BMC Cancer

RESEARCH Open Access

Event-free survival in neuroblastoma with MYCN amplification and deletion of 1p or 11q may be associated with altered immune status

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Abstract

Background Neuroblastoma exhibits substantial heterogeneity, which is intricately linked to various genetic alterations. We aimed to explore immune status in the peripheral blood and prognosis of patients with neuroblastoma with different genetic characteristics.

Methods We enrolled 31 patients with neuroblastoma and collected samples to detect three genetic characteristics. Peripheral blood samples were tested for immune cells and cytokines by fluorescent microspheres conjugated with antibodies and flow cytometry. Event-free survival (EFS) was analyzed using the Kaplan–Meier method.

Results Twenty-two patients had genetic aberrations, including MYCN amplification in 6 patients, chromosome 1p deletion in 9 patients, and chromosome 11q deletion in 14 patients. Two genetic alterations were present in seven patients. The EFS was worse in patients with MYCN amplification or 1p deletion than in the corresponding group, whereas 11q deletion was a prognostic factor only in patients with unamplified MYCN. Changes in immune status revealed a decrease in the proportion of T cells in blood, and an increase in regulatory T cells and immunosuppression-related cytokines such as interleukin (IL)-10. The EFS of the IL-10 high-level group was lower than that of the low-level group. Patients with concomitant genetic alterations and a high level of IL-10 had worse EFS than other patients.

Conclusions Patients with neuroblastoma characterized by these genetic characteristics often have suppressed T cell response and an overabundance of immunosuppressive cells and cytokines in the peripheral blood. This imbalance is significantly associated with poor EFS. Moreover, if these patients show an elevated levels of immunosuppressive cytokines such as IL-10, the prognosis will be worse.

Keywords Neuroblastoma, MYCN, Chromosome 1p, Chromosome 11q, Immune status

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Background

Neuroblastoma (NB) is a childhood malignancy originating from the sympathetic nervous system and is the most common extracranial malignant solid tumor in children, accounting for approximately 12–15% of all childhood cancer-related deaths [1–3]. Approximately 36% of children with NB are younger than 12 months at diagnosis, with a median age of 573 days [4]. NB is characterized by significant heterogeneity in clinical presentation [2, 3]. Some patients with NB have tumors that spontaneously regress [5, 6], while others have recurrent disease progression or recurrence despite a combination of treatment options, such as chemotherapy, surgery, radiation therapy, autologous hematopoietic stem cell transplantation, and immunotherapy, thus becoming relapsed/refractory neuroblastoma [7, 8]. Although multimodal therapies have improved the 5-year overall survival rate of high-risk NB patients from 20 to 50% over the past few decades, the 5-year event-free survival rate remains around 30%, and these patients still need precision medicine to further improve survival [3, 9].

Although most neuroblastomas are sporadic, germline or heritable alterations can also influence the development and progression of neuroblastoma [3]. In contrast to most other solid tumors, somatic mutations in NB are rare, whereas copy number variations in segmental chromosomal regions or entire chromosomes are common, with the former also referred to as segmental chromosomal alterations (SCA) [10, 11]. The type of chromosomal alteration in NB is associated with prognosis; for example, SCAs (including 1p loss, 11q loss, and 17q gain) are associated with poor prognosis [11]. One hypothesis for this condition is that segmental chromosome loss leads to the inactivation of tumor suppressor genes [12, 13]. MYCN alterations are the recurrent somatic mutations of most concern, of which MYCN amplification (MNA) is one of the drivers of neuroblastoma and is also strongly associated with poor prognosis [14, 15].

Tumor cells form their unique tumor microenvironment through communication mechanisms such as paracrine and juxtacrine mechanisms [16, 17]. In this microenvironment, interactions between tumor cells and host cells influence the therapeutic response and disease progression [18]. In addition, immune cells are probably the most involved and functional players in the tumor microenvironment, participating in both antitumor and protumor activities [19]. Tumor cells evade immune attack by downregulating antigen presentation machinery or inducing inhibitory immune checkpoint molecules [20, 21]. Meanwhile, immune cells such as regulatory T cells are used to promote the formation of an immunosuppressive tumor microenvironment to facilitate immune escape [22–24]. These mechanisms allow tumors to have a specific immune microenvironment. However, patients with the same tumor type/subtype may have different changes in the immune microenvironment, resulting in different treatment responses and disease progression [25]. The need to elucidate the mechanisms underlying the heterogeneity of the immune environment in different patients becomes apparent to facilitate rational immunomodulatory strategies in clinical treatment. We herein explored the correlation between molecular subtypes of neuroblastoma and immunophenotypes, to personalize treatment more precisely in future clinical care and further improve the disease outcomes of patients with NB.

Methods

Patients

Patients with NB were newly diagnosed and treated at the Department of Pediatric Oncology, Tianjin Medical University Cancer Hospital from April 2018 to May 2022 and followed up at our hospital between October 2022, and February 2023.

All patients were diagnosed according to the International Neuroblastoma Risk Group Staging System (INRGSS) and treated according to the Chinese Children's Cancer Group (CCCG)-NB-2015 protocol or the CCCG-NB-2021 protocol. The follow-up date ended on February 28, 2023, with a median follow-up time of 22 months (range 9–58 months).

This study was supported by the ethics committee of Tianjin Medical University Cancer Hospital. Informed consent for the biological study was obtained prior to obtaining pathology specimens and peripheral blood samples. Informed consent was signed by the patient's parents or guardians.

Treatment

The CCCG-NB-2021 protocol for patients with NB can be found in Table 1. For high-risk patients, autologous peripheral blood stem cell collection is generally performed after 2 courses of chemotherapy. After 4 courses of chemotherapy, surgical resection of the primary site was performed. After completing conventional chemotherapy, patients received high-dose chemotherapy with sequential ASCT. Radiotherapy to the tumor bed was carried out between the two ASCTs. The maintenance treatment was 13-cis retinoid acid 160 mg/m^2, 14 days/month, for a total of 6 months.

Samples and biologic studies

Peripheral blood samples were collected at the time of the patients' initial admission to the hospital, prior to receiving treatment. Immediately after surgical resection or ultrasound-guided fine needle aspiration, tumor specimens are rapidly frozen or placed in tissue culture media

Table 1 Treatments according to protocol CCCG-NB-2021

Pre-treatment risk stratification	Treatment and medication regimen*	Dosage**
Low risk and very low risk	1. CBP + VP16 2. CBP + CTX + DOXO 3. VP16 + CTX 4. CBP + VP16 + DOXO	CBP, 560 mg/m^2; VP16, 120 mg/m^2; CTX, 1000 mg/m^2; DOXO, 30 mg/m^2
Intermediate risk	1,3,5,7. VCR + CDDP + DOXO + CTX 2,4,6,8. VCR + CDDP + VP16 + CTX	VCR, 1.5 mg/m^2; CDDP, 90 mg/m^2; DOXO, 30 mg/m^2; CTX, 1200 mg/m^2
High risk	1,2. CTX + TOPO 3,5. CDDP + VP16 4,6. CTX + DOXO + VCR + Mesna	CTX, 400 mg/m^2; TOPO, 1.2 mg/m^2; CDDP, 50 mg/m^2; VP16, 200 mg/m^2 CTX, 2100 mg/m^2; DOXO, 25 mg/m^2; VCR, 0.67 mg/m^2; Mesna, 420 mg/m^2

*Numbers represent the order of courses of treatment. 21 days/course

**The individual dosage should be adjusted with reference to the patient's age and body weight

and sent to the pathology department for the study of tumor biology.

Fluorescence in situ hybridization (FISH) analysis of tumor pathology specimens

Determination of the amplification status of MYCN and the allelic status of chromosome arms 1p and 11q using immunohistochemical analysis, fluorescence in situ hybridization assay. MYCN amplification was defined as a > 4-fold increase in MYCN signal relative to chromosome 2. Chromosome 11q deletion is defined as showing only one fusion signal. Chromosome 1p deletion is defined as showing only one fusion signal. At the time of the experiment and assessment, the investigators were blinded to the patient's characteristics and other data.

Flow cytometry analysis of peripheral blood

Peripheral blood samples from NB patients were tested for immune cells and cytokines by flow cytometry. An additional table is available to see details of the antibodies used in the detection [see Additional file 1].

Cytokines were tested using a seven-cytokine test kit (BBJG07A, NZK Biotech, China). In the first step, the sample is mixed with a suspension of microspheres (fluorescent microspheres conjugated with interleukin (IL)-10, tumor necrosis factor (TNF)-α, IL-2, interferon (IFN)-γ, IL-6, IL-4 and IL-17 A antibodies) conjugated with a capture antibody, and the analyte in the sample binds to the specific capture antibody on the microspheres. In the second step, a detection antibody (biotin-labeled antibodies to seven cytokines) is added, which binds specifically to the reaction product of the first step. In the third step, the fluorescein solution is added, and the fluorescein streptavidin binds to the biotin on the detection antibody, forming a "microsphere surface capture antibody-antigen-biotinylated antibody-streptavidin fluorescein" compound. Finally, flow cytometry was performed using APC-Cy7 and APC channels.

FC was performed by BriCyte E6 (TA-12000666, Mindray, China), and analysis was conducted by FlowJo software (version 7.6.1). A minimum of $4*10^6$ events were detected for each sample analyzed.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 software. Tests of association were performed with the use of Fisher's exact test. Significant differences were analyzed using an independent samples T-test. Survival curves were constructed according to the Kaplan–Meier method. Events, or failures, for the event-free survival (EFS) analysis, were defined as disease progression, relapse, or death. The time to event was calculated as the time from the patient's initial diagnosis at our institution to the first event/failure, or to the follow-up cutoff time if no event occurred. Event-free survival rates were calculated as the rates ± SE. In this study, two-sided $p < 0.05$ was considered a statistically significant difference.

Results

MYCN amplification, 1p or 11q deletion are common genetic aberrations in neuroblastoma and these patients had poor EFS

A total of 31 patients with NB were enrolled in this study, including 14 males and 17 females. 77% of patients were diagnosed with NB in the high-risk group. The clinical characteristics of the patients in this cohort can be seen in Table 2. FISH of the pathologic specimens showed genetic aberrations in a total of 22 patients, including MYCN amplification in 6 patients (19%), chromosome 1p chromosome deletion in 9 patients (29%), and chromosome 11q chromosome deletion in 14 patients (45%) (Fig. 1). Two genetic alterations were present in seven patients (22%). None of the patients had both MYCN amplification and 11q deletion, and none of the patients had all three genetic alterations at the same time.

Patients were grouped according to the presence or absence of MYCN amplification, chromosome 1p

Table 2 Statistics on the characteristics of patients with neuroblastoma

Characteristic	Number	% If applicable
Age at diagnosis (m), median (range)	47(9,133)	
> 18	28	90.3
≤ 18	3	9.7
Sex		
Male	14	45.2
Female	17	54.8
Longest diameter of tumor (cm)		
< 10	17	54.8
> 10	5	16.1
Unknown	9	29.0
Bone marrow metastasis		
Yes	24	77.4
No	7	22.6
INRG Pretreatment Risk Group		
Low/Intermediate	7	22.6
High	24	77.4
INRG Stage		
L2	8	25.8
M	23	74.2
Relapse or progression		
Yes	12	38.7
No	19	61.3
Time to relapse or progression from diagnosis(m), median (range)	12(3,30)	

deletion, or chromosome 11q deletion. All patients with 11q deletion were >18 months old at diagnosis. MYCN amplification status was associated with the occurrence of disease recurrence or progression ($p=0.022$), but no association was observed between MYCN status and the patient's age at diagnosis, sex, etc. 1p chromosome status was associated with LDH level at diagnosis ($p=0.006$), whether the disease progressed or recurred ($p=0.041$), and secondary progression or recurrence ($p=0.004$) but was not significantly associated with age or NSE. Chromosome 11q status was associated with the occurrence of bone marrow metastasis in patients ($p=0.009$), but no association was observed with age, sex, NSE, or LDH. In addition, there was a significant negative correlation between MYCN amplification and 11q deletion ($p=0.021$), while there was a positive association between MYCN amplification and 1p deletion ($p=0.004$). An additional file shows this in more detail [see Additional file 2]. The 2-year event-free survival rates in the MYCN amplified and MYCN non-amplified groups were 16.7% and 73.5%, respectively, which were significantly different ($\chi2=4.821$, $p=0.028$). The 2-year event-free survival rates in the groups with and without 1p chromosome deletion were 31.7% and 70.3%, respectively ($\chi2=4.934$, $p=0.026$). The 2-year event-free survival rates in the groups with and without 11q chromosome deletion were 55.7% and 60.4%, respectively, with no significant difference ($\chi2=1.489$, $p=0.222$). However, among patients without MYCN amplification, the 2-year event-free survival rates were 55.7% and 100% in the chromosome 11q deletion and group without chromosome 11q deletion, respectively ($\chi2=6.993$, $p=0.008$), whereas there was no significant difference between the group with chromosome 1p deletion and group without chromosome 1p deletion ($\chi2=2.063$, $p=0.151$) (Fig. 1).

The results of complete blood counts and biochemical tests did not differ significantly between groups of patients with different genetic characteristics

We obtained peripheral blood from the patients on their initial admission to the hospital to test routine blood examination, NSE, ferritin, LDH, and albumin.

White blood cells were in the normal reference interval (4.4–11.9*10^9/L) in 55% of patients. The absolute values of neutrophils were in the range of normal values (1.2-7.0*10^9/L) in 74% of patients. Only 26% of patients had normal absolute values of lymphocytes (1.8–6.3*10^9/L), and 74% of patients had values below the normal reference interval. Monocyte counts were in the normal reference interval (0.12–0.93*10^9/L) in 74% of patients. NSE values were higher than normal (0–16.3 μg/L) in 90% of patients. 68% of patients had ferritin (Ferr) above the normal value interval (13–150 μg/L). LDH was above the normal interval (120–250 U/L) in 48% of patients [see Additional file 3].

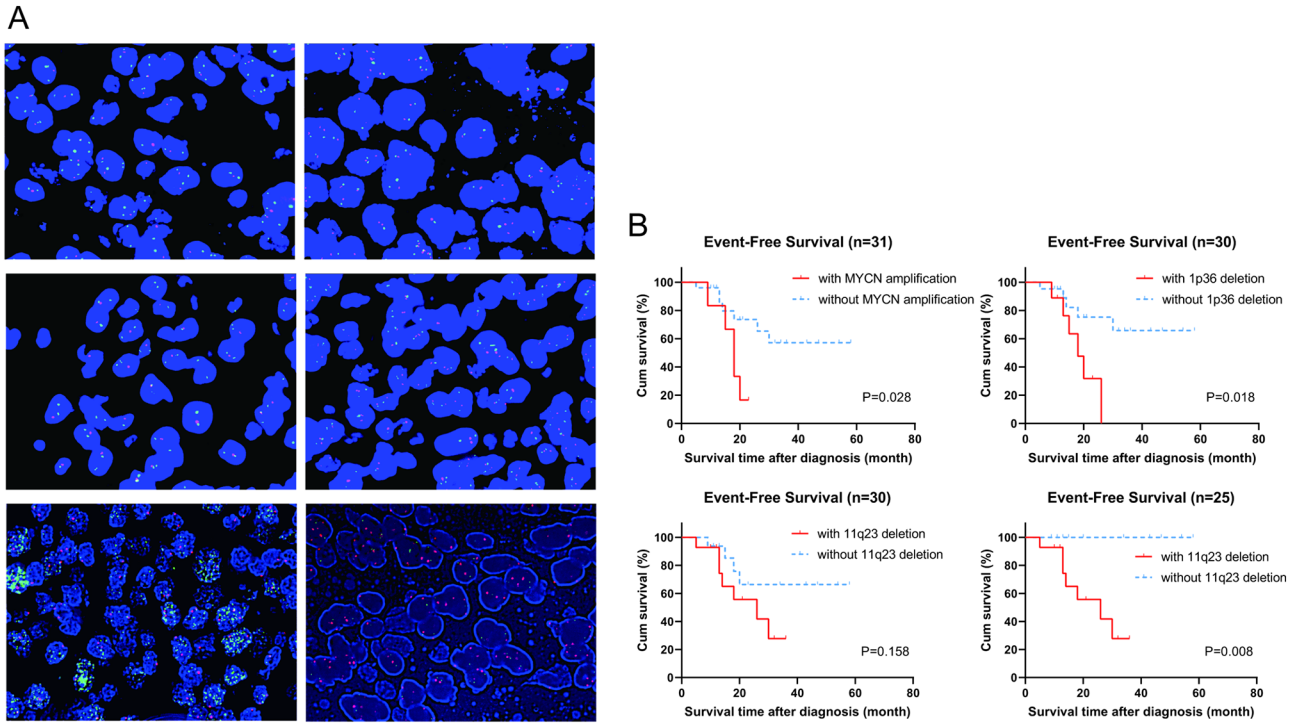

Fig. 1 Diagnosis and prognosis of patients with different genetic alterations. (**A**) Typical fluorescence in situ hybridization (FISH) images of pathological specimens from patients with neuroblastoma. The left picture belongs to patients with genetic alterations, and the right picture belongs to patients without genetic alterations. The status of 11q23 deletion was detected with a dual-color probe. In cells with 11q23 deletion, the pattern is one orange/green fusion signal. Normal cells show two paired orange/green fusion signal patterns **(top)**. The status of the 1p36 deletion was detected with a dual-color probe. In cells with 1p36 deletion, the pattern is one orange/green fusion signal. Normal cells show two paired orange/green fusion signal patterns **(middle)**. The amplification status of the v-myc avian myeloproliferative viral oncogene neuroblastoma-derived homologue (MYCN) was examined using a dual-color probe. The green signals represent a specific probe for MYCN, and the red signals represent a probe for centromeric chromosome 2 **(bottom)**. (**B**) Comparison of event-free survival analyses for different subgroups of genetic alterations. Kaplan–Meier estimates for the MYCN amplification group **(top left)**, chromosome 1p deletion group **(top right)**, chromosome 11q deletion group **(bottom left)**, and chromosome 11q deletion group in patients without MYCN amplification **(bottom right)**

Levels of peripheral blood immune cells differed significantly between groups of patients with different genetic characteristics

The MYCN amplified group had lower total T%, $CD4^+$ T%, and $CD8^+$ T% than the non-amplified group ($p<0.001$, $p<0.001$, $p=0.001$, respectively). The 1p chromosome deletion group had lower total T% and $CD4^+$ T% than the group without 1p chromosome deletion ($p<0.001$ and $p<0.001$, respectively), but there was no significant difference in $CD8^+$ T% ($p=0.062$). The chromosome 11q deletion group had lower total T% and $CD4^+$ T% than the group without chromosome 11q deletion ($p<0.001$ and $p<0.001$, respectively), but there was no significant difference in $CD8^+$ T% ($p=0.516$).

The percentage of Tregs (Treg%) was significantly higher in the MYCN amplified, 1p chromosome deletion, and 11q chromosome deletion groups than in the group without deletion/amplification ($p=0.001$, $p=0.008$, $p=0.017$, respectively). In addition, the absolute values of Treg counts (Treg#) (pcs/μl) were significantly higher in both the MYCN amplified and 1p chromosome deletion groups than in the group without deletion/amplification ($p=0.031$, $p=0.048$, respectively), but there was no significant difference between the 11q chromosome deletion group and the group without deletion/amplification ($p=0.064$).

The B% was statistically higher in the MYCN amplified and 1p chromosome deletion groups than in the group without deletion/amplification ($p=0.016$, $p=0.040$, respectively). There were no significant differences in B% in the 11q chromosome deletion group compared to the group without deletion/amplification ($p=0.208$).

NK% was higher in the chromosome 1p deletion group than in the group without deletion/amplification ($p=0.031$), but there was no significant difference in the MYCN amplified and 11q chromosome deletion groups ($p=0.116$, $p=0.102$, respectively) (Fig. 2).

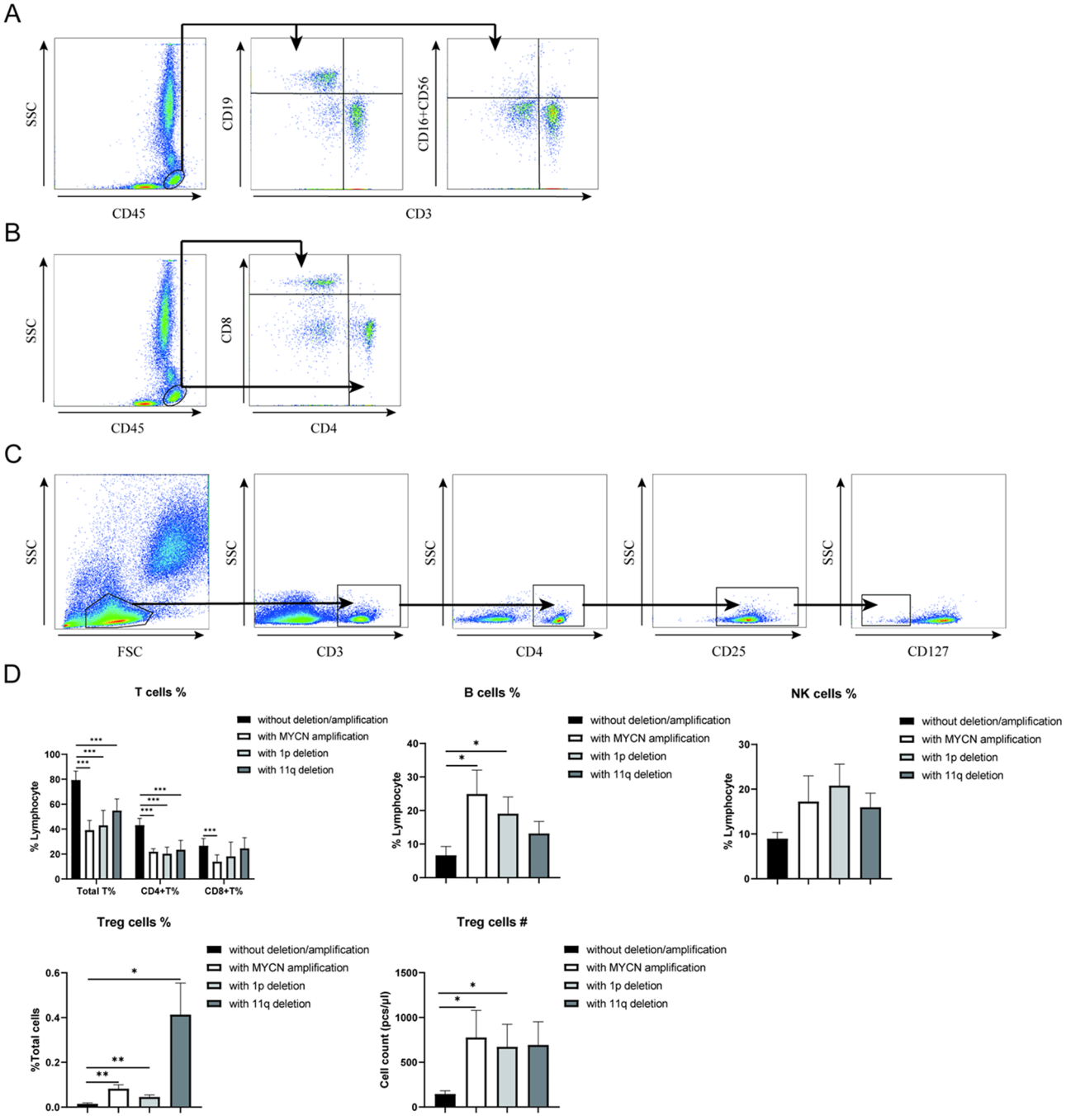

Fig. 2 Flow cytometry assay and gating strategy for peripheral blood immune cells from patients with neuroblastoma. (**A**), (**B**) and (**C**) are gate strategies for B and NK cells, T cells, and Treg cells, respectively. (**D**) Comparison of peripheral blood lymphocytes from different genetic alteration subgroups

Concentrations of IL-10, IL-6, TNF-α, and IFN-γ are upregulated in peripheral blood of patients with genetic alterations

We tested a total of seven cytokines, namely, IL-10, TNF-α, IL-2, IFN-γ, IL-6, IL-4, and IL-17 A, on the patients' peripheral blood samples. IL-10, TNF-α, IL-2, IFN-γ and IL-6 were significantly higher in the MYCN amplified group than in the group without deletion/amplification ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.021$, $p=0.005$, respectively), while no significant differences were observed for any other cytokines. Compared to the group without deletion/amplification, IL-10, TNF-α, IL-2, IFN-γ and IL-6 were significantly higher in the 1p chromosome deletion group ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.027$, $p=0.004$, respectively), while no significant differences were found for any other cytokines. IL-10, TNF-α, IFN-γ,

IL-6 and IL-17 A were significantly higher in the chromosome 11q deletion group than in the group without deletion/amplification ($p<0.001$, $p<0.001$, $p=0.015$, $p=0.004$, $p=0.043$, respectively), while all other cytokines were not significantly different (Fig. 3).

The higher the peripheral blood level of IL-10, the worse the patient's EFS, and when combined with genetic aberrations, their prognosis is even worse

We used the median level of each cytokine in all patients as a criterion to categorize them into high- and low-level groups. The event-free survival rate of the IL-10 high-level group was significantly lower than that of the low-level group ($p=0.001$). There were no significant differences in EFS between the high- and low-level groups for other cytokines. In the IL-10 low-level group, patients with 1p, 11q, or MYCN mutations had significantly lower EFS than group without deletion/amplification ($p=0.043$). Patients with concomitant genetic alterations and a high level of IL-10 had significantly lower EFS than other patients ($p=0.001$) (Fig. 4).

Discussion

Neuroblastoma accounts for 8–10% of malignant tumors in children and has a mortality rate of up to 15% [2, 26]. Because of the marked heterogeneity of

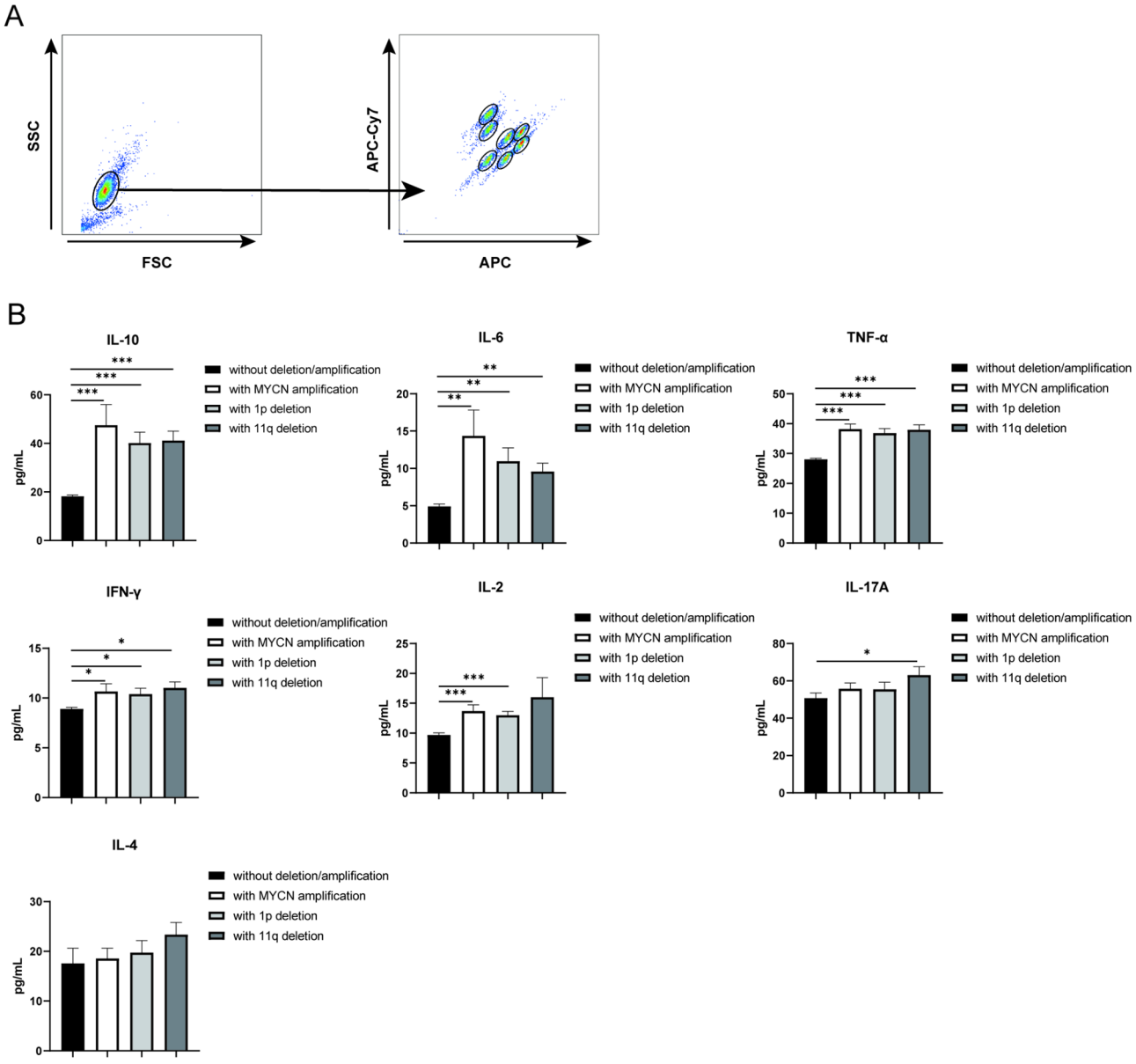

Fig. 3 Flow cytometry assay and gating strategy for peripheral blood cytokines from patients with neuroblastoma. (**A**) The gating strategy for seven cytokines. (**B**) Comparison of peripheral blood cytokines from different genetic alteration subgroups

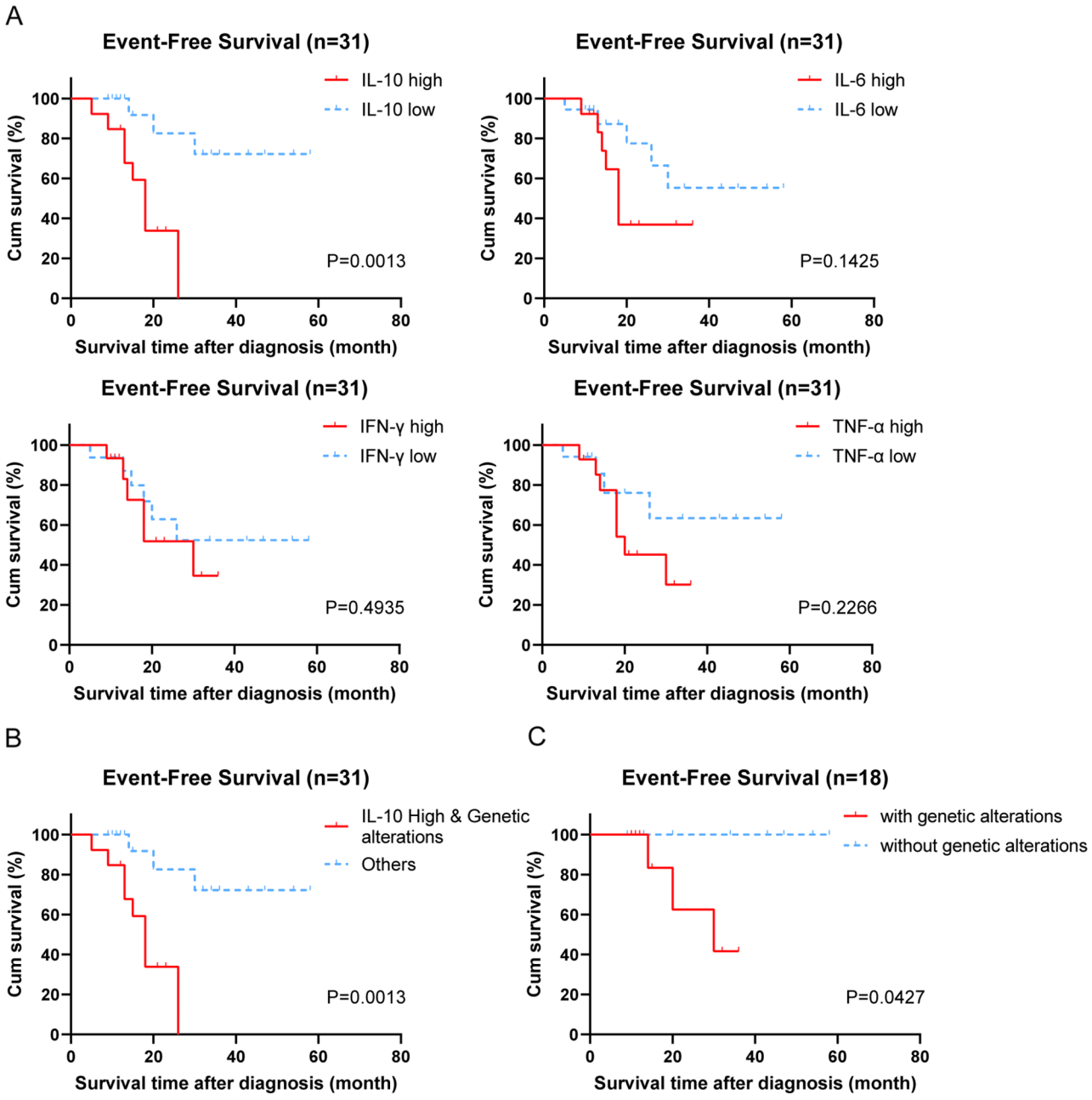

Fig. 4 Comparison of event-free survival analyses for different IL-10 levels and genetic alteration subgroups. Kaplan–Meier estimates for the IL-10, IL-6, IFN-γ and TNF-α level subgroup (**A**), the genetically altered subgroup in the IL-10 low-level group (**B**), and the subgroup with both high levels of IL-10 and genetic alterations (**C**)

NB and the diverse clinical presentations of patients with different biological characteristics, clinical treatment options for NB are currently targeted using a risk stratification approach. The Pretreatment Classification Schema, developed by the International Neuroblastoma Risk Group (INRG) based on image-defined risk factors (IDRFs), is now a commonly accepted and adopted risk stratification system internationally. A number of variables have been applied to risk stratification to predict survival in patients with NB. MYCN gene amplifications and segmental chromosomal aberrations are key genomic features in neuroblastoma, especially in high-risk neuroblastoma. Therefore, MYCN amplification and 11q deletion are currently included in this risk stratification system. There have been many reports on the close association of abnormal MYCN gene amplification with poor prognosis in NB [14, 15]. Segmental chromosome amplification is also associated with an advanced stage

of disease, a higher risk of relapse and a worse prognosis [11, 27–30]. According to Ambros IM and Holly JM et al. [31, 32], the presence of segmental chromosomal aberrations, especially the 11q deletion, significantly reduces survival in patients with NB older than 18 months and is a risk factor for reduced EFS and overall survival (OS) in patients older than 18 months. In patients younger than 18 months, segmental chromosomal aberrations were the only independent risk factor for recurrence, especially the 11q deletion. Chromosome 1p deletion is also a risk factor for neuroblastoma recurrence [33]. To stratify NB patients more accurately, we routinely detected the MYCN gene amplification status and the deletion status of the 1p and 11q chromosome arms in patients with NB.

We divided the patients into the MYCN amplification group, 1p deletion group and 11q deletion group according to genetic characteristics and the corresponding group without deletion/amplification. In this cohort, 19.4% of patients had MYCN amplification, slightly lower than other studies reported [9, 34, 35]. 11q deletions were more common (45.2%) than in the other two groups, consistent with previous reports [28, 36, 37]. No patient had both MYCN amplification and 11q deletion, as the two states appeared to have a mutually exclusive relationship [38, 39]. The coexistence of both states has been reported in only rare cases, which are often very high risk [40]. In addition, patients in the 11q deletion group were all older than 18 months at diagnosis, which is consistent with the older age of onset in NB patients with 11q deletion previously reported [33, 41]. We performed survival analysis on different groups, and the results showed that the event-free survival rate of the MYCN amplification group was significantly lower than that of the group without MYCN amplification, and the EFS of the 1p deletion group was also significantly lower than that of the group without 1p deletion. However, there was no significant difference in EFS between the 11q deletion group and the group without 11q deletion. After excluding patients with MYCN amplification, there was a significant difference in EFS between the 11q chromosome deletion group and the group without 11q deletion. This suggests that MYCN amplification and 1p deletion are prognostic factors in NB patients, but 11q deletion is only a prognostic factor in the MYCN-nonamplified neuroblastoma population.

We further explored the reasons for the poorer EFS of patients with these genetic characteristics. Changes in the immune environment play an important role in influencing treatment response and disease development, and studies have also demonstrated that neuroblastoma has the ability to use the immune system to escape and promote disease metastasis and progression [42]. We focused on changes in the immune environment in NB patients across groups.

In the immune status of neuroblastoma, T cells are the main cells and can be divided into $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T helper cells, regulatory T cells and NKT cells. Tumor-infiltrating lymphocytes ($CD4^+$ and $CD8^+$ T cells) and NKs play an important role in the immune response, while Treg cells and macrophages are also associated with poor prognosis. These factors are involved in the process of immune escape [43]. Therefore, we first analyzed the peripheral blood lymphocyte subtypes of the patients, and found that the total T %, $CD4^+$T % and $CD8^+$T % of the MYCN amplification group were lower than those of the corresponding group without deletion/amplification, and the total T % and $CD4^+$T % of the 1p deletion group and 11q deletion group were also lower, but there was no significant difference in $CD8^+$T %. This suggests that genetic alterations in neuroblastoma appear to be associated with the suppression of tumor-infiltrating lymphocytes. Subsequently, we detected Tregs in the peripheral blood of patients. Compared with the group without deletion/amplification, the Treg% in the MYCN amplification group, the 1p chromosome deletion group and the 11q chromosome deletion group were all higher. Treg# was also significantly higher in the MYCN amplification group and the 1p chromosome deletion group than in the corresponding group without deletion/amplification but not in the 11q chromosome deletion group. In addition, although 41.9% of NB patients had a lower B% than the normal interval, there was no significant difference in B% in the MYCN amplification group, the 1p chromosome deletion group and the 11q chromosome deletion group compared to the group without deletion/amplification. The NK% was higher in the MYCN amplification group and the 11q deletion group than in the group without deletion/amplification. However, the majority of patients in the cohort had NK% in the normal reference interval. Therefore, our results do not present a significant alteration of B cells and NK cells in the peripheral blood that might exist.

Cytokines are known to play an important role in the immune environment, participating in complex and diverse immune responses. Cytokines also interfere directly or indirectly with each other's expression, including interdependent positive and negative feedback mechanisms, thus achieving dynamic homeostasis and immune control. Tumor cells, on the other hand, are able to disrupt this balance through multiple pathways, thereby suppressing the immune response and protecting themselves from potential immune attack [44]. We tested seven cytokines in the peripheral blood of the patients and found that IL-10 and IL-6 were significantly elevated in all three groups compared to the group without deletion/amplification. By further analysis, we found that patients in the IL-10 high-level group had a worse prognosis than those in the IL-10 low-level group.

Furthermore, in the IL-10 low-level group, patients with genetic alterations had a worse prognosis than patients without deletion/amplification. Patients with both high levels of IL-10 and genetic alterations had a worse prognosis than other patients. This suggests that we can use cytokines such as IL-10 to further stratify patients with neuroblastoma who have the same molecular phenotype, thus providing a more accurate prediction of their prognosis. IL-6 and IL-10 are more involved in protumor effects in the tumor microenvironment; for example, they are involved in processes such as promoting immunosuppression and angiogenesis. IL-6 and IL-10 have been found to be positively correlated with tumor size, tumor stage and poor prognosis in many tumor types, such as lung, colorectal and gastric cancers [44]. IL-10 is classified as an immunosuppressive cytokine, and the cellular sources are T helper (Th) 2 cells, Treg cell subsets and Th17 cells [45]. Our results also showed that Treg cells were significantly increased in the three genetically altered groups, which is consistent with the results of increased IL-10.

Th1 and Th2 are two $CD4^+$ helper cell subsets associated with cytokine patterns of immune-promoting and immunosuppressive functions, respectively [46]. Th2 $CD4^+$ T cells express high concentrations of IL-4, IL-6, and IL-10, which inhibit T-cell-mediated cytotoxicity [47]. Treg cells are immunosuppressive and suppress autoimmune responses by releasing suppressive cytokines (e.g., IL-10), inhibiting the maturation of dendritic cells (DCs), and participating in metabolic disorders. In addition, IL-4 and IL-10 are involved in the induction of M2-like macrophage polarization. Alternatively, activated M2 macrophages have protumor and immunosuppressive functions [48]. In the previously reported pan-cancer analysis, $CD8^+$ T cells, NK cells and neutrophils were significantly associated with a better prognosis, while Th2 cells were associated with a poorer prognosis [25]. In the study by Sherif S et al. [49], patients were classified into six immune subtypes, of which S6 was the immune/lymphocyte suppressed subtype, in which both Th2 and Treg were highly enriched. The prognosis of the S6 immune subtype was also relatively poor in the pan-cancer study.

Conclusions

Overall, MYCN amplification and 1p deletion can have adverse effects independently, while 11q deletion functions in the absence of abnormal MYCN amplification. In patients with MYCN amplification, 1p deletion or 11q deletion, immunosuppressive-related cells such as Treg cells are upregulated, and the cytokines IL-6 and IL-10 are correspondingly increased, which together promote the formation of an immunosuppressive microenvironment. An elevated level of IL-10 adversely affects prognosis, and patients with both elevated IL-10 and molecular alterations have the worst prognosis. Therefore, we can not only subgroup patients using molecular typing but also further use cytokines such as IL-10 for more accurate risk stratification, thus enabling precise treatment of patients with neuroblastoma. In addition, in NB with these three genetic characteristics, there may exist some regulatory network/signaling pathways that upregulate Treg cells, Th2 lymphocytes, and M2-like macrophages, downregulate tumor-infiltrating T cells, and promote the secretion of the immunosuppressive cytokines IL-6 and IL-10, creating an immunosuppressive microenvironment that affects the patient's immune response and ultimately leads to a poorer clinical outcome. Therefore, in the future, we need to continue to investigate in depth by which signaling networks MYCN amplification, 1p deletion or 11q deletion are upregulated or inhibited to promote the formation of an immunosuppressive microenvironment that affects patient prognosis. Because of the small size of this cohort, a larger sample size is still needed to further confirm our findings.

Abbreviations

NB	Neuroblastoma
EFS	Event-free survival
Treg	Regulatory T-cell
IL	Interleukin
SCA	Segmental chromosomal alteration
MNA	MYCN amplification
INRGSS	International Neuroblastoma Risk Group Staging System
CCCG	Chinese Children's Cancer Group
CBP	Carboplatin
VP16	Etoposide
CTX	Cyclophosphamide
DOXO	Doxorubicin
VCR	Vincristine
CDDP	Cis-Diaminodichloroplatinum
ASCT	Autologous stem cell transplantation
13-CRA	13-cis retinoid acid
TOPO	Topotecan
FC	Flow cytometry
FISH	Fluorescence in situ hybridization
NK	Natural killer cell
TNF	Tumor necrosis factor
IFN	Interferon
NSE	Neuron specific enolase
LDH	Lactate dehydrogenase
Ferr	Ferritin
ALB	Albumin
INRG	International Neuroblastoma Risk Group
IDRFs	Image-defined risk factors
OS	Overall survival
Th	T helper cell
DC	Dendritic cell

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-13044-5.

Additional file 1: Key Resources Table.

Additional file 2: Distribution and comparison of clinical characteristics of different genetic alteration subgroups.

Additional file 3: Results of routine blood tests, NSE, ferritin, LDH and albumin.

Acknowledgements
We acknowledge Tianjin Medical University Cancer Institute and Hospital for support of samples and excellent conditions. She provides us with a good platform for this clinical research. This work was supported by the National Key Research and Development Program of China, under Grant [number 2018YFC1313000 and 2018YFC1313001], and by the Tianjin health research project, under Grant [number TJWJ2021MS010]. All authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest.

Author contributions
ZW participated in the conceptualization of the study and the design of the work, performed experiments such as flow cytometry, and performed data collection and analysis, wrote the original manuscript, and participated in review and editing. BG were involved in the editing and review of the manuscript. XL performed histological examination of pathological specimens. CC was involved in the conceptualization of the study, the design of the work, and provided some of the data sources, as well as funding acquisition and supervision. QZ was involved in the funding acquisition, supervision and project administration. All authors read and approved the final manuscript.

Funding
This work was supported by the National Key Research and Development Program of China, under Grant [number 2018YFC1313000 and 2018YFC1313001], and by the Tianjin health research project, under Grant [number TJWJ2021MS010].

Data availability
The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study has been supported by the ethics committee of Tianjin Medical University Cancer Hospital. Informed consent for the biological study was obtained from all subjects and/or their legal guardians prior to obtaining pathology specimens and peripheral blood samples.

Consent for publication
Informed consent was signed by the patient's parents or guardians.

Competing interests
The authors declare no competing interests.

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Received: 9 November 2023 / Accepted: 8 October 2024
Published online: 15 October 2024

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