Tumour karyotype discriminates between good and bad prognostic outcome in neuroblastoma

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Summary In 28 patients with neuroblastoma of different stages the karyotype was determined in the primary tumour and/or in the metastases by direct chromosome preparation or short term cell culture. In addition, DNA analysis for the proto-oncogene N-myc was performed for comparison in 10 cases.

Abnormalities (deletions, translocations, derivations) of the short arm of chromosome 1 with the most frequent breakpoint at 1p32 (besides rarer aberrations in other chromosomes) were found in the tumour karyotype of 15 of 18 (83%) patients with metastatic disease (stage IV) and in 2 of 3 patients with stage III, but in none of the 7 patients with stages I, II, IV-S who are all alive with no evidence of disease. These 7 surviving patients with good prognosis had a hyperdiploid tumour karyotype, mainly in the triploid range. Eleven of the 18 (61%) patients with stage IV and 1 of 3 patients with stage III also contained double minutes (DMs) and/or homogeneously staining regions (HSRs) in their tumour karyotypes. N-myc amplification (30 to 60 copies) in the tumour DNA was detected in 2 of 6 (33%) examined cases with stage IV, in 1 out of 2 examined cases with stage III, and correlated with the presence of DMs/HSRs.

Life table analysis showed a 90% probability of surviving in patients lacking the lp abnormality as compared to less than 10% in patients with an aberrant lp chromosome in the tumour cells. We conclude that tumour karyotype, in particular the structure of the short arm of chromosome 1, is the most important factor in determining the different outcome in children with neuroblastoma.

After brain tumours neuroblastoma represents the most common solid tumour in childhood with an annual incidence of 8 cases per million children under 15 years; most of them – in contrast to the more frequent childhood leukaemias – occurring within the first 3 years of life.

The biological behaviour of neuroblastoma is unique and still puzzling to paediatric oncologists in that there is spontaneous regression in congenital tumours (even in the metastatic stage) and an almost 100% survival rate in localized tumours as compared to a less than 10% survival chance in non-localized, disseminated tumours in children over 1 year of age in spite of intensive chemotherapy (Berthold et al., 1986). Besides known prognostic factors such as age and tumour stage additional clinical factors useful in predicting outcome, such as serum ferritin and histologic type, have recently been emphasized (Evans et al., 1987). Also, the DNA properties of neuroblastoma cells such as the increased copy number of the proto-oncogene N-myc (Brodeur et al., 1984; Seeger et al., 1985) or the increased expression of the oncogene (Rosen et al., 1986) were found to be associated with progressive growth of the tumour.

That tumour karyotype may be important in the prognosis of human neuroblastoma was proposed by us after we obtained tumour cytogenetic data in a series of 14 children with neuroblastoma (Franke et al., 1986a). In an extended series of 28 patients with all the different tumour stages we now confirm the presence or absence of the chromosome 1 short arm abnormality as the most decisive factor characterizing the different biological behaviour of neuroblastoma cell clones and thus the clinical outcome.

Materials and methods

Patients were registered and prospectively treated within the consecutive Neuroblastoma Trials NB 79, NB 82, and NB 85 of the German Paediatric Oncology Group which allowed uniform treatment, documentation, pathological evaluation, and continuous control of all clinical parameters (Berthold et al., 1986).

Tumour tissue samples were collected at operation or sent in by express mail, and were taken either prior to or at least 2 months after chemotherapy. Samples were immediately transferred to short-term cultures for 3 h, and/or 2, 3 and at most up to 8 days. Methods pertaining to chromosome preparation from solid tumours have been described previously (Franke et al., 1986b). Chromosome preparations of bone marrow metastases were performed after cell synchronisation with methotrexate. Chromosomes were G-banded with 3% Giemsa solution (pH 6.8) after pre-treatment with trypsin. The best banded metaphases were photographed and karyotyped. In the majority of the patients more than 20 tumour metaphases could be analyzed.

DNA analysis of the N-myc gene from the same tumour cell material that was used for chromosome study could be performed in 10 patients. Isolated DNA was digested with EcoRI, electrophoresed, and transferred on to filter membranes (Du Pont). N-myc amplification was tested with the blotting method (Southern) using the probe NB-1 (Schwab et al., 1983) which detects 2.0 Kb EcoRI fragments. The extent of N-myc amplification was confirmed by dot-blot analyses via serial dilution of the tumour-DNA.

Results

Chromosomes could be successfully prepared and analyzed from neuroblastoma tissue in 28 patients out of a total of about 90 patients examined.

The clinical and the most important cytogenetic data such as the lp abnormality, DMs and HSRs, including N-myc copies in the tumour where DNA analysis was performed, are summarized in Table I. In Table II the complete tumour karyotypes, including the number of cells counted and analyzed are listed. In the bone marrow preparations diploid cells of normal haematopoiesis were also found and could be excluded. The tumour cell preparations showed cell populations of clonal origin. In only a few cases 1 to 3 sub-populations with minor cytogenetic variations were seen (Table II).

Of the 12 infants studied, 4 of the 6 with stage IV disease are dead; the others, including 3 with stage IV-S, 2 with stage II and 1 with stage I tumours, are all alive. All 4 dead infants had a chromosome lp abnormality in the tumour karyotype, and DMs/HSRs in 3. Of the 2 living infants, however, with stage IV none was without structural tumour chromosome aberrations. Patient Mo-K had a 6q-deletion

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but no 1p abnormality, but patient SC-H had a 1p translocation (Figure 1), and thus, prognosis in this case could be bad in regard to the short observation time. All the other infants are alive with no evidence of disease. None of them had a 1p abnormality (Figure 2) and/or DMs/HSRs in the tumour karyotype, but all had hyperdiploid modal chromosome numbers of 58, 70, (82-84), (55-60), (64-68), (56-58), (31-88), i.e. mainly in the triploid range.

In contrast, 13 of the 15 (87%), mainly older, patients with stage IV neuroblastoma who already died, had an 1p aberrant karyotype, and 9 (60%) of them showed DMs and/or HSRs. Modal chromosome numbers were in the diploid range in about half of the patients. In the last 4 patients of the table, initially (probably falsely?) diagnosed as stage III, tumour karyotyping was done later when metastases were prominent.

As to morphology of the tumour chromosomes we purposely selected for demonstration a complete and partial karyotype from hyperdiploid primary tumours of infancy (Figures 1 and 2) as these usually are more difficult to prepare and analyze as compared to pseudodiploid tumour cells from bone marrow metastases.

In the 3 cases where 30-60 copies of the N-myc gene were detected in the genome there were also DMs and/or HSRs in the tumour karyotype. Lack of 1p abnormality was also associated with lack of N-myc amplification except for one (already dead) patient (ME-HER) with stage IV and a derivated chromosome 1. DMs and HSRs never occurred simultaneously in the same metaphase cell but were encountered in different metaphases of the same cell population. HSRs were detected at different chromosomes and on different arms, e.g. at 4p, 6q, 9q, 11q, 15p, 19q, 20q. The only 2 non-surviving patients (JF-GÖ, RB-GI) with stage IV and no 1p abnormality nor DMs/HSRs or N-myc amplification did show, however, other structural aberrations and pseudodiploid tumour karyotype.

Overall, structural aberrations were found in the tumour karyotypes of 23 patients (18 with stage IV, 3 with stage III, 2 with stage II) – whereas in 5 patients (3 with stage IV-S, 1 with stage II, 1 with stage I) only numerical aberrations were present. As listed in Table II structural aberrations of the tumour karyotype mainly affected the short arm of chromosome 1. Other chromosomes not infrequently involved were nos. 6, 11, 12, 17 (long arm).

As to localization of the breakpoint in the abnormal 1p tumour chromosome, 12 patients could be precisely analyzed, and 1p32 was found to be the most frequently involved (Figure 3). The correlation of karyotypic pattern – taking the 1p abnormality as the most characteristic chromosome aberration – with survival could be demonstrated in 26 patients by life table analysis (Kaplan-Meier method, kindly carried out by Dr. P. Kaatjes, Mainz) (Figure 4). The two most recent patients SC-H, FO-ER with stage IV were excluded because of insufficient observation time. The clear-cut distinction between two groups of patients with neuroblastoma is obvious: Patients (n=11)

| Patient | Age at diagnosis (mo) | Sex | Survival (mo)* | Outcome | Stage | Tissue | Modal number (range) | Chromosome-aberrations | No. 1 dms | hsr | N-myc copies** |
|---------|------------------------|-----|----------------|---------|-------|--------|---------------------|------------------------|-----------|-----|---------------|
| JC-H    | 1                      | m   | 35             | alive, NED | I     | tu     | 58                  | 54-64                  | -         | -   | -             |
| RZ-GI   | 73                     | f    | 44             | alive, NED | II    | tu     | (39-78)             | -         | -         | -   | -             |
| TT-DO   | 11                     | m    | 53             | alive, NED | II    | tu     | 70                  | (45-71)              | -         | -   | -             |
| MJ-GI   | pp                     | m    | 10             | alive, NED | II    | tu     | (82-84)             | -         | -         | -   | -             |
| JS-DO   | 2                      | f    | 19             | alive, NED | IV    | tu     | (55-60)             | -         | -         | -   | -             |
| SB-S    | 1                      | f    | 33             | alive, NED | IV    | tu     | (64-68)             | -         | -         | -   | -             |
| JK-NE   | 3                      | m    | 51             | alive, NED | IV    | tu     | (56-58)             | -         | -         | -   | -             |
| MB-MR   | 54                     | m    | 75             | alive     | III   | tu     | 60                  | (54-71)              | -         | -   | -             |
| MB-H    | 17                     | f    | 3              | dead      | III   | tu     | 47                  | (46-64)              | +         | +   | 30            |
| FW-KS   | 43                     | m    | 29             | alive, NED | III   | tu     | (46-64)             | -         | -         | -   | -             |
| MO-K    | 9                      | f    | 25             | alive     | IV    | bm     | 31                  | (31-88)              | -         | -   | -             |
| JF-GÖ   | 35                     | f    | 8              | dead      | IV    | bm     | 46                  | (45-66)              | -         | -   | -             |
| RB-GI   | 45                     | m    | 23             | dead      | IV    | bm     | 46                  | (43-46)              | -         | -   | -             |
| ME-HER  | 23                     | m    | 5              | dead      | IV    | bm     | (30-83)             | -         | +         | -   | -             |
| MB-N    | 119                    | m    | 1              | dead      | IV    | bm     | 57                  | (43-62)              | +         | +   | -             |
| WW-BT   | 17                     | m    | 1              | dead      | IV    | bm     | (80-108)            | -         | -         | -   | -             |
| SS-HD   | 11                     | m    | 6              | dead      | IV    | bm     | 46                  | (26-46)              | -         | -   | -             |
| SC-H    | 10                     | m    | 3              | alive     | IV    | tu     | 73                  | (48-75)              | +         | +   | -             |
| FO-ER   | 15                     | m    | 7              | alive     | IV    | bm     | 46                  | (43-47)              | +         | +   | 30            |
| CR-GI   | 8                      | f    | 5              | dead      | IV    | tu     | 46                  | (44-47)              | +         | +   | -             |
| VS-GI   | 15                     | f    | 7              | dead      | IV    | bm     | 46                  | (44-48)              | +         | +   | -             |
| SB-GI   | 26                     | m    | 16             | dead      | IV    | tu     | 84                  | (82-90)              | +         | +   | -             |
| AG-GI   | 30                     | f    | 17             | dead      | IV    | bm     | (92-174)            | +         | +         | +   | -             |
| MG-N    | 9                      | f    | 16             | dead      | IV    | bm-rel| (46-95)            | +         | +         | +   | -             |
| MH-NOH  | 43                     | f    | 32             | dead      | III>IV| ln     | 61                  | (47-73)              | +         | +   | -             |
| SW-DO   | 35                     | f    | 13             | dead      | III>IV| bm     | 46                  | (42-47)              | +         | +   | 60            |
| MK-WÜ   | 63                     | f    | 16             | dead      | III>IV| tu     | (34-88)             | +         | -         | +   | -             |
| SJ-B    | 9                      | m    | 4              | dead      | III>IV| bm     | (63-84)             | +         | -         | +   | -             |

*Survival, as of 1 July 1987; **Single copy/haploid genome=1. nt: not tested; pp: post partum. NED: no evidence of disease, tu: tumour, bm: bone marrow, ln: lymphnode, rel: relapse.
Table II Tumour cell karyotypes in 28 patients with neuroblastoma

| Patient | Tissue | Number of metaphases counted | Number of metaphases analyzed | Modal chromosome number (range) | Ip aberrations | Ip aberrations: absolute percent |
|---------|--------|-----------------------------|-------------------------------|-------------------------------|---------------|--------------------------------|
| 1. JC-H  | tu     | 20                          | 9                             | 58 (54-64)                    | 0/9           | -                              |
| 2. RZ-GI | tu     | 61                          | 16                            | 39 (78)                       | 0/16          | t(7;21)(p22;p13), t(19;7)(p13.1;p7) |
| 3. TT-DU | tu     | 40                          | 15                            | 70 (45-71)                    | 0/15          | t(19;7)(p13.7)                  |
| 4. MJ-GI | tu     | 6                           | 3                             | 82 (84)                       | 0/3           | -                              |
| 5. JS-DO | tu     | 5                           | 3                             | 55 (60)                       | 0/3           | -                              |
| 6. SB-S  | tu     | 2                           | 2                             | 64 (88 )                      | 0/2           | -                              |
| 7. JK-NE | tu     | 3                           | 2                             | 56 (58)                       | 0/2           | -                              |
| 8. MB-MR | tu     | 37                          | 11                            | 60 (54-71)                    | 0/11          | del(1q), del(5p), t(6;7)(q25;p21), 2 mar |
| 9. MB-H  | tu     | 42                          | 8                             | 47 (46-47)                    | 2/8           | 25% hsr(9)(q22), t(12;17)(q14;p21), t(16;7)(p13.7) |
| 10. FW-KS| tu     | 3                           | 3                             | 46 (64)                      | 3/3           | 100% dupl(1q)(1q32), 2 mar      |
| 11. MO-K | bm     | 11                          | 11                            | 31 (88)                       | 0/11          | del(6)(q23)                     |
| 12. BF-GÖ | bm    | 27                          | 9                             | 46 (45-46)                    | 0/9           | del(11)(q13.21)                |
| 13. RB-GI | bm    | 54                          | 17                            | 46 (43-46)                    | 0/6           | del(11)(q13.21), i(12), t(13;7)(p11.7), 1 mar |
| 14. ME-HER | bm    | 15                          | 9                             | 30 (83)                       | 0/17          | del(3)(p21), del(11)(p11), 1 mar |
| 15. MB-N  | tu     | 84                          | 15                            | 57 (43-62)                    | 9/9           | 100% del(1q)(p32)               |
| 16. WW-BT | bm    | 21                          | 11                            | 80 (108)                      | 15/15         | 100% t(13;7)(q34), 4 mar        |
| 17. SS-HD | bm    | 32                          | 16                            | 46 (26-46)                    | 7/11          | 64%                            |
| 18. SC-H  | tu     | 7                           | 5                             | 73 (48-75)                    | 14/16         | 88% der(3q), der(9p), der(11q), der(12p), der(18q), 1 mar |
| 19. FO-ER | bm    | 17                          | 15                            | 46 (43-47)                    | 5/5           | 100% der(1p), 1 mar, dms        |
| 20. CR-GI | bm    | 178                         | 17                            | 46 (44-47)                    | 6/15          | 100% dms                        |
| 21. VS-GI | bm    | 60                          | 17                            | 46 (44-48)                    | 17/17         | 100% del(2)(p11), del(7p), dms  |
| 22. SB-GI | bm    | 477                         | 31                            | 84 (82-90)                    | 31/31         | 100% del(2)(p21), del(7p), dms  |
| 23. AG-GI | bm    | 70                          | 18                            | 101 (99-103)                  | 15/17         | 88% inv(2)(p3p23), i(7p), dms   |
| 24. MG-N  | bm-re | 43                          | 32                            | 92 (174)                      | 18/18         | 100% inv(5)(p15q11), del(13q2), dms |
| 25. MH-NOH| ln     | 80                          | 21                            | 61 (44-73)                    | 30/32         | 94% der(1p), 3 mar, dms         |
| 26. SW-DO | bm     | 43                          | 17                            | 46 (42-47)                    | 10/10         | 100% der(1p), dms               |
| 27. MK-WU | tu     | 28                          | 12                            | 34 (88)                      | 11/11         | 100% der(2p), der(4q), dms      |
| 28. SJ-B  | bm     | 22                          | 11                            | 63 (84)                       | 12/12         | 100% der(2p), der(4q), dms      |

Tu: tumour, bm: bone marrow, ln: lymph node, rel: relapse.

Discussion

We succeeded in determining the tumour karyotype of 7 children with localized neuroblastoma stages I, II, and stage IV-S, the special congenital form of disseminated neuroblastoma. These patients are alive and have a good prognosis. This case was characterized oncogenically by hyperdiploidy in the triploid range, lack of chromosome 1p abnormality and lack of cytogenetic phenomena of gene amplification such as DMs and/or HSRs (also confirmed by the absence of N-myc amplification). Our observations are in accordance with recent data of Kaneko et al. (1987) who found a near-triploid tumour lacking 1p abnormality and/or DMs, HSRs in 7 children with stage I or II who were alive with no evidence of disease, and also with data of 6 Japanese infants with stage I, II, III neuroblastoma found by mass screening for vanillylmandelic acid in the urine who only had hyperploid tumours with modal chromosome numbers ranging from 67 to 71 as their sole abnormality (Hayashi et al., 1986). By measuring the DNA content of the tumour cells in 35 infants with neuroblastoma (Look et al., 1984) it was also reported that hyperdiploidy was associated with a better response to chemotherapy compared to diploid tumours and that infants (n=4) with IV-S stage had hyperdiploid tumour cells of clonal origin. Another investigation by flow cytometric DNA analysis in 38 cases confirmed the favorable outcome in neuroblastoma patients having an aneuploid tumour stem-line (Gansler et al., 1996).

We would like to emphasize that karyotyping of the tumour cell clone by analysis of individual chromosomes has greater potential in discriminating two distinct prognostic groups in children with neuroblastoma as compared to cytophotometry on one hand, and N-myc determination on the other. In particular, this can be shown in poor prognosis patients, i.e. with stage IV disease: 85% (15 of 18) of our patients with tumour dissemination, being either over or
Figure 1  Complete karyotype of a hyperploid (mn=73) cell from the primary tumour of a 10 month old infant with neuroblastoma stage IV. Note the abnormal short arms of 2 chromosomes 1 with deletion and (unknown) translocation (breakpoint at 1p22), the presence of several DMs and one unidentified marker.

Figure 2  Partial karyotype showing 4 normal chromosomes 1 (a) taken from a hyperploid (mn=82) cell; (b) of the primary tumour of a newborn with neuroblastoma stage II.
under one year, expressed a chromosome 1p abnormality in the tumour karyotype, with a diploid karyotype in only half of the patients, 61% also had DMs and/or HSRs, but only 33% (2 of 6) of the few examined cases had N-myc amplification. In the 13 patients with stage IV examined by Kaneko et al. (1987) 9 (69%) had a 1p abnormality, and 8 (62%) also had DMs/HSRs. Brodeur et al. (1984) found N-myc amplification (more than 3 copies) in neuroblastoma tissue from 13 of 25 (52%) untreated patients with stage IV, and Seeger et al. (1985) in 19 of 40 (48%) patients with stage IV. As one could expect a close correlation between N-myc amplification and presence of DMs/HSRs in the karyotype, as has also proven in one of our patients (MG-N) by in-situ hybridization (Christiansen et al., 1987), multiple copies of N-myc would be present in about 60% of patients with stage IV. As a chromosome 1p abnormality is encountered more frequently in this stage of progressive tumour growth one might speculate that the aberration of chromosome 1 distal to band 1p22 (Brodeur et al., 1981; Gilbert et al., 1984) or 1p13 (our results) is the primary event, and gene amplification is secondary, being rarely associated with morphologic uniformity. As shown by our analysis, HSRs were distributed among several chromosomes and different arms, involving the short arm of chromosomes 4 and 15 and the long arms of chromosomes 6, 9, 11, 19 and 20, and, interestingly, DMs were found in two different size groups in a case of metastatic neuroblastoma (Franke, et al., 1985).

If one takes deletion of certain genes on the short arm of one chromosome 1 as the crucial primary event for aggressive growth in neuroblastomas, our life table analysis can clearly distinguish two groups of patients – regardless of stage and age – one with a less than 10% survival chance versus the other with a 90% survival probability. This is superior to N-myc oncogene determinations in that 55 patients with only a single copy of the gene in their tumour DNA had an estimated survival rate of less than 60% (Seeger et al., 1985).

Neuroblastoma cells with undisturbed homologues of chromosome 1 – probably a completely different entity – might just be temporarily deregulated or because of increased DNA content more vulnerable to chemotherapy.

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References

BERTHOLD, F., BRANDEIS, W.E. & LAMPERT, F. (1986). Neuroblastoma: Diagnostic advances and therapeutic results in 370 patients. Monogr. Paediat., 18, 206 (Karger, Basel).

BRODEUR, G.M., GREEN, A.A., HAYES, F.A., WILLIAMS, K.J., WILLIAMS, D.L. & TSIATIS, A.A. (1981). Cytogenetic features of human neuroblastomas and cell lines. Cancer Res., 41, 4678.

BRODEUR, G.M., SEEGER, R.C., SCHWAB, M., VARMUS, H.E. & BISHOP, J.M. (1984). Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science, 224, 1121.

CHRISTIANSEN, H., FRANKE, F., BARTRAM, C.R. & 5 others (1987). Evolution of tumor cytogenetic aberrations and N-myc oncogene amplification in a case of disseminated neuroblastoma. Cancer Genet. Cytogenet., 26, 235.

EVANS, A.E., D'ANGIO, G.J., PROPERT, K., ANDERSON, J. & HANN, H.L. (1987). Prognostic factors in neuroblastoma. Cancer, 59, 1853.

FRANKE, F., FÖRSTER, W., RUDOLPH, B. & LAMPERT, F. (1985). Metastatic neuroblastoma in an infant: Translocation (1;11), deletion (2) and double minute chromosomes. Eur. J. Pediatr., 143, 305.

FRANKE, F., RUDOLPH, B., CHRISTIANSEN, H., HARBOTT, J. & LAMPERT, F. (1986a). Tumour karyotype may be important in the prognosis of human neuroblastoma. J. Cancer Res. Clin. Oncol., 111, 266.

FRANKE, F., RUDOLPH, B. & LAMPERT, F. (1986b). Translocation (19;?) in two stage II neuroblastomas. Cancer Genet. Cytogenet., 20, 129.

GANSLER, T., CHATTEN, J., VARELLO, M., BUNIN, G.R. & ATKINSON, B. (1986). Flow cytometric DNA analysis of neuroblastoma. Correlation with histology and clinical outcome. Cancer, 58, 2453.

GILBERT, F., FEDER, M., BALABAN, G. & 6 others (1984). Human neuroblastomas and abnormalities of chromosomes 1 and 17. Cancer Res. 44, 5444.

HAYASHI, Y., HABU, Y., FUJI, Y., HANADA, R. & YAMAMOTO, K. (1986). Chromosome abnormalities in neuroblastomas found by VMA mass screening. Cancer Genet. Cytogenet., 22, 363.

KANEKO, Y., KANDA, N., MASEKI, N. & 5 others (1987). Different karyotypic patterns in early and advanced stage neuroblastomas. Cancer Res., 47, 311.

LOOK, A.T., HAYES, F.A., NITSCHKE, R., McWILLIAMS, N. & GREEN, A.A. (1984). Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. N. Engl. J. Med., 311, 231.

ROSEN, N., REYNOLDS, C.P., THIELE, C.J., BIEDLER, J.L. & ISRAEL, M.A. (1986). Increased N-myc expression following progressive growth of human neuroblastoma. Cancer Res., 46, 4139.

SCHWAB, M., ALITALO, K., KLEMPNAUER, K.-H. & 6 others (1983). Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature, 302, 245.

SEEGER, R.C., BRODEUR, G.M., SATHER, H. & 4 others (1985). Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N. Engl. J. Med., 313, 111.