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<th>Nuclear DNA Analysis in Neuroblastoma -Significance of DNA Ploidy as a Prognostic Factor-</th>
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<tr>
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<td>Sakai, Tsutomu</td>
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Nuclear DNA Analysis in Neuroblastoma
—Significance of DNA Ploidy as a Prognostic Factor—

Tsutomu Sakai

First Department of Surgery, Nagasaki University School of Medicine

The nuclear DNA content was measured by using flow cytometer from paraffin-embedded pathological materials in neuroblastomas.

In 55 neuroblastomas, diploidy pattern was found in 26 (47.6%). In contrast, aneuploidy was in 29 (52.7%). The prognosis of aneuploidy pattern is more satisfactory than that of diploidy.

However, aneuploidy pattern included 62% of stage I, II, and IV; patients, 77.8% of less than the first year of age, 20% of N-myc amplification. There was unevenness of the patient's background between diploidy and aneuploidy patterns.

Introduction

Neuroblastoma is one of the infantile representative solid tumors with poor prognosis. It is reported that a part of neuroblastoma accomplishes a tendency toward spontaneous remission. Their prognoses vary so much with patients' ages, disease stages and tumor locations, that understanding of biologic characteristics is required for the treatment of neuroblastoma.

Recent studies were focused on chromosomal aberration and N-myc expression. On the other hand, DNA analytic studies are scanty.

The purpose of this study is to clarify the validity of DNA analysis for neuroblastomas to predict their prognoses.

Material and Method

Material

During the 14 years from 1974 to 1987, 60 surgical specimens were subjected in this study at the First Department of Surgery, Nagasaki University School of Medicine and the Pediatric Surgery, Faculty of Medicine, Kyushu University.

The 180 samples from the 60 neuroblastomas were investigated in this series.

Method

Three or four slices of 50μm thick were cut from a paraffin embedded block and processed by the method of B. Schutte et al and the preparation was stained by Propidium Iodide (PI) by the method of Vindelöv. In this study over seven of coefficient of variation (CV) were excluded.

The DNA index (DI) was determined based on the resultant histogram. Diploidy was defined as DI = 1.0 whereas aneuploid as DI ≠ 1.0. A significant difference was assessed by X²-test and by Wilcoxon test for cumulative survival rates. A difference of p < 0.05 was regarded as statistically significant.

Results

In the six patients DNA analysis was made at varying period of pretreatment, recurrence, metastasis and autopsy. It was convenient for evaluating the response to the prior treatment of chemoterapy. There was no change in DNA patterns among tumor locations.

In this study five cases were excluded for the reasons of unsuitable CV and undeterminable peak delineated on histogram. A suitable DNA histogram (Fig. 1) was obtained
in 40 of neuroblastoma and 15 of ganglioneuroblastoma. The DNA ploidy patterns obtained in this study were evaluated in terms of clinical and histologic backgrounds in accordance with the rule of Japanese pediatric tumor study of pathology. The CV values in 55 patients averaged 4.67 ± 1.48.

1) The incidence of DNA ploidy

Of 55 neuroblastomas, 26 (47.3%) were the diploidy pattern and 29 (52.7%) were aneuploidy. The distribution of DNA ploidy patterns was shown in Fig. 2. Aneuploid distributed from 1.1 to 2.5 of DI. A range from 1.3 to 1.7. Over 2.0 of DI (more than 4C of DNA content) were seen in five cases.

![Fig. 2. Frequency of DNA index](image)

Table 1. Stage and ploidy

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>I</td>
<td>8(8)</td>
<td>5(5)</td>
</tr>
<tr>
<td>II</td>
<td>8(6)</td>
<td>5(5)</td>
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<tr>
<td>III</td>
<td>13(6)</td>
<td>5(4)</td>
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<tr>
<td>IV</td>
<td>17(2)</td>
<td>9(2)</td>
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<tr>
<td>V</td>
<td>4(2)</td>
<td>2(1)</td>
</tr>
<tr>
<td>V</td>
<td>5(3)</td>
<td>3(1)</td>
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Table 2. Stage, ploidy and age

<table>
<thead>
<tr>
<th></th>
<th>D; diploidy</th>
<th>A; aneuploidy</th>
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<tbody>
<tr>
<td>I</td>
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</tr>
<tr>
<td>II</td>
<td>3(1)</td>
<td>5(5)</td>
</tr>
<tr>
<td>III</td>
<td>8(2)</td>
<td>5(4)</td>
</tr>
<tr>
<td>IV</td>
<td>8(0)</td>
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<tr>
<td>V</td>
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<td>2(1)</td>
</tr>
<tr>
<td>V</td>
<td>2(2)</td>
<td>3(1)</td>
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</tbody>
</table>

Table 3. Tumor location and ploidy

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<tr>
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<tr>
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<td>16</td>
</tr>
<tr>
<td>retroperitoneum</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>posterior mediastinum</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>others</td>
<td>1</td>
<td>1</td>
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</table>

Table 4. Relationship between local invasion and metastasis

With respect to the type of local invasion which had been described in the chart, there was no significant pattern between locally invasive types and DNA ploidy patterns.

In cases with n1 of nodal metastasis, the diploidy pattern was seen in 13 (44.8%) out of 29, aneuploidy in 6 (20.7%). On the other hand, in 26 of the origins except for the adrenal gland. There was no significant difference between the incidence of diploidy and aneuploidy patterns as shown in Table 3.

ii) Relationship between ages and disease stages.

Among 18 cases of age under one year, the diploidy pattern was found in four (22.2%), the aneuploidy pattern in 14 (77.8%) with a significant difference. According to disease stages, the diploidy pattern was seen only in two of stage I and IVs. The others were of aneuploidy pattern. In 37 cases over one year old, 22 (59.5%) showed the diploidy pattern, 15 (40.5%) were aneuploidy without statistically significant difference as shown in Table 2.
Table 4. Local invasion, metastasis and ploidy

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>C</td>
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<td></td>
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<tr>
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<td>16</td>
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<td></td>
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</table>

v) Relationship between pathologic types and ploidy patterns.

In 40 neuroblastomas, the incidence of diploidy pattern was equivalent to that of aneuploidy. In contrast, in ganglioneuroblastomas, aneuploidy pattern was seen in nine (60%) out of 15. In particular, aneuploidy pattern was not infrequently seen in poorly differentiated ganglioneuroblastomas.

There was a tendency of demonstrating diploidy pattern in accordance with progression of differentiation. There was no significant trend in the other histological types as shown in Table 5.

Table 5. Histology and ploidy

<table>
<thead>
<tr>
<th></th>
<th>diploidy</th>
<th>aneuploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>20 (6)</td>
<td>20 (12)</td>
</tr>
<tr>
<td>round cell type</td>
<td>2 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>rosette-fibrillary type</td>
<td>18 (6)</td>
<td>19 (12)</td>
</tr>
<tr>
<td>Ganglioneuroblastoma</td>
<td>6 (3)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>poorly differentiated type</td>
<td>1 (0)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>composite type</td>
<td>2 (1)</td>
<td>0</td>
</tr>
<tr>
<td>well differentiated type</td>
<td>3 (2)</td>
<td>1 (0)</td>
</tr>
</tbody>
</table>

( ); alive

vi) Relationship between N-myc amplification and ploidy patterns.

The examinations of N-myc oncogenes were made in 25 specimens. Of 25, ten were amplified in N-myc oncogen in which eight out of 11 had the diploidy pattern and two out of 14 were aneuploidy.

N-myc amplification failed to be found in patients under one year of the first life. All of positive N-myc expressions were over one year old as shown in Table 6.

Table 6. N-myc, DNA ploidy and ages

<table>
<thead>
<tr>
<th></th>
<th>N-myc</th>
<th>ages under 1 year</th>
<th>over 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>amplification</td>
<td>diploidy</td>
<td>aneuploidy</td>
</tr>
<tr>
<td>E</td>
<td>amplification</td>
<td>0 (0)</td>
<td>8 (0)</td>
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<tr>
<td>bm</td>
<td>amplification</td>
<td>0 (0)</td>
<td>8 (0)</td>
</tr>
</tbody>
</table>

2) Relationship between the prognosis and ploidy patterns

The survivors were 27 (49.1%) out of 55 in this series. Fig. 3 shows the cumulative survival curve in accordance with the disease stages. There was no death in stage I. The prognosis had become aggravated with advances in disease stages.

The prognoses for stage I and II were significantly fair (p < 0.05) when compared with those of stage IV.

i) As for ploidy patterns, of 26 with the diploidy pattern, nine (34.6%) survived. In contrast, 18 (62.1%) out of 29 with the aneuploidy pattern were alive.

The cumulative survival curve is shown in Fig. 4. The prognosis of the aneuploidy pattern was significantly satisfactory (p < 0.05).
ii) With respect to disease stages, the prognosis of stage I was fair without any difference between those of diploidy and aneuploidy patterns. On the contrary, the prognoses of stage II, III in aneuploidy patterns were fair (Fig. 5) but it was not a statistically significant difference between the two patterns in stage IV, and IVB (Fig. 6).

iii) Concerning boy’s and girl’s ages, the prognosis of patients under 12 months of the first life was extremely fair in both patterns without a significant difference. On the other hand, in the prognosis of patients over one year old, the prognosis of the aneuploidy pattern was significantly satisfactory \( (p < 0.05) \) when compared with that of the diploidy pattern as shown in Fig. 7.

iv) According to the locations of primary tumors, the prognoses of extra-adrenal origin tumors were better than those of adrenal origin tumors.

There was no constant inclination of ploidy patterns.

v) Relationship between invasive types and their metastasis and ploidy patterns.

There was no specific patterns concerning invasive types and their metastasis in accordance with diploidy and aneuploidy patterns.

vi) Relationship between histologic types and ploidy patterns.

The prognosis of the aneuploidy pattern was fair in every histologic types. There was no close correlation between histologic types and their prognosis.

vii) Relationship between N-myc oncogen expression and ploidy patterns.

In this series amplification of N-myc was seen in 10 in whom no survival was experienced.
Discussion

It is known that the prognosis of neuroblastoma in infant
is divided into the fair prognosis group (under one year of
life, stage I, II and IVs) and the unfair prognosis group
(over one year old, stage III and IVs and stage IVe) through
dates. It is now accepted that a significant difference in
biologic behavior exist in the two groups. Thirty-five subjects
of this study were divided into the two groups. Recently, it has become possible to know the prognosis in
more detail and more accurately.

Trials to elucidate biologic behavior of malignancy have
been made by DNA measurement using microphotograph
spectrometry and flow cytometry.

Hedley7 developed DNA measurement from paraffin-
embedded samples, which contributed to retrospective
study for assessment of malignancy. It is widely ac-
cepted that prognosis of patients with the diploid pattern is much more satisfactory than that with the aneu-
plody one. On the contrary, some investigators6,10 reported
that the aneuploidy pattern in neuroblastoma showed a
good prognosis. In this study, DNA ploidy patterns were
examined in 35 neuroblastomas in analysis of prognostic
factors. Cumulative survival curve revealed a fair survival in the aneuploidy pattern (p < 0.05). In the analysis of the
fair prognosis group, it included the aneuploidy pattern in
babies of less than the first year of life as well as in stage
III, IVs and through the entire ranges of ages.

It is assumed that the aneuploidy pattern is shown in
70% to 80% of stage I, II and IVs and babies under 12
month of birth. There was no close correlation among the
modes of local invasion and metastasis and the ploidy
pattern.

In the somatologic analysis, there was a high incidence
of hyperploidy and also a loss of 1q4 was defined. At the
same time, it has become apparent that Ha-ras expression reflects a fair prognosis.10 In cases with poor prognosis, it is characteristic of demonstration of a diploid pattern and
aberration of 1p.10 It is reported that N-myc expression was
seen in stage III and IV, exceptionally in stage I and II.10
Amplification of N-myc oncogene well correlates with poor
prognosis. Of 25 with N-myc investigation, amplification of N-myc oncogene was seen in 10 in this series in whom
aneuploidy pattern was not revealed. However, it is empha-
sized that the outcome is in association with ages, disease
stages and N-myc expression. The aneuploidy pattern does
not necessarily indicate a fair prognosis. It is not clear as to
what means the deviation to aneuploidy patterns of fair
prognosis. In general, the tumors with aneuploidy pattern
implies activated proliferation of cells. Mass screening in
newborn makes it possible to detect and manage this tumor
early. The tumor indicating the diploidy pattern showed a
low proliferation. On the other hand, chance of treating
advanced case is frequent because it is difficult to be
detected early. There is no great difference in the prognosis
between diploidy and aneuploidy patterns. The possibility
to make a fair prognosis for tumors of the aneuploidy
pattern exist in the use of potent chemotherapy which
reveals a high sensitivity to aneuploid tumors. In conclu-
sion, DNA ploidy analysis has a great value in judging
biologic behavior of the tumors and a further study is
necessary for clarifying heterogeneity and for knowing the
change with the passage of time.

Acknowledgement

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