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Long Telomeres in the Mature Human Placenta

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Abstract

Objective: To investigate whether telomere shortening may play a role in senescence of the placenta.
Study design: Villous tissue was collected from single, random sites of full-term placentas (39–41 weeks of gestation; $n = 10$) as well as multiple, specific sites of the same placenta (39–41 weeks of gestation; $n = 5$). For the latter group of placentas, samples were taken near the umbilical cord and at the periphery on both the maternal and fetal sides (a total of 4 samples per placenta). Cord blood samples were also obtained from all placental donors. Telomerase activity was assessed by the TRAP assay, and telomere length measured by Southern analysis of mean terminal restriction fragment (TRF) length.
Results: We show for the first time that telomeres are longer (~25% longer; $P < 0.001$) in placenta tissue than in cord blood from the same donor.
Conclusion: Telomere shortening is unlikely to have a significant role in senescence or terminal maturation of the placenta.

Keywords: Placenta; Telomere; Telomerase; Cord blood

1. Introduction

The placenta is a complex organ that undergoes continuous and rapid development and maturation during pregnancy. It has been hypothesized that near or at term, the placenta begins to enter a state of senescence [1].

Telomeres are essential genetic elements that cap and stabilize the ends of chromosomes [2]. The replication of telomeres in dividing cells requires a specialized enzyme, telomerase, a ribonucleoprotein complex. Telomerase is composed of an essential RNA component, TR, and an essential catalytic component, telomerase reverse transcriptase (TERT) [3]. In adults, most somatic cells have little or no detectible telomerase activity because TERT expression is very low or absent [4]. As a consequence, telomeres erode to shorter and shorter lengths in human somatic cells grown *in vitro* or during replicative aging *in vivo* [5]. The observation that over-expression of hTERT in normal human cell strains is sufficient to activate telomerase and prevent both telomere shortening and replicative senescence [6] shows that continuous shortening of telomeres is the ultimate cause of senescence of human cells.

Previous studies have demonstrated that telomerase activity in the placenta is higher in the first or second trimester than the third trimester [7–9]. Thus we hypothesized that telomeres may shorten during placental development, and this shortening may account for the senescence of the placenta. Pre-mature senescence or growth arrest of the placenta may be an underlying etiology of a number of perinatal diseases or defects, including fetal growth restriction. Thus knowledge of the dynamics of telomeres during placental development may facilitate our understanding of certain perinatal diseases.

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The goal of this study was to measure and compare telomere length in the placenta at 38–41 weeks of gestation and cord blood samples from the same donor taken at term from women after a normal uncomplicated pregnancy and delivery.

2. Materials and methods

2.1. Placenta and cord blood collection

All samples were collected from anonymous donors at Kapiolani Medical Center. Both cord blood and placenta samples were collected from all donors. A small placental biopsy, approximately 1 cm^3, was taken immediately following birth, frozen in liquid nitrogen, and stored in −80 °C freezer. For 10 of the placentas, biopsies were taken at random sites. Another 5 placentas were biopsied at multiple ($n = 4$) sites, specifically, near the umbilical cord and the periphery on both the fetal and maternal sides. All placenta samples were from full term (39–41 weeks) vaginal deliveries.

Cord samples (1–2 cc.) were transferred to an equal volume of anti-coagulant (PBS plus 10 mM EDTA). White blood cells were harvested the same day. Briefly, red blood cells were precipitated with dextran sulfate, and the residual red blood cells were lysed by treatment with ACK solution (0.15 M NH_4Cl, 0.01 M $KHCO_3$) followed by 2 washes with PBS.

2.2. DNA and RNA isolation

For each placental biopsy and cord sample, approximately half was used for DNA isolation and the other half for telomerase activity assay (TRAP assay) and RNA isolation. DNA was isolated as previously described [10]. All placental DNA extracts were prepared by grinding the frozen tissue and frozen extraction buffer with a mortar and pestle. RNA was isolated using Trizol reagent (GIBCO) from nuclear pellets that were obtained during preparation of telomerase CHAPS extract for each sample.

2.3. TRAP assay

Telomerase extracts were prepared and TRAP assay [11] performed according to manufacturer's protocol. To prepare extracts from frozen placenta samples, a ~2 mm^3 piece of frozen placental tissue was ground up with frozen telomerase CHAPS extract buffer using mortar and pestle. The nuclear pellets that are obtained following telomerase extraction were used for RNA isolation.

2.4. Telomere length analysis

Telomere length was measured by southern analysis of mean terminal restriction fragment (TRF) length as well as dot blot analysis. For TRF length analysis, briefly, high molecular weight DNA was digested with restriction enzymes *Hinf*I and *Rsa*I and 1–1.5 μg of DNA was resolved by electrophoresis

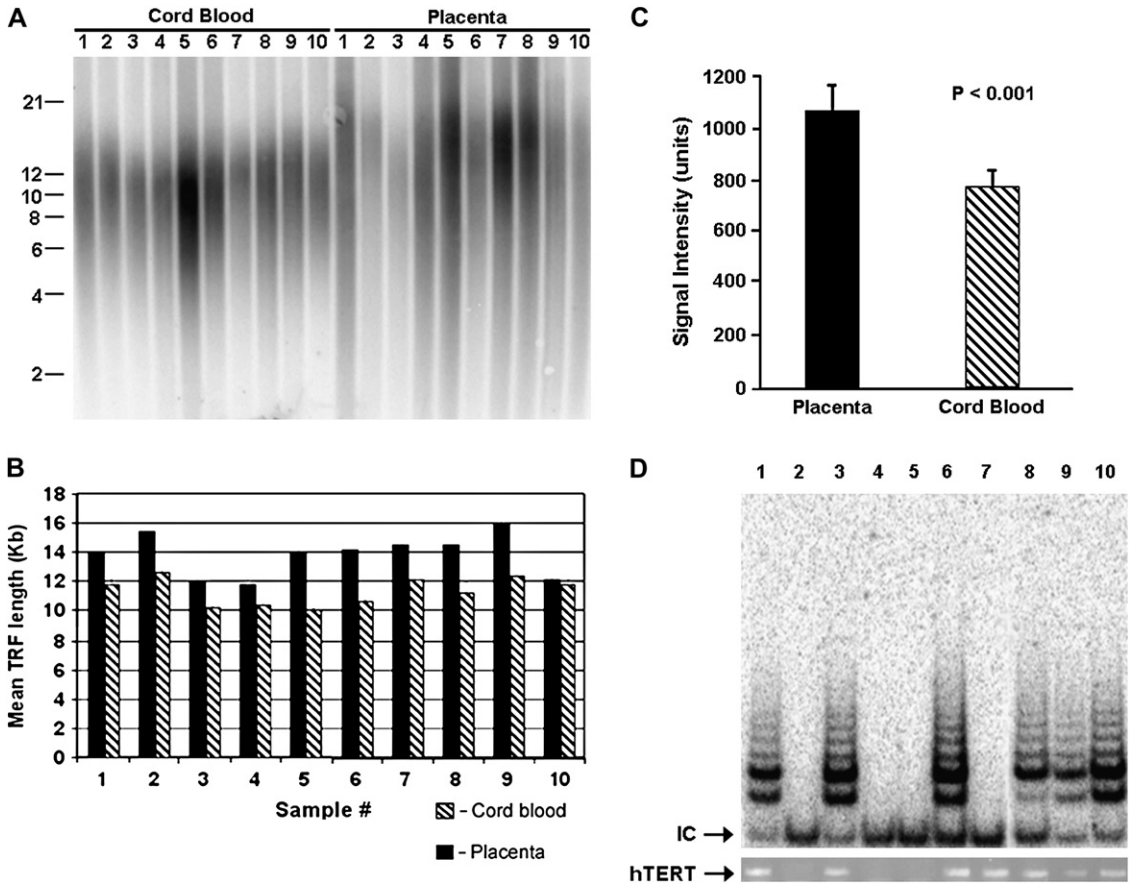

Fig. 1. Analysis of telomere length and telomerase activity and expression in term placenta and cord blood samples biopsied at single, random sites. A: Telomere length was assessed by Southern analysis of TRF length for both placenta and cord blood samples from the same donor. For all cord blood and placenta samples, 1.5 μg and 1ug of DNA was loaded per lane, respectively. Size of molecular weight markers (Kb) is shown at the side. B: Calculation of the mean TRF length for all placenta and cord blood samples ($n = 10$). C: Dot blot analysis and comparison of total telomeric signal intensity for all placenta and cord blood samples. D: Telomerase activity was assessed using the TRAP assay (upper panel) and hTERT expression was assessed by RT-PCR (lower panel). The internal control (IC) for PCR efficiency in the TRAP assay is indicated at the side.