

Clinicopathological Characteristics of TZAP Expression in Colorectal Cancers

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Purpose: The zinc finger protein, ZBTB48, is a telomere-associated protein. It was renamed as telomeric zinc finger-associated protein (TZAP) binding to elongated telomeres. However, its expression level was not measured in cancers.

Patients and Methods: We analyzed TZAP mRNA levels in 60 colorectal cancers (CRC) and its correlation with telomere length and TERT was studied.

Results: TZAP mRNA in CRC was higher statistically than that in paired non-cancerous tissues ($p = 0.033$). Higher TZAP was found in carcinoembryonic antigen (CEA)-positive CRCs (>5 ng/mL) ($p = 0.012$). Shorter telomere was found in CRCs with high TZAP expression than that with low TZAP expression ($p = 0.010$). According to quantitative correlation analysis, TZAP has a correlation with age ($r = -0.349$, $p = 0.007$), TERT ($r = 0.279$, $p = 0.041$) and telomere length ($r = -0.305$, $p = 0.021$). TZAP expression did not harbor prognostic value in CRC. Inhibition of TZAP expression by siRNA suppresses cell growth in HT29 cells; however, it resulted in increased cell viability in HCT116 cells. TZAP inhibition induces a decrease in mRNA levels of TERT in both HT29 and HCT116 cells. TCGA data analysis showed higher expression of TZAP showed poorer overall survival in colon cancer ($p = 0.001$); however, it did not have a significance in rectal cancer ($p = 0.951$).

Conclusion: We suggested that TZAP may be a possible biomarker for CRC.

Keywords: TZAP, telomere, colorectal cancer, ZBTB48

Introduction

Telomeres are composed of 6-bp TTAGGG repeat sequences capping each end of eukaryotic chromosomes.^{1,2} In human somatic cells, an average length of telomere is 5–15 kilobases; however, approximately 30–200 base pairs were shortened at every cell division. It is counteracted by the telomerase reverse transcriptase or the alternative lengthening of telomeres (ALT) mechanism.^{3–5} Overly long telomeres are cut back to normal length by trimming mechanism.^{6–8} Although its precise regulation has not been recognized, a recent study found a novel protein, ZBTB48, regulating telomere length.⁹ As zinc finger protein, it was renamed telomeric zinc finger-associated protein (TZAP). In addition, they found that TZAP overexpression caused extremely short telomere. TZAP is located to chromosome 1p36, a region that is frequently rearranged or deleted in many cancers.^{10–12} Therefore, genetic change of TZAP could have a role in cancer pathogenesis; however, TZAP research has been studied rarely in cancer patients or cells.

Colorectal cancer (CRC) is one of the most common types of cancer in Korea and other developed countries.¹³ CRC carcinogenesis is induced by genetic and

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epigenetic changes and is influenced by tumor–host interactions.^{14,15} Many studies demonstrated molecular markers such as BRAF, KRAS, and MMR for CRC.^{16,17} Previous studies have demonstrated telomere length may be a possible prognostic marker for CRC.^{18,19} However, the regulation of telomere length by TZAP in CRC is uncertain. Here, we evaluate the association between TZAP expression and survival outcomes in CRC and investigate its molecular mechanism. Additionally, TERT mRNA expression and telomere length were also investigated and their results were compared with public available big data, The Cancer Genome Atlas (TCGA). This comprehensive data may suggest molecular mechanism of TZAP expression in colorectal carcinogenesis.

Patients and Methods

Patients and Tissues

A total of 60 male patients (63.6 ± 10.5 years old, 34–83) were diagnosed as CRC and included in this study. Tissue samples, colorectal adenocarcinomas and paired adjacent non-cancerous tissues were provided from Keimyung Human Bio-resource Bank, Korea. It was obtained from the patients undergoing surgery in Dongsan Medical Center (Daegu, Korea) between April 2008 and January 2010. All patients were explained the study purpose, and informed consent was obtained from each study participant. This study was approved by the Institutional Review Board (Keimyung University Dongsan Medical Center No. 2020-07-027).

DNA Isolation and Telomere Length

Analysis

We isolated DNA from samples by QIAamp DNA mini kits (Qiagen, Inc., Valencia, CA, USA). Telomere length was analyzed using qPCR with specific primers for telomere (T) and β -globin (S), based on previous study.¹³ qPCR was performed by a LightCycler 480 II system (Roche Diagnostics, Basel, Switzerland) with the specific primer described in [Table S1](#) and SYBR GREEN Premix (Toyobo, Japan). Relative telomere length was calculated T/S values using the following formula: $T/S = 2^{-\Delta Cq}$, where $\Delta Cq = \text{mean } Cq_T - \text{mean } Cq_S$. β -Actin as a housekeeping gene was used for normalization. Each measurement was repeated in triplicate and five serially diluted control samples were included in each experiment.

RNA Isolation and mRNA Expression Analysis

We extracted RNA from tissues by the TRIzol reagent (Molecular Research Center Inc., Cincinnati, OH, USA). RNA quality was measured by Nanodrop 1000 (Thermo Scientific, Wilmington, Denmark). Each cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA). Then, qPCR was performed with a specific primer in [Table S1](#). Further examination was carried out as previously described.

Cell Culture and siRNA Transfection

Human colon cancer cell lines, HCT116 and HT29, were purchased from the Korean Cell Line Bank (KCLB; Seoul, Republic of Korea). They were maintained in RPMI-1640 medium (Corning Incorporated, Corning, NY, USA) with 10% of fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA) and 1% of penicillin/streptomycin solution (HyClone Laboratories), in a 37°C with 5% CO₂. For MTT and apoptosis assay, cells were plated at 30% density 24 h before transfection. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was mixed with Opti-MEM (Invitrogen) to adjust the final concentration of 15 nM siRNA, then, the medium was replaced 6 h after transfection. TZAP siRNA duplexes (si-TZAP) were chemically synthesized by Ambion (Austin, TX, USA; siRNA ID# s6566, s6567, s6568). The negative control siRNA (si-NC) that does not target any endogenous transcript was used for control experiments. The sequences of si-NC (Bioneer, Daejeon, South Korea) are as follows: 5'-ACGUGA CACGUUCGGAGAA-3' (sense) and 5'-UUCUCCGAA CGUGUCACGU-3' (antisense).

Cell Viability Assay

Cell viability was analyzed by the MTT reagent as stated in the manufacturer's protocol (Duchefa Biochemie, Haarlem, Netherlands). Log phase cells were trypsinized into single-cell suspension and HCT116 (2×10^3 cells) and HT29 (1×10^3 cells) were seeded into 96-well plates. After 24 h, cells were transfected as described above. After 96 h, 90 μL of plain media and 10 μL of MTT were added and cultured for 1–2 h in a 37°C humidified incubator. After 2h, 100 μL of dimethyl sulfoxide (DMSO, SIGMA-ALDRICH, St Louis, MO, USA) solution was added into each well. Then, OD value was measured at a wavelength of 540 nm by an Asys UVM 340 microplate reader (Biochrom, Cambridge, UK).

The Cancer Genome Atlas (TCGA) Data Analysis

We investigated the publically available TCGA datasets and their data were downloaded from the TCGA Data Portal at <https://tcga-data.nci.nih.gov/tcga/>. The microarray and RNA-Seq experiments and clinical data were downloaded directly from the TCGA website in May 2020.

Statistical Analysis

Chi-square, Fisher's exact test and the Mann–Whitney *U*-test were used to analyze the association between variables. Univariate survival analysis constructed by the Log rank test with Kaplan–Meier curve. Overall survival was defined as the time between diagnosis and mortality. Disease-free survival was defined as the time between diagnosis, and disease recurrence or the development of distant metastasis. The correlations between telomere length and mRNA expressions and clinicopathologic parameters were assessed with the Pearson's correlation coefficient analysis. A *P*-value of <0.05 was considered statistically significant.

Results

Expression Levels of TZAP and TERT mRNA and Telomere Length

TZAP and TERT mRNA expression levels were analyzed in CRC patients. The TZAP and TERT expression were normalized to β -actin. Then, the quantitative PCR (qPCR) data were measured by Δ CT values (Figure 1). TZAP mRNA in CRC was statistically higher than that in paired normal tissue ($p = 0.033$). However, TERT mRNA and telomere length were similar in CRC and non-cancerous

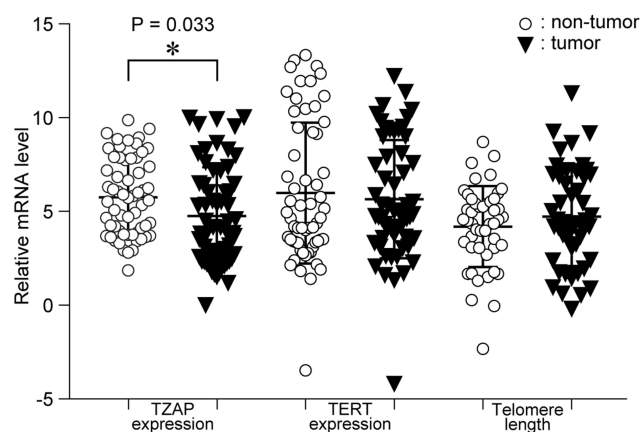


Figure 1 mRNA expression levels of TZAP and TERT, and telomere length in CRCs and paired non-cancerous tissues.

tissues ($p = 0.604$ and 0.288 , respectively). The average of TZAP and TERT expression levels in CRCs were 6.41 ± 1.43 and 4.13 ± 1.09 , respectively, as calculated in CRC compared to that of normal tissues. Telomeres in CRC were an average 3.95-fold (SD = 1.39) longer than that in non-cancerous samples.

Clinicopathological Characteristics of TZAP and TERT mRNA Expressions and Telomere Length in CRC

To identify the association between these changes and the clinicopathological characteristics, patients were divided into two groups by the average values of the Tumor/Non-tumor ratio. Clinicopathological characteristics of telomere length, TZAP, and TERT expression levels are summarized in Table 1. High TZAP and TERT mRNA levels and telomere shortening were shown in 52.5%, 31.6%, and 43.9% of CRCs, respectively. TZAP and TERT expression levels were different according to T stage; however, it did not achieve statistical significance ($p = 0.067$ and 0.072 , respectively). Higher expression levels of TZAP were found in carcinoembryonic antigen (CEA)-positive CRCs (>5 ng/mL) ($p = 0.012$). Telomere length was shorter in CRCs with high TZAP expression than in CRCs with low TZAP expression ($p = 0.010$). Other clinicopathological parameters showed no association with telomere length, TZAP, and TERT mRNA levels.

Quantitative correlation analysis with clinical parameters was performed. The association between age, BMI, CEA, telomere length, TZAP, and TERT is presented in Table 2. Telomere length had no association with age ($r = -0.118$, $p = 0.383$). However, TZAP has a negative correlation with age ($r = -0.349$, $p = 0.007$, Figure 2A) and telomere length ($r = -0.305$, $p = 0.021$, Figure 2B). TZAP and TERT had a weak positive correlation each other ($r = 0.279$, $p = 0.041$, Figure 2C). Other parameters had no quantitative correlation with others.

Prognostic Value of TZAP and TERT mRNA Expressions and Telomere Length in CRC

We evaluated survival analysis of CRC to show prognostic value of TZAP and TERT mRNA expression levels and telomere length. The median follow-up period was 60.2 months (2.5–88.9). Univariate survival analysis showed no significance of TZAP expression for overall survival in CRC (87.3 vs 76.8 months, $p = 0.372$) (Figure 3A).

Table I Clinicopathological Characteristics of TZAP and TERT mRNA Expressions and Telomere Length in CRC

| | TZAP Expression | | | Telomere Length | | | TERT Expression | | |
|--------------------------|-----------------|-----------|-------|-----------------|-----------|-------|-----------------|-----------|-------|
| | Low | High | p | Short | Long | P | Low | High | P |
| Total | 28 (47.5) | 31 (52.5) | | 25 (43.9) | 32 (56.1) | | 39 (68.4) | 18 (31.6) | |
| Age (years) | | | 0.393 | | | 0.847 | | | 0.137 |
| ≤60 | 13 (54.2) | 11 (45.8) | | 10 (45.5) | 12 (54.5) | | 19 (79.2) | 5 (20.8) | |
| >60 | 15 (42.9) | 20 (57.1) | | 15 (42.9) | 20 (57.1) | | 20 (60.6) | 13 (39.4) | |
| BMI (kg/m ²) | | | 0.234 | | | 0.231 | | | 0.633 |
| ≤25 | 22 (52.4) | 20 (47.6) | | 20 (48.8) | 21 (51.2) | | 28 (66.7) | 14 (33.3) | |
| >25 | 6 (35.3) | 11 (64.7) | | 5 (31.2) | 11 (68.8) | | 11 (73.3) | 4 (26.7) | |
| T stage | | | 0.067 | | | 0.107 | | | 0.072 |
| T1 | 3 (100.0) | 0 (0.0) | | 0 (0.0) | 3 (100.0) | | 16 (48.5) | 17 (51.5) | |
| T2 | 1 (12.5) | 7 (87.5) | | 4 (57.1) | 3 (42.9) | | 6 (42.9) | 8 (57.1) | |
| T3 | 17 (44.7) | 21 (55.3) | | 19 (51.4) | 18 (48.6) | | 3 (30.0) | 7 (70.0) | |
| T4 | 7 (70.0) | 3 (30.0) | | 2 (20.0) | 8 (80.0) | | 25 (43.9) | 32(56.1) | |
| N stage | | | 0.107 | | | 0.585 | | | 0.983 |
| N0 | 14 (42.4) | 19 (57.6) | | 16 (48.5) | 17(51.5) | | 22 (68.8) | 10 (31.3) | |
| N1 | 11 (68.8) | 5 (31.3) | | 6 (42.9) | 8 57.1) | | 10 (66.7) | 5 (33.3) | |
| N2 | 3 (30.0) | 7 (70.0) | | 3 (30.0) | 7 (70.0) | | 7 (70.0) | 3 (30.0) | |
| M stage | | | 0.337 | | | 0.797 | | | 1.00 |
| M0 | 25 (45.5) | 30 (54.5) | | 23 (43.4) | 30 (56.6) | | 36 (67.9) | 17 (32.1) | |
| M1 | 3 (75.0) | 1 (25.0) | | 2 (50.0) | 2 (50.0) | | 3 (75.0) | 1 (25.0) | |
| Pathological stage | | | 0.456 | | | 0.720 | | | 0.135 |
| I | 3 (37.5) | 5 (62.5) | | 3 (37.5) | 5 (62.5) | | 3 (37.5) | 5 (62.5) | |
| II | 9 (39.1) | 14 (60.9) | | 12 (52.2) | 11 (47.8) | | 18 (81.8) | 4 (18.2) | |
| III | 13 (54.2) | 11 (45.8) | | 8 (36.4) | 14 (63.6) | | 15 (65.2) | 8 (34.8) | |
| IV | 3 (75.0) | 1 (25.0) | | 2 (50.0) | 2 (50.0) | | 3 (75.0) | 1 (25.0) | |
| CEA (ng/mL) | | | 0.012 | | | 0.528 | | | 0.211 |
| ≤5 | 26 (56.5) | 20 (43.5) | | 18 (40.9) | 26 (59.1) | | 29 (64.4) | 16 (35.6) | |
| >5 | 2 (15.4) | 11 (84.6) | | 7 (53.8) | 6 (46.2) | | 10 (83.3) | 2 (16.7) | |
| Histology | | | 0.610 | | | 0.492 | | | 0.370 |
| Well | 2 (66.7) | 1 (33.3) | | 2 (66.7) | 1 (33.3) | | 2 (66.7) | 1 (33.3) | |
| Moderately | 24 (45.3) | 29 (54.7) | | 21 (41.2) | 30 (58.8) | | 33 (66.0) | 17 (33.) | |
| Poorly | 2 (66.7) | 1 (33.3) | | 2 (66.7) | 1 (33.3) | | 4 (100) | 0 (0) | |
| TZAP | | | | | | 0.010 | | | 0.336 |
| (+) | | | | 18 (60.0) | 12 (40.0) | | 18 (62.1) | 11 (37.9) | |
| (-) | | | | 7 (25.9) | 20 (74.1) | | 20 (74.1) | 7 (25.9) | |
| Telomere | | | 0.010 | | | | | | 0.933 |
| Long | 20 (62.5) | 12 (37.5) | | | | | 21 (67.7) | 10 (32.7) | |
| Short | 7 (28.0) | 18 (72.0) | | | | | 16 (66.7) | 8 (33.3) | |
| TERT | | | 0.336 | | | 0.933 | | | |
| (+) | 7 (38.9) | 11 (61.1) | | 8 (44.4) | 10 (56.8) | | | | |
| (-) | 20 (52.6) | 18 (47.4) | | 16 (43.2) | 21 (56.8) | | | | |

Disease-free survival was not different according to the TZAP expression (82.8 vs 74.8 months, $p = 0.601$). Disease-free survival was not different according to the telomere length (overall survival: 83.4 vs 68.8 months, $p = 0.305$; disease-free survival: 80.7 vs 64.5 months, $p = 0.967$; disease-free survival: 75.7 vs 76.6 months, $p = 0.952$) and telomere length (overall survival: 83.4 vs 68.8 months, $p = 0.305$; disease-free survival: 80.7 vs 64.5 months, $p = 0.967$).

Table 2 Correlation Between TZAP Expression and the Clinical Parameters in CRC

| | | Age | BMI | CEA | Telomere | TZAP | TERT |
|--------------------------|---|--------|-------|--------|----------|--------|--------|
| Age (years) | R | 1 | 0.032 | 0.212 | -0.118 | -0.349 | 0.204 |
| | P | | 0.811 | 0.103 | 0.383 | 0.007 | 0.127 |
| BMI (kg/m ²) | R | 0.032 | 1 | 0.087 | 0.212 | 0.118 | 0.096 |
| | P | 0.811 | | 0.510 | 0.114 | 0.372 | 0.478 |
| CEA | R | 0.212 | 0.087 | 1 | -0.114 | 0.190 | -0.132 |
| | P | 0.103 | 0.510 | | 0.398 | 0.150 | 0.326 |
| Telomere | R | -0.118 | 0.212 | -0.114 | 1 | -0.305 | -0.194 |
| | P | 0.383 | 0.114 | 0.398 | | 0.021 | 0.155 |
| TZAP | R | -0.349 | 0.118 | 0.190 | -0.305 | 1 | 0.279 |
| | P | 0.007 | 0.372 | 0.150 | 0.021 | | 0.041 |
| TERT | R | 0.204 | 0.096 | -0.132 | -0.194 | 0.279 | 1 |
| | P | 0.127 | 0.478 | 0.326 | 0.155 | 0.041 | |

months, $p = 0.313$) did not have any prognostic value in CRCs. When stratified patients by TZAP expression and telomere length, patients with lower TZAP and short telomere length showed poorer prognoses (46.6 vs 83.4 months, $p = 0.003$, [Figure 3B](#)). Other stratification of these markers did not show any significance.

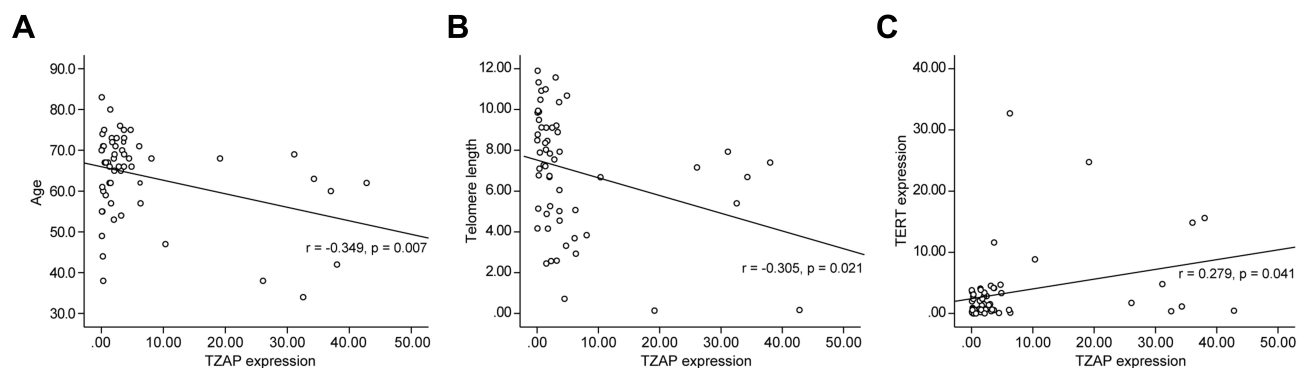
Effect of TZAP Silencing in Cell Viability and TERT mRNA Expression

To show the effect of TZAP silencing on cell survival, HCT116 and HT29 cells were transfected with a negative control (NC) siRNA or TZAP siRNA. Firstly, we determined the efficacy of TZAP siRNA by qPCR. As shown in [Figure 4A](#), TZAP mRNA was significantly lower in HCT116 and HT29 transfected with TZAP siRNA than cells transfected with NC siRNA. Cell viability of the HCT116 and HT29 cells was assessed by using an MTT assay. The viability of the TZAP-siRNA-treated cells was

increased by about 170% in HCT116 cells, when compared to that of the control group. However, in HT29 cells, it was reduced by about 60% ([Figure 4B](#)). To investigate the association between TZAP and TERT, mRNA expression of TERT was analyzed in TZAP silencing HCT116 and HT29 cells. TZAP silencing induces a decrease in mRNA levels of TERT in both cells ([Figure 4C](#)). These findings suggest that the function of TZAP is related to the TERT pathway in CRC progression. However, telomere length was not significantly changed in TZAP silencing HCT116 ($p = 0.48$) and HT29 cells ($p = 0.73$).

The Cancer Genome Atlas (TCGA) Data

We used the publically available colon and rectal cancer dataset in the TCGA database. In colon cancer, TZAP expression was related with lymphatic invasion ($p = 0.039$) and TERT expression ($p = 0.004$) ([Table 3](#)).

**Figure 2** Correlation analysis. (A) Between TZAP expression and age. (B) Between TZAP and telomere length. (C) Between TZAP and TERT expressions.

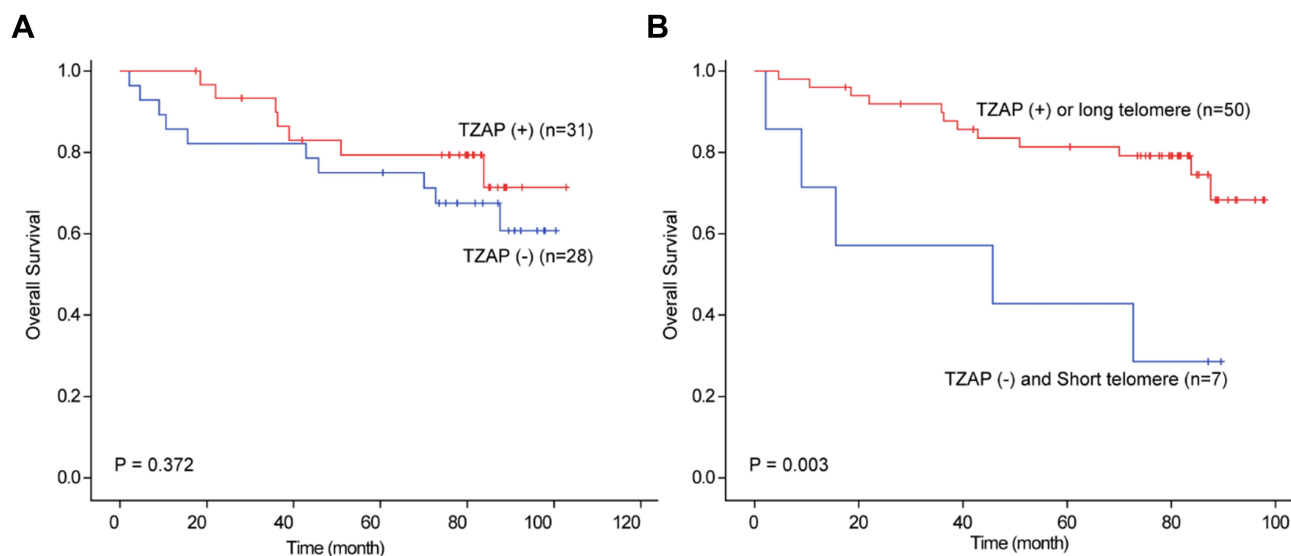


Figure 3 Survival analysis in CRCs. (A) Overall survival according to TZAP expression. (B) Overall survival according to TZAP expression and telomere length.

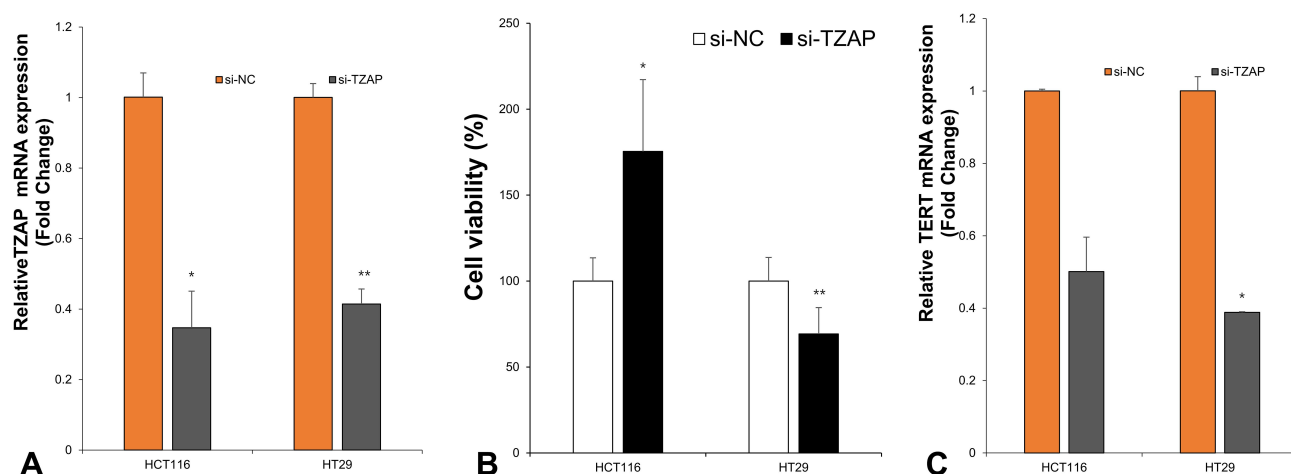


Figure 4 siRNA knockdown of TZAP and growth of colorectal cancer cells. (A) Detection of cell viability after transfection in HCT116 and HT29 cells. (B) Detection of TZAP mRNA expression in HCT116 and HT29 cells at 48 h after transfection. (C) Detection of TERT mRNA expression in HCT116 and HT29 cells at 48 h after transfection. Data represent three independent experiments (* $P < 0.05$ and ** $P < 0.01$ by Student's t-test).

Abbreviations: si-NC, negative control siRNA; si-TZAP, TZAP-specific siRNA.

In rectal cancer, TZAP did not have any clinical characteristics; however, TERT expression had an association with T stage ($p = 0.017$) and lymphatic invasion ($p = 0.17$) (Table 4). TZAP and TERT expression tended to be correlated; however, it did not get a significance ($p = 0.08$).

In our previous report, quantitative correlation analysis also showed that TZAP and TERT mRNA levels were positively correlated in colon cancer ($r = 0.187$, $p < 0.001$) and rectal cancer ($r = 0.186$, $p = 0.020$).²⁰ Survival analysis showed that higher TZAP mRNA levels predicted poorer prognoses in colon cancer ($p < 0.001$, Figure 5A). However, it did not have any prognostic value in rectal cancer ($p = 0.119$, Figure 5B).

Discussion

In this study, we showed clinicopathological significance of TZAP mRNA expression in CRC patients, cell lines, and public data for the first time. Previous studies suggested that TZAP expression might prevent telomere elongation occurring during cancer progression.^{4,9} Therefore, its insufficient expression predicts poorer prognoses in many cancers by TCGA data.²¹ However, higher TZAP expression was associated with poorer survival, resulting in some cancers, especially CRC. In the present study, we analyze TZAP mRNA expression and telomere length in 60 CRC tissues and paired non-cancerous tissues. TZAP

Table 3 TCGA Data of Clinicopathological Characteristics of TZAP and TERT mRNA Expressions in Colon Cancer

| | TZAP Expression | | | TERT Expression | | |
|--------------------|-----------------|-------------|-------|-----------------|-------------|-------|
| | High | Low | p | High | Low | P |
| Age (years) | 66.8 ± 12.7 | 66.4 ± 12.4 | 0.729 | 65.7 ± 13.6 | 67.5 ± 12.5 | 0.137 |
| Gender | | | 0.504 | | | 0.924 |
| Female | 99 (48.1) | 107 (51.9) | | 102 (49.5) | 104 (50.5) | |
| Male | 121 (51.7) | 113 (48.3) | | 118 (50.4) | 116 (49.6) | |
| T stage | | | 0.561 | | | 0.769 |
| T1 | 6 (54.5) | 5 (45.5) | | 6 (54.5) | 5 (45.5) | |
| T2 | 35 (47.3) | 39 (52.7) | | 33 (44.6) | 41 (55.4) | |
| T3 | 148 (48.8) | 155 (51.2) | | 155 (51.2) | 148 (48.8) | |
| T4 | 30 (58.8) | 21 (41.2) | | 25 (49.0) | 26 (51.0) | |
| N stage | | | 0.927 | | | 0.875 |
| N0 | 127 (49.2) | 131 (50.8) | | 127 (50.0) | 131 (50.0) | |
| N1 | 53 (51.0) | 51 (49.0) | | 52 (50.0) | 52 (50.5) | |
| N2 | 40 (51.3) | 38 (48.7) | | 41 (52.6) | 37 (47.4) | |
| M stage | | | 0.612 | | | 0.287 |
| M0 | 159 (48.9) | 166 (51.1) | | 157 (48.3) | 168 (51.7) | |
| M1 | 32 (52.5) | 29 (47.5) | | 34 (55.7) | 27 (44.3) | |
| CEA (ng/mL) | | | 1.00 | | | 0.799 |
| ≤5 | 102 (54.0) | 87 (46.0) | | 94 (49.7) | 95 (50.3) | |
| >5 | 49 (53.8) | 42 (46.2) | | 47 (51.6) | 44 (48.4) | |
| TZAP | | | | | | 0.004 |
| (+) | | | | 125 (60.0) | 95 (40.0) | |
| (-) | | | | 95 (25.9) | 125 (74.1) | |
| Lymphatic invasion | | | 0.039 | | | 0.324 |
| (+) | 86 (56.6) | 66 (43.4) | | 80 (52.6) | 72 (47.4) | |
| (-) | 112 (45.9) | 132 (54.1) | | 116 (47.5) | 128 (52.5) | |
| MSI | | | 0.585 | | | 0.855 |
| (+) | 80 (51.3) | 76 (48.7) | | 76 (48.7) | 80 (51.3) | |
| (-) | 133 (48.5) | 141 (51.5) | | 136 (49.6) | 138 (50.4) | |

expression was significantly higher in CRCs and it was associated with higher CEA and shorter telomere length. To clarify the role of TZAP in telomere regulation, TERT expression inducing telomere elongation was examined. Quantitative correlation analysis showed an age-dependent in TZAP mRNA levels and telomere shortening. Interestingly, TZAP expression was positively correlated with TERT expression. Our previous study using TCGA data showed this correlation in bladder, colorectal, glioblastoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, lung, melanoma, and uterine endometrial carcinomas.²⁰ To confirm their association, TERT mRNA was analyzed in TZAP-silenced HCT116 and HT29 cells. The results showed that TZAP silencing

decreased TERT mRNA expression in both cells. For a first time, this association was confirmed equally in CRC tissues, cell lines, and big data. Although TZAP has been known to compete with TRF1 and TRF2, our results suggested that TZAP and TERT were expressed competitively and proportionally. Unexpectedly, TZAP silencing did not induce the change of telomere length. The paradoxical effect of TZAP and TERT on the regulation of telomere length should be confirmed further.

The public data demonstrated that TZAP mRNA expression in colon cancer was associated with poorer prognoses. It was in agreement with previous hypothesis suggesting telomere shortening via TZAP expression may induce cell death.⁹ In rectal cancer, TZAP mRNA

Table 4 TCGA Data of Clinicopathological Characteristics of TZAP and TERT mRNA Expressions in Rectal Cancer

| | TZAP Expression | | | TERT Expression | | |
|--------------------|-----------------|-------------|-------|-----------------|-------------|-------|
| | High | Low | p | High | Low | P |
| Age (years old) | 64.5 ± 12.1 | 64.7 ± 11.5 | 0.936 | 63.9 ± 11.8 | 65.3 ± 11.8 | 0.467 |
| Gender | | | 0.424 | | | 0.424 |
| Female | 38 (53.3) | 33 (46.5) | | 38 (53.3) | 33 (46.5) | |
| Male | 41 (47.1) | 46 (52.9) | | 41 (47.1) | 46 (52.9) | |
| T stage | | | 0.440 | | | 0.017 |
| T1 | 4 (44.4) | 5 (55.6) | | 2 (22.2) | 7 (77.8) | |
| T2 | 18 (64.3) | 10 (35.7) | | 19 (67.9) | 9 (32.1) | |
| T3 | 51 (47.7) | 56 (52.3) | | 55 (51.4) | 52 (48.6) | |
| T4 | 6 (46.2) | 7 (53.8) | | 3 (23.1) | 10 (76.9) | |
| N stage | | | 0.111 | | | 0.293 |
| N0 | 45 (56.3) | 35 (43.8) | | 37 (46.3) | 43 (53.8) | |
| N1 | 22 (51.1) | 21 (48.8) | | 26 (60.5) | 17 (39.5) | |
| N2 | 11 (34.3) | 21 (65.6) | | 15 (46.9) | 17 (53.1) | |
| M stage | | | 0.291 | | | 0.219 |
| M0 | 66(55.5) | 53(44.5) | | 61(51.3) | 58(48.7) | |
| M1 | 10(43.5) | 13(56.5) | | 15(65.2) | 8(34.8) | |
| CEA (ng/mL) | | | 0.211 | | | 0.148 |
| ≤5 | 33 (56.7) | 29 (43.3) | | 37 (59.7) | 25 (40.3) | |
| >5 | 18 (40.9) | 26 (59.1) | | 20 (45.5) | 24 (54.5) | |
| TZAP | | | | | | 0.08 |
| (+) | | | | 45 (57.0) | 34 (43.0) | |
| (-) | | | | 34 (43.0) | 45 (57.0) | |
| Lymphatic invasion | | | 0.106 | | | 0.017 |
| (+) | 34(59.6) | 23(40.4) | | 35(61.4) | 22(38.6) | |
| (-) | 38(45.8) | 45(54.2) | | 34(41) | 49(59) | |

expression tended to be associated with better survival result; however, it did not have significance. Moreover, TZAP silencing induced cancer cell growth in HCT116 cells; however, it decreased the viability of HT29 cells. Both data and cell line results showed a bipolar effect of TZAP expression; therefore, its pivot factor should be investigated. Considering the genetic difference of HCT116 and HT29 cells, P53 mRNA expression was analyzed. However, there was no significant difference in both cell lines (data not shown). In CRC patients, TZAP did not have prognostic significance; however, the combination of TZAP and telomere length showed powerful prognostic values. We hypothesized that short telomere uncontrolled without TZAP may be important for cancer progression. These controversial results of TZAP expression indicated that many factors might co-operate in cancer progression via telomere regulation. An unknown

physiological mechanism may be present in this process. Moreover, TZAP mRNA level and activity of TZAP protein may be different. The molecular mechanism of TZAP should be confirmed further and its clinical value needed to be discussed.

Taken together, we demonstrated, for the first time, clinical and prognostic values of TZAP in CRC. The correlation between TZAP and TERT was consistent in patients, cell lines, and public data, however, some results were different in these data. Therefore, the issue of telomere regulation in cancer therapy remains to be determined. To date, various molecular targets were exploited to trigger cancer cell death via different mechanisms and pathways. TZAP may be associated with telomere regulation and cancer cell survival in CRCs. Therefore, targeting TZAP may be a new avenue for cancer therapy.

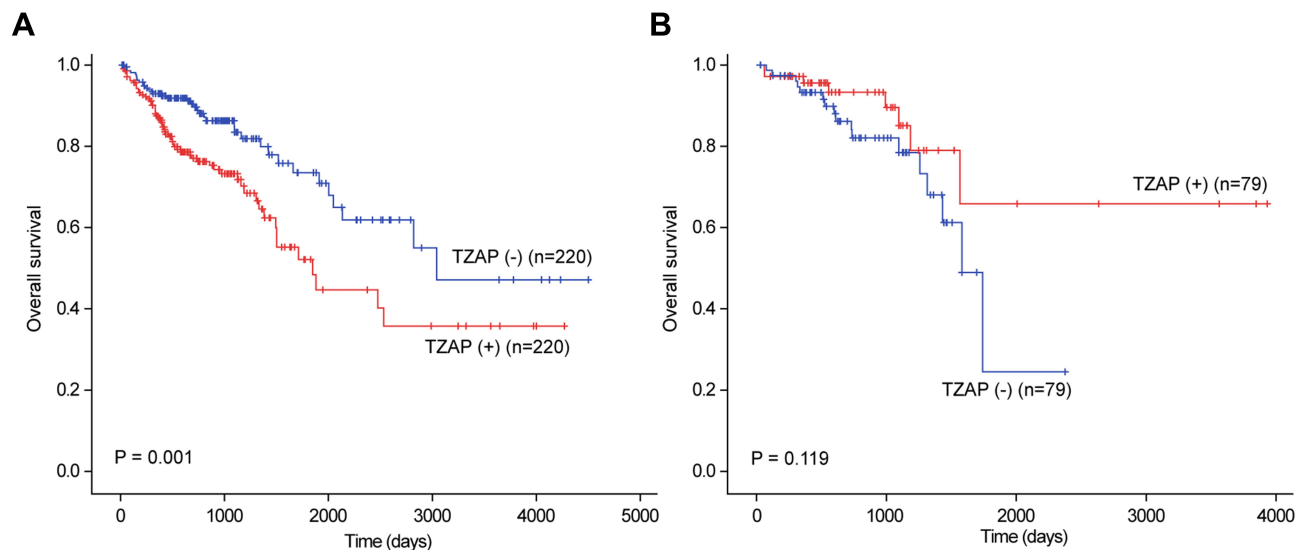


Figure 5 TCGA data of TZAP expression (ZBTB48) in colon cancer (A) and rectal cancer (B).

Conclusion

In conclusion, we demonstrated the clinical implication of TZAP expression in CRC tissues and cells. Big data results also support the significant role of TZAP expression in cancers. Further studies about its functional mechanism are warranted to validate these results.

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Disclosure

The authors declare no conflicts of interest.

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