Telomere Dynamics in Human Health and Disease

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Abstract

Telomere function is critical for genomic stability; in the context of a functional TP53 response, telomere erosion leads to a G1/S cell cycle arrest and the induction of replicative senescence, a process that is considered to underpin the ageing process in long-lived species. Abrogation of the TP53 pathway allows for continued cell division, telomere erosion and the complete loss of telomere function; the ensuing genomic instability facilitates clonal evolution and malignant progression. Telomeres display extensive length heterogeneity in the population that is established at birth, and this affects individual risk of a broad range of diseases including cardiovascular disease and cancer. In this perspective I discuss telomere length heterogeneity at the levels of the population, individual and cell, and consider how the dynamics of these essential chromosomal structures contribute to human disease.

Introduction

Telomeres are structures that cap the ends of eukaryotic chromosomes, preventing the recognition and repair of the natural chromosomal terminus by the cellular DNA damage response (DDR) apparatus (de Lange 2005). As a consequence of end-replication losses, telomeres shorten with on-going cell division. Short telomeres can elicit a TP53-dependent cell cycle arrest (d'Adda di Fagagna et al. 2003), referred to as replicative senescence, that provides a stringent tumour suppressive function in long lived species (Deng et al. 2008). However, this natural limitation on proliferative capacity, together with the induction of replicative senescence, is considered a mechanism that may underlie age-related tissue deterioration and disease.

In the absence of a functional checkpoint response, on-going cell division past the point of replicative senescence results in continued telomere erosion and ultimately the loss of the end-capping function (Counter et al. 1992). Telomeres can then be targeted for DNA repair, resulting in inter- and intra-chromosomal fusion of telomeres with other telomeres and non-telomeric loci (Capper et al. 2007). This can lead to the formation of dicentric chromosomes and the initiation of cycles of anaphase-bridging, breakage and fusion resulting in large-scale genomic rearrangements including non-reciprocal translocations (Artandi et al. 2000). Other repair processes also operate at short dysfunctional telomeres that can lead to more complex mutational outcomes including chromothripsis (Maciejowski et al. 2015; Cleal et al. 2019). This widespread telomere dysfunction, at the end of the replicative lifespan, leads to a replicative crisis and autophagic cell death activated via innate immune responses (Nassour et al. 2019; Nassour et al. 2023). During crisis there is a strong selection pressure for the upregulation of telomere maintenance mechanisms, principally telomerase activity, that facilitates the escape from crisis. The process of crisis escape is considered a key mutational mechanism that drives genomic instability and clonal evolution during malignant progression (Artandi et al. 2000).

Consistent with the concept of antagonistic pleiotropy in long-lived species such as humans, telomeres are considered to play a role in the trade-offs between tumour suppression in early life and age-related disease in later life (Melzer et al. 2020). Consequently, there have been numerous studies associating telomere length with a broad range of human diseases, as well as psychosocial stresses and lifestyle factors, that are considered to be capable of modifying telomere length. From much of this work causality has not been established and the use of

error prone methodologies together with small cohorts has led to potential biases towards the publication of positive associations (Pepper et al. 2018). However recent very large-scale population studies and Mendelian randomisation have started to bring some clarity to this area and demonstrate potential mechanistic links between telomere length and disease phenotypes (Telomeres Mendelian Randomization et al. 2017; Codd et al. 2021). Considering the evolutionary trade-offs that determine species specific telomere length settings, it is apparent that there is an optimal telomere length in humans that is not too long so to lose the limit on replicative capacity and tumour suppression, but not too short to drive age-related disease (Savage 2024). This is exemplified by population studies demonstrating increased risks of cancer from constitutively long telomeres and other conditions including atherosclerosis with shorter telomeres (Aviv 2012; Telomeres Mendelian Randomization et al. 2017). Despite these constraints on telomere length, there is considerable telomere length heterogeneity in the human population. Variants within genes required for telomere maintenance can result in affected individuals born with short telomeres that can lead to a broad range of clinical manifestations that are consistent with telomeres limiting replicative lifespan and the induction of replicative senescence in specific cellular compartments (Armanios 2022). These extreme phenotypes are one end of a predominately genetically determined spectrum of telomere lengths, also influenced by prenatal and early life exposures, that exhibit a range of phenotypes in the human population.

In this perspective I discuss telomere length dynamics and how these impact human health and disease.

Lifelong telomere length heterogeneity is established at birth.

Telomere length exhibits considerable heterogeneity in the adult population. This is apparent from various cross-sectional surveys but was nicely exemplified in a study by Factor-Litvak *et al* who analysed telomere lengths in mother, father and new-born trios using terminal restriction fragment (TRF) analysis. They showed that mean telomere length varied in mothers between 6.19 and 9.81 kb (mean 7.92 kb) and fathers between 5.83 and 9.88 kb (mean 7.70 kb) (Factor-Litvak et al. 2016). These ranges of 3.62 kb and 4.05 kb in mothers and fathers respectively represent around 50% of overall mean telomere lengths. However, it was also apparent from this study, that the telomere length range in new-borns was as great as that observed in the adults, 4.59 kb or 48% of overall mean telomere length (mean 9.50 kb, range 7.01 to 11.6 kb), indeed there were no statistically significant differences in the

variances between mothers, father and new-borns (Factor-Litvak et al. 2016). These observations were also consistent with other cross-sectional studies of telomere length in the human population using different technologies that show a maintenance of telomere length heterogeneity across the age range (De Meyer et al. 2009; Weischer et al. 2013). This fundamentally important observation demonstrates that telomere length heterogeneity is established at birth and is maintained into adulthood. Moreover, these data indicate there is little requirement to invoke additional factors that create further inter-individual telomere length heterogeneity during life. Consistent with these observations, longitudinal studies have shown that telomere length ranking, relative to the age-matched normal length range, changes little during life (Benetos et al. 2013). This is important because the conventional view was that stress and disease during life leads to chronic inflammation and an increase in the turnover of the haematopoietic stem cell (HSC) compartments (Zhang et al. 2016). As telomerase activity in HSCs is insufficient to counteract end-replication losses, increased turnover will be manifested as increased rates of telomere shortening. In this paradigm, telomere length is a biomarker of long-term chronic inflammation (Wong et al. 2014), that may in turn increase risk of disease, including cardiovascular disease (Pusceddu et al. 2020). Importantly also, this paradigm implies the possibility that individual rates of telomere erosion could be modulated by controlling rates of chronic inflammation. In contrast the concept that telomere length, relative to the age-matched normal length range, is set early in life, implies it is not readily modifiable, that it precedes the onset of disease and may thus be causal. This was further exemplified in large-scale analysis of leukocyte telomere length in participants of the UK Biobank using qPCR methodology. Analysis of 422,797 individuals showed that several modifiable behaviours and traits were associated with telomere length, but the effects were too small to modify the association of telomere length and disease (Bountziouka et al. 2022). In this study healthy behaviours accounted for up to just 0.2% of the variation in telomere length; telomere length therefore appears to be set early in life and cannot be significantly changed by lifestyle factors to impact health and disease.

Determinants of telomere length at birth.

Telomere length at birth is underpinned by genetic and epigenetic factors, as well as maternal health during pregnancy and prenatal conditions. Prenatal adversity and intrauterine exposures can modulate telomere length at birth, as well as in the placenta, that may functionally contribute to low birth weight (Davy et al. 2009; Biron-Shental et al. 2010a; Biron-Shental et al. 2010b). These exposures include maternal psychosocial stress (Entringer

et al. 2011), prenatal depression (Garcia-Martin et al. 2021), gestational diabetes mellitus (Xu et al. 2014; Hjort et al. 2018) and metformin treatment (Garcia-Martin et al. 2018). The underlying mechanisms have not been established, but these factors potentially contribute to telomere length heterogeneity at birth.

Telomere length in adult females is longer than in males (Gardner et al. 2014), this has previously been attributed to sex specific differences in rates of telomere attrition with age (Bayne et al. 2007), however it is now apparent that this is established at birth and thus may be genetically or prenatally determined (Factor-Litvak et al. 2016). In addition, the increase in paternal germline telomere length as a function of age (Allsopp et al. 1992; Baird et al. 2006) likely accounts for the correlation between paternal age at conception and telomere length in new-borns, such that older fathers have children with longer telomeres (Unryn et al. 2005; De Meyer et al. 2007; Njajou et al. 2007; Arbeev et al. 2011).

Twin studies have been used to estimate the genetic contribution to telomere length heterogeneity in the population. These studies have consistently shown higher correlations in telomere length between monozygotic twins compared to dizygotic twins, indicating a substantial genetic influence on telomere length. Heritability estimates from these studies vary between 30-80% (Slagboom et al. 1994; Bischoff et al. 2005; Vasa-Nicotera et al. 2005; Andrew et al. 2006; Broer et al. 2013). This variation in heritability estimates across studies is likely to be largely due to methodological variability in study design, sample size, telomere length measurement techniques, and statistical analysis methods. Moreover, the dynamic nature of telomere length as a function of age, together with potential environmental interactions can contribute to differences in heritability estimates of telomere length.

Several genome-wide association studies (GWAS) studies have identified key loci that contribute to telomere length heterogeneity (Codd et al. 2010; Codd et al. 2013; Pooley et al. 2013; Li et al. 2020). One very large GWAS on telomere length in the human population using telomere length data from 472,174 participants of the UK Biobank, identified numerous genetic variants associated with telomere length variation at 138 genomic loci, shedding light on the molecular mechanisms underlying telomere biology and its implications for human health (Codd et al. 2021). GWAS have identified variants in genes directly involved in telomere maintenance pathways, such as those encoding components of the telomerase enzyme and the shelterin protein complex. For example, variants near the TERT and TERC

genes, encoding the catalytic and RNA subunits of telomerase, have been associated with telomere length variation (Codd et al. 2013). Similarly, variants near genes encoding components of the shelterin complex proteins, including *ACD*, *TERF1*, *TERF2* and *POT1* and all three components of the CST complex *CTC1*, *SNT1* and *TEN1*, have all been implicated in telomere length regulation (Codd et al. 2021). As have the *ATRX*, *PML* and *SLX4* genes, whose encoded proteins are implicated in the establishment of the alternative lengthening of telomeres pathway (ALT), and the *UPS7* gene, the protein of which deubiquitinates ACD and POT1. In addition to known telomere maintenance genes, this GWAS uncovered 108 genomic loci not previously associated with telomere length variation. These loci include genes *RPA1* and *RPA2* with roles in DNA replication, including telomeres, genes involved in DNA repair activity *SLX4*, *MCM4* and *SAMHD1* at telomeres, but also genes encoding the translesion polymerases POLI and POLN not previously implicated in telomere metabolism (Codd et al. 2021).

The establishment of telomere length is therefore influenced by multiple genetic and environmental factors. It is clear from these studies that telomere length determination is genetically complex with numerous quantitative trait loci contributing to inter-individual heterogeneity in telomere length at birth, that is modulated by prenatal conditions and parental age. Importantly however, despite this considerable heterogeneity, numerous Mendelian randomisation studies have demonstrated a causal relationship between heritable long or short telomeres and a wide range of diseases.

Telomere dynamics in human cells

As outlined above, telomeres display considerable length heterogeneity in the human population, with the inter-individual range in length representing nearly 50% of overall mean telomere length at birth. At the cellular level telomere length can be considerably more heterogeneous. The full extent of telomere length heterogeneity was revealed using Single Telomere Length Analysis (STELA), a process that allows the full spectrum of telomere lengths to be determined from single chromosome ends, at the single molecule level (Baird et al. 2003). For example, STELA of immune cell subsets revealed single telomeres in normal purified B-cells obtained from healthy adults ranging from 1.6 kb to 22 kb, a range of 20.4 kb with a mean 9.9 kb over 200% of mean telomere length (Lin et al. 2010). Similar ranges were observed with STELA in T-cell subsets (Ahmed et al. 2016; Ahmed et al. 2020; Roger et al. 2023) and other normal tissues including colorectal mucosa, gastric mucosa and squamous

oesophageal epithelium (Roger et al. 2013; Letsolo et al. 2017). At each chromosome end individuals inherit two telomeric alleles of unitary length, however the natural dynamics of telomeres with ongoing cell division means that these individual alleles become progressively more heterogeneous during life.

In the absence of telomerase activity, the end-replication problem, coupled with DNA processing to create 3′ overhangs, leads to the gradual erosion of telomeres with on-going cell division (Deng et al. 2008). Mathematical modelling of this process, with telomeres starting from a single telomere of unitary length, predicts a gradual decrease in the mean and increase in the variance of telomere length distributions with ongoing cell division (Levy et al. 1992). These models are consistent with the dynamics of telomeres observed in human cell cultures using terminal restriction fragment analysis of telomere length (Harley et al. 1990), but was most clearly observed using STELA where the analysis of single telomeric alleles in clonal populations of cells revealed a clear decrease in mean with a commensurate increase in variance of the distributions consistent with telomere erosion occurring primarily by end-replication losses (Baird et al. 2003). Interestingly however, additional rare outlying short telomeres were observed arising because of substantial telomere length changes. These events were detected in young proliferating cells, senescent cells and cells expressing telomerase, as well as normal and malignant tissues and thus appear to be a normal aspect of telomere dynamics (Baird 2008). These apparently stochastic telomeric deletion events were not consistent with end-replication losses and created telomeres shorter than those observed in senescent cells. They did not appear to accumulate in culture with cell division, indicating that these shortened telomeres may have been processed, for example, by being re-lengthened or by being subjected to DNA repair activity to create a fused telomere, alternatively the cells in which telomere deletion arose exited the cell cycle, or were subjected to apoptosis. The underlying mechanism for these specific telomeric deletion events has not been formally established, however they may relate to the phenomenon of telomere trimming, arising from the resolution of T-loops, in a process involving Regulator of Telomere Elongation Helicase 1 (Vannier et al. 2012).

In addition to end-replication losses, terminal processing and stochastic deletion events, telomere dynamics are further complicated by the action of telomerase. Telomerase mediated telomere elongation is dynamic and stochastic, such that different telomeres may receive distinct telomere elongation. During elongation, short telomeres are preferentially elongated

by telomerase, but in equilibrium conditions all telomeres are elongated equally adding approximately 60 nts at each telomere (Britt-Compton et al. 2009; Zhao et al. 2009; Zhao et al. 2011). Higher-order chromatin structures may modify accessibility of the chromosomal terminus to telomerase activity and different replication timings during S-phase may lead to differentials in telomeric elongation (Jady et al. 2006; Tomlinson et al. 2006; Chen et al. 2012; Redon et al. 2013).

Thus, replicative history, telomerase activity and additional telomeric mutation contribute to the considerable inter-cellular telomere length heterogeneity observed in normal human somatic cells.

Telomere dysfunction and fusion: the crisis paradigm

In human cells, with functional DNA damage checkpoint responses, telomere shortening ultimately leads to the loss of the end-protective function and the triggering of a partial DNA damage response referred to as replicative senescence, or mortality stage 1 (Wright and Shay 1992; d'Adda di Fagagna et al. 2003). In addition to a permanent cessation of cell division, cells undergoing replicative senescence typically exhibit characteristic morphological changes such as enlarged and flattened cell morphology, increased granularity, and changes to nuclear morphology (Sikora et al. 2016). Importantly senescent cells exhibit changes in their secretory phenotype, known as the senescence-associated secretory phenotype (SASP)(Coppe et al. 2008). The SASP is characterised by the secretion of pro-inflammatory cytokines, chemokines, growth factors, and proteases that contribute to chronic inflammation, tissue remodelling, and the recruitment of immune cells to sites of senescent cell accumulation (Huang et al. 2022). Whilst the SASP can have beneficial effects in certain contexts, such as promoting tissue repair and immune surveillance, chronic SASP activation can drive age-related pathologies, including cancer, cardiovascular disease, and neurodegenerative disorders (Wang et al. 2024a). Thus, telomere erosion limits replicative lifespan, and this provides a stringent tumour suppressive mechanism, however in long lived species, the accumulation of non-replicating senescent cells, together with the SASP, may facilitate tissue deterioration and disease as a function of age (Campisi and Robert 2014; Schmitt et al. 2022).

Short telomeres in senescent cells are detected as DSBs leading to the activation of the key regulators of the DDR pathway ataxia telangiectasia mutated (ATM) and ATM and Rad3-

related (ATR) kinases (d'Adda di Fagagna et al. 2003; Nassour et al. 2021). ATM and ATR phosphorylate downstream effector proteins involved in cell cycle checkpoints including TP53, which induces the expression of P21, a cyclin-dependent kinase inhibitor that inhibits the activity of cyclin-dependent kinases involved in cell cycle progression (Shiloh and Ziv 2013). This leads to cell cycle arrest at the G1/S checkpoint, preventing the proliferation of damaged cells. Whilst senescent cells are subjected to a cell cycle arrest, the telomeres are not repaired, and thus, despite the presence of short telomeres, telomere fusion in senescence cells is no more common than that observed in young proliferating cells (Counter et al. 1992; Capper et al. 2007; Cesare et al. 2009; Kaul et al. 2012). The underling mechanism for the lack of repair of short telomeres in senescent cells is not clear, however it is likely that these telomeres may retain sufficient TRF2 to inhibit DNA repair activity and fusions (Cesare and Karlseder 2012). Moreover, extensive chromatin remodelling occurs in senescent cells that leads to the formation of senescence-associated heterochromatin foci (SAHF)(Narita et al. 2003). These densely packed heterochromatic regions contribute to the stable repression of cell cycle genes and reinforce the irreversible growth arrest characteristic of senescence. SAHF formation can also lead to repression of genes involved in the DNA damage response and may further prevent access of repair proteins to telomeres (Di Micco et al. 2011).

In the absence of functional TP53, the expression of P21 and other senescence-associated genes is reduced, and the presence of short telomeres does not illicit cell cycle arrest. In this situation cells continue to divide beyond their normal replicative capacity and telomeres continue to shorten (Counter et al. 1992). The extended lifespan of these cultures varies between cell types, but typically human fibroblast cultures exhibit an additional 20-40 population doublings (PD) before entering a state referred to as replicative crisis, or mortality stage 2 (Wright and Shay 1992). During crisis the expansion of the culture slows and stops, over time cell death becomes greater than cell division and the culture comes to an end. Crisis is characterised by the presence of telomere fusion events, widespread genomic instability and autophagy-dependent cell death mediated via cGAS (cyclic GMP-AMP synthase)-STING (stimulator of interferon genes) (Counter et al. 1992; Capper et al. 2007; Nassour et al. 2019). Abrogation of the cGAS-STING pathway allows cells to continue to divide beyond M2, to a third cell growth plateau, M3 (Nassour et al. 2021). M2 and M3 crisis represent the final proliferative lifespan barriers of human cells. Cells can only permanently escape crisis by the activation of a telomere maintenance mechanism to prevent telomere erosion, either via the establishment of telomerase activity, or following the induction of the alternative

lengthening of telomeres pathway. The mechanism underlying the induction of crisis and escape is reviewed elsewhere in this volume.

The mutational impact of telomere dysfunction and fusion.

Telomere crisis represents the final proliferative lifespan barrier that cells must overcome to progress to malignancy. The widespread genome instability during crisis provides the genetic variation on which clonal selection can operate to drive clonal evolution. Thus, crisis is considered to be a critical event in tumorigenesis and is associated with the acquisition of genomic alterations that promote cancer development (Artandi and DePinho 2010).

Sensitive single-molecule PCR techniques allow for the detection and sequence characterisation of single telomere fusion events in a background of 10^5-10^6 cells. Telomere fusion events are rare in normal cells, occurring at similar frequencies irrespective of whether they are young and capable of proliferation or whether they are undergoing replicative senescence (Capper et al. 2007). The DNA sequence of rare telomere fusion events detected in normal cells revealed short telomeres at the fusion point that were considerably shorter than those observed in the bulk distribution, consistent with fusion between stochastic telomeric deletion events in normal cells (Capper et al. 2007). Thus, normal cells with otherwise intact and functional telomeres can be subjected to sporadic telomeric deletion, and these telomeres can undergo telomere fusion which can lead to the induction of large-scale chromosomal mutation.

Following the experimental abrogation of the TP53 pathway, cells continue to divide, and telomeres continue to erode to a point at which they are subjected to DNA repair activity that results in telomere fusion. Telomere fusions can be detected within 5 population doublings (PDs) from the PD point at which the culture would have undergone replicative senescence (Letsolo et al. 2010; Tankimanova et al. 2012). The frequency of telomere fusion, and the diversity of events generated, progressively increases as the telomeres continue to shorten and the cells enter replicative crisis (Capper et al. 2007; Tankimanova et al. 2012; Jones et al. 2014). Interestingly, as cells progress deeper into crisis the diversity of telomere fusion events decreases and becomes dominated by a smaller number of clonal events (Capper et al. 2007; Letsolo et al. 2010). This is presumed to reflect the replicative dynamics of cells in culture, with the clonal fusion events being detected in the longest-lived clones. Importantly

this observation demonstrates that telomere fusion is not necessarily catastrophic for a cell undergoing crisis, instead it can provide a temporary solution to the loss of telomere function and allows for additional replicative cycles.

Dysfunctional telomeres are susceptible to fusion through multiple DNA repair mechanisms. These repair pathways can contribute to genomic instability and chromosomal rearrangements. In the absence of the key shelterin component TRF2, chromosomes are subject to widespread fusions. This dramatic phenotype leads to the formation of long 'trains' of chromosomes joined end to end at the telomeres. The formation of these structures is entirely dependent on LIG4, a key component of the classical Non-Homologous End Joining (NHEJ) pathway (Smogorzewska et al. 2002). These observations inform the mechanism by which TRF2, and the shelterin complex, confers a key function of telomeres, that of distinguishing the natural end of the chromosome from non-telomeric DSBs and the prevention of aberrant DNA repair activity at the chromosomal termini. Telomeres rendered dysfunctional by the experimental loss of TRF2 still contain full lengthened telomere repeat arrays at the fusion points (Smogorzewska et al. 2002; Capper et al. 2007) and thus may not fully recapitulate the nature of naturally occurring dysfunctional telomeres. Indeed, it became apparent that telomeres rendered dysfunctional as a consequence of replicative erosion can be processed differently. The evidence for this came from observations in *S.pombe*, *Arabidopsis* and mice, where fusion of short telomeres was observed in the absence of key components of the classical NHEJ pathway including KU, DNA PKCS and LIG4 (Baumann and Cech 2000; Heacock et al. 2004; Maser et al. 2007; Rai et al. 2010). Moreover, DNA sequence analysis of telomere fusion events obtained from human cells undergoing crisis revealed a distinct mutational profile that was not consistent with classical NHEJ. Instead fused telomeres were characterised by sub-telomeric deletion of one, or both, of the participating telomeres and the presence of DNA sequence microhomology at the fusion point (Capper et al. 2007). This profile is consistent with the microhomology mediated end-joining pathway (MMEJ) revealed experimentally in the absence of classical-NHEJ. MMEJ requires microhomology and is error prone, resulting in extensive DNA resection activity that creates deletions (Boulton and Jackson 1996; Gottlich et al. 1998; Ma et al. 2003; Yu and Gabriel 2003). MMEJ has been defined in physiological roles for mediating class switch recombination (Pan-Hammarstrom et al. 2005; Yan et al. 2007; Robert et al. 2009; Boboila et al. 2010a; Boboila et al. 2010b) and may be required for the processing of DSBs within repetitive DNA elements (Sfeir and Symington 2015).

Further work to establish the genetic requirements for the fusion of short dysfunctional telomeres revealed that LIG4 dependent classical-NHEJ predominantly mediates inter-chromosomal fusion and MMEJ predominates with intra-chromosomal telomere fusion (Jones et al. 2014; Liddiard et al. 2016). Furthermore LIG1, the replicative ligase, was shown to have an essential and non-redundant role in mediating the fusion of sister chromatid telomeres, that was decoupled from its engagement in DNA replication (Jones et al. 2014; Liddiard et al. 2019). DNA polymerase theta (POLQ) has emerged as a critical determinant of MMEJ and this was first established in the context of telomere fusion in mouse embryonic fibroblasts following the depletion of both TRF1 and TRF2. Whole genome sequence analysis revealed non-telomeric insertions within the telomere-telomere fusion breakpoints that were dependent on POLQ (Mateos-Gomez et al. 2015). It is now apparent that POLQ is the key mediator of MMEJ, or what is now referred to as POLQ -mediated end joining (TMEJ), and this is the primary mechanism for end-joining in M-phase (Ramsden et al. 2022; Brambati et al. 2023). Consistent with the roles of classical and MMEJ in mediating inter- and intra-chromosomal telomere fusion, POLQ appears to predominantly mediate intra-chromosomal telomere fusions including events involving centromeric satellite repeats (Liddiard et al. 2022).

Telomere dysfunction and fusion is a key mutational mechanism that can generate large-scale genomic rearrangements. The earliest observations of telomere dysfunction leading to genomic mutation were described by Barbara McClintock in the 1930's who observed cycles of chromosomal breakage, fusion and anaphase bridging (BFB) initiated following the loss of telomeres (McClintock 1941). In these cycles, dicentric chromosomes that arise from telomere fusion fail to properly segregate and instead form bridges between the dividing cells at anaphase. The bridges can break with the resulting daughter cells acquiring an unequal distribution of genetic material that will depend on whether the fusion is inter- or intra-chromosomal (Murnane 2006). Intra-chromosomal fusion will lead to a terminal deletion in one daughter cell and inverted repeat in the other. As the broken chromosome ends lack telomeres they can subsequently fuse with other broken ends; in the case of the daughter cell containing an inverted repeat, subsequent BFB cycles can lead to further amplification, and additional deletion in the cell that acquired the initial deletion (Murnane 2012). BFB cycles can only be stopped following the 'healing' of chromosomes with the acquisition of a new

telomere, either via the addition of telomere repeat sequences *de novo* at broken ends, or by translocation with a pre-existing telomere (Sabatier et al. 2005).

The BFB paradigm has provided a framework understanding of how short dysfunctional telomeres can drive genomic mutation in cancer. However, the advent of genomic sequencing technologies has revealed the astonishing complexity of structural mutation in cancers and subsequent data has implicated telomere dysfunction in the initiation of some of these types of events (Maciejowski et al. 2015; Cleal et al. 2019; Dewhurst et al. 2021). One of the first descriptions of these complex mutational phenomena was provided in 2011 following whole genome sequencing (WGS) of chronic lymphocytic leukaemia (CLL) B-cells from a single patient. This analysis revealed forty-two rearrangements involving chromosome 4 and nine translocations involving three other chromosomes (Stephens et al. 2011). The breakpoints were clustered with an alternating copy number profile arising because of deletions between the breakpoints. The authors coined the term of chromothripsis to describe this mutational pattern, from the Greek '*thripsis*' to shatter, it was considered that the chromosome had 'shattered' and had been re-ligated in an apparently random order with missing sections of DNA. Subsequently, a large body of literature has described chromothriptic like mutational patterns in the majority of cancer types, including glioblastoma, melanoma and lung adenocarcinoma that exhibit chromothripsis in over 50% of cases and up to 100% of liposarcoma cases (Cortes-Ciriano et al. 2020). Coincidently chromothripsis is rare (≈ 1%) in CLL cases, the tumour type in which it was first discovered. Moreover chromothripsis in not confined to cancer, as it has also been observed in some congenital defects (Kloosterman et al. 2011; Gamba et al. 2015). Other mutational processes have been characterised including chromoanasynthesis and chromoplexy (Liu et al. 2011; Baca et al. 2013). These mutational phenomena are referred to under the collective term of chromoanagenesis and may represent a continuum of large-scale mutational events, with distinct, but potentially overlapping, underlying mechanisms (Holland and Cleveland 2012). Chromothripsis is the most common and intensively studied, however the underlying mechanistic basis of chromothripsis and how it can be initiated in the context of a replicative telomere crisis is yet to be fully established and it is possible that multiple mechanisms may have similar mutational outcomes (Liu et al. 2011; Maciejowski et al. 2015; Cleal et al. 2019; Cleal and Baird 2020; Umbreit et al. 2020; Dewhurst et al. 2021).

Telomere dynamics and disease associations

Telomere length heterogeneity in the human population, together with the decline in length as a function of age, has led to considerable interest in the potential association of telomere length and disease. The role that telomere biology plays in human disease is most obviously exemplified in the telomere biology disorders (TBDs). These are a group of rare genetic conditions characterised by abnormalities in telomere length maintenance and function. These disorders result from mutations in genes involved in telomere maintenance pathways, such as telomerase and shelterin complex components. TBDs can affect multiple organ systems and manifest with a wide range of clinical features. Dyskeratosis Congenita (DC) is a TBD characterised by the triad of abnormal skin pigmentation, nail dystrophy, and leukoplakia in the mucous membranes. Individuals with DC are at increased risk of bone marrow failure, pulmonary fibrosis, liver disease, and certain cancers (Alter et al. 2009; Schratz and Armanios 2020). Aplastic Anaemia in which bone marrow failure results in low blood cell counts can also be caused by a TBD (Vulliamy et al. 2002). Idiopathic Pulmonary Fibrosis (IPF) is a progressive lung disease characterised by the formation of fibrotic scar tissue in the lungs, leading to impaired lung function and respiratory symptoms. While most cases of IPF are sporadic, a subset of individuals with familial IPF have mutations in genes associated with telomere maintenance, such as TERT, TERC, and other shelterin complex components (Alder et al. 2008). Whilst TBDs are caused by defined genetic variants, they can also display disease anticipation whereby the severity of the disease increases, and age of onset decreases, between generations (Vulliamy et al. 2004). This can be accounted for by the inheritance of ever shorter telomeres between the generations; in these situations, pedigrees may be observed where grandparental carriers are unaffected but exhibit telomere lengths less than the 50^{th} percentile, a parental carrier with telomeres around 1^{st} – 10^{th} percentile manifests symptoms in adulthood and their offspring with telomeres shorter than the 1^{st} percentile exhibiting severe symptoms in early childhood. A detailed description of TBDs and their underlying biology is provided in more detail elsewhere in this volume.

TBDs provide direct evidence for a role of telomere length in disease but represent the extreme manifestations of telomere dysfunction. As telomere length is a continuous variable in the population the presence of long and short telomeres and their association with disease has been extensively studied, of particular interest is the relationship between telomere length and cancer. Casual relationships between telomere length and health outcomes have been investigated with Mendelian randomisation approaches that utilise variants identified with GWAS that associate with telomere length. These studies have revealed decreased risk of

cardiovascular disease, Alzheimer disease, interstitial lung disease, immunodeficiency and celiac disease, in individuals with genetic determined long telomeres (Haycock et al. 2017; Deng et al. 2022; Wang et al. 2024b) but increased risk of several cancers including glioma, kidney, serous low-malignancy-potential ovarian, bladder, neuroblastoma, melanoma and lung (Walsh et al. 2015; Zhang et al. 2015; Haycock et al. 2017). The association between longer telomere lengths and cancer risk is not absolute as some studies have indicated an increased risk of pancreatic cancer in individuals with short telomeres (Campa et al. 2019) although this is controversial (Antwi et al. 2017). Interestingly, whilst short telomeres confer a decreased risk of cancer, they can also reduce survival from cancer (Weischer et al. 2013).

Telomere length dynamics and dysfunction in cancer

Inherited short telomeres in normal somatic tissues confer a reduced risk of cancer. However, the original observations that telomere lengths in cancers tend to be shorter than that of patient matched normal tissues, implicated telomere dynamics and dysfunction in the progression to malignancy (de Lange et al. 1990; Hastie et al. 1990). It had been assumed that, in the context of limited telomerase activity, the telomere length differentials between normal and tumour tissue arise as a consequence of extensive cell division and replicative telomere erosion from the original cell to the clonal malignant tumour analysed. However, analysis of early-stage lesions, including colorectal adenomatous polyps, reveals short telomeres and fusions occurring prior to disease progression (Roger et al. 2013). In the pre-malignant condition Barrett's Oesophagus, clonal patches of extreme telomere erosion have been observed (Maley et al. 2006; Letsolo et al. 2017). Telomere shortening was detected in colonocytes from patients with ulcerative colitis, a condition that increases the risk of colorectal cancer (Risques et al. 2008), and in early-stage cervical intraepithelial neoplasia (Maida et al. 2006). Telomere shortening was also detected in myelodysplasia that predisposes to acute myeloid leukaemia (Ohyashiki et al. 1999; Williams et al. 2017). CLL B-cell clones can exhibit extreme telomere erosion and fusion, consistent with these cells undergoing a telomere-driven crisis (Lin et al. 2010) and this exacerbated in the context of mutation in the Ataxia Telangiectasia Mutated (ATM) gene (Britt-Compton et al. 2012). Telomere erosion and dysfunction is a feature of late-stage CLL but was also detected in a subset of early-stage (Binet stage A) patients prior to clinical progression, where telomere fusion activity was observed together with large-scale genomic rearrangements that included telomeric regions (Lin et al. 2010). Thus, short dysfunctional telomeres capable of fusion are

detected in early-stage cancer prior to clinical progression and significant telomere shortening has been observed in pre-malignant lesions.

Detailed analysis of the telomere length spectrums in patient-matched normal tissues reveals that the telomere length distributions of normal cells overlap with those observed in cancers (Lin et al. 2010; Roger et al. 2013). In the context of colorectal cancer, these data imply that telomere erosion not only precedes the adenoma/carcinoma transition, but is consistent with being pre-existent in normal cells in which the initiating mutations occur (Roger et al. 2013). In this situation, normal cells with long telomeres give rise to adenomatous polyps that retain their long telomeres and stable genomes, whereas those with short telomeres produce adenomas with increased incidence of telomere fusion and chromosomal instability, and these clones may in turn have a greater probability of transition to carcinoma. Similarly in CLL, the telomere length distributions observed in normal B-cells overlapped with those observed in CLL B-cell clones from early and late stage disease (Lin et al. 2010). Thus, like colorectal adenomas, the telomere-length distributions of early-stage CLL indicated an earlier origin for telomere erosion potentially within normal B-cells. This situation was also observed when comparing patient matched telomere length distributions in multiple myeloma and Barrett's Oesophagus (Hyatt et al. 2017; Letsolo et al. 2017).

Taken together these observations point to an early origin for telomere erosion, with short telomeres being pre-existent in sub-sets of normal cells, and short dysfunctional telomeres being present within pre-malignant and early-stage lesions.

The correlation between telomere length and genomic complexity implicates telomere dysfunction as a potential driver of tumour progression; a premiss that is further strengthened by associations between short telomere length and a poorer clinical outcome in several tumour types including prostate, myelodysplasia, chronic myeloid leukaemia, CLL, breast and colorectal (Bechter et al. 1998; Donaldson et al. 1999; Ohyashiki et al. 1999; Brummendorf et al. 2000; Gertler et al. 2004; Fordyce et al. 2006). The use of telomere length was further refined in CLL by using high-resolution telomere length analysis, coupled with telomere fusion analysis, to functionally define the length below which telomere dysfunction was detected (Lin et al. 2014). Patients with CLL B-cells with telomeres shorter than the telomere fusion threshold had significantly reduced overall survival that was even more prognostic in early-stage disease patients prior to clinical progression. Importantly also,

the same telomere length threshold was predictive of outcome of the standard treatment for CLL, fludarabine, cyclophosphamide, rituximab (FCR)(Strefford et al. 2015; Norris et al. 2019), surpassing all other CLL disease markers currently employed. Combination of telomere length with IGHV mutation status and CD49d allowed the identification of long-term progression free CLL patients treated with FCR (Pepper et al. 2022). These data imply that short telomeres and dysfunction occur early in CLL, thus facilitating clonal evolution and clinical progression, and may provide a clinically useful prognostic and predictive marker.

The nature of the disease and ease of sampling means some of the most detailed analysis of telomere dynamics has been undertaken in CLL, however the telomere paradigm is not specific to CLL. Indeed, the application of high-resolution telomere length analysis together with the telomere fusion threshold defined in CLL, provided prognostic information for overall survival Myelodysplasia (Williams et al. 2017), Multiple Myeloma (Hyatt et al. 2017) and breast cancer (Simpson et al. 2015). Interestingly, whilst these parameters define prognosis in Myelodysplasia, a precursor lesion for acute myeloid leukaemia (AML), no significant prognostic signature was identified in AML (Williams et al. 2017). Moreover, whilst short telomeres and dysfunction were observed in colorectal adenomatous polyps, there was no prognostic signature for telomere length in colorectal carcinoma (Roger et al. 2013). In these situations, it is possible that progression is accompanied by an upregulation of telomerase activity that effectively homogenises telomere length differentials, that were apparent in earlier disease stages, thereby reducing the prognostic signature in these malignancies.

Taken together, the clinical data support the thesis that telomere erosion and dysfunction occur early in the progression to malignancy in several tumour types and may indeed be present in the initiating cell. Importantly telomere-based markers may provide clinically useful tools in diverse cancers to inform the identification of those patients that require, and will benefit from, treatment. These data provide important evidence that the progression to malignancy is accompanied by the period of telomere dysfunction, consistent with a telomere crisis. Telomere crisis represents a situation of cellular vulnerability and as such may provide an opportunity for therapeutic intervention at the earliest stages of tumorigenesis. As the biological underpinnings of telomere crisis are dissected, new or existing agents may be utilised to target tumours and early-stage lesions exhibiting telomere dysfunction.

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