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Telomerase activation induces elongation of the telomeric single-stranded overhang, but does not prevent chromosome aberrations in human vascular endothelial cells

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Abstract

Chromosome aberrations such as loss of chromosome 13 were frequently observed in human endothelial cells from umbilical cord veins (HUVEC). A recent study showed that the length of telomeric single-stranded 3′-overhangs (G-tails) is more important as an essential structure for chromosome maintenance than the net telomere length in telomere t-loop formation. Here, we have examined G-tail length using G-tail telomere HPA in normal and hTERT-transduced HUVECs. We found that forced expression of hTERT in HUVEC induced G-tail as well as total telomere length elongation. G-tail length was well correlated with total telomere length. However, hTERT introduction did not prevent chromosome aberrations such as loss of chromosome 13. Normal characteristics such as morphology, up-regulation of vWF, and tube formation were observed in hTERT-HUVEC as in young normal HUVEC. These results show that chromosome aberrations in HUVEC are independent of telomere G-tail and total telomere attrition.

Keywords: Telomere; Telomerase; TERT; 3′ Telomeric overhang; G-tail; Endothelial cells; Immortalization; Chromosome; Telomere binding protein

Telomeres are specialized structures at the end of eukaryotic chromosomes that protect chromosomes from degradation, recombination, loss of functional genes, and end-to-end joining [1,2]. Human telomeres contain 15–10 kbp of the repeating telomeric DNA sequence, 5′-TTAGGG-3′, followed by a guanine-rich single-stranded 3′-overhang, the so called telomere G-tail [3]. Telomeric DNA shortening was first observed in human fibroblasts grown in culture, and telomere length has been proposed as a counting mechanism of cell division number that controls cellular senescence [4]. In contrast to normal cells, immortalized cells such as cancer cells maintain telomere length by expressing telomerase, which synthesizes telomere repeat sequences at the end of telomeres [5]. Introduction of the catalytic subunit of telomerase, hTERT, sufficiently induced telomerase activity and immortalized human fibroblast, epithelial, and smooth muscle cells [6,7].

Involvement of telomere shortening in cellular senescence is well known, but the relationship between telomere G-tail length and cellular senescence is poorly understood. Reduction of telomere G-tail length was first reported in human fibroblasts [8], but other studies have shown that telomere G-tail length in human fibroblasts does not change with cellular senescence [9]. This contradictory result is possibly caused by technical problems and/or

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culture condition. To overcome these technical problems, we have recently established a technique to measure telomere G-tail length, telomere G-tail HPA [10]. This method has the advantages of being simple to perform, accurate, and highly sensitive for G-tails as short as 20 nucleotides. Culture conditions also presumably affect the G-tail length in cultured cells. One major culture condition, oxidative stress, leads to oxidative DNA damage at the end of telomeres, and induces cellular senescence due to telomere dysfunction [11]. Culture of murine cells in standard culture condition (20% O_2) induces chromosome aberrations and p53 mutations [12]. But, hypoxia (3% O_2) condition prevents both chromosomal aberrations and p53 mutations [12]. These results suggest that oxidative stress influences both telomere dysfunction and chromosome aberrations. However, the effect of oxidative stress on telomere G-tail has not been studied.

Chromosome aberrations rarely occur in normal human cells such as fibroblasts, but they occur often in HUVECs under standard culture condition. Loss of chromosome 13 is a major chromosome aberration in HUVECs [13], but the cause of such chromosome aberrations is still unknown. We speculate that culture conditions such as oxidative stress and telomere dysfunction induced by telomere G-tail shortening are possible reasons. Thus, in this paper, we first tested whether or not the hypoxic condition protects chromosomes from aberrations. Second, we examined the telomere G-tail length in normal and hTERT immortalized HUVECs to determine if telomere dysfunctions by G-tail reduction induce chromosomal aberrations in HUVECs. In this study, we demonstrated that hypoxia condition slightly induces cellular lifespan, but chromosome aberrations still occurred. In addition, telomere G-tail reduction is observed in both normoxia and hypoxia conditions during cellular senescence in HUVECs. We also found that forced expression of hTERT induced telomere G-tail elongation as well as total telomere elongation, but chromosome aberrations could not be prevented by hTERT introduction.

Results

Effect of cultured oxygen on cellular mortality and chromosome aberration in human endothelial cells

Oxidative stress by hyperoxia condition induces cellular senescence *in vitro* [11]. It is possible that these culture conditions may be involved in induction of cellular senescence and chromosome alterations including loss of chromosome 13 in human endothelial cells. Thus, we tested whether or not optimization of culture conditions can extend the lifespan of human umbilical vein endothelial cells and protect chromosomes from alterations. First, we compared the lifespan in endothelial cells at normoxia (20% O_2) and hypoxia (3% O_2) conditions since we previously showed that hypoxia (3% O_2) condition significantly extends the lifespan in human fibroblasts (unpublished data). We used two independent normal human endothelial cells, HUE101-1 and HUE147-1.2, derived from umbilical cord veins. At 20% and 3% oxygen conditions, HUE101-1 ceases to senesce at 54 and 59 PDLs, respectively (Fig. 1A). HUE147-1.2 ceases to senesce at 41 and 47 PDLs at 20% and 3% oxygen conditions, respectively (Fig. 1A). SA-β-gal staining positive cells were found in senescent endothelial cells

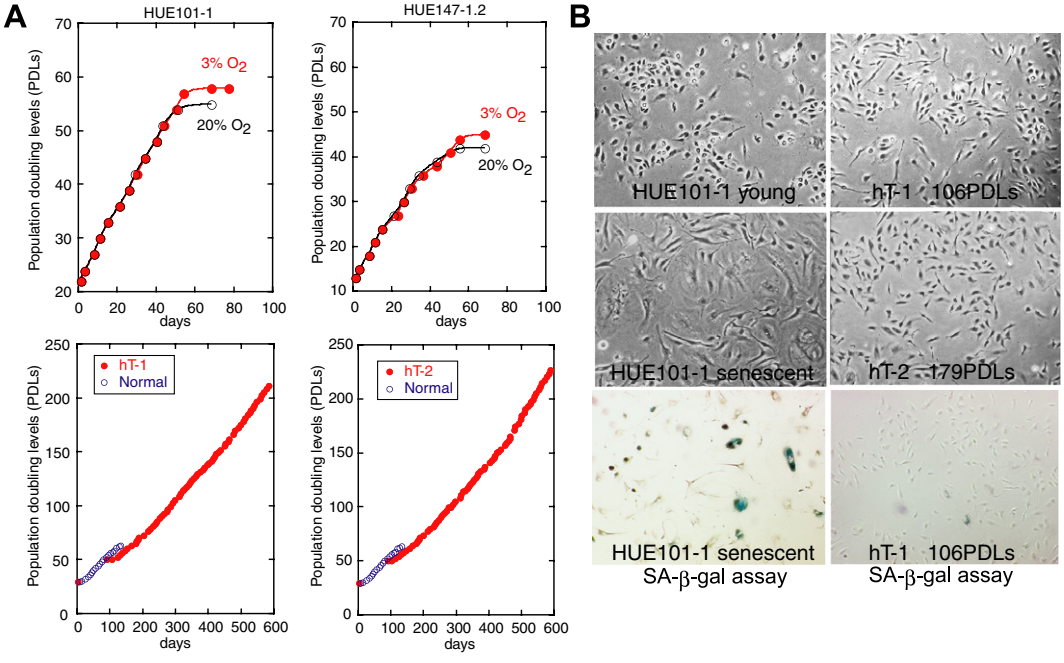

Fig. 1. Morphology and growth curve of HUVECs and hTERT-HUVECs (A) Cumulative growth curve in normal HUVECs (HUE101-1 and HUE147-1.2) and hTERT-HUVECs (hT-1 and hT-2). (B) Morphology of human endothelial cells and hTERT immortalized cells.