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Elevated telomerase activity in essential thrombocythemia with extreme thrombocytosis

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ABSTRACT

Introduction: We performed a comparative analysis of telomerase activity (TA) in patients with myeloproliferative neoplasm (MPN) and myelodysplastic syndrome (MDS). The relationships between TA and known prognostic factors were also analyzed.

Materials and methods: A telomeric repeat amplification protocol was performed with bone marrow hematopoietic cells from 96 normal controls, 44 MPNs, and 40 MDSs.

Result: TA (measured as molecules/reaction) showed no correlation with age in the control group ($R^2 = 0.0057$, $p = 0.464$). MPN showed elevated TA compared with controls (15,537.57 vs. 7775.44, $p = 0.020$). Patients with essential thrombocythemia showed markedly elevated TA (22,688.56, $p = 0.030$), particularly in cases with extreme thrombocytosis versus those without extreme thrombocytosis (34,522.19 vs. 9375.71, $p = 0.041$). MDS patients showed a TA value of 7578.50.

Conclusion: There was no association between age and TA in bone marrow hematopoietic cells. TA was elevated in MPN but borderline in MDS, probably because of differences in the nature of the diseases. Elevated TA in patients with essential thrombocythemia, especially those with extreme thrombocytosis, suggests that an anti-telomerase strategy could be beneficial in the prevention of thrombotic complications.

Introduction

Telomere is a noncoding DNA structure composed of tandem arrays of telomeric repeats (5′-TTAGGG-3′) located at the end of each chromosome [1,2]. By capping the chromosomal end, the telomere preserves the stability of the chromosome and prevents the activation of DNA damage pathways that cause cell senescence and death [1,2]. Telomeres are not replicated by DNA polymerase and progressively shorten during the cycles of cell division [1–3]. Instead, telomerase, a ribonucleoprotein enzyme complex composed of a protein reverse transcriptase (hTERT) and an RNA template for the telomere elongation, catalyzes the synthesis and extension of telomeric DNA by adding single-stranded TTAGGG repeats [1,2]. However, most somatic tissues in adult humans lack telomerase activity (TA); thus, the telomere shortens continuously during cell replications [4].

Telomere shortening has a dual role in cancers [4]. In young organisms, the average telomere length in an organ's cells is long and stable, and telomere shortening occurs only in a few cells, resulting in loss of cell cycle arrest, senescence, or apoptosis. This process works as a tumor suppressor mechanism that prevents continuous proliferation of cells and protects the organism from cancer. During old age, however, a large portion of the cells in an organ show critically short telomeres after repeated cell replications. Damage to the unprotected chromosomal ends increases the rate of cancer, possibly outweighing the tumor suppressor effect. In addition, malignant cells within cancers protect themselves from apoptosis by maintaining the telomere length through the activation of telomerase.

Telomerase activation is observed in many human cancers. Solid tumors including bladder cancer, lung cancer, hepatocellular carcinoma, colorectal and prostate cancer show elevated TA [1,5–9]. Elevated TA works as a tumor marker or prognostic factor: Measurement of TA helps early detection of bladder cancer [8,10]; High telomerase activity is suggested as an independent prognostic indicator for poor prognosis in colorectal cancer and lung cancer [11,12]. Elevated TA is also reported in hematologic malignancies including acute myeloid leukemia (AML), chronic myelogenous leukemia (CML) and multiple myeloma and

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lymphoma also showed elevated TA [13,14]. In CML, TA correlates with disease progression: TA is moderately elevated during the chronic phase (CML-CP) and more pronounced during the blastic phase (CML-BP) [14]. In multiple myeloma, elevated TA is associated with cytogenetic abnormalities associated with poor prognosis [15]. Thus, the regulation of telomeres and telomerase appears to be a promising target for anticancer drug research [1]. Drugs specifically targeting telomere and telomerase are under development including GRN163/GRN163L for myeloma cells and renal and prostatic cells lines, RHSP4 for breast tumor cells and vulva tumor cells, dominant negative hTERT for CML and BIBR1532 for AML [1].

In contrast, little investigation has been done on the potential benefit of an anti-telomerase strategy in relatively uncommon hematologic malignancies, such as essential thrombocythemia (ET) and polycythemia vera (PV). To investigate the potential application of anti-telomerase strategy on those uncommon hematologic malignancies, we performed a comparative analysis of TA in newly diagnosed myeloproliferative neoplasms (MPNs), including CML-CP, ET and PV, and myelodysplastic syndromes (MDSs), by using a telomeric repeat amplification protocol [7]. We also investigated the associations between TA and important factors related to clinical outcome.

Materials and methods

Patients

Data for the study came from 44 newly diagnosed MPN patients and 40 de novo MDS patients without history of treatment, who were diagnosed at Seoul National University Hospital between January 2006 and December 2008. The MPN group consisted of 18 cases of CML-CP, 17 cases of ET and 9 cases of PV. The MDS group included 14 cases of refractory anemia (RA) or refractory anemia with ringed sideroblasts, 5 cases of refractory cytopenia with multilineage dysplasia (RCMD), 10 cases of refractory anemia with excess blasts-1 (RAEB-1), and 11 cases of refractory anemia with excess blasts-2 (RAEB-2). As normal controls, the study included 96 patients who were referred for the staging of lymphoma and were diagnosed as having no evidence of bone marrow (BM) involvement in malignant lymphoma. The study was approved by the Institutional Research Board of Seoul National University Hospital (IRB No. H-1103-129-357).

Telomeric repeat amplification protocol

The nucleated cells from the BM aspiration during the initial diagnosis were stored at −70 °C and used for the study. Protein extraction and quantification were performed according to the manufacturer's instructions. In brief, cell pellets were lysed for 30 min in lysis buffer (Allied Biotech, Ijamsville, MD, USA) on ice. Frequent vortexing was performed during lysis, and the samples were left on ice for an additional hour. The lysates were centrifuged for 30 min at 14,000 rpm at 4 °C. Protein concentrations were determined using the bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockford, NY, USA), and 1 μg of protein from each cell extract was used for the TRAP procedure. To evaluate the TA, we measured the ability of telomerase to synthesize telomeric repeats onto an oligonucleotide substrate by using a quantitative telomerase detection kit (Allied Biotech), an optimized and previously validated SYBR Green real-time PCR method, according to the manufacturer's protocol. All real-time amplifications were performed with an iCycler iQ real-time PCR machine (BioRad Laboratories, Hercules, CA, USA). Calibrations using a serial dilution of TSR control template (Allied Biotech), as well as no-template reactions, were also performed on every plate to obtain the standard curves for quantitation. The real-time PCR conditions were as follows: telomerase reaction for 20 min at 25 °C, PCR initial activation for 10 min at 95 °C, and 3-step cycling (40 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C). The generated PCR products were directly detected by measuring the increase in fluorescence caused by binding of SYBR Green to double-stranded DNA and calculated with iCycler iQ software (BioRad Laboratories).

Statistical analysis

The statistical significance of the differences in TA between the subgroups was analyzed by independent *t*-test. The chi-square test was used to compare the categorical variables. The statistical software package SPSS 19.0 (SPSS, Chicago, IL, USA) was used, and $p < 0.05$ was considered to be significant.

Results

Correlation of telomerase activity with age in normal controls

The median age of the 96 normal controls was 54.9 years (range, 16.5 to 78.7 years). The TA generally decreased with age but showed great individual variation ($y = -46.301x + 10{,}225$, $R^2 = 0.0057$, $p = 0.464$, Fig. 1). The mean TA (as measured in molecules/reaction) of the normal controls was 7775.44. The mean TA in each age group was also calculated: 10–19 years ($n = 2$), 17,858.27; 20–29 years ($n = 7$), 5927.48; 30–39 years ($n = 7$), 9892.49; 40–49 years ($n = 22$), 8198.82; 50–59 years ($n = 22$), 6855.39; 60–69 years ($n = 25$), 8976.14; and 70–79 years ($n = 11$), 4035.49.

Telomerase activity in patients with myeloproliferative neoplasms

The median age of the 44 MPN patients was 52.0 years (range, 19.2 to 83.9 years). MPN patients showed elevated TA compared with normal controls (15,537.57 vs. 7775.44, $p = 0.020$, Fig. 2a). The 17 ET patients showed markedly elevated TA compared with normal controls (22,688.56 vs. 7775.44, $p = 0.030$, Fig. 2b). In contrast, the 18 CML-CP patients and 9 PV patients were likely to show higher TA than the normal controls, but the differences were not statistically significant (10,193.89, $p > 0.05$ and 8401.89, $p > 0.05$).

We investigated the association between TA and the risk factors for thrombohemorrhagic event in patients with ET (Table 1) [16]. Risk factors for thrombohemorrhagic event included age (>60 years), anemia (Hb <11 g/dL), leukocytosis (white blood cell count >11,000/μL), and extreme thrombocytosis (platelet count >1000 × 10^3/μL). Nine

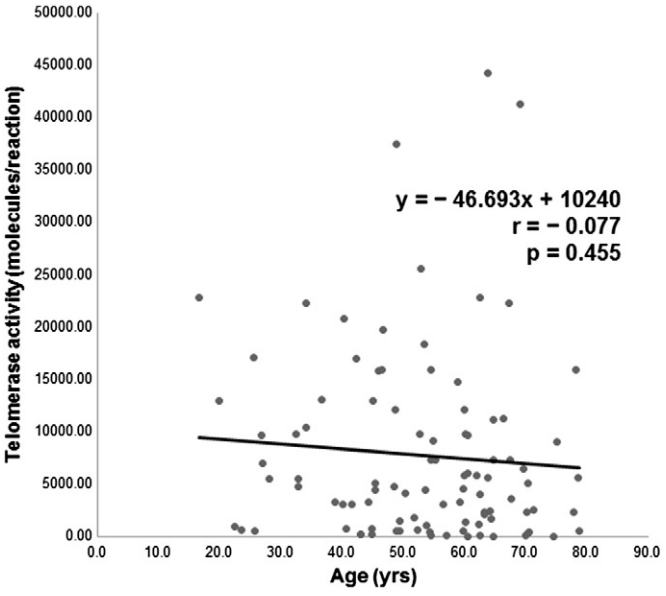

Fig. 1. Telomerase activity and age in 96 normal controls.