Identification and characterization of a novel germ line p53 mutation in familial gastric cancer in the Japanese population

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Germ line mutations of the p53 gene are known to cause Li-Fraumeni syndrome, and a germ line p53 mutation has recently been reported in a small subset of familial gastric cancer (FGC) in Europe and Korea. Although the incidence of gastric cancer is very high in Japan and familial clustering is not uncommon, there has been little information on the genetic factors of FGC. Therefore, to determine the role of germ line p53 mutations in FGC in the Japanese population in this study, we used sequencing analysis to examine 80 individuals from 35 Japanese FGC families without germ line CDH1 mutations for germ line p53 mutations. One missense (c.91G>A; p.Val31Ile) and two intronic germ line mutations were found, and transcriptional activity of the Ile31 mutant on p53-responsive genes was examined to determine the functional effect of the novel p.Val31Ile germ line mutation. A luciferase reporter assay showed that the transcriptional activity of p21 (CDKN1A) and MDM2 promoters but not of the BAX promoter was significantly lower in the Ile31-type p53 than in the wild-type (wt) p53. Next, doxycycline-regulated p53-inducible H1299 cell lines were established by applying a retrovirus-mediated gene transfer system to a p53-null human H1299 cell line. Under similar p53 expression conditions shown by western blot and immunofluorescence analyses, a cell proliferation assay showed that the Ile31-type p53 had significantly lower cell proliferation suppressing activity than wt p53. These results suggest that Ile31-type p53 may be partly involved in FGC because of its low transcriptional activity and low cell proliferation suppressing activity.

Introduction

Gastric cancer is one of the most common cancers worldwide, and although its incidence has decreased in recent years, it is still high in East Asia, including Japan. Most gastric cancers are sporadic and develop in response to the cumulative effect of environmental risk factors and genetic susceptibility of individuals (1), but a small subset are aggregated in families. The risk of individuals in families with gastric cancer aggregation is higher than in individuals without familial aggregation (2,3). Since the report of the existence of germ line mutations in familial aggregation (2,3), since the report of the existence of germ line mutations of the CDH1 gene in New Zealand Maori families with diffuse-type gastric cancer (4), germ line mutations in the CDH1 gene have been found in gastric cancer families in different ethnic groups (5–11). However, the frequency of germ line CDH1 mutations is low, even in families with aggregation of gastric cancer (8–10,12,13). Kim et al. (14) recently reported finding a germ line missense mutation in the MET receptor tyrosine kinase gene in familial gastric cancer (FGC) patients without a germ line CDH1 mutation. The above findings suggest that genetic factors are important to the pathogenesis of gastric cancer with familial aggregation and that germ line mutations of genes other than CDH1 may contribute to the development of FGC.

The tumor suppressor protein p53 mediates growth arrest or apoptosis in response to potentially oncogenic cellular stresses such as DNA damage (15,16), and loss of p53 function plays a central role in the development of cancer and is caused by mutations. Many somatic p53 mutations lead to a loss of p53 function and are detected in gastric cancer (17,18). Germ line p53 mutations are known to cause Li-Fraumeni syndrome (LFS) (19–21), and interestingly, germ line p53 mutations have recently been found in European and Korean FGC patients (22–24). Since, to our knowledge only three FGC cases with a germ line p53 mutation have been reported (22–24) and none have been in Japanese FGC patients, in the present study, we investigated whether germ line p53 mutations are responsible for FGC in a Japanese population by screening 35 Japanese FGC families for p53 germ line mutations. We identified a p53-p.Val31Ile germ line mutation in one family and characterized the p53 mutant.

Materials and methods

Samples, cell lines and ethical considerations

In a previous study, 80 blood samples were collected from 35 Japanese FGC families, and DNA was extracted (10). All the families had been shown to be negative for germ line CDH1 mutation (10). Cell lines H1299 and 293T were purchased from the American Type Culture Collection (Manassas, VA). DNA from specimens of sporadic gastric cancer was collected previously (25,26). The research protocol was approved by the Institutional Review Board of Hamamatsu University School of Medicine (12–14).

Polymerase chain reaction and sequencing analysis

Fragments covering the entire coding exons and boundary regions of the p53 gene were amplified by polymerase chain reaction (PCR) with HotStarTaq DNA polymerase (QIAGEN, Valencia, CA). The primers used were as follows: 5′-TTG GAA GTG TCT CAT GCT G-3′ and 5′-TTG AAG TCT CAT GGA AGC CAG-3′ for exons 2, 3 and 4, 5′-TTG TTC GCC CAT TTC CTA CTC-3′ and 5′-GGG GCT CAA ATA AGC AGC AGG-3′ for exons 5 and 6, 5′-CTC ACG TGC CCT GCT TAT C-3′ and 5′-ACT TCC CAC TGT GTA AAG GGT CCC-3′ for exons 7, 8 and 9 and 5′-ATA CCT ACT TCT CCT CTT CCT CCT CGT-3′ and 5′-TTG AGT CAA AGG AGG GGT CAA-3′ for exons 10 and 11. The PCR products were purified with a PCR purification kit (QIAGEN) and directly sequenced with a BigDye Terminator Cycle Sequencing Reaction Kit and the ABI 3100 Genetic Analyzer (Applied Biosystems, Tokyo, Japan) as described previously (27).

Plasmid construction

Expression vectors for wild-type (wt) and mutant (Ala143) p53 were provided by Dr B. Vogelstein (28,29). Expression vector for Ile31-type p53 was generated by site-directed mutagenesis with a QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) (30) and the primer set: 5′-GCA AGG GCC GGC GGA AGC TAT GCT TAT AAG GGA-3′ for exons 7, 8 and 9 and 5′-ATA CCT ACT TCT CTT CCT CTT CCT CGT-3′ and 5′-TTG AGA AGG GAC ACC TAC TCC CTG GCT-3′. Luciferase reporter plasmids for the p21, BAX and MDM2 promoters were constructed by inserting a DNA fragment containing each promoter amplified from genomic DNA derived from blood samples from a healthy individual into the NheI-BglII site of the expression vector (pBHLuc) (Promega, Madison, WI). The PCR primers used for

Abbreviations: Dox, doxycycline; FGC, familial gastric cancer; LFS, Li-Fraumeni syndrome; PCR, polymerase chain reaction; wt, wild-type.

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amplification were as follows: 5′-CTA GCT AGC TAG CCT CCC AGG AAC ATG CCT G-3′ and 5′-GAA GAT CTT CCT GTC GGC TGC TG-3′ for the p21 promoter, 5′-CTA GCT AGC TAG ACC AAA GCA GGC CTG G-3′ and 5′-GAA GAT CTT CTA TCG CAG CTA TAA GCT-3′ for the BAX promoter and 5′-TCG TAG CTA GCT TTT GCC GAG TTG TTG G-3′ and 5′-AA TCT TCT GGA CAT AAC CAT CCT CTC G-3′ for the MD2M promoter. To construct p53 expression vector with a retroviral-mediated tetracycline-regulation system, pRevTRE-Tight vector was first constructed by inserting the P Tight promoter region amplified from pTRE-Tight vector (Clontech, Mountain View, CA) into the XhoI and BamHI sites of the pRevTRE vector (Clontech). p53 was then inserted into the pRevTRE-Tight vector at the BamHI site, and the expression vector created was named pRevTRE-Tight p53 vector. pQCXIN-Tet-On retroviral vector was constructed by inserting the Tet-On sequence amplified from the pRevTetOn vector (Clontech) into the BamHI site of pQCXIN retroviral vector (Clontech). The following PCR primers were used: 5′-TCC GCC GAT CCA AGC TTG TTA ATT AAT ATG TC-3′ and 5′-TCG ATG TGG ATC CTC GCC CCA CCT ACC CAC-3′. All the plasmid vectors were confirmed by sequencing.

Luciferase reporter assay
A p53-null H1299 human cancer cell line was cotransfected with 1 μg of the p53 expression vector, 300 ng of firefly luciferase reporter vector pGL4.10 containing a fragment of the p21 (CDKN1A), BAX or MDM2 promoter, and 3 ng of Renilla luciferase reporter vector pGL4.74(luciferase/Tk) (Promega) by using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). The cells were harvested 24 h post-transfection. Luciferase activity was quantified by a Dual Luciferase Reporter Assay System (Promega), and relative luciferase activity was calculated according to the manufacturer’s instructions. The statistical comparisons were performed by Dunnett’s multiple comparison.

Establishment of the doubly stable H1299 cell line expressing p53 under doxycycline control
Lipofectamine 2000 reagent (Invitrogen) was used to cotransfect 293T cells with a combination of pQCXIN-Tet-On and pC101A1 plasmid vectors (IMGENEX Corp., San Diego, CA) or pRevTRE-Tight p53 and pC101A1 plasmid vectors. The resulting virus-containing supernatants were used to serially infect H1299 cells-first with pQCXIN-Tet-On virus to produce a 1 mg/ml G418- and 500 μg/ml G418-resistant doubly stable H1299-Tet-On-p53 cell line. Positive clones were confirmed by sequencing. Western blot analysis
Cells were harvested in lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1% Nonidet P-40, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol and 0.1 mg/ml phenylmethylsulfonyl fluoride, and the whole-cell extracts were mixed with an equal volume of 2× sodium dodecyl sulfate sample buffer and boiled. A 25 μg amount of extracts was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and the proteins obtained were electrotransferred to a polyvinylidene difluoride membrane (GE Healthcare Bio-Science Corp., Piscataway, NJ). The membrane was blocked with non-fat milk and incubated with anti-p53 monoclonal antibody (clone D07; Novocastra Laboratories, Newcastle, UK). After washing with phosphate-buffered saline containing 0.1% Tween-20, the membrane was incubated with anti-mouse horseradish peroxidase-conjugated secondary antibody (GE Healthcare Bio-Science Corp.). After washing with phosphate-buffered saline containing 0.1% Tween-20, immunoreactivity was visualized with an ECL chemiluminescence system (GE Healthcare Bio-Science Corp.).

Indirect immunofluorescence analysis
Double stable H1299-Tet-On-p53 cells were cultured on cover slips in the presence of Dox, and after 24 h the cells were washed with phosphate-buffered saline and fixed for 20 min at room temperature with 10% formalin (WAKO, Tokyo, Japan). The cells were then permeabilized with 1% Nonidet P-40 in phosphate-buffered saline for 5 min, and incubated with 10% normal goat serum blocking solution (DAKO, Kyoto, Japan) for 1 h. The cells were probed with anti-p53 monoclonal antibody D07 (Novocastra Laboratories) at room temperature for 1 h, and indirect immunofluorescence labeling was performed at room temperature for 1 h with a Alexa Fluor 488-conjugated goat anti-mouse as the secondary antibody (Molecular Probes, Eugene, OR), and the nuclei were stained with 4′,6-diamidino-2-phenylindole (Sigma, St Louis, MO). The immunostained cells were examined under a fluorescence microscope (Olympus BX-51-FL; Olympus, Tokyo, Japan) equipped with epifluorescence filters and a photometric CCD camera (Sensicam; PCO Company, Kelheim, Germany). The images captured were digitized and stored in the image analysis program (MetaMorph; Molecular Devices, Sunnyvale, CA).

Cell proliferation assay
Double stable H1299-Tet-On-p53 cells were seeded at a density of 7500 cells per well into 96-well plates in medium containing or not containing Dox. After 72 h, the viable cell number was counted by using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer’s instructions. The kit detects mitochondrial nicotinamide adenine dinucleotide dehydrogenase activity in live cells by measuring reduction of the tetrazolium monosodium salt WST-8. The statistical comparisons between the results for wt p53 and Ile31-type p53 were performed by the two-tailed Student’s t-test.

Genotyping of the c.91G>A mutation
PCR–single-strand conformation polymorphism analysis was used to type the c.91G>A mutation. A 103 base nucleotide fragment containing the c.91G>A mutation was amplified by PCR with a primer set consisting of 5′-CTA GCA GAC ACC TGT G-3′ and 5′-CCA ACC CTT GTC TTT ACC AG-3′, and single-strand conformation polymorphism was performed as described previously (27). In brief, PCR products were diluted with loading solution, and after applying them to 8% polyacrylamide gels, the products were electrophoresed at 4°C. The DNA bands obtained were detected by silver staining. Before typing the sporadic gastric cancer cases, bands derived from the DNA of a FGCC patient that had shifted abnormally in comparison with bands derived from the DNA of cases homozygous for the c.91G allele were confirmed to be the c.91A mutation by sequencing.

Results
Identification of the novel c.91G>A germline mutation of the p53 gene in a Japanese FGC family
We screened for p53 germline mutations by PCR and subsequent sequencing analyses in 80 probands or relatives in 35 Japanese FGC families without germline CDH1 mutations, and 11 variations were found in the p53 gene locus. When we defined a germline mutation as a gene variation not registered on the database of single nucleotide polymorphisms (dbSNP) homepage of the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/SNP/), a database of Japanese single nucleotide polymorphisms (31) and the genetic polymorphism data of the International Agency for Research on Cancer TP53 mutation database (http://www-p53.iarc.fr/PolymorphismView.asp) (32); three p53 variations were classified as a germline mutation and each was found in unrelated families (Table I). One of them, the c.91G>A mutation located in exon 2, was detected only in the family with the identification number FC20G2 who was a heterozygote, and the mutation leads to an amino acid substitution of isoleucine for valine in codon 31 (Figure 1A). Two members of the family FGC20 had gastric cancer, and the proband was diagnosed with five synchronous colon cancers at 42 years of age (Figure 1B). Interestingly, there were two members with hepatocellular carcinoma and two members with uterine cancer in the same family. Unfortunately, no samples from family members other than the proband were available for genetic testing for the p53 mutation. Detection of the c.91G>A mutation was previously reported as a somatic mutation in lung cancer (35), and has never been registered on the dbSNP or Japanese single nucleotide polymorphism hompages as described above, implying that it is a novel germline mutation. The following eight p53 gene variations besides the germ line mutation were detected in our analysis: c.74 +1G>C variation, was associated with an amino acid substitution p53,iarc.fr/PolymorphismView.asp) (32); three p53 variations were classified as a germline mutation and each was found in unrelated families (Table I). One of them, the c.91G>A mutation located in exon 2, was detected only in the family with the identification number FC20G2 who was a heterozygote, and the mutation leads to an amino acid substitution of isoleucine for valine in codon 31 (Figure 1A). Two members of the family FGC20 had gastric cancer, and the proband was diagnosed with five synchronous colon cancers at 42 years of age (Figure 1B). Interestingly, there were two members with hepatocellular carcinoma and two members with uterine cancer in the same family. Unfortunately, no samples from family members other than the proband were available for genetic testing for the p53 mutation. Detection of the c.91G>A mutation was previously reported as a somatic mutation in lung cancer (35), and has never been registered on the dbSNP or Japanese single nucleotide polymorphism hompages as described above, implying that it is a novel germline mutation. The following eight p53 gene variations besides the germ line mutation were detected in our analysis: c.74 +1G>C variation, was associated with an amino acid substitution
Identification and characterization of a novel germ line p53 mutation

Table 1. Germ line p53 mutations detected in FGC families

<table>
<thead>
<tr>
<th>Nucleotide changeab</th>
<th>Location</th>
<th>Predicted effect</th>
<th>No. of individuals having the mutation</th>
<th>Homozygous/heterozygous</th>
<th>Family identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.91G&gt;A</td>
<td>Exon 3</td>
<td>p.Val31Ile</td>
<td>1</td>
<td>Heterozygous</td>
<td>FGC20</td>
</tr>
<tr>
<td>c.1100+410G&gt;A</td>
<td>Intron 10</td>
<td>Unknown</td>
<td>1</td>
<td>Heterozygous</td>
<td>FGC34</td>
</tr>
<tr>
<td>c.1101−160G&gt;A</td>
<td>Intron 10</td>
<td>Unknown</td>
<td>1</td>
<td>Heterozygous</td>
<td>FGC17</td>
</tr>
</tbody>
</table>

aMutation nomenclature is according to den Dunnen and Antonarakis (33) and den Dunnen and Paalman (34). Nucleotide +1 is the A of the ATG translation initiation codon.

bIn this paper, we defined a germ line mutation as a gene variation not registered in the single nucleotide polymorphism databases (dbSNP or Japanese single nucleotide polymorphism). The following variations were also found in our analysis, but are not listed in the table because they have been registered in the dbSNP or Japanese single nucleotide polymorphism database: c.74+38G>C, c.96+16_96+31dup16, c.97–29C>A, c.215G>C, c.672+62G>A, c.782+72C>T, c.782+92T>G and c.1101–221G>A.

Low transcriptional activation function of the Ile31-type p53

To evaluate the functional effect of the p.Val31Ile mutation, we first examined the transcriptional activation potential of the Ile31 type, because the mutation is located in the transactivation domain of p53 protein. p53-null human H1299 cells were transiently cotransfected with the p53 expression vector, luciferase reporter vector pGL4.10 containing a fragment of the p21 (CDKN1A), BAX or MDM2 promoter and a transfection control vector pGL4.74, and 24 hr after transfection, luciferase activity was measured by a Dual-Luciferase Reporter Assay System and compared in wt p53 and Ile31-type p53. The Ala143 mutation located in the DNA-binding domain of p53 protein was used as a positive control in the analysis because the mutant has been shown to have low transcriptional activity (38). Empty mammalian expression vector alone was also prepared as a negative control. The p53 responsiveness of the p21, BAX and MDM2 genes and the presence of the p53-binding sequence in the promoter of these genes has been shown previously (39). As expected, p21-, BAX- and MDM2-luciferase activity was much lower level in the cells transfected with Ala143-type p53 expression vector or empty vector than in the cells transfected with wt p53 expression vector (Figure 2). The comparison between wt and Ile31-type p53 showed that p21-luciferase and MDM2-luciferase, but not BAX-luciferase, activity was significantly lower in the cells transfected with the Ile31-type p53 expression vector than in cells transfected with the wt p53 expression vector (Figure 2). These results suggested that Ile31-type p53 has low transcriptional activity in relation to a subset of p53-responsive promoters.

Establishment of p53-inducible cell line

To precisely characterize the p.Val31Ile mutation, we decided to establish cell lines expressing various types of p53 under the control of Dox. First, p53-null human cell line H1299 was infected with virus-containing supernatants from 293T cells cotransfected with pQCXIN-Tet-On and pCL10A1 plasmids, and cells were selected with G418. Next, the stable H1299-Tet-On cell line produced was infected with virus-containing supernatants from 293T cells cotransfected with pRevTRE-Tight p53 and pCL10A1 plasmids, and cells were selected with hygromycin B. These steps resulted in the production of a G418- and hygromycin B-resistant stable H1299-Tet-On-p53 cell lines. p53 protein was expressed in the H1299-Tet-On-p53 cells in the presence of Dox. We adjusted the concentration of Dox in the medium so that the level of p53 protein expression by the wt, Ile31-type and Ala143-type p53-inducible H1299 cells was almost the same according to the results of western blot analysis with anti-p53 monoclonal antibody (Figure 3A). Nuclear expression of each type of p53 was confirmed by immunofluorescence analysis with anti-p53 monoclonal antibody (Figure 3B).

Low ability of the Ile31-type p53 protein to suppress cell proliferation

Cell proliferation suppressing activity is one of the major activities of p53 protein (15,16), and we used the H1299-Tet-On-p53 cell lines to compare the cell proliferation suppressing activity of wt p53 and...
Ile31-type p53. Cells were plated in medium with or without Dox, and 72 h later the number of viable cells was counted by measuring the reduction of the tetrazolium monosodium salt WST-8. In the absence of Dox, the number of viable H1299-Tet-On-wt p53 cells and H1299-Tet-On-Ile31-type p53 cells was similar (Figure 4A). However, in the presence of Dox, there were significantly fewer viable H1299-Tet-On-wt p53 cells than H1299-Tet-On-Ile31-type p53 cells (Figure 4B). These results suggested that Ile31-type p53 has lower cell proliferation suppressing activity than wt p53.

Frequency of the c.91G>A (p.Val31Ile) germ line mutation in sporadic gastric cancer

To more clearly demonstrate that the c.91G>A (p.Val31Ile) germ line mutation associated with low p53 activity is specific to the FGC20 family, we genotyped DNA from the blood of 110 sporadic gastric cancer patients for the mutation by PCR–single-strand conformation polymorphism analysis. The c.91G>A mutation was not found in any of them (data not shown), suggesting that the c.91G>A germ line mutation is specific to a family with gastric cancer clustering.

Discussion

Sequencing analysis for germ line p53 mutations was performed in 80 individuals from 35 Japanese FGC families without germ line CDH1 mutations, and one missense (c.91G>A; p.Val31Ile) and two intronic germ line mutations were found. The missense mutation of p.Val31Ile was detected as a heterozygote in only the proband from one FGC family and is a novel germ line mutation. The functional effect of the p.Val31Ile mutation was then examined to investigate the role of the p.Val31Ile mutation in the familial clustering of cancers. A luciferase reporter assay showed that transcriptional activity on p21 and MDM2 promoters but not the BAX promoter was significantly lower in those with Ile31-type p53 than wt p53. Next, by applying a retrovirus-mediated gene transfer system to p53-null human cell line H1299, we established a stable cell line H1299 expressing p53 under Dox control. Expression of p53 protein was regulated by addition of Dox at a similar level of the western blot analysis, and nuclear p53 expression was confirmed by immunofluorescence analysis. A cell proliferation assay of Ile31-type p53 showed significantly lower cell proliferation suppressing activity than wt p53. These results suggest that Ile31-type p53 may be partly involved in FGC because of its low transcriptional activity and low cell proliferation suppressing activity in comparison with wt p53.

There have been three reports of FGC families in which a germ line mutation of the p53 gene has been identified in Europe and Korea (22–24). One of the families meet the clinical criteria for LFS (24), but, the other two do not (22,23). LFS is a cancer predisposition syndrome associated with sarcoma, breast cancer, brain tumors, leukemia, adrenocortical carcinoma, melanoma and other cancers, including gastric cancer (19–21). However, because of the relatively low frequency of gastric cancer in LFS families with the characteristics of both LFS and FGC are thought to be rare, and in fact, the case described above is the only one even reported (24). On the other hand, when our Japanese case is added to the previous two German and Portuguese cases (22,23), the number of FGC families with a germ line p53 mutation that do not meet the clinical criteria for LFS rise to three. As regards frequency of the detection of FGC with a germ line p53 mutation, according to the previous papers (22,23) and the present paper, one FGC with a germ line p53 mutation was found in 35 Japanese families with FGC, one among 34 German families with FGC and one among 10 Portuguese families with FGC. The results of this and previous studies suggested that the p53 gene should be included in genetic testing of FGC families.

Fig. 2. Low transcriptional activation function of the Ile31-type p53 shown by luciferase assay. p21-, BAX- and MDM2-luciferase activity was measured in p53-null H1299 cells transiently transfected with p53 expression vector, firefly luciferase reporter vector pGL4.10 and a transfection control vector pGL4.74. Luciferase activity was quantified with a Dual-Luciferase Reporter Assay System, and relative luciferase activity was calculated. Values are means ± standard deviations of three independent experiments. In each p53-responsive gene, i.e. p21, BAX and MDM2 the luciferase activity of cells transfected with wt p53 was set equal to 1.0. P values were calculated by Dunnett’s multiple comparison. **P < 0.001; *P < 0.0001.

Fig. 3. Expression of p53 protein in p53-inducible H1299-Tet-On-p53 cell lines in the presence of Dox. (A) Detection of protein expression of wt, Ile31-type and Ala143-type p53 by western blot analysis with anti-p53 antibody D07. (B) Detection of protein expression of wt, Ile31-type and Ala143-type p53 by immunofluorescence analysis. p53 protein (green) was stained with anti-p53 antibody 4’6-diamidino-2-phenylindole (blue).
cell proliferation suppressing activity levels of p53 mutants are not always parallel, as has been shown previously (40). If in addition to the results of the current study, we compared p53 activities of wt p53, Ile31-type p53 and Ala143-type p53 proteins other than their transcriptional activity and cell proliferation suppressing activity, we would be able to evaluate the level of the mutant p53 protein ability more precisely. This might enable us to better understand the role of Ile31-type p53 in the occurrence of FGC.

In this study, we used a retrovirus-mediated gene transfer system to establish Dox-regulated cell lines with inducible, doubly stable expression of wt p53, Ile31-type p53 and Ala143-type p53 proteins other than their transcriptional activity and cell proliferation suppressing activity, we would be able to evaluate the level of the mutant p53 protein ability more precisely. This might enable us to better understand the role of Ile31-type p53 in the occurrence of FGC.

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Conflict of Interest Statement: None declared.

References


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