

Analysis of the *c-myc*, *K-ras* and *p53* Genes in Methylcholanthrene-induced Mouse Sarcomas

Hiroshi Watanabe,^{1,3} Kiyoshi Shimokado,¹ Toshimasa Asahara,¹ Kiyohiko Dohi¹ and Ohtsura Niwa²

¹Second Department of Surgery, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734-0001 and ²Radiation Biology Center, Kyoto University, Yoshida-Konoe, Sakyo-ku, Kyoto 606-0000

We have examined 63 methylcholanthrene (MCA)-induced mouse sarcomas for possible correlations of mutations involving the *c-myc*, *ras* and *p53* genes. The *c-myc* gene was found to be amplified in 18 of these sarcomas (29%). Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis and subsequent direct sequencing identified 18 cases carrying *K-ras* mutation at codons 12, 13 and 61 (29%). No mutation was detected in the *H-ras* and *N-ras* genes. Mutations of the *p53* gene in exons 5 to 8 were found in 45 cases (71%). Comparison of these mutations revealed that out of 18 cases with *c-myc* gene amplifications, 10 carried *K-ras* mutations (56%) and 14 carried *p53* mutations (78%). In contrast, among 45 cases of sarcomas without *c-myc* gene amplification, 8 were found to have *K-ras* mutations (18%). The same 45 cases were found to have 31 *p53* mutations (69%). The present study suggests a strong correlation between *c-myc* gene amplification and *K-ras* gene mutation ($P < 0.01$). *p53* gene mutation was frequently found among MCA-induced mouse sarcomas, indicating the importance of this mutation in the etiology of these tumors. However, *p53* mutations were present in sarcomas regardless of the state of *c-myc* amplification and *K-ras* mutation. Therefore, a defect in the *p53* gene is independent of amplification of the *c-myc* gene or point mutation of the *K-ras* gene.

Key words: *c-myc* — *K-ras* — *p53* — MCA — Mouse sarcoma

The process of carcinogenesis involves a number of genetic changes, including gain-of-function mutation of oncogenes and loss-of-function mutation of tumor suppressor genes.^{1,2} Activations of the *c-myc* gene by amplification and *K-ras* gene by point mutation are the most common of the genetic changes among various cancers of human and animal origins.³⁻⁵ The *ras* and *myc* genes form two large gene families, which seem to cooperate in the transformation of cells *in vitro* and in carcinogenesis *in vivo*.⁶⁻⁸

Recent studies have indicated that the *c-myc* gene has a dual role in normal cellular functions. Its role in proliferation of cells was first recognized, and later studies have revealed that the gene product functions as a transcription factor involved in cell cycle progression. The *c-myc* gene product, MYC, is a bHLH-LZ protein and forms a heterodimer with another bHLH-LZ protein, MAX, which transactivates genes involved in cell cycling. This MYC/MAX heterodimer competes for DNA binding with a heterodimer of MAX and MAD, which suppresses cell cycling.⁹ A more recent discovery is the pivotal role of the *c-myc* gene in apoptosis.¹⁰ Thus, the dual roles of the *c-myc* gene and its frequent activation in cancers are central to

an understanding of the survival and death of neoplastic cells.

There are two modes of activation of the *c-myc* gene. Gene amplification is common in solid tumors such as small cell lung carcinoma,¹¹ breast,¹² and cervical carcinomas.¹³ Activation of the *myc* gene by rearrangement is frequent in lymphoid malignancies.¹⁴ Overexpression of the *c-myc* gene as a result of these gain-of-function mutations has to be accompanied with other mutations which enable the cells to negate the apoptotic function of the *c-myc* gene.¹⁵ Suppression of apoptosis is brought about by several mechanisms. One of them involves survival signals which counteract the death signals. This is well exemplified by the insulin-like growth factor cascade which is transmitted by the receptor tyrosine kinase, RAS, and phosphatidyl inositol 3-kinase, and eventually ends in phosphorylation of BAD¹⁶ to block apoptosis. The *ras* gene products play an essential role in this pathway. The *ras* family consists of three closely related genes, *H-ras*, *K-ras* and *N-ras*, which control cell growth and differentiation by passing the signals to downstream effectors.^{17, 18}

Apoptosis is a safety mechanism for tissues to eliminate cells that escape from normal regulation of cell proliferation and differentiation. It also functions to eliminate cells carrying DNA damage. Therefore, abrogation of the apoptotic pathway frequently results in genetic instability

³ Present address: Sanyou Jin Clinic, 1-7-8 Nogami-cho, Fuku-yama 720-0815. E-mail: nabe3@hiroshima-cdas.or.jp

of precancerous and cancer cells. The *p53* gene is a multifunctional tumor suppressor gene,^{19, 20} and has been implicated in the tumorigenesis of a wide variety of human cancers.²¹ The *p53* gene also has dual functions; one is to maintain stability of the genome and the other is to eliminate rogue cells by apoptosis. Loss-of-function mutation of the gene is supposed to lead to the genetic instability which characterizes many types of cancers.²² Proliferation, apoptosis and genetic instability are key concepts for understanding carcinogenesis. These three processes are mutually interdependent and connected by intricate pathways which are currently the subjects of intensive study.

We demonstrated previously the frequent amplification of the *c-myc* gene²³ and its association with minisatellite instability in methylcholanthrene (MCA)-induced mouse sarcomas.²⁴ In this study, we extended that work and analyzed 63 MCA-induced mouse sarcomas for *c-myc* gene amplification, *K-ras* gene mutation and *p53* gene mutation. The results clearly demonstrate that *c-myc* gene amplification is frequently associated with activation of the *K-ras* oncogene, but not with mutation of the *p53* gene.

MATERIALS AND METHODS

Sarcomas MCA-induced sarcomas analyzed in this study were described previously.²³ Briefly C57BL/6N×C3H/HeN (BCF1) or C3H/HeN×C57BL/6N (CBF1) mice were injected subcutaneously at 6 regions on the back with 0.5–1.0 mg of MCA dissolved in olive oil. When tumors had grown to 1 cm in diameter, they were excised and examined histologically. Sixty-three independent tumors were obtained, of which five were from CBF1 mice (tumors with CB designators), and 26 from BCF1 mice (tumors with BC designators). A portion of each tumor was minced with scissors and transferred to a 3-cm

culture dish. Each sample was grown for 10 days in order to minimize contamination with stromal cells. All of the tumors used in the present study were transplantable to syngeneic mice.

Southern blotting of the *c-myc* oncogene Amplification of the *c-myc* oncogene was assessed as previously reported²³ by Southern blotting of DNA using as a probe a 10 kb *KpnI* fragment of the genomic *c-myc* oncogene. The mouse α -globin gene was used as an internal marker for the amount of DNA applied, with a 2 kb genomic fragment of the mouse α -globin gene as a probe. The intensities of the *c-myc* band and the α -globin band were measured by densitometry and the ratio of the two was taken to represent the extent of amplification of the *c-myc* gene in sarcomas.

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of *ras* and *p53* genes Oligonucleotide primers for exon 1 (codons 12 and 13) and for exon 2 (codon 61) were used for the analysis of H-, K- and N-*ras* oncogenes.²⁵ The sequences are shown in Table I. Mutations of the *p53* gene occur most frequently in exons 5 to 8 and primers for these exons were synthesized as described previously.^{26, 27} All primers of the *p53* gene included a portion of intron in order to avoid amplification of the *p53* pseudogene. PCR-SSCP analysis was performed according to the standard procedure.²⁸ Briefly, primers were end-labeled with [γ -³²P]ATP using T4 polynucleotide kinase. Genomic DNA was amplified for 30 cycles in 10 μ l of reaction mixture containing 100 ng of template DNA, 4 μ M end-labeled primers and 0.05 units of *Taq* DNA polymerase. Each cycle consisted of 94°C for 1 min, 52–60°C for 1 min and 72°C for 30 s. Different annealing temperatures were applied for the best amplification of the fragments. The reaction mixture contained 10 μ l of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cya-

Table I. Oligonucleotide Primers Used for PCR of *ras* Genes

Region amplified (annealing temp.)	Primer sequences (sense/antisense)
H- <i>ras</i> exon 1 (60°C)	5'-ACAGAATACAAGCTTGTGGTGGTG-3' 5'-CTCTATAGTGGGATCATACTCGTC-3'
H- <i>ras</i> exon 2 (60°C)	5'-GACTCCTACCGAAACAGGTGGTC-3' 5'-GGCAAATACACAGAGGAAGCCCTC-3'
K- <i>ras</i> exon 1 (58°C)	5'-TATAAACTTGTGGTGGTTGGAGCT-3' 5'-GTACTCATCCACAAAGTGATTCTG-3'
K- <i>ras</i> exon 2 (58°C)	5'-GACTCCTACAGGAAACAAGTAGTA-3' 5'-TATGGCAAATACACAAAGAAAGCC-3'
N- <i>ras</i> exon 1 (60°C)	5'-ACTGAGTACAACTGGTGGTGGTTGGAGCA-3' 5'-ATCATATTCATCCACAAAGTGGTTCTGG-3'
N- <i>ras</i> exon 2 (58°C)	5'-GATTCTTACCGAAAGCAAGTGGTG-3' 5'-ATTGATGGCAAATACACAGAGGAA-3'

nol, and denaturation was carried out at 90°C for 2 min. An aliquot (1 µl/lane) was applied to a 12% polyacrylamide gel containing 10% glycerol and electrophoresed at 20 W for 5–6 h at room temperature with fan cooling. The gel was dried and exposed to X-ray film.

Direct sequencing of the K-ras gene DNA fragments with abnormal mobility on PCR-SSCP gels were eluted from the dried gel and reamplified by PCR as above. Direct sequencing of the fragments was performed using Takara Cycle Sequencing Kit under the conditions recommended by the manufacturer (Takara Shuzo, Kyoto), as described previously.²⁷⁾

Quantitative reverse transcriptase (RT)-PCR analysis of ras genes The amount of mRNA of each *ras* gene was quantified using RT-PCR analysis. The relationship between the amount of PCR product and the number of PCR cycles was analyzed using a known amount of genomic DNA. Briefly, the genomic DNA (18 ng, 10⁻²⁰ mol) of each gene was amplified using the ³²P-end-labeled primer sets for exon 1 of H-*ras*, K-*ras* and N-*ras* genes. After PCR amplification of 20 to 40 cycles, the reaction mixtures were electrophoresed through polyacrylamide gel and the amounts of the products was estimated by using a BAS 2000™ image analyzer (Fuji Film Co., Ltd., Tokyo). The estimated amount was plotted against the number of cycles and this was used as the standard curve for quantitative RT-PCR of H-*ras*, K-*ras* and N-*ras* genes.

Similar analysis was done for the cDNA from sarcoma lines. Total RNA was extracted using guanidine isothiocyanate lysis of the cells and sedimentation.²⁹⁾ First-strand cDNA was synthesized in a volume of 40 µl with 1.5 µg of the total RNA using SuperScript RNase H⁻ Reverse Transcriptase with NotI-oligo(dT) primer under the conditions recommended by the manufacturer (Life Technologies, Rockville). The reaction mixture was diluted to 80 µl and used as the template for quantitative PCR amplification. The amounts of template cDNA solution were 1 µl for H-*ras* and N-*ras* genes and 2 µl for K-*ras* gene.

RESULTS

Amplification of the c-myc oncogene in MCA-induced sarcomas Southern analysis was performed on DNA from 63 sarcomas, including 5 sarcomas from CBF1 and 58 sarcomas from BCF1 mice. Comparison of the intensity of the bands for the *c-myc* gene and the *α-globin* gene demonstrated that the *c-myc* gene was amplified frequently. Eighteen of 63 sarcomas (29%) were found to carry the *c-myc* gene with varying degrees of amplification. The results are summarized in Table II.

PCR-SSCP analysis and direct sequencing of the K-ras gene and the p53 gene Genomic DNAs from 63 MCA-induced mouse sarcomas were examined by PCR-SSCP analysis of exon 1 (containing codon 12 and codon 13)

Table II. Summary of *c-myc* Amplifications, K-*ras* Mutations and *p53* Mutations

Case No.	Sarcoma	<i>c-myc</i> Amplification	K- <i>ras</i> mutation Codon, Base change	<i>p53</i> mutation Exon
1	CB6296	—	—	7, 8
2	CB6328	—	12-2, G→T (Gly→Val)	7
3	CB6329	—	—	7
4	CB6330	—	—	5
5	BC7199-1	—	—	5
6	BC7199-3	—	—	5
7	BC7200-1	—	—	8
8	BC7210-1	—	—	7
9	BC7211-1	—	—	—
10	BC7211-2	—	—	—
11	BC7211-3	—	—	—
12	BC7212-3	—	—	6, 8
13	BC7213-1	—	—	7
14	BC7213-2	—	—	7
15	BC7214-1	—	—	—
16	BC7214-3	—	—	5
17	BC7274-1	—	—	7
18	BC7352-1	—	—	5
19	BC7353-1	—	13-1, G→T (Gly→Cys)	7
20	BC7353-3	—	—	7, 8
21	BC7354-1	—	—	8
22	BC7354-2	—	—	—
23	BC7354-3	—	—	—
24	BC7354-4	—	—	7
25	BC7371-1	—	—	8
26	BC7371-2	—	12-1, G→T (Gly→Cys)	8
27	BC7372-1	—	12-2, G→A (Gly→Asp)	—
28	BC7373-1	—	13-1, G→C (Gly→Arg)	5, 6
29	BC7412-1	—	12-1, G→T (Gly→Cys)	6
30	BC7412-2	—	—	—
31	BC7413-3	—	—	—
32	BC7414-1	—	13-1, G→C (Gly→Arg)	—
33	BC7415-1	—	—	—
34	BC7415-2	—	—	8
35	BC7415-3	—	—	—
36	BC7421-2	—	12-1, G→T (Gly→Cys)	8
37	BC7422-1	—	—	—
38	BC7422-2	—	—	6
39	BC7422-4	—	—	5
40	BC7423-5	—	—	8
41	BC7424-5	—	—	5
42	BC7425-1	—	—	7
43	BC7425-5	—	—	5, 7
44	BC7426-2	—	—	5
45	BC7426-4	—	—	—
46	CB6334	+	12-2, G→T (Gly→Val)	7
47	BC7200-2	+	—	7
48	BC7214-2	+	—	5
49	BC7273	+	12-1, G→T (Gly→Cys)	8
50	BC7274-2	+	—	—
51	BC7353-2	+	12-1, G→T (Gly→Cys)	7
52	BC7412-3	+	—	6
53	BC7413-1	+	13-1, G→C (Gly→Arg)	5, 6
54	BC7413-2	+	12-1, G→T (Gly→Cys)	8
55	BC7413-4	+	—	8
56	BC7413-5	+	—	—
57	BC7415-4	+	13-1, G→C (Gly→Arg)	8
58	BC7419-4	+	12-2, G→A (Gly→Asp)	8
59	BC7421-3	+	61-2, A→T (Gln→Leu)	—
60	BC7421-4	+	—	5
61	BC7422-5	+	—	—
62	BC7423-3	+	12-1, G→C (Gly→Arg)	5
63	BC7424-4	+	13-1, G→C (Gly→Arg)	5

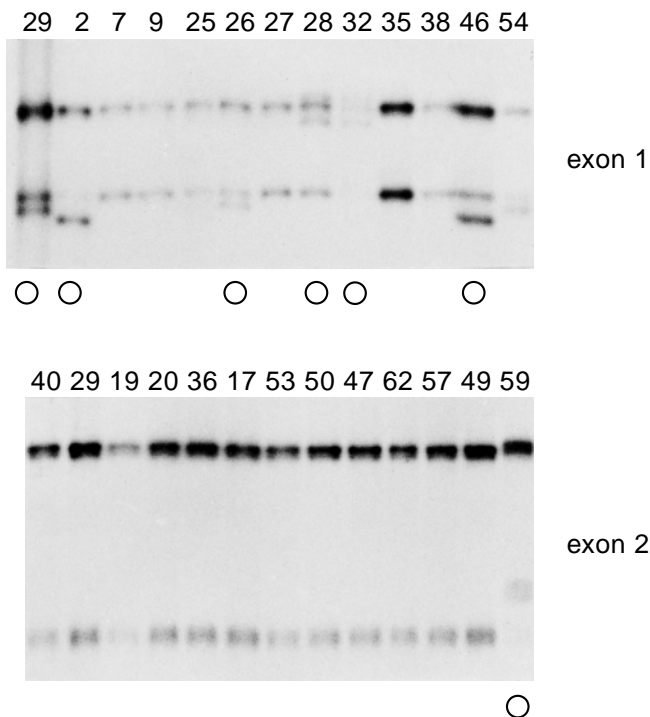


Fig. 1. PCR-SSCP analysis of the *K-ras* gene in MCA-induced sarcoma lines. Upper panel; PCR-SSCP analysis of exon 1, which contains codons 12 and 13. Bands with aberrant mobility were identified for case No. 29, 2, 26, 28, 32 and 46. Lower panel; PCR-SSCP analysis of exon 2, which contains codon 61. Case 59 carries an aberrant band. Putative mutants are marked with an open circle at the bottom of the panels.

and exon 2 (containing codon 61) of the *H-ras*, *K-ras* and *N-ras* genes. Fig. 1 shows one such analysis and illustrates some of the mutations in exon 1 and exon 2. DNA fragments showing aberrant mobility on SSCP gels were eluted from the gel and subjected to direct sequencing. Fig. 2 shows the sequencing data of mutations at codon 12 and codon 13. No mutation was detected in the *H-ras* and *N-ras* genes. Out of 63 sarcomas, 18 cases (29%) were identified to carry *K-ras* mutations at codon 12 (11 cases), codon 13 (6 cases) or codon 61 (one case); the results are summarized in Table II. The mutations of the *p53* gene were also examined by PCR-SSCP analysis of exons 5 to 8 (Fig. 3). Aberrant bands were extracted and mutations were confirmed by direct sequencing. The results of direct sequencing have already been published.²⁷⁾ Forty-five of 63 sarcomas carried mutations of the *p53* gene (71%). Among these 45 cases, 53 mutations were identified (Table II).

Quantitative RT-PCR analysis of sarcoma lines for *H-ras*, *K-ras* and *N-ras* genes We were curious as to why only the *K-ras* gene was mutated in these sarcoma lines. It is possible that the *K-ras* gene is the only *ras* gene

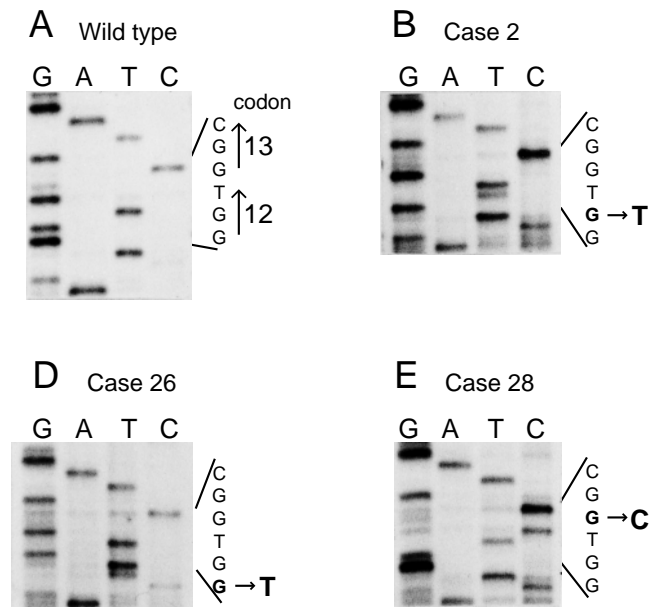


Fig. 2. Direct sequencing of *K-ras* codons 12 and 13. Panel A represents the sequence of the wild-type allele of the *K-ras* gene around codons 12 and 13 and panels B, C and D are mutant sequences at these codons.

expressed in these cells so that mutation and activation of the other *ras* genes are irrelevant to the development of sarcomas. Therefore, we quantified expression of the three *ras* genes. Two sarcomas, BC7210-1 without *ras* mutation and BC7413-1 with *K-ras* mutation, were used for quantitative RT-PCR of the three *ras* oncogenes. Each was cultured to near-confluence, total RNA was extracted, and quantitative RT-PCR was performed as described in "Materials and Methods."

Fig. 4 shows the result of such an analysis. These curves enabled us to estimate the amount of cDNA. The estimated amounts of cDNA for *H-ras*, *K-ras*, *N-ras* in BC7210-1 sarcoma were 16, 11, 43×10^{-20} mol per 1 μ g of total RNA, respectively. Those in BC7413-1C sarcoma were 16, 16, 52×10^{-20} mol per 1 μ g of total RNA, respectively. These results suggest that all three *ras* genes were actively expressed in these sarcomas, regardless of the presence of mutation in the *K-ras* gene.

Correlation analysis Table III summarizes the frequencies of and the correlation between *c-myc* gene amplification, *K-ras* gene mutation and *p53* gene mutation. Among 45 sarcomas with the normal *c-myc* gene, 8 cases of *K-ras* mutation (18%) and 31 cases of *p53* mutation were identified. In contrast, among 18 with the amplified *c-myc* gene, 10 carried *K-ras* mutation (56%) and 14 carried *p53* mutation (78%). The results shown in Table III demonstrate that the association of *c-myc* gene amplification

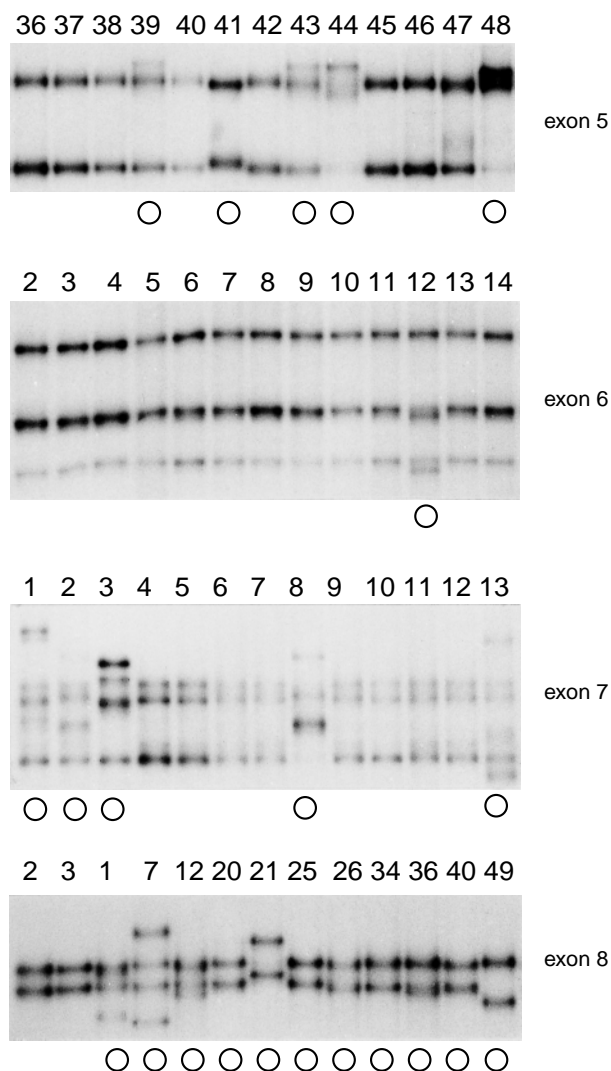


Fig. 3. PCR-SSCP analysis of the *p53* gene. Each panel represents exons 5 to 8 and putative mutants are marked with an open circle at the bottom of the panels.

with *K-ras* gene mutation is statistically significant ($P < 0.01$). In contrast, statistical analysis indicated that *p53* gene mutation was not associated with *c-myc* gene amplification. In accordance with these results, *K-ras* mutation was not associated with *p53* gene mutation.

DISCUSSION

In this study, we have demonstrated that amplification of *c-myc* gene and point mutation of the *K-ras* gene are frequent, and both occurred in 18 of 63 sarcoma lines (29%). In addition, the frequency of point mutation in the *p53* gene was as high as 71% among 63 sarcomas. There-

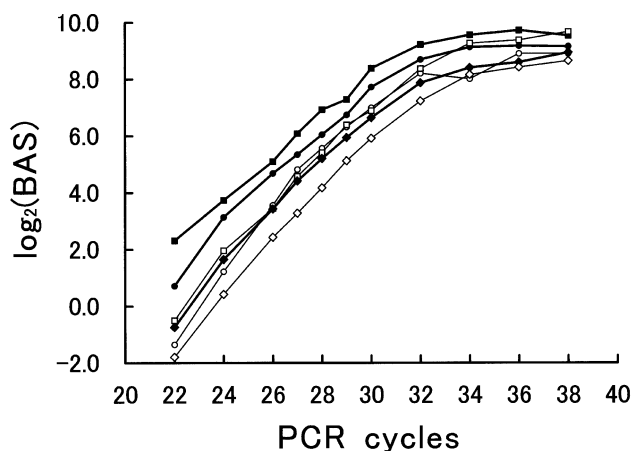


Fig. 4. Quantitative RT-PCR analysis of MCA-induced sarcomas. Total cellular RNA isolated from BC7210-1 and BC7413-1 was subjected to quantitative RT-PCR analysis. The number of PCR cycles was plotted against log values of the amplified bands. The band intensity was measured by using a BAS2000 as described in "Materials and Methods." ● *H-ras* (BC7210-1), ○ *H-ras* (BC7413-1), ■ *K-ras* (BC7210-1), □ *K-ras* (BC7413-1), ◆ *N-ras* (BC7210-1), ◇ *N-ras* (BC7413-1).

Table III. Mutations in MCA-induced Mouse Sarcomas

Genes	Frequency
<i>c-myc</i> amplification	18/63 (29%)
<i>K-ras</i> mutation	18/63 (29%)
<i>p53</i> mutation	45/63 (71%)
Correlation between <i>K-ras</i> and <i>c-myc</i> ^{a)}	
<i>K-ras</i> (+) among <i>c-myc</i> (-)	8/45 (18%)
<i>K-ras</i> (+) among <i>c-myc</i> (+)	10/18 (56%)
Correlation between <i>p53</i> and <i>c-myc</i> ^{b)}	
<i>p53</i> (+) among <i>c-myc</i> (-)	31/45 (69%)
<i>p53</i> (+) among <i>c-myc</i> (+)	14/18 (78%)
Correlation between <i>K-ras</i> and <i>p53</i> ^{b)}	
<i>K-ras</i> (+) among <i>p53</i> (-)	3/18 (17%)
<i>K-ras</i> (+) among <i>p53</i> (+)	15/45 (33%)

a) $P < 0.01$ by χ^2 test.

b) Not significant by χ^2 test.

fore, these mutations may play critical roles in the development of MCA sarcomas. Analysis of the data demonstrated that *c-myc* amplification tends to be associated with point mutation of the *K-ras* gene, though mutations of these two oncogenes had no association with that of the *p53* gene. Our previous studies on 14 cases of MCA-induced sarcomas demonstrated strong association of *c-myc* gene amplification, instability of a minisatellite sequence and malignancy of MCA-induced mouse sarcomas.²⁴⁾

There was also a possible association of *c-myc* gene amplification and point mutation of the *K-ras* gene.²⁴⁾ The present results with 63 sarcomas are in complete agreement with those of the previous study and confirm the association between gain-of-function mutations of the *c-myc* and *K-ras* oncogenes.

The *c-myc* gene is frequently amplified in a variety of tumors, especially in soft tissue tumors.³⁰⁾ The *c-myc* gene encodes a transcription factor with the HLH/leucine zipper motif that forms a complex with MAX. MYC acts as an upstream regulator of cyclin-dependent kinases and plays an important role in cell cycle regulation.³¹⁾ In addition, inappropriate expression of the *c-myc* gene induces apoptosis.³²⁾ The *p21 ras* gene serves as a survival signal and suppresses apoptosis in hematopoietic as well as epithelial cells.³³⁻³⁵⁾ This suppression of apoptosis is supposed to be mediated by increased expression of the *bcl-2* gene.³⁶⁾ In our present analysis, amplification of the *c-myc* oncogene was frequently associated with activation of the *K-ras* oncogene. Therefore, it is reasonable to assume that the activated *K-ras* gene functions to suppress apoptosis by overexpression of the *c-myc* gene, and contributes the survival of malignant tumor cells. In fact, RAS-mediated signaling was shown to suppress MYC-mediated apoptosis in fibroblast cells.¹⁶⁾

Quantitative RT-PCR analysis of two sarcoma lines with and without *K-ras* mutation revealed that all three *ras* genes were expressed at more or less similar levels, indicating that a mutation at any one of these genes may participate in tumorigenesis. However, in the present study of 63 sarcomas, activation was specific to the *K-ras* gene. We still do not know why only the *K-ras* gene is activated, and not other two, in MCA-induced sarcoma lines. Previous studies on MCA-induced transformation of a human cell line revealed activation of the *H-ras* oncogene at codon 61.³⁷⁾ Another study on MCA-induced fibrosarcomas in BALB/c mice demonstrated that some carried mutation of the *K-ras* gene at codons 12 and 13, and the *N-ras* gene at codon 61.³⁸⁾ *In utero* exposure to MCA induced liver tumors in mice all of which carried mutation of the *K-ras* gene exclusively at codon 13.³⁹⁾ The *K-ras* gene is indispensable to mouse development and homozygous knockout mice die at 12 to 14 days of gestation, though the other two genes can be ablated without the appearance of lethal phenotypes in mice.⁴⁰⁾

The *K-ras* gene mutations observed in this study were predominantly transversion of G to T (9 among 18 sarcoma lines, i.e., 50%). The G-to-C transversion, G-to-A transition and A-to-T transversion were less prominent. We have previously reported that the spectrum of *p53* gene mutations in the same MCA-induced mouse sarcoma lines was also predominantly of G-to-T transversions (21 among 45 cases, i.e., 47%), and G-to-C, G-to-A and A-to-T mutations were observed less frequently.²⁷⁾ Therefore,

we can conclude that the spectra of mutation at the *K-ras* gene and at the *p53* gene are similar in these sarcoma lines and that the mutation is likely to be caused directly by DNA adducts of MCA. Indeed, the G-to-T transversion was shown to be the predominant mutation induced by MCA in murine fibrosarcoma.³⁸⁾ The same G-to-T transversion was predominant in mutation of the *K-ras* gene in MCA-induced murine lung tumors.⁴¹⁾ However, the G-to-C transversion was also reported to be predominant for the *K-ras* gene in MCA-induced murine lung tumors.^{42,43)} The same G-to-C mutation occurred, but exclusively at codon 13 of the *K-ras* gene.⁴⁴⁾ *K-ras* mutations of our study involve codons 12, 13 and 61. These studies differ in the stage and timing of MCA administration and also in the strains of mice used, so further work is needed to clarify the spectrum of MCA-induced *K-ras* gene mutations in mouse sarcomas.

As discussed above, the MYC/MAX heterodimer functions as a transactivator which binds to a CA(C/T)GTG element. This element is also found in the *p53* promoter, and an elevated level of MYC leads to higher expression of the gene in some tumors.⁴⁵⁾ Therefore there is some connection between the *c-myc* gene and the *p53* gene. Besides, the role of *p53* gene as a guardian of the genome is well established, and mutation of the *p53* gene was shown to destabilize the genome, leading to gene amplification.^{46,47)} However, in our present study, we found no correlation between amplification of the former and point mutation of the latter. Nor could we find any association between mutations of the *K-ras* gene and the *p53* gene. Recent findings indicated that *p53* mutation was not associated with *c-myc* gene amplification in human sarcoma⁴⁸⁾ or with *N-myc* gene amplification in neuroblastoma.⁴⁹⁾ Our previous study indicated that overexpression of the *c-myc* gene rather than mutation of the *p53* gene contributes to genomic instability.²⁴⁾ Indeed, amplification of the *c-myc* gene led to a higher frequency of mutation at the dihydrofolate reductase gene in lymphoid and in non-lymphoid cell lines of a variety of species.^{50,51)} It is highly likely that there are at least two pathways to genetic instability, one involving the loss of function of the *p53* gene and the other, amplification of the *c-myc* gene. Further analysis is necessary to elucidate the roles of these two genes in genetic instability and in carcinogenesis.

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