An increased NM23H1 copy number may be a poor prognostic factor independent of LOH on 1p in neuroblastomas

O Takeda1, M Handa2, T Uehara3, N Maseki4, A Sakashita4, M Sakurai5, Y Kanda6 and Y Kaneko1

1Department of Cancer Chemotherapy, 2Second Clinical Department, 3Department of Clinical Pathology and 4Third Clinical Department, Saitama Cancer Center Hospital, Ina, Saitama 362; 5Department of Anatomy and Developmental Biology, Tokyo Women’s Medical College, Shinjuku-ku, Tokyo 162; 6Radiotherapy Division, National Cancer Center Research Institute, Chuo-ku, Tokyo 104, Japan.

Summary  In a study of 154 neuroblastomas, loss of heterozygosity (LOH) was observed on 1p (13%, 19/145), 11q (19%, 11/59), 14q (15%, 15/97), 17p (5%, 5/105) and 17q (17%, 9/52). We also found an increase in NM23H1 copy number in 14% (13/95) of neuroblastomas. All except one tumour with an increased copy number stained positive with anti-NM23H1 monoclonal antibody. Event-free survival (EFS) was significantly shorter in 19 patients with LOH on 1p than in 128 without (41% vs 77% 4 year EFS, P=0.0093), and in 13 patients with increased NM23H1 copy numbers than in 82 with normal copy numbers of the gene (61% vs 84% 4 year EFS, P=0.0103). LOH on 11q, 14q or 17q did not affect EFS. Most tumours with LOH on 1p, increased NM23H1 copy numbers or MYCN amplification occurred in patients aged 12 months or more, those with advanced stage disease, and those who showed near diploidy or pseudodiploidy. However, LOH on 1p was found in only 1 of the 13 tumours with increased NM23H1 copy numbers, and MYCN amplification of four copies occurred in only one other such tumour. These findings suggest that the increased NM23H1 copy number may be a predictor for poor prognosis, independent of LOH on 1p, and probably also of MYCN amplification.

Keywords: neuroblastoma; loss of heterozygosity; NM23H1; prognostic factors

Molecular genetic studies of neuroblastoma have suggested the presence of a tumour-suppressor gene on 1p36 (Fong et al., 1989, 1992; Weith et al., 1989). We and other investigators have recently reported that another tumour-suppressor gene on 1p32–1p34 may be closely associated with a biologically aggressive subtype of neuroblastoma (Takeda et al., 1994; Schleiermacher et al., 1994). Furthermore, two other tumour-suppressor genes associated with the development and/or progression of neuroblastoma are thought to be located on 11q and 14q (Fong et al., 1992; Srivatsan et al., 1993; Suzuki et al., 1989; Takayama et al., 1992). The NM23 gene was identified as a metastasis-suppressor gene by differential hybridisation between two murine melanoma sublines with low and high metastatic potential (Steeg et al., 1988). The gene codes for the nucleoside diphosphate kinase protein, and is located on 17q21–22 (Gilles et al., 1991; Varesco et al., 1992). Reduced expression of NM23 was associated with lymph node metastases and poor prognosis in breast cancer (Bevilacqua et al., 1989). In contrast, overexpression, mutation and amplification of NM23 were reported in aggressive neuroblastomas (Haillet et al., 1991; Leone et al., 1993; Chang et al., 1994).

We studied the loss of heterozygosity (LOH) on 1p, 11q, 14q, 17p and 17q in 154 neuroblastomas and found LOH on 1p, 11q, 14q and 17q in incidences ranging between 13% and 19%, but that on 17p, where a tumour-suppressor gene TP53 is located (Human Gene Mapping, 1991), in only 5%. In addition, we examined NM23H1 copy markers in 95 neuroblastomas and found that there was an increase in the copy number in 14% of the tumours. Event-free survival (EFS) was examined between patients with LOH on 1p, 11q, 14q or 17q and those without, and between patients with increased NM23H1 copy numbers and those without. The results indicated that only allelic loss on 1p and increased NM23H1 copy number predicted an adverse treatment outcome.

Materials and methods

Tissue samples

Tumours were obtained from 154 Japanese infants and children aged between 10 days and 9 years who were consecutively admitted to various institutions (listed in the Acknowledgements) between May 1985 and December 1993. One-hundred and twenty-five tumours were obtained at diagnosis, 25 after induction therapy and four at relapse. One-hundred and forty tumours were obtained from the primary sites and 14 from metastatic sites (five from bone marrow, seven from metastatic lymph nodes and two from pleural effusion). Normal tissues were obtained from peripheral blood of the same patients. Of the 154 tumours, 152 were histologically classified as neuroblastoma or ganglioneuroblastoma, and two as ganglioneuroma. Patients were staged according to the Evans staging system (Evans et al., 1971). Patients in stage I or II were treated with either surgery alone or surgery plus chemotherapy consisting of cyclophosphamide and vincristine; those in stage III or IV were treated with multidrug chemotherapy consisting of cyclophosphamide, vincristine, irinotecan, cisplatin and etoposide with or without surgery.

Molecular studies

Genomic DNA was extracted from the tumour tissue and peripheral blood using standard phenol/chloroform procedures. An aliquot of 3–8 μg of DNA from each sample was digested with appropriate restriction enzymes, electrophoresed through 0.6–0.8% agarose gels and transferred onto nylon membranes (Hybond N+, Amersham, Tokyo) by alkaline blotting.

The 22 probes used to detect allelic loss included D1S7 (MS1) on 1p, D11S146 (pHBlS9), D11S29 (L7), CD3D (pPGBC9), CD3E (pDJ4), PBGD (PBGD), D11S147

Correspondence: Y Kaneko, Department of Cancer Chemotherapy, Saitama Cancer Center Hospital, 818 Komuro, Ina, Saitama 362, Japan

Received 21 September 1995; revised 22 March 1996; accepted 1 July 1996
copy number as an increase in the radioactivity of the NM23H1 fragments of tumour tissue relative to both D81B2.0 and D14S23 to more than 150% compared with that of the corresponding fragments of normal tissue (Figure 1).

PCR – SSCP (polymerase chain reaction – single strand conformation polymorphism) analysis was performed on three tumours that showed LOH on 17p, using pairs of primers to detect mutations of exons 5, 6, 7 and 8 of TP53 (Murakami et al., 1991).

Immunohistochemical studies

Avidin – biotin complex (ABC) immunoperoxidase assay was performed on 4 μm sections from formalin-fixed, paraffin-embedded tissues. Tissue sections were deparaffinised, rehydrated and exposed to 0.3% hydrogen peroxide in methanol to eliminate endogenous peroxidase activity. Sections were incubated with monoclonal anti-human NM23H1 antibody (Seikagaku, Tokyo, Japan) diluted 1:300 in phosphate-buffered saline (PBS) for 18 h at 4°C. The specificity of the antibody was proved by immunoprecipitation and immunoblotting (Urano et al., 1993). The ABC assay was performed using CSA kits (Dako, CA, USA). The end-products were visualised by treating the sections with diaminobenzidine tetrahydrochloride. Negative controls were performed with normal mouse or rabbit serum instead of the primary antibody. The slides were scored without knowledge of the NM23H1 gene analysis and before compilation of the clinical data. Three categories were used in scoring the slides: strong staining (+), weak staining (+), no staining (−) of tumour cells. Only tumours with strong staining were considered to have a positive reaction.

Chromosome studies

The tumour tissue was minced with scissors and was cultured in plastic flasks containing ES medium (Nissui, Seiyaku, Tokyo) with 15% fetal calf serum. The cells were harvested within 96 h from the start of culture. Bone marrow cells were cultured for 24 h in plastic flasks containing RPMI-1640 medium with 20% fetal calf serum, and were harvested. Chromosomes were analysed by regular Giemsa staining and Q- and/or G-band techniques. We defined abnormal clones and chromosome ploidies according to ISCN (1991). When we found only normal diploid metaphase cells in tumour tissues, the examination was considered to have failed to detect malignant mitotic cells and, hence, to be inadequate.

Statistical analyses

The EFS for each group of patients was estimated by the Kaplan – Meier method (Kaplan and Meier, 1958) on the data updated on 30 April, 1995; and differences in EFS curves were assessed using the generalised Wilcoxon and log-rank tests (Gehan, 1965; Peto and Peto, 1972). Significance of the differences in various clinical and biological aspects of the disease between patients with LOH on 1p and those without, and between patients with an increased NM23H1 copy number and those without, was examined by the chi-square or Fisher’s exact test.

Results

Allelic loss on 1p, 11q, 14q, 17p or 17q, and NM23H1 copy numbers

One hundred and forty-seven of the 154 patients were informative at D1S7 on 1p, and 19 of the 147 informative patients (13%) showed LOH on 1p. Fifty-nine of the 102 patients were informative at one or more loci on chromosome 11q, and 11 of the 59 informative patients (19%) showed LOH on 11q. The results for the 11 tumours are summarised in Figure 2. As tumours 694 and 804 retained heterozygosity at
D11S383 and D11S146 respectively, the commonly deleted region on 11q was distal to D11S146 and proximal to D11S383. Ninety-six of the 107 patients were informative at one or more loci on chromosome 14q, and 15 of the 96 informative patients (16%) showed LOH on 14q. The results for the 15 tumours are summarised in Figure 3. As tumours 882 and 955 retained heterozygosity at D14S13 and tumour 998 retained that at D14S1, the consensus deletion on 14q

Figure 2  LOH on 11q and 11p in 11 neuroblastomas. The closed and open circles indicate LOH and no LOH respectively, and the shaded circle indicates 'uninformative.'

Figure 3  LOH on 14q in 15 neuroblastomas. The closed and open circles indicate LOH and no LOH respectively, and the shaded circle indicates 'uninformative.'
encompassed the region distal to D14S13 and proximal to D14S1. One hundred and five of the 122 patients were informative at D17S30 on chromosome 17p, and only 5 of the 105 (5%) showed LOH at the D17S30 locus. Fifty-two of the 97 patients were informative at the NM23H1 locus on 17q, and 9 of the 52 patients (17%) showed LOH on 17q.

PCR–SSCP analysis was performed on three tumours (nos. 841, 1152 and 1185) with LOH on 17p, and showed abnormal mobilities of fragments, representing exon 7 of TP53 in polyacrylamide gel, in one (no. 1152) of them.

The NM23H1 copy number was examined in 95 patients, and 13 of the 95 (14%) showed increased copy numbers ranging from 1.5 to 4.4 (Figure 1).

**Immunohistochemical staining with anti-NM23H1 monoclonal antibody**

NM23H1 protein expression was demonstrated in 11 of the 29 patients whose tumour tissues were examined (Figure 4), in 7 of 8 tumours with the increased NM23H1 copy number, and in 4 of 21 tumours with the normal copy number ($P = 0.0014$ by Fisher's exact test) (Table III).

**Event-free survival**

One hundred and fifty-four patients were classified into groups on the basis of age, stage, outcome of mass screening (Sawada *et al.*, 1984) and ploidy of tumours. They were also classified by presence or absence of MYCN amplification, LOH on 1p, 11q, 14q and 17q, and increased NM23H1 copy numbers. EFS at 4 years in each group of patients is shown in Table I. There were significant differences in the survival time between the patients classified by age, stage, outcome of mass screening, ploidy of tumours, presence or absence of MYCN amplification, LOH on 1p and the increased

![Figure 4](image-url) Tumour 482 stained with anti-NM23H1 monoclonal antibody. The nucleus was strongly stained. The tumour cells had an increased copy number (×2) of the NM23H1 gene (ABC method, ×100).

### Table 1 Clinical and biological features of 154 children with neuroblastoma

| Age | No. (%) of patients | Four year %EFS | S.E. | Log-rank P | Generalised Wilcoxon P |
|-----|---------------------|----------------|------|------------|-----------------------|
| A. <12 months | 92 (60) | 99 | 1 | <0.0001 | A vs B | <0.0001 |
| B. >12 months | 62 (40) | 57 | 8 | | | |
| Stage | | | | | | |
| A. I, II, IVs | 74 (48) | 100 | 0 | <0.0001 | A vs B | <0.0001 |
| B. III | 31 (20) | 63 | 11 | A vs C | 0.0050 |
| C. IV | 49 (32) | 54 | 9 | B vs C | 0.0215 |
| Mass screening | | | | | | |
| A. Positive | 87 (56) | 99 | 1 | <0.0001 | A vs B | <0.0001 |
| B. Negative | 43 (30) | 55 | 9 | A vs C | 0.0005 |
| C. Not undergone | 24 (16) | 65 | 17 | B vs C | N.S. |
| Ploidy of tumours | | | | | | |
| A. 2n | 31 (20) | 57 | 12 | 0.0012 | A vs B | 0.0002 |
| B. 3n | 68 (44) | 94 | 3 | A vs C or D | N.S. |
| C. 4n | 6 (4) | 75 | 22 | B vs C or D | N.S. |
| D. No mitotic cells | 49 (32) | 81 | 8 | | | |
| MYCN amplification | | | | | | |
| A. Present | 15 (10) | 31 | 18 | 0.0002 | A vs B | 0.0082 |
| B. Absent | 138 (90) | 87 | 3 | | | |
| LOH on 1p | | | | | | |
| A. Present | 19 (12) | 41 | 13 | 0.0022 | A vs B | 0.0093 |
| B. Absent | 128 (83) | 77 | 4 | A vs C | 0.0285 |
| C. Not informative | 7 (5) | 100 | 0 | B vs C | N.S. |
| LOH on 11q | | | | | | |
| A. Present | 11 (11) | 73 | 13 | N.S. | A vs B or C | N.S. |
| B. Absent | 48 (47) | 76 | 7 | B vs C | N.S. |
| C. Not informative | 43 (42) | 71 | 7 | | | |
| LOH on 14q | | | | | | |
| A. Present | 15 (14) | 74 | 11 | N.S. | A vs B or C | N.S. |
| B. Absent | 81 (76) | 74 | 5 | B vs C | N.S. |
| C. Not informative | 11 (10) | 82 | 12 | | | |
| LOH on 17q | | | | | | |
| A. Present | 9 (9) | 75 | 21 | N.S. | A vs B or C | N.S. |
| B. Absent | 43 (44) | 91 | 5 | B vs C | N.S. |
| C. Not informative | 45 (47) | 76 | 9 | | | |
| NM23H1 copy number | | | | | | |
| A. Increased | 13 (14) | 61 | 15 | 0.0693 | A vs B | 0.0103 |
| B. Normal | 82 (86) | 84 | 7 | | | |

*Three year %EFS is shown. EFS, event-free survival; S.E., standard error; N.S., not significant.
Table II Clinical characteristics, chromosome ploidy and MYCN amplification in neuroblastomas classified by presence or absence of LOH on 1p or increased NM23H1 copy numbers

| Group of patients | No. of patients | Age | Stage of disease | Chromosome ploidy | MYCN amplification |
|-------------------|----------------|-----|------------------|-------------------|-------------------|
|                   |                |     |                  |                   |                   |
| 1pLOH + b         | 19             | 4   | +                | N+1+1+1 | +              |
| 1pLOH - b         | 128            | 82  | -                | N+1+1+1 | +              |
| NM23H1 + c,d      | 13             | 3   | -                | N+1+1+1 | +              |
| NM23H1 - c,d      | 82             | 52  | -                | N+1+1+1 | +              |

*Mass screening: +, undergone the mass screening with a positive result; -, undergone the mass screening with a negative result; N, not undergone the mass screening. There is a significant difference in the incidence of patients under 12 months of age (P = 0.0006), in the incidence of patients found by mass screening (P = 0.0136), in the stage distribution (P = 0.0142), in the ploidy distribution (P = 0.0071) and in the incidence of patients with MYCN amplification (P < 0.0001) between the patients with LOH on 1p and those without. NM23H1 +, with an increased NM23H1 copy number; NM23H1 -, with a normal NM23H1 copy number. There is a significant difference in the incidence of patients under 12 months of age (P = 0.0129), in the stage distribution (P = 0.0158) and in the ploidy distribution (P = 0.0121) between the patients with an increased NM23H1 copy number and those without.

Table III Clinical, cytogenetic and genetic characteristics of 13 patients with increased NM23H1 copy numbers

| Patient | Age (months) | Mass screening | Stage | Primary site | Ploidy | MYCN copy numbers | NM23H1 immuno-staining | Present status | Event-free survival (months) |
|---------|--------------|----------------|-------|--------------|--------|-------------------|------------------------|----------------|----------------------------|
| 482     | 12           | +              | IV    | Adr.         | NM     | 1                 | +                      | NED            | 74+                        |
| 786     | 6            | +              | IV    | Ret.         | 46     | +                 | +                      | NED            | 50+                       |
| 790<sup>a</sup> | 43     | -              | IV    | Ret.         | NM     | 1                 | +                      | NED            | 50+                       |
| 792     | 34           | -              | IV    | Ret.         | NM     | 1                 | +                      | DOD            | 24                        |
| 797     | 67           | N              | IV    | Adr.         | 46     | +                 | NE                     | DOD            | 26                        |
| 860     | 12           | +              | IV    | Adr.         | 78     | 1                 | NE                     | NED            | 36+                       |
| 909<sup>a</sup> | 72     | +              | IV    | Adr.         | 44     | 1                 | NE                     | DOD            | 0                         |
| 912     | 18           | +              | -     | IV           | NM     | 4                 | +                      | NED            | 47+                       |
| 927     | 60           | N              | IV    | Adr.         | 45     | 1                 | NE                     | DOD            | 11                        |
| 1036    | 8            | +              | I     | Adr.         | 67     | 1                 | +                      | NED            | 31+                       |
| 1089    | 7            | +              | III   | Ret.         | 55     | 1                 | +                      | NED            | 27+                       |
| 1126    | 54           | N              | IV    | Pelv.        | 50     | 1                 | +                      | AWD            | 6                         |
| 1152    | 60           | N              | III   | Ret.         | 53     | 1                 | NE                     | NED            | 22+                       |

*Mass screening: +, undergone the mass screening with a positive result; -, undergone the mass screening with a negative result; N, not undergone the mass screening. * + after the number of months indicates that the patient was still alive. *Karyotypes are described in Table IV. The tumour also showed LOH on 1p, 14q and 17q. *The tumour also showed LOH on 1q4, Adr, adrenal; Ret, retroperitoneum; Pelv., pelvic cavity; NM, no good metaphases obtained; NED, no evidence of disease; DOD, died of disease; AWD, alive with disease; NE, not examined.

Figure 5 Event-free survival curves for two groups of patients classified by presence or absence of an increased NM23H1 copy number (P = 0.0103). - - - , Normal NM23H1 copy number (n = 82); ---, increased NM23H1 copy number (n = 13).

 NM23H1 copy number (Figure 5), but no differences were detected between patients classified by presence or absence of LOH on 11q, 14q or 17q.

Clinical and biological characteristics of patients with LOH on 1p or an increased NM23H1 copy number (Tables II and III)

There was a significant difference in the incidence of patients under 12 months of age (P = 0.0006), in the stage distribution (P = 0.0142), in the incidence of patients found by mass screening (P = 0.0136), in the ploidy distribution (P = 0.0071) and in the incidence of tumours with MYCN amplification (P < 0.0001) between patients with LOH on 1p and those without. There was a significant difference in the incidence of patients under 12 months of age (P = 0.0129), in the stage distribution (P = 0.0158) and in ploidy distribution (P = 0.0121) between patients with increased NM23H1 copy number and those without. Clinical, cytogenetic and genetic characteristics of 13 patients with increased NM23H1 copy number are shown in Table III. Only 1 (no. 790) of the 13 patients with the increased NM23H1 copy number showed LOH on 1p in the tumour.

Ploidies and karyotypes of tumours with an increased NM23H1 copy number (Tables III and IV)

Modal chromosome numbers were determinable in 10 of the 13 tumours with increased NM23H1 copy number; seven had near-diploidy or pseudodiploidy, two had near-triploidy and the other had hypotetraploidy. Karyotypes were successfully analysed in three of the ten tumours (Table IV). All three tumours had hypo- or pseudodiploidy; two of them apparently had a normal pair of chromosomes 17, and the

Table IV Karyotypes of neuroblastomas with increased NM23H1 copy numbers

| Tumour number | Representative karyotype |
|---------------|--------------------------|
| 786           | 46,XY,del(3)(q13q25),del(11)(q12q25),add(15) (p13),add(17)(q22),add(19)(q13),−20,+mar |
| 797           | 46,XY,del(2p)23,−5,del(13) (q14q22),add(13) (q34),−15,add(18)(q23),−20,+mar |
| 909           | 44,XX, dic(1;20)(p36q13),−15,−18,−22,+mar |
other showed an abnormal chromosome 17 with an unknown fragment on 17q22.

Discussion

We found allelic loss on 1p, 11q, 14q, 17p and 17q in 13% (19/147), 19% (11/59), 16% (15/95), 5% (5/105) and 17% (9/52) of neuroblastomas respectively. In previous studies on neuroblastomas, the incidence of LOH on 1p ranged from 25% to 89%, on 11q was 28%, on 14q ranged from 22% to 40% and on 17p was 0% (Fong et al., 1989, 1992; Weith et al., 1989; Takeda et al., 1994; Schlieermann et al., 1994; Srivastan et al., 1993; Suzuki et al., 1988; Takayama et al., 1992). The incidence of LOH on 17q has not been reported. The incidences of LOH on 1p, 11q or 14q in our series were lower than those previously reported on these three chromosomal regions. The different incidences may have been caused by the inclusion in our series of a large number of patients found by mass screening. Our study defined the locations of putative tumour-suppressor genes of 11q and 14q in the region distal to D11S146 (11q13) and proximal to D11S383 (11q24–25) and in the region distal to D14S13 and proximal to D14S1 respectively. Both of the D14 loci were mapped in 14q32, and the distance between D14S13 and D14S1 was 8 Mb (Nakamura et al., 1991).

We also found increased NM23H1 copy numbers in 14% (13/95) of neuroblastomas. The results were confirmed by immunohistochemical staining using anti-NM23H1 monoclonal antibody. The previous study reported increased NM23H1 copy numbers in 23% (7/31) of neuroblastomas (Leone et al., 1993). The same study reported no increase in the copy number of NM23H2, which is located next to NM23H1 on 17q21–22. Another study showed 17q polyom in 38% (20/53) of neuroblastomas using polymorphic DNA markers on 17q other than NM23H1 (Caron, 1995). Our cytogenetic study on the three tumours showed no polyom, and suggested that the limited chromosomal region including the NM23H1 locus may have amplified in the tumours.

We compared EFS of different groups of patients classified by presence or absence of LOH in each of the four chromosomal regions (i.e. 1p, 11q, 14q and 17q) in the tumour and by presence or absence of an increased NM23H1 copy number. Only LOH on 1p and an increased NM23H1 copy number proved to be predictors for adverse treatment outcome. Most tumours with an increased NM23H1 copy number occurred in patients aged 12 months or more with advanced stage disease and who showed near-diploidy or pseudodiploidy; these characteristics are similar to those tumours with LOH on 1p or with MYCN amplification (Takeda et al., 1994). However, LOH on 1p was found in only 1 of the 13 tumours with an increased NM23H1 copy number, and MYCN amplification of four copies was found in only one other tumour (Table III). These findings indicate that an increased NM23H1 copy number may be a predictor for poor prognosis independent of LOH on 1p and probably also of MYCN amplification. Thus, by using various genetic markers, including the copy numbers of MYCN and NM23H1 and presence or absence of LOH on 1p, we may be able to predict the prognosis of neuroblastoma patients more precisely than otherwise. Therapy should be intensified in patients with positive results for these specified genetic markers.

Acknowledgements

This work was supported in part by a Grant-in-Aid for a Creative Basic Research (Human Genome Program) from the Ministry of Education, Science and Culture, and by a Grant-in-Aid from the Ministry of Health and Welfare (Japan). We acknowledge Drs H Shiku and T Kozu for providing the NM23H1 and D81B2.0 probes respectively. All other probes in our study were obtained through the Japanese Cancer Research Resources Bank or the American Type Culture Collection. We also acknowledge T Matsuki and A. T. Amura for expert technical assistance in the immunohistochemistry.

We thank Dr T Oka, Asahikawa Medical College (Asahikawa, Hokkaido); Dr Y Hatae, National Sapporo Hospital (Sapporo, Hokkaido); Dr T Hiramura, Hokkaido Children’s Medical Center (Hokkaido); Dr A Watanabe, Akita University (Akita, Japan); Dr A Kikuta, Fukushima Medical College (Fukushima, Fukushima); Dr Y Tsunematsu, National Children’s Hospital (Setagaya-ku, Tokyo); Dr M Iwata, Nihon University (Itabashi-ku, Tokyo); Dr J Yokoyama, Keio University (Shinjuku-ku, Tokyo); Dr T Takayama, National Cancer Center (Chou-ku, Tokyo); Dr A Hayashi, Kiyose Children’s Hospital (Kiyose, Tokyo); Drs H Nishihira and Y Tanaka, Kanagawa Children’s Medical Center (Yokohama, Kanagawa); Dr S Koizumi, Kana- kawa University (Kanazawa, Ishikawa); Dr T Sakajari, Fukushima Hospital (Fuku, Fukushima); Dr S Y Horikoshi and Y Hamazaki, Shizuoka Children’s Hospital (Shizuoka, Shizuoka); Dr H Kitou, Seirei Hamamatsu Hospital (Hamamatsu, Shizuoka); Dr Y Hanji, Ichinomiya Municipal Hospital (Ichinomiya, Aichi); Drs S Mabuchi and Y Imai, Hyogo Children’s Hospital (Kobe, Hyogo); Drs M Sakurai and H Kawasaki, Mie University (Tsu, Mie); Dr Y Nakamura, Uwajima Municipal Hospital (Uwajima, Ehime); Dr Y Ishida, Ehime University (Onsen-gun, Ehime); Dr K Matsu- naga, Nagasaki University (Nagasaki); Dr H Eguchi, Kurume University (Kurume, Fukuoka); Dr G Uroho, University of Occupational and Environmental Health (Kitakyushu, Fukuoka); and Dr J Ikamura, Kyushu Cancer Center (Fukuoka, Fukuoka) for providing samples, pathology slides and clinical data.

References

BEVILACQUA G, SOBEL M, LIUTTA LA AND STEEG PS. (1989). Association of low mm23 RNA levels in human primary infiltration ductal breast carcinomas with lymph-node involvement and other histopathological indicators of high metastatic potential. Cancer Res., 49, 5185–5190.

CARON H. (1995). Allelic loss of chromosome 1 and additional chromosome segments are both unfavorable prognostic markers in neuroblastoma. Med. Pediatr. Oncol., 24, 215–221.

CHANG, CL, ZHU X, THORAVAL DH, UNGAR D, RAWWAS J, HORA N, STRAHLER JR AND HANASH SM. (1994). nm23H1 mutation in neuroblastoma. Cancer Res., 54, 370, 371–377.

CRISPER AE, D’ANGIO DJ AND RANDOLPH J. (1971). A proposed staging for children with neuroblastoma. Cancer, 27, 374–378.

FEINBERG AP AND VOGELSTEIN B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 132, 6–13.

FONG CT, DRACOPOLI NC, WHITE PS, MERRILL PGT, GRIFFITH RC, HOUSMAN DE AND BRODEUR GM. (1989). Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastomas: correlation with NMyc amplification. Proc. Natl Acad. Sci. USA, 86, 3753–3757.

FONG CT, WHITE PS, PETERSON K, SAPIENZA C, CAVENEKE WK, KERM SE, VOGELSTEIN B, CANTOR AB, LOOK AT AND BRODEUR GM. (1992). Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. Cancer Res., 52, 1780–1785.

GEHAN E. (1965). A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika, 52, 203–224.

GILLES AM, PRESECAN E, VONICA A AND LASCU L. (1991). Nucleoside diprophosphate kinase from human erythrocytes. J. Biol. Chem., 266, 8784–8789.

HAYASHI T, KIEM DR, A, DRAMMLICHTNER RF, ZHU X, ECKERKORN C, BRODEUR GM, REYNOLDS CP, SEGER RC, LOTTSTEIPECH, STRAHLER JR AND HANASH SM. (1991). High levels of p19

HUMAN GENE MAPPING 11. (1991). Cytogenet. Cell Genet., 58, 1440–1569.

ISCN 1991. (1992). Guidelines for Cancer Cytogenetics: Supplement to an International System for Human Cytogenetic Nomenclature. Karger: Basel.
KAPLAN EL AND MEIER P. (1958). Nonparametric estimation for incomplete observations. J. Am. Stat. Assoc., 53, 457 – 481.

LEONE A, SEEGER RC, HONG CM, HU YY, ARBOLEDA MJ, BRODEUR GM, STRAM D, SLAMON DJ AND STEEG PS. (1993). Evidence for nm23 RNA overexpression, DNA amplification and mutation in aggressive childhood neuroblastomas. Oncogene, 8, 855 – 865.

MURAKAMI Y, HAYASHI K AND SEKIYA T. (1991). Detection of aberrations of the p53 alleles and the gene transcript in human tumor cell lines by single-stranded conformation polymorphism analysis. Cancer Res., 51, 3356 – 3361.

NAKAMURA Y, LATHROP M, O’CONNELL P, LEPPERT M, KAMBOH MI, LALOUEL JM AND WHITE R. (1989). Frequent recombination is observed in the distal end of the long arm of chromosome 14. Genomics, 4, 76 – 81.

OKADA K, URANO T, GOIT BABA H, YAMAGUCHI A, FURUKAWA K AND SHIKU H. (1994). Isolation of human nm23 genomes and analysis of loss of heterozygosity in primary colorectal carcinomas using a specific genomic probe. Cancer Res., 54, 3979 – 3982.

PETRO R AND PETO J. (1972). Asymptotically efficient rank invariant test procedures. J. R. Stat. Soc., (a), 135, 185 – 206.

SAWADA T, HIRAYAMA M AND NAKATA T. (1984). Mass screening for neuroblastoma in infants in Japan. Lancet, 2, 271 – 273.

SCHLEIERMACHER G, PETER M, MICHON J, HUGOT J, VIELH P, ZUCKER J, MAGDELENAT H, THOMAS G AND DELATTRE O. (1994). Two distinct deleted regions on the short arm of chromosome 1 in neuroblastoma. Genes Chrom. Cancer, 10, 275 – 281.

SRIVATSA ES, YING KL AND SEEGER RC. (1993). Deletion of chromosome 11 and of 14q sequences in neuroblastoma. Genes Chroms. Cancer, 7, 32 – 37.

STEEG PS, BEVILACQUA G, KOPPER L, THORGEIRSSON UP, TALMADGE JE, LIOTTA LA AND SOBEL ME. (1988). Evidence for a novel gene associated with low tumor-metastatic potential. J. Natl Cancer Inst., 80, 200 – 204.

SUZUKI T, YOKOTA J, MUGISHIMA H, OKABE I, OOKUNI M, SUGIMURA T AND TERADA M. (1989). Frequent loss of heterozygosity on chromosome 14q in neuroblastoma. Cancer Res., 49, 1094 – 1098.

TAKAYAMA H, SUZUKI T, MUGISHIMA T, FUJISAWA T, OOKUNI M, SCHWAB M, GEHRING M, NAKAMURA Y, SUGIMURA T, TERADA M AND YOKOTA J. (1992). Deletion mapping of chromosome 14q and 1p in human neuroblastoma. Oncogene, 7, 1185 – 1189.

TAKEDA O, HOMMA C, MASEKI N, SAKURAI M, KANDA N, SCHWAB M, NAKAMURA Y AND KANEKO Y. (1994). There may be two tumor suppressor genes on chromosome 1p closely associated with biologically distinct subtypes of neuroblastoma. Genes Chrom. Cancer, 10, 30 – 39.

URANO T, FURUKAWA K AND SHIKU H. (1993). Expression of nm23/NDP kinase proteins on the cell surface. Oncogene, 8, 1371 – 1376.

VARESCO L, CALIGO MA, SIMI P, BLACK DM, NARDINI V, CASARINO L, ROCCHI M, FERRARA G, SOLOMON E AND BEVILACQUA. (1992). The nm23 gene maps to human chromosome band 17q22 and shows a restriction fragment length polymorphism with BgII. Genes Chrom. Cancer, 4, 84 – 88.

WEITH A, MARTINSSON T, CZIEPLUCH C, BRUDERLEIN S, AMLER LC, BERTHOLD F AND SCHWAB M. (1989). Neuroblastoma consensus deletion maps to 1p36.1 – 2. Genes Chrom. Cancer, 1, 159 – 166.