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Original Article

Analysis of the relationship between MYCN gene and serum NSE and urinary VMA levels and neuroblastoma pathological features and prognosis

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Abstract

The purpose of this study was to explore the relationship between the MYCN gene, serum neuron-specific enolase (NSE), urinary vanillylmandelic acid (VMA) levels, and neuroblastoma pathological features and prognosis. Ninety-four children with neuroblastoma treated in the hospital were selected to compare the differences in MYCN gene amplification, serum NSE, and urinary VMA levels in children with different clinicopathological features and prognoses. The proportion of children with MYCN gene copy number ≥10 in INSS stage 3-4 was higher than that of children with INSS stage 1-2 ($P < 0.05$); the proportion of children with MYCN gene copy number ≥10 in high-risk children in the COG risk stratification was higher than that of children with intermediate and low risk ($P < 0.05$); the serum NSE of children aged >12 months higher than that of children aged ≤12 months ($P < 0.05$); serum NSE of children with tumors >500 cm3 higher than that of children with tumors ≤500 cm3 ($P < 0.05$); serum NSE and urinary VMA of children with INSS staging of stages 3-4 were higher than that of children with stages 1 to 2 ($P < 0.05$); serum NSE and urinary VMA in children with lymph node metastasis were higher than that of children without lymph node metastasis ($P < 0.05$); serum NSE of children with MYCN gene copy number ≥10 was higher than that of children without lymph node metastasis ($P < 0.05$); the proportion of children with MYCN gene copy number ≥10 who died, and the percentages of serum NSE and urinary VMA were higher than those of the surviving children ($P < 0.05$). MYCN gene amplification and serum NSE and urinary VMA levels were related to the age, tumor size, INSS stage, COG stage, lymph node metastasis, and prognosis of the children with neuroblastoma.

Keywords: MYCN gene, Serum NSE, Urinary VMA, Neuroblastoma.

1. Introduction

Neuroblastoma (NB) is a complex and diverse solid tumor that originates in the sympathetic nervous system. Most commonly, NB tumors form in the abdomen, particularly in the adrenal gland [1]. This type of cancer accounts for 7-8% of childhood malignancies, with approximately 650 new cases diagnosed annually in the United States. Despite its relatively low incidence, NB contributes to roughly 15% of all pediatric cancer deaths.

Survival rates vary significantly depending on the stage and risk level of the disease. Patients with low or intermediate-risk NB have survival rates approaching 100%, while those with high-risk NB have a 5-year survival rate of less than 50% [2]. There are also ethnic disparities in NB prevalence, as it is more common among individuals of European ancestry, and African-American children tend to present with higher-risk forms of the disease [3].

Despite significant improvements in treatment outcomes against neuroblastoma over the past decades, there are still some high-risk patients with poor outcomes and poor prognoses. Therefore, the search for biomarkers associated with the pathological features and prognosis of neuroblastoma, and thus the early identification of different types of NB, is of great importance in guiding clinical treatment. NB develops from the cells of the sympathetic nervous system, particularly sympathoadrenal progenitor cells, which differentiate into adrenal chromaffin cells and sympathetic ganglion cells. The transformation process involves several factors, such as overexpression of neural growth factor (NGF) and MYCN, SRY-related HMG-box gene 10 (Sox10), and mammalian achaete-scute homolog 1 (MASH1), all induced by bone morphogenetic proteins (BMPs) [4]. The transformation of persistent resting progenitor cells into NB cells requires anaplastic lymphoma kinase (ALK) mutations and MYCN amplification. It involves transcription factors including Sox11, nescient helix-loop-helix protein 2 (NHIH2), Twist-related protein 1 (TWIST1), achaete-scute family bhlh transcription factor 1 (ASCL1), insulinoma-associated 2 (INSM2), and transcription factor 3 (TCF3). Several transcriptional regulators are involved in determining the fate of cells with sympathetic lineages, such as MASH1, inhibitor of DNA binding 2 (ID2), dHAND, hypoxia-inducible factor (HIF), and paired-like homeobox 2b (PHOX2), all of which likely play roles in the pathogenesis of NB. Elevated levels of N-Myc protein produced due to MYCN amplification play an essential role in the pathogenesis of NB. The MYCN locus encodes MYCNOS (antisense transcript), which encodes

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N-CYM. Inhibition of GSK3β-driven N-Myc degradation leads to N-CYM, stabilizing N-Myc. ALK also plays a significant role in transforming sympathoadrenal cells into NB cells. The expression of ALK is associated with a poor prognosis, and it is thought that activated ALK works together with MYCN to accelerate NB growth significantly [5].

MYCN gene is one of the critical genes in neuroblastoma development and progression [6]. MYCN gene amplification is closely associated with the occurrence and poor prognosis of high-risk neuroblastoma [7]. MYCN gene amplification is strongly associated with the development of high-risk neuroblastoma and poor prognosis. In addition, serum neuron-specific enolase (NSE) and urinary levels of the catecholamine metabolite vanillylmandelic acid (VMA) have been suggested as potential prognostic indicators for neuroblastoma [8,9]. Few studies have been reported on the correlation between the indicators above and pathological features of NB. Based on this, the present study investigated the relationship between the MYCN gene and NSE, urinary VMA levels, and neuroblastoma pathological features and prognosis.

2. Materials and Methods

2.1. Patients

Ninety-four children with neuroblastoma treated in our hospital from January 2010 to January 2023 were selected: 23 children aged ≤12 months and 71 children aged >12 months; 45 male and 49 female children; 10 cases of ganglion cell neuroma, 23 cases of ganglion cell neuroblastoma and 61 cases of neuroblastoma. Inclusion criteria: (1) all confirmed by surgery or biopsy pathology; (2) children diagnosed for the first time; (3) receiving MYCN gene, serum NSE, and urinary VMA examination; (4) complete clinicopathological and follow-up data. Exclusion criteria: (1) having received anti-tumor treatment such as radiotherapy; (2) having other malignant tumors; (3) having other organ diseases such as heart, liver and lung. This study was approved by the ethics committee of Shenzhen Children's Hospital. Signed written informed consents were obtained from the patients and guardians.

2.2. Experimental methodology

All the specimens were collected for sectioning, and the means of detecting NMYC gene amplification by FISH technology was as follows: Wax blocks were prepared into slides, aged at 56°C for 30 min, dehydrated in 70%, 85% and 100% ethanol for 2 min each, and then added with probe and hybridization buffer at 2:8, sealed, denatured at 72°C for 5 min, and then hybridized for 16 h at 46°C in a wet box. After hybridization, NP-40 buffer was applied, washed, and dried under light, and 4',6-diamidino-2-phenylindole (DAPI) was added. After hybridization, the samples should be washed with NP-40 buffer, dried and protected from light, then re-stained with 4',6-diamidino-2-phenylindole (DAPI) for 20 min, and then observed under a fluorescence microscope, and 200 interphase cells were counted in each specimen (Figure 1).

All the test subjects collected peripheral venous blood in the early morning fasting state for examination, and collected morning urine for examination, and used enzyme-linked immunosorbent assay (ELISA) to detect serum NSE and urine VMA levels. All kits were purchased from Shanghai Roche (Shanghai, China).

2.3. Treatment and follow-up methods

For treatment, refer to the Neuroblastoma (Figure 2) CCCG-NB-2015 Consensus Selection, which was made according to the tumor stage, and surgical treatment is preferred for early cases. For cases where the tumor cannot be removed entirely, low-dose VAC regimen and OPEC regimen are given according to age when the tumor can be removed entirely, surgery was performed, and chemotherapy is continued after surgery. Second-line regimens such as topotecan and cyclophosphamide were used for disease progression or recurrence after treatment. The patient's basic information was collected by reviewing the electronic medical record system and the results of auxiliary examinations, including age, gender, tumor size, pathological type, and INSS staging [10], COG risk stratification [11], tumor site, and lymph node metastasis.

2.4. Statistical analysis

Data were analyzed using Statistic Package for Social Science (SPSS) 22.0 software (IBM, Armonk, NY, USA), non-normally distributed measurements were expressed as M (Q25, Q75), and differences between groups were compared using the rank-sum test; count data were expressed as n (%) ratios, and the χ^2 test was used to analyze the differences between the groups; the criterion for statistical

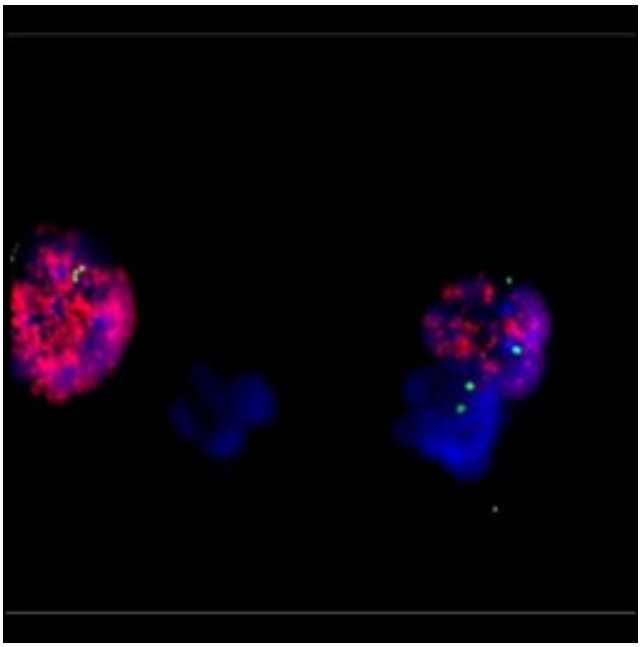

Fig. 1. Positive images for MYCN gene test (copy number ≥ 10).

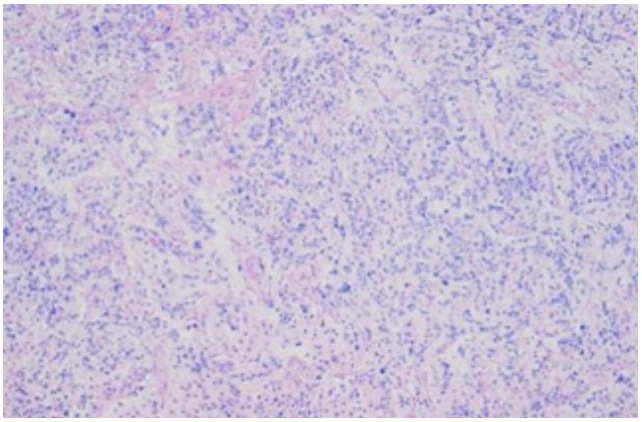

Fig. 2. Pathological picture of neuroblastoma (HE, ×200).

significance was: $P < 0.05$.

3. Results

3.1. Comparison of MYCN gene amplification in children with different pathological characteristics

The proportion of children with MYCN gene copy number ≥10 was higher than that of children with INSS stage 3-4 than that of children with INSS stage 1-2 (P<0.05, Table 1); the proportion of children with MYCN gene copy number ≥10 was higher than that of children with low or intermediate risk in the COG risk stratification (P<0.05, Table 1); the proportion of children with MYCN gene copy number ≥10 was not statistically significant in the comparison of the proportion of children with different ages, genders, tumor bodies, pathologic types, tumor sites, and metastasis of lymph nodes (P>0.05, Table 1). The difference in proportion was not statistically significant (P>0.05, Table 1).

3.2. Comparison of serum NSE and urine VMA levels in children with different pathological features

Serum NSE was higher in children aged >12 months than in children aged ≤12 months (P<0.05, Table 2); serum NSE was higher in children with tumors >500 cm^3 than in children with tumors <500 cm^3 (P<0.05, Table 2); serum NSE and urinary VMA were higher in children with INSS stage 3-4 than in children with stage 1-2 (P<0.05, Table 2); serum NSE and urinary VMA were higher in children with lymph node metastases than in children without lymph node metastases (P<0.05, Table 2). Children with lymph node metastasis had higher serum NSE and urine VMA than children without lymph node metastasis (P<0.05, Table 2).

3.3. Comparison of serum NSE and urinary VMA levels in children with different MYCN gene amplification

Serum NSE of children with MYCN gene copy number ≥10 was higher than that of children with MYCN gene copy number <10 ($P < 0.05$, Table 3); the difference in urinary VMA between children with MYCN gene copy number ≥10 and <10 was not statistically significant ($P > 0.05$, Table 3).

3.4. Comparison of MYCN gene amplification, serum NSE and urine VMA levels in children with different prognoses

As of November 2023, 39 children died and 55 survived; the proportion of MYCN gene copy number ≥10, serum NSE, and urinary VMA were higher in deceased children than in surviving children ($P < 0.05$, Table 4).

4. Discussion

Neuroblastoma originates from neural crest cells, which form part of the autonomic nervous system during embryonic development. Under normal circumstances, neural crest cells undergo a process of differentiation and maturation that culminates in forming different types of neuronal cells, such as neurons and glial cells [12]. Howe-

Table 1. Comparison of MYCN gene amplification in children with different pathological features.

Clinicopathological features	number of examples	Proportion of MYCN gene copy number ≥10 (%)	c^2	*P*
age				
≤ 12 months	23	10 (43.48)	0.937	0.333
>12 months	71	23 (32.39)		
sex				
male	45	16 (35.56)	0.008	0.930
women	49	17 (34.69)		
Tumor volume				
≤500 cm^3	71	26 (36.62)	0.292	0.589
>500 cm^3	23	7 (30.43)		
pathological type				
ganglioneuroma	10	4 (40.00)		
ganglion cell neuroblastoma	23	9 (39.13)	0.413	0.814
neuroblastoma	61	20 (32.79)		
INSS staging				
Phase 1 to 2	32	6 (18.75)	5.697	0.017
Phase 3-4	62	27 (43.55)		
COG risk stratification				
low to medium risk	35	5 (14.29)	10.611	0.001
high risk	59	28 (47.46)		
tumor site				
retroperitoneum	67	22 (32.84)	0.528	0.468
rest	27	11 (40.74)		
lymphatic node transfer				
yes	60	18 (30.00)	1.899	0.168
not	34	15 (44.12)		

Table 2. Comparison of serum NSE and urinary VMA levels in children with different pathological features.

Clinicopathological features	number of examples	Serum NSE (ng/ml)	Urinary VMA (mmol/24h)
age			
≤ 12 months	23	102.23 (65.54,155,27)	60.23 (41.13,98.27)
>12 months	71	189.28 (120.03,273.22)	51.14 (35.56,110.02)
Z		-4.454	-0.543
P		0.000	0.887
distinguishing between the sexes			
male	45	143.34 (98.72,167.76)	64.45 (50.60,105.54)
women	49	160.02 (87.70,160.02)	58.80 (40.50,87.72)
Z		-1.021	-1.112
P		0.601	0.545
Tumor volume			
≤500 cm^3	71	135.50 (78.40,180.02)	59.23 (40.50,98.27)
>500 cm^3	23	210.02 (162.12,265.56)	60.23 (35.56,105.54)
Z		-5.543	-1.323
P		0.000	0.512
pathological type			
ganglioneuroma	10	165.50 (110.32,210.01)	54.45 (35.56,70.20)
ganglion cell neuroblastoma	23	148.87 (98.82, 198.22)	60.23 (40.50,110.02)
neuroblastoma	61	156.65 (104.45, 205.54)	58.80 (41.13,98.27)
χ^2		0.892	0.998
P		0.773	0.712
INSS staging			
Phase 1 to 2	32	110.43 (76.67,178.22)	40.50 (32.23,75.60)
Phase 3-4	62	201.12 (162.12,265.56)	89.29 (60.40,150.50)
Z		-6.012	-4.454
P		0.000	0.000
COG risk stratification			
low to medium risk	35	130.50 (98.82, 198.22)	46.60 (30.02,68.80)
high risk	59	210.02 (178.22,273.22)	82.50 (55.45,135.54)
Z		-5.851	-6.676
P		0.000	0.000
tumor site			
retroperitoneum	67	156.65 (104.45, 190.20)	55.80 (40.50,87.72)
rest	27	148.87 (98.82, 205.54)	54.45 (35.56,98.27)
Z		-0.778	-0.721
P		0.791	0.812
lymphatic node transfer			
yes	60	220.03 (178.22,273.22)	90.30 (78.87,140.50)
not	34	156.65 (98.82, 205.54)	60.23 (41.13,98.27)
Z		-3.443	-4.443
P		0.000	0.000

Table 3. Comparison of serum NSE and urinary VMA levels in children with different pathological features.

MYCN gene	number of examples	Serum NSE (ng/ml)	Urinary VMA (mmol/24h)
Copy number ≥ 10	33	210.02 (162.12,265.56)	58.80 (41.13,98.27)
Number of copies <10	61	110.43 (65.54,160.02)	64.45 (50.60,87.72)
Z		-7.874	-1.443
P		0.000	0.332

Table 4. Comparison of MYCN gene amplification, serum NSE, and urinary VMA levels in children with different prognoses.

groups	number of examples	Proportion of MYCN gene copy number ≥10 (%)	Serum NSE (ng/ml)	Urinary VMA (mmol/24h)
dead	39	22 (56.41)	220.03 (162.12, 273.22)	90.30 (78.87,135.54)
survive	55	11 (20.00)	102.23 (78.40,155.27)	54.45 (35.56,70.20)
χ^2/Z		13.279	-6.676	-8.154
P		0.000	0.000	0.000

ver, due to genetic and environmental factors, neural crest cells may undergo abnormal proliferation and differentiation, leading to neuroblastoma formation. Neuroblastoma is a common malignant tumor in children, which can cause compression or invasion of the nervous system, leading to neurological symptoms such as headaches, vision changes, motor or sensory deficits, etc. Additionally, some neuroblastomas have endocrine functions, which can cause endocrine disruption [13]. In addition, some neuroblastomas have endocrine function and can cause endocrine disorders. Studies have shown that the prognosis of different clinical types of neuroblastoma is different [14]. Low-risk neuroblastoma Low-risk neuroblastoma usually has a low degree of differentiation and has a good prognosis; intermediate-risk and high-risk neuroblastoma has a relatively high degree of malignancy and a poorer prognosis, and it needs comprehensive treatments such as chemotherapy, radiotherapy, and targeted therapy. Therefore, early identification of different neuroblastoma types is essential to guide clinical treatment. In this study, we compared the differences in MYCN gene amplification, serum NSE, and urinary VMA levels among children with different clinicopathological features and prognoses to provide a basis for more accurate diagnosis and individualized treatment strategies.

The International Neuroblastoma INSS Staging System (INSS) is used to assess the severity and spread of disease in neuroblastoma patients, including the local spread of the tumor and lymph node involvement, and the higher the stage, the more malignant the tumor. The COG risk stratification is a scoring system for assessing the risk of the tumor tissues, which is classified as low risk, intermediate risk, and high risk. The results of this study showed that the proportion of children with MYCN gene copy number ≥10 was higher than that of children with INSS stage 3-4 than that of children with INSS stage 1-2, and the proportion of children with MYCN gene copy number ≥10 was higher than that of children with low and intermediate risk. The correlation between MYCN gene expression and INSS stage and COG risk stratification in children with neuroblastoma suggests that the MYCN gene is a gene encoding a transcription factor, which plays a vital role in neuroblastoma development and progression, and the amplification of the MYCN gene promotes the proliferation and progression of tumor cells. Studies have shown that neuroblastomas with MYCN gene amplification usually have higher cell proliferation capacity, infiltration, and metastasis, leading to increased INSS staging COG risk stratification [15].

NSE is an enzyme found predominantly in neurons and neuroendocrine cells, and it plays an essential role in the development, function, and damage of the nervous system. Serum NSE levels can be used to assess neurological disease damage [16]. Some neuroblastoma cells have endocrine function, which can synthesize and secrete catecholamines, and VMA is an essential metabolite of catecholamines. A 24-hour urinary VMA is a diagnostic marker for neuroblastoma [17]. VMA is an essential metabolite of catecholamines. This study showed that age, tumor size, INSS stage, and lymph node metastasis were correlated with serum NSE and urinary VMA in children. Older children usually have faster tumor growth and produce relatively more NSE and catecholamines. In addition, the larger size of the tumor reflects the active proliferation of tumor cells, which also causes an increase in the release of NSE and catechols from the tumor cells, which in turn causes an increase in blood NSE and urine VMA levels. Lymph node metastasis is one of the common ways of metastasis in neuroblastoma. The presence of lymph node metastasis may indicate tumor progression and increased malignancy, resulting in a corresponding increase in tumor INSS stage. In contrast, highly staged neuroblastomas usually have a more significant tumor load, with more proliferative activity and malignancy of the tumor cells. More tumor cells release NSE and catechins into the bloodstream, leading to elevated serum NSE and urinary VMA levels.

In addition, this study also showed that serum NSE was higher in children with MYCN gene copy number ≥10 than in children with MYCN gene copy number <10, suggesting that MYCN gene amplification is associated with elevated serum NSE [18]. An increase in the number of MYCN gene copies suggests that the expression of this gene is enhanced in children. When the copy number of the MYCN gene increases, the production of MYCN protein in the cells also increases, and the protein encoded by the MYCN gene is a transcription factor, which is involved in the regulation of the critical processes of cell proliferation, differentiation, and apoptosis, etc. The increase of MYCN protein may promote the proliferation of the cells and inhibit their apoptosis, which may lead to an increase in the degree of malignancy of the tumor and then cause the release of NSE from the tumor cells to be elevated.

This study also showed that the proportion of MYCN gene copy number ≥10, serum NSE, and urinary VMA were higher in children who died than in those who survived. This indicates that MYCN amplification, serum NSE, and urinary VMA levels are closely related to the prognosis of children with neuroblastoma. The reasons for this are that when MYCN gene amplification occurs, it leads to overexpression of MYCN protein, which in turn promotes the development and progression of neuroblastoma, and high serum NSE levels as well as urinary VMA levels are usually associated with increased tumor load and malignancy. Therefore, MYCN amplification, elevated serum NSE, and urinary VMA levels lead to increased tumor malignancy and cause poor prognosis to occur.

5. Conclusion

In conclusion, MYCN gene amplification and serum NSE and urinary VMA levels are related to age, tumor size, INSS stage, COG stage, lymph node metastasis, and prognosis of children with neuroblastoma.

Conflict of Interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
This study was approved by the ethics committee of Shenzhen Children's Hospital.

Informed Consent
Signed written informed consents were obtained from the patients and guardians.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions
Huan Zhang and Xianping Jiang designed the study and performed the experiments, Xiaoxiao He and Qiuling Miao collected the data, Li Li and Jianming Song analyzed the data, Huan Zhang and Xianping Jiang prepared the manuscript. All authors read and approved the final manuscript.

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