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

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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.

Materials and Methods

Patients and samples. Primary NB from 92 untreated patients, who underwent biopsy or surgery at various institutions in Japan, 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. 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 (CCCC7817).

Genomic grouping. Genomic signature grouping according array comparative genomic hybridization resulted in three excellent survival, whereas the presence of segmental alterations was the strongest predictor of relapse. Shimada et al. 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.

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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. 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
variables were assessed using χ²-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* amplification...
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 GGP3 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 TrkA 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

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. |
Among the genomic groups in FH, shown) died, although OS was not significantly different been proposed in risk-group stratification. The deletion of tic factor, and hence chromosome 11q deletion has recently induced genomic instability might have triggered NB genesis in the progenitor or stem cells of a sympathetic cell lineage. As GGP tumors show multiple chromosomal aberra-
tions 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. Of the FH tumors, five were GGP with a single copy of MYCN. Of 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 GOW, 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 age at diagnosis, INPC, tumor DNA index, and amplification of the MYCN oncogene within tumor tissue (Children’s Oncology Group Neuroblastoma Risk Grouping). 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 charac-
ters. 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 prac-
tice. 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.

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