

p53

Study on the expression and detection of the p53 mutation in Korean colon cancer cell lines

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Background: Inactivation in p53 tumor suppressor gene through a point mutation and deletion is one of the most frequent genetic changes found in human cancer, with 50% of an incidence. This high rate of mutation mostly suggests that the gene plays a central role in the development of cancer and the mutations detected so far were found in exons 5 to 8. Mutation of p53 locus produced accumulation of abnormal p53 protein, and negative regulation of cell proliferation and transcriptional activation as a suppressor of transformation were lost. In addition, inhibition of its normal cellular function of wild-type by mutant is an important step in tumorigenesis. **Method:** 4 colon cancer cell lines (SNU C1, C2A, C4, C5) were examined for mutation in exons 5 to 8 of the p53 tumor suppressor gene by PCR-SSCP analysis and expression pattern by western blotting and immunoprecipitation. p53-mediated transactivation ability were examined by CAT assay and base substitution of p53 in SNU C2A cell were detected by DNA sequencing. **Results:** 1) SNU C2A cell and SNU C5 cell were detected mobility shifts each in exon 5 and exon 7 of p53 gene by the PCR-SSCP method, implicating being of p53 mutation. 2) 3 colon cancer cell lines (SNU C1, SNU C2A, SNU C5) expressed wild type and mutant type p53 protein. 3) In northern blot experiment, SNU C2A and SNU C5 cell expressed high level of p53 mRNA. 4) Results of p53-mediated transactivation in colon cancer cell lines by CAT assay represented only SNU C2A cell has transcriptional activity. 5) DNA sequencing in SNU C2A cell showed missense mutation in codon 179 of one allele, histidine to arginine and wild type p53 in the other allele. **Conclusion:** Colon cancer cell lines showed correlation with mutation in p53 gene and accumulation of abnormal p53 protein. Colon cancer cell SNU C2A retained p53-mediated transactivation as heterozygous p53 with one mutant allele in 179 codon and the other wild-type allele.

Key Words: Tumor suppressor gene, p53 mutation, colon cancer cell line, PCR-SSCP, SNU C2A cell

, p53
(1). , 가 missense
retinoblastoma (Rb) p53 mutation (20), 가
가 (2, 3). (hybrid cell), 가 (conserved region), 130-290 ,
, (loss of heterozygosity) residue 117-142, 171-181, 234-258 270-286
가 (21).
(4). p53 175, 248, 273 hot spot
가 p53
(5). .
p53 17p 13.1 p53
11 exon 20Kb DNA , 가 , p53 가
mRNA 2.8Kb p53
가 53kDa (6). (16). , 3
p53 simian virus 40 (SV40) 가 ,
SV40 large T antigen (monoclonal antibody)
(7). (22).
p53 p53 p53 가
가 . G1 ,
S (transition)
(check point) ,
(oncogene) cell trans- (23).
formation . p53
(differentiation) (apoptosis)
(cell death) (8, 9).
p53
DNA sequence (GAAD45, MDM2 WAF1/ cip1) p53 75%가 allele
(10-12). , 90%
p53 (24).
 ,
 . p53 p53
(transcriptional activator)
p53 p53 DNA sequence p53
가 (13, 14), (gene trans-
cription) , p53 p53 p53
p53 p53 가 , p53 가
(15, 16). , p53 ,
 , p53
 .
p53 , , , (single strand conformation polymorphism)
(3, p53 ,
17-19). 가

isothiocyanate (28) formamide (0.26 %)/ formaldehyde (0.2%)/sample buffer (0.13%) 65 , 15 RNA foraldehyde (5%)/ agarose (1%) gel . Mytran membrane capillary transfer 1200E, 90 UV crosslinking (Hoeffer UVC 1000, UV crosslinker) RNA . Membrane hybridization oven 68 , 2 prehybridization P³²-labeling p53 probe 100 10 denature 가 . probe ml 2 × 10⁶cpm 가 65 2xSSC, 0.1% SDS 15 3 , 65 0.5xSSC, 0.1% SDS 1 Kodak XAR .

6. CAT (Chloramphenicol acetyl transferase)

p53
CAT assay calcium phosphate mammalian cell transfection kit (5 prime 3 prime, Inc.) . 6 human colorectal carcinoma 1 × 10⁶ 10cm dish 5μg Cosx1 CAT reporter plasmid CaPO₄ transfection CAT 48 phosphate buffer saline(PBS) 2 STE buffer (40mM Tris, pH 7.4, 150mM NaCl, 1mM EDTA) 1ml rubber policeman . 3000rpm 1 0.25M Tris-hydrochloride (pH7.5) . protein extract 5 freezing (-75) thawing (37) lysis . 4 , 12000 rpm 20 tube Bradford assay (Bio-Rad) . 100μg (protein extract) 4μl [¹⁴C] -chloramphenicol (56.0mci/mmol, Amersham), 30μl 2mM acetyl-CoA, 70μl 0.25M Tris (pH 8.0) 154μl가 가 . 37 1 1ml 가 ethyl acetate tube . 25μl ethyl acetate chloramphenicol thin-layer chromatography plates (Merck) spot chloroform-methanol (95:5)

, Fuji film phosphoimager 24 48 radioactivity .

7.

PCR-SSCP 가 SNU C2A exon 5 nucleotide sequence . PCR DNA TA cloning vector(Invitrogen) *E. coli* DH5 , ampicillin LB . DNA “GeneClean II” kit . DNA dideoxy-chain termination Sequi-Therm cycle sequencing kit (Epicentre technologies, USA)

primer T₇ promoter primer, 5'-d (TATACGACT CACTATAGGG)-3'; T₃ promoter primer, 5'-d (ATTA ACCCTCACTAAAGGGA)-3' (Promega, USA) .

1. PCR - SSCP p53 4 (SNU C1, SNU C2A, SNU C4, SNU C5) p53 11 exon 5(codons 126-186), 6(codons 187-224), 7(codons 225-261) 8(codons 262-306) . p53 exon 5, 6, 7, 8(amino acids 126-306) DNA 4 primer . PCR DNA 4% polyacrylamide gel single strand DNA가 2 가 1 DNA (mobility) 가 . 4 exon 5, 6, 7, 8 SSCP SNU C2A SNU C5 exon 5 exon 7 DNA band가 (Table 1, Fig. 1B).

2. p53 (27).
p53 p53 p53
p53 가 p53
p53 PAb421 p53

Table 1. Properties of p53 in colon cancer cell lines

Cell lines	Immuno blot	Northern blot	Immunoprecipitation		Mutation by PCR-SSCP
	Ab421 ^{a)}		Ab3 ^{b)}	Ab5 ^{c)}	
SV80	+	ND	ND	ND	ND
SaOS2	-	-	ND	ND	p53(-/-)
U2OS	+	+	ND	ND	p53(+/-)
MCF 7	+	+	-	+	p53(+/-)
SNU C1	+	+	+	+	-
SNU C2A	+	+	+	+	exon 5
SNU C4	±	+	-	+	-
SNU C5	+	+	+	+	exon 7

Abbreviations and symbols: (+) Epitope present, (-) Epitope absent, ND, not determined. ^{a)}PAb421 detects both mutant and wild-type p53 protein from many species. ^{b)}p53(Ab-3) recognizes many mutants p53 protein but not the wild type protein. ^{c)}p53(Ab-5) recognizes wild type p53 protein.

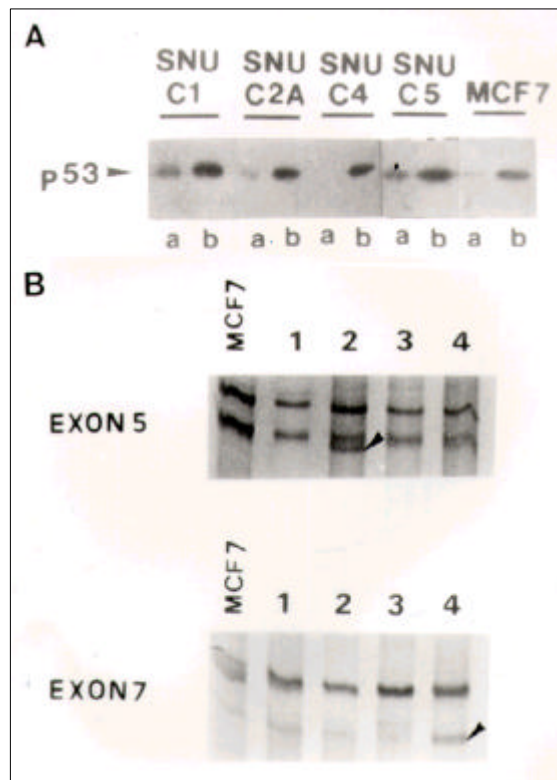


Fig. 1. PCR-SSCP analysis for p53 mutation and immunoprecipitation of p53 in colon cancer cell lines. (A) Immunoprecipitation of p53. Protein extract (400µg) from the various cancer cell lines was immunoprecipitated with anti p53 monoclonal Ab-3 and Ab-5. The resulting immunoprecipitates were immunoblotted with PAb421 as described under materials and methods, Lane a : immunoprecipitated with p53 (Ab-3) ; Lane b : immunoprecipitated with p53 (Ab-5). (B) PCR-SSCP analysis. PCR-SSCP analysis of DNA from cancer cell lines was performed as described in the text. Aberrantly immigrated DNA fragments were indicated by arrows. MCF 7 cell is normal control. The numbers above each lane correspond to specific small cell carcinoma (1:SNU C1, 2:SNU C2A, 3:SNU C4, 4:SNU C5).

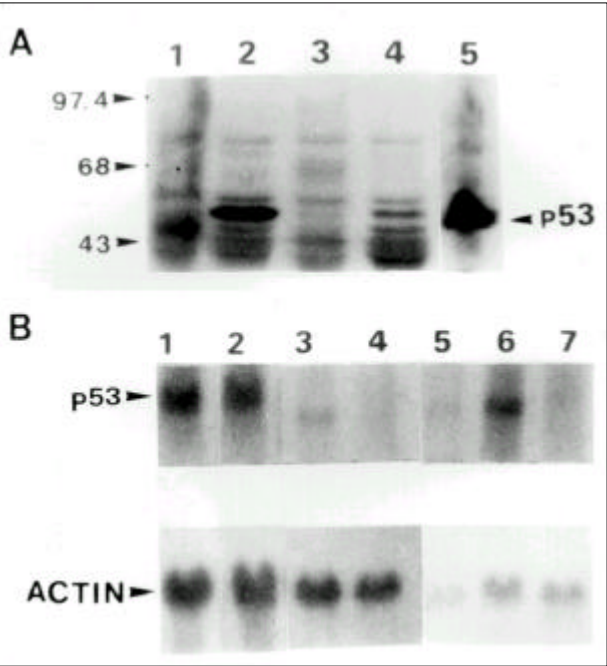


Fig. 2. Expression level of p53 in colon cancer cell lines. (A) Western blot using anti p53 monoclonal antibody(PAb421). Immunoblot was performed with PAb421 as described under "Materials and Methods". SV80 cell(lane 5) is positive control for high level p53 expression. The numbers above each lane correspond to specific small cell carcinoma(1:SNU C1, 2:SNU C2A, 3:SNU C4, 4:SNU C5). (B) Northern analysis of p53 mRNA expression. Control hybridization was done with a probe for the α -actin gene. U2OS(lane 4) and MCF7(lane 1) cell is positive control and SaOS2(lane5) cell is negative control for p53 expression. The numbers above each lane correspond to specific small cell carcinoma(2:SNU C5, 3:SNU C4, 6:SNU C2A, 7:SNU C1).

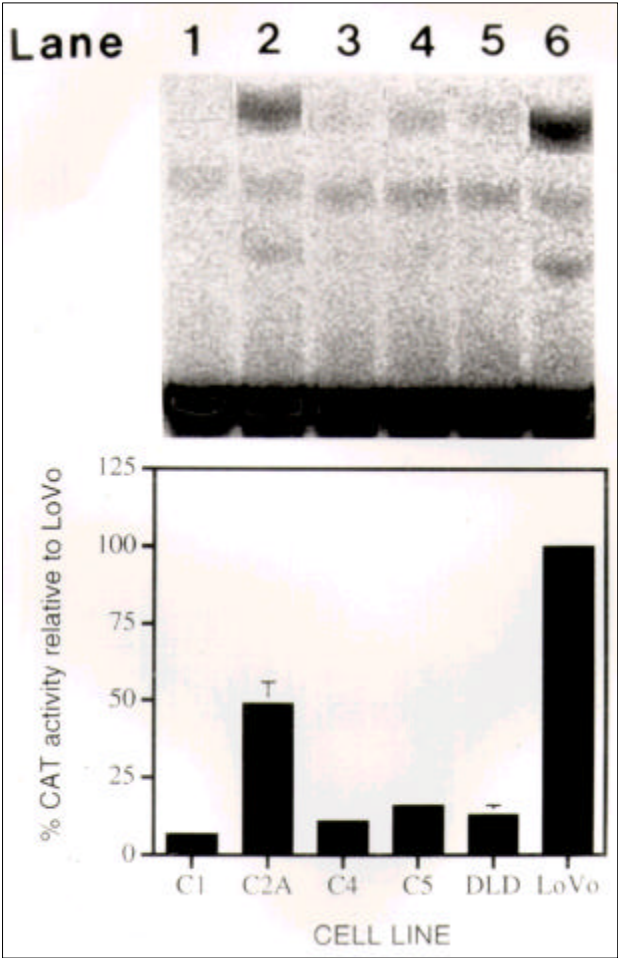


Fig. 3. Transcriptional activation of the p53-responsive reporter plasmid Cosxl CAT in human colorectal carcinoma cell lines. Human colorectal carcinoma cell lines were transfected with 5 μ g of Cosxl CAT reporter, using the calcium phosphate precipitation method. Cells were harvested and analyzed for CAT activity as described in materials and methods. DLD-1 cell (lane 5) is negative control and LoVo(lane 6) cell is positive control for transcriptional ability of p53. Graphic data represented transcriptional activation of the p53-responsive reporter plasmid Cosxl CAT in human colorectal carcinoma cell lines. The data were normalized to the value (100%) obtained with LoVo cell. The numbers above each lane correspond to specific small cell carcinoma (1:SNU C1, 2:SNU C2A, 3:SNU C4, 4:SNU C5).

, 4 3
(SNU C1, SNU C2A, SNU C5) p53
(Fig. 2A), PCR-
SSCP 7† SNU C1, SNU C5
(Table 1).
p53 p53(Ab-3)
4 3 (75%)
(SNU C1, SNU C2A, SNU C5)
, SNU C4
p53 4
(Fig. 1A).

4
(28) RNA guanidinium thiocyanate
p53 Northern blot
band α -actin
PCR-SSCP

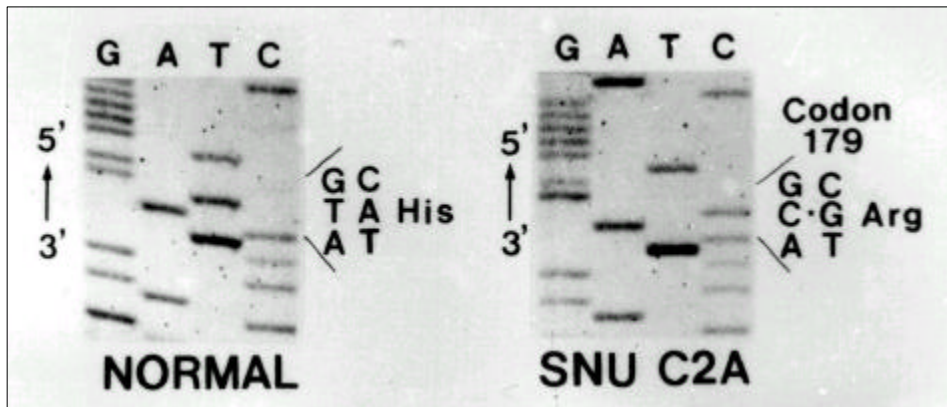


Fig. 4. Sequence analysis of p53 mutations in SNU C2A cell. The sequencing ladders of mutated DNA samples were shown together with those of normal control DNAs. SNU C2A cell showed a CAT to CGT (histidine to arginine) mutation at codon 179 in exon 5.

가 SNU C2A, SNU C5 p53 4. SNU C2A p53
mRNA
MCF 7 mRNA , PCR-SSCP exon 5
Immuno blot , p53
p53
SNU SNU C2A
C1 mRNA (Fig. 2B). PCR
3. p53 exon 5 TA cloning vector
p53
CAT가 CGT
SNU C2A 가 가 p53
(30). p53 (Fig. 4).
p53
(SNU C1, SNU C2A, SNU C4, SNU C5, DLD-1, LOVO) CAT assay . p53
Cosx1 CAT reporter plasmid
CaPO₄ 6 가
CAT . p53 (30).
LOVO CAT 100% SNU
C2A (60%) ,
SNU C1, SNU C4 SNU C5 ras, Rb p53 ,
negative control (Fig. 3). DLD-1 가 , (MHC
gene)가 , 가
가 p53 (31). p53

PCR SSCP .

(32). SSCP p53 4 가 2 (SNU 50% p53 가 C2A, SNU C5) DNA가 band (Fig. 2B.) (5). p53 DNA 가 2 가 p53 band , 가 DNA sample band missense mutation 가 (17), (37, 38). SNU C2A SNU C5 (allele), 가 p53 2 가 . p53 .

11 exon p53 , ELISA, , exon 5 9 가 exon 5 9 p53 p53 . PCR-SSCP (33). , p53 exon 5 exon 7 가 2 p53 SNU C2A SNU C5 p53 , p53 p53 DNA .

DNA sequencing DNA (SNU C2A, SNU C5) p53 mRNA (Fig. 1B). p53 20 가 , 가 p53 6 8 가 .

PCR-SSCP 가 (27). p53 (34, 35). 가 가 (35). p53 . PCR -SSCP PCR DNA fragment 가 (single missense mutation strand) , (33). 가 가 (strand) nucleotide p53 가 p53 , 2 가 . p53(Ab-3) 3 (SNU 가 , C1, SNU C2A, SNU C5) p53 SSCP 400bp DNA fragment (Fig. 2A). SNU 4 C5 SNU C2A 가 가 DNA exon 가 가

- (13,14). SNU C2A p53 p53 p53 p53
- 4 PCR-SSCP , p53 p53 p53
- 가 , 179 (histidine) (arginine)
- 2 p53 가 ,
- p53 p53 가
- SNU C1 PCR-SSCP (33),
- 가 . Coles (34) (16). p53 가
- p53 DNA SNU C2A p53
- 가 , p53
- 가 DNA 10% p53
- exon 5-8 (37)
- p53 가 가
- (36). Thompson p53 (38), 가
- (37) p53 mRNA, DNA p53
- , DNA p53 , 가
- 가 data p53
- DNA sequencing p53
- p53
- CAT SNU
- C2A DNA
- p53
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