Two Cases of Neuroblastoma with Genotype–Phenotype Discordance: Clinical Management According to Histological Subtype and N-myc Expression

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Abstract

MYCN-amplified neuroblastoma demonstrating favorable histology, which is genotype–phenotype discordant, is extremely rare. This study reports two cases of peripheral neuroblastic tumors with genotype–phenotype discordance: a 3-month-old female and 10-month-old male patients with stage 4S and 2B neuroblastoma, respectively, harboring MYCN-amplification and favorable histology. Immunohistochemical staining was negative for N-myc. Both patients were treated with conventional chemotherapy and 13-cis-retinoic acid without autologous stem-cell rescue, and have been disease-free for 74 and 38 months post-resection, respectively. Nevertheless, chemotherapy could have been optimized on the basis of histological features of the tumors, showing no expression of N-myc.

INTRODUCTION

Peripheral neuroblastic tumors (pNTs), which comprise neuroblastoma (NB), ganglioneuroblastoma, and ganglioneuroma, have well-established prognostic factors such as age at diagnosis, clinical stage (International Neuroblastoma Staging System), *MYCN* status, chromosomal abnormalities (Chromosome 1p and 11q deletions), DNA ploidy pattern, and histopathology classification (International Neuroblastoma Pathology Classification; INPC). Based on these factors, NB cases can be classified into four categories: very low-risk, low-risk, intermediate-risk, or high-risk; which will in turn determine the treatment protocol applied.[1-5] *MYCN* -amplification (*MYCN* -A) demonstrate is a common histologic characteristic of NB, whereas undifferentiated/poorly differentiated NB subtype harbor a high mitosis-karyorrhexis index (MKI), which is considered unfavorable histology (UH) in INPC classification.

According to INPC, the *MYCN* -A is rarely found in a tumor showing favorable histology (FH: pathologically good prognosis group). Tumors within the *MYCN* -A and FH group account for only 1.1% of all NB cases, and their treatment remains controversial. Suganuma et al. reported a case of *MYCN* -A+FH genotype–phenotype discordant and analyzed its histopathological nature. Their analysis revealed that such discordant cases could be divided into good or poor prognosis groups based on the nuclear morphology and the presence of N-myc expression, resulting in subclassification of "conventional" ("salt and pepper" nucleoli) and "Bull's eye" (prominent nucleoli) tumors.

Herein, we present two genotype–phenotype discordant NB cases, which further support the relevance of histopathology, along with N-myc expression analysis, as important factors for selecting an appropriate

treatment for pNTs.

CASE REPORT

Case 1. A 3-month-old female infant presented with left adrenal gland origin NB, stage 4S. Her urine homovanillic acid (HVA) and vanillylmandelic acid (VMA) levels were 6,612 μg/mg Cr (<18 μg/mg Cr) and 3,200 μg/mg Cr (<23 μg/mg Cr). Because the case was complicated with inferior vena cava syndrome, irradiations to the liver (1.5 G/dose, for 3 days) and one cycle of chemotherapy (vincristine and cyclophosphamide) were administered prior to tumor biopsy for avoiding any oncology emergency. The biopsy image of the metastases of the liver after the irradiation showed that the NB was poorly differentiated with intermediate MKI (192/5000). Histology data after chemotherapy led to a classification of conventional NB as per Suganuma classification, which is equivalent to FH of INPC (Fig. 1A). DNA ploidy analysis showed that the tumor was diploid, and real time polymerase chain reaction (RT-PCR) data showed that it harbored 20 copies of *MYCN* . *MYCN* -A was also detected by fluorescence*in situ* hybridization (FISH) (Fig. 1B). Immunohistochemical staining with an anti-N-myc rabbit polyclonal antibody (Proteintech Group, Rosemont, IL, USA) revealed that the tumor was negative for N-myc (Fig. 1C). She received six cycles of chemotherapy (cisplatin, vincristine, cyclophosphamide, and pirarubicin), underwent total resection of the primary tumor and 13-cis-retinoic acid maintenance therapy without autologous stem cell rescue and radiation therapy in the tumor bed. She remained disease-free for the 74 months following tumor resection.

Case 2. A 10-month-old male infant presented with left retroperitoneal NB, stage 2B. Urine HVA and VMA levels were of 168.2 μg/mg Cr and 101.3 μg/mg Cr. The biopsy showed that the NB was poorly differentiated with a low MKI (26/5000), fitting within the FH classification (Fig. 1D). The tumor showed to be a mixture of diploid and hyperdiploid. Moreover, it harbored 5.6 copies of *MYCN* , which was not considered to be*MYCN* -A but genetic gain.[7] *MYCN* -A detected by FISH (Fig. 1E) helped interpret the clinical data as a discordance case. The tumor histology showed that the NB was a conventional type as per the Suganuma classification, since N-myc staining was negative (Fig. 1F). He received six cycles of chemotherapy (cisplatin, carboplatin, etoposide, vincristine, cyclophosphamide, and pirarubicin), underwent total resection of the primary tumor and received 13-cis-retinoic acid maintenance therapy without autologous stem cell rescue and radiation therapy. He remained disease-free for the 38 months following the tumor resection.

DISCUSSION

Although a high-risk treatment is generally recommended for NB cases of Stage 2,3, and 4S with *MYCN* -A in young patients (within 18 months),[8] these patients often suffer with late adverse-effects of radiotherapy and high-dose chemotherapy. Therefore, the treatment strategy is commonly decided according to several prognostic factors. A tumor with *MYCN* -A and FH, which represents genotype–phenotype discordance, is very rare and has been characterized based on specific features and prognosis.[6] In the present study, we reported two NB discordant pediatric cases (under 18-months-old; Stages 4S and 2B) with negative results for N-myc staining, and good prognosis under conventional therapy.

We reviewed seven previously published cases of Stage 4S NB with discordance features (Table 1).[9-12] Four cases survived for more than 24 months with conventional therapy without autologous stem cell rescue or radiotherapy. Because N-myc expression finding was not reported in any of these cases, an association between N-myc expression and prognosis in Stage 4S NB remained controversial until Suganuma report.[6]

Goto et al. classified the discordance feature of *MYCN* -A and*MYCN* -nonamplified (*MYCN* -NA) NBs into four categories (*MYCN* -A+FH, *MYCN* -NA+UH, *MYCN* -A+UH, and*MYCN* -NA+FH), and discussed their specific clinical and pathological features, and prognosis. Both of our cases involved*MYCN* -A+FH tumors.[13] In the Children's Cancer Group and Children's Oncology Group Neuroblastoma Study,[6] the prognosis of *MYCN* -A+FH (51 of 5962 cases registered) was reported as 5-year event-free survival (5yEFS) of 65.2±11.6% and an overall survival (OS) of 72.6±11.0%. This was better than *MYCN* -A+UH cases, where 5yEFS was estimated of 41.4±3.2% and OS of 48.2±3.2%. When discordance cases are further categorized into "conventional" or "Bull's eye" tumors by nucleoli form,[6] "conventional" tumors (5yEFS: 85.7±12.2%, OS: 89.3±10.3%) had significantly better outcomes than "Bull's eye" tumors (5yEFS: 31.3±13.6%, OS:

42.9±16.2%), and the prognosis was similar to*MYCN* -A+FH group. They concluded that among *MYCN* -A+FH tumors, two prognostic subgroups could be identified based on the nuclear morphology and immunohistochemistry. In particular, "conventional" tumors with undetectable N-myc expression were associated with an excellent prognosis. The NBs in both our cases were*MYCN* -A+FH and "conventional" tumors; therefore, good prognosis was inferred.

Despite of *MYCN*- A was observed, N-myc was not detected in both our cases and the nucleoli, where *MYCN* transcripts are located, were inconspicuous (not visible). Inconspicuous nucleoli in "conventional" tumors may be due to a lack of mRNA synthesis. The downregulation of N-myc in the "conventional" tumor promotes cell differentiation, blocks cell proliferation and, therefore, accelerates tumor maturation. Indeed, a differentiated tumor, such as intermixed subtype of ganglioneuroblastoma and maturing subtype of ganglioneuroma, were included in the subgroup of "conventional" tumors in older children. On the contrary, it is believed that excessive N-myc expression in "Bull's eye" tumors results in the formation of the MYC-MAX heterodimer that is responsible for the aggressive behavior of this type of NB.[6]

Elizabeth et al. recently validated that high MKI remains a valuable prognostic factor and that it can be treated as an independent risk factor.[14] Moreover, MKI along with other histologic features can replace the INPC score, which is believed to be a cofounding factor to define risk.[14] Therefore, because our two cases had low/intermediate MKI, it was expected for them to have a good prognosis.

In conclusion, morphology and immunohistochemical expression of N-myc should be evaluated to identify different prognostic groups, i.e., "conventional" and "Bull's eye" tumor, in genotype-phenotype discordant NB (*MYCN* -A+FH) cases. These pathological genetic and cellular features should be considered to optimize the treatment approach applied in such cases.

Ethical Statement: The parents of the infants included in this study provided written informed consent for publication.

Conflict of Interest Statement: The authors have no conflict of interest to declare.

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Figure Legends

Figure 1. Histological and immunostaining finding on two NB genotype–phenotype discordant cases. Representative images of Case 1 (A–C) and Case 2 (D–F) are shown. (**A**) Hematoxylin and eosin (H&E) staining: status after chemotherapy, equivalent to favorable histology (FH) of the International Neuroblastoma Pathology Classification (INPC); Neuroblastoma (Schwannian stroma-poor), poorly differentiated subtype with "conventional" type nuclei. (**B**) Fluorescence *in situ* hybridization (FISH):*MYCN* -amplification was detected (Vysis LSI N-MYC (2p24) Spectrum Green/ CEP 2 Spectrum Orange Probe). (**C**) Immunohistochemical staining for N-myc was negative. (**D**) H&E: FH of INPC; Neuroblastoma (Schwannian stroma-poor), poorly differentiated subtype with "conventional" type nuclei. (**E**) Not all, but some cells showed more than 4-fold increase in *MYCN* signal compared to*CEP2* . (**F**) Immunohistochemical staining for N-myc was negative.

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