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RESEARCH ARTICLE

# The *hTERT*-VNTR2-2nd alleles are involved in genomic stability in gastrointestinal cancer

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**Abstract**

**Background** *hTERT* contains a high density of minisatellites, of which rare alleles of *hTERT*-VNTR2-2nd have been reported to be associated with prostate cancer. This shows an association between VNTR and cancer, but this repeat sequence is likely to be associated with genomic instability. Therefore, we investigated the effects of *hTERT*-VNTR2-2nd on gastrointestinal cancer and the relationship between repeated sequence and chromosome instability.

**Methods** A case–control study was performed using DNA from 818 cancer-free controls, 539 cases with gastric cancer, 275 cases with colon cancer and 274 cases with rectal cancer. To determine whether minisatellites affect gene expression, expression levels were examined using *TERT*-reporter vectors in cell lines. In addition, the length of the *hTERT*-VNTR2-2nd alleles were determined in blood and cancer tissues from 107 gastric cancers, 112 colon cancers and 76 rectal cancers patients to determine whether the repeat sequence was associated with genomic instability during cancer development.

**Results** No statistically significant association between *hTERT*-VNTR2-2nd and risk of gastrointestinal cancer was detected. However, it has been shown that VNTRs inserted into the enhancer region can regulate the expression of *TERT* in gastrointestinal cancer cells. Moreover, *hTERT*-VNTR2-2nd was analyzed in matched blood and cancer tissue from patients with gastrointestinal cancer and in seven among 294 subjects, and *hTERT*-VNTR2-2nd was found to be rearranged.

**Conclusions** We suggest that minisatellites are associated with genomic instability in cancer and that the *hTERT*-VNTRs region may increase *hTERT* expression in gastrointestinal cancer cells.

**Keywords** *TERT* · Gastrointestinal cancer · Minisatellite polymorphism · Genome instability

## Introduction

The ends of eukaryotic chromosomes undergo degradation and end-to-end fusion problem during cell division (Blackburn 2000). To protect the coding region from this event, telomeres that are non-coding repeat region are specialized at the ends of eukaryotic chromosomes (Blackburn 2001). Maintaining chromosome terminal length by telomerase is necessary for immortal proliferation in stem cells or most malignancies (Shay and Bacchetti 1997). Template-containing RNA components (TR) and human telomerase reverse transcriptase (*hTERT*) are major components for chromosome end synthesis (Jiang et al. 1999; Morales et al. 1999), of which the telomerase catalytic subunit, *hTERT*, has been found to be abnormal in many human cancers (Ito et al. 1998; Kanaya et al. 1998; Kyo et al. 1998, 1999; Takakura et al. 1998).

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In previous studies, we identified five variable number tandem repeat (VNTR, mini-satellite) regions present in the intron region of *hTERT*, of which rare alleles of *hTERT*-VNTR 2-2$^{nd}$ were associated with prostate cancer (Kim et al. 2003; Leem et al. 2002). The region of *hTERT*-VNTR2-2$^{nd}$ found that the repeat unit was 61-bp long, with 44 repeated VNTRs being the most common allele (Yoon et al. 2010a). In particular, 28 and 37 repeated VNTR alleles were detected only in a group of prostate cancer patients, and the rare *hTERT*-VNTR2-2$^{nd}$ allele containing these cancer specific alleles was statistically associated with prostate cancer risk (Yoon et al. 2010a). And we confirmed that the *hTERT*-VNTR2-2$^{nd}$ allele region could influence the expression of *hTERT* in prostate cancer (Yoon et al. 2010a). In addition, rearrangement occurrence in the VNTR6-1 and VNTR6-2 regions present in intron six was confirmed by comparison of normal and cancerous tissues (Leem et al. 2002). Our previous study demonstrated the relationship between minisatellite variants and several cancers (Ahn et al. 2009; Jeong et al. 2007; Seol et al. 2008; Yoon et al. 2008, 2010b), and also revealed that VNTR alleles influences gene expression (Kwon et al. 2010; Yoon et al. 2010a).

In this study, we investigated the allelic variation and genetic rearrangement of *hTERT*-VNTR2-2$^{nd}$ in gastrointestinal cancer and their association with *hTERT* expression. A case control study using a PCR-based method was performed and compared the allelic distribution of *hTERT*-VNTR2-2$^{nd}$ in DNA samples from 818 cancer-free controls and 1088 patients with gastrointestinal cancer. In sequence, we identified the effect of the VNTRs on the transcriptional levels of *hTERT*-promoter driven reporter gene in gastrointestinal cancer cell lines. In addition, the length of the *hTERT*-VNTR2-2$^{nd}$ allele was determined in the blood and cancer tissue of gastrointestinal cancer patients to determine whether the repeat sequence was associated with genomic instability during cancer development. Here, we report that *hTERT*-VNTR2-2$^{nd}$ alleles may be related to genomic instability and regulation of expression level of *hTERT* in gastrointestinal cancer.

## Materials and methods

### Preparation of case–control study and genomic DNA from peripheral blood lymphocytes and cancer tissues

We performed a case–control study with genomic DNA from the 818 cancer-free controls and 539 cases with gastric cancer, 275 cases with colon cancer and 274 cases with rectal cancer (Table 1). The controls consist of similar proportion of sex and age range to the cases (Table 1). A total of 818 individuals in the control group with no personal history of

**Table 1** Age and sex distribution of cases and controls

| Characteristic | Controls, *N* (%) | | | Gastric cancer cases, *N* (%) | | | Colon cancer cases, *N* (%) | | | Rectal cancer cases, *N* (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total | Males | Females | Total | Males | Females | Total |
| Age (years) | | | | | | | | | | | | |
| 20–29 | 3 (0.7) | 10 (2.5) | 13 (1.6) | 2 (0.6) | 5 (2.5) | 7 (1.3) | – | 3 (2.4) | 3 (1.1) | 1 (0.6) | 2 (2.1) | 3 (1.1) |
| 30–39 | 20 (4.8) | 36 (9.1) | 56 (6.8) | 12 (3.6) | 18 (8.8) | 30 (5.6) | 3 (2.0) | 4 (3.1) | 7 (2.5) | 7 (4.0) | 7 (7.2) | 14 (5.1) |
| 40–49 | 27 (6.4) | 76 (19.1) | 103 (12.6) | 55 (16.4) | 38 (18.6) | 93 (17.3) | 20 (13.5) | 19 (15.0) | 39 (14.2) | 23 (13.0) | 12 (12.4) | 35 (12.8) |
| 50–59 | 121 (28.7) | 98 (24.7) | 219 (26.8) | 96 (28.7) | 48 (23.5) | 144 (26.7) | 41 (27.7) | 29 (22.8) | 70 (25.5) | 56 (31.6) | 22 (22.7) | 78 (28.5) |
| 60–69 | 166 (39.4) | 112 (28.2) | 278 (34.0) | 119 (35.5) | 56 (27.5) | 175 (32.5) | 48 (32.4) | 34 (26.8) | 82 (29.8) | 55 (31.1) | 27 (27.8) | 82 (29.9) |
| 70–79 | 79 (18.8) | 54 (13.6) | 133 (16.3) | 48 (14.3) | 34 (16.7) | 82 (15.2) | 31 (20.9) | 34 (26.8) | 65 (23.6) | 33 (18.6) | 25 (25.8) | 58 (21.2) |
| ≥80 | 5 (1.2) | 11 (2.8) | 16 (2.0) | 3 (0.9) | 5 (2.5) | 8 (1.5) | 5 (3.4) | 4 (3.1) | 9 (3.3) | 2 (1.1) | 2 (2.1) | 4 (1.5) |
| Average | 60.5 | 56.1 | 58.5 | 58.6 | 56.7 | 57.9 | 61 | 60.7 | 60.9 | 58.9 | 59.5 | 59.1 |
| Median | 61 | 58 | 59 | 60 | 58 | 59 | 62 | 63 | 62 | 59 | 63 | 60 |
| *N* | 421 | 397 | 818 | 335 | 204 | 539 | 148 | 127 | 275 | 177 | 97 | 274 |

cancers or current cancer were recruited and completed an interview. Cases with gastrointestinal cancer and controls were recruited from three different hospitals in two different cities. The bioethics committees of Dong-A University Hospital, Inje University Paik Hospital and Chungbuk National University Hospital approved the study plan and procedures: [Dong-A University Hospital (#IRB-07-10-7; Busan, Korea), Inje University Paik Hospital (#IRB11-011) and the Chungbuk National University Hospital (#IRB-2006-1; Cheongju, Korea)].

For PCR experiments, genomic DNA was isolated from 400 μL whole blood, using a Blood DNA Mini Kit (Qiagen, CA). A total of 295 cancerous tissues and their respective non-cancerous tissues were obtained from patients with gastrointestinal cancer and were immediately frozen in liquid nitrogen. Cancer and normal cells were laser captured and microdissected using a Pix Cell II LCM system and stained by the HistoGen LCM Frozen Section Staining Kit (Arturus, USA). Malignant cells were captured and their genomic DNA was isolated by using the PicoPure DNA extraction kit (Arturus, USA) (Jeong et al. 2007).

## PCR analysis of *hTERT*-VNTR2-2$^{nd}$

Primer sequences used for amplification of *hTERT*-VNTR2-2$^{nd}$ are as follows: *hTERT*-VNTR2-2$^{nd}$ forward 5′-TGGGAG CATCACTCACAGGA, *hTERT*-VNTR2-2$^{nd}$ reverse 5′-GGA ACACAGCCAACCCCTTA (Leem et al. 2002). PCR analysis of human DNA samples was performed using the *Go Taq* polymerase (Promega, USA) with 100 ng genomic DNA. Genomic DNA was amplified using primers under the following standard PCR conditions: 50 mM KCl, 10 mM Tris–HCl, pH 9.0, 3 mM $MgCl_2$, 0.2 mM dTTP, dCTP, dGTP and dATP in a final volume of 30 μl. Cycle conditions were 94 °C for 2 min, then 30 cycles consisting of 45 s at 94 °C, then 2 min 30 s at 69 °C in a 9700 Thermalcycler (Perkin-Elmer, CA, USA). The last elongation step was extended to 7 min at 72 °C. PCR products were analyzed by gel electrophoresis (1 V/cm) in TAE buffer through 0.8% agarose gel.

## Cells and luciferase assay

Human cell lines were examined for the effect of the *hTERT*-VNTR2-2$^{nd}$ on *hTERT* expression: 293T (human embryonic kidney cell line; obtained from Korean Cell Line Bank (KCLB), South Korea), MCF7 (breast cancer cell line; obtained from KCLB, South Korea), AGS (gastric cancer cell line; obtained from KCLB, South Korea) and HCT116 (colon cancer cell line; obtained from KCLB, South Korea). For the luciferase assay, cells ($1 \times 10^5$) were seeded in 12-well plates, cultured overnight and transfected with the *hTERT* promoter–luciferase plasmids (0.5 μg per well; constructed as described in (Yoon et al. 2010a)) by use of FuGENE6 transfection reagent (Roche Diagnostics, USA) and the ratio of DNA to FuGENE6 was 1:3 (Yoon et al. 2010a). The cells were analyzed using a dual-luciferase reporter assay system (Promega) 48 h after completion of the transfection procedure. The *Firefly* luciferase activity was normalized according to *Renilla* luciferase activity and expressed as relative luciferase units to reflect the promoter activity. Triplicate transfections per each construct were examined for one experiment, and final results were calculated by four independently repeated experiments.

## RNA isolation and reverse transcription PCR

Total RNA was isolated from cancer cell lines using QIAGEN RNeasy Mini Kit. For the reverse transcription reaction, a mixture of total RNA (3 μg), an oligo$(dT)_{20}$ (50 nM), and 10 mM dNTP was incubated at 65 °C for 5 min. A mixture of 1X RT buffer, 25 nM $MgCl_2$, 0.1 M DTT and RNaseOUT™ was incubated at 42° C for 2 min. After incubation at 42 °C for 2 min, Invitrogen SuperScript III (200 U/μl) was added. This mixture was then incubated at 42 °C for 50 min followed by 70 °C for 15 min. RNase H (2 U/μl) was then added and incubated at 37 °C for 20 min in a 9700 Thermocycler (Perkin-Elmer) with the following primer sequences (exon 2): *hTERT* forward 5′-AGTGACCGTGGT TTCTGTGTGGTG-3′ and *hTERT* reverse 5′-GCCTGGAAC CCAGAAAGATGGTCT-3′; glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) forward 5′-CCCTTCATTGAC CTCAACTACATGG-3′ and *GAPDH* reverse 5′-CCTGCT TCACCACCTTCTTGATGTC-3′. PCR products containing *hTERT* and *GAPDH* were amplified using gene specific primers under standard PCR conditions: 50 mM KCl, 10 mM Tris–HCl, pH 9.0, 3.0 mM $MgCl_2$, 0.2 mM dTTP, dCTP, dGTP, and dATP in a final volume of 40 μl. Thermocycling conditions were as follows: one cycle of initial denaturation for 2 min at 94 °C, 30 cycles of 30 s at 94 °C, annealing for 15 s at 62 °C and extension for 45 s at 72 °C, followed by a final 7 min extension at 72 °C in a 9700 Thermocycler. Cosmo G Taq DNA polymerase was used for amplification. PCR products were analyzed by gel electrophoresis (1 V/cm) in TAE buffer through 1.2% agarose gel.

## Statistical analysis

Regression analyses were performed to determine the odds ratios (ORs) in the association between control and case groups. ORs were estimated using the natural logarithm and its standard error. Where relevant, we used a Chi squared test with 1° of freedom to assess statistical significance. Differences were considered significant for confidence intervals (CIs) of 95%. All tests were two-sided, with $p < 0.05$ being considered statistically significant. Statistical analyses were

performed using MS Excel with CHITEST and R statistical software (v2.5.1, www.r-project.org) with chisq.test for the calculation of Chi squared values.

## Results

### Analysis of *hTERT*-VNTR2-2nd allelic polymorphism in controls and cases with gastrointestinal cancer

Using the PCR amplification with diagnostic primers on human genomic DNA samples isolated from 818 cancer-free unrelated individuals (412 males and 397 females), we analyzed the degree of polymorphism within the *hTERT*-VNTR2-2nd (Table 1). A similar distribution of allelic frequency of *hTERT*-VNTR2-2nd was revealed in the male and female controls (Table 2). The five alleles of *hTERT*-VNTR2-2nd ranged from 2605 to 2910 bp in length and contained 39–44 copies of the repeat unit, with 44 copies being present in the most common allele (total controls, 61.31%; male controls, 61.05%; female controls, 61.59%) (Table 2) (Yoon et al. 2010a). Seven different genotypes with the five alleles were found for *hTERT*-VNTR2-2nd in controls (Fig. 1a).

We analyzed the frequency and distribution of the polymorphic *hTERT*-VNTR2-2nd alleles between controls and cancer cases for evaluation of a correlation between *hTERT*-VNTR2-2nd variants and gastrointestinal cancer. To compare DNA obtained from the 818 controls and from 539 patients with gastric cancer (335 males and 204 females), 275 patients with colon cancer (148 males and 127 females) and 274 patients with rectal cancer (177 males and 97 females), we conducted a case–control study (Table 2). *hTERT*-VNTR2-2nd had seven haploid types in controls (Fig. 1a), seven haploid types in gastric cancer patients (Fig. 1b), six haploid types in colon cancer patients (Fig. 1c), seven haploid types in rectal cancer patients (Fig. 1d). Although there was no statistically significant difference between controls and cases, three rare alleles (35, 37 and 50 repeats) were detected in gastric and rectal cancer (Table 2, Fig. 1).

For analysis of susceptibility for cancer according to rare alleles of *hTERT*-VNTR2-2nd, each *hTERT*-VNTR2-2nd allele was divided into two groups (common and rare alleles) based on their frequency in the controls. Rare alleles were grouped through threshold frequency, ≤ 1%. The six alleles of *hTERT*-VNTR2-2nd were grouped as three common alleles (42, 43 and 44 repeats) and five rare alleles (35, 37, 39, 40 and 50 repeats) (Table 2; bold represents the rare alleles). Consistent with comparison of allele frequency between controls and cases, these data showed no significant association between rare alleles and OR of gastrointestinal cancer (data not shown).

**Table 2** Allelic genotypes and frequency of *hTERT*-VNTR 2-2nd in controls and gastrointestinal cases

| Genotype | Controls, *N* (%) | | | Gastric cancer cases, *N* (%) | | | Colon cancer cases, *N* (%) | | | Rectal cancer cases, *N* (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total | Males | Females | Total | Males | Females | Total |
| **35**/44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.0056) | 0 | 1 (0.0036) |
| **37**/44 | 0 | 0 | 0 | 1 (0.0030) | 0 | 1 (0.0019) | 0 | 0 | 0 | 0 | 0 | 0 |
| **39**/42 | 1 (0.0024) | 2 (0.0050) | 3 (0.0037) | 1 (0.0030) | 0 | 1 (0.0019) | 0 | 0 | 0 | 0 | 0 | 0 |
| **40**/44 | 1 (0.0024) | 1 (0.0025) | 2 (0.0024) | 0 | 0 | 0 | 1 (0.0068) | 0 | 1 (0.0036) | 0 | 0 | 0 |
| 42/42 | 55 (0.1306) | 51 (0.1285) | 106 (0.1296) | 36 (0.1075) | 17 (0.0833) | 53 (0.0983) | 13 (0.0878) | 8 (0.0630) | 21 (0.0764) | 19 (0.1073) | 12 (0.1237) | 31 (0.1131) |
| 42/43 | 7 (0.0166) | 7 (0.0176) | 14 (0.0171) | 6 (0.0179) | 0 | 6 (0.0111) | 6 (0.0405) | 2 (0.0157) | 8 (0.0291) | 0 | 2 (0.0206) | 2 (0.0073) |
| 42/44 | 185 (0.4394) | 175 (0.4408) | 360 (0.4401) | 128 (0.3821) | 91 (0.4461) | 219 (0.4063) | 59 (0.3986) | 65 (0.5118) | 124 (0.4509) | 75 (0.4237) | 39 (0.4021) | 114 (0.4161) |
| 42/**50** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.0103) | 1 (0.0036) |
| 43/44 | 16 (0.0380) | 9 (0.0227) | 25 (0.0306) | 14 (0.0418) | 3 (0.0147) | 17 (0.0315) | 5 (0.0338) | 0 | 5 (0.0182) | 6 (0.0339) | 2 (0.0206) | 8 (0.0292) |
| 44/44 | 156 (0.3705) | 152 (0.3829) | 308 (0.3765) | 149 (0.4448) | 93 (0.4559) | 242 (0.4490) | 64 (0.4324) | 52 (0.4094) | 116 (0.4218) | 76 (0.4294) | 41 (0.4227) | 117 (0.4270) |
| *N* | 421 | 397 | 818 | 335 | 204 | 539 | 148 | 127 | 275 | 177 | 97 | 274 |

Bold numbers represent the rare alleles of *hTERT*-VNTR 2-2nd

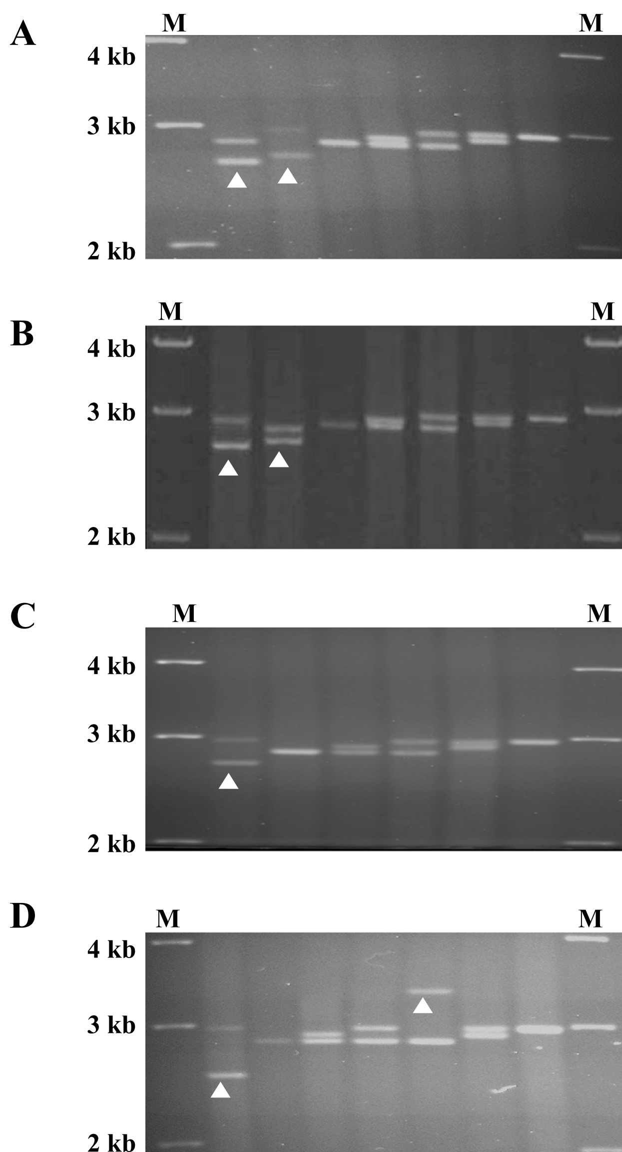


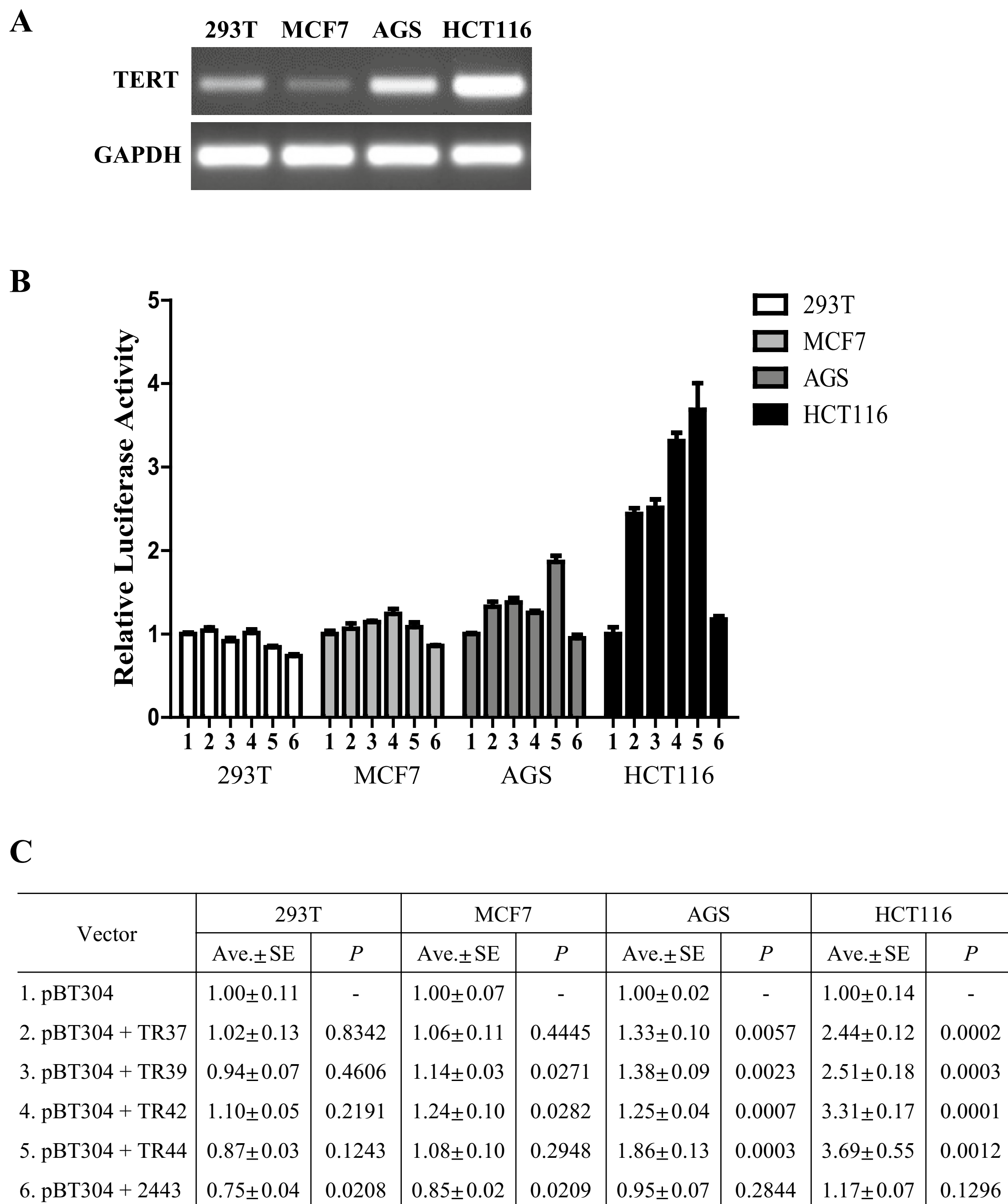

**Fig. 1** Allele typing at *hTERT*-VNTR2-2$^{nd}$ in cancer-free controls and gastrointestinal cancer patients. Electrophoretic patterns of PCR products of *hTERT*-VNTR2-2$^{nd}$ in controls and patients. Seven haplotype patterns of *hTERT*-VNTR2-2$^{nd}$ were detected in DNA from 818 cancer-free controls (**a**) and 539 gastric cancer (**b**). Six haplotype patterns were detected in DNA from 275 colon cancer patients (**c**). Seven haplotype patterns were detected in DNA from 274 rectal cancer patients (**d**). Three rare haplotypes were detected in DNA from patients with gastric cancer (37/44) and rectal cancer (35/44, 42/50). The first and last lanes correspond to a 1-kb marker (M)

## Regulation of the VNTR polymorphism on the *hTERT* promoter within gastrointestinal cancer

To investigate whether *hTERT*-VNTR2-2$^{nd}$ alleles affect *hTERT* expression levels, we used reporter vectors which contained the *hTERT* promoter, luciferase gene and four different sizes of *hTERT*-VNTR2-2$^{nd}$ alleles were published in our study (Fig. 1a) (Yoon et al. 2010a). We analyzed VNTRs carry between 37 and 44 copies of the 61 bp repeats unit, and they were positioned in the enhancer region of the pGL3-Basic vector with *hTERT* core promoter which was published (Yoon et al. 2010a) in the promoter region. Transfection of control vector (pBT304) and vectors with each VNTR repeat variant (37, 39, 42, 44 copies) was used for estimation of expression level in 293T, MCF7, AGS and HCT116 cells. Expression of *hTERT* was checked by RT-PCR through each cell line before the luciferase assay began. RT-PCR analysis revealed that 293T, MCF7, AGS and HCT116 cells expressed the *hTERT* gene (Fig. 2a). Then, the luciferase activity between the control pBT304 and pBT304 with each VNTR repeat did not show statistically significant difference in 293T and MCF7 cells (Fig. 2b, c). AGS and HCT116 cells were also transiently transfected with pBT304 or pBT304 + each length of VNTR. Interestingly, as shown in Fig. 2b, luciferase assays revealed that each VNTRs stimulated the activity of the *hTERT* promoter, representing approximately 1.3–3.7-fold increases in AGS and HCT116, respectively (Fig. 2c). Although we could not find a statistically significant difference based upon VNTR length, we identified a potential of *hTERT*-VNTR2-2$^{nd}$ within *hTERT* gene expression regulation. Furthermore, when we used the pBT304 with 2443 bp of unrelated genomic region, there was no significant difference in luciferase activity (Fig. 2b, c).

## Analysis of minisatellite instability in gastrointestinal cancer tissues

*hTERT,* which located at the subtelomeric region at 15p3.3, contains a high density of minisatellites that may play a role in its chromosomal instability (Yoon et al. 2010a). This possibility was examined by comparing the polymorphic alleles of *hTERT*-VNTR2-2$^{nd}$ minisatellites in the blood and cancer tissues from 107 gastric cancers, 112 colon cancers and 76 rectal cancers patients. These cancer tissues were not included in determination of rare alleles for each minisatellite because of their small sample size (Fig. 3). In DNA obtained from both blood and cancer tissue of patients with gastrointestinal cancer, there were four cases of small deletions or loss of heterozygosity (LOH) in *hTERT*-VNTR2-2$^{nd}$ in DNA obtained from cancer tissues (Fig. 3a). Among the 107 gastric cancer patients, the frequency of rearrangement was 3.74% (4/107) in *hTERT*-VNTR2-2$^{nd}$ (Fig. 3a). The frequencies of rearrangement of *hTERT*-VNTR2-2$^{nd}$ in colon and rectal cancer tissues were 0.89% (1/112) in colon cancer tissues and 2.63% (2/76) in rectal cancer tissues (Fig. 3b, c).




| Vector | 293T | | MCF7 | | AGS | | HCT116 | |
|---|---|---|---|---|---|---|---|---|
| | Ave.±SE | *P* | Ave.±SE | *P* | Ave.±SE | *P* | Ave.±SE | *P* |
| 1. pBT304 | 1.00±0.11 | - | 1.00±0.07 | - | 1.00±0.02 | - | 1.00±0.14 | - |
| 2. pBT304 + TR37 | 1.02±0.13 | 0.8342 | 1.06±0.11 | 0.4445 | 1.33±0.10 | 0.0057 | 2.44±0.12 | 0.0002 |
| 3. pBT304 + TR39 | 0.94±0.07 | 0.4606 | 1.14±0.03 | 0.0271 | 1.38±0.09 | 0.0023 | 2.51±0.18 | 0.0003 |
| 4. pBT304 + TR42 | 1.10±0.05 | 0.2191 | 1.24±0.10 | 0.0282 | 1.25±0.04 | 0.0007 | 3.31±0.17 | 0.0001 |
| 5. pBT304 + TR44 | 0.87±0.03 | 0.1243 | 1.08±0.10 | 0.2948 | 1.86±0.13 | 0.0003 | 3.69±0.55 | 0.0012 |
| 6. pBT304 + 2443 | 0.75±0.04 | 0.0208 | 0.85±0.02 | 0.0209 | 0.95±0.07 | 0.2844 | 1.17±0.07 | 0.1296 |

**Fig. 2** Effect of allelic variation of *hTERT*-VNTR2-2nd in *hTERT*-promoter activity. **a** *hTERT* mRNA expression in 293T, MCF7, AGS and HCT116 cells were confirmed by RT-PCR. **b** The effect of VNTR regions on *hTERT* gene expression were analyzed by the luciferase reporter system. 293T, MCF7, AGS and HCT116 cell lines were transfected with 6 different plasmids. **c** The average value of the promoter activity and *p* value were schematized. Two rare alleles TR (37 and 39 copies) and two common alleles TR (42 and 44 copies) were inserted in the pBT304 plasmid: pBT304+TR37, pBT304+TR39, pBT304+TR42, and pBT304+TR44. The pBT304+2443 plasmid was made by insertion of an irrelevant 2443-bp fragment instead of TR

## Discussion

In particular, if the gene is located in the terminal region of the chromosome and contains high density repeats such as VNTR, certain VNTR alleles present in the gene may be involved in rearrangement or susceptibility to cancer (Ahn et al. 2009; Jeong et al. 2007; Kwon et al. 2010; Seol et al. 2008; Yoon et al. 2008, 2010a, b). *hTERT*, located at the end

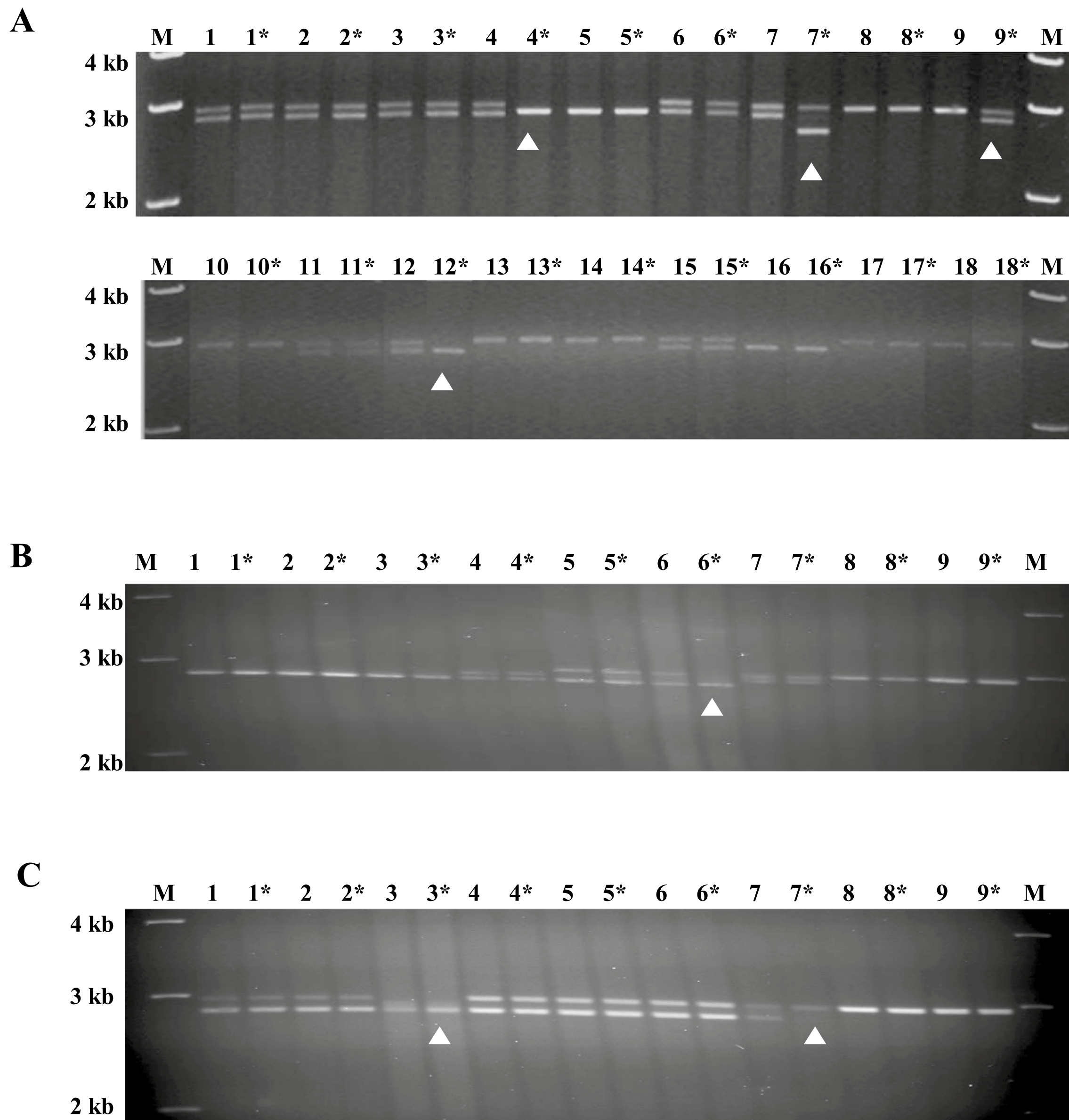



**Fig. 3** Rearrangement of *hTERT*-VNTR2-2nd in cancer patient tissue. Instability of *hTERT*-VNTR2-2nd in paired peripheral blood and cancer tissue from gastric (**a**), colon (**b**) and rectal (**c**) cancer patients were detected. Cancer tissue samples are indicated by asterisks and rearrangements in cancer tissues are indicated by arrows. The first and last lanes correspond to a 1-kb marker (M)

of chromosome 5, has these characteristics, genetic mutations involving important carcinogenic pathways have been identified and are associated with susceptibility to cancer (Carpentier et al. 2007). Telomeres roles as a cap for the protection of the DNA from exonuclease degradation and recombination events at telomeric regions could lead to genomic instability (Blackburn 2001). Therefore, we were interested in rearrangement and susceptibility in cancer of specific VNTR regions for the *hTERT*.

We previously reported that the short rare *hTERT*-VNTR2-2nd alleles are correlated with prostate cancer susceptibility when compared with common alleles (Yoon et al. 2010a). In this study, we examined association with rare alleles for gastrointestinal cancer, but no significant differences were found. In addition, previous results showed a significant difference in prostate cancer, while no significant difference was found in breast cancer, a female cancer (data not shown). These results suggest that the cancer susceptibility of rare *hTERT*-VNTR2-2nd alleles vary according to the cancer type and is not applied in gastrointestinal cancer.

And then we hypothesized that the *hTERT*-VNTR2-2nd polymorphism which affects the expression of *hTERT* in gastrointestinal cancer. One of the characteristics of gastric cancer cells is telomerase expression and telomerase

activity is frequently up-regulated by a variety of mechanisms during gastric cancer development (He et al. 2010). In most embryonic and adult tissues, the RNA component of telomerase is expressed (Feng et al. 1995); on the contrary, expression of *hTERT* is highly regulated and correlates with telomerase activity (Kilian et al. 1997; Meyerson et al. 1997; Nakamura et al. 1997).

We also analyzed the putative binding sites for the transcription factors, estrogen receptor 1 (ER/ESR1; 5), v-Erb1 (5 sites) and NF-kappaB (3 sites) in the *hTERT*-VNTR2-$2^{nd}$ (Yoon et al. 2010a). Several study showed that cell and tissue level in gastrointestinal cancer revealed the presence of estrogen receptor a (ERa) and estrogen receptor b (ERb) (Messa et al. 2000; Singh et al. 1997; Takano et al. 2002). Furthermore, poor prognosis of gastric cancer patients is related with expression of ERa and the absence of ERb expression, and both of ERa and ERb could use as a marker for gastric cancer (Xu et al. 2010). In addition, NF-kappaB is constitutively activated in gastric carcinoma tissues and NF-kappaB activation is correlated with clinicopathological features of tumor aggression of gastric carcinoma (Sasaki et al. 2001). Coincidence with luciferase assay, this analysis suggests that *hTERT*-VNTR2-$2^{nd}$ is a potential region for regulation of *hTERT* gene expression even though it located in non-coding region, intron.

It has been suggested that cancer cell development accelerates the accumulation of genetic variation, causing genomic instability, which plays an important role in cancer development and progression (Lengauer et al. 1998). In addition, *hTERT*, located at the end of the chromosome, contains a high-density minisatellite that may play an important role in chromosome instability (Yoon et al. 2010a). We examined the *hTERT*-VNTR2-$2^{nd}$ in genomic DNA from blood and cancer tissue derived from 295 patients with gastrointestinal cancer and detected seven cases with small deletions or LOH in *hTERT*-VNTR2-$2^{nd}$ (Fig. 3); frequency of rearrangement was 3.9%. This frequency is higher than in the previously known minisatellite region of H-ras (Krontiris et al. 1985).

Thus, these results strongly suggest that *hTERT*-VNTR2-$2^{nd}$ alleles may be associated with chromosomal instability in cancer. Based on these findings, further investigations, such as large-scale epidemiological studies of the association between minisatellite and cancer risk, could provide a useful reference for understanding gene function and complex genomic characteristics.

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## Compliance with ethical standards

**Conflict of interest** Jeong-Ah Kwon, Mi-So Jeong, Se-Lyun Yoon, Jeong-Yeon Mun, Min-Hye Kim, Gi-Eun Yang, Seong-Hwan Park, Jin-Woong Chung, Yung Hyun Choi, Hee-Jae Cha and Sun-Hee Leem declare that they have no competing interests.

**Ethical approval** This study was conducted with informed written consent from participants and after approval by the bioethics committees of Dong-A University Hospital (#IRB-07-10-7; Busan, Korea), Inje University Paik Hospital (#IRB11-011) and the Chungbuk National University Hospital (#IRB-2006-1; Cheongju, Korea).

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