

Short Communication

Microsatellite Instability Is Infrequent in Neuroblastoma¹

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Abstract

Neuroblastoma (NB) is a childhood cancer of the autonomic nervous system. The molecular pathology of NB is not yet well understood. Both amplification of the proto-oncogene *N-myc* and loss of heterozygosity of several chromosomal loci occur in NB, representing genetic instability. In this study, we examined another type of genetic instability, microsatellite instability. Five chromosomal loci known to exhibit this alteration in colon, gastric, and pancreatic cancers were used in a PCR-based assay to examine 30 matched normal and tumor DNAs, which included all stages of tumor progression. Among these 30, only 2 (7%) manifested microsatellite instability. There was no correlation between the occurrence of microsatellite instability and the amplification of the *N-myc* gene. These data show that microsatellite instability is infrequent in neuroblastoma tumors.

Introduction

NB³ is a relatively common solid tumor of the autonomic nervous system of children, affecting 1 in 7000 children before the age of 5 years (1). It is unusual in that, for unknown reasons, it has a high rate of spontaneous regression. The proto-oncogene *N-myc* is amplified in 22% of NB patients and is associated with metastasis and fatal outcome (2). Loss of heterozygosity has been observed at several chromosomal loci, although no genes have yet been identified at these loci (3-5). Recently we have shown allelic imbalance at the *APC* locus in 38% of NB tumor DNAs (6). These data show that genetic instability, manifested as amplification of the *N-myc* gene, loss of heterozygosity at several loci, and allelic imbalance, occurs in a relatively small proportion of neuroblastoma patients.

Microsatellites, which are short nucleotide sequence repeats occurring approximately 100,000 times throughout the human genome, tend to be polymorphic among different individuals but stable within one individual (7, 8). Instability in microsatellite DNA, manifested as differences in the number of

repeats between normal and tumor DNA from the same patient, has been observed at high frequencies in hereditary nonpolyposis colon cancer (79%), pancreatic cancer (67%), bladder cancer (41%), and gastric cancer (31-39%), among other tumor types (9-16). The frequency of microsatellite instability is not uniformly high in all types of cancer, however. It is exhibited in only 13-22% of sporadic colon, uterine, esophageal and ovarian cancers, and occurs in 0-4% of cancers of the breast, liver, lung, and testis (10, 12, 14, 15, 17). One cause of microsatellite instability appears to be mutation in mismatch repair genes. Human genes identified thus far include *hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2* (18-23). Because little is known about the molecular biology of NB, we examined 30 neuroblastomas and matched normal tissues for microsatellite instability.

Materials and Methods

Patient Samples. Purified DNA or tissue from 30 paired normal and tumor samples was obtained from the Pediatric Oncology Group neuroblastoma tissue bank. DNA was extracted by using standard methods (24). Matching data including patient age, site of primary tumor, stage, *N-myc* amplification, DNA index, survival, and follow-up time were available for each patient.

Analysis of Microsatellite Instability. Normal and tumor DNAs were PCR-amplified at microsatellite repeat loci *D2S123*, *D2S147*, *D2S119*, *D10S197*, and *D11S904*, which show relatively high instability rates in colorectal and gastric tumors (10, 13). We used multiplex PCR, in which more than one locus is amplified simultaneously in the same reaction tube. Loci *D2S123* and *D10S197* were coamplified, as were loci *D2S147*, *D2S119*, and *D11S904*. PCR conditions consisted of 33 cycles at 95°C × 50 s, 58°C × 90 s, and 72°C × 90 s. PCR was performed by using 0.2 μCi of [³²P]dCTP incorporated into a 10-μl reaction volume. After denaturation in 95% formamide, PCR products were electrophoresed on denaturing polyacrylamide gels and visualized by autoradiography. Instability was manifested as alteration in DNA fragment lengths in tumor relative to normal.

Results

Of 30 matched samples analyzed at each of the 5 loci, 2 (7%) exhibited microsatellite instability (Table 1). The DNA pattern of one of the positives, patient 747, is shown in Fig. 1. In the tumor DNA there was a novel band at locus *D2S123*, a shorter band containing a decreased number of repeats at *D2S147*, and a longer band suggesting an increased number of repeats at *D2S119* and *D11S904*. The normal and tumor DNAs were derived from the same patient, as evidenced by the absence of band changes at locus *D10S197*, which was amplified in the same tube as *D2S123* (see "Materials and Methods"). Furthermore, there were no differences between normal and tumor DNAs from this patient revealed by PCR primers from a region

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³ The abbreviation used is: NB, neuroblastoma.

Table 1 Microsatellite instability in NB: patients showing genetic instability

Patient	POG no. ^a	D2S2119	D2S123	D2S147	D10S197	D11S90	N-myc ^b
34	106003						200
254	109444				+		
326	110590						50
598	113528						3
678	114403						100
716	114598						75
747	114792	+	+	+		+	75

^a POG, Pediatric Oncology Group.

^b The copy number of the N-myc gene is given only if amplified.

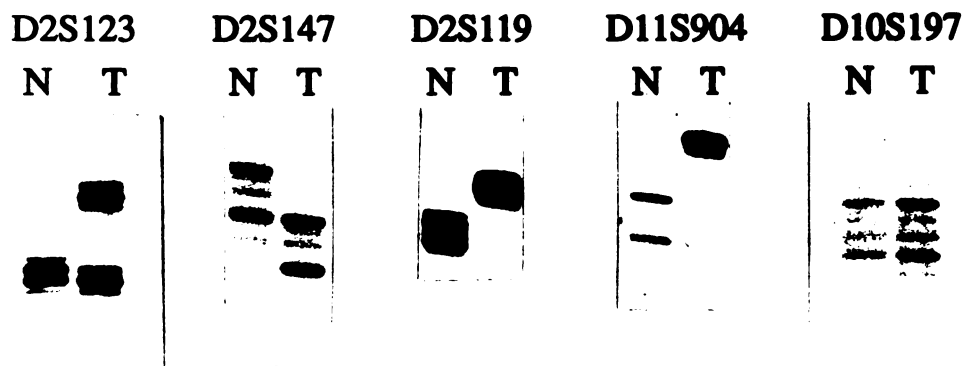


Fig. 1. Example of microsatellite instability in a neuroblastoma tumor. Genomic DNA from matched normal (N) and tumor (T) tissues were compared at the dinucleotide repeat markers indicated.

Table 2 Microsatellite instability in NB: clinical data of patients showing microsatellite instability

Patient	N-myc copy no.	DNA index	Age Dx ^a (month)	Clinical stage ^b	Site of primary ^c	Follow-up time (month)	Outcome
254	1	ND ^d	19	B	A/P/RP	51	Alive
747	75	1.0	14	D	Adrenal	25	Alive ^e

^a Age Dx, age at diagnosis.

^b Staging according to POG: B, incomplete gross resection of primary tumor with negative lymph nodes and liver; D, metastases beyond intracavity nodes.

^c A, abdomen; P, peritoneum; RP, retroperitoneum.

^d ND, not determined.

^e Alive but relapsed.

containing a variable number of tandem repeats within the *p53* gene.⁴

Patient 254 exhibited instability at locus *D10S197*, consisting of an abnormally long band suggesting a gain of repeats (data not shown).

Clinical and molecular data for the two patients manifesting microsatellite instability are displayed in Table 2. Among the 30 patients analyzed in this study, 6 (20%) showed amplification of the N-myc gene (Tables 1 and 2), which is representative of the frequencies reported in previous studies of NB patients (2). Patient 747, who exhibited microsatellite instability, showed amplified N-myc and a DNA index of 1.0, both indicators of a poor prognosis. This patient was stage D and is alive but has relapsed. In contrast, patient 254 exhibited microsatellite instability, but only a single copy of N-myc, was stage B, and the patient is alive after 51 months.

Discussion

We analyzed 30 matched normal/tumor DNAs at 5 loci shown previously to exhibit microsatellite instability (10) and detected two patients (7%) with altered numbers of repeats. This rate lies between the 0–4% prevalences reported for cancers of the lung, breast, liver, and testis and the 13–22% reported for sporadic colon, uterine, esophageal, and ovarian cancers (9, 11, 13, 14). These data suggest that microsatellite instability is an infrequent occurrence in neuroblastoma tumors.

The genetic instability often manifested by tumor cells can consist of DNA amplification, loss of heterozygosity, and aneuploidy (25), all of which are known to occur in NB. Recent studies show that microsatellite instability may constitute another type of genetic instability (9–17). The interrelationships among these various types of genetic instability are not yet known. Of the six NB tumors known to exhibit an amplified N-myc gene in this study, only one manifested microsatellite instability; conversely, only one of the two tumors exhibiting microsatellite instability showed N-myc amplification. These

⁴ J. Liu and P. Berg, unpublished observations.

preliminary data suggest there is no apparent correlation between microsatellite instability and *N-myc* amplification in these tumors.

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References

1. Woods, W. G., and Tuchman, M. The case for screening infants in North America. *Pediatrics*, 79: 869–873, 1987.
2. Seeger, R. C., Brodeur, G. M., Sather, H., Dalton, A., Siegel, S. E., Wong, K. Y., and Hammond, D. Association of multiple copies of the *N-myc* oncogene with rapid progression of neuroblastomas. *N. Engl. J. Med.*, 313: 1111–1116, 1985.
3. Fong, C.-T., White, P. S., Peterson, K., Sapienza, C., Cavenee, W. K., Kern, S. E., Vogelstein, B., Cantor, A. B., Look, A. T., and Brodeur, G. M. Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. *Cancer Res.*, 52: 1780–1785, 1992.
4. Fong, C.-T., Dracopoli, N. D., White, P. S., Merrill, P. T., Griffith, R. C., Housman, D. E., and Brodeur, G. M. Loss of heterozygosity for chromosome 1p in human neuroblastomas: correlation with *N-myc* amplification. *Proc. Natl. Acad. Sci. USA*, 86: 3753–3757, 1989.
5. Suzuki, T., Yokota, J., Hideo, M., Okabe, I., Ookuni, M., Sugimira, T., and Terada, M. Frequent loss of heterozygosity on chromosome 14q in neuroblastoma. *Cancer Res.*, 49: 1095–1098, 1989.
6. O'Doherty, S. P., Meltzer, S. J., Frantz, C. N., Yin, J., Cantor, A. B., Brodeur, G. M., and Berg, P. E. Loss of heterozygosity of the APC gene in neuroblastoma. *Proc. Am. Assoc. Cancer Res.*, 35: 186, 1994.
7. Weber, J. L., and May, P. E. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.*, 44: 388–396, 1989.
8. Jeffreys, A. M., Wilson, V., Neumann, R., and Keyte, J. Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. *Nucleic Acids Res.*, 16: 10953–10971, 1988.
9. Thibodeau, S. N., Bren, G., and Schaid, D. Microsatellite instability in cancer of the proximal colon. *Science (Washington DC)*, 260: 816–819, 1993.
10. Aaltonen, L. A., Peltomaki, P., Leach, F. S., Sistonen, P., Pylkkanen, L., M. J.-P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. *Science (Washington DC)*, 260: 812–816, 1993.
11. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature (Lond.)*, 363: 558–561, 1993.
12. Han, H.-J., Yanagisawa, A., Kato, Y., Park, J.-G., and Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.*, 53: 5087–5089, 1993.
13. Rhyu, M.-G., Park, W.-S., and Meltzer, S. J. Microsatellite instability occurs frequently in human gastric carcinoma. *Oncogene*, 9: 29–32, 1994.
14. Peltomaki, P., Lothe, R. A., Aaltonen, L. A., Pylkkanen, L., Nystrom-Lahti, M., Seruca, R., David, L., Holm, R., Ryberg, D., Haugen, A., Brogger, A., Borresen, A.-L., and de la Chapelle, A. Microsatellite instability is associated with tumors that characterize the hereditary nonpolyposis colorectal carcinoma syndrome. *Cancer Res.*, 53: 5853–5855, 1993.
15. Lothe, R. A., Peltomaki, P., Meling, G. I., Aaltonen, L. A., Nystrom-Lahti, M., Pylkkanen, L., Heimdal, K., Andersen, T. I., Moller, P., Rognum, T. O., Fossa, S. D., Haldorsen, T., Langmark, F., Brogger, A., de la Chapelle, A., and Borresen, A.-L. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res.*, 53: 5849–5852, 1993.
16. Orlow, I., Lianes, P., Lacombe, L., Dalbagni, G., Reuter, V. E., and Cordon-Cardo, C. Chromosome 9 allelic losses and microsatellite alterations in human bladder tumors. *Cancer Res.*, 54: 2848–2851, 1994.
17. Meltzer, S. J., Yin, J., Manin, B., Rhyu, M.-G., Cottrell, J., Hudson, E., Redd, J. L., Krasna, M. F., Abraham, J. M., and Reid, B. J. Microsatellite instability occurs frequently and in both diploid and aneuploid cell populations of Barrett's-associated esophageal adenocarcinomas. *Cancer Res.*, 54: 3379–3382, 1994.
18. Peltomaki, P., Aaltonen, L. A., Sistonen, P., Pylkkanen, L., Mecklin, J.-P., Jarvinen, H., Green, J. S., Jass, J. R., Weber, J. L., Leach, F. S., Petersen, G. M., Hamilton, S. R., de la Chapelle, A., and Vogelstein, B. Genetic mapping of a locus predisposing to human colorectal cancer. *Science (Washington DC)*, 260: 810–812, 1993.
19. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell*, 75: 1027–1038, 1993.
20. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L. A., Nystrom-Lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K. M., Fourmier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J.-P., Jarvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell*, 75: 1215–1225, 1993.
21. Papadopoulos, N., Nicolaides, N. C., Wei, Y.-F., Ruben, S. M., Carter, K. C., Rosen, C. R., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Petersen, G. M., Watson, P., Lynch, H. T., Peltomaki, P., Mecklin, J.-P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutation of a mutL homolog in hereditary colon cancer. *Science (Washington DC)*, 263: 1625–1629, 1994.
22. Nicolaides, N. C., Papadopoulos, N., Liu, B., Wei, Y.-F., Carter, K. C., Ruben, S. M., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Dunlop, M. G., Hamilton, S. R., Petersen, G. M., de la Chapelle, A., Vogelstein, B., and Kinzler, K. W. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature (Lond.)*, 371: 75–80, 1994.
23. Liu, B., Parsons, R. E., Hamilton, S. R., Petersen, G. M., Lynch, H. T., Watson, P., Markowitz, S., Willson, J. K. V., Freen, J., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res.*, 54: 4590–4594, 1994.
24. Goelz, S. E., Hamilton, S. R., and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem. Biophys. Res. Commun.*, 130: 118–126, 1985.
25. Tlsty, T. D. Normal diploid human and rodent cells lack a detectable frequency of gene amplification. *Proc. Natl. Acad. Sci. USA*, 87: 3132–3136, 1990.