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 trans-activates 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
of precancerous and cancer cells. The p53 gene is a multifunctional tumor suppressor gene,18,20 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.21 Briefly C57BL/6N×C3H/HeN (BCF1) or C3H/HeNxC57BL/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 BC designators), and 26 from BCF1 mice (tumors with CB designators), and 26 from BCF1 and examined histologically. Sixty-three independent tumors had grown to 1 cm in diameter, they were excised with 0.5 mm of MCA dissolved in olive oil. When tumors had grown to 1 cm in diameter, they were excised and immediately 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 polymerase. Genomic DNA was amplified for 30 cycles in 10 µl of reaction mixture containing 100 ng of template DNA, 4 µM 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 µl 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-ras exon 1 (60°C)               | 5′-ACAGAATACAAAGCCTTGTTGGTG-3′ |
|                                   | 5′-CTCTATAGGGATCATACTCGTC-3′    |
| H-ras exon 2 (60°C)               | 5′-GACTCTTACCCGAAACAGTGGTCT-3′ |
|                                   | 5′-GGCAAAATACACAGAGGAAAGCCCTC-3′ |
| K-ras exon 1 (58°C)               | 5′-TATAAAGCTTCTTGGGTGGTCTG-3′   |
|                                   | 5′-GTACTCATCCACAAAAGTGATTCTG-3′ |
| K-ras exon 2 (58°C)               | 5′-GACTCCTACAGGAAAACAGTATAGA-3′ |
|                                   | 5′-TATGGCAAATACACAAAAAGGAAAAGCC-3′ |
| N-ras exon 1 (60°C)               | 5′-ACTGAGTACAAACTGGTGTTGGGTGCAC-3′ |
|                                   | 5′-ATCATATATCATCCAAAGTGTTCTGG-3′ |
| N-ras exon 2 (58°C)               | 5′-GATTCTTACCGAAAGCAGTGTTG-3′   |
|                                   | 5′-ATTGATGGCAATACACAGAGGAA-3′   |
Amplification of the c-myc oncogene in MCA-induced sarcomas Southern analysis was performed on DNA from 63 sarcomas, including 5 sarcomas from CBFI and 58 sarcomas from BCF1 mice. Comparison of the intensity of the bands for the c-myc gene and the \( \alpha \)-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)
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 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 µg of total RNA, respectively. Those in BC7413-1C sarcoma were 16, 16, 52×10⁻²⁰ mol per 1 µg 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
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. Therefore, 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.

---

**Table III. Mutations in MCA-induced Mouse Sarcomas**

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

$^a$ $P<0.01$ by $\chi^2$ test.
$^b$ Not significant by $\chi^2$ test.
There was also a possible association of c-myc gene amplification and point mutation of the K-ras gene. 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. 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. In addition, inappropriate expression of the c-myc gene induces apoptosis. The p21 ras gene serves as a survival signal and suppresses apoptosis in hematopoietic as well as epithelial cells. This suppression of apoptosis is supposed to be mediated by increased expression of the bcl-2 gene. 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.

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 a 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. 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. In vivo exposure to MCA induced liver tumors in mice all of which carried mutation of the K-ras gene exclusively at codon 13. 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.

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. 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. The same G-to-T transversion was predominant in mutation of the K-ras gene in MCA-induced murine lung tumors. However, the G-to-C transversion was also reported to be predominant for the K-ras gene in MCA-induced murine lung tumors. The same G-to-C mutation occurred, but exclusively at codon 13 of the K-ras gene. 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. 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 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 sarcoma or with N-myc gene amplification in neuroblastoma. Our previous study indicated that overexpression of the c-myc gene rather than mutation of the p53 gene contributes to genomic instability. 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. 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.

ACKNOWLEDGMENTS

We thank A. Kinomura for excellent technical assistance. We also thank T. Nishioka for the photographic work. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan.

(Received September 16, 1998/Revised October 29, 1998/ Accepted November 2, 1998)
REFERENCES

1) Bishop, J. M. The molecular genetics of cancer. *Science*, 235, 305–310 (1987).
2) Kinzler, K. W. and Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell*, 87, 159–170 (1996).
3) Field, J. K. and Spandidios, D. A. The role of ras and myc oncogenes in human solid tumours and their relevance in diagnosis and prognosis (review). *Anticancer Res.*, 10, 1–22 (1990).
4) Smith, D. R., Elnatan, J., Myint, T. and Goh, H. S. Association of activated proto-oncogenes ras and myc in colorectal carcinomas. *Ann. Acad. Med. Singapore*, 24, 393–398 (1995).
5) Malkinson, A. M. Primary lung tumors in mice: an experimentally manipulable model of human adenocarcinoma. *Cancer Res.*, 52 (Suppl.), 2670S–2676S (1992).
6) Thompson, T. C., Southgate, J., Kitchener, G. and Land, H. Multistage carcinogenesis induced by ras and myc oncogenes in a reconstituted organ. *Cell*, 56, 917–930 (1989).
7) Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallance, R. and Leder, P. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergetic action of oncogenes in vivo. *Cell*, 49, 465–475 (1987).
8) MacAuley, A. and Pawson, T. Cooperative transforming activities of ras, myc, and src viral oncogenes in nonestablished rat adrenocortical cells. *J. Virol.*, 62, 4712–4721 (1988).
9) Austen, M., Cerni, C., Henriksson, M., Hilfenhaus, S., Luscher-Firzlaff, J. M., Menkel, A., Seelos, C., Sommer, A. and Luscher, B. Regulation of cell growth by the Myc-Max-Mad network: role of Mad proteins and YY1. *Curr. Top. Microbiol. Immunol.*, 224, 123–130 (1997).
10) Harrington, E. A., Famidi, A. and Evan, G. I. Oncogenes and cell death. *Curr. Opin. Genet. Dev.*, 4, 120–129 (1994).
11) Gazdar, A. F., Carney, D. F., Nau, M. M. and Stehelin, D. Characterization of variant subclasses of cell line derived from small cell lung cancer having distinctive biochemical, morphological and growth properties. *Cancer Res.*, 45, 2924–2930 (1984).
12) Guerin, M., Barrois, M., Terrier, M. J., Spielman, M. and Riou, G. Overexpression of either c-myc or c-erbB-2/neu proto-oncogenes in human breast carcinomas: correlation with poor prognosis. *Oncogene Res.*, 3, 21–31 (1988).
13) Ocadiz, R., Sauceda, R., Cruz, M., Graef, A. M. and Garglio, P. High correlation between molecular alterations of c-myc oncogene and carcinoma of the uterine cervix. *Cancer Res.*, 47, 4173–4177 (1987).
14) Aisenberg, A. C. Utility of gene rearrangements in lymphoid malignancies. *Annu. Rev. Med.*, 44, 75–84 (1993).
15) Hueber, A. O. and Evan, G. I. Traps to catch unruly oncogenes. *Trends Genet.*, 14, 364–367 (1998).
16) Kauffmann-Zeh, A., Rodriguez-Viciana, P., Ulrich, E., Gilbert, C., Coffer, P., Downward, J. and Evan, G. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature*, 385, 544–548 (1997).
17) Grand, R. J. A. and Owens, D. The biochemistry of ras p21. *Biochem. J.*, 279, 609–631 (1991).
18) Gomez, J., Martinez, A. C., Gonzalez, A. and Rebollo, A. Dual role of Ras and Rho proteins: at the cutting edge of life and death. *Immunol. Cell. Biol.*, 76, 125–134 (1998).
19) Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C. C. p53 mutations in human cancers. *Science*, 253, 49–53 (1991).
20) Levine, A. J., Momand, J. and Finlay, C. A. The p53 tumor suppressor gene. *Nature*, 351, 453–456 (1991).
21) Greenblatt, M. S., Bennet, W. P., Hollstein, M. and Harris, C. C. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, 54, 4855–4878 (1994).
22) Wahl, G. M., Linke, S. P., Paulson, T. G. and Huang, L. C. Maintaining genetic stability through TP53 mediated checkpoint control. *Cancer Surv.*, 29, 183–219 (1997).
23) Niwa, O. and Kominami, R. Lack of allelic preference in amplification and loss of the c-myc oncogene in methylcholanthrene-induced mouse sarcomas. *Jpn. J. Cancer Res.*, 83, 1192–1197 (1992).
24) Niwa, O., Kamiya, K., Furihata, C., Nitta, Y., Wang, Z., Fan, Y.-J., Ninomiya, Y., Kotomura, N. and Kominami, R. Association of minisatellite instability with c-myc gene amplification and K-ras mutation in methylcholanthrene-induced mouse sarcomas. *Cancer Res.*, 55, 5670–5676 (1995).
25) Manam, S. and Nichols, W. Multiple polymerase chain reaction amplification and direct sequencing of homologous sequences: point mutation analysis of the ras genes. *Anal. Biochem.*, 199, 106–111 (1991).
26) Bienz, B., Zakut-Houri, R., Givol, D. and Oren, M. Analysis of the gene coding for the murine cellular tumour antigen p53. *EMBO J.*, 3, 2179–2183 (1984).
27) Shimokado, K., Watanabe, H., Sumii, M., Miyagawa, K., Kamiya, K., Dohi, K. and Niwa, O. p53 gene mutation and loss of heterozygosity of chromosome 11 in methylcholanthrene-induced mouse sarcomas. *Jpn. J. Cancer Res.*, 89, 269–277 (1998).
28) Orita, M., Iwahara, H., Kanasawa, H., Hayashi, K. and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. *Proc. Natl. Acad. Sci. USA*, 86, 2766–2770 (1989).
29) Tsukiyama, T., Ueda, H., Hmoire, S. and Niwa, O. Embryonal long terminal repeat-binding protein is a murine homolog of FTZ-F1, a member of the steroid receptor superfamily. *Mol. Cell. Biol.*, 12, 1286–1291 (1992).
30) Barrios, C., Castresana, J. S., Ruiz, J. and Kreichbergs, A. Amplification of the c-myc proto-oncogenes in soft tissue sarcomas. *Oncology*, 51, 13–17 (1994).
31) Steiner, P., Rudilph, B., Muller, D. and Eiler, M. The functions of Myc in cell cycle progression and apoptosis. *Prog. Cell Cycle Res.*, 2, 73–82 (1996).
c-myc, K-ras and p53 in MCA Sarcoma

32) Thompson, E. B. The many roles of c-Myc in apoptosis. *Ann. Rev. Physiol.*, 60, 575–600 (1998).

33) Dumenil, D., Neel, H., Lacout, C. and Dautry, F. Infection with a Kirsten-retrovirus can induce a multiplicity of tumorigenic phenotypes in the interleukin-3-dependent FDC-P1 cells. *Exp. Hematol.*, 22, 178–185 (1994).

34) Billadeau, D., Jelinek, D. F., Shah, N., Tucker, W., LeBien, T. W. and Vanness, D. The many roles of c-Myc in apoptosis. *Mol. Cell. Biol.*, 22, 178–185 (1994).

35) Ward, R. L., Todd, A. V., Santiago, F., O’Connor, T. and Hawkins, N. J. Activation of the K-ras oncogene in colorectal neoplasms is associated with decreased apoptosis. *Cancer*, 79, 1106–1113 (1997).

36) Kinoshita, T., Yokota, T., Arai, K. and Miyajima, A. Regulation of Bel-2 expression by oncogenic Ras protein in hematopoietic cells. *Oncogene*, 10, 2207–2212 (1995).

37) Rhim, J. S., Fujita, J. and Park, J. B. Activation of H-ras oncogene in 3-methylcholanthrene-transformed human cell line. *Carcinogenesis*, 8, 1165–1167 (1987).

38) Carbone, G., Borrello, M. G., Molla, A., Rizzetti, M. G., Pierotti, M. A., DellaPorta, G. and Parmiani, G. Activation of ras oncogenes and expression of tumor-specific transplantation antigens in methylcholanthrene-induced murine fibrosarcomas. *Int. J. Cancer*, 47, 619–625 (1991).

39) Gressani, K. M., Rollins, L. A., Leone-Kabler, S., Cline, J. M. and Miller, M. S. Induction of mutations in Ki-ras and INK4a in liver tumors of mice exposed in utero to 3-methylcholanthrene. *Carcinogenesis*, 19, 1045–1052 (1998).

40) Johnson, L., Greenbaum, D., Cichowski, K., Mercer, K., Murphy, E., Schmitt, E., Bronson, R. T., Umanoff, H., Edelmann, W., Kucherlapati, R. and Backs, J. K-ras is an essential gene in the mouse with partial functional overlap with N-ras. *Genes Dev.*, 11, 2468–2481 (1997).

41) Leone-Kabler, S., Wessner, L. L., McEntee, M. F., D’Ago- stino, R. B. Jr. and Miller, M. S. Ki-ras mutations are an early event and correlate with tumor size in transplacently-induced murine lung tumors. *Carcinogenesis*, 18, 1163–1168 (1997).

42) Wang, X. and Witschi, H. Mutations of the Ki-ras proto-oncogene in 3-methylcholanthrene and urethan-induced and butylated hydroxytoluene-promoted lung tumors of strain A/J and SWR mice. *Cancer Lett.*, 91, 33–39 (1995).

43) Mass, M. J., Jeffers, A. J., Ross, J. A., Nelson, G., Galati, A. J., Stoner, G. D. and Nesnow, S. Ki-ras oncogene mutations in tumors and DNA adducts formed by benz[a]cycloanthrene and benzo[a]pyrene in the lungs of strain A/J mice. *Mol. Carcinog.*, 8, 186–192 (1993).

44) Gressani, K. M., Rollins, L. A., Leone-Kabler, S., Cline, J. M. and Miller, M. S. Induction of mutations in Ki-ras and INK4a in liver tumors of mice exposed in utero to 3-methylcholanthrene. *Carcinogenesis*, 19, 1045–1052 (1998).

45) Roy, B., Beamon, J., Balint, E. and Reisman, D. Reactivation of the human p53 tumor suppressor gene by c-Myc/Max contributes to elevated mutant p53 expression in some tumors. *Mol. Cell. Biol.*, 14, 7805–7815 (1994).

46) Livingstone, L. R., White, A., Sprouse, J., Livannos, E., Jacks, T. and Tlsty, T. D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell*, 70, 923–935 (1992).

47) Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C. and Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell*, 70, 937–948 (1992).

48) Castresana, J. S., Barrios, C., Gomez, L. and Kreicbergs, A. No association between c-myc amplification and TP53 mutation in sarcoma tumorigenesis. *Cancer Genet. Cytogenet.*, 76, 47–49 (1994).

49) Imamura, J., Bartram, C. R., Berthold, F., Harms, D., Nakamura, H. and Koeffler, P. Mutation of the p53 gene in neuroblastoma and its relationship with N-myc amplification. *Cancer Res.*, 53, 4053–4058 (1992).

50) Taylor, C. and Mai, S. c-Myc-associated genomic instability of the dihydrofolate reductase locus in vivo. *Cancer Detect. Prev.*, 22, 350–356 (1998).

51) Taylor, C., Jalava, A. and Mai, S. c-Myc dependent initiation of genomic instability during neoplastic transformation. *Curr. Top. Microbiol. Immunol.*, 224, 201–207 (1997).