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Association of Minisatellite Instability with c-*myc* Amplification and K-*ras* Mutation in Methylcholanthrene-induced Mouse Sarcomas[1]

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ABSTRACT

Instability of microsatellite sequences are frequently found in human tumors. In addition, minisatellite sequences, another group of highly unstable sequences, serve as sensitive markers of genetic instability. We have studied minisatellite instability in methylcholanthrene-induced mouse sarcomas. These sarcomas frequently carry the amplified c-*myc* gene. Seven sarcomas without the amplification and seven others with the amplification were selected randomly. Regardless of the state of the c-*myc* gene amplification, these sarcomas exhibited a varying degree of transplantability in syngeneic mice. The hypervariable mouse minisatellite locus *Ms6hm* was found to be highly unstable, specifically among sarcomas with the amplified c-*myc* gene. However, chromosome instability, as analyzed by micronucleus assay, was observed similarly for two groups of sarcomas. In addition, transversion of G to C and A to T was detected at the K-*ras* gene in four of the seven sarcomas with the amplified c-*myc* gene, and these mutations are thought to be induced directly by methylcholanthrene. Thus, concomitant occurrence was observed for three seemingly unrelated mutations, amplification of the c-*myc* locus, point mutation of the K-*ras* gene, and instability at the hypervariable mouse minisatellite locus. The present study indicates a possible involvement of K-*ras* mutation and c-*myc* amplification in induction of genetic instability in methylcholanthrene-induced mouse sarcomas.

INTRODUCTION

Phenotypic instability of tumor cells is thought to play an important role in the development and progression of cancer (1). Phenotypic instability is brought about by two mechanisms: epigenetic instability and genetic instability. A variety of cellular processes is involved in keeping the stability of the genome at the chromosome level as well as at the DNA sequence level. Disruption of any of these steps leads to destabilization of the cellular genome. Genetic instability at the chromosome level leads to aneuploidy and chromosome aberrations in cancer cells (2, 3). Instability also manifests at the DNA sequence level (4). Mutator phenotype as shown by unstable microsatellite sequences was recently found to be associated with the hereditary nonpolyposis colorectal cancer syndrome (5–7). Mismatch repair genes of *MSH2, MLH1, PMS1*, and *PMS2* were subsequently cloned and shown to be involved in microsatellite instability and development of the disease (8–12). These gene products form a complex which binds to heteroduplex sites and initiates correction of the sequence (13).

Instability of microsatellite sequences has also been found in a variety of human neoplasia other than colorectal cancer (14, 15). Microsatellite instability was shown to be associated with elevated mutation frequencies at selectable loci in colorectal cells (16). However, we still do not have knowledge on the target gene whose mutation by malfunctioning mismatch repair genes leads to development and progression of cancer.

We have demonstrated previously that a MCA[3]-induced mouse sarcoma line was phenotypically unstable and underwent clonal evolution *in vitro* and *in vivo* (17, 18). MCA-induced mouse sarcomas were shown to exhibit genetic instability as revealed by high frequencies of mutation at a hypervariable mouse minisatellite locus (19). It was also shown that as many as 35% of MCA-induced primary mouse sarcomas exhibited amplification of the c-*myc* gene and concomitant loss of the normal allele (20).

In this communication, we have analyzed these sarcomas in detail and report here that amplification of the c-*myc* oncogene was accompanied by the instability of a mouse minisatellite locus. In addition, sarcomas with the amplified c-*myc* gene frequently carried mutation at the K-*ras* gene. To our knowledge, this is the first study to demonstrate an association of minisatellite instability and mutations of oncogenes.

MATERIALS AND METHODS

Sarcomas and Cloning of Cells. Sarcomas analyzed in this study were described previously (20). Briefly, (C57BL/6N × C3H/He) F_1 mice were given s.c. injections of 0.5–1 mg MCA dissolved in olive oil. When tumors had grown to 1 cm in diameter, they were excised and examined histologically. A portion of the tumor was minced with scissors and transferred into a 3-cm culture dish. The cells were grown for 10 days and then stored for further analysis.

Sarcomas were tested for their transplantability in syngeneic mice. Varying numbers of cells were injected s.c. on the backs of mice, and mice were examined at 1.5 months after transplantation. Mice that did not show tumor growth were kept for 3 months, and observation was terminated thereafter. Transplantability was also tested in BALB/c *nu/nu* mice by injection of 1×10^6 cells/site.

Subclones of sarcomas were isolated for the analysis of the frequency of mutation at the *Ms6hm* locus. Briefly, sarcomas were first single cloned, and the clones were grown in 3-cm dishes for about 10 days. The cells were then seeded at low density for subcloning. DNA was extracted when subclones grew to confluency in 6-cm dishes.

Micronucleus Assay. The micronuclei assay was carried out according to the procedure described elsewhere, with slight modifications (21). Briefly, sarcoma cells in culture were incubated for 24 h in the presence of 3 μg/ml cytochalasin B (Sigma Chemical Co., St. Louis, MO). Cultures were then rinsed with PBS and fixed in Carnoy's solution (methanol, 3 volumes; glacial acetic acid, 1 volume). The cells were stained with 0.05 μg/ml Hoechst 33268 in PBS for 30 min and washed with fresh PBS. The number of micronuclei per cell was scored under microscope in 1000 binucleated cells.

Oligonucleotides and the Minisatellite Probe. Oligonucleotide primers used for PCR-SSCP analysis and cycle sequencing of *ras* gene mutations were designed according to the published sequences (22), and they are as follows: H-*ras*12S (ACAGAATACAAGCTTGTGGTGGTG) and H-*ras*12AS (CTCTATAGTGGGATCATACTCGTC) for the H-*ras* codon 12 region; H-*ras*61S (GACTCCTACCGGAAACAGGTGGTC) and H-*ras*61AS (TATGGCAAATA-

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[3] The abbreviations used are: MCA, methylcholanthrene; SSCP, single-strand conformation polymorphism.

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Table 1 *Characterization of MCA-induced mouse sarcomas*

		Tumor take											
		c-*myc*		Syngenic mice					Nude mouse	Micronuclei/cell			
Sarcoma	Histology	Amplif.[a]	LOH	10^4	10^5	10^6	10^7	meta.	10^6	1	2	3	4
BC7210-1	ne	–	–	–	–	–	–	–	–	0	0	0	0
BC7423-5	MFH	–	–	–	–	–	–	–	–	3	0	0	0
BC7412-1	MFH	–	–	–	–	–	+	–	nt	8	0	0	0
BC7353-1	ne	–	–	–	–	+	+	–	nt	1	0	0	0
BC7353-3	ne	–	–	–	–	+	+	–	nt	1	0	0	0
BC7421-2	MFH	–	–	–	+	+	+	–	nt	0	0	0	0
BC7274-1	ne	–	–	+	+	+	+	–	nt	13	1	1	0
				1/7	2/7	4/7	5/7	0/7					
BC7413-1	MFH	+	+	–	–	–	–	–	+	0	0	0	0
BC7274-2	FS	+	–	–	–	–	–	–	+	1	0	0	0
BC7200-2	ne	+	+	–	+	+	+	–	nt	6	0	0	0
BC7423-3	MFH	+	+	–	+	+	+	+	nt	0	0	0	0
BC7415-4	ne	+	+	–	+	+	+	–	nt	0	0	0	0
BC7273	ne	+	+	+	+	+	+	–	nt	3	0	0	0
BC7421-3	MFH	+	+	+	+	+	+	–	nt	4	1	0	0
				2/7	5/7	5/7	5/7	1/7					

[a] Amplif., amplification; LOH, loss of heterozygosity; ne, not examined; nt, not tested; MFH, malignant fibrous histiocytoma; FS, fibrosarcoma.

CACAAAGAAAGCC) for the H-*ras* codon 61 region; K-*ras*12S (TATAAACT-TGTGGTGGTTGGAGCT) and K-*ras*12AS (GTACTCATCCACAAAGTGAT-TCTG) for the K-*ras* codon 12 region; K-*ras*61S (GACTCCTACAGG-AAACAAGTAGTA) and K-*ras*61AS (TATGGCAAATACACAAAGAAA-GCC) for the K-*ras*61 region; N*ras*12S (ACTGAGTAC-AAACTGGTGG-TGGTTGGAGCA) and N*ras*12AS (ATCATATTCAT-CCACAAAGTGGT-TCTGG) for the N-*ras* codon 12 region; and N-*ras*61S (GATTCTTAC-CGAAAGCAAGTGGTG) and N*ras*61AS (ATTGATGGC-AAAACACAGA-GGAA) for the N*ras* codon 61 region.

A locus-specific probe of Pc-1 was used for Southern blot analysis of the hypervariable minisatellite *Ms6hm* locus (23).

Analysis of DNA. DNA was extracted from sarcoma cells in culture using the procedure described previously (20). DNA from normal mouse spleen was used as a control.

Mutation in *ras* genes was studied using PCR-SSCP analysis according to the standard procedure (24). Briefly, PCR reaction was carried out in 10 μl mixture containing 20 pmol ^{32}P-labeled primers, 10 ng DNA, 50 mM KCl, 25 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.001% gelatin, 0.2 mM of each deoxynucleotide, and 0.5 units Taq DNA polymerase. DNA was amplified through 30 cycles at 94°C for 30 s, 55°C for 30 s, and 74°C for 60 s. The reaction products were denatured in a formaldehyde buffer, electrophoresed in 6% polyacrylamide gel with 10% glycerol, and autoradiographed on X-ray film. Mutant bands were excised, and their sequences were determined using the Takara Cycle Sequencing kit under the condition recommended by the manufacturer (Takara Shuzo, Kyoto, Japan).

Length change mutations of the mouse *Ms6hm* locus were analyzed by Southern blotting using the Pc-1 probe. Briefly, 5 μg DNA were digested with *Hae*III or *Hin*fI and electrophoresed in 1.2% agarose gel. After transfer, the filter was prehybridized and then hybridized in a buffer containing the ^{32}P-labeled Pc-1 probe, 4 × SSC, and 1% SDS at 65°C overnight. Filters were washed in 0.01 × SSC, 0.5% SDS at 65°C for 30 min, and autoradiographed on X-ray film.

The χ^2 test was used for the statistical analysis of the data.

RESULTS

Biological Properties of MCA-induced Sarcomas. We have previously analyzed 48 mouse primary sarcomas induced by MCA and found that 17 of them carried the amplified c-*myc* gene. The amplification was frequently accompanied by the loss of the normal c-*myc* allele (20). Seven sarcomas used for the present study were selected randomly from each of the two groups, and they are listed in Table 1.

Transplantability of these sarcomas was examined by injecting varying numbers of cells into syngeneic mice. Table 1 demonstrates that sarcomas with the amplified c-*myc* gene had a tendency to form tumors with less cells than those without amplification. However, this was not absolute, and BC7274-1 without the amplification was transplantable at as low as 1×10^4 cells. In contrast, two sarcomas of each group were not transplantable even at 1×10^7 cells. Among them, primary tumors of BC7423-5 and BC7413-1 were histologically typed as malignant fibrous histiocytoma and BC7274-2 as fibrosarcoma. BC7210-1 and BC7423-5 with the normal c-*myc* gene were not transplantable even in nude mice when 1×10^6 cells were injected s.c. In contrast, BC7413-1 and BC7274-2 carrying the amplified c-*myc* gene formed tumors in nude mice. Three lung metastatic nodules were found in a mouse transplanted with 1×10^5 cells of BC7423-3. These results suggest that amplification of the c-*myc* gene contributed, but not sufficiently for higher malignancy.

Micronucleus Assay. Chromosome instability is frequently associated with malignancy of cancers. Here, we have studied chromosome instability using micronucleus assay. Micronuclei were scored among 1000 binucleated cells, and the results of the analysis are summarized in Table 1. Among sarcomas without c-*myc* amplification, BC7210-1 was not transplantable to syngeneic mice, and no micronucleus was scored among 1000 cells of this sarcoma. BC7421-2, which was transplantable at 1×10^4 cells, was also devoid of micronucleus. Similarly, among sarcomas with c-*myc* amplification, micronucleus was not found in BC7413-1, BC7423-3, and BC7415-4. These three sarcomas exhibited a varying degree of transplantability in syngeneic mice. In contrast, BC7423-5 and BC7274-2 were not transplantable to syngeneic mice but had micronuclei. These results indicate that the number of micronuclei per cell does not correlate with the state of c-*myc* amplification nor does it correlate with the malignancy of MCA-induced mouse sarcomas.

Analysis of K-*ras* Mutation. Sarcomas were analyzed for mutation of *ras* genes using the PCR-SSCP method. No mutation was found at codon 12 and codon 61 of H-*ras* and N-*ras* genes among 14 sarcomas. Mutations of the K-*ras* gene were detected in four of the seven sarcomas carrying the amplified c-*myc* gene (Table 2). Sequence analysis revealed that mutation of BC7413-1 and BC7415-4 was transversion of G to C, leading to a change of glycine to arginine at codon 13. Similarly, mutation of BC7423-3 was G to C transversion, leading to glycine to arginine change at codon 12. Mutation found in BC7421-3 was A to T transversion, with a gluthamine to leucine change at codon 61. None of the sarcomas with the normal c-*myc* gene were found to carry the mutation of the K-*ras* gene. The association of K-*ras* gene mutation with amplification of the c-*myc*

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Table 2 *ras gene mutation and minisatellite mutation in MCA-induced mouse sarcomas*

Sarcoma	Presence of mutation			Type of mutation		*Ms6hm* locus mutation
	N-*ras*	H-*ras*	K-*ras*	codon	Nucleotide change	(mutant/total)
BC7210-1	–	–	–			0/40
BC7423-5	–	–	–			1/40
BC7412-1	–	–	–			0/40
BC7353-1	–	–	–			0/40
BC7353-3	–	–	–			0/40
BC7421-2	–	–	–			0/40
BC7274-1	–	–	–			0/40
BC7413-1	–	–	+	13	G to C	6/40
BC7274-2	–	–	–			2/40
BC7200-2	–	–	–			1/40
BC7423-3	–	–	+	12	G to C	6/40
BC7415-4	–	–	+	13	G to C	6/40
BC7273	–	–	–			4/40
BC7421-3	–	–	+	61	A to T	5/40

gene was statistically significant as analyzed by the χ^2 test ($P = 0.018$).

Southern Blot Analysis of the *Ms6hm* Minisatellite Locus. Genetic instability of sarcomas at the DNA sequence level was studied at the mouse hypervariable *Ms6hm* locus. This locus consists of a stretch of a GGGCA repeat and has been mapped at mouse chromosome 4 (25). Fig. 1 shows the results of Southern blotting of *Hae*III-digested DNA from 14 sarcomas used in the present study. The locus-specific probe of Pc-1 detected two *Ms6hm* alleles in DNA from B6C3F$_1$ mice, the 8-kb band of the *C57BL* allele, and the 3-kb band of the *C3H* allele.

It is our routine to make analysis of the minisatellite sequence using several four cutter restriction enzymes (26). Digestion with *Hin*fI instead of *Hae*III gave almost identical results, indicating that differences in the band length among sarcomas were not due to mutations at the restriction sites, but rather due to changes in the number of repeats in the alleles (data not shown).

Length change mutation of the *Ms6hm* alleles yielded a variety of band patterns among 14 sarcomas. BC7210-1, BC7421-2, and BC7274-1 carried a single band of about 3 kb, indicating a possible loss of the *C57BL* allele. BC7200-2 and BC7421-3 carried three bands, indicating either trisomy of chromosome 4 or existence of subpopulations of the cells, each carrying different length change mutation at one of the parental alleles.

Analysis of Subclones. Each sarcoma was single cloned and expanded to around 1×10^5 cells. The cells were then seeded at low density, and 40 subclones were obtained subsequently. Examples of Southern blot analysis of subclones are shown in Fig. 2. As for sarcomas with the normal c-*myc* allele, BC7353-3 carried two *Ms6hm* bands corresponding to the *C57BL* and *C3H* alleles, and the same band pattern was shared by all 40 subclones. Similar analyses of sarcomas with the amplified c-*myc* gene revealed that the *Ms6hm* locus was highly unstable, and examples of such analyses are shown for BC7412-1, BC7423-2, and BC7421-3 (Fig. 2). Subclones with new bands were detected frequently among sarcomas with c-*myc* amplification. The length change mutations detected using Southern blot analysis of subclones were rather large and spanned a range from a few hundred to a few thousand bases.

Table 2 summarizes the analysis of 14 sarcomas. Among sarcomas with the normal c-*myc* gene, only one mutant was identified during single-step subcloning of BC7423-5. No mutant subclone was identified in the other six sarcomas in this group. Even for highly malignant BC7274-1 sarcoma, no mutation occurred at the *Ms6hm* locus during subcloning. In contrast, instability of the *Ms6hm* locus was evident for sarcomas with c-*myc* amplification. BC7413-1, which was not transplantable to syngeneic hosts, exhibited the instability phenotype at the *Ms6hm* locus, and five mutants were identified among 40 subclones. BC7423-3 with moderate malignancy and BC7421-3 with

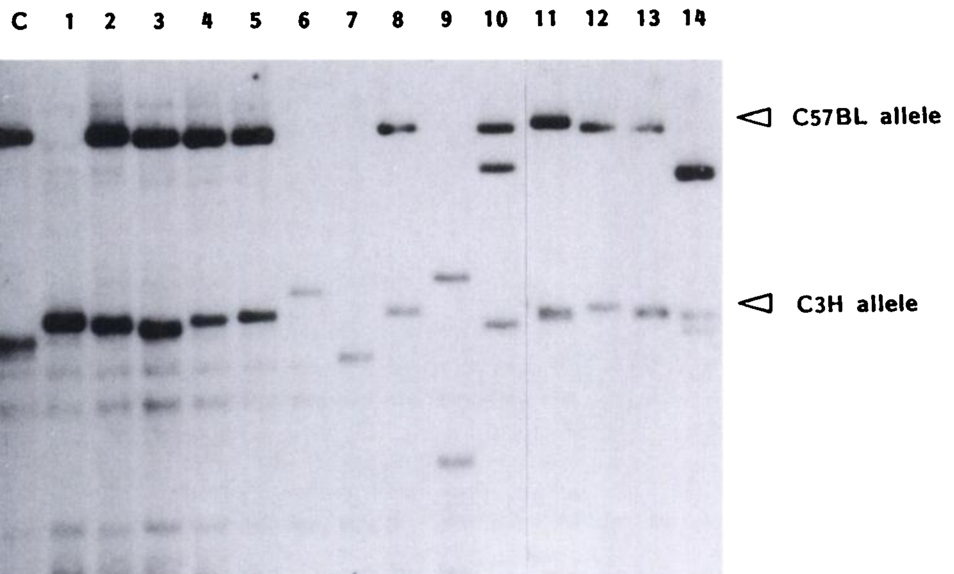

Fig. 1. Southern blot analysis of the *Ms6hm* locus was performed on DNA from 14 sarcomas. Briefly, 5 μg DNA were digested with *Hae*III and analyzed. The order of samples is the same as that listed in Tables 1 and 2. DNA from normal spleen of B6C3F$_1$ Southern blot analysis was used as a control *(lane C)*. *Lanes 1–14* are BC7210-1, BC7423-5, BC7412-1, BC7353-1, BC7353-3, BC7421-2, BC7274-1, BC7413-1, BC7274-2, BC7200-2, BC7423-3, BC7415-4, BC7273, and BC7421-3, respectively.

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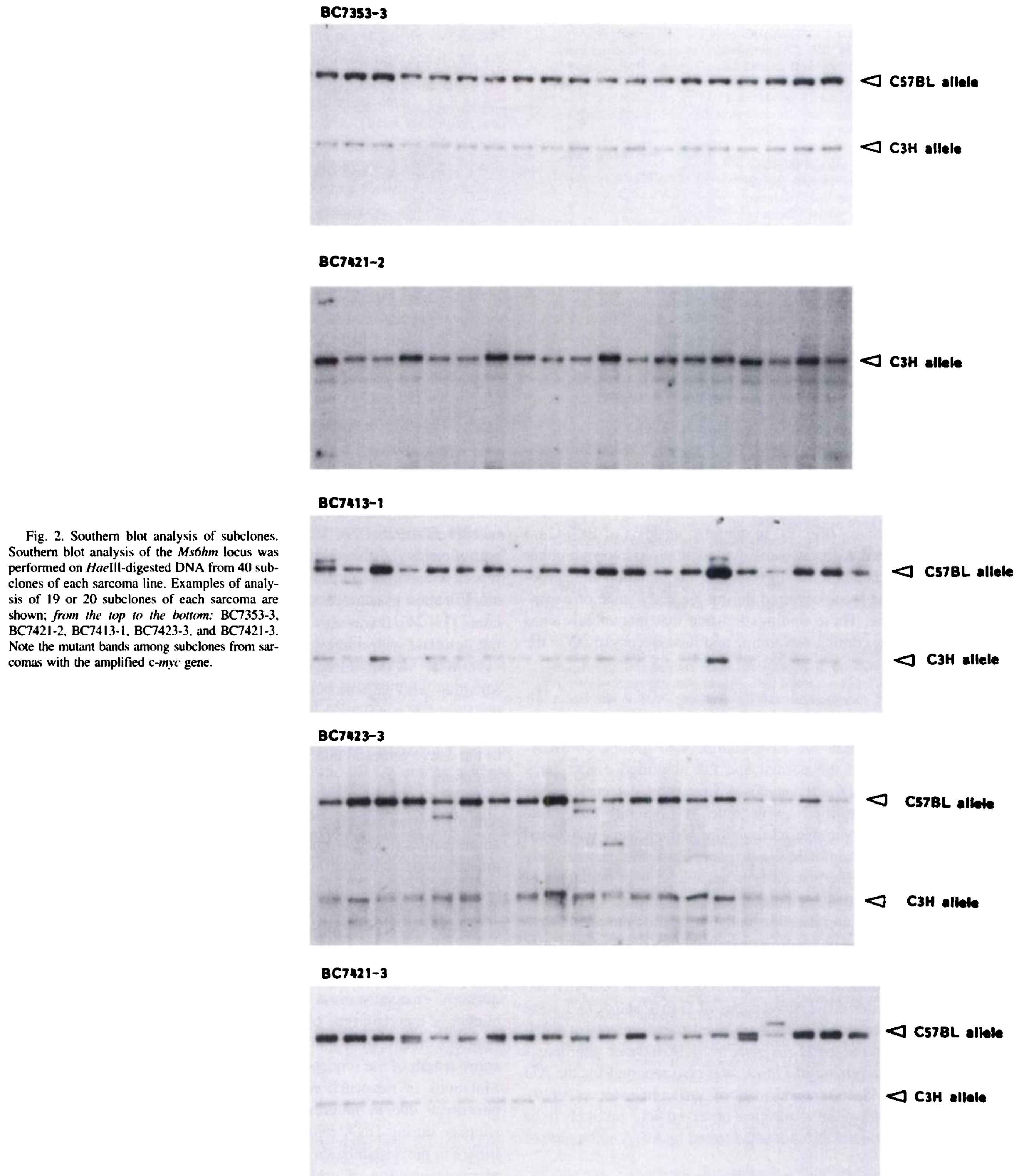

Fig. 2. Southern blot analysis of subclones. Southern blot analysis of the *Ms6hm* locus was performed on *Hae*III-digested DNA from 40 subclones of each sarcoma line. Examples of analysis of 19 or 20 subclones of each sarcoma are shown; *from the top to the bottom:* BC7353-3, BC7421-2, BC7413-1, BC7423-3, and BC7421-3. Note the mutant bands among subclones from sarcomas with the amplified c-*myc* gene.

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high malignancy exhibited similar degrees of instability of the *Ms6hm* locus. Thus, instability of the *Ms6hm* locus correlated well with the state of the c-*myc* gene amplification, but not with the malignancy of sarcomas. The correlation was statistically significant as analyzed by the χ^2 test ($P = 0.012$).

Analysis of Mutant Subclones. Recloning of subclones carrying aberrant bands was performed to elucidate the origin of mutant cells. BC7421-3 subclone 5 carried three bands, and five clones derived from this clone exhibited the same banding pattern, indicating that acquisition of the mutation and subsequent trisomy of the locus

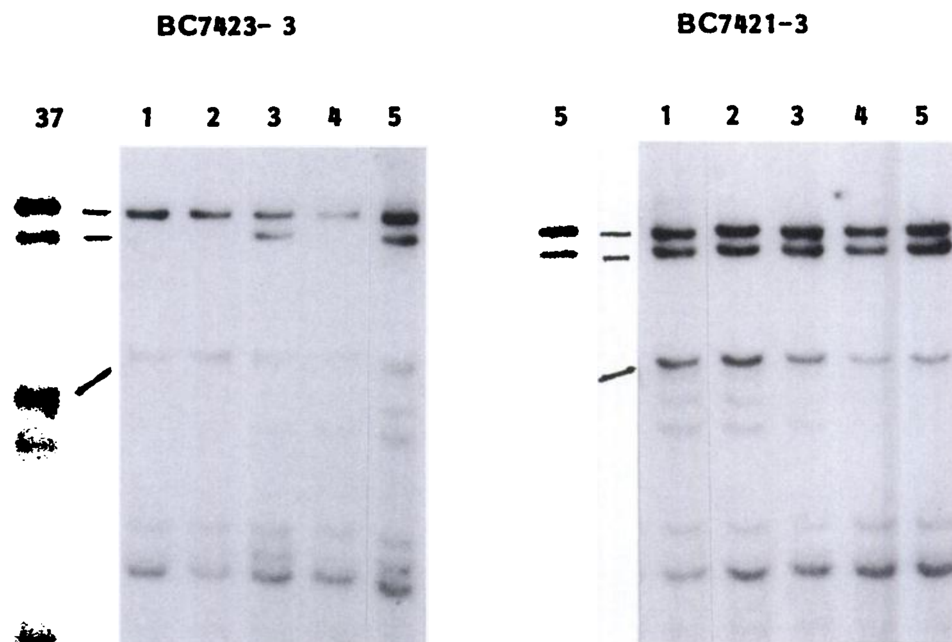

Fig. 3. Analysis of clones isolated from mutant subclones. Southern blot analysis of the *Ms6hm* locus was performed on *Hae*III-digested DNA of clones isolated from mutant subclones. Five clones were analyzed for subclone 37 of BC7423-3 and subclone 5 of BC7421-3. Note that subclone 37 of BC7423-3 consists of a mixture of two subpopulation carrying different length change mutations, while subclone 5 of BC7421-3 consists of one population with a trisomy of chromosome 4.

preceded the subcloning (Fig. 3). In contrast, analysis of BC7423-3 subclone 37 indicated segregation of two populations carrying either two bands or three bands. This suggests that the mutation and subsequent trisomy of the locus occurred during the early stage of expansion of the subclone. These findings indicate that mutant subclones arose at various times during subcloning and subsequent growth of the cells.

DISCUSSION

In this communication, we have studied two groups of MCA-induced sarcomas with the normal and the amplified c-*myc* gene. Point mutation of the K-*ras* gene was frequently detected among sarcomas with the amplified c-*myc* gene. A high rate of genetic instability of the hypervariable minisatellite *Ms6hm* locus was noted for sarcomas with the amplified c-*myc* gene. Thus, the present study indicates that minisatellite instability is associated with mutations of dominant oncogenes in MCA-induced mouse sarcomas. In contrast, K-*ras* mutation, c-*myc* amplification, and minisatellite mutations were found to have no bearing on chromosome instability of these sarcomas as assessed by micronuleus formation.

K-*ras* gene mutations were transversions of G to C at codon 12 and 13 and A to T at codon 61. Transversions of G to T and A to T were reported at the K-*ras* gene in MCA-induced transformants of C3H/10T1/2 cells (27) and at the H-*ras* gene in MCA-induced papillomas (28). Similarly, transversion of G to C was also reported for the *p53* gene in the MCA-induced transformant of Syrian hamster cells (29). Thus, point mutations of the K-*ras* gene observed here are likely to be the primary event due to DNA adducts formed by MCA at the time of the drug injection.

Point mutations of the K-*ras* gene are changes of nucleotide sequences while amplification of the c-*myc* gene and length change mutation at the *Ms6hm* locus involve a block of sequence without changing the nucleotide sequences. It is difficult to envisage how the molecular mechanism responsible for generation of these large mutations also participates in induction of point mutation at the K-*ras* gene. It is possible that the activated K-*ras* gene may have contributed in the generation of large mutations. Amplification of the *dhfr* gene was observed after induction of expression of the mutated H-*ras* gene in NIH-3T3 cells (30). Mutation of the *p53* gene was implicated in amplification of the c-*myc* gene (31, 32). However, it has been reported that *p53* mutation was not associated with N-*myc* gene amplification in neuroblastoma and c-*myc* gene amplification in sarcoma (33, 34). It was also reported that mutation of the *p53* gene did not correlate with microsatellite instability and karyotype instability (15). In the MCA-induced mouse sarcomas studied here, five of seven sarcomas with the normal c-*myc* gene and six of seven sarcomas with the amplified c-*myc* gene were found to carry the mutated *p53* gene.[4] Therefore, *p53* gene mutation may have no relevance to amplification of the c-*myc* gene and minisatellite instability in MCA-induced mouse sarcomas.

Amplification of the c-*myc* oncogene was reported to be associated with aggressive cancers (35). Aneuploidy was shown to correlate with amplification of the N-*myc* oncogene in neuroblastomas as well as musculoskeletal neoplasms (36, 37). It is interesting to note that chromosome instability, as analyzed using the micronucleus assay, was not associated with amplification of the c-*myc* gene nor did it correlate with minisatellite instability in MCA-induced mouse sarcomas studied here.

Recent studies have indicated that mutation in microsatellite sequences, especially those with CA repeat, can serve as a sensitive marker of a certain type of genetic instability in cancer cells. Microsatellites consist of a short stretch of repeats of 1–5 bases; and the entire length of the sequences ranges up to a few hundred bases (38). Mutations in mismatch repair genes lead to the replication error phenotype due to failures of repairing heteroduplexes formed by slippage during DNA replication (13). Other factors may also contribute to the destabilization of the microsatellite, and the association of overexpression of erbB-2 and microsatellite instability was reported recently in gastric carcinomas (39).

Minisatellite sequences usually consist of longer repeat units spanning longer lengths than microsatellites. The repeat unit of the *Ms6hm* locus is 5 bases and rather small for a minisatellite. However, the entire length spans 3–8 kb, and therefore the locus was classified as minisatellite (23, 25).

[4] O. Niwa, unpublished results.

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Another important distinction between the minisatellite and microsatellite is that the mechanisms of the length change mutations are different. The sizes involved in length change mutation of microsatellite sequences observed in cancer cells are rather small, such as the gain or loss of a few repeat units (6–8, 15). These mutations are caused by slippage as discussed above. In contrast, the length change mutation of the *Ms6hm* minisatellite locus observed in the present study was large and spanned a few hundred to a few thousand bases. Length change mutations of minisatellite sequences are reported to occur through gene conversion or recombination between homologous alleles (25, 40). Thus, minisatellite instability and microsatellite instability are distinct entities.

We have made a preliminary analysis using the locus-specific D2MIT30 PCR primer set for the detection of microsatellite instability. Analysis of 40 subclones of BC7413-1, which had minisatellite instability, revealed that the CA repeat was stable in this line.[5] These results indicate that there are multiple pathways of genetic instability leading to different mutations. It was indeed reported that a colon cancer cell line which did not show microsatellite instability, nevertheless, exhibited a high mutation frequency at the *hprt* locus (41).

The mouse *Ms6hm* locus undergoes a high rate of mutation in germ cells and during early embryogenesis (23, 25, 42). The locus is stable in normal somatic cells, but becomes highly unstable in cancer cells (19). Germ cells, early embryonal cells, and cancer cells are known for their high proliferative capacities. The present study demonstrated that instability of the *Ms6hm* minisatellite locus is associated with amplification of the c-*myc* gene. c-myc protein forms a heterodimer with Max protein and binds to a sequence motif of CACGTG (43). The heterodimer activates genes involved in G_0–G_1 transition and contributes to the continuous proliferation of cells (44). Therefore, it is possible that instability of the *Ms6hm* locus in sarcomas might have resulted from the high rate of proliferation of sarcoma cells carrying the amplified c-*myc* gene. It was reported that overexpression of the c-*myc* gene leads to amplification of the *dhfr* gene, which has a sequence element for Myc-Max binding (45, 46). A similar mechanism may contribute to instability of the *Ms6hm* locus in sarcomas carrying the amplified c-*myc* gene. Another possibility is that mutation of a yet to be identified gene might have induced genetic instability in MCA-induced sarcomas, which in turn lead to both amplification of the c-*myc* gene and instability of the minisatellite sequence. We are currently testing these two possibilities by analyzing the effect of overexpression of the c-*myc* gene on the stability of the *Ms6hm* locus.

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