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Telomere length heterogeneity in placenta revealed with high-resolution telomere length analysis

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ABSTRACT

Introduction: Telomeres, are composed of tandem repeat sequences located at the ends of chromosomes and are required to maintain genomic stability. Telomeres can become shorter due to cell division and specific lifestyle factors. Critically shortened telomeres are linked to cellular dysfunction, senescence and aging. A number of studies have used low resolution techniques to assess telomere length in the placenta. In this study, we applied Single Telomere Length Analysis (STELA) which provides high-resolution chromosome specific telomere length profiles to ask whether we could obtain more detailed information on the length of individual telomeres in the placenta.

Methods: Term placentas (37–42 weeks) were collected from women delivering at University Hospital of Wales or Royal Gwent Hospital within 2 h of delivery. Multiple telomere-length distributions were determined using STELA. Intraplacental variation of telomere length was analysed (N = 5). Telomere length distributions were compared between labouring (N = 10) and non-labouring (N = 11) participants. Finally, telomere length was compared between female (N = 17) and male (N = 20) placenta.

Results: There were no significant influences of sampling site, mode of delivery or foetal sex on the telomere-length distributions obtained. The mean telomere length was 7.7 kb ranging from 5.0 kb to 11.7 kb across all samples (N = 42) and longer compared with other human tissues at birth. STELA also revealed considerable telomere length heterogeneity within samples.

Conclusions: We have shown that STELA can be used to study telomere length homeostasis in the placenta regardless of sampling site, mode of delivery and foetal sex. Moreover, as each amplicon is derived from a single telomeric molecule, from a single cell, STELA can reveal the full detail of telomere-length distributions, including telomeres within the length ranges observed in senescent cells. STELA thus provides a new tool to interrogate the relationship between telomere length and pregnancy complications linked to placental dysfunction.

1. Introduction

Telomeres are present at the ends of all mammalian chromosomes [1] and are known to maintain genomic stability avoiding degradation and fusion events [2]. In humans and other vertebrates, telomeres consist of the hexameric DNA sequence TTAGGG tandemly repeated into arrays varying in length up to 25 kb dependent on the individual or tissue analysed [1] [3]. Telomeric DNA is associated with a specific multiprotein structure called 'shelterin', which plays a key role in the control of telomere length and end-protection [4] [5]. Telomere repeats are synthesized by telomerase, the cellular reverse transcriptase enzyme that adds telomeric repeats to the 3′ ends of each chromosome in those cell types in which it is expressed [6] [7]. In the absence of telomerase, each time a cell divides, telomeres progressively lose TTAGGG repeats ultimately reaching a length at which they trigger a Tp53 dependent G1-S cell cycle arrest referred as replicative-senescence [8]. In the absence of a functional Tp53 response, continued cell division can result in critical telomere shortening, the induction of telomere fusion events and genomic instability that can drive

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tumour progression [9]. Thus telomere shortening is considered to contribute to the development of cancer, several age-related diseases and premature ageing syndromes (reviewed in Ref. [10]).

The telomere length composition at birth within the foetal genome may be important for life long health [11]. Starting life with shorter telomeres may increase disease susceptibility later in life [12]. Studies in animals and humans suggest that intrauterine exposure to adverse conditions contribute to shorter telomeres at birth [13]. The placenta is exposed to the same environmental insults as the foetus and may provide a tool to assess the effect of environmental exposures on telomeres in pregnancy [14]. Critically, shortened placental telomeres may functionally contribute to low birth weight [15–21].

Several methods have been developed to estimate telomere length [22]. The two most widely used methods, terminal restriction fragment analysis (TRF) and quantitative polymerase chain reaction (q-PCR) are suitable for estimation of mean telomere length or telomere repeat content respectively. TRF is relatively low throughput and includes not only telomeric repeats, but also variable number of sub-telomeric sequences. Q-PCR constitutes a straightforward and suitable technique for high throughput studies, but suffers from high measurement errors [23]. High resolution telomere length measurements are obtained when used Q-FISH (Quantitative fluorescence in situ hybridization), a technique primarily used in the haematopoietic tissue that provides cell average length using metaphases [24]. Single telomere length analysis (STELA; Fig. 1), a single-molecule PCR based telomere length analysis technology that can determine the full spectrum of telomere lengths from specific chromosome ends [25]. Whilst STELA is comparatively low throughput, it is high-resolution and can detect the presence of telomeres within the length ranges that can lead to senescence, apoptosis and telomere fusion [26,27].

STELA has the potential to provide a richer and more detailed picture of telomere length in the placenta in relation to foetal growth restriction and perhaps provide more information on the role of adverse maternal lifestyles in telomere shortening. Here we examined the patient of XpYp telomere length analysis, using STELA, with respect to placental sampling site, mode of delivery and foetal sex.

2. Materials and methods

2.1. Placental biopsies and participant selection

Study participants (N = 42) were recruited prior to delivery at University Hospital Wales and Royal Gwent Hospital as described previously [28]. Written informed consent was obtained and the study was approved by the South East Wales Research Ethics committee (REC number: 10/WSE02/10). Placenta were collected within 2 h of an elective caesarean section (N = 32), an emergency caesarean section (N = 3) or a vaginal delivery (N = 7) from 42 term pregnancies (37–42 weeks). Chorionic villous samples were taken from the maternal side of the placenta at five different sites midway between the cord and lateral edge. To avoid contamination of the sample with maternal decidual cells, the top surface of the cotyledon was removed and the villous trophoblast tissue below sampled. Placental samples were washed in phosphate buffered saline and stored in RNAlater at −80 °C until needed. To analyse Intraplacental variation of telomere length, three placental samples (near to the cord insertion, middle and lateral edge) were biopsied

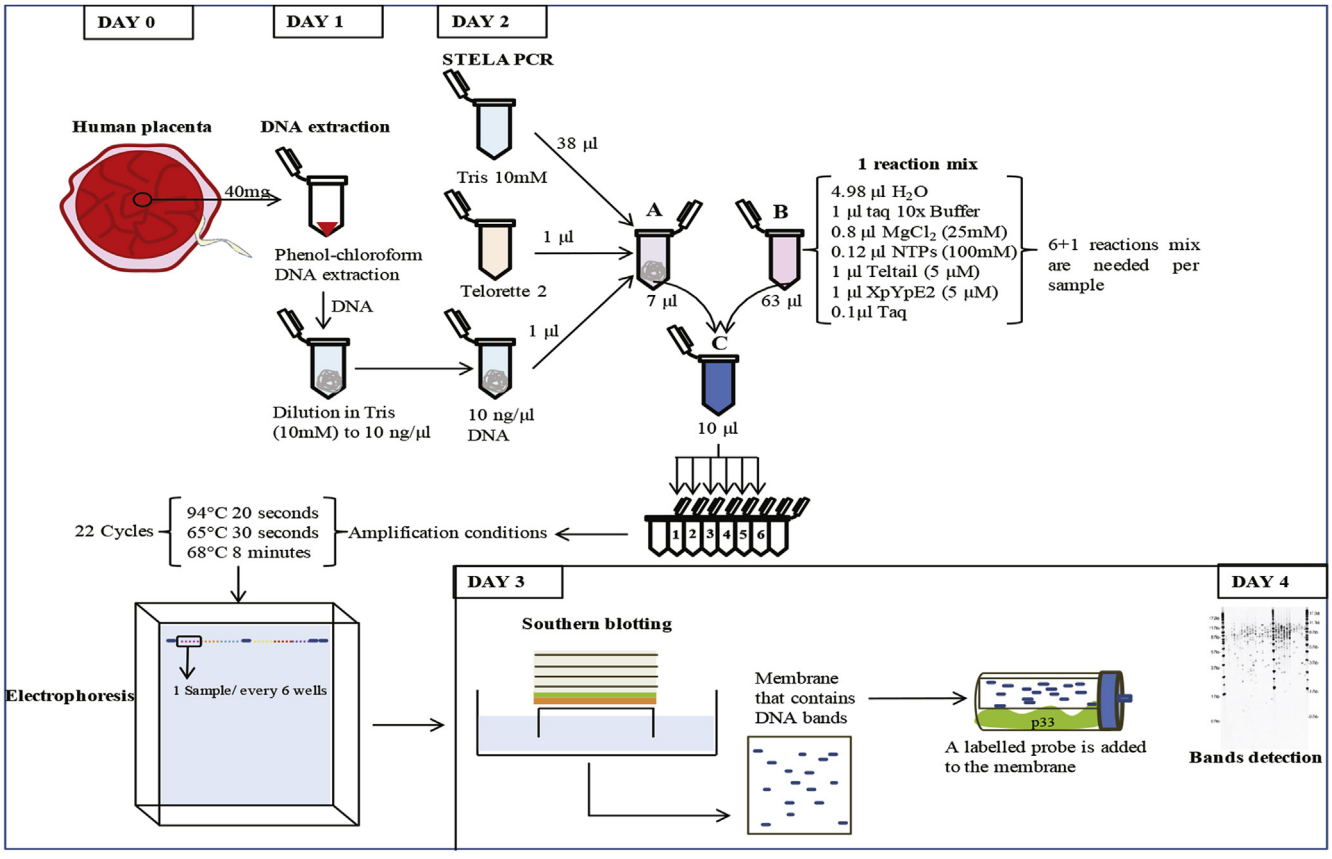

Fig. 1. Flow diagram of STELA protocol using placental samples. Timeline to perform STELA technique on placental samples.