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A Mouse Erythroleukemia Cell Line Possessing Friend Spleen Focus-forming Virus gp55 Transgene and Temperature-sensitive Mutant p53 Gene

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Two different erythroleukemia cell lines have been established from the splenic lesions of transgenic mice possessing the Friend spleen focus-forming virus (F-SFFV) gp55 gene. One showed a near-diploid karyotype and a temperature-sensitive (ts) p53 mutation, and the other, a hyper-triploid karyotype with double p53 mutations found by single-strand conformation polymorphism (SSCP) analysis. The cell lines both retained No.11 chromosomes on which p53 genes are localized. Another p53 allele in the cell line with the ts-p53 mutation appeared intact in the SSCP analysis of the genomic exon 5. The cells with the ts-mutant p53 gene showed no apparent change with temperature shift in their growth or dimethylsulfoxide-induced differentiation, although the wild-type p53 gene on the other allele was not expressing. This ts-p53$^{Val\text{-}135}$ gene made p53-deficient fibroblasts anchorage-independent at 37°C but not at 32°C. This non-virus-producing, mouse erythroleukemia cell line will be useful for the study of mutated p53 function during the induction of erythrodifferentiation or apoptotic change.

Key words: Murine erythroleukemia — Friend spleen focus-forming virus (F-SFFV) gp55 gene — Temperature-sensitive p53 mutant — Erythrodifferentiation

Friend erythroleukemia cell lines have been extensively used to investigate molecular events during induced differentiation,[1-3] and to study the genetic alterations necessary for fixation of a fully leukemic state, including p53 mutation and *Spfi*-1 activation.[4,5] They have also been useful in research on via-EPOR[7] signaling, because the virion envelope-derived glycoprotein with the molecular weight of 55kDa (gp55) encoded by F-SFFV binds to EPOR on the cell surface and sends signals for the growth of the erythroid target cells in susceptible mice.[6-8]

Most Friend erythroleukemia cells are superinfected with replication-competent F-MuLV, and are producing FLV complex consisting of F-SFFV and F-MuLV,[9] causing *de novo* erythroleukemia when inoculated into mice and thus making it difficult to study the behavior of those inoculated leukemic cells alone.

To avoid this complex situation, we previously attempted to induce Friend erythroleukemia, also known as Friend disease, in mice using transgenic technology with F-SFFV gp55 gene as the transgene driven by F-SFFV LTR or human β-actin promoter.[10] This experiment has also shown that the gp55 gene alone could induce Friend erythroleukemia, and that the sporadically developed splenic erythroleukemic lesions did not show apparent mutations of the p53 gene, which had at one time been considered to be a direct transforming gene in Friend virus-induced leukemogenesis.[11] p53 mutations were observed, however, in the cultured or transplantable erythroleukemia cell lines from the transgenic mice with Friend disease.[10]

In this report, we describe biological characteristics of these erythroleukemia cell lines, including their differentiation inducibility, p53 mutations, karyotypes and so forth. We also report the *de novo* acquisition in the unique temperature-sensitive mutant p53 gene of the capacity to convert p53-deficient mouse fibroblasts to grow in soft agar.

MATERIALS AND METHODS

Establishment of erythroleukemia cell lines from F-SFFV gp55-transgenic mice We previously introduced the F-SFFV gp55 gene driven by F-SFFV LTR or by human β-actin promoter into the male pronucleus of

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[7] Abbreviations: EPOR, erythropoietin receptor; F-SFFV, Friend spleen focus-forming virus; F-MuLV, Friend murine leukemia virus; FLV, Friend leukemia virus complex; Mo-MuLV, Moloney murine leukemia virus; DMSO, dimethylsulfoxide; RT-PCR-SSCP, reverse transcription-polymerase chain reaction-single strand conformation polymorphism; LTR, retroviral long terminal repeat.

fertilized eggs of C57BL/6 mice and obtained F-SFFV gp55-transgenic mice.[10] They were crossed with DDD mice with an *Fv*-2^{ss} background. Those descendant mice with a stronger *Fv*-2^{ss} genetic background sporadically developed Friend erythroleukemia-like disease. The rapidly growing erythroid cells in the splenic lesions were readily maintained *in vitro* using RPMI-1640 medium supplemented with 10% fetal bovine serum. Among the 14 cell lines established from those gp55-transgenic mice, 11 showed altered bands in Southern blotting analyses. Two erythroleukemia cell lines, EL-Tg-gp55-1-2 and EL-Tg-gp55-2-2 (or 1-2 and 2-2) were stably maintained *in vitro*. T3-K-1 (or K-1), a Friend leukemia cell line was used for comparison.

Southern blotting to search for p53 mutations Genomic DNAs were prepared from the respective erythroleukemia cell lines or liver by the SDS-proteinase K method.[12] The DNA (10 μg) digested by *Eco*RI was separated in 0.9% agarose gel, blotted onto a nylon membrane and hybridized with the random-primed *Pst*I fragment of p53 cDNA clone pp53-176.[13] Washing and autoradiography were carried out as described.[10]

RT-PCR-SSCP analysis and direct DNA sequencing RT-PCR-SSCP analysis of the p53 gene was carried out according to the method described previously.[14, 15] Total cellular RNA (2 μg) obtained from the respective cell lines, normal spleen and liver of C57BL/6 mice by the AGPC extraction method[16] was subjected to RT reaction using various synthetic primers (A2, B2 C2, D2, E2 and F2 shown in Table I). A portion of the reaction mixture (1 μl) was subjected directly to PCR (10 μl). The 6 pairs of primers were used to cover 1.2 kb of p53 cDNA (Table I, Fig. 2a). PCR was carried out with 25 cycles in the presence of 5 μCi of [α-^{32}P]dCTP (Amersham). For the analysis of genomic DNA by SSCP, genomic DNA (100 ng) was similarly subjected to PCR to amplify exon 5 of the p53 gene. Individual SSCP bands of the particular DNA fragments (Fig. 2b, 2c) directly cut from the dried gel were placed in 20 μl of distilled water and incubated at 80°C for 15 min, then briefly centrifuged to remove debris. An aliquot (5 μl) of the supernatant was put into 50 μl of the PCR mixture. PCR products (100 ng) purified by two centrifugation-washes with a Centricon 100 (Amicon) were subjected to direct DNA sequencing as described.[15]

Cytogenetic analysis Logarithmically growing cells of the respective cell lines were treated with colcemid (0.1 μg/ml) for 40 min at 37°C. Cells collected by centrifugation were treated with 0.075 *M* KCl for 30 min at 37°C, then fixed in methanol:acetic acid (3:1 v/v), and spread on slides for air-drying. Chromosomes were stained with quinacrine and 33258 Hoechst[17] and examined under a fluorescence microscope. Karyotypes were analyzed in accordance with the standard nomenclature.[18] Chromosome numbers were counted on approximately 50 metaphase cells after preparation by conventional Giemsa staining method.

Table I. Primers Used for Amplification of the p53 cDNA

Amplified fragment	Primers	
	Name	Sequence
cDNA fragment		
A	A1	5′ CACGT GCTCA CCCTG GCTAA 3′
	A2	5′ ACAGA TCGTC CATGC AGTGA 3′
B	B1	5′ GAAGA TATCC TGCCA TCACC 3′
	B2	5′ GGAGA GTACG TGCAC ATAAC 3′
C	C1	5′ AGTCT GGGAC AGCCA AGTC 3′
	C2	5′ CAAAT TTCCT TCCAC CCGGA 3′
D	D1	5′ GCTCC TCCCC AGCAT CTTA 3′
	D2	5′ TTTCT TCTTC TGTAC GGCGG 3′
E	E1	5′ ACAGC TTTGA GGTTC GTGT 3′
	E2	5′ AGTAG ACTGG CCCTT CTTG 3′
F	F1	5′ TCAGT CCAGC TACCT GAAGA 3′
	F2	5′ AAGGG ACCGG GAGGA TTGT 3′

Growth rate and differentiation induction The cell lines were maintained like Friend leukemia cell lines[19] in petri dishes containing RPMI-1640 medium with 10% fetal bovine serum. The cells were seeded (5×10^4 cells/ml) and the medium was changed every 3 days. The growth rate was measured in two parallel cultures by counting the number of cells cultured at 37°C or 32°C every day for 6 days. DMSO (1.5% v/v) was added to the medium to induce erythrodifferentiation. Hemoglobin-positive cells were counted by using the benzidine reaction in a chemical-hazard container. The dying cells were stained with methylene blue. We repeated this experiment 3 times with similar results.

Construction of vectors expressing mutant p53 proteins and transfection of the mutant p53 gene into p53-deficient mouse fibroblasts A recombinant plasmid, pLTRp53cGwt, which encodes wild-type mouse p53, was described previously.[20] It contains a chimera of mouse p53 cDNA and genomic DNA, including introns 2–9, under the transcriptional control of a Harvey murine sarcoma virus (HaMSV) LTR. The mutant p53 cDNAs containing the entire open reading frame obtained from 1-2 and 2-2 cells, respectively, by the RT-PCR technique were digested with *Xho*I and *Sac*II. The generated *Xho*I-*Sac*II fragment of the respective p53 cDNA was subcloned into the pLTRp53cGwt expression vector which had been cleaved with *Xho*I and *Sac*II. The entire length of p53 cDNA subcloned was sequenced to confirm its coding frame.

The p53-deficient cells (5×10^5), proven to lack the wild-type p53,[21] were co-transfected with 5 μg of recombinant DNA (mutant p53 cDNA or expression vector only) and 0.5 μg of pY3 DNA (a selection marker gene

for hygromycin B) using Lipofectin reagent (Boehringer Mannheim) in 9 cm tissue culture dishes. The transfected cells were cultured in DMEM, supplemented with 0.4 mg/ml of hygromycin B (Wako Junyaku Kogyo) and 10% FCS at 37°C for about 14 days. Each individual cell clone was ring-isolated and expanded in the presence of hygromycin B (0.4 mg/ml) to maintain its drug-resistant phenotype. The drug was removed during the experimental procedure.

p53 protein expression in the transfected cells The transfected cells (1×10^7) cultured at 37°C or 32°C were lysed for 1 h on ice in 1 ml of high-salt lysis buffer.[21] The cell debris was removed by centrifugation, and the remaining supernatant was pretreated by incubation at 4°C for 2 h with 5 μg of nonspecific IgG2a murine monoclonal antibody followed by addition of 20 μl of 50% protein G-Sepharose (Pharmacia). The supernatant was immunoprecipitated at 4°C for 6 h with 0.5 μg of the following antibodies for p53: PAb421, PAb240 or PAb246 (Oncogene Science). Immune complexes were collected with 20 μl of 50% Protein G-Sepharose, washed twice in cold NET-gel buffer and dissolved in 10 μl of 3×sample buffer[21] by heating at 100°C for 3 min. After centrifugation, the supernatants were subjected to 10% SDS-PAGE and electrophoretically transferred to a nylon filter. The filter was probed with 0.5 μg of anti-p53 PAb240 at room temperature for 4 h, and incubated with horseradish peroxidase-conjugated goat anti-mouse Ig (diluted 600:1) at room temperature for 3 h. The p53 protein was detected by 0.05% 3,3′-diaminobenzidine (Sigma) staining.

Anchorage-independence assay Single p53-deficient cells (1×10^4) or cells transfected with the mutant p53 cDNA were seeded in duplicate in 0.36% soft agar/10% fetal calf serum (FMC BioProducts) in 6 cm plastic petri dishes (Falcon). All cells were cultured at 37°C or 32°C. Colonies were pipetted and trypsinized, and those colonies containing 50 or more cells were scored after 16 days. We repeated this experiment 3 times under the same conditions.

RESULTS

Obvious p53 gene mutation in an F-SFFV gp55-transgenic erythroleukemia cell line The primary splenic erythroleukemia lesions of the F-SFFV gp55-transgenic mice showed no apparent p53 gene mutation in Southern blotting, but one of the two erythroleukemia cell lines, 2-2, derived from the lesion showed a distinct altered band in the p53 DNA which was digested by *Eco*RI (Fig. 1).

Point mutations observed by RT-PCR-SSCP and direct DNA sequencing The RT-PCR-SSCP analysis and direct DNA sequencing of p53 gene of the 1-2 and 2-2 cell lines showed unique p53 point mutations: single Ala to Val (GCG→GTG) mutation in exon 5 at codon 135 (Figs. 2b, 3) in the 1-2 cell line; double Ala to Asp (GCC→GAC) and Met to Ile (ATG→ATA) mutations in exons 5 and 6 at codons 158 and 234, respectively (Figs. 2b, 2c, 3). The 1-2 cells did possess the temperature-sensitive mutant p53 gene which had been described by Oren's group.[22] This mutant p53 shows a mutated p53 function at 37.5°C and a wild-type p53 function at 32.5°C.

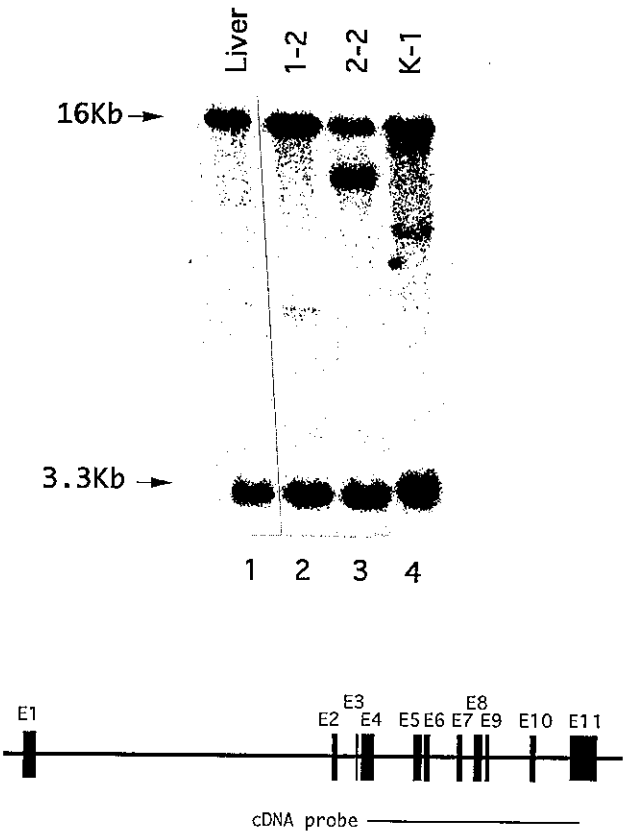

Fig. 1. Southern blot analysis of the p53 gene. Genomic DNA from cell lines 1-2, 2-2, K-1 (a Friend leukemia cell line) and normal liver cell of a C57BL/6 mouse were digested with *Eco*RI. The positions of the fragments expected from the normal gene and pseudogene are indicated by arrows: 16 kb for the normal p53 gene, and 3.3 kb for a pseudogene. The probe used was a random-primed *Pst*I fragment to murine p53 cDNA pp53-176,[13] covering the underlined exons.

No apparent effect of the endogenous ts-p53$^{Val\text{-}135}$ gene on the growth of 1-2 cells We wanted to know the effect of the endogenous ts-p53$^{Val\text{-}135}$ gene on an allele on the growth of the 1-2 cells caused by the temperature shift, because this gene expresses a mutant p53 at 37.5°C and a wild-type p53 at 32.5°C.[22] As shown in Fig. 4a, the temperature shift showed no apparent change in the growth pattern. This is quite distinct from the results

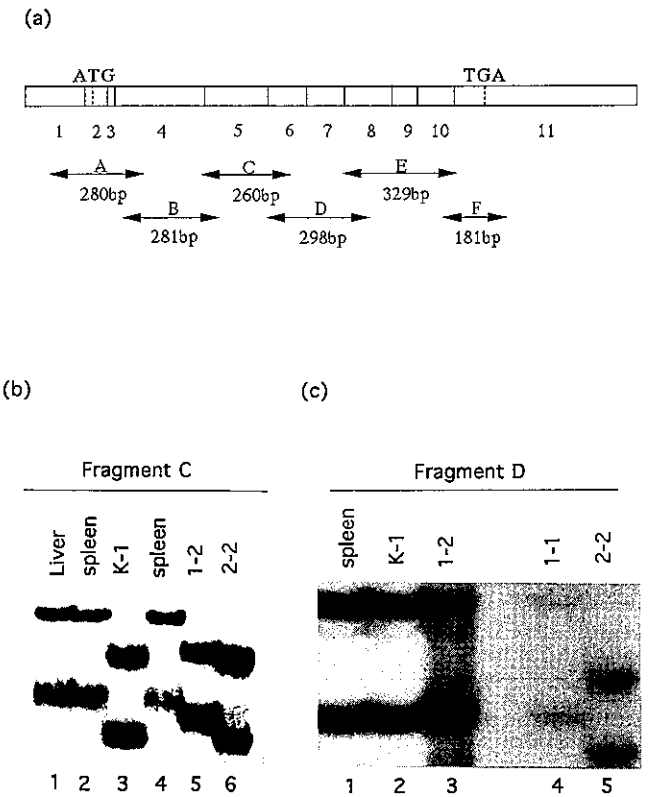

Fig. 2. RT-PCR-SSCP analysis of exon 5 and exon 6 of the p53 gene of the respective cell lines. (a) Regions of p53 cDNA subjected to RT-PCR-SSCP analysis. The coding regions of p53 cDNA were divided into 6 fragments (A to F) which overlapped each other and covered the whole coding sequence of the p53 cDNA. Their positions are indicated by bars with arrows at both ends, and their nucleotide lengths are shown under the bars. ATG and TGA are the initiation and termination codons, respectively, of the open reading frame of the p53 cDNA. The p53 gene from liver and spleen cells of C57BL/6 mouse was used as the normal control. (b) Mobility shift of fragments C (exons 5 and 6). (c) Mobility shift of fragments D (exons 6 and 7). Cell line 1-1 was lost in a later passage.

reported by Benchimol *et al.*,[23] who put the $p53^{Val-135}$ gene into p53-deficient Friend erythroleukemia cells, and observed erythrodifferentiation and increase of erythropoietin sensitivity.

No apparent effect of temperature shift in DMSO-induced differentiation The presence of ts-$p53^{Val-135}$ did not enhance erythrodifferentiation at a lower temperature by inducing apoptotic change. Accordingly, we wanted to know whether or not the p53 gene on the other allele was intact, and so carried out a cytogenetic analysis of the 1-2 and 2-2 cells, and SSCP analysis of the genomic p53 DNA from 1-2 cells.

Karyotypic profiles of 1-2 and 2-2 cells The chromosome number of cell line 1-2 ranged from 34 to 83, with a mode of 41. Structural abnormalities involving chromosomes 1, 2, 5, 17 and 18 were identified in all of the metaphases analyzed, and trisomies of chromosomes 8 and 14 were also observed in some cells. Cell line 2-2 showed hypertriploidy with a modal chromosome number of 62, ranging from 44 to 129. Structural changes derived from chromosomes 1, 3, 5, 9, 10 and 17 were identified in almost all of the cells analyzed. We found karyotypes of both cell lines which had a seemingly normal set of No. 11 chromosomes on which p53 genes are located (data not shown).

Wild-type pattern of exon 5 in genomic p53 of 1-2 cells observed by SSCP In addition to the mutated allele, the wild-type allele of the p53 gene was observed in the SSCP analysis of p53 exon 5 using a pair of nucleotides as primers existing within exon 5, although this does not exclude the possibility of point mutations which do not cause conformational change of the DNA fragment. This wild-type p53 gene, however, was not expressed in the 1-2 cells (Fig. 5, lane 5).

Characterization of p53 protein expressed in the transfected cells at 37°C and 32°C The p53-deficient (p53 −/−) cells derived from lung fibroblasts of null p53 gene transgenic mice were cotransfected with mutant ts $p53^{Val-135}$ cDNA or $p53^{Asp-158, Ile-234}$ cDNA and pY3, and

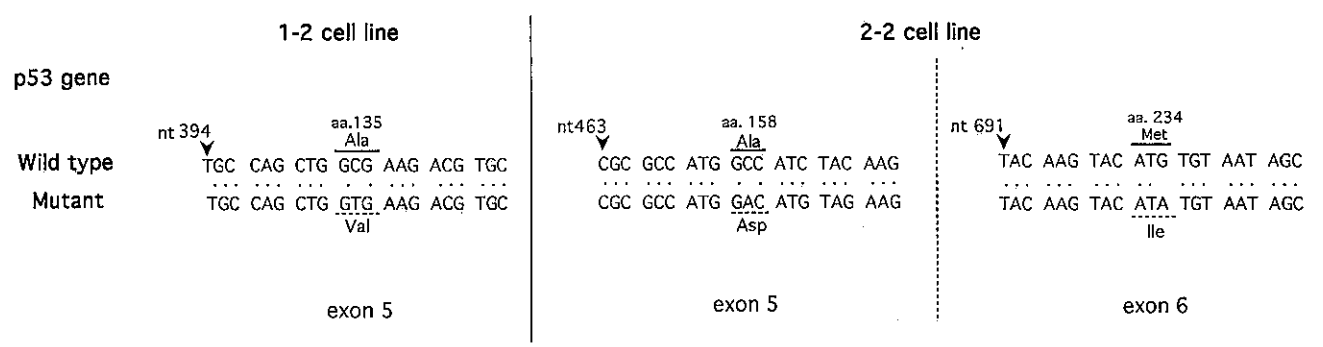

Fig. 3. Nucleotide sequence analysis of exons 5 and 6 of the p53 cDNA from fragments C and D.

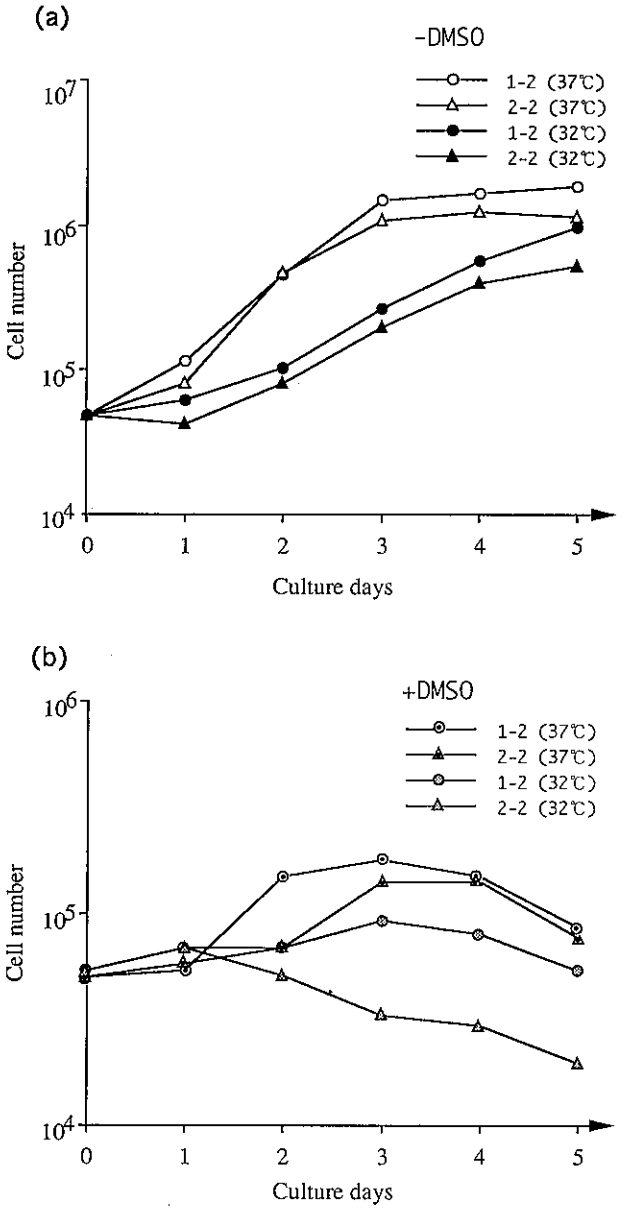

Fig. 4. Growth rates and differentiation of 1-2 and 2-2 cells at different temperatures. (a) Cells growing at 37°C and 32°C, respectively, in the absence of DMSO. (b) Cells growing at 37°C and 32°C, respectively, in the presence of 1.5% DMSO.

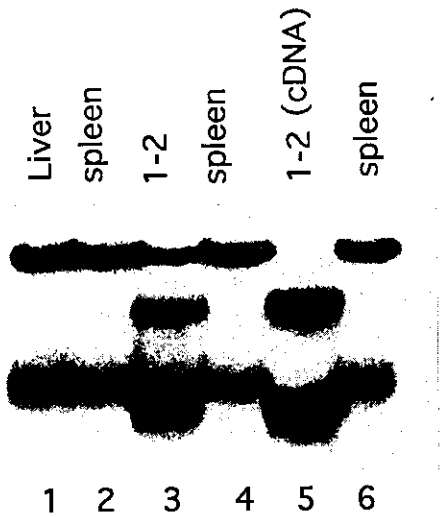

Fig. 5. PCR-SSCP analysis of genomic p53 DNA of exon 5 from 1-2 cells. Genomic p53 DNA (lane 3) and p53 cDNA (lane 5) from 1-2 cells were used.

then selected at 37°C in culture containing hygromycin B as described in "Materials and Methods." The clonal hygromycin B-resistant cells were grown and analyzed for p53 gene expression at 37°C. Two representative clones were chosen for investigating p53 protein expression. The reactivity of the $p53^{Val\text{-}135}$ and $p53^{Asp\text{-}158,\ Ile\text{-}234}$ proteins was observed with PAb421 (a panspecific monoclonal antibody for p53) as shown in Fig. 6a. The $p53^{Val\text{-}135}$ cells and $p53^{Asp\text{-}158,\ Ile\text{-}234}$ cells were both positive for PAb240 (a monoclonal antibody recognizing the mutant form of p53 protein) at 37°C (Fig. 6b). A similar experiment was performed after these cells had been growing at 32°C for 24 h. The $p53^{Val\text{-}135}$ cells were negative for PAb240, but positive for PAb246 (a monoclonal antibody recognizing a conformation-dependent epitope normally found only on the wild-type p53 protein), while $p53^{Asp\text{-}158,\ Ile\text{-}234}$ cells were negative for PAb246 (Fig. 6b).

Introduction of mutant p53 cDNA to induce anchorage independence of p53-deficient fibroblasts We previously described the highly proliferative nature of cell lines derived from a p53-deficient mouse.[21] Homozygous p53-deficient lung fibroblastic cells (p53−/−) were readily immortalized, but could not grow in the soft agar and did not produce tumors when transplanted subcutaneously into nude mice, that is to say, the p53-deficient cells showed behavior characteristic of normal cells. To determine whether or not these mutant p53 protein-expressing cells have great capacity to progress to a more transformed phenotype, a soft agar assay was performed. The assay showed that the p53-deficient cells yielded almost no soft agar colonies, but a significant number of soft agar colonies were produced by those mutant p53 protein-expressing cells at 37°C. We then carried out a similar soft agar assay with the same cells cultured at 32°C for 16 days. The $p53^{Asp\text{-}158,\ Ile\text{-}234}$ protein-expressing cells still retained the capacity to produce colonies, while the $p53^{Val\text{-}135}$ protein-expressing cells had lost the capacity of soft agar colony formation (see Table II). We thus

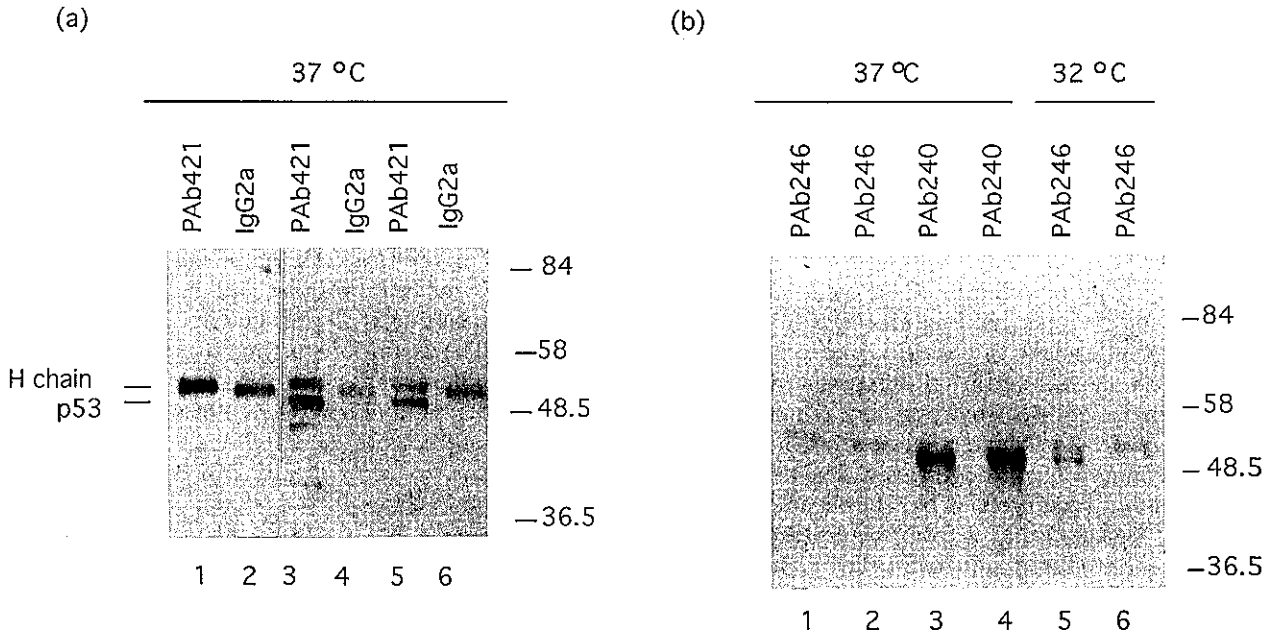

Fig. 6. Protein analysis of cells expressing $p53^{Val-135}$ or $p53^{Asp-158, Ile-234}$ at various temperatures. (a) Lysates were prepared from p53−/− cells (lanes 1 and 2), $p53^{Asp-158, Ile-234}$ cells (lanes 3 and 4) and $p53^{Val-135}$ cells (lanes 5 and 6) cultured at 37°C. PAb421 (lanes 1, 3 and 5) is a panspecific monoclonal antibody for p53. Mouse IgG2a antibody was used here as a control. (b) The $p53^{Val-135}$ cells (lanes 1, 3 and 5) and $p53^{Asp-158, Ile-234}$ cells (lanes 2, 4 and 6) growing at 37°C or 32°C for 24 h were lysed for protein analysis. PAb246 (lanes 1, 2, 5 and 6) used here recognizes a conformation-dependent epitope normally present only on wild-type p53 protein. PAb240 (lanes 3 and 4) is a monoclonal antibody recognizing mutant forms of p53 protein. The positions of the p53 protein and H chain of mouse IgG2a antibody are indicated.

Table II. Colony Formation of p53-deficient Mouse Fibroblasts on Soft Agar at 37°C and 32°C

Transfected with	Expt. 1		Expt. 2		Expt. 3		Mean±SD	
	37°C	32°C	37°C	32°C	37°C	32°C	37°C	32°C
None	0	0	0	0	0	0	0	0
Vector only	0	0	0	0	0	0	0	0
$p53^{Val-135}$ cDNA	84	0	73	0	68	0	75±8.19	0
$p53^{Asp-158, Ile-234}$ cDNA	126	98	134	101	145	86	135±9.54	95±7.94

Colonies that consisted of 50 or more cells were counted 16 days after seeding.

demonstrated that mutated type p53 induced *de novo* in the p53-deficient lung fibroblasts the capacity to grow in soft agar.

DISCUSSION

It was not easy to establish cell lines from the splenic erythroleukemic lesions induced by FLV complex, while erythroleukemic cell lines were readily established from the splenic lesions of our F-SFFV gp55-transgenic mice. This is partly because the latter lesions are non-virus producing and not highly immunogenic. The mouse erythroleukemia cell lines we have described here are examples of the cell lines thus established and provide a good experimental system in which multi-step genetic alterations can be observed during F-SFFV-gp55 leukemogenesis. The behavior *in vivo* of these non-FLV-producing erythroleukemia cells can be well studied without inducing *de novo* Friend leukemia.

In the F-SFFV gp55-transgenic mice, Friend disease-like erythroleukemia developed sporadically but more frequently in those mice with a stronger *Fv*-2^{ss} background.[10] These mice also provide a suitable system to search for the second and third genetic changes which advance the leukemic state. When these mice were infected with Mo-MuLV, the development of erythroleu-

kemia reached 100%, and one of the common integration sites of the Mo-MuLV proviral DNA was in the p53 locus.[11] p53 gene mutation is thus considered important to establish a leukemic state in Friend disease.[24, 25]

The p53 gene mutations observed in the mouse erythroleukemia cell lines with the F-SFFV gp55 transgene were interesting. One line possessed two mutations, and the other showed the temperature-sensitive p53 135 Ala→Val mutation.

It was previously reported by Johnson *et al.* that the introduction of this temperature-sensitive mutant p53 gene into a p53-deficient Friend leukemia cell line suppressed growth, caused erythroid differentiation and increased erythropoietin-sensitivity at low temperature.[23] Our EL-Tg-gp55-1-2 cell line possesses this mutation of the p53 gene on an allele endogenously. The karyograms show apparently normal sets of No. 11 chromosomes.

The $p53^{Val\text{-}135}$ gene, isolated from the erythroleukemic cell line, was introduced into p53-deficient lung fibroblastic cells to show its *de novo* acquisition of anchorage independence-inducing capacity in a temperature-sensitive manner. This ts-mutant p53 gene on an allele in the 1-2 cells may show subtle changes with temperature shift. One or two days after the temperature shift, slight DNA fragmentation occurred (M. V. Kato, unpublished observation). PCR-SSCP analysis of the p53 gene on the other allele in these 1-2 cells showed the normal mobility shift of exon 5, suggesting the presence of the intact p53 allele, although the possible presence of point mutations causing no conformational change should be checked by complete DNA sequencing.

These two non-virus-producing mouse erythroleukemia cell lines can be readily maintained *in vitro* and were shown to be adaptable for use similarly to the usual Friend leukemia cell line. They are readily transplantable to athymic nude mice without causing splenomegaly in the subcutaneous or ascites form. These erythroleukemic cell lines will be useful for studying the molecular biology of erythrodifferentiation, as well as wild-type and mutated p53 function. They will also provide an experimental model for biotherapy.

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REFERENCES

1) Ikawa, Y., Ross, J., Leder, P., Gielen, J., Packman, S., Ebert, P., Hayashi, K. and Sugano, H. Erythrodifferentiation of cultured Friend leukemia cells. *In* "Differentiation and Control of Malignancy of Tumor Cells," ed. W. Nakahara, T. Ono, T. Sugimura and H. Sugano, pp. 515–547 (1973). University of Tokyo Press, Tokyo.
2) Rifkind, R. A., Chen, Z. X., Banks, J. and Marks, P. A. Commitment: a multi-step process during induced MELC differentiation. *Adv. Exp. Med. Biol.*, **158**, 367–373 (1982).
3) Oishi, M. and Watanabe, T. The early reactions and factors involved in *in vitro* erythroid differentiation of mouse erythroleukemia (MEL) cells. *In* "Mechanism of Differentiation," ed. Fisher and B. Paul, Vol. 2, pp. 129–141 (1990). CRC Press, Boca Raton, FL.
4) Wendling, F., Moreau-Gachelin, F. and Tambourin, P. Emergence of tumorigenic cells during the course of Friend virus leukemias. *Proc. Natl. Acad. Sci. USA*, **78**, 3614–3618 (1981).
5) Ikawa, Y., Suda, Y., Furuta, Y., Tsukata, T., Takai, S. and Aizawa, S. Search for genes cooperating with SFFV-gp55 transgene in Friend erythroleukemia development. *In* "Molecular Approaches to the Study and Treatment of Human Diseases," ed. T. O. Yoshida and J. M. Wilson, pp. 229–237 (1992). Elsevier Science Publishers B. V., Amsterdam.
6) D'Andrea, A. D., Yoshimura, A., Youssoufian, H., Zon, L. I., Koo, J.-W. and Lodish, H. F. The cytoplasmic region of the erythropoietin receptor contains nonoverlapping positive and negative growth-regulatory domains. *Mol. Cell. Biol.*, **11**, 1980–1987 (1991).
7) Dusanter-Fourt, I., Casadevall, N., Lacombe, C., Muller, O., Billat, C., Fischer S. and Mayeux, P. Erythropoietin induces the tyrosine phosphorylation of its own receptor in human erythropoietin-responsive cells. *J. Biol. Chem.*, **267**, 10670–10675 (1992).
8) Yamamura, Y., Noda, M. and Ikawa, Y. Activated Ki-ras complements erythropoietin signaling in CTLL-2 cells, including tyrosine phosphorylation of a 160 kDa protein. *Proc. Natl. Acad. Sci. USA*, **91**, 8866–8870 (1994).
9) Ikawa, Y., Yoshida, M. and Yoshikura, H. Identification of proteins specific to Friend strain of spleen focus-forming virus (SFFV). *Proc. Jpn. Acad. Ser. B*, **54**, 651 (1978).
10) Aizawa, S., Suda, Y., Furuta, Y., Yagi, T., Takeda, N., Watanabe, N., Nagayoshi, M. and Ikawa, Y. *env*-derived gp55 gene of Friend spleen focus-forming virus specifically induces neoplastic proliferation of erythroid progenitor cells. *EMBO J.*, **9**, 2107–2116 (1990).
11) Mowat, M., Cheng, A., Kimura, N., Bernstein, A. and Benchimol, S. Rearrangements of the cellular p53 gene in erythroleukemic cells transformed by Friend virus. *Nature*,

314, 633–636 (1985).
12) McKnight, G. S. The induction of ovalbumin and conalbumin mRNA by estrogen and progesterone in chick oviduct explant cultures. *Cell,* **14,** 403–413 (1978).
13) Zakut-Houri, R., Oren, M., Bienz, B., Lavie, V., Hazum, S. and Givol, D. A single gene and a pseudogene for the cellular tumor antigen p53. *Nature,* **306,** 594–597 (1983).
14) Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl. Acad. Sci. USA,* **86,** 2766–2770 (1989).
15) Murakami, Y., Katahira, M., Makino, R., Hayashi, K., Hirohashi, S. and Sekiya, T. Inactivation of the retinoblastoma gene in a human lung carcinoma cell line detected by single-strand conformation polymorphism analysis of the polymerase chain reaction product of cDNA. *Oncogene,* **6,** 37–42 (1990).
16) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156–159 (1987).
17) Yoshida, M. A., Ikeuchi, T. and Sasaki, M. Differential staining of parental chromosomes in interspecific cell hybrids with a combined quinacrine and 33258 Hoechst technique. *Proc. Jpn. Acad.,* **51,** 184–187 (1975).
18) Committee on Standardized Genetic Nomenclature for Mice. Standard karyotype of the mouse, *Mus musculus. J. Hered.,* **63,** 69–72 (1972).
19) Ikawa, Y., Aida, M. and Inoue, Y. Isolation and characterization of high and low differentiation-inducible Friend leukemia lines. *Gann,* **67,** 767–770 (1976).
20) Kramer, W., Drutsa, Y., Jansen, H. W., Kramer, B., Pflugfelder, M. and Fritz, H. J. The gapped duplex DNA approach to oligonucleotide-directed mutation construction. *Nucleic Acids Res.,* **12,** 9441–9456 (1984).
21) Tsukada, T., Tomooka, Y., Takai, S., Ueda, Y., Nishikawa, S., Yagi, T., Tokunaga, T., Takeda, N., Suda, Y., Abe, S., Matsuo, I., Ikawa, Y. and Aizawa, S. Enhanced proliferative potential in culture of cells from p53-deficient mice. *Oncogene,* **8,** 3313–3322 (1993).
22) Michalovitz, D., Halevy, O. and Oren, M. Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant pf p53. *Cell,* **62,** 671–680 (1990).
23) Johnson, P., Chung, S. and Benchimol, S. Growth suppression of Friend virus-transformed erythroleukemia cells by p53 protein is accompanied by hemoglobin production and is sensitive to erythropoietin. *Mol. Cell. Biol.,* **13,** 1456–1463 (1993).
24) Munroe, D. G., Peacock, J. W. and Benchimol, S. Inactivation of the cellular p53 gene is a common feature of Friend virus-induced erythroleukemia: relationship of inactivation to dominant transforming alleles. *Mol. Cell. Biol.,* **10,** 3307–3313 (1990).
25) Ben-David, Y. and Bernstein, A. Friend virus-induced erythroleukemia and the multistage nature of cancer. *Cell,* **66,** 831–834 (1991).