Acta Med. Nagasaki 38:144 – 149

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_S 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.[1] 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.[2-5] On the other hand, DNA analystic studies are scanty.[6-9]

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[11] and the preparation was stained by Propidium Iodide (PI) by the method of Vindelϕv.[12] 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 $\neq$ 1.0. A significant difference was assessed by X^2-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

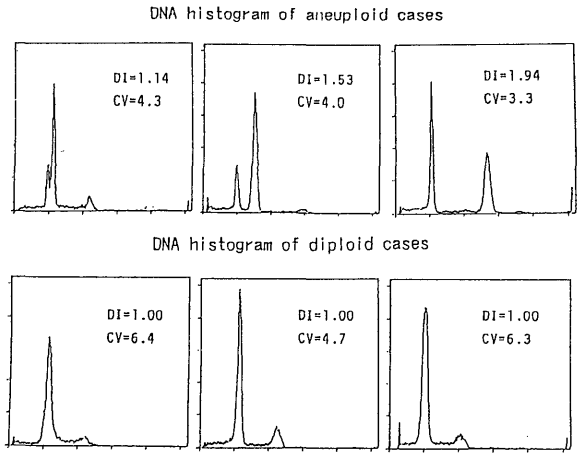

Fig. 1. DNA histogram

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.

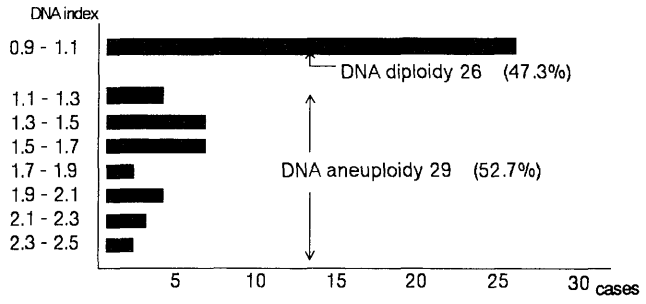

Fig. 2. Frequency of DNA index

i) The incidence according to disease stages.

The diploidy pattern of 21 belonging to stage I, II, IV_S was seen in eight (38.0%). On the other hand, the aneuploidy pattern was shown in 13 (62.0%). In stage III, the diploidy pattern was in 61.5%, aneuploidy in 38.5%.

In stage IV_A and IV_B, a similar distribution was shown. However, the incidence of the diploidy pattern had become higher with advances in disease stages as shown in Table 1.

Table 1. Stage and ploidy

	diploidy	aneuploidy	
I	3 (3)	5 (5)	8 (8)
II	3 (1)	5 (5)	8 (6)
III	8 (2)	5 (4)	13 (6)
IV_A	8 (0)	9 (2)	17 (2)
IV_B	2 (1)	2 (1)	4 (2)
IV_S	2 (2)	3 (1)	5 (3)
	26 (9)	29 (18)	55 (27)

( ); alive

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 IV_S. 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 2. Stage, ploidy and age

	under 1 year age		over 1 year of age	
	D	A	D	A
I	2	5	1	0
II	0	2	3	3
III	0	3	8	2
IV_A	0	1	8	8
IV_B	0	1	2	1
IV_S	2	2	0	1
	4	14	22	15

D; diploidy
A; aneuploidy

iii) Association with tumor location.

In case of the origin of the adrenal gland, the diploidy pattern was seen in 13 (44.8%) out of 29, aneuploidy in 16 (55.2%). 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.

Table 3. Tumor location and ploidy

	diploidy	aneuploidy	
adrenal	13	16	29
retroperitoneum	8	7	15
posterior mediastinum	4	5	9
others	1	1	2

iv) 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 n_2 of nodal metastasis, the diploidy pattern was seen in 62.5%, in contrast, aneuploidy in 37.5% in case with n_3 of nodal involvement. The incidence of aneuploidy pattern had become higher and there is a high tendency toward occurring distant metastasis. In contrast, there is no close correlation between the types metastasis and ploidy patterns as shown in Table 4.

Table 4. Local invasion, metastasis and ploidy

		diploidy	aneuploidy
C	0		
	1	3	8
	2	3	5
	3	10	8
N	0	5	10
	1	1	2
	2	8	2
	3	2	6
	x		1
B	0	12	17
	1	4	4
E	0	16	20
	1	0	1
V	0	15	19
	1	1	2
bm	0	14	18
	1	2	3
H	0	15	18
	1	0	0
	2	0	0
	3	1	3
D	0	16	19
	1	0	2

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

	diploidy	aneuploidy
Neuroblastoma	20 (6)	20 (12)
round cell type	2 (0)	1 (0)
rosette-fibrillary type	18 (6)	19 (12)
Ganglioneuroblastoma	6 (3)	9 (6)
poorly differentiated type	1 (0)	8 (6)
composite type	2 (1)	0
well differentiated type	3 (2)	1 (0)

( ); 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 amplificated 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

N-myc	ages under 1 year		over 1 year	
	diploidy	aneuploidy	diploidy	aneuploidy
amplification (–)	2 (2)	8 (8)	1 (1)	4 (1)
amplification (+)	0	0	8 (0)	2 (0)

( ); alive

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_A.

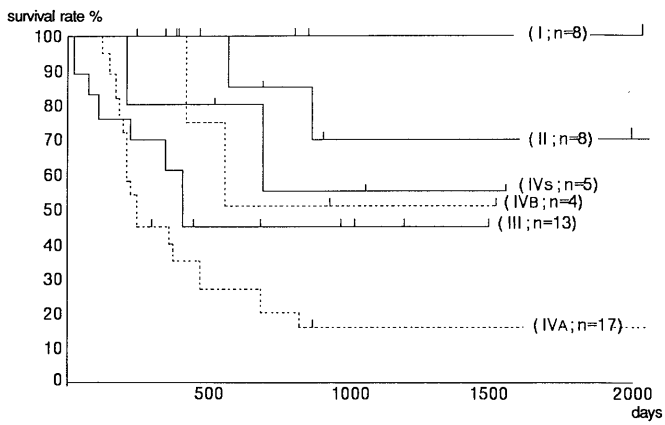

Fig. 3. Cumulative survival curve according to disease stages

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 ($p < 0.05$).

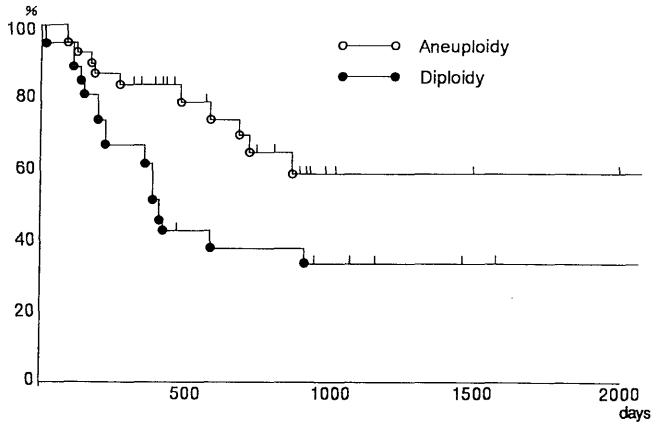

Fig. 4. Cumulative survival curve according to ploidy patterns

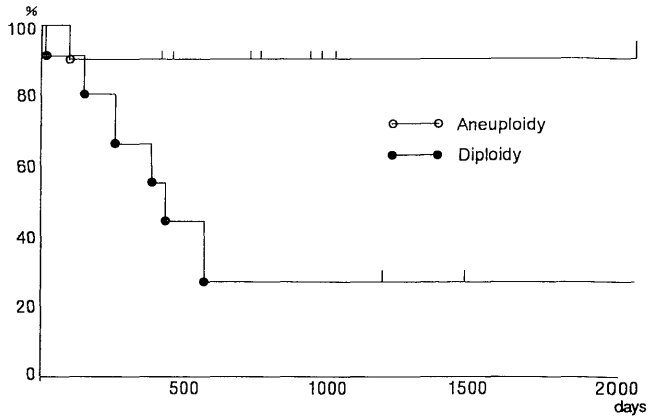

Fig. 5. Cumulative survival curve according to ploidy patterns in stage II and III

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_A and IV_B (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.

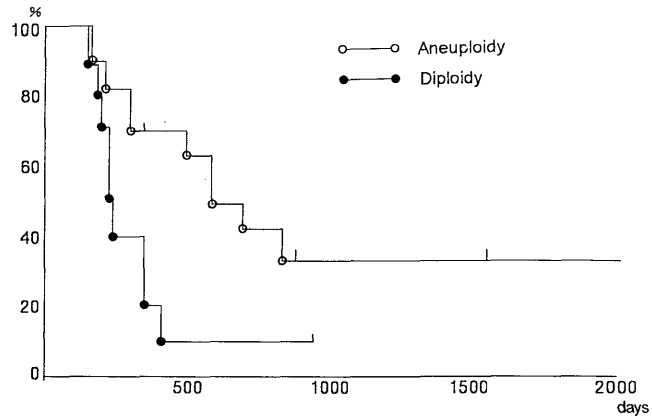

Fig. 6. Cumulative survival curve according to ploidy patterns in stage IV AB

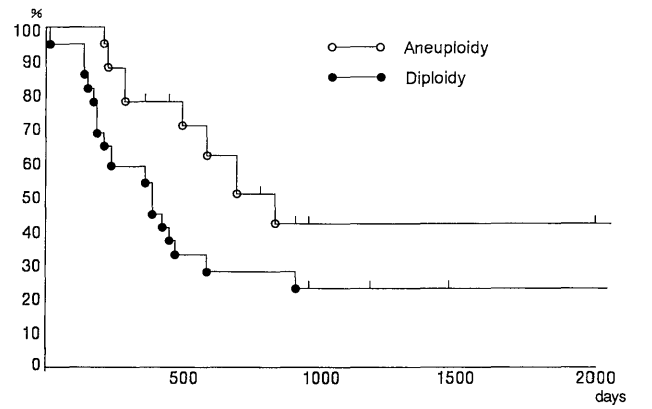

Fig. 7. Cumulative survival curve according to ploidy patterns in babies under 1 year of age

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 IV_S) and the unfair prognosis group (over one year old, stage III and IV_B and stage IV_A) through ages. It is now accepted that a significant difference in biologic behavior exist in the two groups.[2] Fifty-five subjected to this study were divided into the two groups. Recently, it has become possible to know the prognosis in more detail and more accuracy.

Trials to elucidate biologic behavior of malignancy have been made by DNA measurement using microphotospectrometry[14, 15] and flow cytometry.[16]

Hedley[17] developed DNA measurement from paraffin-embedded samples, which contributed to retrospective study for assessment of malignancy. It is widely accepted[18-20] that prognosis of patients with the diploidy pattern is much more satisfactory than that with the aneuploidy one. On the contrary, some investigators[6-10] reported that the aneuploidy pattern in neuroblastoma showed a good progosis. In this study, DNA ploidy patterns were evaluated in 55 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, IV_B 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 IV_S 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 chromosomal analysis, there was a high incidence of hyperploidy and also a loss of 14q was defined. At the same time, it has become apparent that Ha-ras expression reflects a fair prognosis.[21] In cases with poor prognosis, it is characteristic of demonstration of a diploidy pattern and aberration of 1p.[22] It is reported that N-myc expression was seen in stage III and IV, exceptionally in stage I and II[23]. Amplification of N-myc oncogen well correlates with poor prognosis.[24] Of 25 with N-myc investigation, amplification of N-myc oncogen was seen in 10 in this series in whom aneuploidy pattern was not revealed. However, it is emphasized 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 conclusion, 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

The author would like to extend my sincere appreciation to Prof. Masao Tomita, The First Department of Surgery, Nagasaki University School of Medicine, who kindly instructed and advised me and to Prof. Keiichi Ikeda and Instructor Akira Nakagawara for providing a cases and also to Hiroshi Ishikawa for cooperation. A part of the present study was supported by a grant in Aid of the Scientific Research of a assembly to protect children from cancer.

References

1) Schwartz AD, Dadash-Zadeh M, Lee H, et al: Spontaneous regression of disseminated neuroblastoma. J Pediatr 85:760-763, 1974.
2) Japanese Pediatric Surgical Society: Malignant tumor committee Nationwide analysis of pediatric surgical malignant tumor. J Jap Soc Ped Srug 26:143-150,1990.
3) Kanda H, Tsuchida Y: Amplification of N-myc oncogene in neuroblastoma. Tissue Culture 12:161-163, 1986.
4) Nakagawara A, Sasazuki T, Akiyama H, et al: N-myc oncogene and stage IVs neuroblastoma: preliminary observations on ten cases. Cancer 65/9:1960-1967, 1990.
5) Nagao M, Ishizaka Y, Nakagawara A, et al: Expression of ret proto-oncogene in human neuroblastoma. Jap J Cancer Res (GANN) 81/4:309-312, 1990.
6) Gansler T, Chatten J, Varello M, et al: Flow cytometric DNA analysis of neuroblastoma: correlation with histology and clinical outcome. Cancer 58:2453-2458, 1986.
7) Taylor SR, Blatt J, Costantino JP, et al: Flow cytometric DNA analysis of neuroblastoma and ganglioneuroma: a 10-year retrospective study. Cancer 62:749-754, 1988.
8) Oppedal BR, Storm-Mathisen I, Lie OS, et al: Prognostic factors in neuroblastoma: clinical, histopathologic, and immunohistochemical features and DNA ploidy in relation to prognosis. Cancer 62:772-780, 1988.
9) Look AT, Hayes FA, Nitschke R, et al: Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. N Engl J Med 311:231-235,1984.
10) Brenner DW, Barranco SC, Wilson BH, et al: Flow cytometric analysis of DNA content in children with neuroblastoma. J Pediat Surg 24:204-207, 1989.
11) Schutte B, Reynders MMJ, Bosman FF, et al: Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry 6:26-30, 1985.
12) Vindelφv LL, Christensen IJ, Nissen NI: A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 3:323-327, 1983.
13) Committee on Histological Classification of Childhood Tumors, Japanese Pathological society: Histological classification and atlas of tumors in infancy and childhood (I) Hepatoblastoma, Nephroblastoma, Neuroblastoma Kanahara, 1979.

14) Fujita T: Microspectrophotometry. Japanese Histochemistry 261-276, 1986.
15) Bohm N, Sandritter W: DNA in human tumors. Curr Top Pathol 60:151-219, 1975.
16) Van Dilla MA, Trujillo TT, Mullancy PF, et al: Cell microfuluorometry: A method for rapid fluorescence measurement. Science 163:1213-1214, 1969.
17) Hedley DW, Friedlander ML, Taylar IW, et al: Method for analysis of cellular DNA content of paraffin-embedded pathological materials using flow cytometry. J Histochem Cytochem 31:1333-1335, 1983.
18) Emdin SO, Steinling R, Roos G: Prognostic value of DNA content in colorectalcarcinoma. A flow cytometric study with some methodologic aspect. Cancer 60:1282-1287, 1987.
19) Hattori T, Hosokawa Y, Fukuda M, et al: Analysis of DNA ploidy patterns of gastric carcinoma of Japanese. Cancer 54:1591-1597, 1984.
20) Atkin WS, Cuzick J, et al: Value of flow cytometric determination of ploidy as guide to progres in operable rectal cancer. Int J Colorect Dis 2:17-21, 1987.
21) Tanaka T, et al: Clinical significance of HA-ras oncogen expression: As fair prognostic factor of HA-ras p21 in tumors. J Jap Soc Ped Surg 93:2620-2624, 1989.
22) Kaneko Y, Tsuchida Y, Maseki N, et al: Chromosome findings in human neuroblastoma xenografied in nude mice. Jap J Cance Res (Gann) 76:359-362, 1985.
23) Brodeur Gm, Seeger RC, Schwal M, et al: Amplification of N-myc in untreated human neuroblastoma correlates with advanced disease stage. Science 224:1121-1125, 1984.
24) Tsuchida K: Multimodality therapy for neuroblastoma based on tumor oncogen analysis. A Grant-in-Aid of Cancer Research of Ministry of Public Welfare Tokyo, 1990.