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Measurement of Short Telomere Load in Individual Cells

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Abstract: Increasing evidence demonstrates that shortest more than mean telomere length predicts telomere dysfunction and genomic instability in association with a number of conditions, including cell senescence, aging and tumorigenesis. We developed Universal Single Cell Single Telomere Length Analysis (USC-STELA), based on a PCR-amplification and southern blotting, to measure short telomeres in individual cells. The mean short telomere length measured in individual cells by USC-STELA correlates with that from bulk cells, measured by Universal STELA (U-STELA). The validation and reproducibility of USC-STELA was confirmed using different cell types with known telomere lengths, as well as by using paired sister-cells from human embryos and cultured cells. Interestingly, individual cells known to elongate telomeres via alternative lengthening of telomeres (ALT) have more short telomeres, yet longer mean telomere length than control cells. Moreover, individual senescent fibroblasts carry more short telomeres compared to human embryonic stem cells (hESCs), consistent with the notion that short telomeres contribute to cellular senescence. Additionally, we found a greater load of short telomeres in polar bodies than in matching oocytes, providing further insights into the accelerated polar body DNA degradation following extrusion from the oocyte. USC-STELA provides a new method to study telomere dysfunction in individual cells, with potential to improve our understanding of the role of telomere dynamics in cancer, developmental biology and reproductive medicine.

Keywords: Single Cell, Telomere Length, Telomere Dysfunction, Cell Senescence, Genomic Instability

1. Introduction

Telomeres consist of repeated DNA sequences ((TTAGGG) n) with the protein complex known as shelterin. Together telomere repeats and shelterin form protective loop structures at the ends of linear mammalian chromosomes. Telomeres help chromosome ends evade DNA damage response pathways [1, 2]. They also spare genetic information from loss associated with incomplete DNA replication during the cell cycle. The lack of complete replication of the lagging DNA strand causes telomere shortening over many cell divisions and ultimately results in cellular senescence or apoptosis, the so called end replication problem [3, 4]. Oncogenic transformation occurs when cells escape cellular responses to critically short telomeres. The rate of telomere shortening can be modulated by many factors, including telomerase activity, reactive oxygen species, heavy metals, and other environmental factors [5-8].

Recent studies demonstrate that mean telomere length decreases nearly linearly with cell divisions, but the presence of even a small number of critically short telomeres is sufficient to trigger cell fate decisions [9]. Traditionally, telomere length in cells is measured using Terminal Restriction Fragment analysis (TRF) or semi-quantitative Fluorescence in situ Hybridization (qFISH) [10-12]. The load of short telomeres can be inferred by TRF, but this method only measures telomere length in large populations of cells. QFISH can measure the shortest telomeres in individual cells, but requires metaphase spreads, which can be challenging in many cells types. U-STELA was invented to study the load of short telomeres in populations of cells [13]. By digesting the genome, enriching the subtelomeric region and ligating adaptors, U-STELA measures the shortest telomeres within a population of cells. This new tool has yielded new insights into tissue aging and cell fate decisions within large populations of cells [14].

Genome amplification, bisulfite sequencing, RNA sequencing, and amplification of telomere repeats by PCR (SCATR) now extend molecular analysis to single cell resolution. Measurement of short telomere load in individual cells would further research in cell and developmental biology, oncology and reproductive medicine [15-18]. Measurement of the shortest telomeres in individual cells is not feasible with current technology. Here, we present a reliable and accurate method to measure the load of short telomeres within single cells.

2. Methods

2.1. Materials

Lysis buffer was made according to a published protocol [19], and stored at -20°C. Reagents were from Sigma, and protease from Qiagen.

The carrier oligo DNA, GT23VN (GTTTTTTTTTTTTTTTTTTTTTTTVN), was synthesized by Life Technologies, and dissolved in nuclease-free water to 0.2 nmole/µl of working solution and stored at -20°C. Other oligos (15) used in U-STELA were synthesized by Integrated DNA Technologies and reconstituted with nuclease-free water to constitute a 100 µM stock solution.

Restricted endonucleases, buffer, T4 DNA ligase, ATP and BSA were from New England BioLabs. Failsafe PCR enzyme and buffer were from Epicentre. TeloTTAGGG telomere length assay kits were from Roche.

2.2. Single Cell Isolation and Lysis

Single cell isolation from cell lines was performed according to a published protocol [15]. Specially, sister-cell pairs were obtained by synchronization into S phase with nocodazole (Sigma) then cultured in media without nocodazole for 2 hours. Sister-cell pairs were separated by trypsin and placed into PCR tubes with 1µl of PBS.

Discarded, cryopreserved, human, cleavage stage embryos, donated by consented patients for research were obtained from New York University Langone Fertility Center (NYULFC) with approval by IRB of the NYU School of Medicine. Single blastomeres from cleavage embryos (4-8 cell stage) were disassociated using PGD Biopsy media (Life Global), by manual pipetting after zona pellucida removal with 0.33% pronase. Single blastomeres were transferred into PCR tubes with 1ul of PBS/PVP.

4 µl of lysis buffer was added to Isolated, single blastomeres in 1µl of PBS. After spinning tubes were placed into a thermocycler set to a lysis program at 50°C for 3 hours and 75°C for 30 min. Single cell lysate tubes were left on ice for the next step.

2.3 Single Cell STELA

2.3.1. Digestion of Single Cell DNA with Carrier Oligo DNA

To setup reaction, 5 µl of single cell lysate with 100 pmole of carrier DNA was digested in a 10 µl reaction by adding 0.5 µl each of MseI and NdeI, 1 µl of NEBuffer 2 (10X), 0.1 µl of BSA (100X) and water. Thermocycler was set for digestion at 37°C for 16 hours followed by an inactivation step run at 65°C for 25 min.

2.3.2. Ligation of Single Cell Digested DNA

10 µl of digested DNA from the single cells plus 0.5 µl each of 42-mer and 11+2-mer oligo (100 µM) were combined in PCR tubes, set to ramp down from 65°C to 16°C over 1 hour. 11 µl of this mixture was ligated by adding 0.3 µl of T4 DNA ligase with 0.5 µl of NEBuffer 2 (10X) and 1.5 µl of ATP (10X) in a 15 µl reaction at 16°C overnight. This mixture was ligated with 0.5 µl of telorette mix (0.01µM) by adding 0.3 µl of T4 DNA ligase with 0.5 µl of NEBuffer2 and 0.5 µl of ATP in a 20 µl reaction at 35°C for 16 hours followed by a 25 minute inactivation step at 65°C.

2.3.3. Purification by AMPure XP Beads

To achieve the highest recovery, we added 2 volumes (40 µl) of Agencourt AMPure XP beads (Beckman Coulter) to 20 µl of the ligation mixture. After pipetting up and down more than 20 times in order to mix thoroughly, the mixture ,was incubated at room temperature for 15 min. The mixture was placed into a magnetic plate and allowed to stand for 5 minutes. Following two washes with 200 µl of fresh 80% ethanol, the separated beads were dried at RT for 3 minutes (making sure not to over dry). DNA was eluted with 7.2 µl of water by pipetting gently up and down 50 times, then left standing for 10 minutes to release the DNA. After returning the mixture to the magnetic plate for 5 minutes, the eluate is pipetted out carefully into a new PCR tube.

2.3.4. Single Cell STELA PCR

The STELA PCR reactions were run in a 16 µl volume containing 7.2 µl of single cell ligated DNA, 8 µl of Failsafe PCR Pre-Mix H (2X), 0.2 µl of Adapter (10 µM), 0.2 µl of Teltail (10 µM) and 0.4 µl of Failsafe enzyme. The thermal cycler reaction program was set at 68°C for 5 min and 95°C for 2 min, followed by 29 cycles of 95°C for 15 s, 58°C annealing for 30 s and extension at 72°C for 12 min, with a

final extension for 15 min at 72°C.

2.3.5. Southern Blot Analysis of Short Telomere Loading

STELA PCR product was loaded on 0.8% agarose gel and separated in 1X TAE buffer for 2 hours at 120V. Following the instruction of the TeloTTAGGG telomere length assay, we are able to perform Southern blotting using a telomere probe. The short telomere distribution was analyzed using software of Bio-Rad image analysis system.

2.4. Measurement of Short Telomere in Bulk Cells by Universal STELA

Genomic DNA from bulk cells (about 5 x 10^6 cells from each cell line) was extracted by quick-gDNA Miniprep kit (Zymo Research, # D3024) following the manufacturer's instructions. The quality and concentration of DNA was measured using Nanodrop Lite (Thermo Scientific) and Qubit dsDNA BR assay kit. High DNA quality is indicated by a ratio of A280/A260 between 1.8-1.9.

1 µg of genomic DNA is digested by 2 µl of enzyme (1: 1 mixture of MseI and NdeI) in 20 µl reaction volume mixed with 2 µl of Cutsmart buffer (10X) at 37°C for overnight. 10 ng of digested DNA with 0.5 µl each of 42-mer and 11+2-mer oligos (100 µM) in a 10 µl volume is ramped down from 65°C to 16°C over 1 hour. Next, the mixture is ligated by adding 0.3 µl of T4 DNA ligase with 1.5 µl of NEBuffer2 (10X) and 1.5 µl of ATP (10X) in a 15 µl volume at 16°C for overnight. The mixture is ligated with 0.5 µl of telorette mix (0.01µM) by adding 0.3 µl of T4 DNA ligase with 0.5 µl of NEBuffer2 (10X) and 0.5 µl of ATP (10X) in a 20 µl volume at 35°C for 16 hours followed by a 20 minute inactivation step at 65°C.

Ligated DNA is diluted to a final concentration of 40 pg/µl by adding water. Reactions are set up in 12 µl volume containing 40-500 pg of template DNA, 6 µl of Failsafe PCR PreMixH (2X), 0.12 µl of Adapter (10 µM), 0.12 µl of Teltail (10 µM) and 0.3 µl of Failsafe enzyme. Thermal cycler reaction conditions are set at 68°C for 5 min and 95°C for 2 min followed by 26 cycles of 95°C for 15 s, 58°C annealing for 30 s and extension at 72°C for 12 min, with a final extension for 15 min at 72°C.

Short telomere loading analysis is performed as described above.

2.5. Statistics Analysis

We use the software GraphPad Prism6 to perform statistical analyses.

3. Results

3.1. Methodology of Universal Single Cell Single Telomere Length Analysis (USC-STELA)

We adapted the principle of universal STELA [13] to the constraints of single cell genomic DNA. To minimize DNA loss, all procedures are performed in the same PCR tube, from lysis to final ligation. After purification, eluted DNA is transferred to a new PCR tube, and then STELA PCR is performed.

The first challenge to apply the universal STELA methodology to the single cell level is enabling sufficient lysis to release intact DNA from one cell without negatively impacting subsequent steps in the process, especially restriction enzyme digestion. After testing a number of lysis buffers we found one that uses a serine protease to digest most proteins. Previous studies showed it does not disrupt the activity of restriction endonucleases [19]. Next, we designed custom oligos to serve as carrier DNA, whose sequences are different from those used in subsequent STELA ligation steps. The carrier DNA ($GT_{23}VN$) worked better than two other custom oligos (T_{23} and A_{23}) (Figure 1a), as judged by the number and intensity of bands upon blotting. Spiking in carrier DNA during the digestion reaction did not interfere with the subsequent steps, so it was used in all experiments. Due to the many steps requiring the addition of enzymes and buffers, the final STLEA PCR using the ligation mixture as template was unsuccessful. Commercial magnetic beads proved the best method to purify the ligation mixture before STELA PCR, presumably because of the low elution volume and high DNA recovery.

Only 10-40 pg of template per PCR is sufficient to produce discrete bands. Higher amounts of DNA template cause smearing instead of discrete bands. STELA PCR did not require pre-amplification. We performed STELA PCR on two different cell lines (U2OS and RuES2) with various PCR cycle numbers. The number and density of bands increased proportionally with PCR cycle number from 26 to 30 (Figure 1c). Following 30 PCR cycles, bands appeared as large smears because of excess PCR product. After 28 cycles, many bands were too faint. So 29 cycles was chosen for the USC-STELA PCR.

Next, we validated that USC-STELA PCR only amplifies genomic DNA and does not result in false positives in negative controls. When water or carrier DNA from the lysis step were added to the final PCR, the USC-STELA produced no bands compared with single cell DNA plus carrier DNA performed in parallel (Figure 1a and b).

To demonstrate the scalability and sensitivity of the method, we tried multi-cell reactions compared with one cell. We performed the USC-STELA method using 1, 3 and 10 cells in 1µl of PBS from two cell lines (U2OS and RuES2). As a result, the number of bands increased with increasing cell numbers (Figure 1d), suggesting that our method is as sensitive as universal STELA to template amount and that the appearance of bands depends on the amount of the starting material. In particular, the telomere bands started smearing with 10 cells per reaction, consistent with universal STELA in that a smear appears when using more than 100 pg of DNA. Overall, our modifications of universal STELA enabled the generation of a sensitive, scalable method for single cell resolution of measurement of short telomeres.

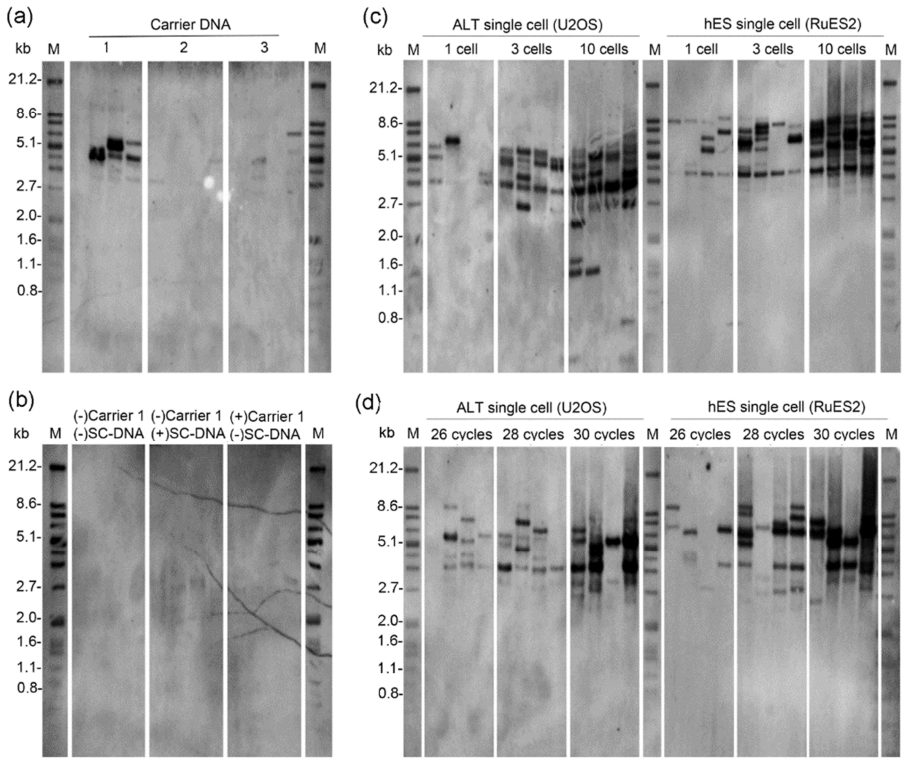

Figure 1. *Universal single cell STELA (USC-STELA). (a) Selection of carrier DNA. 1, 2 and 3 represent different sequences of carrier DNAs. (b) Negative controls in the USC-STELA assay. [20] and (-) represent addition and deletion in the USC-STELA assay respectively. (c) Impact of PCR cycle number on USC-STELA assay. (d) Sensitivity of DNA template in USC-STELA assay. The more cells (>1 cell) are assayed by USC-STELA, the more bands are loaded on the southern blot membrane.*

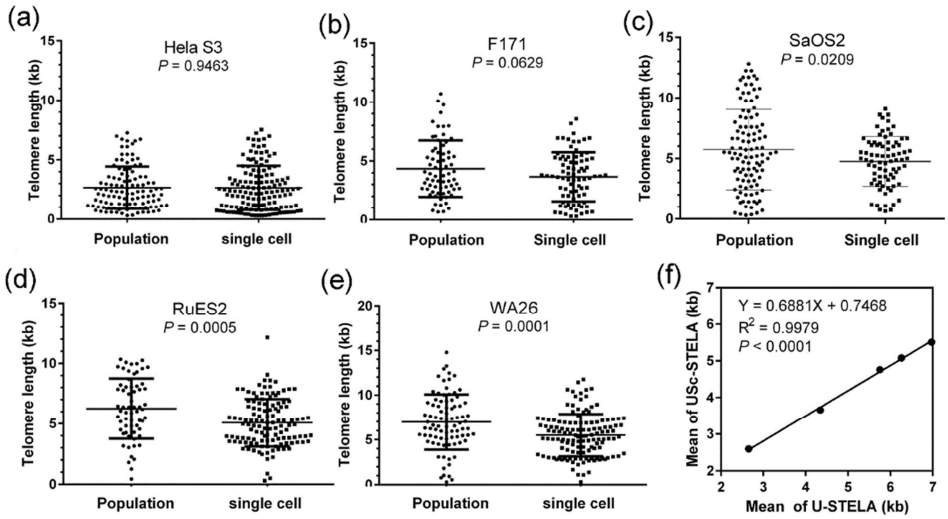

Figure 2. *Validation of USC-STELA by comparing single short telomeres length between individual cells and population cells. (a), (b), (c), (d) and (e) Scatter plots show loading of short telomeres in five human cell lines. Short telomeres from bulk cells were measured by U-STELA assay and those of single cells by USC-STELA assay. (f) Analysis of linear correlation of average short telomere length between bulk cells and single cells.*

3.2. Accuracy of USC-STELA by Comparing Short Telomere Loading Between Single Cells and Bulk Cells

Both USC-STELA and universal STELA (U-STELA) measure the load of short telomeres, so the mean of short telomeres measured by USC-STELA in individual cells, sampled from a population of cells, should correlate with the results of U-STELA measured from the same population of cells. First, we measured short telomeres with U-STELA in five human cell lines with different average telomere length (SaOS2 > WA 26 > RuES2 > F171 > Hela S3) [15]. We also collected individual cells from these populations and performed USC-STELA. Since each reaction of USC-STELA contains the DNA content from one cell (about 7pg) and that of U-STELA uses 20 pg DNA as template per PCR, we performed USC-STELA on 36 individual cells (36 reactions) for each cell line, to match universal STELA PCR

(12 reactions). Theoretically, the longest PCR products are less than 12 kb because of the 12 minutes extension setting in the PCR program. Thus, the telomere bands that appear on the membrane tend to be less than 12 kb, even in those cell lines with telomere lengths exceeding 12 kb. We took the mean value of short telomere length for each cell line to compare USC-STELA and U-STELA by an unpaired T-test as significant if $P < 0.05$. The mean length of short telomeres in bulk cells by U-STELA in three cell lines (SaOS2, WA26 and RuES2) was longer than that of individual cells by USC-STELA ($P < 0.05$) (Figure 2c, d and e). We interpret this as resulting from low recovery of magnetic beads when applied to large DNA fragments (>10 kb) during the purification step. Concordantly, when USC-STELA was performed on individual cells from Hela S3 and F171, which had average telomere lengths of about 7 kb, the load of short telomere did not differ between bulk and individual cells (Figure 2a and b). Linear regression demonstrated a strong relationship between the two methods ($R^2 = 0.9979$, $P < 0.0001$) (Figure 2f). This demonstrates that USC-STELA represents an accurate estimate of the load of short telomeres within individual cells.

3.3. Reliability of USC-STELA by Analyzing Short Telomere Loading on Pairs of Sister-cell

The task of finding a model to establish the reliability of our method is daunting as no studies to date report the distribution of single telomere lengths among individual cells. However, our previous work indicated sister cells from mouse 2-cell or 4-cell embryos had very similar average telomere length, so we assume that single telomere length within human blastomeres should be more homogeneous relative to those from cultured individual cells. Based on this hypothesis, we obtained surplus, cryopreserved human embryos from NYU Fertility Center, which had been donated for research, via an IRB approved protocol, and cultured them to day 3. We separated single blastomeres, and performed USC-STELA to investigate the distribution of short telomeres within cleavage stage, human embryos. Not all blastomeres from each embryo could be recovered. We discarded dead or fragmented cells. As expected, the load of very short telomeres (< 4kb) among sister blastomeres within individual embryos was conserved (Figure 3a). Using Bio-Rad Image Lab software we converted bands appearing on the membrane into telomere length by using standard molecular weight DNA markers. We compared the mean length of very short telomeres (< 4 kb) between blastomeres by unpaired T-test or one-way ANOVA. The load of very short telomeres within blastomeres from the same embryo did not differ significantly ($P > 0.05$) (Figure 3c), although the load of longer telomeres (> 4 kb) did differ (Figure 3a), possibly due to telomere length heterogeneity among blastomeres.

We applied USC-STELA to eight pairs of cells from human embryonic stem (RuES2, R) and osteosarcoma cells (U2OS, U), after separation using trypsin-EDTA and micro-pipetting. Each pair of sister-cells exhibited nearly identical distribution of short (< 4 kb) telomeres (Figure 3b), and the mean value of very short telomeres (< 4 kb) did not differ significantly between paired sister-cells ($P > 0.05$, T-test) (Figure 3d). Intriguingly, two pairs of sister-cells (R3 and U1) had ultra-short telomere loading (0.7 kb and 0.3 kb). Presumably, in spite of the capacity for unlimited proliferative capacity characteristic of embryonic stem and cancer cells, *in vitro* culture still promotes telomere dysfunction in a subset of cells.

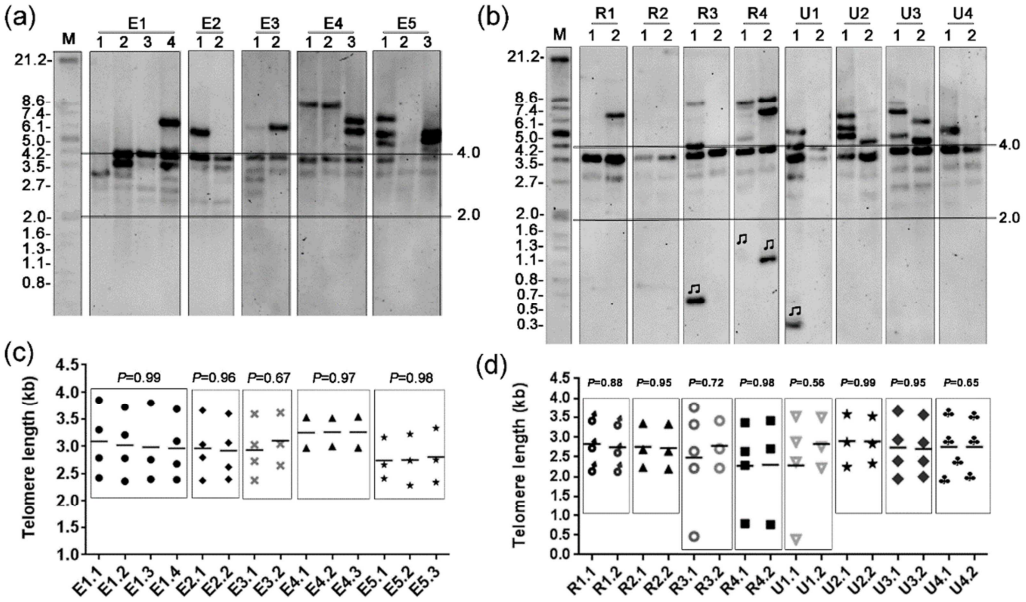

Figure 3. *Reliability of USC-STELA as determined by comparing short telomeres on paired sister-cells from human blastomeres and cultured cells. (a) and (b) Southern blot assay showed distribution of single, short telomere loading in 5 human embryos (E1-E5) and eight pairs of sister-cells (R1-R4 and U1-U4). (c) and (d) Loading pattern of single short telomeres among blastomeres and between sister-cells was analyzed by comparing average short telomere length. Threshold is set below 4 kb.*

3.4. USC-STELA Identifies Short Telomere Loading in ALT Cells and Senescent Fibroblasts

Telomere length heterogeneity is one of the hallmarks of ALT cell lines. Telomeres can range from very short to extremely long (up to 20 kb) [21]. We used USC-STELA to evaluate heterogeneity of single chromosome telomere length at the single cell level. We compared the load of short telomeres in 36 individual cells from each of the following cell lines- ALT cells U2OS and SaOS2, human embryonic stem cells (WA26) and fibroblasts (F171 and F200). Consistent with previous reports, individual ALT cells (U2OS) showed significantly higher loads of very short telomeres (< 1 kb) compared to WA26 cells (Figure 4b), (P < 0.0001, *Chi*-square) (Figure 4c and d). The frequency of telomeres less than 2 kb also was significantly higher in SaOS2 compared to WA26 cells (P < 0.01, Fisher's exact test) (Figure 4).

Critically short telomeres (chromosomal uncapping) trigger cell senescence and apoptosis [22, 23]. We investigated two different senescent fibroblast cells, F171 (p28) cultured *in vitro* until senescence, and F200, derived from a 76 year old patient. On the single cell level, some senescent fibroblasts showed ultra-short telomeres (<1 kb) compared to human embryonic stem cells (WA26) (Figure 4), (P = 0.0007 *Chi*-square). Ultra-short telomeres (<1 kb) and normal length telomeres (> 4 kb) can coexist in one cell, consistent with the notion that a few, critically short telomeres can promote cell senescence [9], even in the setting of normal mean telomere length.

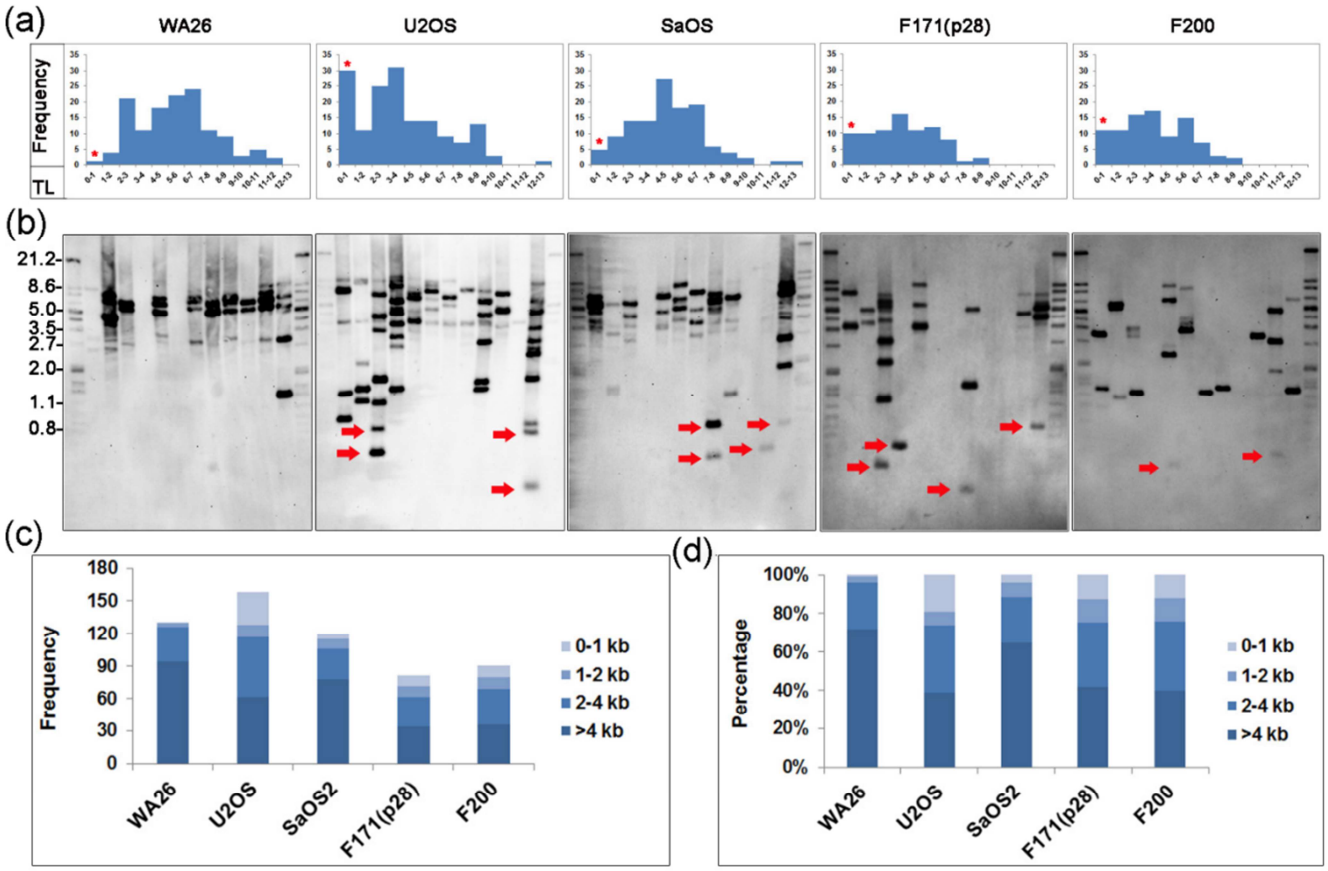

Figure 4. *Measurement of single short telomeres by USC-STELA in five human cell lines. (a) Charts showing distribution of single short telomeres in individual cells. TL represents telomere length. (b) Representative southern blotting data showing load of short telomeres. Thirty-six individual cells from bulk cells were performed by USC-STELA assay for each cell line. Each membrane had 12 wells of individual cell loading, plus 2 wells of molecular weight (M) loading. Red arrows indicate single short telomeres are below 1000bp of length which is considered as critical short telomeres. (c) Summary of single short telomeres distribution in different ranges. (d) Comparison of percentage of single short telomeres loading in different ranges.*

3.5. Short Telomeres in Polar Bodies Compared with Matched MII Oocytes

Recent studies have found that mean telomere lengths are highly concordant between the human oocyte and its matching polar body, though they have different fates during development [15, 24]. During meiotic maturation, the first polar body disintegrates within a few hours following extrusion. Few studies deal with the mechanism of degeneration of the first polar body, and we believe that telomere dysfunction might be at least part of the cause. We performed USC-STELA on pairs of first polar bodies and matched MII oocytes, donated by consenting patients from the NYUFC. In all eight polar bodies/oocyte pairs assessed polar bodies had shorter telomeres compared to their

matching oocytes (Figure 5a and d) ($P = 0.0182$, Unpaired T-Test). Polar body five showed ultra-short telomeres (0.3 kb and 0.5 kb) (Figure 5a and b) compared to its matching oocyte. Critically short telomeres might contribute to polar body apoptosis. Though more short telomeres are present within polar bodies compared to oocytes, mean short telomere length did not differ in each pair of polar body and oocyte (Figure 5b) ($P > 0.05$, Unpaired T-Test). Moreover, there was no correlation between polar bodies and oocytes in short telomere loading ($P = 0.2271$, Pearson correlation $r = -0.4815$) (Figure 5c).

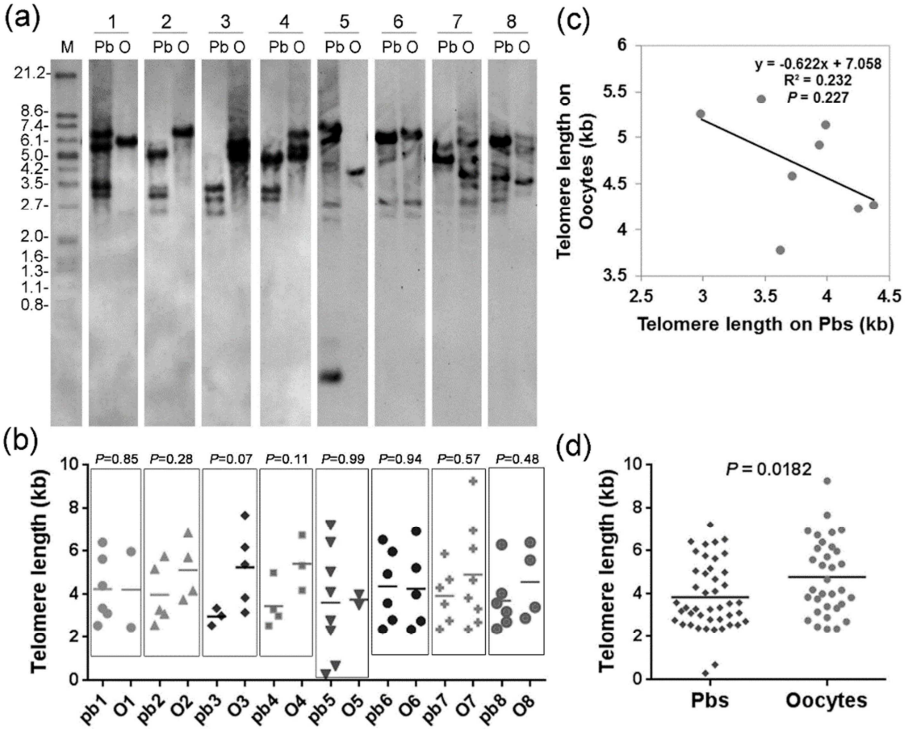

Figure 5. *Comparison of load of short telomeres, measured by USC-STELA, human polar body- oocyte pairs. (a) Southern blot assay showing short telomeres from eight human polar body-oocyte pairs. Polar body number 5 shows critically short telomeres, whose length is less than 0.8kb. (b) Distribution of short telomeres in paired polar bodies and oocytes. Single telomere loading band was analyzed by Image Lab software and converted to absolute length (kilo base). Comparison between polar body and matched oocyte was analyzed by unpaired T-test, and significance indicated by $P < 0.05$. (c) Pearson correlation analysis shows nonlinear relationship between polar body and oocytes short telomere length. (d) Scatter plot showing distribution of short telomere length from 8 polar body-oocyte pairs. Unpaired T-test shows $P = 0.0182$ which indicates single short telomere loading in polar bodies exceeds that in oocytes.*

4. Discussion

To our knowledge, USC-STELA is the first assay to measure single telomere length loading patterns at the single cell level. By using this method, we were able to analyze the load of short telomeres in individual human cells including pluripotent stem cells, immortal cancer cells, senescent fibroblasts and blastomeres from human embryos. Moreover, with USC-STELA, we show a disproportionate bulk of short telomeres in polar bodies, suggesting telomere dysfunction which may contribute to the rapid degeneration of polar bodies following their extrusion during meiotic maturation.

We adapted the principles of universal STELA to generate a method scalable to single cells. USC-STELA shares some common features with universal STELA. First, they both require digestion, ligation and PCR steps, which can produce artifacts because of variable efficiency of each step. Second, they both are sensitive to template loading- the pattern of short telomeres depends on the starting amount of DNA. This in turn depends on the number of individual cells analyzed. Third, for both PCR product size is limited by the kinetics of DNA polymerase. This is beneficial for the measurement of short telomeres, but limits the ability to measure mean telomere length.

Because of these shared characteristics, we were able to validate our method by demonstrating a linear relationship between short telomere loading in individual cells, measured by USC-STELA and bulk cells, measured by U-STELA. Both methods share a similar disadvantage in that they are time consuming and labor intensive. From start to finish each USC-STELA or U-STELA assay takes six days. Moreover, error during any single step produces the absence of bands on the membranes. USC-STELA and U-STELA, therefore, are not high-throughput techniques.

Another disadvantage of USC-STELA is its sensitivity to extremes of telomere size. According to the manufacturers' instructions for AMPure XP beads, the efficiency of recovery is influenced by three factors-fragment size, elution volume and input concentration. Individual telomeres in single cells cannot exceed 10 kb nor can they be shorter than 1 kb, especially in embryonic stem and ALT cells. During purification, recovery of large fragments, especially over 10 kb, is remarkably low and results in significant differences in loading of short telomere

between individual cells and bulk cells (Figure 2c, d and e). On the other hand, when USC-STELA is applied to individual cells with short telomere (<7 kb), such as fibroblasts or HelaS3, it shows great consistency of loading of short telomeres between the two methods. Compared with U-STELA, our method has an advantage in that the results from USC-STELA represents the true pattern of short telomere loading, because it uses whole cell genomic DNA as templates instead of aliquots from bulk cells.

In theory, diploid individual cells have 46 chromosomes and consequently 92 individual telomere ends contribute to the signal on southern blot. Our data however, typically shows fewer bands per cell. This can be explained by the low resolution of agarose gel electrophoresis with a large range of nucleic acid loading [25]. Individual cells with more homogenous telomere length, such as human embryonic stem cells, may have telomere lengths varying by 100-1000 bp, but agarose gel electrophoresis is not able to separate them sufficiently when their length exceeds 3 kb. In such cases fewer than expected bands appear. Another explanation is the difference of PCR efficiency with different DNA template size. In general, the efficiency of PCR is greater for smaller size templates than high molecular weight DNA [26]. Presumably, STELA PCR preferentially amplifies shorter DNA fragments when different size telomeres co-exist within a cell. Regardless, our method shows increased heterogeneity in telomere length within ALT cells, such as U2OS and SaOS2 cells.

5. Conclusion

In conclusion, our new method, USC-STELA promises to improve understanding of the relationships between telomere biology and cell fate. With the ability to directly examine the telomere architecture of single cells, we can now study telomere biology in rare, hard-to-acquire cell types, such as individual gametes or blastomeres from early embryos.

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