lower risk. Recently, high *TrkC* mRNA expression in PNET was identified as a powerful independent predictor of favourable clinical outcome [26,27]. In our study, the prognostic influence of the factors metastatic stage, patient's age and extent of surgical resection did not reach significance, possibly due to the low number of patients. In comparison, the expression of *Apo-3* and *Apo-3L* was of prognostic significance, even in this small study cohort. Future studies in larger patient cohorts will be required to determine whether the expression of *Apo-3* and/or *Apo-3L* is predictive of neuroblastoma and/or PNET outcome and might serve as an independent prognostic factor.

Deletions and/or translocations of the 1p36 region have been associated with a number of human malignancies and developmental defects [28–30]. These include neuroblastoma, germ cell tumour, pheochromocytoma, astrocytoma, medullary thyroid carcinoma, ductal breast carcinoma, colon carcinoma and familial malignant melanoma. Many of these tumours are associated with the loss of varying portions of the 1p35-p36 region, as well as unbalanced translocations often involving chromosome 17. Our results indicate that expression of *Apo-3* is significantly higher in *MYCN* single copy human neuroblastoma cell lines without 1p deletion. However, we detected *Apo-3* mRNA in every cell line and *Apo-3* protein expression could be detected in most cell lines. Thus, it unlikely that *Apo-3* is the putative neuroblastoma tumour suppressor gene on chromosome 1p36.2-3. In the neuroblastoma tumours, the correlation of *Apo-3* expression with the *MYCN* status only approached significance. This might be due to the low number of *MYCN* amplified tumours in our study. Unfortunately, data about 1p deletion was not available for many of the tumours analysed.

Expression of *Apo-3* and *Apo-3L* might not only be a molecular marker for a more favourable subgroup of neuroblastomas and PNETs, but also might promote apoptotic signals that modulate responsiveness to therapy. It is possible that multiple, linked genes encoding proteins with apoptotic/antiproliferative functions are selectively lost in neuroblastomas characterised by *MYCN* amplification and/or 1p deletion. Gene deletion and/or translocation may globally affect the ability of these tumour cells to undergo apoptosis induced by intra- and extracellular signals, including chemotherapeutic drugs, and provide important growth advantages to these tumour cells.

Taken together, our results suggest that expression of the Apo-3 receptor and ligand system have a biological role in neuroblastomas and PNET. Together with other death receptors of the TNF family, they might contribute to a more favourable tumour biology with a better response to therapy. Induction of apoptosis via activation of cell death receptors might be a promising experimental strategy to eliminate human neuroblastoma cells *in vivo*.

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