

Heterozygous *TP53*_{stop146/R72P} fibroblasts from a Li-Fraumeni syndrome patient with impaired response to DNA damage

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Abstract. The Li-Fraumeni syndrome (LFS) is a rare autosomal dominant hereditary cancer syndrome, characterized by a wide spectrum of neoplasms, occurring in children and young adults. The identification of germline *TP53* mutations in LFS has given rise to a number of *in vitro* studies using cultures of cancer cells and non-tumoral fibroblasts presenting germline *TP53* mutations. In the present study, we performed a detailed documentation of the pedigree of an LFS family with a comprehensive analysis of genotype-phenotype correlations. We sequenced the *TP53* gene and verified that the proband carries a germline nonsense mutation in codon 146 in one allele, the *TP53*_{Arg72Pro} polymorphism in the second, and other intronic polymorphisms in the *TP53* gene. In order to investigate the disruption of the p53 function in a patient presenting this mutation and the *TP53*_{Arg72Pro} polymorphism who had so far suffered five malignant tumors and a benign meningioma, we tested her fibroblasts in response to DNA damage by evaluating the proliferation rate, apoptosis, and disruption of the *TP53* pathway. The proband's heterozygous fibroblasts were not as efficient as control fibroblasts or those of her mother, who carried only the *TP53*_{Arg72Pro} polymorphism, in causing cell arrest and cell death after DNA damage, which was correlated with diminished TP21 protein levels.

Introduction

Li-Fraumeni syndrome (LFS) is a rare autosomal disorder characterized by a familial clustering of cancer cases with an early onset and a predominance of sarcomas, breast cancers, cerebral tumors, leukemia, and adrenocortical carcinomas. Classical LFS is defined as a proband developing a sarcoma before age 45 who has a first-degree relative <45 years old diagnosed with any cancer as well as one additional first- or second-degree relative who developed any cancer within 45 years of age or who was diagnosed with a sarcoma at any age (1).

Approximately 75% of families classified as classical LFS present a germline mutation in the tumor suppressor gene *TP53* localized in chromosome 17p13.1 (2,3). *TP53* protein is a transcription factor constitutively expressed in most cell types that is involved in cell cycle control, DNA repair, and apoptosis (4,5). Cellular stresses, such as exposure to ionising or ultraviolet radiations, DNA-damaging drugs, or hypoxia (6), trigger the activation and the accumulation of *TP53* proteins that translocate into the nucleus and guide the transcription of genes involved in the cellular process (5).

The activity of p53 thus prevents the accumulation of mutations which may lead to tumorigenesis. Accordingly, sporadic cancers present somatic mutations in *TP53* with a frequency as high as 60% depending upon the cancer type, and this gene is one of the more frequently altered ones in human neoplasms (7). In contrast to other tumor suppressor genes that are frequently inactivated by frameshift or nonsense mutations, the majority of *TP53* mutations in cancer are missense and generate stable and sub-functional proteins (8).

Missense mutations in the DNA-binding domain have been associated with the appearance of brain tumors and with a high frequency or early onset of breast cancers, while mutations outside this region have been associated with adrenocortical carcinomas (9). Nonsense mutations are not reported to predispose to particular tumor types, but are generally associated with an early onset of tumors (10).

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Apart from the mutations, it has been proposed that polymorphisms in the *TP53* gene could alter its biological properties. One common single-nucleotide polymorphism (SNP) occurs at amino acid 72 of the *TP53* gene resulting in either an arginine (*TP53*^{Arg}) or a proline residue (*TP53*^{Pro}). This polymorphism is located in a proline-rich region of the *TP53* protein that is required for growth suppression and apoptosis. The Poly-Pro region has been shown to be essential for apoptosis but not for cell cycle arrest (11).

The *TP53* protein with 72^{Pro} is structurally different from the *TP53*^{Arg}, as shown by its altered electrophoretic mobility (12), suggesting that it may have a functional impact. Storey *et al* (13) found that *TP53*^{Arg} was more susceptible to E6-mediated degradation than the *TP53*^{Pro} variant, and individuals carrying the Arg allele were more susceptible to HPV (human papillomavirus)-associated tumorigenesis than individuals carrying the Pro/Arg genotype.

Studies have shown that although both variants do not differ in the ability to bind to DNA, they display distinct biological properties of interaction with basic components of the transcriptional machinery, which could influence their transcriptional abilities (14).

Whether the *TP53* codon 72 polymorphism has an important influence on cancer development is still uncertain due to the paradoxical functions of both variants (15). Several studies have demonstrated that *TP53*^{Arg} represses more efficiently the transformation of primary cells (14) and induces apoptosis better than the corresponding *TP53*^{Pro} variant (16,17). In contrast, the *TP53*^{Pro} form is more effective than *TP53*^{Arg} in inducing cell-cycle arrest (18) and inactivating several *TP53*-dependent DNA-repair target genes in several cellular systems (19). Additionally, reports suggest that the *TP53*^{Pro} variant promotes genomic stability because it exhibits reduced micronuclei formation (19).

The molecular mechanism of some observations has not yet been established; however, it is known that mutations on one *TP53* allele are often accompanied by loss of heterozygosity (LOH) (3) that favors tumoral evolution (20). Studies of widely diverse types of tumors have demonstrated the predominant loss of *TP53*^{Pro} and the presence of mutations preferentially in the *TP53*^{Arg} allele, suggesting that although the *TP53*^{Arg} variant is better in inducing apoptosis, the mutations could affect its function in preventing tumor development.

The present study was designed to investigate the molecular status of the *TP53* gene in a family with Li-Fraumeni syndrome and to functionally characterize the patient's fibroblast rate of proliferation and apoptosis after DNA-damage. The proband was previously investigated as a carrier of a germline nonsense mutation in codon 146 (TGG146TGA) of the *TP53* gene (21). This is the first study characterizing the response of heterozygous fibroblasts in the presence of the *TP53*^{Arg72Pro} polymorphism and in the absence of the wild-type protein.

Patients and methods

Case history. A 57-year-old Caucasian female proband and her family members were under molecular investigation to evaluate her record of familial cancers and disruption of the *p53* function. At the age of 38 years, the patient was

diagnosed with colonic adenocarcinoma (T₃N₀M₀) which was completely removed, and at the same age she underwent an excisional biopsy of a dysplastic nevus. At age 39, another primary tumor was diagnosed and classified as a mucinous adenocarcinoma of the ovary (stage III), and the patient underwent bilateral salpingoophorectomy and hysterectomy. At age 40, a ductal carcinoma *in situ* was diagnosed in the proband's left breast, which reappeared despite tamoxifen treatment for 2 years. The patient underwent left breast quadrantectomy and radiotherapy. A similar lesion developed at age 42 in the right breast associated with ductal invasive carcinoma (grade II-III). Complete bilateral mastectomy was suggested, but the patient chose quadrantectomy of the right breast with axillary dissection. At age 49, she was diagnosed with cancer of the pancreas and underwent surgical resection. A metastatic focus of the pancreatic tumor was surgically resected from the patient's liver 3 years later, but lesions reappeared at both 4.5 and 5 years later. Each liver lesion was diagnosed as a pancreatic metastasis and subjected to complete surgical resection associated with chemotherapy and radiotherapy. After the third and last relapse (in lymph nodes and liver), she was administered chemotherapy (capecitabine and oxaliplatin) and underwent radical peri-hilar and retro-peritoneal lymph node dissection associated with hepatectomy. Despite the excellent response to this treatment, one lesion remained active and chemotherapy was resumed (capecitabine and erlotinib). After 5 primary malignant cancers and 4 relapses of the last one (pancreas), she presented with a benign meningioma.

The family pedigree revealed the presence of several paternal relatives who had been diagnosed with tumors, the majority of which appeared at ages ≤46 years (Fig. 1). The proband's father had been diagnosed with cancer of the intestine at age 46, and her paternal uncle presented with prostate cancer at the same age; both survived only for 1 year after the diagnoses. The paternal grandmother had died due to breast cancer at age 29. The proband's older sister had succumbed to leukemia at 2 years of age, and her younger sister had died at 31 years of age from breast cancer and melanoma.

This study was approved by the Medical Research Ethics Committee of the Hospital de Clínicas of Universidade Federal do Paraná, and all participants provided informed consent. Peripheral blood samples were obtained from the proband, her husband, her two children, her cousin, and her mother; skin biopsies were acquired from the proband, her mother, and a healthy donor.

***TP53* sequencing.** The DNA was extracted from fibroblasts and the *TP53* genomic sequence was analyzed by the Clinical Molecular Diagnostic Laboratory of the City of Hope, California, USA. For samples from the proband and her mother, the gene was amplified in 5 segments, covering all the coding exons (2-11) as well as the adjacent intronic splicing sequences. All deviations from the control sequence were confirmed by DNA sequence analysis of the strand from an independently amplified fragment. All other siblings were examined for the presence of any mutation found in the proband and/or her mother. This analysis has a reported sensitivity of 95% and a specificity of 98%. None of the relatives presented any mutation in the *TP53* gene.

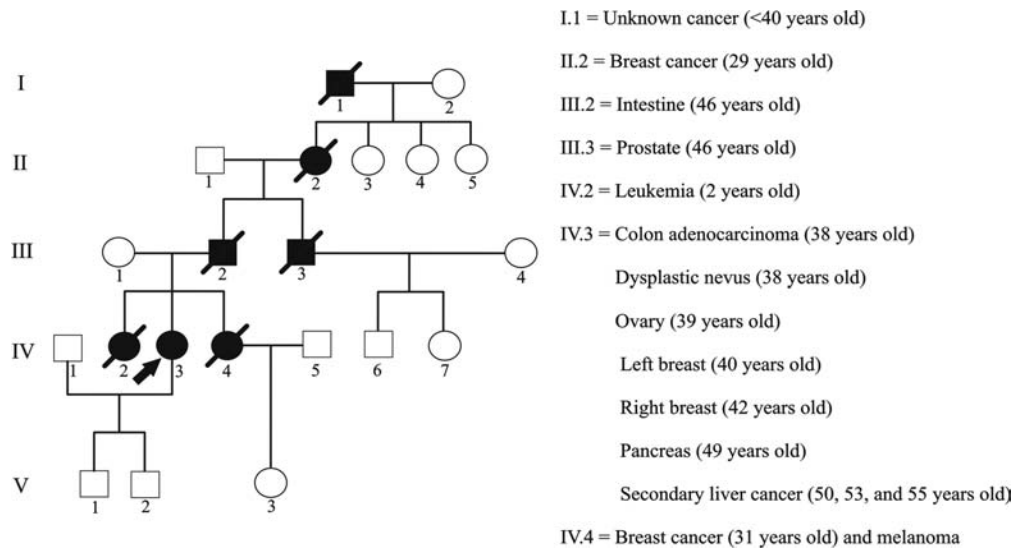


Figure 1. Family hereditogram. The proband is indicated by an arrow; oblique line symbolizes deceased subjects and filled and open symbols represent symptomatic and asymptomatic subjects, respectively. The tumor pathology, site, and age at cancer diagnosis are indicated for each affected individual.

To confirm that the 13116G→A mutation and the 12139G>C polymorphism were present on different alleles, DNA samples from the patient's and her mother's fibroblasts were amplified with primers 11724-F1 (AGCCGCAGTCAGATCCTAGC) and 13200-R1 (CTCACAACTCCGTCATGTG), amplicons were cloned into pGEM-T Easy vector (Promega Corp., Madison, WI), and positive clones were sequenced bidirectionally.

Proliferation and apoptosis assays. Skin biopsies obtained from the proband, her mother, and the healthy donor were washed three times with culture medium (85% RPMI-1640 supplemented with 15% fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulphate). After removal of the adipose tissue, the biopsies were sliced into ~4-mm² fragments that were distributed into 25-cm² culture flasks. The culture medium was changed every 3 days. After 1 week of incubation in humidified air and 5% CO₂ at 37°C, when the fibroblasts visibly adhered to the culture flask, the skin fragments were removed. Cells from the third passage were resuspended in culture medium containing FBS and 10% DMSO and frozen in liquid nitrogen for future use. All the subsequent tests were performed on cells from the fourth passage.

Fibroblasts were seeded (1×10⁵ cells/well) and cultured for 24 h on poly-L-lysine-coated round slides placed in 24-well culture plates. Test plates were irradiated (5 Gy) with a ⁶⁰Co source yielding 68.23 cGy/min at 80 cm, while control plates received no irradiation. Test and control cells were then cultured for 6 h post-irradiation and used for further studies. Proliferation was assessed by the immunofluorescent detection of 5-Bromo-2'-deoxy-uridine (BrdU) incorporation using the *In Situ* Cell Proliferation Kit, FLUOS (Roche, Meylan, France). The *in situ* evaluation of apoptosis was performed by the Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay using the *In Situ* Cell Death Detection Kit, Fluorescein (Roche). For proliferation and apoptosis tests, cells were evaluated on a fluorescence microscope (Axioskop 2 mot plus; Carl Zeiss, Heidelberg, Germany)

and image acquisition was performed with the Expo 2.1.1 software (Applied Spectral Imaging, Germany). Five slides were captured for each experiment, including at least 200 cells/experimental or control group. Results of the percentage of stained cells were presented as mean ± SD. Statistical comparisons were made using Student's t-test, with P<0.05 being statistically significant.

Western blotting. Etoposide is a topoisomerase II inhibitor known to induce DNA double-strand breaks leading to the activation of the TP53 pathway and apoptosis in a manner similar to that of irradiation. In this experiment, irradiation (5 Gy) treatment with a ⁶⁰Co source yielding 68.23 cGy/min at 80 cm or 100 µM etoposide was used for inducing cellular damage in the cell culture. For this objective, subject fibroblasts were cultured in 25-cm² flasks as described above. After 6, 12, 18, or 24 h of the treatment, the cells were washed with PBS, scraped into 200 µl of hot Laemmli sample buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, and 0.125 M Tris HCl; pH 6.8) and then boiled for 5 min.

Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. Immunoblotting was performed using a chemiluminescence system for protein detection (ECL Plus, GE Healthcare). Primary antibodies used were as follows: rabbit anti-p21 antibody (1:200 dilution; Santa Cruz); mouse monoclonal anti-β-tubulin (1:1000 dilution; Sigma-Aldrich). The images were obtained by Luminescent Image Analyzer LAS-3000 (Fujifilm, Japan).

Results

TP53 sequencing. Sequencing of the proband's TP53 gene revealed a G→A transition mutation at nucleotide no. 13116 (GenBank entry X54156), resulting in a nonsense mutation at codon 146 of the p53 protein (13116 G→A, W146X; Fig. 2). Five heterozygous polymorphisms were also identified, four of which were intronic (IVS02+37 G→C; IVS03+40ins16 bp;

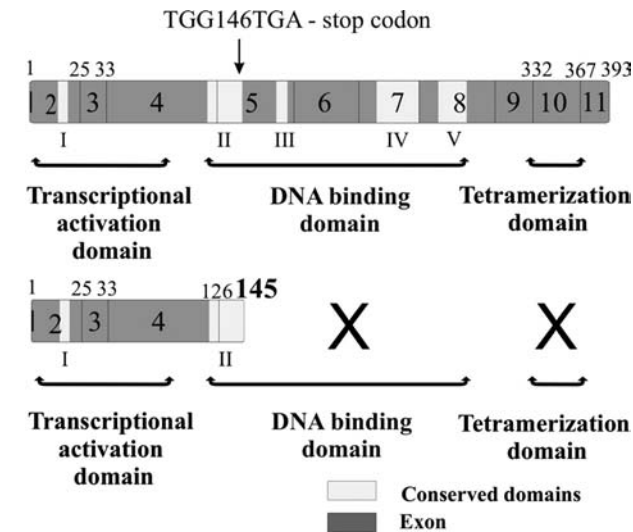


Figure 2. Structure of TP53 protein. The upper figure shows the three functional domains: the amino terminal domain (aa 1-42) involved in transcriptional activation; the hydrophobic central DNA-binding core (aa 102-292); and the tetramerization domain (aa 323-356). In the lower figure, the nonsense mutation at codon 146 (TGG146TGA, W146X) leads to a truncated TP53 protein of 145 residues.

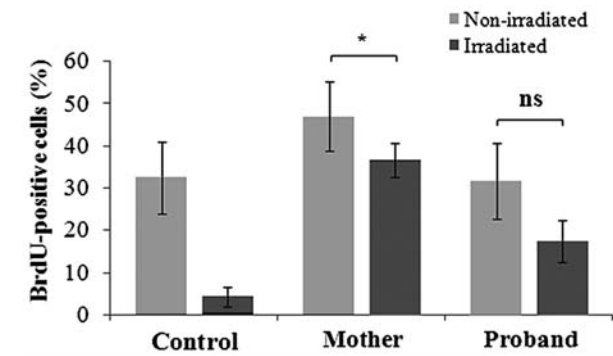


Figure 3. Proliferation monitored by BrdU incorporation. The percentage of BrdU-positive fibroblasts was observed at 6 h after the irradiation treatment. The radiation led to a decrease in the proliferation of cells from the healthy donor as well as of fibroblasts from the proband's mother. The reduction in cell proliferation of the patient's fibroblasts before and after irradiation was not statistically significant (ns). The results represent the means \pm SD of five experiments expressed as a percentage of total cells counted; * $P < 0.05$ ($P = 0.033$) Student's t-test, in comparison with non-irradiated cells.

IVS10-221 G→A; IVS10+60 T→C) and one exonic (12139 G→C, Arg72Pro). None of the fifth-generation members and individuals III.1 and IV.1 (Fig. 1) possessed the W146X mutated allele. In addition, the exonic polymorphism at codon 72 encoded proline inherited from the proband's mother was found in the wild-type *P53* gene copy.

The W146X mutated allele is predicted to encode for a 145-aa TP53 protein containing the transcriptional activation domain but missing the DNA binding and tetramerization domains.

Cellular proliferation and apoptosis assays. Cell proliferation was measured by immunofluorescence detection of BrdU incorporation 6 h after exposure to radiation. The decrease

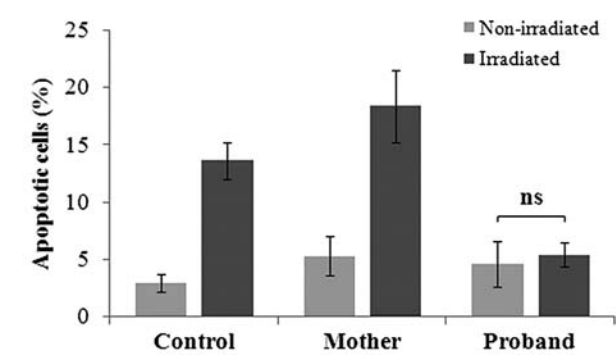


Figure 4. Apoptosis evaluation after radiation. The percentage of TUNEL-positive fibroblasts observed at 6 h after radiation exposure. The treatment was able to induce apoptosis in fibroblasts from the healthy donor and from the patient's mother; the increase in apoptosis observed in the patient's fibroblasts was not statistically significant (ns). In the absence of radiation, the apoptotic rate was comparable for the patient, her mother, and the control samples. The results represent the means \pm SD of five experiments expressed as a percentage of total cells counted.

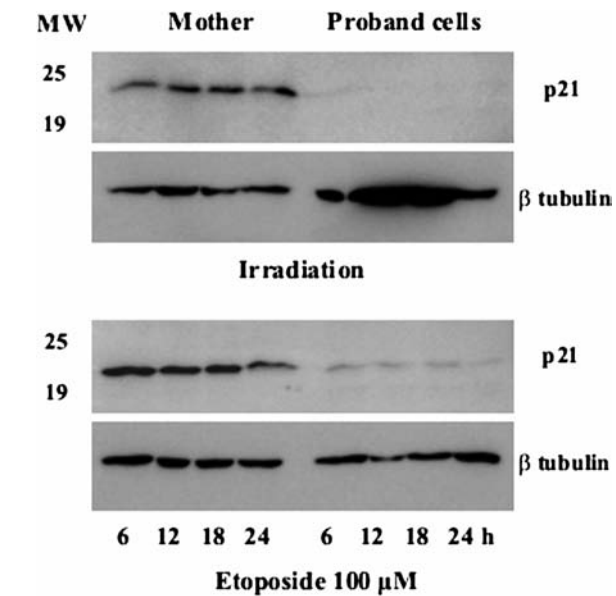


Figure 5. Expression of *TP21* in fibroblasts from the proband and the wt-p53 control after treatment. Fibroblasts were harvested after 6, 12, 18, and 24 h of exposure to 5 Gy γ irradiation or 100 μ M etoposide as indicated. Total protein extracts were loaded and submitted for Western blotting analysis using anti TP21 antibodies. The protein samples were normalized by reblotting the same membranes using anti β -tubulin antibodies.

in proliferation upon exposure to radiation was evident on fibroblasts from the healthy donor and on cells from the proband's mother ($P = 0.033$) who carried only the exonic *TP53*_{Arg72Pro} polymorphism. The proband's fibroblasts did not present a statistically significant reduction in proliferation when compared to their non-irradiated cells ($P = 0.07$) (Fig. 3).

The death rate of the proband's fibroblasts upon exposure to ionizing radiation, as measured by the TUNEL assay, was markedly inhibited as compared to cells from her mother and the healthy donor. However, the apoptosis rate of the proband's fibroblasts did not change significantly after the irradiation (Fig. 4).

Background cell proliferation and apoptosis rates in the absence of radiation-induced DNA damage were similar in the proband, her mother, and control sample cells, indicating no modification in basal cellular proliferation and death rate in the proband.

TP21 protein levels after DNA damage caused by etoposide treatment and irradiation. Exponentially growing fibroblasts were irradiated or treated with etoposide, and samples were collected for analysis at 6, 12, 18, and 24 h following treatment. We examined whether the proband's cells (TGG146TGA) lost their ability to stimulate the endogenous expression of the *TP21/WAF1* gene using Western blot analysis.

Normal fibroblasts presenting a wild-type *TP53* allele and the *TP53*_{Arg72Pro} polymorphism in the second allele induced increased *TP21* expression in the first 6 h after treatment. In contrast, in the proband's fibroblasts (13116 G→A, W146X and *TP53*_{Arg72Pro}), these levels did not change. In addition, a markedly reduced expression of p21 was found after 24 h of treatment (Fig. 5). These results indicate that *TP53* from the proband's cells was unable to induce downstream target promoters.

Discussion

The vulnerability of LFS families to the early development of cancer could be due to negative dominant activity inhibiting the transactivation of its target genes (22) owing to the loss of the remaining *TP53* wild-type allele. The role played by some polymorphic proteins in the pathogenesis remains under debate (15,23-25).

Several clinical studies have proposed the hypothesis that the SNP within the proline-rich region of *TP53* at codon 72 (arginine or proline), is involved in cancer predisposition (Table I). Unfortunately, an appropriate genetically modified homologous mouse model does not exist; therefore, this hypothesis cannot be appropriately tested *in vivo* (39).

In the present study, we describe a human cell line expressing a truncated *TP53* protein with 145 residues and a wild-type copy presenting the polymorphism *TP53*_{Arg72Pro}. The primary question was whether fibroblasts expressing only a single full copy of the wild-type *TP53* protein, which presents proline in codon 72, would have a diminished response to DNA damage. To this end, two approaches were tested, i.e., DNA damage caused by exposure to 5 Gy or to etoposide. In contrast to the response of the normal proband's mother's fibroblasts that presented normal *TP53* copies and the *TP53*_{Arg72Pro} polymorphism, the proband's cells presented a low-level of *TP21* expression, suggesting a diminished function of the polymorphic *TP53*_{72Pro} in activating *TP53*-responsive promoters and in controlling the cell cycle. The expression of *TP21* in response to the used concentration of etoposide was less intense than to radiation exposure, and it remained low for at least 24 h. The resistance of the proband's cells to radiation was also noted through the proliferation rate. There was no statistically significant reduction in the cell proliferation rate after radiation.

Similarly, the data obtained using the TUNEL method showed that the radiation-treated fibroblasts from the proband were inefficient in activating the apoptosis pathway. Previous

studies demonstrated that *TP53*_{Arg} activates cell death more efficiently than the *TP53*_{Pro} form (16,40). Until recently, very little was known about the mechanism involved, other than the greater ability of *TP53*_{Arg} to localize to the mitochondria (16). This difference could also be explained by studies showing that regulators of *TP53*, particularly iASPP (inhibitor of apoptosis stimulating proteins of p53), selectively bind and inhibit the apoptotic potential of the *TP53*_{Pro} variant by the reduction of expression of ASSP1 and ASSP2 (17).

Although some studies (Table I) indicate that the codon 72 polymorphism confers vulnerability to cancer development by altering the p53 biological function, the effect of each variant on the evolution and prognosis depends upon the cancer type (15). The molecular mechanism of this event remains controversial; however, studies have shown individuals with germline *TP53*_{Arg72Pro} present with mutations preferentially in the *TP53*_{Arg} allele (Table I). Moreover, there is a higher LOH frequency related to the *TP53*_{Arg72Pro} than to the *TP53*_{Arg72Arg} genotype, predominantly with the loss of *TP53*_{Pro} in heterozygous genotypes.

Our results suggest that tumorigenesis is caused by the association of the germline-truncated p53 and the *TP53*_{Arg72Pro} polymorphism. Furthermore, it has been proposed that patients presenting with a mutated *TP53*_{Arg} allele are more resistant to antitumoral drugs than those expressing *TP53* protein encoding proline in codon 72 (41). Biochemical analyses have demonstrated that the *TP53*_{Arg} mutant efficiently inhibits p73, a structural and functional homologue of p53 (27).

Alternatively, the impaired p21 expression and alteration in cell proliferation and apoptosis observed in the present study could be due to *TP53* haplo-insufficiency. The *TP53* haplo-insufficiency reported by Lynch and Milner (42) in *TP53*^{+/-} human colorectal carcinoma cells identified a 4-fold decrease in *TP53* mRNA and protein levels as compared with *TP53*^{+/+} cells under both stressed and non-stressed conditions. These authors also pointed out that the attenuated *TP53* stress response was correlated with reduced G₁ arrest and apoptosis. One possibility to be considered is that the mutated allele is inherited and there is somatic loss of part of or the entire second allele (43) during passaging (44) or in response to DNA-damaging agents due to chromosome instability (45).

Although sequencing of the *TP53* gene was performed using DNA from the fibroblasts, it is possible that *in vivo* exposure to radiation (breast cancer radiotherapy received by the proband) or the γ radiation restricted to the fibroblasts (on passage 2) after DNA sequencing (5 Gy) could have hit other targets in the *TP53* or other genes, resulting in loss of function or chromosomal instability (45). However, in the present case, this does not appear to have occurred because fibroblasts from the proband's mother presenting only the *TP53*_{Arg72Pro} polymorphism were similarly prone to any type of damage. This finding is in line with the observation of the lower susceptibility of normal fibroblasts to loss of cell cycle arrest function following radiation (46).

Thus, it is possible that the *in vitro* DNA damage induced by the radiation exposure or treatment with etoposide could have promoted a synergism between the nonsense *TP53* mutation and the *TP53*_{Arg72Pro} polymorphism resulting in LOH and loss of its tumor suppressor activity.

Table I. *TP53*_{Arg72Pro} studies: correlation between high frequency of mutation and LOH.

Cancer type (refs.)	Arg72Pro (n)	Mutated allele/ Arg72Pro		LOH/ Arg72Pro		Mutated allele/LOH		Comments
		72Arg	72Pro	72Arg	72Pro	72Arg	72Pro	
Vulva cancer (26)	18/31	10/18	1/18	2/18	13/18	8/15	1/15	<i>TP53</i> LOH is more common in <i>TP53</i> _{Arg72Pro} than in <i>TP53</i> _{Arg72Arg} individuals with preferential retention of the Arg72 allele
Metastasis (26)	3/5	2/3	1/3	0/3	2/3	2/2	0/2	
Vulva cancer (27)	21/36	12/21	2/21	1/21	10/21	10/11	1/11	
Non-melanoma skin cancer (27)	9/16	5/9	0/9	0/9	5/9	5/5	0/5	All mutated <i>TP53</i> bind to p73 in cell lines containing the Arg72 allele
Head and neck cancer (27)	10/26	4/10	3/10	3/10	0/10	0/3	3/3	
Brain tumor (28)	39	25/39	14/39	NE	NE			
Liver cancer (28)	8	5/8	3/8					Only recessive mutants showed selection of Arg72
Colon cancer (28)	11	8/11	3/11					
Breast cancer (28)	17	13/17	4/17					
Pancreatic cancer (28)	7	5/7	2/7					
Oral cancer (28)	17	13/17	4/17					
Skin cancer (28)	1	0/1	1/1					
Urinary tract carcinoma (29)	11/40	8/11	3/11	1/11	5/11	5/6	1/6	The mutant Arg72 allele may be preferentially selected during tumorigenesis
Skin cancer (30)	14/70	6/14	2/14	0/14	7/14	5/7	0/7	Preferential loss of Pro72 in heterozygous individuals is independent of the presence or absence of mutation in the second allele
Lung cancer (31)	27/54	NE	NE	3/27	11/27	NE	NE	The Arg72 allele is preferentially retained in Arg72Pro patients
Hepatocellular carcinoma (32)	47/97	NE	NE	0/47	15/47	NE	NE	
Breast carcinoma (33)	29/67	NE	NE	2/29	7/29	NE	NE	Arg72 was the preferentially mutated allele
Colorectal cancer (34)	26 ^a /61	8/26	0/26	0/26	10/26	10/10	0/10	Retention of the Arg72 allele is associated with reduced disease-free and overall survival
Liver metastasis (34)	8 ^a /28	4/8	0/8	0/8	4/8	4/4	0/4	
Squamous cell carcinomas of the head and neck (35)	55/122	NE	NE	10 ^b /55	16 ^b /55	NE	NE	Preferential loss of Pro72 or Arg72 mutation is associated with malignant potential
Non-small cell lung cancer (36)	118/293	34/118	5/100 ^c	33/100 ^c	16/38	2/38		Arg72 was the preferentially mutated allele
Breast cancer (37)	68/204	11/68	24/68	16/68	5/40	5/40		
Breast carcinoma (38)	199/677	NE	NE	21/199	103/199	12/124	3/124	Worst prognosis with mutation on the Pro72 allele
								Retention of the Pro allele is associated with reduced disease-free survival
								Preferential loss of Pro72 and LOH of Arg72 is associated with an early age diagnosis and worse survival

NE, not evaluated; ^aOne primary tumor and its metastasis were investigated; ^bLoss of *TP53* transcription; ^cLOH was determined in 100 of 118 Arg72Pro.

Although it appears clear that LFS patients with *TP53* mutations have a 90% risk of developing cancer until the seventh decade of life (47), the 57-year-old proband investigated in this study had already presented with five types of cancer and three recurrences of pancreatic cancer in the last two decades of her life.

It is known that the SNP309 T→G in the promoter of the *MDM2* gene increases the expression level of *MDM2*, thereby causing an impairment in the TP53 tumor suppressor activity. A study by a French group verified that the *MDM2* G allele associated with the *TP53*_{Arg} variant was correlated with rapid cancer development in carriers of germline *TP53* mutations (48). Additionally, it was reported that individuals carrying both the *TP53*_{Pro} and *MDM2* SNP G alleles were at increased risk of therapy-related acute myeloid leukemia after alkylating therapy (49). A similar situation could have occurred in an LFS patient who had accumulated a number of mutations, explaining the 17 different cancer types he suffered following different treatments (50).

In contrast to the other deceased family members, the patient investigated in the present study was diagnosed early and received immediate treatment for all the cancers she suffered, which may have contributed to her survival.

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