p53 protein expression affected by TP53 polymorphism is associated with the biological behavior and prognosis of low rectal cancer

GUANGZHE ZHANG[1,2], QIAN XU[1,2], ZEYANG WANG[1,2], LIPING SUN[1,2], ZHI LV[1,2], JINGWEI LIU[1,2], CHENGZHONG XING[1,2] and YUAN YUAN[1,2]

[1]Department of Tumor Etiology and Screening, Cancer Institute and General Surgery, The First Hospital of China Medical University; [2]Key Laboratory of Cancer Etiology and Prevention in Liaoning Education Department, The First Hospital of China Medical University, Shenyang, Liaoning 110001, P.R. China

Received March 21, 2018; Accepted November 29, 2018

DOI: 10.3892/ol.2019.10999

Abstract. Low rectal cancer is a subtype of colorectal cancer at a special anatomic site with distinct biological behavior. TP53 is one of the most important cancer suppressor genes, and its structural variation and abnormal expression has been revealed to be associated with multiple cancer types. However, to the best of our knowledge, the association of p53 protein expression with its gene polymorphism, biological behavior and prognosis in low rectal cancer has not been clarified. Therefore, the current study aimed to explore these associations. In the present study, 347 patients with low rectal cancer and 353 controls were enrolled. Kompetitive Allele-Specific Polymerase Chain Reaction was used to detect five polymorphic sites of the TP53 gene (rs1042522, rs12947788, rs1625895, rs2909430 and rs12951053), while immunohistochemistry was used to detect the protein expression of TP53. The associations between p53 protein expression and TP53 polymorphism, biological behavior and prognosis in low rectal cancer were systematically analyzed. In low rectal cancer, p53 protein expression was markedly higher in TP53 rs1042522 mutant carriers compared with that in other genotypes where expression was higher in poorly differentiated, III-IV phase and T3-4 phase tumors, and in III-IV phase female patients. The survival time of patients with low p53 protein expression was evidently longer in females, non-smokers and patients >60 years old. In summary, p53 protein expression was identified to be affected by TP53 rs1042522 polymorphism, and was associated with the biological behavior and prognosis of low rectal cancer. TP53 rs1042522 and the associated protein expression could be used as indicators for biological behavior and prognosis in low rectal cancer.

Correspondence to: Dr Yuan Yuan, Department of Tumor Etiology and Screening, Cancer Institute and General Surgery, The First Hospital of China Medical University, 155 NanjingBei Street, Shenyang, Liaoning 110001, P.R. China
E-mail: yuanyuan@cmu.edu.cn

Key words: cellular tumor antigen p53, expression, polymorphism, low rectal cancer, prognosis

Introduction

Low rectal cancer (LRC) is located in an area that is 6-8 cm away from the rectum (1). LRC is a type of colorectal cancer that occurs at a specific anatomical site and exhibits a specific biological behavior. Compared with middle and upper rectal cancer, LRC possesses different pathological types, clinical outcomes and surgical options (2,3). Despite advancements in treatment options for LRC and an improved understanding of its biological characteristics, LRC remains a challenge to human health due to its high local recurrence risk (4). The accurate classification of molecular phenotype may significantly contribute to monitoring the biological behavior of LRC and improve the personalized prognosis for the disease.

The TP53 gene, located at the 17p13.1 locus of the short arm of the human chromosome, covers an overall length of 16-20 kb and consists of 11 exons and 10 introns (5). The TP53 gene encodes an intranuclear phosphorylated protein that consists of 393 amino acids, with a 25-kb mRNA transcription product (6,7). Wild-type TP53 is a cancer suppressor gene that serves a crucial role in multiple cellular processes, including the cell cycle, cell apoptosis, cell aging, gene stability and the inhibition of angiogenesis (8-10). By contrast, mutated TP53 can stimulate cell division and function as an oncogene. It is well understood that mutation of the TP53 gene and dysfunction of the TP53 pathway is a characteristic hallmark of various types of human malignancy (11). In addition to mutations, polymorphisms in the TP53 gene may occur in coding and non-coding sequences. According to previous studies, at least eight polymorphic sites have been detected in the promoter region of the TP53 gene, as well as in the first, second, third, sixth, seventh and tenth intron regions, and in the seventh exon region. Among these polymorphisms, three polymorphic sites have been associated with genetic susceptibility to multiple cancer types. These include a CD72 Arg/Pro polymorphism, a repetitive sequence inserted in 16 bp of the third intron region

and a polymorphism of the restriction enzyme digestion site of MspI in the sixth intron (12-14). As one of these functional TP53 single nucleotide polymorphisms (SNPs), the CD72 Arg/Pro polymorphism (rs1042522) has been studied in colon cancer. One study reported that there was no evident association between rs1042522 and colorectal cancer (15), while two study groups identified that the rs1042522 polymorphic genotype was associated with increased colon cancer risk (16,17).

With structural variation of the TP53 gene, abnormal protein expression of p53 has also been revealed to be associated with multiple cancer types, including colorectal cancer. A literature review revealed that the overexpression of p53 is an independent predictor for cancer survival (18). However, another study did not identify a prognostic value of p53 in colorectal cancer (19). A further study demonstrated that p53 protein expression is associated with short-term prognosis in colorectal cancer, since a significant association between p53 expression and rectal carcinoma was identified and the percentage of p53 positive cells was associated with clinicopathological variables (20).

Although the association between p53 and colorectal cancer has been studied for a number of years, the majority of previous studies failed to investigate colorectal cancer based on the position of the lesion site or only divided colorectal cancer into colon cancer and rectum cancer. Furthermore, the conclusions of these previous studies have been contradictory. To the best of our knowledge, the association between p53 and LRC has not been investigated in previous studies. Therefore, it remains unclear whether p53 protein expression is associated with TP53 gene polymorphisms in LRC, and whether p53 protein expression is associated with the biological behavior and prognosis of LRC.

Based on patients with or without LRC, associations between the five most common polymorphic sites of the TP53 gene (rs1042522, rs12947788, rs1625895, rs2909430 and rs12951053) and p53 protein expression were investigated in the present study. In addition, the associations between p53 protein expression and biological behavior and the prognosis of LRC were systematically studied. The overall aim of the current study was to provide information that may be useful for the development of individualized therapeutic strategies prior to surgery, and to improve the biological behavior and prognosis of patients with LRC in clinical practice.

Materials and methods

Patients. The current study was approved by the Medical Ethics Committee of the First Hospital of China Medical University (Shenyang, China) and written informed consent for use of samples was obtained from all participants. A total of 347 patients diagnosed with LRC (within 8 cm from the anal verge), treated by surgery at the Department of Anus and Intestine Surgery of the First Hospital of China Medical University (Shenyang, China) between December 2011 and June 2016, were included in the present study. A total of 353 patients with an anal benign lesion, but no colorectal cancer, as determined by colonoscopy and rectal examination, were hospitalized during the same period and used as controls. The mean ages of patients with LRC and patients with an anal benign lesion were 61.4±11.0 and 59.6±14.4 years, respectively. The sex distribution (male vs. female) in patients group and control group were 203:144 and 185:168, respectively.

Table I. Sequences of the primers used for the Kompetitive allele specific polymerase chain reaction.

SNPs	Primer sequences
rs1042522	
Forward	GGGTCTTACGGTCTCCGACGAGGGG
Reverse	GCACCGGGGACGTGGTCGTCGAGGA
rs12947788	
Forward	CCTCTGCTTGCCTCTGACCCCTGGG
Reverse	CCACCTCTTACCGATTTCTTCCATA
rs1625895	
Forward	ATTCCCACCAACAGTCACCGGGAGG
Reverse	CCACTCGTCATCCCCCCGAAAGAGG
rs2909430	
Forward	GATCACCCAACGTCCTCCACGAATG
Reverse	GTACAAACAAAGAAACGACGGCAGA
rs12951053	
Forward	CTGGGCCCACCTCTTACCGATTTCT
Reverse	CCATACTACTACCCATCCACCTCTC

Table II. Thermocycling conditions for the polymerase chain reaction.

Steps	Temperature	Duration	Number of cycles
1			
Activation	94˚C	15 min	1
2			
Denaturation	94˚C	20 sec	10
Annealing/Elongation	55-61˚C	60 sec	
3			
Denaturation	94˚C	20 sec	26
Annealing/Elongation	55˚C	60 sec	

The inclusion criteria were as follows: i) Rectal cancer diagnosed within 8 cm of the anal verge; and ii) age >18 years old. The clinical diagnostic criteria for LRC were defined according to the literature (21). The exclusion criteria were as follows: i) Patients with an immune system disease; ii) patients with an infectious disease; iii) patients with primary tumors on other visceral organs prior to surgery; and iv) patients who received neoadjuvant chemoradiotherapy prior to surgery.

Sample and patient history collection. The peripheral blood of each individual included in the present study was collected prior to surgery for patients or prior to colonoscopy for controls for genomic DNA extraction. Each sample was immediately frozen and kept at -80˚C until further use. The basic information of each

Table III. Associations between polymorphisms of the TP53 gene and p53 protein expression.

p53 protein expression	Genotype			Heterozygous vs.wild-type	Mutant vs. wild-type	Dominant model	Recessive model
	Wild-type, n	Heterozygous, n	Mutant, n	P-value	P-value	P-value	P-value
rs1042522				0.027	0.239	0.032	0.905
Positive	64	135	49				
Negative	37	43	19				
rs12947788				0.203	0.990	0.278	0.659
Positive	97	125	26				
Negative	45	42	12				
rs1625895				0.280			
Positive	221	27					
Negative	92	7					
rs2909430				0.204			
Positive	216	32					
Negative	91	8					
rs12951053				0.154	0.787	0.191	0.905
Positive	116	108	24				
Negative	54	35	10				

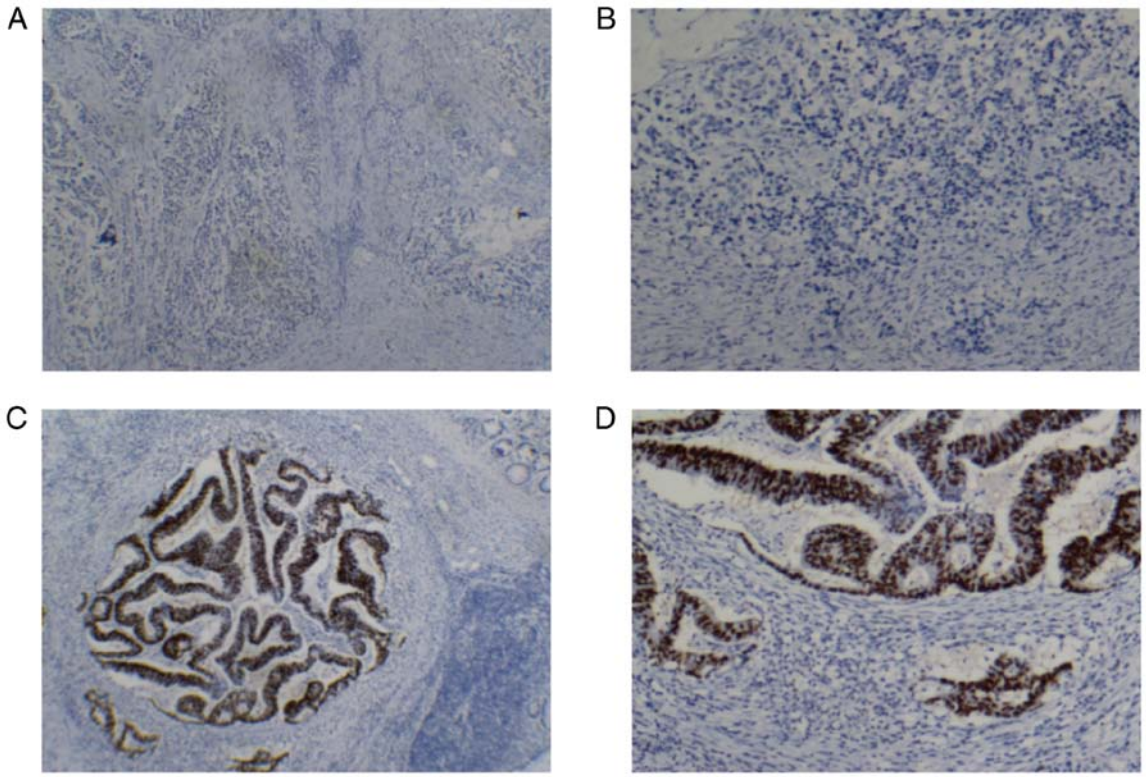

Figure 1. Determination of p53 protein expression by immunohistochemistry. (A) p53 negative expression (magnification, x40). (B) p53 negative expression (magnification, x200). (C) p53 positive expression (magnification, x40). (D) p53 positive expression (magnification, x200).

individual was collected using a questionnaire, which included their sex, age, and smoking status and alcohol consumption. Data regarding the Tumor-Node-Metastasis (TNM) system classification, depth of invasion, growth pattern, histological type, paracancerous lymphocyte infiltration status, peripheral ganglion violation status, cancer embolus in vascularization, lymph node metastasis and implantation in extra nodes were extracted from the medical records of patients with LRC. The overall survival (OS) of individuals following diagnosis or treatment was assessed until August 2016.

Table IV. Clinical characteristics and overall survival time of patients with low rectal cancer.

Characteristic	Low rectal cancer, n	Mortality, n	Median survival time	P-value
Sex	304	44		0.193
Male	178	29	38.504	
Female	126	15	38.908	
Age, years	304	44		0.827
≤60	138	20	39.656	
>60	166	24	38.362	
TNM stage	303	44		$3.246x10^{-10}$
I-II	181	8	43.197	
III-IV	122	36	33.081	
Depth of infiltration	304	44		0.003
T1+T2	87	4	42.988	
T3+T4	217	40	37.261	
Lymph node metastasis	303	44		$2.406x10^{-11}$
Negative	193	8	43.288	
Positive	110	36	32.490	
Histological type	304	44		$4.1911x10^{-8}$
Well-differentiated	195	13	42.412	
Poorly differentiated	109	31	32.060	
Peripheral lymphocyte infiltration	283	41		0.619
Negative	24	4	40.125	
Positive	259	37	35.165	
Peripheral ganglion violation	266	39		0.002
Negative	72	4	38.586	
Positive	194	35	32.952	
Vascular cancer embolus	293	44		0.001
Negative	222	25	40.574	
Positive	71	19	33.467	
Implantation in extra nodes	264	39		<0.001
Negative	246	30	35.886	
Positive	18	9	23.202	

TNM, Tumor-Node-Metastasis.

Candidate TP53 gene SNP selection. To explore the association between TP53 gene polymorphisms and p53 protein expression, a total of 5 SNPs (rs1042522, rs12947788, rs1625895, rs2909430 and rs12951053) with a minimum allele frequency <5% in the Chinese population were selected based on the tagging information from the NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp) and International HapMap Project (www.hapmap.org) in 2016.

Kompetitive allele-specific polymerase chain reaction (KASP™) genotyping assay. Genomic DNA was prepared from peripheral blood mononuclear cells collected from patients using the QIAamp DNA Blood Mini kit (Qiagen China Co., Ltd., Shanghai, China) according to the manufacture's protocol and stored at -80˚C. SNP genotyping was performed applying KASP with an SNPLine platform (LGC Genomics). The steps of the PCR were as follows: i) The extracted DNA samples were diluted in 30 μl TE buffer (concentration ≥60 ng/μl) in 96-well plates, and transferred into 384-well plates and 1536-well plates by Replikator (final concentration ~10 ng/μl); ii) the 1536-well plates containing DNA samples were dried in an oven at 65˚C for 30 min; iii) the PCR reaction system (1 μl) was constructed and the sequences of primers used were presented in Table I; iv) plates with reaction system were sealed and centrifuged at 12,000 x g; v) PCR was performed in water bath after centrifugation according to the thermal cycling conditions presented in Table II; vi) plates with completed reaction were cooled down and read with a microplate reader Pherastar (BMG Labtech GmbH); and vii) additional PCR would be performed to double check the genotyping results if necessary.

Immunohistochemistry assay. Tissue specimens were fixed with 10% formalin at room temperature for 24 h and embedded

Table V. Overall association analysis between p53 protein expression and characteristics of low rectal cancer.

	p53 protein expression			p53 protein expression level				
Characteristic (n)	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis (n=346)			0.851					0.182
Positive	100	39		66	30	4	39	
Negative	147	60		97	33	16	60	
Histological type (n=347)			0.990					0.056
Well-differentiated	158	63		114	32	11	63	
Poorly differentiated	90	36		50	31	9	36	
TNM stage (n=347)			0.879					0.304
III-IV	108	44		72	31	5	44	
I-II	140	55		92	32	15	55	
Depth of infiltration (n=347)			0.254					0.165
T3+T4	127	57		82	37	7	57	
T1+T2	122	47		83	26	13	42	
Growth mode (n=347)			0.384					0.308
Nested growth	143	52		93	41	9	52	
Infiltration growth	105	47		71	22	11	47	
Vascular cancer embolus (n=336)			0.368					0.672
Positive	58	27		38	17	3	27	
Negative	184	67		124	45	14	67	
Extranodal implantation (n=305)			0.507					0.685
Positive	13	7		10	3	0	7	
Negative	205	80		137	52	15	80	
Ganglion violation (n=307)			0.595					0.260
Positive	163	67		111	43	8	67	
Negative	57	20		37	13	7	20	
Peripheral lymphatic infiltration (n=326)			0.454					0.829
Positive	214	87		146	52	15	87	
Negative	16	9		10	5	1	9	

TNM, Tumor-Node-Metastasis.

with paraffin and cut into 4-μm sections. Immunohistochemical staining was performed using Ultra Sensitive™ SP kit (cat. no. KIT-9709/9719; Maixin, Fuzhou, China) according to the manufacturer's protocol. Sections were deparaffinized and rehydrated through ethanol gradient (100, 95 and 75% ethanol for 5 min each), incubated in 10 mM citrate buffer (pH 6.0) and heated in a microwave oven for 5 min. After cooling, slides were incubated with blocker of endogenous peroxidase activity (buffer A in the kit) at room temperature for 1 h, and blocked with normal goat serum (one drop; buffer B in the kit) for 30 min at room temperature. Sections were washed with PBS, incubated with anti-p53 rabbit polyclonal antibody (1:100; cat. no. ab131442; Abcam, Cambridge, UK) for 1 h r at room temperature, with biotin-conjugated secondary antibody (one drop, buffer C in the kit) for 10 min at room temperature, and with HPR-Streptomycin (one drop, buffer D in the kit) for 10 min at room temperature. Signal was visualized with the 3'-diaminobenzidine visualization kit. (cat. no. dab-0031; Fuzhou Maixin Biotech Co., Ltd.). Slides were observed with an inverted microscopy (Olympus Corporation, Tokyo, Japan).

p53 protein expression was independently read and scored by two pathologists, in accordance with the double-blind principle. A senior pathologist was consulted with regard to inconsistent scores in order to arrive at a consensus. Positive p53 protein expression was located in the nuclei of cancer cells and appeared as stronger brown granules under a microscope with high magnification (x40). Subsequently, the positive p53 protein expression area was detected under a microscope with low magnification (x10). A total of 10 fields of each slide were randomly selected under a microscope with high magnification and 100 cancer cells were counted in each field. The percentage of cancer cells with positive p53 protein expression was calculated. The scores for positive p53 expression were determined according to the percentage of p53-positive cells in each sample as follows: Negative, <10%; positive +, 10-30%); ++, 30-50%; and +++, 50-100%.

Table VI. Stratified association analysis between p53 protein expression and characteristics of low rectal cancer.

A, Male sex

	p53 protein expression			p53 protein expression level				
Characteristic	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.412					0.567
Positive	57	26		42	14	1	26	
Negative	88	31		60	22	5	31	
Histological type			0.171					0.136
Well-differentiated	97	32		73	19	4	32	
Poorly differentiated	49	25		30	17	2	25	
TNM stage								
III-IV	62	28		48	13	1	28	
I-II	84	29		55	23	5	29	
Depth of infiltration			0.755					0.317
T3+T4	81	33		54	24	2	33	
T1+T2	65	24		49	12	4	24	
Growth mode			0.857					0.057
Nested growth	84	32		57	26	1	32	
Infiltration growth	62	25		46	10	5	25	
Vascular cancer embolus			0.506					0.447
Positive	35	16		22	12	1	16	
Negative	108	39		80	23	4	39	
Extranodal implantation			0.786					0.934
Positive	9	4		7	2	0	4	
Negative	120	45		85	30	4	45	
Ganglion violation			0.335					0.335
Positive	98	40		68	27	2	40	
Negative	33	9		25	6	2	9	
Peripheral lymphatic infiltration			0.325					0.582
Positive	126	49		91	6	2	49	
Negative	9	6		8	30	4	6	

B, Female sex

	p53 protein expression			p53 protein expression level				
Characteristic	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.210					0.054
Positive	43	13		24	16	3	13	
Negative	59	29		37	11	11	29	
Histological type			0.112					0.104
Well-differentiated	61	31		41	13	7	31	
Poorly differentiated	41	11		20	14	7	11	
TNM stage								
III-IV	46	16		24	18	4	16	
I-II	56	26		37	9	10	26	
Depth of infiltration			0.155					0.458
T3+T4	45	24		27	13	5	24	
T1+T2	57	18		34	14	9	18	
Growth mode			0.262					0.717
Nested growth	59	20		36	15	8	20	
Infiltration growth	43	22		25	12	6	22	

Table VI. Continued.

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Vascular cancer embolus			0.542					0.716
Positive	23	11		16	5	2	11	
Negative	76	28		44	22	10	28	
Extranodal implantation			0.442					0.773
Positive	4	3		3	1	0	3	
Negative	85	35		52	22	11	35	
Ganglion violation			0.819					0.423
Positive	65	27		43	16	6	27	
Negative	24	11		12	7	5	11	
Peripheral lymphatic infiltration			0.992					0.294
Positive	88	38		55	22	11	38	
Negative	7	3		2	4	1	3	

C, Age ≥60 years

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.212					0.166
Positive	50	23		33	16	1	23	
Negative	95	29		64	22	9	29	
Histological type			0.127					0.002
Well-differentiated	96	28		73	16	7	28	
Poorly differentiated	50	24		25	22	3	24	
TNM stage			0.073					0.049
III-IV	55	27		36	18	1	27	
I-II staging	91	25		62	20	9	25	
Depth of infiltration			0.130					0.034
T3+T4	72	32		44	25	3	32	
T1+T2	74	20		54	13	7	20	
Growth mode			0.729					0.227
Nested growth	83	31		55	25	3	31	
Infiltration growth	63	21		43	13	7	21	
Vascular cancer embolus			0.158					0.482
Positive	29	15		20	8	1	15	
Negative	114	35		77	29	8	35	
Extranodal implantation			0.199					0.529
Positive	5	4		3	2	0	4	
Negative	125	42		85	32	8	42	
Ganglion violation			0.107					0.205
Positive	93	38		63	26	4	38	
Negative	39	8		26	9	4	8	
Peripheral lymphatic infiltration			0.336					0.551
Positive	130	46		90	33	7	46	
Negative	6	4		4	1	1	4	

Table VI. Continued.

D, Age <60 years

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.087					0.166
Positive	50	16		33	14	3	16	
Negative	52	31		33	11	7	31	
Histological type			0.103					0.184
Well-differentiated	62	35		41	16	4	35	
Poorly differentiated	40	12		25	9	6	12	
TNM stage			0.073					0.245
III-IV	53	17		36	13	4	17	
I-II	49	30		30	12	6	30	
Depth of infiltration			0.977					0.760
T3+T4	54	25		37	12	4	25	
T1+T2	48	22		29	13	6	22	
Growth mode			0.107					0.375
Nested growth	60	21		38	16	6	21	
Infiltration growth	42	26		28	9	4	26	
Vascular cancer embolus			0.805					0.855
Positive	29	12		18	9	2	12	
Negative	20	32		47	16	6	32	
Extranodal implantation			0.737					0.585
Positive	8	3		7	1	0	3	
Negative	80	38		52	20	7	38	
Ganglion violation			0.270					0.358
Positive	70	29		48	17	4	29	
Negative	18	12		11	4	3	12	
Peripheral lymphatic infiltration			0.967					0.554
Positive	84	41		56	19	8	41	
Negative	10	5		6	4	0	5	

E, Smoker

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.032					0.095
Positive	26	16		18	7	1	16	
Negative	54	13		40	8	5	13	
Histological type			0.198					0.608
Well-differentiated	58	17		43	10	4	17	
Poorly differentiated	23	12		16	5	2	12	
TNM stage								
III-IV	31	16		24	6	1	16	
I-II	50	13		35	9	5	13	
Depth of infiltration			0.135					0.375
T3+T4	40	19		30	7	2	19	
T1+T2	41	10		29	8	4	10	

Table VI. Continued.

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Growth mode			0.608					0.381
Nested growth	43	17		29	11	3	17	
Infiltration growth	38	12		30	4	3	12	
Vascular cancer embolus			0.882					0.114
Positive	18	6		10	7	1	6	
Negative	61	22		48	8	4	22	
Extranodal implantation			0.226					0.578
Positive	4	0		3	1	0	0	
Negative	70	26		51	13	5	26	
Ganglion violation			0.625					0.392
Positive	54	20		40	11	2	20	
Negative	21	6		14	4	3	6	
Peripheral lymphatic infiltration			0.269					0.642
Positive	77	26		56	15	5	26	
Negative	2	2		2	0	0	2	

F, Non-smoker

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.102					0.109
Positive	74	23		48	23	3	23	
Negative	93	47		57	25	11	47	
Histological type			0.400					0.047
Well-differentiated	100	46		71	22	7	46	
Poorly-differentiated	67	24		34	26	7	24	
TNM stage								
III-IV	77	28		48	25	4	28	
I-II	90	42		57	23	10	42	
Depth of infiltration			0.695					0.237
T3+T4	86	38		51	30	5	38	
T1+T2	81	32		54	18	9	32	
Growth mode			0.161					0.284
Nested growth	100	35		64	30	6	35	
Infiltration growth	67	35		41	18	8	35	
Vascular cancer embolus			0.259					0.529
Positive	40	21		28	10	2	21	
Negative	123	45		76	37	10	45	
Extranodal implantation			0.202					0.478
Positive	9	7		7	2	0	7	
Negative	135	54		86	39	10	54	
Ganglion violation			0.774					0.676
Positive	109	47		71	32	6	47	
Negative	36	14		23	9	4	14	
Peripheral lymphatic infiltration			0.812					0.912
Positive	137	61		90	37	10	61	
Negative	14	7		8	5	1	7	

Table VI. Continued.

G, Alcohol consumption

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.816					0.499
Positive	11	4		7	4	0	4	
Negative	21	9		16	3	1	9	
Histological type			0.458					0.243
Well-differentiated	24	8		19	4	0	8	
Poorly differentiated	9	5		5	3	1	5	
TNM stage								
III-IV	12	4		8	4	0	4	
I-II	21	9		16	3	1	9	
Depth of infiltration			0.818					0.718
T3+T4	19	7		14	4	0	7	
T1+T2	14	6		10	3	1	6	
Growth mode			0.818					0.575
Nested growth	19	7		14	5	0	7	
Infiltration growth	14	6		10	2	1	6	
Vascular cancer embolus			0.452					0.363
Positive	8	2		5	3	0	2	
Negative	23	11		18	3	1	11	
Extranodal implantation			0.220					0.496
Positive	3	0		2	1	0	0	
Negative	25	13		19	4	1	13	
Ganglion violation			0.226					0.130
Positive	23	8		17	5	0	8	
Negative	6	5		5	0	1	5	
Peripheral lymphatic infiltration			0.314					0.613
Positive	25	13		19	4	1	13	
Negative	2	0		2	0	0	0	

H, No alcohol consumption

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Lymph node metastasis			0.912					0.275
Positive	89	35		59	26	4	35	
Negative	126	51		81	30	15	51	
Histological type			0.792					0.127
Well-differentiated	134	55		95	28	11	55	
Poorly differentiated	81	31		45	28	8	31	
TNM stage								
III-IV	96	40		64	27	5	40	
I-II	119	46		76	29	14	46	
Depth of infiltration			0.189					0.166
T3+T4	107	50		67	33	7	50	
T1+T2	108	36		73	23	12	36	
Growth mode			0.398					0.455
Nested growth	124	45		79	36	9	45	
Infiltration growth	91	41		61	20	10	41	

Table VI. Continued.

Characteristic	p53 protein expression			p53 protein expression level				
	Positive, n	Negative, n	P-value	+++, n	++, n	+, n	-, n	P-value
Vascular cancer embolus			0.209					0.608
Positive	50	25		33	14	3	25	
Negative	161	56		106	42	13	56	
Extranodal implantation			0.212					0.461
Positive	10	7		8	2	0	7	
Negative	180	67		118	48	14	67	
Ganglion violation			0.277					0.349
Positive	140	59		94	38	8	59	
Negative	51	15		32	13	6	15	
Peripheral lymphatic infiltration			0.265					0.599
Positive	189	74		127	48	14	74	
Negative	14	9		8	5	1	9	

TNM, Tumor-Node-Metastasis.

Statistical analysis. Statistical analysis was performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Independent sample t-test was used to compare the differences between two groups, and one-way ANOVA followed by Tukey post-hoc analysis was used to compare the differences among multiple groups (>2). Parameters that reflected the behavior and prognosis of LRC in each genotype were represented by hazard ratios (HR) and 95% confidence intervals (CIs). The HR values were calculated by multivariate Cox proportional hazards regression analysis. The χ^2 test was used to evaluate the association between TP53 gene polymorphism and p53 protein expression, or between p53 protein expression and the clinical pathological parameters of LRC. The log-rank test was used to compare the survival times between the groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association between TP53 gene polymorphism and p53 protein expression. To study the influence of TP53 gene polymorphism on the protein expression of p53, five polymorphic loci of the TP53 gene (rs1042522, rs12947788, rs1625895, rs2909430 and rs12951053) and the protein expression level of p53 in the 347 patients diagnosed with LRC were detected. Results revealed that the TP53 rs1042522 polymorphism was associated with p53 protein expression [CG (heterozygous) vs. GG (mutant), P=0.027; CC (wide-type) + CG vs. GG, P=0.032], indicating that positive p53 protein expression was significantly higher compared with other genotypes in the heterozygous and dominant models (Table III and Fig. 1). The other four polymorphic loci were not identified to be associated with p53 protein expression.

Protein expression of p53 is associated with the biological characteristics of LRC. In the present study, the association between time survival and clinicopathological parameters of LRC, including TNM classification, depth of invasion, histological type, paracancerous lymphocyte infiltration, ganglion infiltration, vascular cancer embolus, lymph node metastasis and extranodal implantation status were analyzed (Table IV). The χ^2 test revealed that the protein expression of p53 was not significantly associated with the clinicopathological parameters in the whole population (Table V). However, following stratification of the patients by classic risk factors, including sex, age, smoking status and alcohol consumption, the results revealed a significant association between the protein expression of p53 and the clinicopathological parameters of LRC. In female patients, the protein expression of p53 in stage III-IV was significantly higher compared with that in stage I-II (P=0.044). Furthermore, in patients with an age ≥60 years, histological type, TNM stage and depth of tumor invasion were all associated with the protein expression of p53 (P=0.002, P=0.049 and P=0.034, respectively). The protein expression of p53 was significantly higher in poorly-differentiated tumors compared with well-differentiated tumors, in stage III-IV compared with stage I-II, and in the T3-4 stage compared with the T1-2 stage. In patients with a history of smoking, the p53 protein expression was significantly associated with the occurrence of lymph node metastasis (P=0.032). In contrast to smokers, the p53 protein expression level in poorly-differentiated tumors was significantly higher compared with well-differentiated tumors in non-smokers (P=0.047; Table VI).

p53 protein expression is associated with the prognosis of LRC. To further determine whether p53 protein expression is an independent prognostic factor for LRC, univariate and multivariate Cox proportional hazards regression analyses were conducted (Table VII). Univariate survival analysis revealed a significant association between the protein

Table VII. Association analysis between p53 protein expression and the prognosis of low rectal cancer.

A, Overall analysis

p53 protein expression	Low rectal cancer, n	Mortality, n	Median survival time, months	Univariate			Multivariate		
				P-value	HR	95% CI	P-value	HR	95% CI
Low expression	137	13	41.235		1 (ref.)			1 (ref)	
High expression	167	31	37.137	0.028	2.071	1.083-3.958	0.195	1.580	0.791-3.154

B, Stratification analysis

p53 protein expression	Low rectal cancer, n	Mortality, n	Median survival time, months	Univariate			Multivariate		
				P-value	HR	95% CI	P-value	HR	95% CI
Sex									
Male									
Low expression	71	9	39.821		1 (ref.)			1 (ref.)	
High expression	107	20	36.890	0.312	1.501	0.683-3.298	0.750	1.153	0.481-2.764
Female									
Low expression	66	4	40.939		1 (ref.)			1 (ref.)	
High expression	60	11	36.694	0.042	3.280	1.043-10.311	0.139	2.890	0.708-11.792
Age, years									
≥60									
Low expression	71	6	40.783		1 (ref.)			1 (ref.)	
High expression	95	18	36.734	0.083	2.268	0.900-5.716	0.021	3.425	1.208-9.712
<60									
Low expression	66	7	40.983		1 (ref.)			1 (ref.)	
High expression	72	13	36.819	0.182	1.871	0.745-4.696	0.848	0.908	0.339-2.431
Smoking status									
Smoker									
Low expression	38	5	38.281		1 (ref.)			1 (ref.)	
High expression	56	7	38.217	0.896	1.079	0.342-3.410	0.815	0.852	0.223-3.256
Non-smoker									
Low expression	99	8	41.879		1 (ref.)			1 (ref.)	
High expression	111	24	36.564	0.014	2.724	1.223-6.066	0.081	2.185	0.909-5.254

Table VII. Continued.

p53 protein expression	Low rectal cancer, n	Mortality, n	Median survival time, months	Univariate			Multivariate		
				P-value	HR	95% CI	P-value	HR	95% CI
Alcohol consumption									
Consumption									
Low expression	15	0			1 (ref.)			1 (ref.)	
High expression	28	4		0.371	41.001	0.132-1.987	1.000	1.000	0.094-10.593
No consumption									
Low expression	122	13	40.841		1 (ref.)			1 (ref.)	
High expression	139	27	36.914	0.054	1.918	0.990-3.718	0.224	1.559	0.762-3.189

HR, hazard ratio; CI, confidence interval; ref., reference.

expression of p53 and the OS for LRC; the survival time of patients with low p53 expression was significantly longer compared with that of patients with high p53 expression [hazard ratio (HR), 2.071; 95% CI, 1.083-3.958; P=0.028]. However, the multivariate survival analysis revealed that the protein expression level of p53 was no longer associated with the survival time in all patients with LRC (HR, 1.580; 95% CI, 0.791-3.154; P=0.195) (Table VII). Following stratification of the patients according to risk factors of LRC, including sex, age, smoking status and drinking status, the results revealed that the survival time of patients with low p53 protein expression was significantly longer compared with patients with high p53 protein expression in female patients (HR, 3.280; 95% CI, 1.043-10.311; P=0.042) and non-smokers (HR, 2.724; 95% CI, 1.223-6.066; P=0.014). Multivariate analysis for patients with an age ≥60 years revealed that patients with low p53 protein expression had a longer survival time compared with patients with high p53 protein expression (P=0.021; HR, 3.425; 95% CI, 1.208-9.712).

Discussion

As a subtype of colorectal cancer at a special anatomical site, LRC is characterized by its specific biological behavior. TP53 is one of the most important cancer suppressor genes, and structural variation and abnormal expression of p53 have been identified to be associated with numerous cancer types (22-25). However, the associations between TP53 gene polymorphisms and protein expression, and the association of p53 protein expression with the biological behavior and prognosis of LRC have not been clearly investigated. Understanding these associations is important for the preoperative assessment of LRC and the development of individualized treatments. The present study investigated the associations between TP53 gene polymorphisms and p53 protein expression, and the associations between p53 protein expression and the biological behavior and prognosis of LRC. The overall aim was to address the role of TP53 gene polymorphisms and p53 protein expression in the biological behavior and prognosis of LRC.

Genetic polymorphisms are a common genetic variation, which may affect the expression of proteins and protein function (26-29). In the present study, the associations between the five most common TP53 SNPs (rs1042522, rs12947788, rs1625895, rs2909430 and rs12951053) and p53 protein expression were evaluated. The results revealed that the TP53 rs1042522 polymorphism was associated with p53 protein expression, which was evidenced by the significantly higher p53 protein expression in TP53 rs1042522 mutant carriers compared with that in the other genotypes. No associations were identified between p53 protein expression and the other TP53 SNPs. Among the five polymorphic loci selected in the present study, only rs1042522 was located in the exon region, whereas the other four polymorphic loci were located in the intron region. This indicated that the rs1042522 polymorphism may be present in the coding sequence of the TP53 gene, affecting therefore the protein expression of p53 (30). However, this does not indicate that other polymorphisms are not functionally important, since SNPs that are not located in protein coding regions

may affect other processes, including gene splicing, which requires further investigation.

Although previous studies have been conducted to investigate the protein expression of p53 and its association with the clinical biological behavior and prognosis of colorectal cancer, results from these studies have been inconsistent. Furthermore, to the best of our knowledge, systematic studies focusing on LRC have not previously been performed. Therefore, the association between p53 expression and LRC at 6-8 cm from the anal margin was investigated in the present study. Overall analysis results revealed that there was no significant association between p53 protein expression and the clinicopathological parameters of LRC. However, following stratification analysis, an association was identified between lymphatic metastasis in smokers and p53 protein expression. Furthermore, histological type, TNM stage and tumor infiltration depth were associated with p53 expression level in patients ≥60 years old. In addition, p53 expression was markedly higher in poorly-differentiated, III-IV phase or T3-4 phase tumors, and a significant association was revealed between p53 expression level and TNM stage in female patients, which was evidently higher in III-IV phase female patients. Additionally, an association was identified between p53 expression and the histological type of LRC among non-smokers.

The survival time of patients with low p53 protein expression was significantly longer in females, non-smokers and patients ≥60 years old. These results indicate that p53 protein expression may be used as an indicator for the prognosis of LRC, particularly for patients ≥60 years old, non-smokers, patients with III-IV phase tumor or female patients with T3-4 phase tumors. Although the exact mechanism requires further exploration, the current findings indicate that p53 protein expression should be regularly screened in the aforementioned subgroups of patients to enable individualized treatments that improve clinical outcomes in future clinical practice.

In conclusion, the TP53 rs1042522 polymorphism affects the p53 protein expression in LRC, and p53 protein expression is associated with the biological behavior and prognosis of LRC. Therefore, the TP53 rs1042522 polymorphism and p53 protein expression may serve as indicators to predict the biological behavior and prognosis of LRC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Science and Technology Support Program (grant no. 2015BAI13B07) and the National Key R&D Program (grant no. 2016YFC1303202).

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

YY designed the study. CX, GZ, ZW and LS collected the samples and performed the experiments. QX, ZL and JL performed the statistical analysis. GZ and QX drafted the manuscript. YY revised the manuscript. All authors approved final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the First Hospital of China Medical University (Shenyang, China) and written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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