Reduced Expression of CAMTA1 Correlates with Adverse Outcome in Neuroblastoma Patients

Kai-Oliver Henrich,1 Matthias Fischer,5 Daniel Mertens,2 Axel Benner,4 Ruprecht Wiedemeyer,1 Benedikt Brors,3 André Oberthuer,5 Frank Berthold,5 Jun Stephen Wei,6 Javed Khan,6 Manfred Schwab,1 and Frank Westermann1

Abstract

Purpose: A distal portion of 1p is frequently deleted in human neuroblastomas, and it is generally assumed that this region harbors at least one gene relevant for neuroblastoma development. A 1p36.3 commonly deleted region, bordered by DIS2731 and DIS214 has been defined. The present study surveys whether expression of genes mapping to this region is associated with tumor behavior.

Experimental Design: Candidate genes localized within the deleted region were identified by sequence data analysis. Their expression was assessed in a cohort of 49 primary neuroblastomas using cDNA microarray analysis. Gene expression patterns associated with known prognostic markers and patient outcome were further evaluated by quantitative real-time reverse transcription-PCR in a cohort of 102 neuroblastomas.

Results: The commonly deleted region spans 261 kb and encompasses two genes, FLJ10737 and CAMTA1. We found no evidence for an association of FLJ10737 expression with established prognostic variables or outcome. In contrast, low CAMTA1 expression characterized tumors with 1p deletion, MYCN amplification, and advanced tumor stages 3 and 4. Moreover, low CAMTA1 expression was significantly associated with poor outcome (P < 0.001). In multivariate analysis of event-free survival, the prognostic information of low CAMTA1 expression was independent of 1p status, MYCN status, tumor stage, and age of the patient at diagnosis (hazard ratio, 3.52; 95% confidence interval, 1.21-10.28; P = 0.02).

Conclusions: Our data suggest that assessment of CAMTA1 expression may improve the prognostic models for neuroblastoma and that it will be important to define the biological function of CAMTA1 in this disease.

Neuroblastoma, a tumor of neural crest–derived undifferentiated neuroectodermal cells (1), is the most common cancer in infancy and accounts for 9% of all childhood cancers. This heterogeneous disease exhibits patterns of clinical behavior ranging from rapid progression to spontaneous regression. In light of this heterogeneity, understanding the biological behavior of an individual tumor is critical for the selection of risk-adapted therapies. Currently, prognostic evaluation is predominantly based on clinical stage of disease and age of the child at diagnosis. In addition, certain genetic alterations, like amplification of the oncogene MYCN (2, 3), allow the classification of tumors into subsets with distinct biological features, and thus, help to choose the appropriate therapy intensity. However, treatment failure occurs in patients of all subgroups, suggesting that additional parameters are needed to predict the biological course of the disease. Research directed at sites of genetic alterations should provide insights into the pathogenesis of neuroblastoma and help to establish new molecular targets for diagnosis and therapy.

Deletions within 1p occur in ~30% of all neuroblastomas, constitutional translocations and deletions have been reported as well (4, 5). Introduction of 1p chromosomal material into neuroblastoma cells resulted in reduced tumorigenicity (6), providing functional evidence for the role of 1p in neuroblastoma. Furthermore, deletion of 1p36 is associated with adverse prognostic markers such as amplified MYCN and has been identified as an independent predictor of decreased event-free survival (7). Thus, it is widely assumed that distal 1p harbors neuroblastoma-related genetic information.

In pursuit of defining the identity of the putative 1p neuroblastoma gene(s), substantial efforts have been undertaken to confirm and refine a 1p smallest region of overlapping...
deletions (SRO) in neuroblastoma. Early analyses (8–13) led to a preliminary composite SRO of about 5 Mb between the first retained distal marker D1S47 (9) and the first retained proximal marker D1S244 (12, 14). Subsequent studies have attempted to reduce the genomic complexity of the deleted region. A promising step in this direction was suggested by the identification of a homozygous deletion in a neuroblastoma cell line spanning ~500 kb at D1S244 (15). However, thus far, extensive analysis of the six genes mapping to this region gave no solid evidence for any of them functioning as a neuroblastoma suppressor gene (15–22).

Recent studies have made it possible to considerably narrow down a smallest region of overlapping heterozygous deletions in 1p36.3 mapping distal to the homozygous deletion at D1S244. The borders of a SRO that spans a region between D1S2660 (distal) and D1S214 (proximal) have been identified (23–25). This region partially overlaps with a 754 kb SRO defined by D1S2731 (distal) and D1S2666 (proximal; ref. 26). Collectively, these results define a 261 kb composite SRO between D1S2731 and D1S214. We examined the expression of candidate genes mapping to this region in neuroblastomas to determine whether aberrant expression of these genes might play a role in neuroblastoma development. Potential prognostic information in these expression profiles was investigated by correlating them to clinicobiological variables and outcome in a large representative cohort of patients with neuroblastoma.

Materials and Methods

Patients

All patients were enrolled in the German Neuroblastoma Trial and diagnosed between 1991 and 2002. Informed consent was obtained from the patients’ parents. The cohort available for cDNA microarray analysis consisted of 49 patients, the cohort available for quantitative real-time reverse transcription-PCR (QPCR) consisted of 102 patients (Table 1). These cohorts overlapped by 17 tumors allowing us to compare expression profiles derived by the two methods. The only criterion for patient selection was the availability of sufficient amounts of tumor material. The composition of the cohorts in terms of tumor stage, MYCN status, and age at diagnosis was in agreement with the composition of an unselected cohort of 1,741 patients diagnosed between 1990 and 2003 in Germany (ref. 27; data not shown). The clinical stage of disease was evaluated according to the International Neuroblastoma Staging System. Status of MYCN and 1p36 in each tumor was determined by fluorescence in situ hybridization as described previously (28). 1p36 deletion was defined as, D1Z1 signal number > 4-fold MYCN signal number. MYCN amplification was defined as >4-fold MYCN signal number in relation to the copy number of chromosome 2. Patients were treated according to the guidelines established by the German Neuroblastoma Trial, with risk stratification criteria as described elsewhere (29).

Sample preparation

Total RNA to be used for cDNA microarray analysis was extracted as described previously (30). Total RNA to be used for real-time reverse transcription-PCR was extracted after homogenization of the tumor material. The composition of the cohorts in terms of tumor stage, MYCN and 1p36 in each tumor was determined by fluorescence in situ hybridization as described previously (28). 1p36 deletion was defined as, D1Z1 signal number > 4-fold MYCN signal number. MYCN amplification was defined as >4-fold MYCN signal number in relation to the copy number of chromosome 2. Patients were treated according to the guidelines established by the German Neuroblastoma Trial, with risk stratification criteria as described elsewhere (29).

cDNA microarray analysis

Labeling of cDNA, fabrication of microarrays, hybridization, and image analysis were done as described previously (31). Briefly, mRNA was amplified with one round of a modified Eberwine RNA amplification procedure (32), and used for indirect fluorescent-labeling cDNA synthesis (33). Microarrays were fabricated printing a total of 42,578 cDNA clones (libraries purchased from Research Genetics, Huntsville, AL) on glass slides using a BioRobotics MicroGrid II spotter (Harvard Bioscience, Holliston, MA). Hybridization and washing of microarrays were done as described elsewhere (33). Images were acquired by an Agilent DNA Microarray scanner (Agilent) and analyzed using the Microarray Suite program (IPLab, Scantometrics, Fairfax, VA). Raw microarray data were normalized using the variance stabilization method (34), which has proven to be robust and reliable for normalization of microarray data (35). Briefly, values from each probe were transformed using the variance stabilizing asinh function, which is asymptotically equal to log(2$x$). For each chip, two parameters representing an offset and a scaling factor were estimated by the maximum likelihood method. Values were obtained after normalization on a logarithmic scale. Calculations were done with R (36) and Bioconductor (37) software using the package vsn (version 1.6.3). In order to check performance of the normalization and assess the quality of hybridizations, all data were subjected to quality assessment methods implemented in the Bioconductor package arrayMagic version 1.5.8 (38).

QPCR

Single-stranded cDNA was generated from total RNA of primary neuroblastomas using the Superscript II First-Strand Synthesis System (Invitrogen) according to the manufacturer’s directions. QPCR was done on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) with SYBR Green chemistry using the standard curve method (user bulletin no. 2, ABI PRISM 7700 SDS). To prevent amplification from contaminating genomic DNA, primer sequences were selected allowing intron-spanning amplification. PCR reactions were run as duplicates for each sample and as triplicates for determination of standard curves. For normalization, the expression level of the target gene was divided by the geometric mean of expression levels of the

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<td><strong>Microarray cohort</strong></td>
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<tr>
<td><strong>International Neuroblastoma Staging System</strong></td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td><strong>MYCN amplification</strong></td>
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<tr>
<td>Total</td>
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NOTE: Microarray cohort and QPCR cohort overlapped by 17 tumors. Thus, 134 tumors were investigated in total.
housekeeping genes hypoxanthine phosphoribosyltransferase 1 (HPRT1) and succinate dehydrogenase complex, subunit A (SDHA; ref. 39). Primer sequences for CAMTA1 were: 5′-AGTGCAGAAAAT-GAAAGATGCCG-3′ (forward) and 5′-CAAAAATTCTCCTGCTTGATTCG-3′ (reverse).

**Statistics**

**Analysis of cDNA microarray expression data.** To test the association between candidate gene expression and established prognostic factors, the Wilcoxon rank sum test was used. Univariate survival analysis was done using Cox proportional hazards regression. For each candidate gene, the estimated hazard ratio and its 95% confidence interval (CI) were determined for an increase of expression levels from the lower to the upper quartile to obtain comparable results for all genes analyzed. A generalized likelihood ratio test was done to determine whether these changes in gene expression in relation to event-free survival and overall survival were statistically significant.

**Analysis of QPCR expression data.** To test the association between candidate gene expression and established prognostic factors, the Wilcoxon rank sum test was used. Correlation between the corresponding cDNA microarray and QPCR expression measurements was estimated by Pearson’s product moment correlation coefficient. To identify a model describing the relationship between survival and candidate gene expression, the functional form of this relationship was tested by multivariable fractional polynomials (40) and maximally selected log-rank statistics (41). The resulting model was applied in further survival analyses. Multivariate Cox regression was used to investigate the prognostic power of candidate gene expression adjusting for established prognostic variables. To correct for overestimation of the hazard ratio estimate due to selection of the functional form of candidate gene expression variables, shrinkage of the parameter estimate was applied (42). Univariate survival analysis for established prognostic variables was done by means of the log-rank test. The Kaplan-Meier method was used to estimate event-free survival and overall survival curves.

Event-free survival was measured from date of diagnosis until occurrence of disease progression, relapse, or death due to neuroblastoma. Event-free survival times of patients who have not experienced an event until last follow-up were censored. Overall survival was measured from date of diagnosis until death due to neuroblastoma. Overall survival times of patients that were alive at the last follow-up were censored. An effect was considered as statistically significant if the P value of its corresponding test statistic was ≤5%. The statistical analyses were done using the software package R, version 2.0.1 (36).

**Results**

**Genomic complexity of the SRO.** We initially determined the DNA sequence representing the SRO defined by Bauer et al. (26) using a biocomputational approach: sequence-tagged site markers from a contig encompassing the SRO (26) were used for BLAST searches against human genomic databases. This revealed that the sequence representing the SRO, bordered proximally by DIS2666 and distally by DIS2731, is fully known and part of the National Center for Biotechnology Information contig NT_028054 (http://www.ncbi.nlm.nih.gov). The physical length of this SRO is 753.7 kb. The partial overlap of this region with a deleted region characterized by another group (23–25) defines a composite SRO that spans an interval of 260.6 kb, bordered proximally by DIS214 and distally by DIS2731 (Fig. 1).

**Genes within the composite SRO.** BLAST searches cross-referencing the genomic sequence of the composite SRO to transcript databases identified two genes, CAMTA1 and FLJ10737. Gene structure and orientation of both genes were derived from the alignment of their cDNA sequences (CAMTA1, GenBank AB020640; FLJ10737, GenBank AK001599) to the genomic contig sequence NT_028054. The 6,582 bp cDNA of CAMTA1 consists of 23 exons encompassing 982.5 kb of genomic DNA. The 3,199 bp cDNA of FLJ10737 consists of 16 exons encompassing 67.6 kb of genomic DNA. DIS214, bordering the composite SRO proximally, maps to CAMTA1 intron 3. DIS2731, bordering the composite SRO distally, maps to FLJ10737 intron 10 (Fig. 1).

**CAMTA1 and FLJ10737 expression as determined by microarray analysis.** Expression patterns of CAMTA1 and FLJ10737 were derived from a cDNA microarray analysis7 of 49 neuroblastomas (Table 1) and correlated to clinicobiological data and outcome of the respective patients.

We found no significant association of FLJ10737 expression with MYCN status (Wilcoxon rank sum test, P = 0.61), stage (P = 0.23), or 1p status (P = 0.57). To determine whether


![Diagram of CAMTA1 and FLJ10737 genes](image-url)
FLJ10737 expression levels were related to differences in survival time distribution, hazard ratios were estimated by Cox proportional hazards regression comparing the upper to the lower quartile of the expression range. Differences in FLJ10737 expression were not statistically significant in relation to event-free survival (hazard ratio, lower quartile versus upper quartile: 1.25; 95% CI, 0.73-2.17; \( P = 0.41 \)) or overall survival (hazard ratio, lower quartile versus upper quartile: 1.37; 95% CI, 0.61-3.03; \( P = 0.45 \)).

CAMTA1 expression was significantly lower in MYCN-amplified tumors (\( P < 0.001 \)) and advanced stages (stages 3 and 4, \( P = 0.005 \)), and tumors with 1p deletion (\( P < 0.001 \); Fig. 2A). Furthermore, low CAMTA1 expression was significantly associated with decreased event-free survival (hazard ratio, lower quartile versus upper quartile: 2.94; 95% CI, 1.64-5.26; \( P < 0.001 \)) and decreased overall survival (hazard ratio, lower quartile versus upper quartile: 5.88; 95% CI, 2.13-16.67; \( P < 0.001 \)).

CAMTA1 expression as determined by QPCR. To further explore the value of CAMTA1 expression as a prognostic variable, we applied CAMTA1-specific QPCR in a panel of 102 neuroblastomas (Table 1). Relative expression values for CAMTA1 were normalized to the geometric mean of HPRT1 and SDHA expression values (39). Of the 102 tumors, 17 were investigated both by cDNA microarray analysis and QPCR. Considering that these two methods were done on independent RNA preparations of the respective tumors, we found a good concordance between microarray and QPCR expression values for these samples (Pearson’s \( r = 0.78; 95\% \) CI, 0.47-0.92; \( P < 0.001 \)). QPCR analysis confirmed the association of low CAMTA1 expression with markers of poor prognosis: expression of CAMTA1 was significantly lower in MYCN-amplified tumors (\( P < 0.001 \)), advanced stages (stages 3 and 4, \( P < 0.001 \)), and tumors with 1p deletion (\( P < 0.001 \); Fig. 2B).

The search for a model describing the relationship between survival and CAMTA1 expression using fractional

![Fig. 2. Expression of CAMTA1 in (A) 49 primary neuroblastomas as determined by cDNA microarray analysis (A) 102 primary neuroblastomas as determined by QPCR (described in Materials and Methods). Data are represented as box plots: horizontal boundaries of the box represent the 25th and 75th percentiles. The 50th percentile (median) is denoted by a horizontal line in the box and whiskers above and below extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Statistical comparisons are for MYCN amplified versus MYCN single-copy tumors, advanced tumors of stages 3 and 4 versus tumors of stages 1, 2, and 4s, and tumors with 1p-deletion versus tumors without 1p-deletion. Data on 1p status were not available for all tumors. Differential expression was assessed by Wilcoxon rank sum tests. Of 134 tumors in total, 17 were analyzed both by cDNA microarray analysis and QPCR.](https://www.clincancerres.aacrjournals.org/article-pdf/12/1/134/4247951/clincancerres-2006-12-01-134.pdf)
polynomials and maximally selected log-rank statistics identified a cut point model to be most suitable: a CAMTA1 QPCR cutoff value of 4.44 separated two patient subgroups with significantly different outcome (maximally selected log-rank statistics, \( P < 0.001 \)). Applying this CAMTA1 expression cutoff to Kaplan-Meier survival curve estimation revealed decreased survival probability and increased recurrence rate for patients with tumors expressing low levels of CAMTA1 (Fig. 3).

As CAMTA1 maps to the 1p SRO and its expression is significantly correlated with 1p deletion, we asked whether CAMTA1 expression is only a surrogate marker for 1p deletion or whether it adds prognostic information independent of 1p status. To address this question, and to determine whether CAMTA1 expression provides additional predictive power over other established prognostic markers, multivariate survival analysis was done. The risk factors included in the model were all associated with decreased event-free survival in univariate survival analysis: MYCN amplification (\( P = 0.002 \)), stages 3 and 4 (\( P < 0.001 \)), 1p deletion (\( P = 0.02 \)), and age at diagnosis \( \geq 1 \) year (\( P = 0.002 \)). Cox proportional hazards models were built based on 94 patients with complete data on these prognostic variables. Low CAMTA1 expression was selected as the only independent predictor for decreased event-free survival (Table 2). To further illustrate the prognostic independence of CAMTA1 expression from 1p deletion, multivariate survival analysis was done in the subgroup of 71 tumors with normal 1p status. Even within this cohort, CAMTA1 expression, together with tumor stage, emerged as an independent prognostic factor (Table 3).

**Discussion**

Deletion within 1p is one of the most common chromosomal rearrangements observed in neuroblastomas. By combining recent loss of heterozygosity studies, a 261 kb smallest region of overlapping heterozygous deletion at 1p36.3, bordered distally by D1S2731 (26) and proximally by D1S214 (23–25), has been defined. Two genes, FLJ10737 and CAMTA1, map to this region. To evaluate whether their deregulation might contribute to neuroblastoma development, we measured their expression in tumors of a large representative neuroblastoma cohort and correlated the resulting expression profiles to established prognostic variables and patient outcomes.

Expression analysis of 49 neuroblastomas by cDNA microarray gave no evidence for an association of FLJ10737 expression with known variables of poor outcome like MYCN amplification, advanced stages (3 and 4), or 1p deletion. Furthermore, we found no significant association of FLJ10737 expression with outcome. An absence of a correlation between FLJ10737 expression and 1p status might be explained by the existence of homeostatic mechanisms that compensate for copy number changes of this gene. Together, these results do not provide evidence for a contribution of deregulated FLJ10737 expression to neuroblastoma development.

<p>| Table 2. Cox proportional hazards regression for event-free survival (n = 94; 23 events) |</p>
<table>
<thead>
<tr>
<th>Factors</th>
<th>Effect</th>
<th>Hazard ratio (95% confidence limits)</th>
<th>( P )</th>
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<tbody>
<tr>
<td>CAMTA1 expression*</td>
<td>low vs. high</td>
<td>3.52 (1.21, 10.28)</td>
<td>0.02</td>
</tr>
<tr>
<td>1p deletion</td>
<td>yes vs. no</td>
<td>0.65 (0.19, 2.29)</td>
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<td>Stage</td>
<td>3, 4 vs. 1, 2, 4s</td>
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<tr>
<td>Age</td>
<td>( \geq 1 ) vs. (&lt; 1 )y</td>
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<tr>
<td>MYCN amplification</td>
<td>yes vs. no</td>
<td>1.05 (0.29, 3.83)</td>
<td>0.94</td>
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</tbody>
</table>

*The estimated shrinkage factor for the effect of CAMTA1 expression was 0.81, yielding a corrected estimated hazard ratio of 2.78. The cutoff value for dichotomization of CAMTA1 expression was estimated by maximally selected log-rank statistics. High CAMTA1, QPCR \( \geq 4.44 \); low CAMTA1, QPCR \(< 4.44 \).
expression to neuroblastoma pathogenesis. However, sequence analysis will be necessary to clarify whether FLJ10737 mutations or sequence variants exist that exert influence on the disease.

Expression analysis of 49 neuroblastomas by cDNA microarray revealed significantly lower CAMTA1 expression in MYCN-amplified tumors, tumors of stages 3 and 4, and tumors with 1p deletion. The prognostic relevance of CAMTA1 expression indicated by the association with these variables of poor prognosis was confirmed by univariate analysis for both overall survival and event-free survival. The use of QPCR corroborated these results in a cohort of 102 patients.

However, these findings alone were not sufficient to indicate that CAMTA1 could add supplementary information to the existing risk stratification. Approximately 15% of 1p35-36 encoded genes are expressed at significantly lower levels in 1p-deleted compared with 1p-normal neuroblastomas (43). Low expression of such a gene, even if not involved in neuroblastoma development, is likely to be associated with poor outcome as it can act as a surrogate marker for 1p deletion [which itself might be a surrogate marker for inactivation or repression of other 1p gene[s]]. However, CAMTA1 expression contained prognostic information that was not covered by the variable “1p status.” Multivariate survival analysis identified CAMTA1 expression as a predictor variable that was independent not only of 1p status but also of MYCN status, stage, and age at diagnosis. The prognostic independence of CAMTA1 expression from 1p deletion was further shown by multivariate survival analysis within the subgroup of 1p normal neuroblastomas.

All of the analyzed established prognostic variables (MYCN status, 1p status, stage, and age at diagnosis) were predictive of outcome in univariate survival analysis. This indicates that our group of patients was representative of an unselected neuroblastoma cohort and that the supplementary prognostic value of CAMTA1 expression reflects that in the general neuroblastoma population. Thus, the measurement of this variable should allow an additional biological stratification of tumors and help to assign patients to the appropriate therapy. The fact that the differential CAMTA1 expression with respect to survival probability could be detected by two independent methods, QPCR and cDNA microarray analysis, indicates the robustness of this prognostic marker. The prognostic value of CAMTA1 expression is further supported by a genome-wide microarray expression study that identified CAMTA1 as one of the 47 top-ranked differentially expressed transcripts separating progressive from regressive neuroblastoma phenotypes.7

CAMTA1 is a member of a recently characterized protein family designated as calmodulin-binding transcription activators (CAMTA; ref. 44). The physiologic roles of these proteins remain to be elucidated but it has been shown for plant CAMTAs that they (a) bind double-stranded DNA, (b) are predominantly localized in the nucleus, (c) bind calmodulin, and (d) could activate transcription. The highly conserved domain structure within this protein family suggests similar functions for CAMTA1 and its role as transcription activator could be confirmed in a yeast reporter system (44). These data indicate that CAMTA1 is a transcription factor which possibly responds to calcium signaling by direct binding of calmodulin. Such activities would well figure into pathways of tumorigenesis as many calcium-binding proteins are involved in cell cycle control and/or apoptotic cell death, and modulation of calmodulin levels has been recognized as a feature of cell transformation and of malignant cells (45, 46).

The CAMTA1 expression pattern found in neuroblastomas lets us speculate that down-regulation of CAMTA1 mediates a selective advantage in the course of neuroblastoma development. A variety of mechanisms could be responsible for low expression of CAMTA1 in unfavorable tumors.

A high proportion of this tumor entity displays 1p deletion. The significantly lower CAMTA1 levels in 1p-deleted tumors suggests a decrease of the gene’s expression due to a reduction of its copy number. There is a growing body of literature on genes whose dosage reduction accounts for phenotypic changes that contribute to tumor development (47, 48). Whether reduced CAMTA1 gene dosage as a consequence of 1p deletion brings about such functional haploinsufficiency needs to be clarified. Low CAMTA1 expression in the absence of 1p deletion calls for additional regulation mechanisms in the respective tumors. Dereegulation of upstream elements and transcription factors might account for differential CAMTA1 expression independent of 1p deletion. CAMTA1 levels were significantly lower in MYCN-amplified neuroblastomas and a direct or indirect regulation of CAMTA1 by MYCN might be a possible explanation for this observation. However, monitoring CAMTA1 levels in the MYCN-inducible neuroblastoma cell line Tet21N gave no evidence supporting this hypothesis (data not shown). In many tumor types, methylation of cytosine residues in gene-associated CpG islands is a common mechanism suppressing transcription of growth-regulating genes (49, 50). Whether transcription of CAMTA1 is suppressed by methylation remains to be investigated.

A potential role for CAMTA1 in tumor development is strongly supported by the findings of a recent loss of heterozygosity study on 205 gliomas: a 1p minimal deleted region was identified that spans a region of 150 kb between D1S2694 and D1S2666. Thus far, the only gene mapped to

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Table 3. Cox proportional hazards regression for event-free survival in patients without 1p deletion (n = 71; 13 events)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Effect</th>
<th>Hazard ratio (95% confidence limits)</th>
<th>P</th>
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<td>CAMTA1 expression*</td>
<td>low vs. high</td>
<td>3.78 (107,13.37)</td>
<td>0.04</td>
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<td>Stage</td>
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<td>Age</td>
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*The estimated shrinkage factor for the effect of CAMTA1 expression was 0.76, yielding a corrected estimated hazard ratio of 2.76. The cutoff value for dichotomization of CAMTA1 expression was estimated by maximally selected log-rank statistics. High CAMTA1, QPCR ≥ 4.44; low CAMTA1, QPCR < 4.44.
this region is CAMTA1 (51). Further support comes from a query of the microarray database Oncomine (52). A CAMTA1-specific search highlighted a study classifying subgroups of melanoma by genome-wide expression analysis (53). CAMTA1 expression was significantly reduced in melanoma of soft parts, a rare and aggressive melanoma subtype. This finding is intriguing considering that melanomas, as neuroblastomas, arise from neural crest cells, and suggests the need to investigate the prognostic relevance of CAMTA1 expression in this tumor.

Taken together, our data show that the expression of CAMTA1, a gene mapping to a 1p36 region commonly deleted in neuroblastoma, can represent a powerful prognostic variable that may complement the predictive value of established risk factors in neuroblastoma. We suggest that CAMTA1 reduction might contribute to the effect of 1p36 deletion on event-free survival (7). An understanding of the function and regulation of this gene and its product should give further insight into the biological features of neuroblastoma. Sequence analysis should address the question of whether CAMTA1 mutations or sequence variants exist that are associated with neuroblastoma.

Acknowledgments

We thank Yvonne Kahler for excellent technical assistance.

References


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