Original Article
Expression of p53β and Δ133p53 isoforms in different gastric tissues

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Abstract: This study aims to detect the mRNA of p53β and Δ133p53 isoforms in three gastric carcinoma cell lines and tissues of superficial gastritis, atrophic gastritis, gastric carcinoma, or paracancerous area. Nested reverse transcription PCR was used to detect the mRNA of p53β and Δ133p53 isoforms in tissues of superficial gastritis, chronic atrophic gastritis, gastric cancer cell lines (SGC-7901, MKN45, KATO III), gastric adenocarcinoma, and paracancerous lesion. The amplified products were shown by agarose gel electrophoresis. The expression difference among various tissues was analyzed by x^2 tests. The positive rates of Δ133p53 mRNA were 73.3% (11/15) in gastric adenocarcinoma and 20% (3/15) in paracancerous tissue, whereas the positive rates of p53β mRNA were 20% (3/15) in gastric adenocarcinoma and 66.7% (10/15) in paracancerous tissue. The difference between adenocarcinoma and paracancerous tissues was significant ($P<0.05$). The positive rates of Δ133p53 mRNA were 25% (5/20), 50% (15/30), and 75% (15/20), respectively, in superficial gastritis, atrophic gastritis, and gastric adenocarcinoma; the positive rates of p53β mRNA were 65% (13/20), 33.3% (10/30), and 25% (5/20), respectively, in superficial gastritis, atrophic gastritis, and gastric adenocarcinoma. The difference between adenocarcinoma and superficial gastritis samples was significant ($P<0.05$). Both p53β and Δ133p53 mRNAs were positive in MKN45; only p53β mRNA was detected in SGC7901; neither p53β nor Δ133p53 mRNA was detected in KATO III. Δ133p53 and p53β, which are possible indicators for the diagnosis and biological therapy of gastric carcinoma, were expressed differentially in different gastric tissues.

Keywords: Gastric carcinoma, p53β, Δ133p53, p53, isoforms

Introduction

p53 was first described in 1979 as a protein binding to the simian virus large T antigen. This protein is the product of a pivotal tumor-suppressor gene whose inactivation existed in more than half of various malignant tumors. Thus, p53 has been regarded as "the guardian of the genome." The inactivation of p53 may not only be caused by the mutations or deletion of the TP53 gene itself but also by alternative splicing (**Figure 1A** and **1C**) [1-7]. p53 isoforms were reported to appear in different tissues, including various types of normal, precancerous, and malignant tissues. These isoforms work together with wild-type p53 or other pathways and are involved in the process of carcinogenesis [8-20]. Therefore, treating various types of cancer without the knowledge of p53 isoforms is questionable. Gastric cancer has

caused severe fatality and mortality in China and other Asian countries. However, no information on p53 isoforms had been reported in this popular malignancy. Thus, we need to clarify the status of p53 isoforms in both diagnostic and therapeutic manipulation of the p53 pathway.

Materials and methods

Patients

Cancer and corresponding paracancerous samples were taken from 20 patients with advanced gastric carcinoma via gastrectomy from April 2009 to May 2011. Another 10 gastric carcinoma, 20 superficial gastritis, and 30 atrophic gastritis samples were taken via gastroscopic biopsy at the Affiliated Hospital of Weifang Medical University. The patients enrolled in this
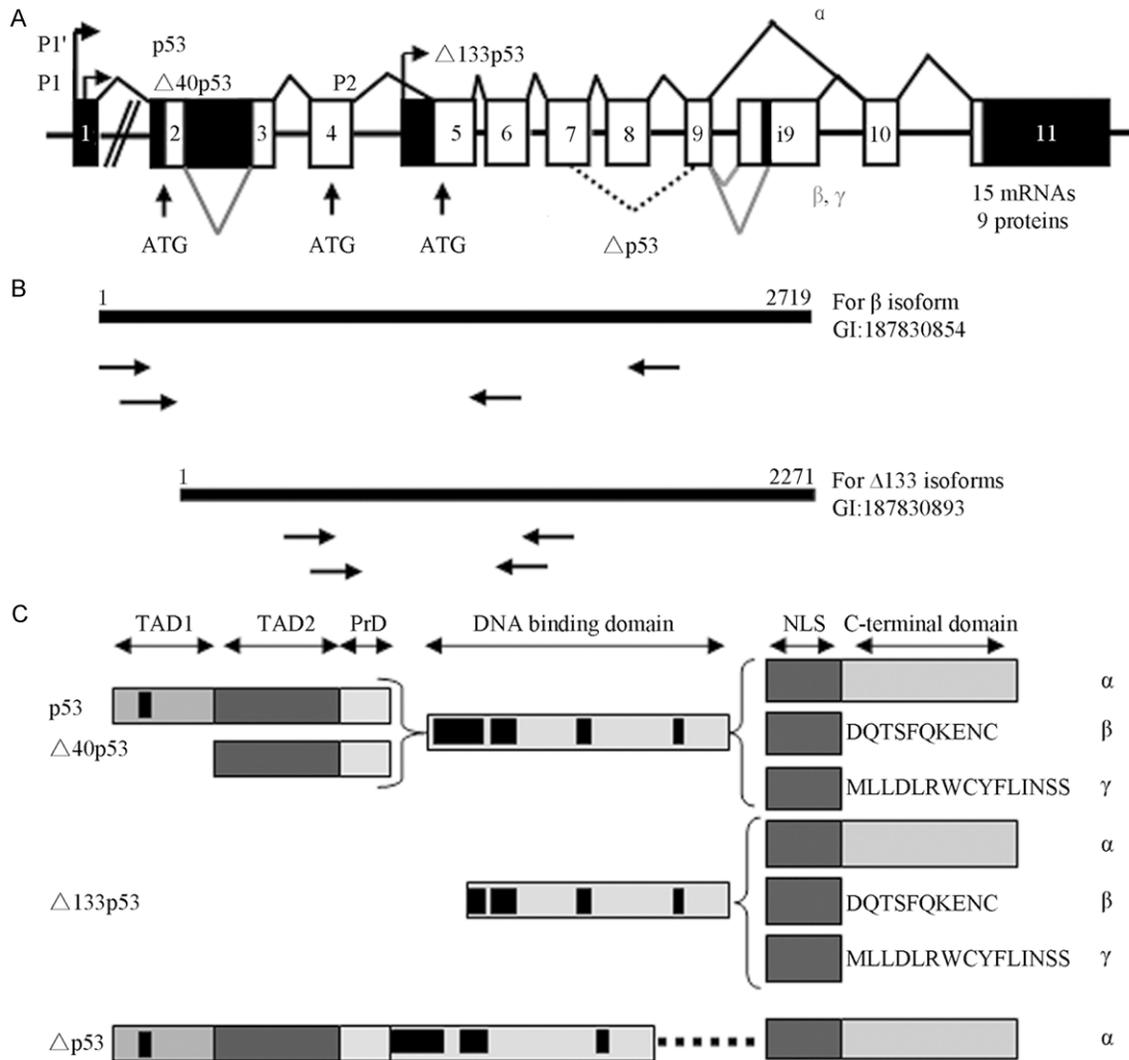
Figure 1. p53 gene structure, isoforms and primer design. A. Human p53 gene structure; B. p53 transcripts variants and site of primers; C. p53 protein isoforms.

investigation had never been treated with chemotherapy or radiotherapy. The patients offering gastroscopic samples had no history of antibiotics and aspirin administration at least two weeks before biopsy. All samples were frozen immediately after gastroscopy or surgery and stored at -70°C. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Affiliated Hospital of Weifang Medical University. Written informed consent was obtained from all participants.

Cell lines

SGC-7901 was stored in our laboratory. KATO III and MKN45 gastric cancer cell lines were given by Dr. Wang Xin (State Key laboratory of Oncology, Xijing Hospital, Fourth Military Medical University, Xi'an, China). These cell lines had been passaged four times at the time of harvesting for RNA isolation. All human cell lines were cultured in DMEM with 5% fetal bovine serum and maintained in exponential growth at 37°C and 5% CO_2.

RT-PCR

A series of keys was used in total RNA isolation, cDNA synthesis, and PCR amplification (Sangon Biotech Shanghai Co., Ltd., Shanghai, China). RNA was isolated from snap frozen tumor tissue. The concentration of total RNA was determined by Thermo EV0300 spectrophotometer.

Table 1. Primers applied in the amplification of p53β or Δ133p53 mRNA

Molecules		Primer sequence	Length
p53β	Outer	F: 5'-GTCACTGCCATGGAGGAGCCGCA-3'	1012
		R: 5-GACGCACACCTATTGCAAGCAAGGGTTC-3'	
	Internal	US: 5'-ATGGAGGAGCCGCAGTCAGAT-3'	
		DS: 5'-TTTGAAAGCTGGTCTGGTCCTGA-3'	
Δ133p53	Outer	US: 5'-CTGAGGTGTAGACGCCAACTCTCTCTAG-3'	750
		DS: 5'-AGTCAGTCTGAGTCAGGCCCTTCTGTC-3'	
	Internal	US: 5'-GCTAGTGGGTTGCAGGAGGTGCTTACAC-3'	
		DS: 5'-CTCACGCCCACGGATCTGA-3'	
β-actin		US: 5'-GTGGGGCGCCCCAGGCACCA-3'	539
		DS: 5'-CTCCTTAATGTCACGCACGATTTC-3'	

Note: nested PCR applied in the amplification of p53β and Δ133p53.

Table 2. Expression of p53β and Δ133p53 mRNA in different gastric biopsy tissues (positive samples/total samples, %)

Isoforms	Carcinoma	Atrophic gastritis	Superficial gastritis	P
p53β	5/30, 16.7%	10/30, 33.3%	17/20, 85.0%	0.033
Δ133p53	21/30, 70.0%	15/30, 50.0%	5/20, 25.0%	0.008

Note: χ^2 tests were used to analyze the data in this contingency table. Significance level: α=0.05.
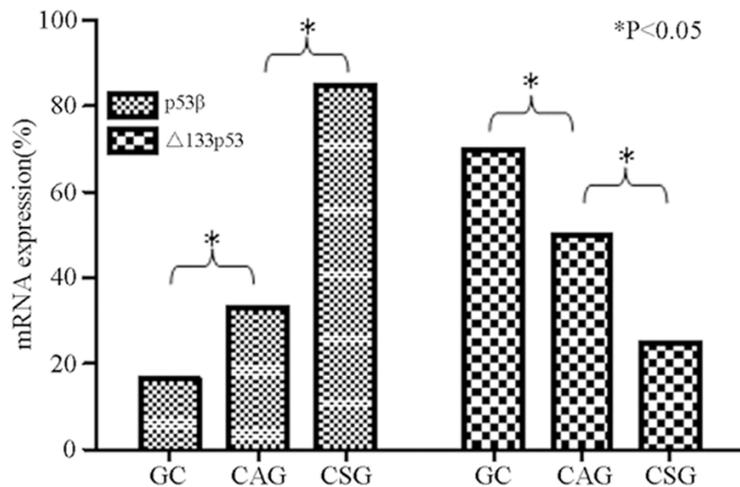
Figure 2. Diagram of expression rate (percentage) p53β and Δ133p53 mRNA in different gastric biopsy tissues.

M-MuLV first chain synthesis key was used in reverse transcription (RT) of RNA. A 20 μl reaction contained the following: 4 μl of 25 mM MgCl$_2$ solution; 2 μl of 10× PCR Buffer II; 1 μl of H$_2$O; premixed deoxyribonucleoside triphosphates: 2 μl of dGTP, 2 μl of dATP, 2 μl of dTTP, 2 μl of dCTP (10 mM each), 1 μl of RNA inhibitor (20 U/μl); 1 μl of random hexamers; and 1 μl of MuLV reverse transcriptase as a master mix.

Approximately 2 μl of total RNA was added prior to the start of reaction. On the basis of a previous photometric measurement, the total RNA template concentration was below the reaction capacity of ≤1 μg of RNA per reaction. The following adapted time and temperature profiles for the RT were used: incubation for 10 min at 25°C, 30 min at 42°C for RT of RNA, 5 min at 95°C for denaturation, and 5 min at 5°C to cool down the reaction. Negative controls were added to ensure contamination-free consumables for the RT reaction in each series of cDNA reactions. Subsequently, the RT reaction samples underwent 1:4 dilution to obtain a final concentration of 10 ng/μl of cDNA.

PCR reaction was under the guidelines of the PCR Amplification Key (Sangon Biotech Shanghai Co., Ltd., Shanghai, China). Nested PCR was done briefly via the PE-5700 My-Cyder (Applied Biosystems, Inc., USA) in a final volume of 25 μl under the following conditions: 35 cycles at 94°C for 1 min, 58°C for 50 s, and 72°C for 1 min. Primers for the p53β and Δ133p53 mRNA sequences are shown (**Figure 1B**; **Table 1**). The sequence of various primers were listed as follows: external primers for p53β, forward: 5'-GTC ACT GCC ATG GAG GAG CCG CA-3', reverse: 5-GAC GCA CAC CTA TTG CAA GCA AGG GTT C-3'; internal primers for p53β, forward: 5'-ATG GAG GAG CCG CAGTCA GAT-3', reverse: 5'-TTT GAA AGC TGG TCT GGT CCT GA-3'; external primers for Δ133p53, forward: 5'-CTG AGG TGT AGA CGC CAA CTC TCT CTA G-3', reverse: 5'-AGT CAG TCT GAG TCA GGC CCT TCT GTC-3'; internal primers for Δ133p53, forward: 5'-GCT AGT GGG TTG CAG GAG GTG CTT ACA C-3', reverse: 5'-CTC ACG CCC ACG GAT
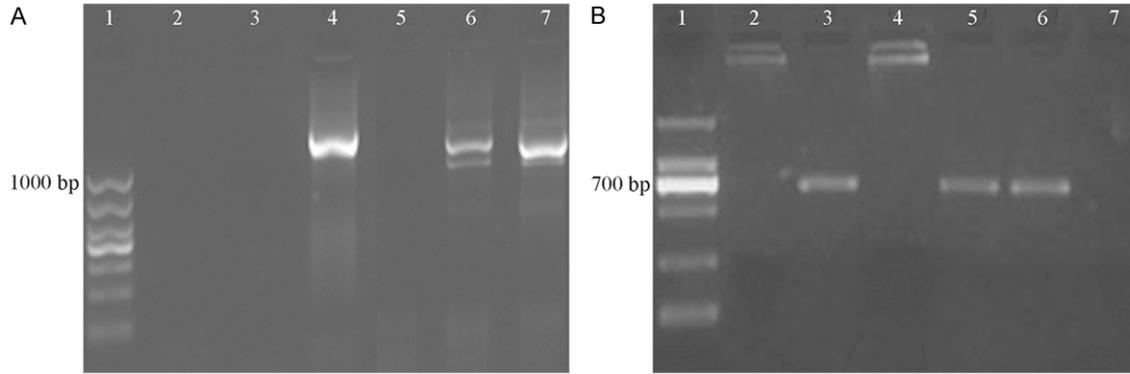
Figure 3. Electrophoresis results of p53β and Δ133p53 in different gastric biopsy tissues. Left: p53β amplified fragment (1050 bp); right: Δ133p53 amplified fragment (750 bp). Lane 1: DNA Marker; lane 2 and 5: tissues of gastric carcinoma; lane 3 and 6: tissues of atrophic gastritis; lane 4 and 7: tissues of superficial gastritis.

Table 3. Expression of p53β and Δ133p53 mRNA in gastric carcinoma or paracancerous tissues (positive samples/total samples, %)

Isoforms	Carcinoma	Paracancerous lesion	χ^2	P
p53β	3/15, 20%	10/15, 66.7%	4.884	0.00427
Δ133p53	11/15, 73.3%	3/15, 20.0%	6.652	0.01140

Note: *Fisher Exact* Test was applied in the analysis of the data in this table. Significance level: α=0.05.

CTG A-3'; primers for β-actin, forward: 5'-GTG GGG CGC CCC AGG CAC CA-3', reverse: 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'. The PCR product was run in 1% agarose electrophoresis under the level electrophoresis apparatus (Gulf Gene Group Company, USA). The result was scanned and recorded by the Biospectrum AC gel imaging system (Alpha Innotech Corporation, USA).

Statistical analysis

SPSS16.0 Statistical Software Package was used to analyze experimental data. Data in contingency table were analyzed by x^2 tests. Fisher's exact test was used to analyze the differential expression of p53 isoforms between cancer and paracancerous tissue. *P<0.05* was taken as the significance level.

Results

Differential expression of Δ133p53 and p53β in tissues of superficial gastritis, atrophic gastritis, and gastric adenocarcinoma

The positive rates of Δ133p53 mRNA were 25% (5/20), 50% (15/30), and 75% (15/20), respectively, in superficial gastritis, atrophic gastritis, and gastric adenocarcinoma; the difference between superficial gastritis and gastric carcinoma tissues was significant (P<0.05). The positive rates of p53β mRNA were 65% (13/20), 33.3% (10/30), and 25% (5/20), respectively, in tissues of superficial gastritis, atrophic gastritis, and gastric adenocarcinoma; the difference between superficial gastritis and gastric carcinoma tissues was significant (P<0.05). The following trend accompanied the decreased positive rate of p53β and increased positive rate of Δ133p53: gastric carcinogenesis from superficial gastritis → atrophic gastritis → gastric adenocarcinoma. The difference among tissues was significant (**Table 2**; **Figures 2** and **3**).

Differential expression of Δ133p53 and p53β in tissues of paracancerous lesion and gastric adenocarcinoma

Table 3 and **Figure 4** show that the positive rates of Δ133p53 mRNA were 73.3% (11/15) in gastric adenocarcinoma and 20% (3/15) in paracancerous tissue, and the difference was significant (P=0.00427); the positive rates of p53β mRNA were 20% (3/15) in gastric adenocarcinoma and 66.7% (10/15) in paracancerous tissue, and the difference was significant (P=0.01140).

Differential expression of Δ133p53 and p53β in gastric cancer cell lines

In gastric cancer cell lines, both p53β and Δ133p53 mRNA were positive in MKN45; only

Figure 4. Expression of p53β and Δ133p53 mRNA in tissues of gastric cancer or paracancerous lesion. Upward: p53β amplified fragment (1050 bp); downward: Δ133p53 amplified fragment (750 bp). Lane 1: DNA Marker; lane 2, 3 and 4: tissues of paracancerous lesion; lane 5, 6, 7, and 8: tissues of gastric cancer.
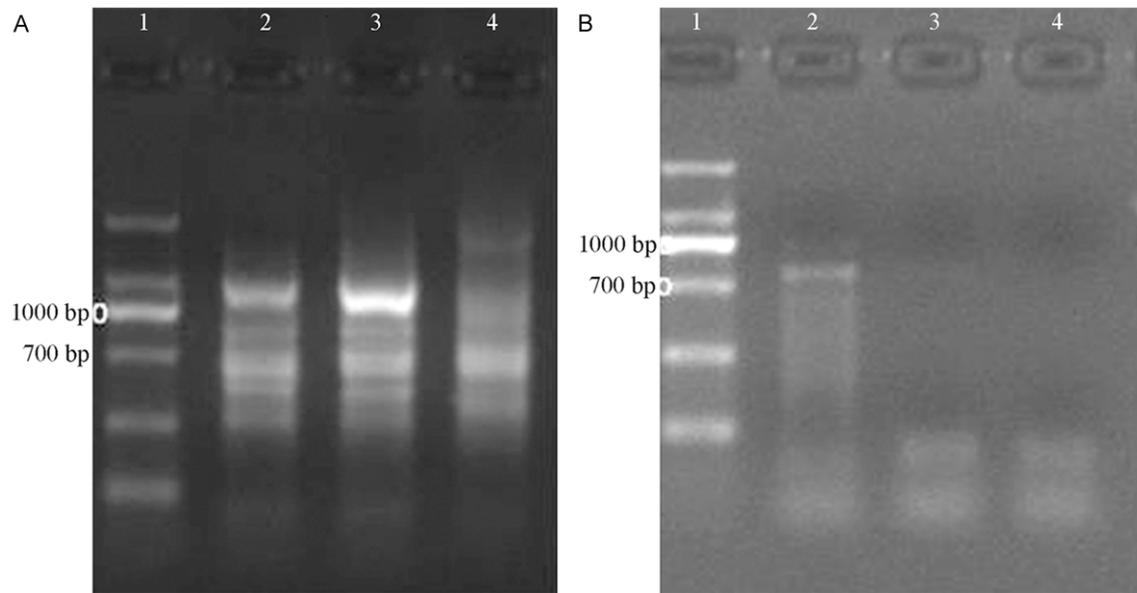
Figure 5. Expression of p53β and Δ133p53 mRNA in gastric cancer cell lines. Left: p53β expression in three gastric cell lines; right: Δ133p53 expression in three gastric cell lines. Lane 1: DNA marker, lane 2: MKN45, lane 3: SGC7901, and lane 4: KATO III.

p53β mRNA was detected in SGC7901; neither p53β nor Δ133p53 mRNA was detected in KATO III (**Figure 5**).

Discussion

Gastric carcinogenesis is accepted as a multiple-step process with accumulating genetic alterations, in which the loss of p53 function plays a pivotal role. The mutation and deletion of TP53 gene in gastric carcinoma and cell lines were reported in detail [21, 22], however, no research exists on p53 isoforms in gastric diseases. Analyzing p53 isoform expression in the tissues of gastric carcinoma and other gastric diseases is necessary to decipher the role of p53 isoforms in gastric carcinogenesis.

In these experiments, the expression rate of Δ133p53 mRNA went down significantly in gastric carcinoma and the p53β expression rate arose significantly compared with gastritis and paracancerous lesion. The following trend accompanied the decreased p53β and increased Δ133p53 expressions: gastric carcinogenesis from superficial gastritis → atrophic gastritis → gastric adenocarcinoma. Although Δ40p53 (Hafsi et al.) and p53γ (Bourdon et al.)

were reported to be correlated with cancer development [9, 10, 14, 23, 24], the results in the present study suggested that p53β and Δ133p53 might be possible indicators for gastric carcinogenesis.

Boldrup et al. showed that p53β was detected in the majority of tissues of normal epithelium and squamous cell carcinoma in head and neck and called for further research to declare the correlation between the p53 status and clinical outcome [18]. Goldschneider et al. proved that p53β was the only isoform detected in a neuroblastoma cell line SK-N-AS, making the protein relevant to tumor development [16]. Avery-Kiejda et al. reported that p53β could enhance p53-dependent transcription of p21 and PUMA in human melanoma cells [17]. In another study, Hofstetter et al. proved that Δ133p53 is an independent prognostic marker in p53 mutant advanced serous ovarian cancer [13]. The study from Moore et al. indicated that Δ133p53 inhibited the p53-dependent transcription from the p21 promoter by competing with p68 [11]. Results from previous studies and in the present experiment indicated that p53 isoforms work as p53 coordinators in a tissue-specific manner and may play different roles in different tissues.

As proven in previous research, Δ133p53 shows an oncogenic (anti-apoptotic) character, whereas p53β works as a pro-apoptotic assistant [25-27]. In the current experiment, both p53β and Δ133p53 mRNA were positive in MKN45, only p53β mRNA was detected in SGC7901, but neither p53β nor Δ133p53 mRNA was detected in KATO III. Intriguingly, the result suggested that the complicated p53 is a background for gastric carcinoma development. Aside from the deletion and mutation of the *TP53* gene, the mechanism of malignant transformation should also consider the profiles of decreasing pro-apoptotic isoforms and increasing anti-apoptotic isoforms.

We discovered significant changes of p53 isoforms in the development of gastric carcinoma. However, more information is required before the actual clinical application. Additional cases should also be enrolled in the next step. Moreover, the actual mechanism of isoforms remained controversial because of the complicated regulation of p53 functions. Therefore, further study should focus on the role of p53

isoforms in chronic gastritis and other precancerous lesions. The exploration of isoforms should be placed under the background of the entire p53 signal pathway and the cross-talk among other pathways (NF-κB pathway for example).

In summary, Δ133p53 and p53β, which are possible indicators for the diagnosis and biological therapy of gastric carcinoma, were expressed differentially in different gastric tissues and might be involved in the chronic process of gastric carcinogenesis.

Disclosure of conflict of interest

None.

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