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AMPLIFIED ALLELE OF THE HUMAN N-*myc* ONCOGENE IN NEUROBLASTOMAS

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The N-*myc* amplification unit was investigated using two restriction fragment length polymorphisms for *Sph*I and *Pvu*II which we previously found to occur in the 2nd intron and in the 3′ region of the human N-*myc* oncogene, respectively. All the three haplotypes which are dominant in the Japanese population were observed in the amplified DNA after analyses of a total of 22 DNA samples of fresh neuroblastomas with N-*myc* amplification. We conclude that only one of the alleles was amplified and that either allele could be amplified with respect to both the *Sph*I and *Pvu*II polymorphisms.

Key words: N-*myc* oncogene — Gene amplification — Neuroblastoma — Allele

Restriction fragment length polymorphism (RFLP) is a reflection of some changes in nucleotide sequences and is a powerful marker to detect genetic differences between individuals as well as heterogeneity in the population.[1] In the hope of detecting possible genetic factors involved in cancer susceptibility, RFLPs in or near cellular oncogenes have been extensively studied. Several minor alleles of the human Ha-*ras* oncogene have been found only in tumor patients, although contradictory results have been reported.[2–4] It has been suggested that genotypes of L-*myc* are correlated with lung cancer metastases.[5] The human N-*myc* oncogene is amplified in some embryonal tumors.[6–10] It is expressed in tumors carrying amplified N-*myc* as well as in Wilms' tumors, which usually have a single copy of the gene. It is not expressed in normal tissue except during early development.[11] N-*myc* gene amplification is associated with progressive stages and poorer prognoses in neuroblastoma patients.[12, 13] We recently reported two RFLPs in the human N-*myc* gene.[14] One of the RFLPs was caused by the presence (allele S1) or absence (S2) of an *Sph*I site in the second intron, and the other was caused by the presence (P1) or absence (P2) of a *Pvu*II site in the 3′ region of the gene. Analysis of a limited number of DNA samples isolated from neuroblastomas (including one rhabdomyosarcoma) with N-*myc* amplification showed that the amplified allele was random with respect to the *Pvu*II polymorphism and that the S2 allele was amplified in all the samples examined. We pursued this study to see if there is an association of a specific allele with gene amplification.

The procedures used for DNA isolation and hybridization analyses were described previously.[14] The extent of amplification was estimated by the Southern hybridization of *Eco*RI digests with a 2.0 kb *Eco*RI-*Eco*RI fragment of pMY 816 (probe A in ref. 14). To determine the haplotype of the amplified DNA, the amount of each DNA sample to be used was reduced in a reciprocal manner to the extent of amplification. Probes used to detect the *Sph*I and *Pvu*II polymorphisms were a 2.0 kb *Eco*RI-*Eco*RI fragment of pMY 816 (probe A in ref. 14) and a 2.3 kb *Bgl*II-*Eco*RI fragment of pMY820 (probe B in ref. 14), respectively.

In our previous study,[14] we found 5 neuroblastomas carrying the amplified N-*myc* gene after screening 52 fresh neuroblastomas. We have since collected an additional 39 fresh neuroblastomas with the help of collaborators

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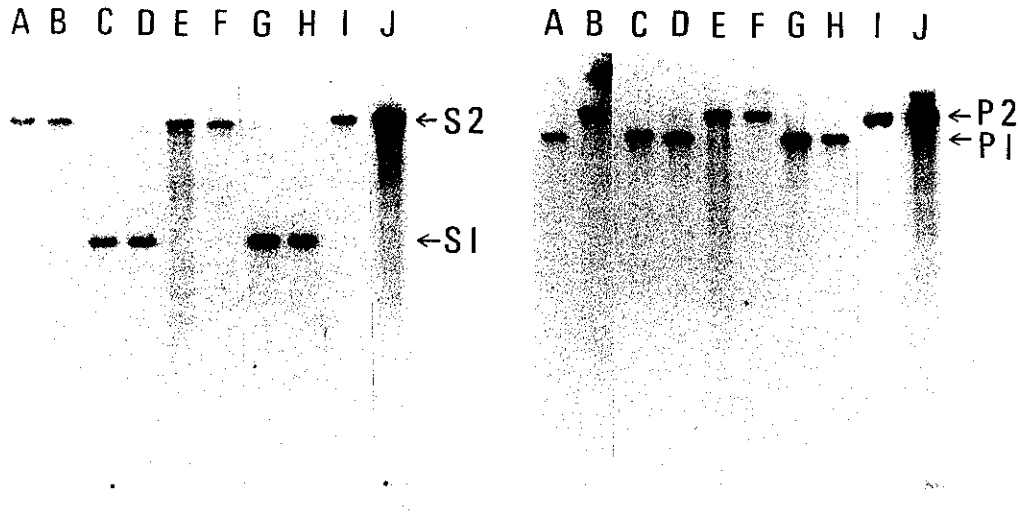

Fig. 1. Detection of the amplified alleles of N-*myc* in neuroblastomas. DNA was digested with *Sph*I (left) and *Pvu*II (right) and analyzed by Southern hybridization with N-*myc* probes. Since the amount of each DNA sample to be used was reduced in a reciprocal manner to the extent of amplification, only the amplified allele was detected in these autoradiograms. Lanes: A=UOEH 1; B=UOEH 5; C=UOEH 26; D=UOEH 28; E=UOEH 32; F=UOEH 33; G=UOEH 35; H=UOEH 36; I=221 and J=UOEH 34.

in hospitals and found that 5 of them carried the amplified N-*myc* gene. Additional DNA samples of neuroblastomas with the N-*myc* amplification were chosen from a collection of neuroblastoma DNA samples described previously,[15] and are indicated in this report with the prefix UOEH followed by the same number used in that report. A total of 22 neuroblastomas including the five previously reported were analyzed for the allele in the amplified DNA. Some of the results obtained by Southern hybridization analysis are shown in Fig. 1. The amplified haplotype is summarized in Table I with the stages and extent of amplification. Amplification of the P1 allele was found in 13 cases and that of the P2 allele in 9 cases. These results are in accord with the previous findings in which amplification of N-*myc* was random with respect to the P allele. However, in contrast to the previous findings, the S1 allele was observed in 8 cases of the amplified DNA, and the S2 allele in 14 cases. Thus, N-*myc* amplification was not associated with the S2 allele. Due to an extreme linkage disequilibrium between the two RFLP loci, the S1P2 haplotype was hardly observed in the Japanese population, as previously reported.[14] Therefore, all the three haplotypes which are dominant in the Japanese population were observed as amplified DNA; S1P1 in 8 cases, S2P1 in 5 cases and S2P2 in 9 cases. Amplification of the S2P1 haplotype was frequently observed in neuroblastomas which were collected from the Tokyo area, while that of the S1P1 haplotype was frequently observed in the UOEH collection that was derived from the Kyushu area. We have not yet confirmed, however, whether the N-*myc* haplotype is distributed unevenly in different areas of Japan.

Gene amplification was studied in tumor cells as well as in relation to drug resistance.[16] It has been reported that two polymorphic genes for dihydrofolate reductase in Chinese hamster cells can be amplified with approximately equal probability.[17] Both alleles of the L-*myc* oncogene, 6.6 kb and 10 kb fragments by *Eco*RI digestion, have been observed in the amplified DNA of some small cell lung carcinomas.[18] Our results on N-*myc* amplification are in accord with those results. According to our knowledge of all these systems, either

Table I. Amplified Alleles of the N-*myc* Gene in Neuroblastomas

DNA samples	Stage	Extent of amplification	Amplified haplotype
50[a]	Not known	30	S2P2
87[a]	IV	50	S2P1
173[a]	III	100	S2P1
221[a]	Not known	10	S2P2
712[a]	IV	15	S2P1
802	IV	100	S2P1
846	IV	30	S2P2
861	III	100–200	S2P2
863	IV	10	S1P1
876	IV	100	S2P2
UOEH 1[b]	IV	92	S2P1
UOEH 5	IV	112	S2P2
UOEH 8	IV	130	S1P1
UOEH 18	IVs	32	S1P1
UOEH 19	III	37	S1P1
UOEH 26	IV	36	S1P1
UOEH 28	IV	20	S1P1
UOEH 32	IV	37	S2P2
UOEH 33	II	71	S2P2
UOEH 34	III	113	S2P2
UOEH 35	IV	84	S1P1
UOEH 36	IV	113	S1P1

a) Results were reported in ref. 14.
b) The stages and extent of amplification for the DNA samples beginning with UOEH have been reported in ref. 15.

allele can be amplified. However, no case has been reported in which both alleles were amplified in a single cell. To elucidate the molecular mechanism of gene amplification, RFLP studies of oncogenes are useful since oncogenes are frequently amplified in cancer.

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