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RESEARCH

Expression of Tankyrase 1 in Gastric Cancer and Its Correlation with Telomerase Activity

Jinbo Gao · Jinghui Zhang · Yaoping Long · Yuan Tian · Xiaoming Lu

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Abstract Tankyrase 1, which enhances telomerase access to telomeres, plays an important role in telomere maintenance. The aim of this study was to determine the expression and potential role of tankyrase 1 in gastric cancer development and progression. We examined the expression of tankyrase 1 by RT-PCR and Western blotting, and assessed telomerase activity by TRAP-ELISA method in gastric cancer and adjacent normal tissues. We found that tankyrase 1 expression was significantly up-regulated in gastric cancer tissues compared to normal corresponding tissues. Tankyrase 1 over-expression by gastric cancerous tissue was significantly associated with tumor histology differentiation and tumor stage. Moreover, tankyrase 1 expression was significantly correlation with telomerase activity. Our results indicate that tankyrase 1 over-expression may play an important role in gastric cancer development and progression. Tankyrase 1 may be used as a biomarker of gastric cancer and may serve as a target for cancer therapy.

Keywords Tankyrase 1 · Gastric cancer · Telomerase · Telomere

Introduction

Telomeres are the nucleoprotein structures that cap the end of chromosomes, playing an important role in chromosomal protection and genome stability. Human telomeres are composed of up to 15 kb of tandem repeats of the hexanucleotide TTAGGG telomeric DNA and associated telomere binding proteins [1, 2]. Because of the "end replication problem", gradual telomere loss occurs with each cell division. When telomeres reach a critical length, cells are triggered into replicative senescence. Therefore, normal somatic cells have a limited lifespan [3]. In contrast, cancer cells have limitless replicative potential, or the immortal phenotype, which is one of the six cancer hallmarks [4]. The maintenance of telomeres contributes to the immortal phenotype. Telomeres can be maintained by their associated proteins as well as the telomerase complex [5, 6].

Telomerase is a ribonucleoprotein that adds telomeric DNA repeats onto chromosome ends. The human telomerase consists of two essential components: human telomerase RNA component (hTERC), which serves as a template for the synthesis of telomere repeat sequence and human telomerase reverse transcriptase (hTERT), which is the catalytic component of the enzyme [7, 8]. Telomerase activity is repressed in somatic cells, but is reactivated in most cancer cells. Therefore, telomere loss in the presence of low or nondetectable telomerase may account for the finite replicative capacity of normal human cells, while constitutive telomerase activation in cancer cells may account for the immortality of tumor cells [9–11].

Furthermore, telomere maintenance is also associated with the action of telomere-specific binding proteins. The enzyme tankyrase 1, a member of the growing family of poly(ADP-ribose) polymerases, was identified as a component of the human telomeric complex [12]. Tankyrase 1 ADPribosylates TRF1, preventing TRF1 from binding telomeric DNA, and then enhances telomerase access to telomere. It has been demonstrated that tankyrase 1 promotes telomere elongation [13]. Over-expression of

J. Gao (✉) · J. Zhang · Y. Long · Y. Tian · X. Lu
Department of Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology,
1277 Jiefang Avenue,
Wuhan, Hubei 430022, China
e-mail: drgaojinbo@gmail.com

tankyrase 1 in the nucleus releases TRF1 from telomeres and induces telomere elongation, indicating that tankyrase 1 is a positive regulator of telomere length [14]. It has been shown that tankyrase 1 expression is up-regulated in several human cancers, including multiple myeloma [15], non-Hodgkins's lymphomas [16], breast cancer [17], colon cancer [18] and bladder cancer [19]. Moreover, tankyrase 1 expression correlates significantly with highly aggressive disease and poor prognosis in some types of cancer, such as breast, colon, and bladder cancer [17–19]. However, the clinical significance of tankyrase 1 expression has not been well studied in gastric adenocarcinoma. In this study, we evaluated the level of tankyrase 1 expression in gastric cancers and their adjacent normal tissues. We also assessed the relationship between tankyrase 1 expression and clinicopathological findings of tumors. Finally, we examined the association between tankyrase 1 expression and telomerase activity.

Materials and Methods

Tissue Samples Gastric cancer tissue samples and paired adjacent nonmalignant tissue samples were obtained from 36 Chinese patients who underwent surgery in Surgical Department of Union Hospital of Tongji Medical College, between 2005 and 2007. The study was approved by the hospital ethics committee, and all patients gave informed consent before surgery. The patients were composed of 24 males and 12 females, ranging in age from 37 to 75 years (mean, 57 years). None of the patients had received preoperative treatment. All patients were staged according to the TNM classification of malignant tumors, proposed by the International Union against Cancer in 2002 [20]. Tissue samples were immediately snap-frozen after surgery and stored in −80°C. Histological examination was performed to verify the collected tissues in all samples.

Real- Time Quantitative RT-PCR Total RNA was extracted with Trizol reagent (Gibco BRL, Grand Island, NY, USA) according to manufacturer's instructions, and the concentration was measured by UV spectrophotometry, and its quality was examined by formaldehyde denatured agarose gel electrophoresis. The following primes were used for tankyrase 1 (forward 5′-TCGCAGCAGGCTACAACCGC-3′ and reverse 5′-GGCAGCCCTTCTTGGCAGCA-3′), and beta-actin (forward 5′-ACAGAGCCTCGCCTTTGCCG-3′ and reverse 5′-TGGGCCTCGTCGCCCACATA-3′). The qRT-PCRs were carried out using iScript One-Step RT-PCR Kit with SYBR Green (Bio-Rad, Hercules, CA, USA). The reactions contained 1× SYBR green QPCR reaction mix, each primer at 300 nM, 100 ng RNA, and 1 μl iScript reverse transcriptase mixture in a 25 μl reaction. The reaction mixture was first incubated at 50°C for 10 min to allow for reverse transcription. PCR was initiated with one cycle of 95°C for 5 min to activate the Taq polymerase followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The dissociation curve analysis was carried out at the end of amplification to confirm PCR product specificity. On each experiment no-template controls and no-RT controls were also performed at the same time. No signals were detected in no-template controls and no-RT controls. The average threshold cycle for each sample was determined from triplicate reactions and the expression level was normalized to β-actin. The relative amount of messenger RNA (mRNA) was calculated as the average $2^{-\Delta ct}$, where $\Delta Ct = Ct - Ct_{actin}$.

Western Blot Analysis Tissues were homogenized in a lysis buffer (20 mmol/L Tris (pH 7.4), 1% NP40, 5 mmol/L EDTA, 10% glycerol, 0.1% SDS, and 150 mmol/L NaCl) containing protease inhibitor mixture (Sigma, St. Louis, MO, USA) on ice. The homogenates were cleared by centrifugation for 30 min at 13,000 *g* at 4°C, and protein concentration was measured with BCA Protein Assay Kit (Beyotime Biotechnology, Jiangsu, China). Equal amounts of tissue protein lysates (60 μg) were separated by 8% SDS-PAGE, and then electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in TBST (20 mM Tris–HCl, 150 mM NaCl, and 0.1% Tween 20) for 1 h, and incubated with the primary antibody [anti-tankyrase 1 mouse IgG] (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. This was followed by 3×5 min washes with TBST, and then incubated with the second antibody (a horseradish peroxidase-conjugated rabbit anti-mouse IgG) for 1 h at room temperature. Signal detection was performed using an enhanced hemiluminescence reaction (Pierce, Rockford, IL, USA). To ensure that equal amounts of protein were loaded, the membranes were then stripped and probed with β-actin antibodies to confirm equal sample loading.

Telomerase Activity Assays Telomerase activity was determined by the telomeric repeat amplification protocol (TRAP assay) using the telomerase PCR ELISA kit (Boehringer Mannheim Corporation, Mannheim, Germany) according to manufacturer's instructions. Briefly, tissue specimens were homogenized in pre-cooled lysis buffer. After incubating the lysate on ice for 30 min, they were centrifuged at 16,000 g for 20 min at 4°C. Supernatants were collected, and protein concentrations were determined. For the TRAP reaction, 30 μg tissue extract was added to 25 μl of reaction mixture with the appropriate amount of sterile water to make a final volume of 50 μl. PCR was carried out as follows: primer elongation (30 min, 25°C), telomerase inactivation (5 min, 94°C), and product amplification by the repeat of 30 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 90 s). To quantitate telomerase activity, the

PCR product was denatured and then hybridized with the digoxigenin-labeled telomeric repeat-specific probe. The PCR product was visualized using peroxidase-conjugated antibodies against digoxigenin and measured at 450 nm using an ELISA reader.

Statistical Analysis

Data are shown as mean ± S.D. Comparisons between groups were analyzed using analysis of variance (ANOVA) or *t*-test. The correlation between tankyrase 1 expression and telomerase activity was analyzed by using the Pearson correlation. All tests were two-sided. A P value of less than 0.05 was considered to indicate statistical significance. All analyses were performed with the use of the SPSS software package (SPSS, Chicago, IL, USA) .

Results

Expression of Tankyrase 1 in Human Gastric Carcinomas The mRNA levels of tankyrase 1 were measured in gastric cancer tissue samples and their paired adjacent normal tissues by quantitative real-time RT-PCR. Tankyrase 1 mRNA was expressed in all tumor samples and matched normal tissues. Tankyrase 1 mRNA expression was elevated in 33 of 36 (91.7%) gastric cancers compared with expression in individually matched normal mucosa. The mean level of tankyrase 1 mRNA was significantly higher in gastric cancers (0.00913±0.00315) in comparison to their normal counterpart (0.00168±0.00129) (P<0.001) (Fig. 1).

We then performed Western blot to evaluate tankyrase 1 expression at protein level. As shown in Fig. 2, expressions of tankyrase 1 protein were significantly higher in gastric cancer than normal tissues.

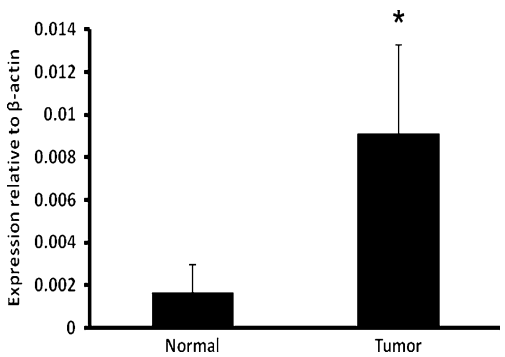

Fig. 1 The level of tankyrase 1 mRNA in gastric cancer. The mRNA levels of tankyrase 1 were determined by real-time RT-PCR in gastric cancer tissues and their normal counterparts. All mRNA levels were normalized to the level of β-actin mRNA. * P<0.001

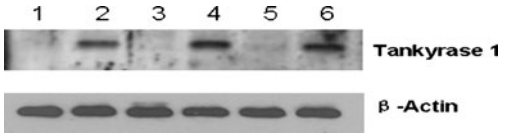

Fig. 2 Tankyrase 1 protein expression in gastric cancer. Western blot analysis revealed up-regulated tankyrase 1 expression in gastric cancer samples (*lane 2,4,6*) when compared with that in normal gastric tissues(*lane 1,3,5*). Depicted are 3 individual matched pairs of gastric normal–cancer samples

Clinicopathological Significance of Tankyrase Expression To investigate whether the expression of tankyrase 1 is correlated with any clinical behavior of gastric cancer, we summarized the relationships between tankyrase 1 expression and various clinicopathological variables (Table 1).

Tankyrase 1 expression had significant associations with tumor histology differentiation (P=0.001) and tumor stage (P=0.014). Well or moderately differentiated cancers had lower expression of tankyrase 1, whereas poorly differentiated ones was associated with higher expression of tankyrase 1 [P=0.001(well VS poorly), P=0.002 (moderately VS poorly), P=0.33(well VS moderately)]. Moreover, tankyrase 1 expression was significantly higher in tumors with late stages (III,IV) than those with early stages (I,II)(P=0.014). However, no significant relationships were found between tankyrase 1 expression and age, sex, tumor location, tumor size, tumor depth and lymph node metastasis.

Correlation Between Tankyrase and Telomerase Activity To investigate whether the expression of tankyrase 1 is correlated with telomerase activity, we also analyzed telomerase activity at the same time. Telomerase activity was significantly higher in gastric cancers in comparison to corresponding normal tissues (Fig. 3). We then calculated the correlation between tankyrase 1 mRNA expression and telomerase activity. As shown in Fig. 4, a clear positive association between tankyrase 1 expression and telomerase activity was found(r=0.553, P<0.01).

Discussion

The maintenance of telomeres plays a pivotal role in cellular immortality and tumorigenesis. Tankyrase 1 was identified as a component of the telomere complex. It has been reported to be critical for telomere length maintenance. Tankyrase 1 was up-regulated in some kinds of cancer [15–19]. However, there is little known about the possible role of tankyrase 1 in gastric cancer, and the results is still controversial. Matsutani et al. reported that tankyrase 1 was up-regulated in gastric cancers [21]. In contradiction,

Table 1 Correlation between tankyrase 1 expression and clinicopathological characteristics in gastric cancer

Variable	No. cases	Tankyrase 1 mRNA level (mean ± SD)	*P* value
Age (years)			
<59	21	0.00902±0.00455	0.853
≥60	15	0.00929±0.00350	
Sex			
Male	24	0.00906±0.00384	0.888
Female	12	0.00927±0.00473	
Tumor location			
Upper	10	0.01011±0.00460	0.628
Middle	7	0.00818±0.00531	
Lower	19	0.00897±0.00342	
Tumor size			
<5 cm	16	0.00948±0.00390	0.656
≥5 cm	20	0.00885±0.00432	
Tumor depth			
Into the wall	13	0.00778±0.00487	0.137
Out of serosa	23	0.00990±0.00347	
Histology			
Well differentiated	6	0.00565±0.00415	0.001
Moderately differentiated	12	0.00731±0.00226	
Poorly differentiated	18	0.01151±0.00367	
Lymph nodes			
Negative	11	0.00715±0.00341	0.053
Positive	25	0.01000±0.00412	
Stage			
I, II	12	0.00682±0.00320	0.014
III, IV	24	0.01029±0.00405	

Yamada et al. failed to find the over-expression of tankyrase 1 in gastric cancer [22]. In this study, we examined the level of tankyrase 1 expression in gastric cancers and their adjacent normal tissues. Our results showed that the normal and neoplastic gastric tissues express tankyrase 1, and tankyase 1 expression was significantly up-regulated in gastric cancer

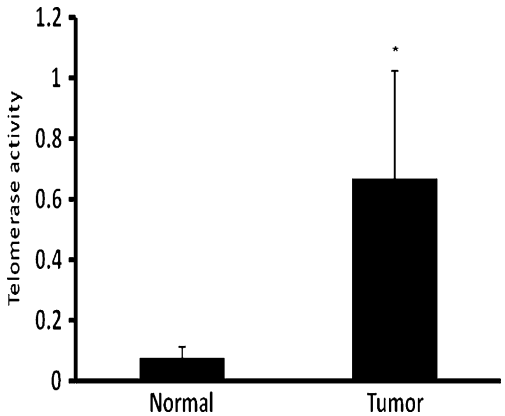

Fig. 3 Telomerase activity in gastric cancer. Telomerase activity was measured by TRAP-ELISA method in gastric cancer tissues and their normal counterparts. Height of the bars represents mean value and error bars represent standard deviation. * $P<0.001$

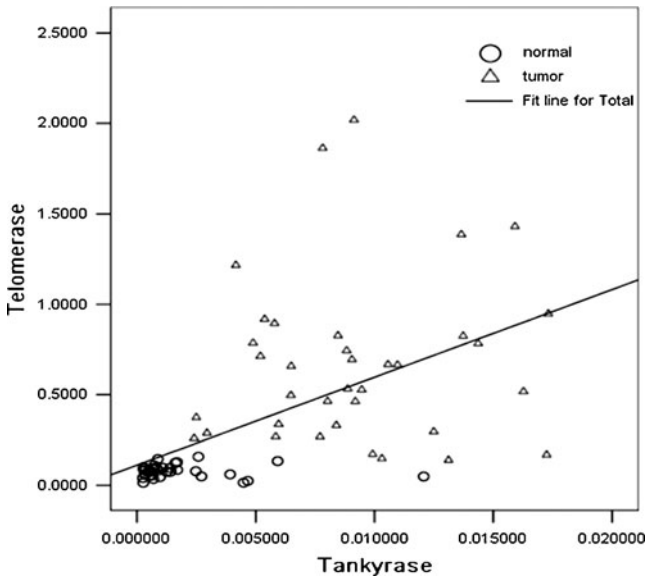

Fig. 4 Correlation between tankyrase1 and telomerase activity. The mRNA levels of tankyrase 1 correlated with telomerase activity in gastric cancer and their normal adjacent tissues. There is a significant positive correlation between tankyrase 1 and telomerase activity ($r=0.553$, $P<0.01$)

tissue compared to normal corresponding tissue. This indicates that tankyrase 1 over-expression may play an important role in gastric carcinogenesis.

Abnormal tankyrase 1 expression and activation may contribute to tumor development and progression. Gelmini et al. reported that tankyrase mRNA level correlates inversely with a cancer progression stage, patient survival and disease-free interval in colon cancer [18]. However, there was not a significant correlation between tankyrase 1 expression and the clinical and pathological features in breast cancer patients [17]. In the present study, we showed that tankyrase 1 over-expression by gastric cancerous tissue was significantly associated with tumor histology differentiation and tumor stage, but did not correlate with any other factors, such as age, sex, tumor size, tumor location, tumor depth and lymph node metastasis. These results indicate that tankyrase 1 over-expression may be involved in tumor development, by telomere length maintenance through enhancing telomerase access to telomeres in patients with gastric carcinomas. It is possible, then, that the over-expression of tankyrase 1 may contribute not only to tumorigenesis, but also to the clinical aggressiveness of a tumor. Clearly, further investigation should be warranted to determine whether and, if so, how tankyrase 1 plays different roles in the development and progression of tumors from different histological origins.

Human tankyrase 1 have been reported to be critical for telomere length maintenance. Tankyrase 1 releases TRF1 from telomeric complexes in a PARP activity-dependent manner, resulting in a telomeric conformation that provides greater access of telomerase to telomeres. Telomere elongation is induced by over-expression of exogenous tankyrase 1 in telomerase-positive cells, but not in telomerase-negative cells [13]. This shows that tankyrase 1 works as a positive regulator for telomere elongation by regulating telomerase. Moreover, it has been shown that tankyrase 1 regulates sister telomere separation [23], mitotic progression [24] and glucose metabolism [25].

The mechanism for the impact of tankyrase 1 alteration on tumor development and progression is still unclear. The most extensively studied aspect of tankyrase 1 function has involved the role of tankyrase 1 in regulation of telomerase access to telomere. Therefore, we also examined telomerase activity in gastric cancer and their adjacent normal tissues at the same time. We found that telomerase activity was significantly higher in gastric cancers in comparison to corresponding normal tissues. This is in accordance with most studies that previously investigated in gastric cancer [26, 27]. Furthermore, we showed that tankyrase 1 expression was significantly correlation with telomerase activity. This finding further confirms tankyrase 1 elongate telomere through regulating telomerase activity.

In summary, we showed that tankyrase 1 expression was up-regulated in gastric cancer and was associated with tumor histology differentiation and tumor stage. We also showed that tankyrase 1 expression was significantly correlation with telomerase activity. Our results indicate that tankyrase 1 over-expression may play an important role in gastric cancer development and progression. In addition, tankyrase 1 may be used as a biomarker of gastric cancer and may serve as a cellular target for the development of novel therapeutic agent.

Conflicts of interest The authors declare no conflict of interest.

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