Comparing Tribbles Homolog 3 (TRIB3) Protein Expression Levels with Clinicopathological Characteristics and Survival Among Neuroblastoma Patients

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ABSTRACT

BACKGROUND: Tribbles Homolog 3 (TRIB3) is a member of the pseudokinase family of tribbles and acts as an adaptor protein to regulate different cellular processes. Upregulation of TRIB3 expression was shown either as a favorable or an adverse prognostic factor in various adult malignancies. However, TRIB3 expression has not been examined in pediatric cancers. Neuroblastoma is the most common malignant solid tumor of childhood, which affects mostly children under 5 years old. Risk stratification of patients defined by International Neuroblastoma Risk Group was used to determine prognosis and treatment of the disease. This study aimed to examine the relationship between TRIB3 protein expression levels and clinicopathological features and survival of patients.

METHODS: TRIB3 protein expression was analyzed using immunohistochemical staining on formalin-fixed paraffin-embedded tissue samples of neuroblastoma patients (n = 56). Survival analyses were performed with Kaplan-Meier method and log-rank tests. Association between TRIB3 expression and clinicopathological characteristics were analyzed with Spearman's correlation.

RESULTS: Of the patients, 32.1% were in the low-risk group, 21.4% in the medium-risk group, and 46.4% in the high-risk group. Survival analysis was performed in the entire neuroblastoma patient group and sub-risk groups of neuroblastoma patients. In the entire patient group, there was no significant difference in overall survival ($P = .202$) and event-free survival ($P = .172$) between TRIB3-positive and -negative patients. However, when survival analyses were performed in each risk group, TRIB3 expression was significantly associated with higher overall survival ($P = .034$) and event-free survival ($P = .032$) in low-risk group neuroblastoma patients. Nevertheless, no association was found between TRIB3 expression and overall survival ($P = .799$) and event-free survival ($P = .448$) in high-risk neuroblastoma patients. Furthermore, a significant correlation was identified between 1p36 loss-of-heterozygosity and TRIB3 expression ($P = .030$). However, TRIB3 expression did not correlate with other clinicopathological features.

CONCLUSION: TRIB3 expression is a potential predictive biomarker for low-risk neuroblastoma patients.

KEYWORDS: Neuroblastoma, TRIB3, prognosis, biomarker, survival

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Introduction

Neuroblastoma (NB) is an embryonal tumor that affects the normal development of the adrenal medulla and paravertebral sympathetic ganglia in early childhood. NB is the most common solid tumor in children, accounting for ~15% of childhood cancer-related mortality.[1] NB exhibits diverse clinical behaviors such as early age of onset, a tendency for spontaneous regression of tumors in infants, and a higher risk for metastatic disease in patients over 1 year of age.[2] NB is a very heterogeneous disease, yet its clinical behavior can be reliably predicted based on analyzing a panel of prognostic variables.[3] International Neuroblastoma Risk Group (INRG) was established to stratify patients based on presenting clinicopathological characteristics and tumor biology for guiding better and more effective treatment strategies.[4] The risk stratification of patients is defined by considering the factors including age, stage of the tumor, histology of the tumor, presence of *MYCN* (2p24) gene amplification, tumor cell ploidy, presence of segmental chromosomal abnormalities (including 11q23 deletions, 17q23 amplification) and these factors are being modified with the results of clinical trials and development of novel treatment strategies.[5] Patients with low-risk diseases are often managed with surgical resection or observed with spontaneous regression, and patients with intermediate-risk diseases receive courses of chemotherapy in addition to surgical resection.[6] Patients with high-risk disease have a poor prognosis despite having intensive and multimodal treatments [including surgical resection, intensive chemotherapy, radiotherapy, autologous stem cell transplantation (ASCT), and the application of differentiating agents such as iso-retinoic acid]. The five-year event-free survival (EFS) of patients with high-risk disease is still between 30% and 50% compared with 90% and 100% for

patients with low- or intermediate-risk disease.[7,8] New targeted therapies (ALK inhibitors), immunotherapies (anti-GD2 antibodies), and CAR-T therapy are currently applied, especially for eliminating minimal residual disease after intensive chemotherapy and surgery.[9] Nevertheless, new prognostic factors are required to refine therapeutic approaches and develop more effective therapies with reduced side effects.

Tribbles protein was first identified in Drosophila and played a role in coordinating cell division and morphogenesis.[10,11] Tribbles (TRIB) protein family are serine-threonine kinases lacking ATP-binding catalytic region. Without possessing an enzymatic activity, they could interact with signaling proteins or act as a scaffold or adaptor proteins.[12] Tribbles Homolog 3 (TRIB3) is one of the members of the TRIB protein family. TRIB3 plays a role in many different cellular signaling pathways, including endoplasmic reticulum stress, insulin regulation, cell proliferation, and differentiation.[13] Interestingly, hypoxia was found to increase TRIB3 expression in breast cancer patients, which was correlated with favorable prognosis in patients. While breast cancer cells expressing high levels of TRIB3 protein were more susceptible to hypoxia, cancer cells expressing low levels of TRIB3 protein were more tolerant to hypoxic conditions.[14] In another study, TRIB3 was found to be a master regulator of Notch through the MAPK-ERK and TGFβ pathways in breast cancer, establishing TRIB3 as a potential therapeutic target.[15] However, in a recent article, TRIB3 was positively associated with breast cancer progression, metastasis, and relapse.[16] Furthermore, TRIB3 expression levels were also examined in other cancer types. TRIB3 was found to be upregulated in non-small cell lung cancer (NSCLC) samples and was correlated with tumor metastasis, disease recurrence, and poor survival in patients.[17] TRIB3 protein expression was found at higher levels in colorectal carcinoma (CRC) cells than in corresponding normal regions and also closely related to metastasis (except lymphatic metastasis) and correlated with poorer prognosis among CRC patients.[18] TRIB3 gene expression was also found upregulated in hepatocellular carcinoma tissue samples compared with paired normal tissues from the same patient and highly correlated with tumor size in this patients.[19]

Even though TRIB3 protein expression levels were previously studied in various adult cancers, TRIB3 expression has never been evaluated in pediatric cancers. Therefore, this study aimed to examine TRIB3 protein expression levels in NB samples and to determine whether its expression levels are associated with patients' clinicopathologic features and prognosis. This study showed that TRIB3 expression correlated with better survival in low-risk NB patients.

Materials and Methods

Clinicopathological features of neuroblastoma patients

In this study, archival formalin-fixed paraffin-embedded (FFPE) tissue samples, which were collected from 56 patients diagnosed with NB from 2018 to 2023, were used. Clinical data of the patients were obtained from electronic records. Patients were clinicopathologically categorized into low-risk, intermediate-risk, and high-risk groups according to criteria (tumor stage, histology, age of patient, *MYCN* amplification, 1p36 deletion, 17q23 amplification, DNA ploidy status) defined by INGR and Turkish Pediatric Oncology Group (TPOG) guidelines[4,20] (see Table 1). Patient FFPE samples from both low-risk and high-risk NB groups were chosen for the study. All unstained tissue sections and hematoxylin and eosin (H&E) stained histological slides of the tumors were reviewed by an expert pathologist and the optimal tissue sections were selected for the study. The immunohistochemical (IHC) staining and microscopic analysis of the staining were performed blindly.

IHC staining of samples:

Before performing the IHC staining on samples, tissue sections were treated to remove paraffin. The slides were incubated at 60°C for an overnight period. The slides were then put in xylene for 30 min at room temperature. Following this, the slides were washed with graded concentrations of ethanol from high to low and finally washed with distilled water. In the antigen retrieval step, slides were placed in vented plastic tubes, and 20 mM citrate buffer (pH 6.0) was poured into each plastic tube. The tubes were placed in a microwave and boiled for 2 min at low power and 3 min at high power, then removed and cooled at room temperature. After cooling down, the slides were washed with distilled water. The slides were placed in 3% hydrogen peroxide (H_2O_2) to block the endogenous peroxide activity. After the 10 min incubation in H_2O_2, the slides were washed with distilled water and 1X phosphate-buffered saline (PBS; pH 7.4). The slides were blocked with blocking reagent Inhibitor CM for 5 min at room temperature. Primary antibodies against TRIB3 (catalog (Cat.) No. DF7844, dilution, 1:100 to 1:200 in distilled water) were from Affinity Bioscience, Inc. (Cincinnati, OH, USA). After removing excessive Inhibitor CM from slides, primary antibodies were added on each slide and incubated overnight at 4°C. Next day; the slides were washed with 1X PBS and incubated in 1X PBS for 15 min. Horseradish peroxide (HRP) conjugated secondary anti-rabbit antibody (Cat. No. S0001, dilution, 1:200 to 1:400 in distilled water) from Affinity Bioscience, Inc. (Cincinnati, OH, USA) was prepared. The slides were treated with secondary antibody and incubated at room temperature for 90 min. After incubation, slides were washed with 1X PBS and incubated in 1X PBS for 15 min. 3′,3-Diaminobenzidine (DAB) reagent was diluted with 3% H_2O_2 and added onto each slide drop by drop. The slides were incubated with DAB and examined under a microscope until the desired color change happened. Then, the slides were washed with distilled water two times. The slides were placed in hematoxylin for 1 min and immediately removed and rinsed with tap water several times. Slides were placed in distilled water and gradually washed with

Table 1. Risk stratification criteria for NB patients enrolled in this study.

RISK GROUP TABLE	INSS STAGE	AGE (YEARS)	*MYCN* AMPLIFICATION	SHIMADA HISTOLOGY	DNA PLOIDY
Low-risk	1	0-21	Any	Any	Any
	2A-2B	<1	Any	Any	Any
	2A-2B	$\geqslant 1$	(–)	Any	Any
	2A-2B	$\geqslant 1$	(+)	Good	Any
	4S	<1	(–)	Good	>1
Intermediate-risk with good histology	3	<1	(–)	Good	>1
	3	$\geqslant 1$	(–)	Good	>1
	4	<1	(–)	Good	Any
Intermediate-risk with poor histology	3-4	<1	(–)	Good	1
	3-4	<1	(–)	Poor	>1
	4S	<1	(–)	Poor	Any
	4S	<1	(–)	Good	1
High-risk	2A-2B	$\geqslant 1$	(+)	Poor	–
	3	0-21	(+)	Any	Any
	3	$\geqslant 1$	(–)	Poor	Any
	4	>1	Any	Any	Any
	4	<1	(+)	Any	Any
	4S	<1	(+)	Any	Any

Abbreviation: INSS, International Neuroblastoma Staging System.

ethanol series (from low concentration to high concentration) and finally placed in xylene for at least 2 h. In the final step, slides were removed from the xylene and left to air-dry. Later on, a mounting medium was poured on each slide, and each section was covered with a coverslip.

Microscopic examination

A pathologist examined the slides blindly under an inverted microscope (Olympus, Tokyo, Japan). Each of the sections was examined, and stained NB cells were counted and compared to the total NB cells. Hence the percentage of positivity in each section was scored.

Statistical analysis

The data were analyzed with SPSS Statistics v22.0 program (SPSS, Chicago, IL, USA), and a P-value $< .05$ was accepted as a statistically significant difference. The overall survival (OVS) and EFS were analyzed by log-rank test, and the Kaplan-Meier curve was drawn. Spearman's correlation analysis determined the relationship between TRIB3 protein expression and the clinicopathological characteristics of patients.

Results

Patient characteristics

In total, 56 NB patients (30 female and 26 male) enrolled in this study (see Table 2). The mean age of the subjects was 37.94 ± 42.11 months (min: 1 month and max: 191 months). The mean of EFS was 36.25 ± 30.39 months, and the mean of OVS was 40.24 ± 29.10 months. The survival rates between low-risk and high-risk patients are quite different in NB. Hence, there is variation in survival rates. International Neuroblastoma Staging System (INSS) was used for classifying tumor stages. Stage 4S patients were not included in the study since stage 4S represented a group of exceptional cases less than 1-year old. The stage of tumors varied between Stage I to IV (Stage I: 21.4%, Stage II: 8.9%, Stage III: 14.3%, Stage IV: 55.4%, respectively). While 32.1% of patients were classified in the low-risk group, 21.4% were in the intermediate-risk group, and 46.4% were in the high-risk group. *MYCN* amplification was

Table 2. Histopathological characteristics of NB patient cohort in the study.

FEATURES	TOTAL (N = 56)		
Age (mean, maximum, minimum)	37.94 ± 42.11 (months)	191.0	1.0
Event-free survival (EFS) (mean, maximum, minimum)	36.25 ± 30.39 (months)	150	0.30
Overall survival (OVS) (mean, maximum, minimum)	40.24 ± 29.10 (months)	150	0.30
Sex (number, frequency)			
Female	30	53.6%	
Male	26	46.4%	
Stage (number, frequency)			
I	12	21.4%	
II	5	8.9%	
III	8	14.3%	
IV	31	55.4%	
Risk group (number, frequency)			
Low	18	32.1%	
Intermediate	12	21.4%	
High	26	46.4%	
MYCN amplification			
Yes	12	21.4%	
No	44	78.6%	
1p LOH			
Yes	25	44.6%	
No	30	53.6%	
Undefined	1	1.8%	
11q deletion			
Yes	18	32.1%	
No	38	67.9%	
17q gain			
Yes	25	44.6%	
No	30	53.6%	
Undefined	1	1.8%	

Abbreviations: LOH, loss of heterozygosity; NB, neuroblastoma.

detected in 21.4% of patients. Loss of heterozygosity of the 1p36 arm was observed in 44.6% of patients. While deletion of 11q23 was observed in 32.1% of patients, a gain of 17q23 was observed in 44.6% of cases.

Analysis of TRIB3 expression in NB tissues

NB tissue sections were analyzed for TRIB3 protein expression (see Figure 1). While 20.9% of NB patients' tumors were stained positive for TRIB3 protein expression, 79.1% were negative.

Survival analysis of TRIB3

The relationship between TRIB3 expression and the prognosis of the disease was examined with survival analysis. Kaplan-Meier graphs were made, and log-rank analysis was performed to calculate the statistically significant difference between

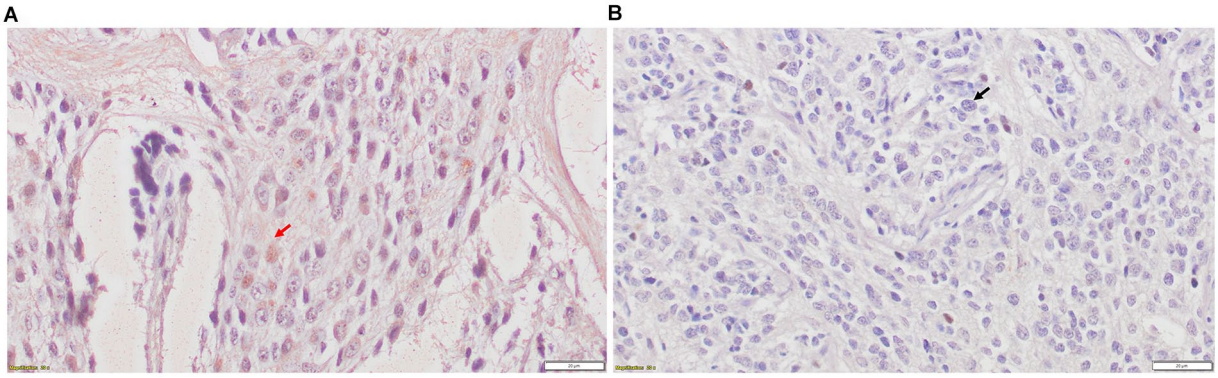

Figure 1. Immunohistochemical staining of TRIB3 in NB patient specimens. TRIB3 positive staining showed cytoplasmic staining. (A) TRIB3 positive differentiating NB. The red arrow indicated the TRIB3 positive cell, which was brown in color. (B) TRIB3 negative differentiating NB. The black arrow indicated TRIB3 negative cell, which was blue in color. (×100 magnification, Bar: 20 μm). NB indicates neuroblastoma; TRIB3, tribbles homolog 3.

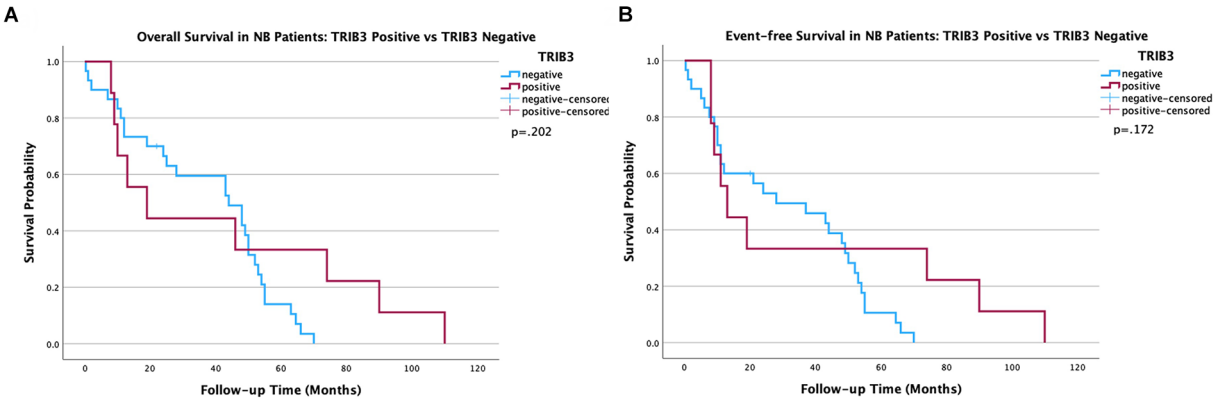

Figure 2. OVS and EFS analysis of TRIB3-positive and TRIB3-negative in entire NB patient population (A and B). EFS indicates event-free survival; NB, neuroblastoma; OVS, overall survival; TRIB3, tribbles homolog 3.

TRIB3-positive and -negative patients. In the entire patient group, no significant change was observed between TRIB3-positive and -negative patients in terms of OVS ($P = .202$) and EFS ($P = .172$; see Figure 2A and B).

Since the survival rates of NB patients were quite different between risk groups, we decided to perform survival analysis separately in each risk group. TRIB3 expression was positively correlated with OVS ($P = .034$) and EFS ($P = .032$) in low-risk NB patients (see Figure 3A and B). However, there was no association found between TRIB3 expression and OVS ($P = .799$) or EFS ($P = .448$) in high-risk NB patients (see Figure 3C and D). Intermediate-risk patients were not included in the survival analysis since only one intermediate-risk patient was positive for TRIB3 expression.

Subsequently, we studied TRIB3 expression and survival in patients with different tumor stages. Although the tumor stage is one of the factors contributing to the risk group, it is not the same. Therefore, we also analyzed the survival of patients according to their stage. Stage I and Stage II tumors were considered low-stage tumors, while Stage III and Stage IV tumors were considered high-stage tumors. Kaplan-Meier analyses were performed in patients with low-stage and high-stage tumors. TRIB3 expression levels were also positively correlated with OVS ($P = .046$) and EFS ($P = .046$) in patients with low-stage tumors. However, no association between TRIB3 expression and OVS ($P = .799$) and EFS ($P = .448$) of patients with high-stage tumors (see Supplementary Figure 1).

Interestingly, in the high-stage group, patients with positive TRIB3 expression had lower OVS and EFS than patients who did not have TRIB3 expression. Therefore, we analyzed Stage IV patients separately and observed a negative association between TRIB3 expression and patient OVS and EFS in Kaplan-Meier graphs. Nevertheless, we did not find a significant relationship between TRIB3 and patients' OVS and EFS (see Supplementary Figure 2).

Correlation analysis of TRIB3 expression with clinicopathological characteristics of patients

Spearman's correlation analysis was performed to examine the relationship between TRIB3 expression and the clinicopathological characteristics of the patients (see Table 3). TRIB3

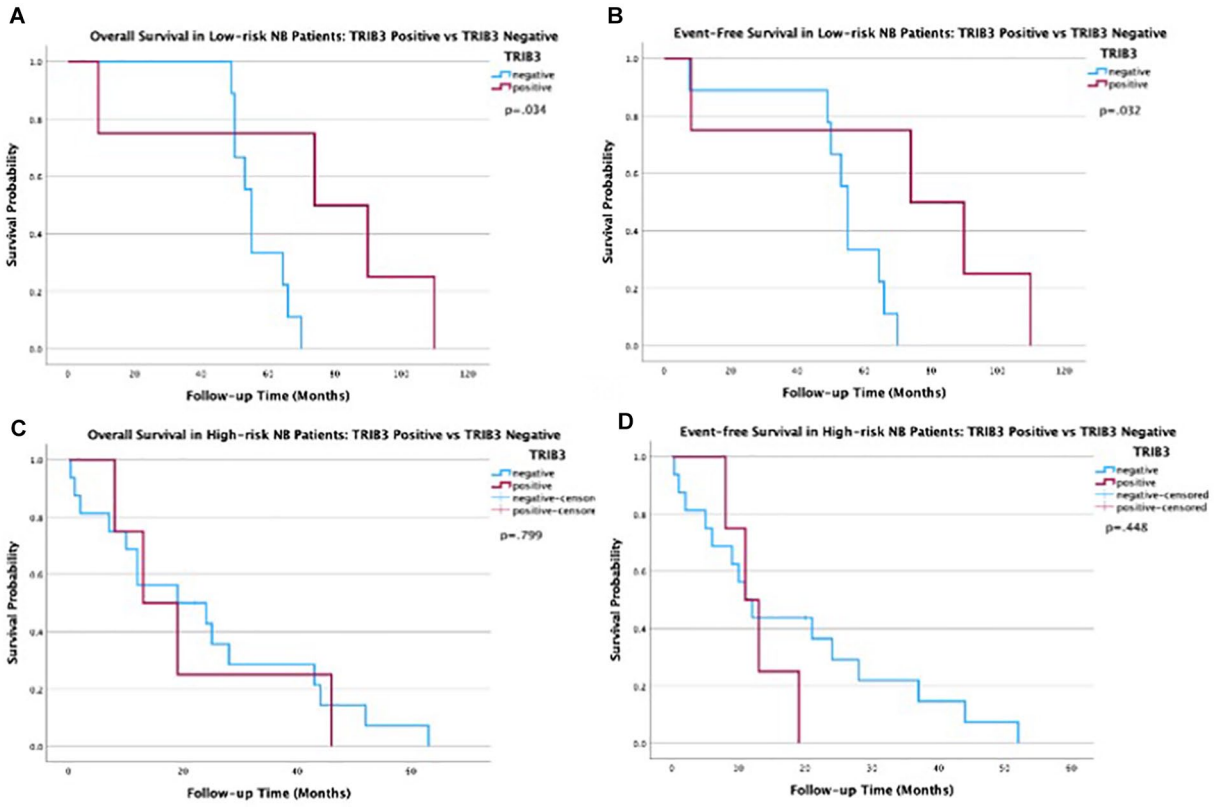

Figure 3. OVS and EFS analysis of TRIB3-positive and TRIB3-negative patients in low-risk group of patients (A and B) and high-risk group of patients (C and D). EFS indicates event-free survival; NB, neuroblastoma; OVS, overall survival; TRIB3, tribbles homolog 3.

Table 3. Correlation analysis of TRIB3 expression with clinicopathological characteristics of patients.

CORRELATIONS OF TRIB3	*P*-VALUE	CORRELATION COEFFICIENT
MYCN	.428	.124
1p LOH	.030*	.335
11q Del	.957	−.009
17q gain	.601	.083
Stage	.517	−.101
Risk group	.523	−.100
Sex	.075	−.274
Age	.102	.256
Relapse	.259	.162
Metastases	.369	−.154

Abbreviations: TRIB3, tribbles homolog 3; LOH, loss of heterozygosity.
*$P < .005$ was assumed as statistically significant.

expression was found to be correlated with 1p36 LOH ($P = .030$). However, no correlation was found between TRIB3 expression and *MYCN* amplification, 17q23 amplification, 11q23 deletion, age, sex, and relapse, presence of metastases (see Table 3).

Discussion

TRIB3 interferes with a broad range of cellular processes through non-catalytic mechanisms. It plays a role in many diverse cellular signaling processes due to various interacting protein partners.[21] TRIB3 partners include kinase-dependent proteins, transcription factors, ubiquitin ligases, ER-stress-related proteins, and spliceosome machinery components.[22] TRIB3 and its interacting partners play a role in cancer pathophysiology and chemotherapy resistance. TRIB3 was assumed to be a good or bad prognostic factor for various human cancers by regulating different cell signaling mechanisms.[23] Nevertheless, the role of TRIB3 has never been studied in childhood cancers, especially in neuroblastoma.

NB is a very heterogeneous disease. NB prognosis and recurrence risk depend on various clinical and biological factors such as age, stage, histopathology, DNA ploidy, *MYCN* amplification, and other chromosomal aberrations.[24] Risk classification system is used to determine the prognosis of the disease and the most appropriate treatment for NB patients; therefore, it aims to treat patients most effectively with fewer side effects.[25] This study aimed to evaluate TRIB3 expression as a candidate prognostic factor in NB patients. TRIB3 expression and patient survival were analyzed with log-rank analyses and shown with Kaplan-Meier graphs. We found a significant relationship between TRIB3 expression and OVS and EFS of low-risk NB patients. TRIB3 expression was found to be correlated with better survival in low-risk patients. In addition, we studied

TRIB3 expression and survival in patients with low-stage tumors. A significant correlation was found between TRIB3 expression and OVS and EFS in low-stage patients. Nevertheless, we did not observe a significant correlation between TRIB3 expression and OVS and EFS in patients with high-risk disease or with high-stage tumors.

MYCN amplification is a well-established prognostic marker for NB related to advanced disease phenotype.[26] However, no significant correlation between TRIB3 expression and *MYCN* amplification was detected in our patient cohort. Instead, a significant correlation between TRIB3 and 1p36 LOH was identified among patients. 1p36 region is known to harbor several tumor suppressor genes and mostly deleted in NB.[27] Previous studies observed a strong correlation between *MYCN* amplification and 1p36 LOH.[28] However, we did not observe a correlation between *MYCN* amplification and 1p36 LOH among patients. Loss of heterozygosity at 1p36 affected disease progression yet did not decrease overall survival in NB patients; therefore, it was assumed as a marker for disease-free survival.[29] This study showed a significant correlation between TRIB3 and 1pLOH for the first time in NB. We still do not know how this co-occurrence contributes to tumor formation and prognosis of the disease.

Nevertheless, the limitations of this study are that this study was conducted in a single center, and the number of cases participating in the study is low. We propose that our study is the first to investigate TRIB3 expression in patients with NB, and we found that TRIB3 expression was associated with good prognosis in low-risk patients. However, confirming the findings in larger patient cohorts would have been better. We are planning to conduct new studies in larger patient cohorts to solidify TRIB3's role as a predictive biomarker in NB progression and cell culture studies to examine TRIB3's role and interaction with other proteins in NB progression.

Conclusions

Treatment of NB benefited from prognostic markers for risk stratification. TRIB3 expression was positively correlated with OVS and EFS in low-risk NB patients. Therefore, TRIB3 expression is proposed as a potential predictive biomarker for NB patients in low-risk groups.

Author Contributions

BB, GS, and SKÖ performed tissue sections and IHC staining's; DK collected the clinical data; SA performed microscopical examination as an expert pathologist; BB, and SA performed statistical analysis; BB, ZA, and NO were involved in the concept of the idea and designed the study and wrote the article. We hereby confirm that "All authors made a substantial contribution to the concept or design of the work, acquisition, analysis or interpretation of data. All authors have read and revised the manuscript critically for important intellectual content and approved the latest version of the manuscript to be published. Each author in the study participated sufficiently in the work to take public responsibility for appropriate portions of the content."

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Ethics Approval and Consent to Participate

The study was conducted under the ethical guidelines of the institution's local ethical committee. The study was approved by the Ethics Committee of the University (with the accession number 2022/16-03). All the patients have provided their written informed consent.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

1. Zafar A, Wang W, Liu G, et al. Molecular targeting therapies for neuroblastoma: progress and challenges. *Med Res Rev*. 2021;41:961-1021. doi:10.1002/med.21750
2. Nakagawara A, Li Y, Izumi H, Muramori K, Inada H, Nishi M. Neuroblastoma. *Jpn J Clin Oncol*. 2018;48:214-241. doi:10.1093/jjco/hyx176
3. Maris JM. The biologic basis for neuroblastoma heterogeneity and risk stratification. *Curr Opin Pediatr*. 2005;17:7-13. doi:10.1097/01.mop.0000150631.60571.89
4. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol*. 2009;27:289-297. doi:10.1200/JCO.2008.16.6785
5. Irwin MS, Naranjo A, Zhang FF, et al. Revised neuroblastoma risk classification system: a report from the Children's Oncology Group. *J Clin Oncol*. 2021;39:3229-3241. doi:10.1200/JCO.21.00278
6. Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and treatment. *Cell Tissue Res*. 2018;372:195-209. doi:10.1007/s00441-018-2821-2
7. Smith V, Foster J. High-risk neuroblastoma treatment review. *Children (Basel)*. 2018;5:114. doi:10.3390/children5090114
8. Sokol E, Desai AV. The evolution of risk classification for neuroblastoma. *Children (Basel)*. 2019;6:27. doi:10.3390/children6020027
9. Qiu B, Matthay KK. Advancing therapy for neuroblastoma. *Nat Rev Clin Oncol*. 2022;19:515-533. doi:10.1038/s41571-022-00643-z
10. Grosshans J, Wieschaus E. A genetic link between morphogenesis and cell division during formation of the ventral furrow in Drosophila. *Cell*. 2000;101:523-531. doi:10.1016/s0092-8674(00)80862-4
11. Seher TC, Leptin M. Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during Drosophila gastrulation. *Curr Biol*. 2000;10:623-629. doi:10.1016/s0960-9822(00)00502-9
12. Mayoral-Varo V, Jiménez L, Link W. The critical role of TRIB2 in cancer and therapy resistance. *Cancers (Basel)*. 2021;13:2701. doi:10.3390/cancers13112701
13. Richmond L, Keeshan K. Pseudokinases: a tribble-edged sword. *FEBS J*. 2020;287:4170-4182. doi:10.1111/febs.15096
14. Wennemers M, Bussink J, Grebenchtchikov N, Sweep FC, Span PN. TRIB3 protein denotes a good prognosis in breast cancer patients and is associated with hypoxia sensitivity. *Radiother Oncol*. 2011;101:198-202. doi:10.1016/j.radonc.2011.05.057
15. Izrailit J, Reedijk M. Developmental pathways in breast cancer and breast tumor-initiating cells: therapeutic implications. *Cancer Lett*. 2012;317:115-126. doi:10.1016/j.canlet.2011.11.028
16. Yu JM, Sun W, Wang ZH, et al. TRIB3 supports breast cancer stemness by suppressing FOXO1 degradation and enhancing SOX2 transcription. *Nat Commun*. 2019;10:5720. doi:10.1038/s41467-019-13700-6

17. Zhou H, Luo Y, Chen JH, et al. Knockdown of TRB3 induces apoptosis in human lung adenocarcinoma cells through regulation of Notch 1 expression. *Mol Med Rep*. 2013;8:47-52. doi:10.3892/mmr.2013.1453
18. Miyoshi N, Ishii H, Mimori K, et al. Abnormal expression of TRIB3 in colorectal cancer: a novel marker for prognosis. *Br J Cancer*. 2009;101:1664-1670. doi:10.1038/sj.bjc.6605361
19. Wang XJ, Li FF, Zhang YJ, Jiang M, Ren WH. TRIB3 promotes hepatocellular carcinoma growth and predicts poor prognosis. *Cancer Biomark*. 2020;29:307-315. doi:10.3233/CBM-201577
20. Aksoylar S, Varan A, Vergin C, et al. Treatment of high-risk neuroblastoma: national protocol results of the Turkish Pediatric Oncology Group. *J Cancer Res Ther*. 2017;13:284-290. doi:10.4103/0973-1482.183205
21. Stefanovska B, André F, Fromigué O. Tribbles pseudokinase 3 regulation and contribution to cancer. *Cancers (Basel)*. 2021;13:1822. doi:10.3390/cancers13081822.
22. Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science*. 2003;300:1574-1577. doi:10.1126/science.1079817
23. Arif A, Alameri AA, Tariq UB, et al. The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. *Int Immunopharmacol*. 2023;114:109581. doi:10.1016/j.intimp.2022.109581
24. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. *Pediatr Clin North Am*. 2015;62:225-256. doi:10.1016/j.pcl.2014.09.015
25. Pinto NR, Applebaum MA, Volchenboum SL, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol*. 2015;33:3008-3017. doi:10.1200/JCO.2014.59.4648
26. Bartolucci D, Montemurro L, Raieli S, et al. MYCN impact on high-risk neuroblastoma: from diagnosis and prognosis to targeted treatment. *Cancers (Basel)*. 2022;14:4421. doi:10.3390/cancers14184421
27. Kuick CH, Tan JY, Jasmine D, et al. Mutations of 1p genes do not consistently abrogate tumor suppressor functions in 1p-intact neuroblastoma. *BMC Cancer*. 2022;22:717. doi:10.1186/s12885-022-09800-0
28. Maris JM, Guo C, Blake D, et al. Comprehensive analysis of chromosome 1p deletions in neuroblastoma. *Med Pediatr Oncol*. 2001;36:32-36. doi:10.1002/1096-911X(20010101)36
29. Attiyeh EF, London WB, Mossé YP, et al. Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med*. 2005;353:2243-2253. doi:10.1056/NEJMoa052399