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Differential shortening rate of telomere length in the development of human fetus

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ABSTRACT

Telomeres play an important role in the maintenance of genomic stability/integrity and are synthesized by the RNA-dependent polymerase telomerase. Progressive telomere shortening contributes to both in vitro and in vivo aging, and telomere length dynamics and telomerase expression profile in human tissues during extrauterine life have been well characterized. However, little is known about these changes in the early stage of gestation. In the present study, we determined telomere length and the expression of telomerase core units (telomerase reverse transcriptase, hTERT, and telomerase RNA component, hTERC) in human fetus tissues from 6 to 11 weeks of gestational age. A sharp decline in telomere length occurred between 6 and 7 weeks of gestational age, and a relatively stable or slightly shortened telomere length was thereafter maintained until birth. The inverse correlation between TERT or TERC expression and gestational age was steadily observed in these fetus tissues. Taken together, there is a rapid reduction followed by a slow erosion of telomere length in human fetus from gestational age 6–11 weeks, while hTERT and hTERC expression decreases steadily during this period. The present findings not only contribute to better understandings of telomere/telomerase biology in human embryonic development, but also are implicated in telomere/telomerase-related diseases or problems.

1. Introduction

Human telomeres at the termini of chromosomes are the nucleoprotein complex consisting of tandemly repeated TTAGGG sequences and associated proteins [1,2]. It has long been appreciated that the telomere structure form protective caps on chromosome ends, and are essential to maintenance of genomic stability/integrity [1,2]. Although telomere length is affected by multiple elements, one of the major players in controlling telomere length is telomerase, an RNA-dependent DNA polymerase that elongates TTAGGG sequences [3,4]. Telomerase core enzyme includes telomerase reverse transcriptase (hTERT) and RNA template (hTERC) [3,4], and the enzymatic activity is silent in the majority of normal human somatic cells, which, together with "the end replication problem", results in progressive telomere shortening with each round of cell divisions [1]. Cells are triggered to enter a permanent growth arrest stage named "senescence" when their telomeres reach a critical size or become dysfunctional [1]. Telomere shortening also occurs in vivo with increased age and has been shown to be associated with the aging process and onset of age-related diseases [1,2].

Although dynamics in telomere length and expression profile of hTERT and hTERC have been well characterized from infants to elderly individuals, little is known about these in human fetus. In a previous study, Youngren et al. examined telomere length in human fetus with the gestational age from 15 to 19 weeks, and found lack of the relationship between telomere length and gestational ages [5]. It was thus suggested that synchrony in telomere length existed in human fetus [5]. To further address this issue, we determined telomere length in human fetus between 6 and 11 weeks, and compared it with that in cord blood cells. In the meanwhile, we analyzed hTERT and hTERC expression in these tissues. Our results show that there is a rapid reduction followed by a slow ero-

Abbreviations: hTERC, human telomerase RNA component; hTERT, human telomerase reverse transcriptase; qPCR, quantitative PCR; qRT-PCR, quantitative RT-PCR.

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sion of telomere length in human fetus from gestational age 6–11 weeks, while hTERT and hTERC expression decreases steadily during this period.

2. Materials and methods

2.1. Human fetal samples

A total of 105 human fetal tissues were obtained from pregnant women who had elective abortion using uterine curettage. The pregnancy was terminated due to failed contraception and gestational ages were from 6 to 11 weeks. Samples were washed twice with PBS and then subjected to DNA and RNA extraction. Cord blood was collected from full-term normal newborns and mononuclear cells were isolated using with red blood cell lysis buffer (Tiangen, China). The study was reviewed and approved by the Shandong University Hospital ethics committee. Informal consent was obtained from all the participants.

2.2. DNA and telomere length assessment

Genomic DNA was extracted using a kit from Qiagen. Telomere length was determined using quantitative real-time PCR (qPCR) as described [6–8]. Two nanograms of DNA were used for each PCR reaction and PCR was carried out in an ABI7700 sequence detector (Applied Biosystems, Foster City, CA). The primer sequences for human telomere (Tel 1b and Tel 2b) and β-globin (HBG3 and HBG4) were: Tel1b: 5′-CGGTTTGTTTGGGTTTGGGT-TTGGGTTT-GGGTTTGGGTT-3′; Tel2b: 5′-GGCTTGCCTTACCCTTACCCTTACCC-TTACCCTTACCCT-3′; HBG3: 5′-TGTGCTGGCCCATCACTTTG-3′, and HBG4: 5′-ACCAGCCA-CCACTTTCTGATAGG-3′. T/HBG values were determined using the formula $T/S = 2^{-\Delta Ct}$, where ΔCt = average $Ct_{telomere}$ − average $Ct_{\beta\text{-globin}}$. The *T*/*S* ratio was arbitrarily expressed as TL [6–8].

2.3. RNA extraction and quantitative RT-PCR (qRT-PCR)

Trazol kits were used for isolation of the total RNA from fetal tissues. cDNA was synthesized using random primers (N6) (Pharmacia, Uppsala, Sweden). The following PCR primers were used: hTERT: 5′-CGGAAGAGTGTCTGGAGCAA-3′ (Forward) and 5′-GGATGAAGCGGAGTCTGGA-3′ (Reverse); hTERC: 5′-TCTAACCC-TAACTGAGAAGGGCGTAG-3′ (Forward) and 5′-GTTTGCTCTAGAAT-GAACGGTGGAAG-3′ (Reverse). β2-M expression was used as a control for RNA loading and RT efficiency and amplified. qRT-PCR was carried out in an ABI7700 sequence detector (Applied Biosystems, Foster City, CA) using a SYBR Green kit (Applied Biosystems). Levels of target mRNA were calculated based on the threshold cycle (CT) values and normalization of human β2-M expression. A renal cell carcinoma cell line 786-O was used as a reference and hTERT and hTERC expression level in these cells was arbitrarily defined as 100.

2.4. Statistical analyses

The comparison of hTERT mRNA and hTERC RNA, and telomere length among different week groups was made using a Student *t*-test or Mann–Whitney *U*-test. All the tests were two-tailed and computed using SigmaStat3.1® software (Systat Software, Inc., Richmond, CA). *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Alterations in telomere length in the fetal tissues of gestational ages from 6 to 11 weeks

Genomic DNA from 105 fetal samples and 13 cord blood cells were analyzed for telomere length using qPCR. Telomere length

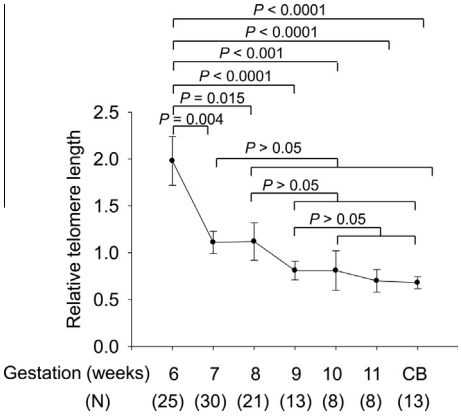

Fig. 1. Telomere length dynamics in human fetal tissues at gestational ages of 6–11 weeks and cord blood cells. Telomere length was assessed using qPCR as described in Section 2 and arbitrarily expressed as the ratio of telomere and reference gene HBG signals. The number analysed in each gestational age group and cord blood cells was indicated.

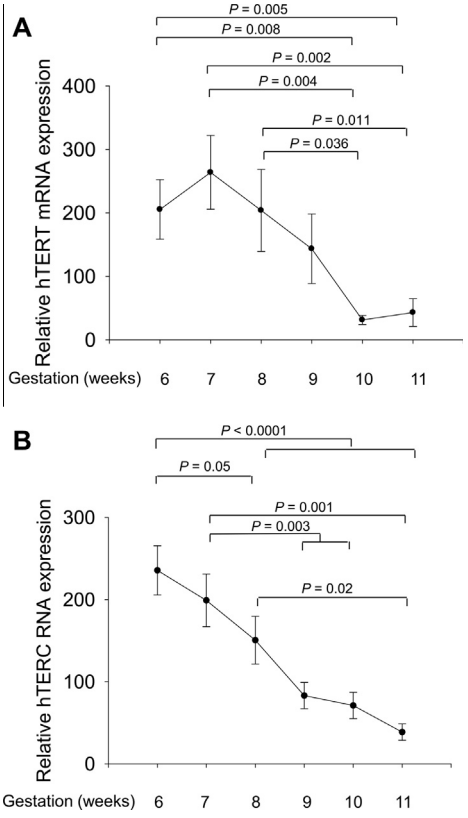

Fig. 2. hTERT and hTERC expression profiles in human fetal tissues at gestational ages of 6–11 weeks. Total RNA was extracted from the same set of fetal samples used for telomere length assessment, and then analysed for hTERT and hTERC expression using qRT-PCR. hTERT and hTERC expression in a renal cell carcinoma line 786-O were included in the assay and their levels were regarded as 100. The levels of hTERT and hTERC mRNA in fetal tissues were calculated based on their abundance in 786-O cells.

varied from individual samples at the same gestational age and as well among those with different gestational ages. As shown in Fig. 1, mean telomere length was 1.98 ± 0.26 (mean ± SEM) at gestational age week 6, and a sharp decline already occurred by week 7 (1.11 ± 0.12, weeks 6 vs 7, $P = 0.004$). A highly significant difference in telomere length was also observed between weeks 6 and 8–10 or weeks 11 of gestational age. However, telomeres only slightly shortened since then, and there was no significant difference in the length from weeks 7 to 11 of gestational ages. We further determined telomere length in mononuclear cells from 13 cord blood samples, and surprisingly, telomere length did not differ significantly between fetal tissues at week 7 and cord blood cells (week 7 vs CB, 1.11 ± 0.12 vs 0.68 ± 0.064, $P > 0.05$).

3.2. hTERT mRNA expression in the fetal tissues of gestational ages from 6 to 11 weeks

Transcriptional hTERT expression is in general closely correlated with telomerase activity in human cells or tissues [3,4]. We thus wanted to determine whether hTERT expression level is coupled with telomere dynamics in the fetal tissues. hTERT mRNA level at week 6 was 205 ± 46.9, and slightly lower than that at week 7 (263.7 ± 58.1, $P = 0.449$) (Fig. 2A). The steady decline in hTERT transcripts was observed in the fetal tissues since week 7 and only 1/5 remained by week 11 (Fig. 2A, weeks 7 vs 11, $P = 0.004$). Similarly, a statistically significant difference in hTERT mRNA expression was seen in the tissues between week 6 or weeks 8 and 11 (Fig. 2A).

3.3. Progressive decline in hTERC expression in the fetal tissues with increased gestational ages

hTERC is one of two telomerase core components [3,4], and we were therefore interested in its expression in fetal tissues. Fig. 2B showed the alteration in hTERC expression from weeks 6 to 11 of gestational ages. A progressive decline in hTERC RNA levels occurred with its highest level at week 6 (235 ± 30) and lowest expression at week 11 (38.6 ± 10, weeks 6 vs 11, $P < 0.0001$). In addition, hTERC levels were also significantly lower in the fetal tissues at week 11 than those at week 7 or week 8 (weeks 7 and 8: 198.97 ± 32 and 150.5 ± 29, respectively; $P = 0.012$ for weeks 7 vs 11, and 0.016 for weeks 8 vs 11).

4. Discussion

Numerous studies have been performed to characterize telomere length dynamics and telomerase expression profile in postnatal human life, and the accumulated evidence suggests multiple biological functions of telomeres and telomerase. However, little is known about such information in human embryonic development or fetal life. In the present study, we determined telomere length and the expression of two telomerase core components hTERT and hTERC in embryonic/fetal tissues at gestational ages weeks 6–11. Our results showed a rapid decline in telomere length between weeks 6 and 7 of gestational age, and since then, telomere did not shorten significantly. hTERT and hTERC expression displayed in general a progressive reduction in these tissues with increased gestational ages.

Youngren et al. studied telomere length in fetal tissues from gestational ages 15 to 19 weeks, and they did not find a significant difference in telomere length among the samples derived from different gestational weeks [5]. The authors thus suggested the existence of synchrony in telomere length in human fetus. Based on our present data, a significant telomere shortening occurred between weeks 6 and 7, a narrow window period that missed in Youngrens' study. Nevertheless, our observation after gestational age of week 7 was consistent with their results. However, another publication reported diverse telomere length kinetics in hematopoietic stem cells (HSCs) from same foetuses with gestational ages of 23–36 weeks. The kinetics differed from fetus to fetus with increased gestational ages: Some were largely unchanged or reduced, while more than 1/3 of them had longer telomeres at late week points than at early ones [9]. These findings indicate a complicated telomere length dynamics during human fetal development, and further studies are required to elucidate this issue and underlying mechanisms.

In normal human cells, telomere length is maintained by telomerase [3,4]. However, intriguingly, the expression dynamics of hTERT, a key component of the telomerase complex, were not coupled with the alteration in telomere length in the examined fetal tissues. The longest telomeres were seen at gestational age of 6 weeks, whereas the highest level of hTERT mRNA occurred at week 7. Thereafter, a progressive decline in hTERT expression with increased gestational ages was in contrast to relatively stable telomere length in this same period. It is currently unclear whether telomerase is the only mechanism elongating telomeres in human fetus. A previous study showed a rapid increase in telomere length during the early cleavage development in both wild type and telomerase-null mice, and there was the evidence that a recombination-based mechanism rather than telomerase was responsible for telomere lengthening during the early cleavage cycles following fertilization [10]. It remains to be defined whether this mechanism is operative in human embryonic development and when it is switched off.

Unlike hTERT, a rate-limiting component for telomerase activity, hTERC is ubiquitously expressed in all normal human cells [3,4]. Unexpectedly, we observed a 5-fold difference in hTERC expression between weeks 6 and 11 of gestational ages. hTERC up-regulation has also been found in activated lymphocytes and cancer cells [11,12], and is believed to contribute to telomerase activation. Thus, it is likely that high levels of hTERC expression observed in fetal tissues at week 6 are required to maintain high telomerase activity.

In summary, we define the dynamics of telomere length, hTERT and hTERC expression in human fetal tissues at gestational ages from weeks 6 to 11. The findings not only contribute to better understandings of telomere/telomerase biology in human embryonic development, but also are implicated in telomere/telomerase-related diseases or problems.

Conflict of interest

Nothing to be declared.

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