Int J Clin Exp Pathol 2016;9(2):1758-1763
www.ijcep.com /ISSN:1936-2625/IJCEP0018236

Original Article

TERT promoter mutation, telomere length, and TERT expression in gastric cancer

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Received October 21, 2015; Accepted December 22, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Objective: The mutation in the promoter region of telomerase reverse transcriptase (TERT) has been focused in various cancers. In present study, the frequency and clinical characteristics of TERT promoter mutation, telomere length, and TERT expression in gastric cancers were studied. Materials and Methods: We sequenced TERT promoter region in 104 gastric cancers. And telomere length and TERT expression was analyzed by using real-time PCR. Results: TERT promoter mutation was found in 10.6% (11/104) of gastric cancers and associated with advanced cancers (P = 0.02). The incidence of telomere shortening and TERT expression was 60.6% and 16.3%, respectively. Diffuse type of gastric cancer tended to show higher expression of TERT (P = 0.07) and telomere-length maintenance (P = 0.09). However, these telomere markers did not have prognostic value. Conclusion: These data demonstrated that telomere status contribute to gastric carcinogenesis, however, it may be not a potential molecular marker for predicting the prognosis.

Keywords: Gastric cancer, telomere, TERT promoter mutation, survival curve

Introduction

Gastric cancer (GC) is highly prevalent in Asia and is the one of the leading causes of death worldwide. Its development has been shown as a multi-step process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia, and finally, invasive cancer (Correa et al., 1994). Most of GCs are adenocarcinomas, classified by histological phenotype as intestinal type, diffuse type, and mixed/unclassifiable according to Lauren's classification. Previous molecular studies have provided that intestinal and diffuse types of GC evolve by different genetic pathways. However, their clinical and prognostic significances in GC was ambiguous until now.

Telomeres, composed of 6-bp TTAGGG repeat sequences, are the nucleoprotein complexes capping the each end of the eukaryotic chromosome. Telomeres are shortened with each cell division in normal cells, whereas they are continuously elongated by telomerase in cancers. Telomerase reverse transcriptase (TERT) gene encodes the catalytic subunit of telomerase which is responsible for elongation of telomeric DNA at the termini of linear chromosomes. Telomerase is responsible for contributing to infinite proliferation potential of malignant cells by regulating telomere length. Recent studies showed high frequency of TERT promoter mutation in various cancers [1-3]. It increased telomerase expression by generating new E-twenty-six (ETS) binding motifs (TTCCGG). It is reported that, however, GC had low frequency of TERT promoter mutation by previous studies [2, 4]. According to these data, TERT promoter mutation was absent or rare in GC. In present study, we identified TERT promoter mutation, TERT expression, and telomere length in Korean patients with GCs and their clinical and prognostic values were studied.

Material and methods

Patients and DNA/RNA extraction

We recruited 109 patients who underwent gastrectomy for treating gastric adenocarcinoma from archives of paraffin blocks at Keimyung University Dongsan Hospital from October 1999 to December 2001. All cases were reviewed by an expert panel of two pathologists

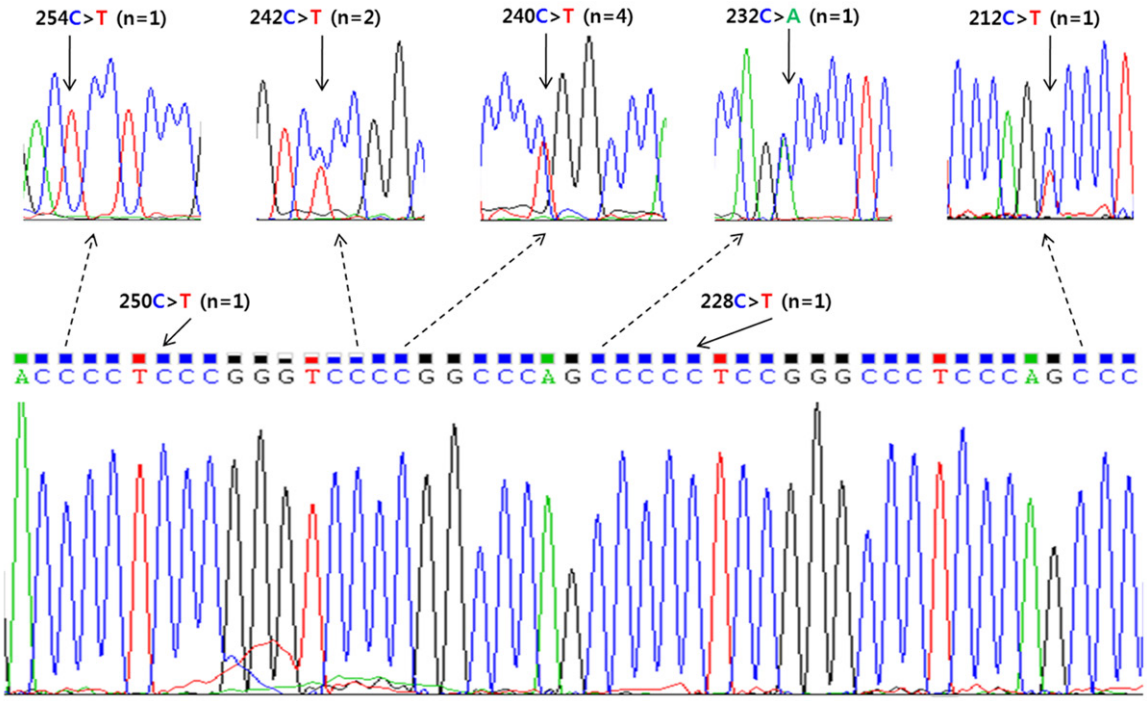

Figure 1. Mutations of TERT promoter except -C250T and -C228T in gastric cancers.

and tumor area and adjacent normal tissue were selected from slide according to hematoxylin and eosin stained sections. Tumor area and adjacent normal mucosa were selected from slide according to hematoxylin and eosin stained sections. Subsequently, the selected areas from paraffin embedded tissues were used for DNA and RNA extraction. DNA and RNA were isolated by using DNA extraction Kit (Absolute™ DNA extraction Kit, BioSewoom, Korea) and RNeasy Kit (Qiagen, Hilden, Germany), respectively, according to the manufacturer's instructions. Samples were then quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) analysis.

TERT promoter mutation

The polymerase chain reaction (PCR) amplification of the TERT promoter region was performed as described previously with minor modification [1, 2, 5]. PCR was done using AmpliTaq Gold (Applied Biosystems, USA). The PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide to confirm the size of the bands. And then, direct DNA sequencing was performed using the ABI 3730 DNA sequencer by Bionics Inc, Korea.

Telomere length and TERT expression

Telomere length and TERT expression were analyzed by quantitative real-time (qRT) PCR. For the quantitative determination of telomere length relative to nDNA, primers for specific amplification of telomere (T) and nDNA-encoded ß-globin (S) were selected according to previous study [6]. TERT (T) and ß-globin (S) mRNA expression levels were analyzed and relative TERT expression was calculated. Real-time PCR was then carried out on a LightCycler 480 II system (Roche Diagnostics, Germany). Relative telomere length and TERT expression were determined by calculating T/S values using the formula $T/S = 2^{-\Delta Ct}$, where ΔCt = average $Ct_{telomere\ or\ TERT}$-average $Ct_{ß\text{-}globin}$. Each measurement was repeated in triplicate and 5 serially diluted control samples were included in each experiment.

Statistical analysis

Chi-square and Fischer' exact tests were used to analyze the relationship between variables. Survival curves, estimated with the Kaplan-Meier method (Univariate analysis), were compared by log-rank test. Overall survival was defined as the time between diagnosis and

Table 1. Clinicopathological characteristics of TERT promoter mutation in gastric cancers

	Telomere length (%, n)			TERT mutation (%, n)			TERT expression (%, n)		
	High	Low	P	(+)	(-)	P	(+)	(-)	P
Total	39.5 (43)	60.5(66)		10.6 (11)	89.4 (93)		16.3 (17)	83.7 (87)	
Age			0.23			0.65			0.25
< 60	37.0 (20)	63.0 (34)		12.0 (6)	88.0 (44)		20.4 (11)	79.6 (43)	
≥ 60	41.8 (23)	58.2 (32)		9.3 (5)	90.7 (49)		12.0 (6)	88.0 (44)	
Gender			0.23			0.28			0.24
Male	42.7 (35)	57.3 (47)		13.3 (10)	86.7 (65)		13.9 (11)	86.1 (68)	
Female	29.6 (8)	70.4 (19)		3.4 (1)	96.6 (28)		24.0 (6)	76.0 (19)	
pT			0.41			0.17			0.60
I/II	42.3 (30)	57.7 (41)		7.5 (5)	92.5 (62)		14.8 (9)	85.2 (52)	
III/IV	34.2 (13)	65.8 (25)		16.2 (6)	83.8 (31)		18.6 (8)	81.4 (35)	
pN			0.82			0.56			0.92
0/I	40.3 (27)	59.7 (40)		9.2 (6)	90.8 (59)		16.7 (10)	83.3 (50)	
II/III	38.1 (16)	61.9 (26)		12.8 (5)	87.2 (34)		15.9 (7)	84.1 (34)	
Lauren classification			0.09			0.62			0.08
Diffuse	53.8 (14)	46.2 (12)		8.0 (2)	92.0 (23)		28.0 (7)	82.0 (18)	
Intestinal	35.4 (29)	64.6 (53)		11.5 (9)	88.5 (69)		12.8 (10)	87.2 (68)	
Depth of invasion			0.95			0.02			0.11
Early	39.1 (18)	60.9 (28)		2.3 (1)	97.7 (42)		8.1 (3)	91.9 (34)	
Advanced	39.7 (25)	60.3 (38)		16.4 (10)	83.6 (51)		20.9 (14)	79.1 (53)	

either death from disease or death from other causes. All *P*-values < 0.05 were considered statistically significant.

Results

Among 109 patients of GC, TERT promoter regions were successfully amplified in 104 GCs. The mean age of the 109 patients with gastric adenocarcinoma was 56.2 years (range, 25-82 years). TERT promoter mutations were found in 10.6% (11/104) of GC patients. Previous studies reported that C228T and C250T were hot spots of this mutation, however, our study demonstrated that other mutations (C254T, C242T, C240T, C232A, and C212T) in TERT promoter region were more frequently found in GCs (**Figure 1**).

To clarify the mechanism of telomere in GC, TERT expression and telomere length were also investigated. Relative TERT expression and telomere length were calculated from quantified data split by T/N ratio (tumors (T) divided by that in normal tissue (N) × 100%). As results, TERT expression and telomere length was 0.87 ± 0.53 and 1.27 ± 0.83, respectively.

Telomere length and TERT expression was divided into two groups to examine their clinicopathological characteristics. All cases in which the T/N ratio was less than 1 were included in the group of telomere shortening or negative TERT expression. When T/N was at least 1, tumors were included in the group of telomere maintenance or positive TERT expression [7]. The frequency of TERT expression and telomere-length maintenance was 16.3% (17/104) and 39.5% (43/109), respectively. TERT mutation, TERT expression, and telomere length were investigated in GCs and their associations with clinicopathological characteristics were presented in **Table 1**. Analysis of clinical characteristics of telomere markers showed that TERT promoter mutation was significantly associated with advanced cancers (P = 0.02). And TERT expression and telomere-length maintenance tended to be associated with diffuse-type GC, though it did not reach statistical significance (P = 0.08 and P = 0.09, respectively). Other clinicopathological characteristics had no association with these telomere markers in GC.

We then assessed overall survival to clarify prognostic significance of TERT mutation, telomere length, and TERT expression in GCs. The median follow-up of patients for survival analysis was 80.4 months (1-123). Kaplan-Meier

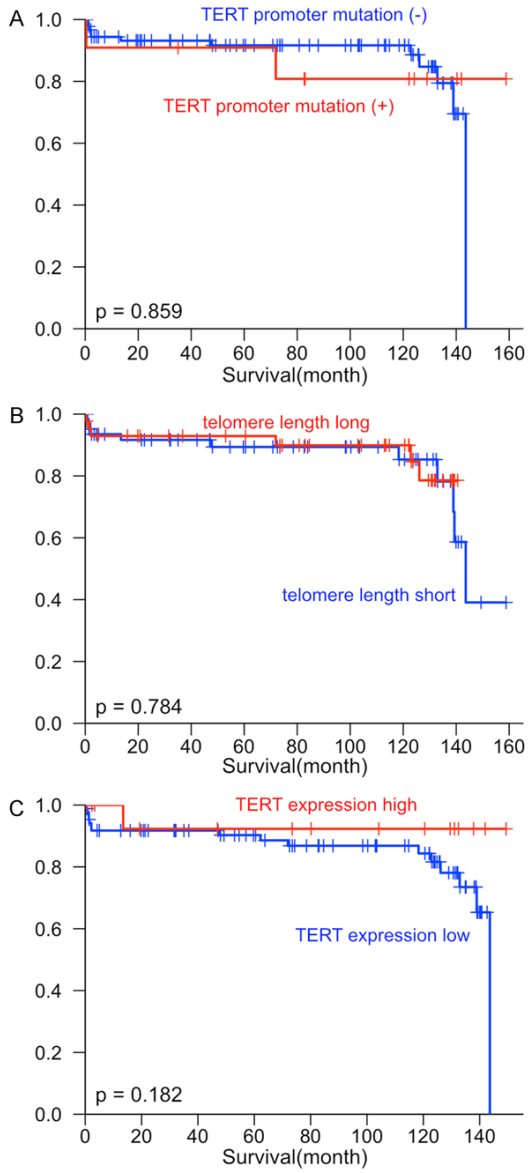

Figure 2. Survival analysis of TERT promoter mutation (A), telomere length (B), and TERT expression (C) in gastric cancers.

curve revealed that TERT promoter mutation, telomere length, and TERT expression was not associated with prognosis of GC patients (**Figure 2**). When stratifying for variables, their prognostic value had no statistical significance.

Discussion

Maintenance of telomere length, most frequently by activation of telomerase, is a hallmark of cancer [8]. The mutations in the promoter of the TERT gene, which was discovered recently, may be an important mechanism contributing to maintenance of telomeres in cancer [1, 9]. Our study identified clinicopathological characteristics of telomere markers in GCs. Telomere length and TERT expression level of our results were in agreement of previous studies, which indicates the similarity of pathological character of GC [7, 10].

However, the frequency of TERT promoter mutation in GC had been reported in several studies with discrepancy. Majority of mutation in TERT promoter was found in two hot spot, C228T (chr 5: 1295228) and C250T (chr 5: 1295250) [1-5, 9, 11-18]. Huang, et al. [2] found C228T mutation in one (5%) of 20 GC cell lines, which is compatible with our study (4.8%). Qu, et al. [4], which searched only hot spot mutations by using pyrosequencing, showed only C250T mutation in two (0.7%) of 268 patients with GC. Though these studies demonstrated low frequency of TERT promoter mutation in GC [2, 4, 19], our data showed contradictory result (10.6%, 11/104) including novel mutations. Moreover, the frequency of hot spot mutation C228T and C250T) was only 4.8% (5/104), other novel mutations were found more frequently. Among those novel mutation, C242T mutation was already introduced as double mutation C242T/C243T, because the base change at -139 bp has been reported as a rare polymorphism (rs35550267) [1, 2]. In skin squamous cell carcinomas, one case with C254T mutation has been reported [20]. Recent study showed some novel mutations rather than hot-spot of TERT promoter region in non-small cell lung cancers [21]. However, other mutations of TERT promoter (C254T, C240T, C232A, and C212T) in our study have not been reported in any cancers. The disagreement suggested that TERT promoter mutation have an association with various pathogenesis of GC. This is a preliminary finding that may be also worthy of further investigation.

It is recently reported that TERT mRNA and protein highly expressed in GC and its precancerous lesion, which suggested that the event of TERT overexpression might occur in early stage of carcinogenesis of the stomach [22]. However, our results showed that TERT mRNA is highly detected in the diffuse type of GC, which has more aggressive character. Furthermore, clinicopathological analysis showed that the advanced stage of GC had significant correlation with the TERT promoter mutation. Our results suggests that high expression of TERT by its

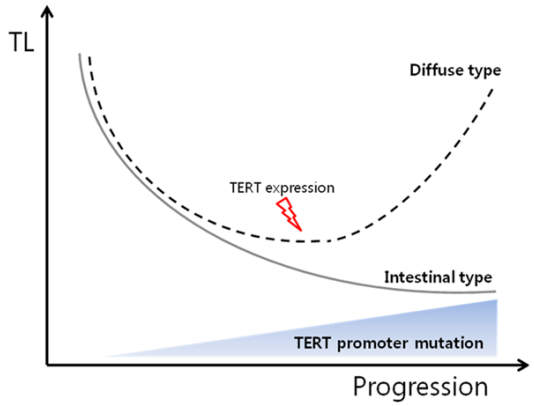

Figure 3. Schematic diagram of the role of telomere status in gastric carcinogenesis. Among GC progression, TERT expression level is increased in diffuse-type of GC elongating telomere length, and the frequency of TERT promoter mutation is also increased.

promoter mutation might play role of sustaining the tumorous cell character in GCs. Based on these data, we deduced the role TL and TERT in the progression of GC, as presented in **Figure 3**. Changes of TERT expression may drive the course of GC to diffuse or intestinal type and also cause differences in TL.

However, survival result of our data did not show a statistical difference between the groups which were compared by TERT promoter mutation, TERT expression, and telomere length. It is well known that high expression of TERT resulted by TERT promoter mutation is correlated poor prognosis in various cancers [5, 13, 23]. Therefore, detail mechanism of telomere markers in GC progression should be studied with larger samples.

In conclusion, it is needed to investigate the underlying tumorigenic mechanism of the TERT promoter mutation including the novel site in further studies.

Acknowledgements

This work was supported by Health fellowship Foundation (2014).

Disclosure of conflict of interest

None.

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