Analysis of Tumor Suppressor Gene *p53* in Chicken Lymphoblastoid Tumor Cell Lines and Field Tumors

Michihiro TAKAGI, Kazuhiko OHASHI, Toshifumi MORIMURA, Chihiro SUGIMOTO and Misao ONUMA*

Laboratory of Infectious Diseases, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

(Received 7 January 1998/Accepted 22 April 1998)

ABSTRACT. To determine whether there is any abnormalities of the *p53* gene in chicken lymphoblastoid tumor cell lines derived from Marek's disease (MD), lymphoid leukosis, reticuloendotheliosis, and field tumors, some portions of *p53* cDNA corresponding to core and C-terminal domains (nucleotide positions 277–1104 in the *p53* open reading frame (ORF)) were sequenced. Several mutations were identified in both cell lines and field tumors. However, none of these mutations is localized at the "hot spot", which has been reported as the site for transformation-activating mutations. Moreover, partial cDNA clones with a 122-bp deletion in the *p53* ORF were identified in two cell lines, MSB1 and MTB1 derived from MD tumors. Southern blot analysis showed that no deletion occurred in the genome of *p53* in MSB1, indicating that deletion occurred at the transcriptional level. This deletion could cause a frame shift of the encoding *p53* protein, possibly resulting in the generation of a functionally different *p53* protein. However, we confirmed that *p53* mRNA without deletion is also present in each of these cell lines. These mutations of the *p53* gene and deletion in the *p53* transcript may be ones of molecular changes specific to the transformation induced by MD virus. — **KEY WORDS:** Marek's disease, *p53*, RT-PCR, transformation.

J. Vet. Med. Sci. 60(8): 923–929, 1998

The tumor suppressor gene *p53* plays a role in mediating the cellular response to DNA damage caused by γ -irradiation, UV, drugs and viral transforming proteins [5, 14, 33]. *p53* can act as a transcriptional regulator and transactivate cellular genes through sequence-specific interactions with DNA containing the sequence, 5'-PuPuPuC(A/T)(T/A) GPyPyPy-3', typically in the context of two such sequence motifs separated by up to 13bp [4, 11]. At least two important biological pathway are regulated by *p53* in connection with its function as a tumor suppressor. These are the induction cell cycle arrest at the G1 phase [10], and the induction of apoptosis [34].

The function of *p53* is inactivated in many human and other mammalian tumors by several point mutations within the *p53* gene itself. Most of these point mutations, causing conformational changes, are associated with a loss of DNA binding ability of *p53*. On the other hand, viral oncoproteins, such as simian virus 40 large T antigen [15, 21], adenovirus type 5 E1B protein [32], human papilloma virus type 16/18 E6 protein [24], and hepatitis B virus X-protein [28], inactivated *p53* through their formation of complexes with *p53*. The mutations and deletion of the *p53* gene have been found in many kinds of cancers in mammalian species [12, 16, 19].

Marek's disease virus (MDV) is a herpesvirus that causes a lymphoproliferative disease of chickens, which is characterized by malignant T-cell lymphoma and

demyelination of peripheral nerves [23]. The molecular mechanism of transformation by MDV has not been fully understood. Unlike retroviruses, MDV does not have any apparent oncogenes, suggesting that interaction of viral proteins with cellular oncoproteins or activation of cellular oncogenes are important for the transformation mechanism. Recently, it has been reported that Meq, which has a homology with members of the *jun/fos* oncoprotein family, could be a viral oncogene based on its potential role as a transcription factor [30]. In addition, the Meq protein has been shown to have a potential to interact with *p53* [2], suggesting possible involvement of this interaction in the transformation process by MDV. However, no information is available concerning the changes in the *p53* gene of host cells related to transformation by MDV. Thus, to determine whether *p53* mutations are involved in the neoplastic process of chicken lymphoma, we analyzed the molecular structure of chicken *p53* genes and its transcripts in chicken tumor cell lines derived from MD, avian leukosis (AL), reticuloendotheliosis (RE), and field tumors. We here reported that tumor cell lines possess mis-sense mutations and deletion in the *p53* transcript.

MATERIALS AND METHODS

Cell lines and tumor samples: Ten chicken cell lines established from chicken lymphoma were used in this study, MD cell lines: MSB1 [1], MTB1 [9], JP1, JP2 [31], HP1, HP2 [20] and RP1[18]; AL cell lines: 1104B1 [6] and CU10 [3] an RE cell line: Ku7 [22]. All cell lines were grown at 40°C in RPMI1640 medium supplemented with 10% fetal bovine serum, penicillin G (200 U/ml), streptomycin (200 mg/ml) and 2×10^{-5} M 2-mercaptoethanol. Tumor samples

* CORRESPONDENCE TO: ONUMA, M., Laboratory of Infectious Diseases, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

Table 1. Primers used to amplify chicken *p53* cDNA

Primer	Nucleotide sequence	Annealing temperature (°C)	Size of fragments amplified (location) ^{a)}
p53-5	5'-ATGTGCAACAGTTCCTGCAT-3'	55	157 bp
p53-6	5'-AGTTCTCCTCCTCGATCTTG-3'		(667-823)
p53-8	5'-AAAAGGGGGCGTCGTCAGT-3'	50	680 bp
p53-9	5'-GTCTATAAGAAATCAGAGCA-3'		(439-1119)
p53-11	5'-TGCTCTGATTCTTATAGAC-3'	46	182 bp
p53-12	5'-GACTTCCGGGTGGGGTTCGT-3'		(277-458)

a) Numbers indicate the locations in the *p53* open reading frame.

were obtained from the spleens and livers of 5 chickens which showed clinical signs related to MD in a commercial poultry house. Tumor samples were also obtained from 2 chickens purchased from Hokuren Co., Ltd., Sapporo, which were experimentally infected with a very virulent MDV, strain Md5, in our laboratory.

Preparation of total cellular RNA: Total RNA was isolated from cell lines and tumor samples using TRIzol (GIBCO-BRL, Gaithersburg, MD) following the manufacturer's instructions.

Reverse transcription and polymerase chain reaction: Extracted total cellular RNA (10 µg) treated with DNaseI (Boehringer Mannheim GmbH, Germany) was dissolved in 30 µl of reaction buffer containing 50 mM Tris-HCl (pH8.3), 40 mM KCl, 1 mM DTT, 3 mM MgCl₂, 0.5 mM each of dATP, dCTP, dGTP and dTTP, 40 U of RNase inhibitor (Promega, Madison, WI, U.S.A.), 200 pmol Oligo (dT)₁₅ and 20 U of reverse transcriptase (RAV-2, Takara, Kyoto, Japan). The mixture was incubated at 42°C for 60 min for reverse transcription. Then, newly synthesized first strand cDNA (30 µl) was mixed with 70 µl of distilled water. For polymerase chain reaction (PCR), 10 µl of cDNA template was added to 40 µl of reaction buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.8 mM each of dATP, dCTP, dGTP and dTTP, 30 pmol sense/antisense primers (Table 1, Fig. 1A) designed from the previously reported chicken *p53* cDNA sequence [26], and 2.5 U of Taq polymerase (Pharmacia Biotech, Uppsala, Sweden). The PCR were performed on a thermal cycler (PTC-100, MJ Research, Watertown, MA) under the following conditions: one cycle of 4 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1.5 min at 46, 48 or 55°C, and 1.5 min at 72°C, and a final 72°C 10 min extension cycle. The PCR products (10 µl) were separated on 2% agarose gels and visualized by staining with ethidium bromide.

DNA sequencing: The PCR products were purified by using GeneClean II kit (Bio 101, La Jolla, CA) and cloned into pGEM-T vector (Promega Corp., Madison, WI). Cloned inserts were sequenced on a 373A automatic sequencer (Applied Biosystems, Foster City, CA) using the Taq dye terminator cycle sequencing kit (Perkin-Elmer, Foster City, CA).

Northern blot analysis: For the detection of *p53* mRNA, total cellular RNA (15 µg) from tumor cell lines and normal

spleen were fractionated on a 1.5% agarose gel containing 1% formaldehyde in MOPS buffer, transferred to a nylon membrane filter, (Hibond-N+, Amersham, Buckinghamshire, UK), and hybridized with ³²P-labeled a *NcoI-NdeI* fragment of *p53*/8-9 cDNA in hybridization buffer (5XSSC, 5XDenhardt's reagent, 0.5% SDS, 50% formamide) at 42°C for overnight. The filter was washed twice with 2XSSC/0.1% SDS at room temperature, three times with 0.1XSSC/0.1% SDS at 65°C, and subjected to autoradiography.

Isolation of DNA and Southern blot analysis: High molecular weight genomic DNA was isolated from normal spleen and MSB1. Cells were lysed in lysis buffer (20 mM Tris-HCl, 25 mM EDTA, 100 mM NaCl, 0.5% SDS, pH 8.0) containing proteinase K (1 mg/ml) for overnight at room temperature. The lysate was extracted with phenol and chloroform, then DNA was precipitated with ethanol. Approximately 10 µg of genomic DNA was digested with *EcoRI*, *BamHI*, or *PstI*, fractionated on a 0.8% agarose gel, and transferred to a nylon membrane filter. The filter was hybridized with *p53*/8-9 cDNA probe in buffer (0.5 M Sodium-pyrophosphate buffer, 1 mM EDTA, 7% SDS, pH 7.2) at 65°C for overnight. The filter was washed in washing buffer (40 mM Sodium-pyrophosphate buffer, 1% SDS, pH 7.2) at 65°C, and subjected to autoradiography.

RESULTS

PCR and DNA sequencing: PCR amplification of cDNA prepared from all cell lines, tumor samples, normal spleen as a control, using primer pairs p53-5 and p53-6, p53-11 and p53-12 resulted in production of 157-bp and 182-bp cDNA fragments, respectively (Fig. 1). By using p53-8 and p53-9 primers, a 681-bp band was detected in tumor samples, normal spleen and all examined tumor cell lines except for MSB1 and MTB1. Instead, a approximately 600-bp fragment was amplified from MSB1 and MTB1 (Fig. 1).

In the next experiment, we determined if the *p53* transcripts from these chicken cell lines and tumor samples had any mutations (Table 2). The nucleotide sequences of these amplified DNA fragments of *p53* cDNA corresponding to the positions 277 to 1119 in the *p53* open reading frame from all cell lines, tumor samples and normal spleen were compared with that of chicken wild-type *p53* cDNA reported previously. Mutations were found in 9 out of 10 cell lines

A

Fig. 1. (A) Schematic representation of the *p53* open reading frame and locations of primers used in RT-PCR. PCR was performed by using three different pairs of *p53*-specific primers (Table 1). Highly conserved regions are indicated with roman numbers, I, II, III, IV and V, and positions of these domains are indicated with both nucleotide and codon numbers. (B) RT-PCR of *p53* transcripts expressed in chicken tumor cell lines. RNA samples were prepared from Marek's disease (MD), avian leukosis (AL) and reticuloendotheliosis (RE) tumor cell lines, and normal spleen as a control. Resultant PCR products were resolved on the agarose gel, and analyzed by ethidium bromide-staining. Arrows indicate the positions of the amplified *p53*-specific products.

Table 2. Mutations of *p53* genes in chicken lymphoblastoid cell lines and tumor samples

Cell line and		Mutation analysis				
tumor sample	Origin	Codon	Nucleotide		Amino acid	
MSB1	MD	146	GCC	GTC	Alanine	Valine
MTB1	MD	188	CAG	CCG	Glutamine	Proline
		202	GTC	CTC	Valine	Leucine
		146	GCC	GTC	Aline	Valine
JP2	MD	253	CGG	TGG	Arginine	Tryptophan
HP1	MD	146	GCC	GTC	Alanine	Valine
RP1	MD		ND			
1104B1	AL	146	GCC	GTC	Alanine	Valine
CU10	AL	146	GCC	GTC	Alanine	Valine
KU7	RE	146	GCC	GTC	Alanine	Valine
		356	AGG	ATC	Serine	Isoleucine
G1	spleen	146	GCC	GTC	Alanine	Valine
G2	liver		ND			
G3	spleen		ND			
G4	spleen		ND			
G5	spleen		ND			
H1	spleen		ND			
H2	spleen		ND			

Tumor samples G1 to G5 were obtained from 5 chickens in a commercial poultry house, and H1 and H2 were obtained from chickens experimentally infected with MDV in our laboratory. ND, mutations were not detected.

and one out of 7 tumor samples examined. A nucleotide C to T transition in codon 146 was found for MSB1, JP1, HP1, HP2, 1104B1, CU10 and Ku7 cell lines and one field tumor sample (G1), and this codon was changed from alanine to valine. For Ku7, codon 356 was also changed from G to T, resulting in transition of the codon from serine to isoleucine. Two-base-pair mutations were identified in MTB1, which resulted in the substitutions of glutamine for proline at codon 188 (transition from CAG to CCG), of valine for leucine at codon 202 (transition from GTC to CTC). A transition from C to T occurred within codon 253 (CGG to TGG) in JP2, resulting in a change of the encoded amino acid from arginine to tryptophan.

A short form of *p53* cDNA detected in MSB1 and MTB1: Since RT-PCR resulted in the amplification of a shorter *p53* cDNA fragment than wild-type cDNA in two MD cell lines, MSB1 and MTB1 (Fig. 1), the nucleotide sequences of the short forms were determined. These fragments were 559 bp in length, and the sequence encoding of the C-terminal part of the core domain including domain V was deleted (Fig. 2). This deletion could cause a frame shift, possibly generating a different *p53* molecules consisting of 268 amino acids. However, random sequencing of the amplified fragments from MSB1 and MTB1 showed that the *p53* transcript without deletion is also present in each of these cell lines although the 681-bp fragment was not visible by the staining with ethidium bromide (Fig. 1B). This short form of the *p53* transcript was not detected in tumor samples obtained from chickens in a commercial poultry house or from chickens experimentally infected with MDV.

Northern blot analysis: To determine whether two types of *p53* mRNA were expressed in MSB1 and MTB1,

Northern blot analysis was performed (Fig. 3). A *p53* transcript of 1.8 kb was found in all tested cell lines and normal spleen as a control. However, it was impossible to detect two types of *p53* transcripts in MSB1 and MTB1 because they are only 122-base different from each other.

Southern blot analysis: To estimate whether genomic alterations related to *p53* gene expression occurred in the MSB1 cell line, Southern blot analysis was carried out (Fig. 4). The genomic DNA from normal spleen and MSB1, digested with *EcoRI*, *BamHI* and *PstI*, and probed with *p53*/8-9 cDNA, had a 4.2- kb, and 5.3- and 3.7- kb bands, respectively. Similar to the result of Northern blot analysis, no deletion was occurred in the *p53* gene of MSB1.

DISCUSSION

Mutations in the *p53* gene has been shown in many kinds of mammalian tumors. These mutations were found within or around five conserved regions I, II, III, IV and V [26]. "Hot spots", in which mutations were frequently occurred, exist in these regions [7]. In this study, several mutations were found in ten cell lines derived from MD, AL and RE, and one field tumor sample (Table 2). However, these mutations were not localized at the "hot spots". These results suggested that mutations of the *p53* gene in chicken tumors may occur out of conserved regions, or that these mutations may not change the functional properties of *p53*.

It is of interest that a short form of *p53* transcript was detected in MSB1 and MTB1 derived from MD. This short form was not detected in cell lines from AL and RE, suggesting that the presence of the short form of the *p53* transcript may be one of molecular changes specific to MDV

Fig. 2. Comparison of the nucleotide sequences between the wild type and short form of *p53* cDNA. Nucleotide sequences corresponding a part of exons 8 and 9 and deduced amino acid sequences are shown. Dashes (-) represent the deleted sequence in the short form of *p53* cDNA.

Mutations or deletion in the *p53* transcript may be caused by viral protein(s) of MDV, or the deletion may result from alternative splicing which might be dependent on cell cycle progression [13]. MSB1 and MTB1, from which the short form of cDNA was recovered, originate from primary T-cell lymphoma and are not transplantable, while other MD cell lines are derived from chickens transplanted with tumor

ACKNOWLEDGMENT. This work was supported by Research Fellowship of the Japan Society for the Promotion of Science.

1. Akiyama, Y. and Kato, S. 1974. Two cell lines from lymphomas of Marek's disease. *Biken J.* 17: 105-116.
2. Brunovskis, P., Qian, Z., Li, D., Lee, L. F. and Kung, H-J. 1996. Functional analysis of the MDV basic-leucine zipper product, meq. pp. 265-270. *In: Current Research on Marek's*

Fig. 3. Northern blot analysis of *p53* transcripts expressed in tumor cell lines. Total RNA samples prepared from Marek's disease (MSB1, MTB1, JP1, JP2, HP1, HP2 and RP1), avian leukosis (1104B1 and CU10) and reticuloendotheliosis (Ku7) tumor cell lines were analyzed. Arrow heads indicates the positions of *p53* transcripts, 18S and 28S ribosomal RNAs.

Disease (Silva, R.F., Cheng, H.H., Coussens P.M., Lee, L.F. and Velicer, L.F. eds.), American Association of Avian Pathologists, Kennett Square.

3. Calnek, B.W., Murthy, K.K. and Schat, K.A. 1978. Establishment of Marek's disease lymphoblastoid cell lines from transplantable versus primary lymphoma. *Int. J. Cancer* 21: 100–107.
4. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W.

Fig. 4. Southern blot analysis of the chicken *p53* gene. Cellular DNA samples were prepared from MSB-1 (M) and normal splenocytes (S). These DNA samples were digested with either *EcoRI*, *BamHI* or *PstI*, fractionated on a agarose gel, and transferred to a membrane filter. Then, the filter was probed with a *p53*-specific probe. Numbers and arrow heads indicate the sizes (kb) and locations of molecular standard markers.

and Vogelstein, B. 1992. Definition of a consensus binding site for p53. *Nature Genetics* 1: 45–49.

5. Finlay, C.A., Hinds, P. W. and Levine, A. J. 1989. The *p53* proto-oncogene can act as a suppressor of transformation. *Cell* 57: 1083–1093.
6. Hihara, H., Shimizu, T. and Yamamoto, H. 1974. Establishment of tumor cell lines cultured from chickens with avian lymphoid leukosis. *Natl. Inst. Anim. Health Q.* 14: 163–173.
7. Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C. C. 1991. *p53* mutations in human cancers. *Science* 253: 49–53.
8. Hupp, T.R., Meek, D. W., Midgley, C. A. and Lane, D.P. 1992. Regulation of the specific DNA binding function of p53. *Cell* 71: 875–886.
9. Ikuta, K., Nakajima, K., Kanamori, A., Maotani, K., Mah, J.S., Ueda, S., Kato, S., Yoshida, M., Nii, S., Naito, M., Nishida-Umehara, C., Sasaki, M. and Hirai, K. 1985. Establishment and characterization of a T-lymphoblastoid cell line MDCC-MTB1 derived from chicken lymphocytes infected *in vitro* with Marek's disease virus serotype I. *Int. J. Cancer* 39: 514–520.
10. Kastan, M.B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. and Fornace, A. J. Jr. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* 71: 587–597.

11. Kern, S. E., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. W. and Vogelstein, B. 1992. Oncogenic form of *p53* inhibit *p53*-regulated gene expression. *Science* 256: 827–830.
12. Komori, H., Ishiguro, N., Horiuchi, M., Shinagawa, M. and Aida, Y. 1996. Predominant *p53* mutations in enzootic bovine leukemic cell lines. *Vet. Immunol. Immunopathol.* 52: 53–63.
13. Kulesz-Martin, M.F., Lisafeld, B., Huang, H., Kisiel, N. D. and Lee L. 1994. Endogenous *p53* protein generated from wild type alternatively spliced *p53* RNA in mouse epidermal cells. *Mol. Cell. Biol.* 14: 1698–1708.
14. Lane, D.P. 1992. *p53*, guardian of the genome. *Nature (Lond.)* 358: 15–16.
15. Lane, D.P. and Crawford, L.V. 1979. T antigen is bound to a host protein in SV40-transformed cells. *Nature (Lond.)* 278: 261–263.
16. Mayr, B., Heczko, U., Schellander, K., Schleger, W. and Reifinger, M. 1994. Sequence of an exon of the canine *p53* gene-mutation in a papilloma. *Br. Vet. J.* 150: 81–84.
17. Meek, D. W. 1994. Post-translational modification of *p53*. *Cancer Biol.* 5: 203–210.
18. Nazerian, K., Stephens, E. A., Sharma, J. M., Lee, L. F., Gailitis, M. and Witter, R. L. 1976. A nonproducer T lymphoblastoid cell line from Marek's disease transplantable tumor (JMV). *Avian Dis.* 21: 69–76.
19. Nigro, J. M., Baker, S. J., Presinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C. and Vogelstein, B. 1989. *p53* gene mutations occur in diverse human tumor types. *Nature (Lond.)* 342: 705–708.
20. Powell, P. C., Payne, L. N., Frazier, J. A. and Rennie, M. 1974. T lymphoblastoid cell lines from Marek's disease lymphomas. *Nature (Lond.)* 251: 79–80.
21. Sarnow, P., Ho, Y. S., Williams, J. and Levine, A. J. 1982. Adenovirus E16-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54kd cellular protein in transformed cells. *Cell* 28: 387–394.
22. Sasaki, T., Koyama, H., Yoshikawa, H. and Sasahara, J. 1981. Establishment of tumor cell lines cultured from chick embryos and chickens inoculated with oncogenic reticuloendotheliosis virus. p. 170. *In: Proceedings of the 92nd Meeting of the Japanese Society for Veterinary Science.*
23. Schat, K. A. 1987. Marek's disease: a model for protection against herpesvirus-induced tumors. *Cancer Surv.* 6: 1–37.
24. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. and Howley, P. M. 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of *p53*. *Cell* 63: 1129–1136.
25. Shaulsky, G., Ben-Ze'ev, A. and Rotter, V. 1990. Subcellular distribution of the *p53* protein during the cell cycle of Balb/c 3T3 cells. *Oncogene* 5: 1707–1711.
26. Soussi, T., Caron de Fromentel, C. and May, P. 1990. Structural aspects of the *p53* protein in relation to gene evolution. *Oncogene* 5: 945–952.
27. Sturzbecher, H.-W., Brain, R., Addison, C., Rudge, K., Remm, M., Grimaldi, M. and Keenan, E. 1992. A C-terminal α -helix plus basic region motif is the major structural determinant of *p53* tetramerization. *Oncogene* 7: 1513–1523.
28. Wang, X. W., Forrester, K., Yeh, H., Feitelson, M. A. and Gu, J.-R. 1994. Hepatitis B virus X protein inhibits *p53* sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc. Natl. Acad. Sci. U.S.A.* 91: 2230–2234.
29. Wang, Y., Reed, M., Wang, P., Stenger, J. E., Mayr, G., Anderson, M. E., Schwedes, J. F. and Tegtmeyer, P. 1993. *p53* domains: identification and characterization of two autonomous DNA-binding regions. *Genes Dev.* 7: 2575–2586.
30. Xie, Q., Anderson, A. S. and Morgan, R. W. 1996. Marek's disease virus (MDV) ICP4, *pp38*, and *meq* genes are involved in the maintenance of transformation of MDCC-MSB1 MDV-transformed lymphoblastoid cells. *J. Virol.* 70: 1125–1131.
31. Yamaguchi, S., Kawamura, H., Matsuda, H. and Kato, S. 1979. Establishment of Marek's disease lymphoblastoid cell lines from chickens with BABK of B blood groups. *Biken J.* 22: 35–40.
32. Yew, P. R. and Berk, A. J. 1992. Inhibition of *p53* transactivation required for transformation by adenovirus early 1B protein. *Nature (Lond.)* 357: 82–85.
33. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C. and Wahl, G. M. 1992. Wild-type *p53* restores cell cycle control and inhibits gene amplification in cell with mutant *p53* alleles. *Cell* 70: 937–948.
34. Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. 1991. Wild-type *p53* induces apoptosis of myeloid leukemic cells that is inhibited by interleukin-6. *Nature (Lond.)* 352: 345–347.