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Correlation between the International Neuroblastoma Pathology Classification and genomic signature in neuroblastoma

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Key words

Array comparative genomic hybridization, neuroblastoma, pathology, risk assessment, risk factors

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The International Neuroblastoma Pathology Classification (INPC) has a prognostic impact that distinguishes two categories of neuroblastoma: favorable histology (FH) and unfavorable histology (UH). We analyzed 92 cases of neuroblastoma with the INPC evaluation and genomic grouping to investigate the correlation between the INPC and genomic signature, together with their prognostic significance. The correlation of UH tumor and partial gains and/or losses (GGP), as well as the correlation of FH tumor and whole gains and/or losses (GGW), was statistically significant. Both UH and GGP were late-onset (median age at diagnosis was 36 and 48 months, respectively) and had poor prognosis (overall survival rate [OS], 43.1% and 42.4%, respectively). In contrast, both FH and GGW were early-onset (median age at diagnosis, 4 and 9.5 months, respectively) and had favorable prognosis (OS, 88.6% and 87.1%, respectively). Unfavorable histology and GGP had significantly inferior OS compared to FH and GGW. Overall survival was not significantly different among the genomic groups in FH; however, it was inferior in UH with GGP. In UH with a single copy *MYCN*, genomic subgroups GGP2s (both 1p and 11q losses) and GGP3s (partial 11q loss but not 1p loss) indicated significantly poor prognosis compared to GGP4s (no partial 1p and 11q loss). As INPC and *MYCN* amplification were found to be the most powerful prognostic biological factors, they should be included with genomic grouping as treatment stratification for patients with UH and single copy of *MYCN*.

Neuroblastoma (NB) is known for its unpredictable behavior; some spontaneously regress, some mature, whereas others develop into aggressive forms despite intensive multimodality treatment.[1,2] A combination of prognostic variables, such as age at diagnosis, clinical stage, *MYCN* gene amplification, histology (International Neuroblastoma Pathology Classification; INPC), and DNA index, has been used for risk-group assignment and treatment stratification.[2,3] The INPC has a prognostic impact that distinguishes two categories of NB: favorable histology (FH) and unfavorable histology (UH).[4]

Recently, comprehensive genome-wide genetic alterations in NB were revealed by microarray-based comparative genomic hybridization analysis.[5–7] We had previously shown that NB with whole chromosome gains and losses (GGW) had good prognosis, whereas NB with partial chromosome gains and/or losses (GGP) had poor prognosis.[7] The International Neuroblastoma Staging System (INSS) stages 1, 2, and 4s were significantly correlated with GGW, whereas INSS stages 3 and 4 were significantly correlated with GGP. It was found that GGP also correlated with older age (1 year and older), primary adrenal NB, diploidy, and low *Trk A* expression; GGW was correlated with infants aged <1 year and aneuploidy.[7] Janoueix-Lerosey *et al.*[5] also indicated that exclusively whole-chromosome copy number variations were associated with excellent survival, whereas the presence of segmental alterations was the strongest predictor of relapse. Shimada *et al.*[8] found that *MYCN* amplification is associated with characteristic histopathological features which, together with molecular signature, have been linked to the underlying molecular mechanisms of oncogenesis. The aim of this study was to investigate the correlation between the INPC and genomic signature, as well as their prognostic significance in NB.

Materials and Methods

Patients and samples. Primary NB from 92 untreated patients, who underwent biopsy or surgery at various institutions in Japan,[7] were histologically evaluated based on the INPC. Clinical information was obtained from the database of Chiba Cancer Center (Chiba, Japan). Six patients without follow-up data were excluded from further analysis. Patients were treated between 1995 and 2003 according to standard protocols in Japan.[9,10] Follow-up data were obtained from 86 patients. The median follow-up period was 103 months (range, 0–199 months). The study was approved by the Institutional Review Board of the Chiba Cancer Center (CCC7817).

Genomic grouping. Genomic signature grouping according array comparative genomic hybridization resulted in three

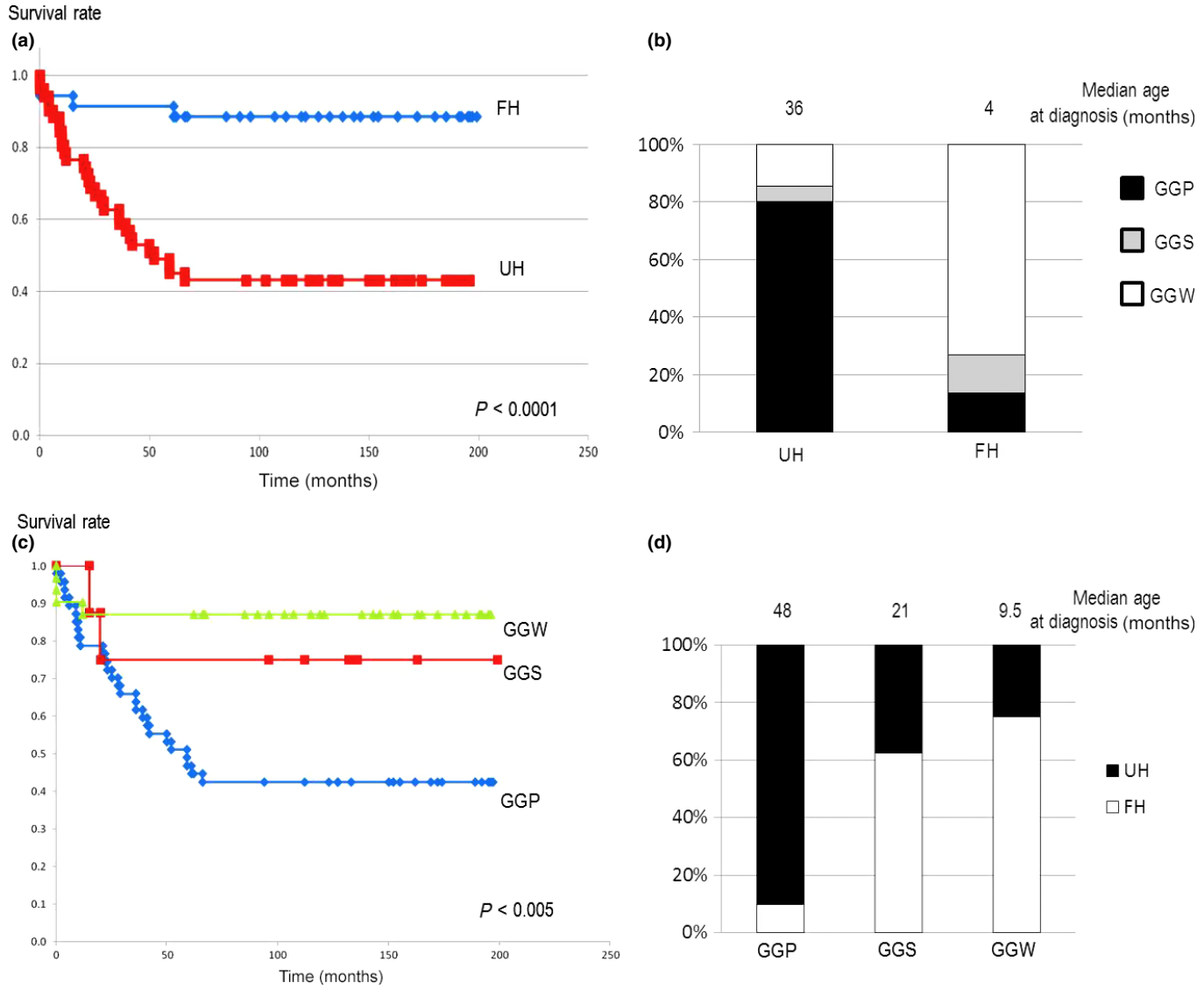

Fig. 1. (a) Kaplan–Meier overall survival curves for patients with neuroblastoma, subdivided by favorable histology (FH) *versus* unfavorable histology (UH). (b) Genomic group distribution in FH and UH. (c) Kaplan–Meier OS curves for patients with NB subdivided by whole (GGW) and silent gains and/or losses (GGS) *versus* partial gains and/or losses (GGP). (d) International Neuroblastoma Pathology Classification categories (FH and UH) in genomic groups GGP, GGS, and GGW.

major genomic groups of chromosomal aberrations: silent (GGS), GGP, and GGW, corresponding to no gain of either chromosome 17 or 17q, gain of chromosome 17q, and gain of whole chromosome 17, respectively. Each genomic group was divided by the status of the *MYCN* gene, a single copy of *MYCN* (s) and *MYCN* amplification (a).

The GGP groups were further categorized into four subgroups according to the presence and/or absence of 1p loss and 11q loss as described previously[7]: subgroup 1 (GGP1) has 1p loss but not partial 11q loss; subgroup 2 (GGP2) has both 1p and 11q losses; subgroup 3 (GGP3) has partial 11q loss but not 1p loss; and subgroup 4 (GGP4) has neither partial 1p nor 11q loss. The criteria for categorization of genomic group/subgroup and their prognostic difference were described in our previous report.[7]

Tumor evaluation. Neuroblastoma tumors were evaluated based on the INPC as a prognostic indicator, taking into account the grade of neuroblastic differentiation (undifferentiated, poorly differentiated, differentiating) and the mitosis karyorrhexis index (low, intermediate, high) in the context of age at diagnosis.[4] The pathology review focused on the presence or absence of pleomorphic cells, which have enlarged nuclei with diameters more than twice that of other tumor cells, and/or bizarre nuclei. These pleomorphic cells were easily recognizable under low-power magnification. In FH tumors, however, it can sometimes be difficult to distinguish pleomorphic cells, which have scanty or unrecognizable cytoplasm, from multinuclear cells, which have abundant cytoplasm, at low-power magnification. *MYCN* gene copy number analysis was carried out on 92 tumors using FISH and compared with a reference probe located on chromosome 2.[11]

Statistical analysis. A survival analysis was made based on Kaplan–Meier and log–rank tests. The relationships between

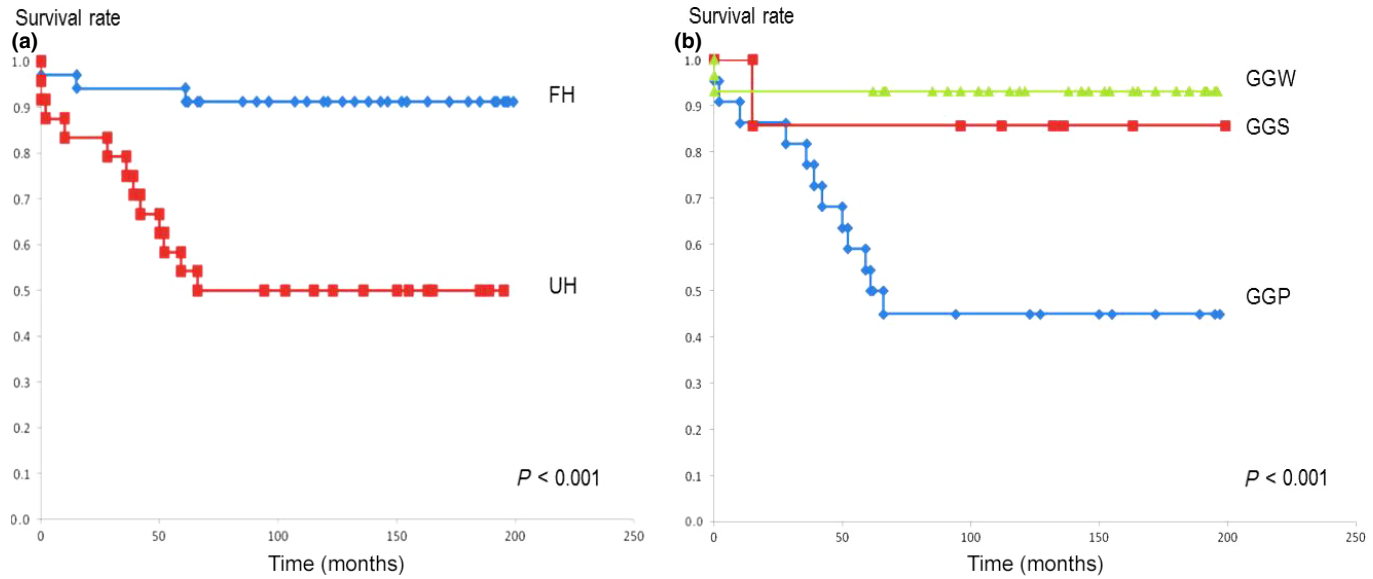

Fig. 2. Kaplan–Meier overall survival curves for patients with neuroblastoma with single copy of *MYCN*, subdivided by favorable histology (FH) *versus* unfavorable histology (UH) (a) and whole (GGW) and silent gains and/or losses (GGS) *versus* partial gains and/or losses (GGP) (b).

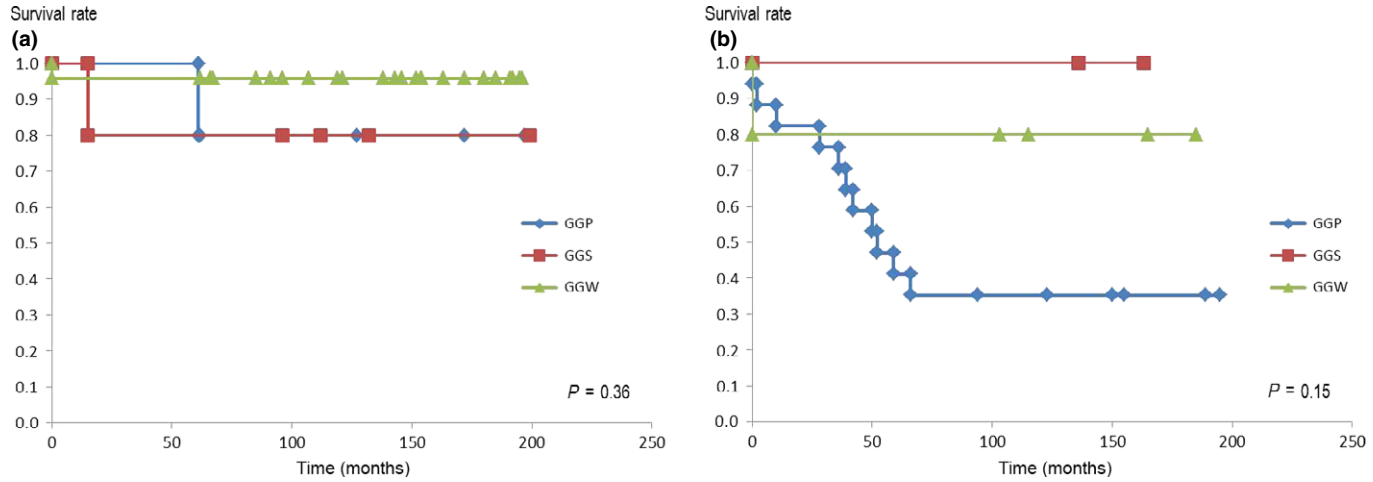

Fig. 3. Kaplan–Meier overall survival curves for patients with neuroblastoma with single copy of *MYCN*, subdivided by genomic group in favorable histology (FH) (a) and unfavorable histology (UH) (b). GGP, partial gains and/or losses; GGS, silent gains and/or losses; GGW, whole gains and/or losses.

variables were assessed using χ^2-tests. *P*-values of <0.05 were considered to indicate statistical significance.

Results

Unfavorable histology associated with inferior overall survival. Thirty-seven NB tumors were classified to the FH group and 55 to the UH group. The median age at diagnosis was older in patients with UH compared to patients with FH (36 *vs* 4 months). Patients with UH showed significantly inferior overall survival rate (OS) compared to patients with FH (43.1% *vs* 88.6%, $P < 0.001$) (Fig. 1a). In the FH group, 27 (73%) of the NB were GGW, five were GGS, and five were GGP. In the UH group, 44 (80%) of the NB were GGP, three were GGS, and eight were GGW (Fig. 1b). Neuroblastomas with FH were significantly classified as GGW ($P = 0.005$); those with UH were significantly classified as GGP ($P < 0.001$).

Thirty-five (38.0%) NB tumors were classified as GGW, 49 (53.3%) were classified as GGP, and eight (8.7%) were classified as GGS. Patients with GGP showed significantly inferior OS compared to patients with GGW (42.4% *vs* 87.1%, $P < 0.005$) (Fig. 1c). The median age at diagnosis was 9.5 months in patients with GGW, 21 months in patients with GGS, and 48 months in patients with GGP. The median age at diagnosis was significantly different between patients with GGW and patients with GGP. In GGP, 44 (90%) were UH; in GGW, 27 (77%) were FH (Fig. 1d). Neuroblastoma with GGW was significantly classified into FH ($P = 0.001$) and NB with GGP was significantly classified into UH ($P < 0.001$).

***MYCN* amplification associated with inferior OS.** Patients with *MYCN* amplification showed more inferior OS compared to patients without *MYCN* amplification (35.7% *vs* 74.1%, $P < 0.001$). *MYCN* amplification was detected in 29 (48%) of 60 UH tumors and in 27 (59%) of 46 UH tumors with GGP. In contrast, *MYCN* amplification was found in only 3.3% (1/30) of FH tumors.

GGP and GGP subtype (GGP2s and GGP3s) associated with inferior OS in UH NB with single copy *MYCN*. As *MYCN* ampli-

fication is a known indicator of poor prognosis, we focused on NB without *MYCN* amplification, that is, NB with single copy *MYCN*. Both UH and GGP were significantly associated with poor prognosis in NB with single copy *MYCN*, as well as in all other types of NB (Fig. 2). The survival rate was not significantly different among genomic groups in FH NB, but in UH NB, it was inferior in GGP compared to GGW/GGS (Fig. 3). Next, we analyzed the survival rate among GGP subtypes in UH NB. The GGP2s and GGP3s subtypes showed significantly poorer prognosis (OS, 16.7%) than GGP4s (OS, 80%) which, together with GGWs, had better survival rate ($P = 0.02$) (Fig. 4) (Table 1). *MYCN* amplification was found to have no prognostic impact on UH NB with GGP; OS of *MYCN* amplified NB was 40.0%, whereas OS of *MYCN* non-amplified NB was 35.3% ($P = 0.84$) (Fig. 5). In GGP with single copy *MYCN* (GGPs) subtypes, there was no significant prognostic difference between the presence and absence of pleomorphic cells (OS, 38% *vs* 50%, $P = 0.481$). Among the 15 GGPs cases having pleomorphic cells, 10 cases were GGP3s, 3 were GGP4s, and 2 were GGP2s. Pleomorphic cells were observed most prominently in GGP2s and GGP3s cases compared to GGP4s cases ($P = 0.002$) (Fig. 6).

Discussion

Peripheral neuroblastic tumors including NB, ganglioneuroblastoma, and ganglioneuroma are biologically heterogeneous. They show variable clinical and histopathological phenotypes, such as spontaneous regression, maturation, and aggressiveness. The main concept of the INPC is based on whether the tumor has any potential of age-linked maturation.[4] Tumors with FH exist in a framework of age-linked maturation, from poorly differentiated NB to differentiating NB, to ganglioneuroblastoma (intermixed subtype), and finally ganglioneuroma. Tumors with UH are less mature than age-linked maturation sequence and/or have a high mitosis karyorrhexis index, which was found to have a reproducible correlation with *MYCN* amplification.[8] Notably, *MYCN* amplification exists in approximately 40% of UH tumors.[4] With the exception of *MYCN* amplification and *Trk A* expression, the genetic background of FH or UH tumors has not been analyzed in detail.[4,8,12] Although recent studies have provided a comprehensive

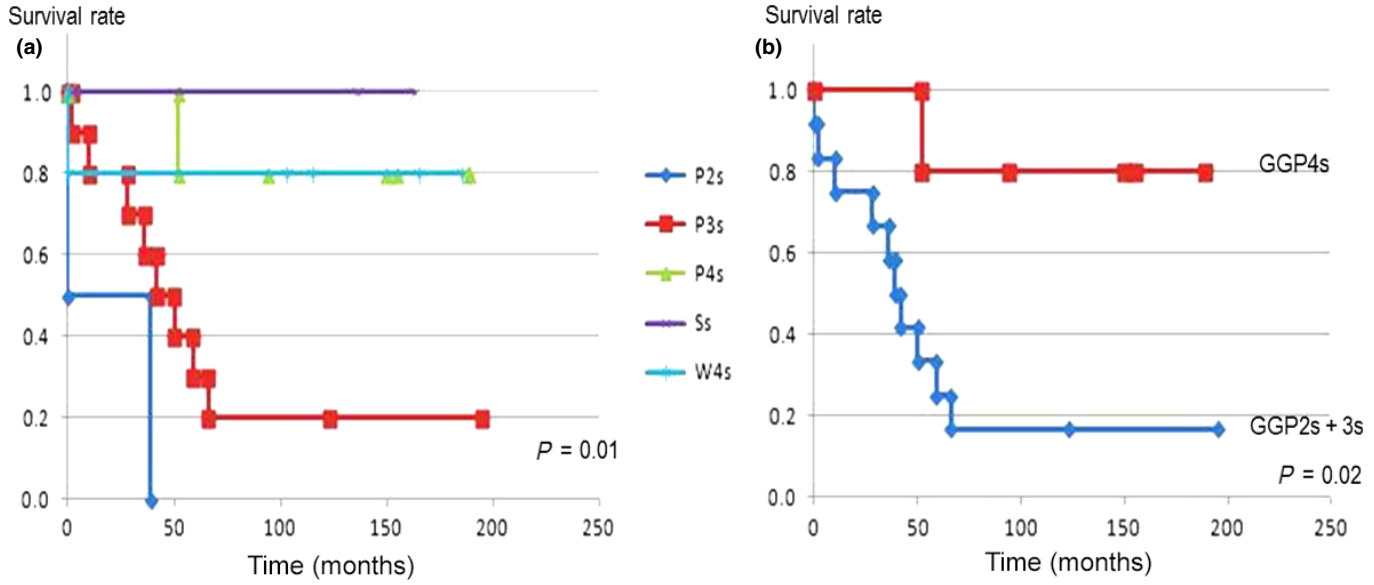

Fig. 4. Kaplan–Meier overall survival curves for patients with neuroblastoma with single copy of *MYCN* and unfavorable histology, subdivided by genomic group (a) and partial gains and/or losses (GGPs) subgroup (b). P2s–4s, GGPs subgroups; Ss, silent gains and/or losses subgroup; W4s, whole gains and/or losses subgroup.

Table 1. Number of cases of neuroblastoma and overall survival (%) subdivided by the International Neuroblastoma Pathology Classification, favorable histology (FH) and unfavorable histology (UH), genomic group, and *MYCN* status

		GGP						
	GGS	Single *MYCN* 22 cases (45%)			Amplified *MYCN* 25 cases (40%)			GGW
		GGP1	GGP2+GGP3	GGP4	GGP1	GGP2+GGP3	GGP4	
FH								
35 cases (88.6%)	5 cases (80.0 %)			5 cases (80.0%)				25 cases (92.0%)
UH								
51 cases (43.1%)	3 cases (66.7 %)			42 cases (38.1%)				6 cases (66.7%)
		0 case	12 cases (16.7%)	5 cases (80.0%)	17 cases (47.1%)	7 cases (28.6%)	1 cases (0%)	

GGP, partial gains and/or losses; GGS, silent gains and/or losses; GGW, whole gains and/or losses.

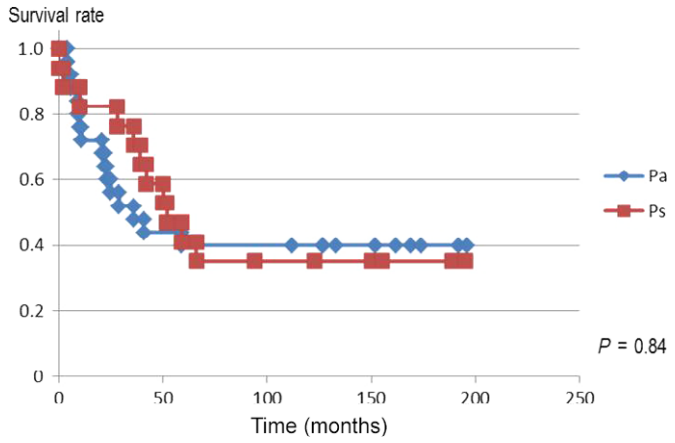

Fig. 5. Kaplan–Meier overall survival curves for patients with neuroblastoma with unfavorable histology and partial gains and/or losses, subdivided by *MYCN* status. Pa, GGP with amplified *MYCN*.; Ps, GGP with single copy *MYCN*.

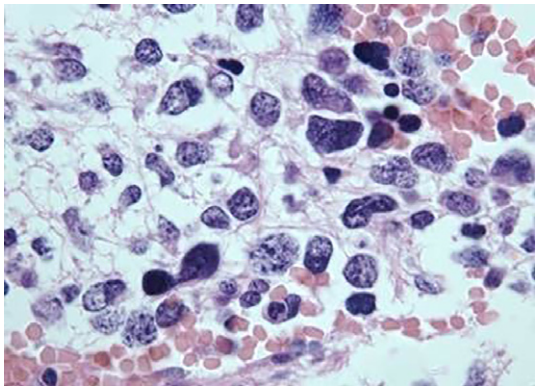

Fig. 6. Pleomorphic neuroblastoma cells have hyperchromatic, enlarged nuclei with diameters more than twice the size of those of other tumor cells. Magnification, ×400.

overview of genetic alterations and their prognostic impact in NB, the reports did not mention the INPC. In our study, we highlighted the relationship between the INPC and the genomic groups.

Among the NB tumors in our study, 53% were GGP, which is consistent with the range found in other studies (38–65%).[5–7] As GGP tumors show multiple chromosomal aberrations with partial gains and/or losses, unknown causes that induced genomic instability might have triggered NB genesis in the progenitor or stem cells of a sympathetic cell lineage.[7] Of the FH tumors, five were GGP with a single copy of *MYCN*. One of the patients (P5s with *ALK* mutation; data not shown) died, although OS was not significantly different among the genomic groups in FH.

MYCN amplification alone is an incomplete genetic prognostic factor, and hence chromosome 11q deletion has recently been proposed in risk-group stratification.[3] The deletion of 11q is an independent prognostic factor for poor outcome. The outcome of NB with 11q deletion is comparable to that of *MYCN* amplified NB, but has a later onset (median patient age at diagnosis, 42 *vs* 21 months).[13] Our data also confirmed that NB with single copy of *MYCN* and 11q deletion (GGP2 and GGP3) had poorer prognosis than *MYCN* amplified tumor, with the OS being 18% and 35.7%, respectively.

Pediatric cancers do not require as many genetic alterations as typical adult cancers, as epigenetic alterations mainly contribute to the initiation or progression of NB.[14] We identified eight GGS; of these, seven had single copy of *MYCN* and one had amplified *MYCN*. In the seven with a single copy of *MYCN* (GGSs), five were FH and OS was 85.7% (all but one patient survived). It is interesting to investigate the difference between GGSs and GGW, most of which showed FH and favorable outcome, with comprehensive epigenetic analysis. It allows us to add to the growing knowledge of the INPC concept of "FH means a tumor with in age-linked maturation sequence". The important connection between genetic/epigenetic pathways and INPC categories should be considered in the management of patients with NB.

Genomic group classification provides additional important prognostic information and can contribute to the improvement of current therapeutic risk assignment schemes. Patients with NB are assigned to a low-risk, intermediate-risk, or high-risk groups based on the following: tumor stage as defined by the INSS,[15] age at diagnosis, INPC, tumor DNA index, and amplification of the *MYCN* oncogene within tumor tissue (Children's Oncology Group Neuroblastoma Risk Grouping).[16] Our data indicated that some high-risk group patients with UH and single copy of *MYCN* may in fact be downgraded to intermediate risk according to the genomic analysis. If the patient is classified into GGP4s, risk assignment could be downgraded to intermediate. In GGP4s, "pleomorphic cells" were less frequent compared to GGP2s and GGP3s, although we could not find definite morphologic characters. As comprehensive genome-wide analyses require higher cost and more sophisticated technology, it is necessary minimize the use of genomic factors for risk-group assignment in clinical practice. Moreover, as the INPC and *MYCN* amplification are found to be powerful prognostic biological factors, they should be included with genomic grouping as treatment stratification of patients with UH and single copy of *MYCN*. Further analysis through clinical trials is required to establish better risk-group stratification.

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Disclosure Statement

The authors have no conflict of interest.

References

1 Maris JM, Hogarty MD, Bagatell R *et al.* Neuroblastoma. *Lancet* 2007; **369**: 2106–20.
2 Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003; **3**: 203–16.
3 Cohn SL, Pearson ADJ, London WB *et al.* The international neuroblastoma risk group (INRG) classification system: an INRG task force report. *J Clin Oncol* 2009; **27**: 289–97.
4 Shimada H, Ambros IM, Dehner LP *et al.* The International Neuroblastoma Pathology Classification (the Shimada System). *Cancer* 1999; **86**: 364–72.

5 Janoueix-Lerosey I, Schleiermacher G, Michels E *et al.* Overall genomic pattern is a predictor of outcome in neurpblastoma. *J Clin Oncol* 2009; **27**: 1026–33.
6 Spitz R, Oberthuer A, Zapatka M *et al.* Oligonucleotide array–based comparative genomic hybridization (aCGH) of 90 neuroblastomas reveals aberration patterns closely associated with relapse pattern and outcome. *Genes Chromosom Cancer* 2006; **45**: 1130–42.
7 Tomioka N, Oba S, Ohira M *et al.* Novel risk stratification of patients with neuroblastoma by genomic signature, which is independent of molecular signature. *Oncogene* 2008; **27**: 441–9.
8 Shimada H, Stram DO, Chatten J *et al.* Identification of subsets of neuroblastoma by combined histopathologic and N-myc analysis. *J Natl Cancer Inst* 1995; **87**: 1470–6.
9 Kaneko M, Tsuchida Y, Mugishima H *et al.* Intensified chemotherapy increases the survival rates in patients with stage 4 neuroblastoma with MYCN amplification. *J Pediatr Hematol Oncol* 2002; **24**: 613–21.
10 Matsumura T, Michon J. Treatment of localized neuroblastoma. In: Brodeur GM, Sawada T, Tsuchida Y, Voûte PA, eds. *Neuroblastoma*. Amsterdam: Elsevier, 2000; 403–15.
11 Ambros PF, Ambros IM, Brodeur GM *et al.* International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee. *Br J Cancer* 2009; **100**: 1471–82.
12 Shimada H, Nakagawa A, Peter J *et al.* TrkA expression in peripheral neuroblastic tumors. Prognostic significance and biological relevance. *Cancer* 2004; **101**: 1873–81.
13 Caren H, Kryh H, Nethander M *et al.* High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. *Proc Natl Acad Sci U S A* 2010; **107**: 4323–8.
14 Sausen M, Leary RJ, Jones S *et al.* Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. *Nat Genet* 2013; **45**: 12–7.
15 Brodeur GM, Pritchard J, Berthold F *et al.* Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol* 1993; **11**: 1466–77.
16 Children's Oncology Group Neuroblastoma Risk Grouping. [Cited 15, December, 2014] Available from URL: http://www.cancer.gov/cancertopics/pdq/treatment/neuroblastoma/healthProfessional/Page4/