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Telomere dysfunction, genome instability and cancer

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1. ABSTRACT

Telomeres are highly specialized structures at the ends of chromosomes that are made up of tandem 5'-TTAGGG-3' repeats and a number of telomere associated proteins. By forming loop structures, the very end of a telomere is concealed and distinguished from a DNA break, thus protecting chromosomes from end-to-end fusions, misrepair and degradation. Telomere length is maintained by an enzyme called telomerase which is very weak or undetectable in most normal human somatic cells. In telomerase-negative cells, telomeric DNA is progressively lost with cell divisions until the cells undergo replicative senescence, which serves as an intrinsic mechanism to prevent normal somatic cells from replicating indefinitely. In checkpoint defective cells, telomere dysfunction resulting from excessive telomere attrition or disruption of telomere structure may initiate chromosomal instability through end-to-end fusion of unprotected chromosomes. Through propagation of breakage-fusion-bridge (BFB) cycles, genetic aberrations characteristic of cancers, including aneuploidy, loss of heterozygosity, gene amplification and gene loss can be generated. *In vitro*, cells with extensive chromosomal instability succumb to crisis which is characterized by wide-spread cell death. It has been reported that cells surviving crisis either have activated telomerase, or use an alternative telomere lengthening (ALT) mechanism to stabilize the existing telomeres and alleviate chromosome instability. The immortalized post-crisis cells have the potential to acquire additional genetic alterations for malignant transformation. In this review, we summarize our knowledge on the association between telomere dysfunction, genomic instability and cancer development.

2. INTRODUCTION

Cancer has been described as a disease of genomic instability (1). As reviewed by Lengauer *et al.* (2), there are two distinct levels of genomic instability: microsatellite instability (MIN) involving subtle base substitutions, deletions or insertions of a few nucleotides, and chromosomal instability (CIN) which involves losses and gains of whole or large portions of chromosomes. The latter is present in most cancers. Manifested as increased rate of acquiring new structural and numerical chromosomal aberrations during cell proliferation, chromosome instability imparts oncogenic potential to cells through creation of fusion genes or deregulated oncogenes at chromosome breakpoints, or loss or gain of chromosome elements which alters gene dosage. Evidence is emerging to show that multiple chromosome aberrations already exist at the pre-malignant stage (3-5) and largely persist with the progression to invasive stage, although new aberrations are also acquired. Defects in chromosome segregation, cell cycle checkpoints, DNA damage response and telomere function can cause chromosomal instability (6-8).

Telomeres are highly specialized structures at the ends of chromosomes which function to stabilize and protect the ends of linear chromosomes. In this review, we begin with a synopsis of the structure, maintenance and function of human telomeres, and the effects of telomere shortening or dysfunction on human somatic cells. As cellular immortalization is an early and indispensable step towards cancer, the role of telomere dysfunction in initiating and promoting chromosomal instability will be discussed mainly in the context of cellular immortalization to understand the early events of carcinogenesis. The role

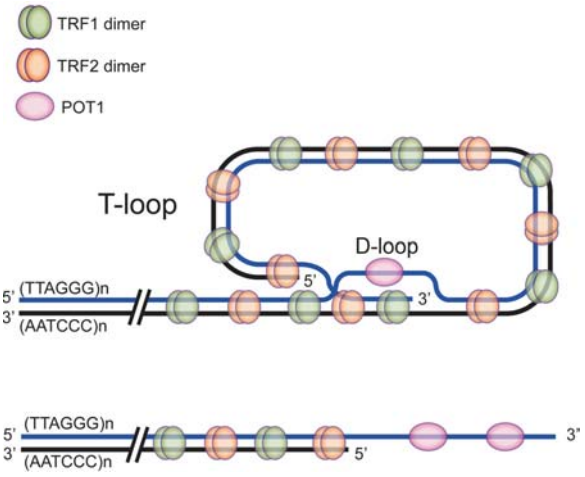

Figure 1. Structure of human telomere.

of telomerase deficiency-mediated chromosomal instability in carcinogenesis in mouse models will be briefly reviewed. We will also discuss the significance of telomere length profile of individuals in shaping the karyotype outcomes of immortalized cells, and speculate on how telomere length heterogeneity may contribute to the generation of non-random chromosome aberrations found in many types of carcinomas.

3. WHAT ARE TELOMERES?

About seventy years ago, two geneticists, Herman J Muller and Barbara McClintock, working with *Drosophilia melanogaster* and *Zea mays*, respectively, independently proposed that the natural ends of chromosomes have special features that distinguish them from broken chromosome ends, and thereby protect them from chromosome end-to-end fusions (9,10). Extensive research in the last two decades revealed that each linear chromosome end has a telomere structure consisting of repetitive DNA sequences and associated proteins that are essential for chromosome stability (11-14).

Amongst eukaryotes, telomere lengths and sequences vary among species. Human telomeres are typically 10-15 kb long in the germ cells, and the average telomere lengths in normal somatic cells are substantially shorter (15,16). Telomere lengths differ between individual human chromosomes, and even between chromosome arms of the same chromosome (17-19). The human telomere DNA sequence consists of tandem 5'-TTAGGG-3' repeats with a single-stranded G-rich 3' overhang of about 50-210 bases (20). In mammalian telomeres, the single-stranded 3'-end overhang invades the duplex telomeric DNA repeats to form a large duplex telomere loop (T-loop) and a smaller single-stranded displacement loop (D-loop) *in vitro* and *in vivo* (21,22) (Figure 1). This configuration, together with a number of telomere associated proteins, creates a telomere cap which protects the chromosome end and distinguishes it from a double-strand break (DSB). The presence of the G-rich single-stranded 3' overhang readily creates other secondary DNA conformations *in vitro*, such as intramolecular G-quadruplexes (23) which may occur in the T-loop (24). Recent reports suggest that such conformations also exist *in vivo* (25,26).

To date, three proteins are known to bind directly to the human telomeric DNA sequence. These telomeric DNA-binding proteins are TRF1 (27) and TRF2 (28) (telomeric repeat binding factors 1 and 2), each in the form of homodimers binding to the double-stranded region (29); and POT1 (protection of telomeres 1) binding to the single-stranded 3' overhang and the single-stranded displaced DNA at the base of the T-loop (i.e. D-loop) (30) (Figure 1). Both TRF2 and POT1 are involved in maintaining the integrity of the T-loop (21,31). In addition, there is an increasing list of other proteins including DNA repair factors that interact with and form complexes with TRF1, TRF2 or POT1. Some of these proteins are known to modulate telomere function [reviewed in (32-34)]. The term shelterin was coined to describe a complex consisting of six proteins (TRF1, TRF2, POT1, hRap1, TIN2 and TPP1) that mediates telomere end-capping (14,35).

Although telomere end-capping is essential for chromosome integrity, it has been proposed that some degree of transient uncapping is also required for telomere length regulation (11). This is because the transient uncapping state allows access of telomerase (an enzyme that adds telomeres onto the ends of linear chromosomes; see Section 5) or telomere recombination to regenerate telomeres to compensate for telomere loss (see the following section). The higher-order telomeric DNA-protein complex facilitates a dynamic switch between the capped and uncapped states. Yet the two physical states have to be well regulated. It has long been recognized that normal mammalian telomeric regions are heterochromatic, implying that they are tightly packed. This feature is thought to limit telomere elongation by hindering the access of telomerase and other telomere elongation activities, thus favoring negative regulation of telomere lengths. In line with this, losing heterochromatic features at telomere chromatin leads to unprecedented abnormal elongation of telomeres in a mouse model (36,37).

4. TELOMERE SHORTENING OCCURS NATURALLY IN HUMAN SOMATIC CELLS

Extensive studies have shown that telomeres in normal human somatic cells shorten with cell divisions *in vitro* as well as *in vivo*. In cultured human fibroblasts, this progresses at a rate of 50-200 bp per population doubling (16,38,39). *In vivo* studies have also shown that the average telomere lengths in normal somatic cells shorten at an estimated rate of 15-40 bp per year (15,40,41), and that telomere erosion declines with age (41).

One of major mechanisms leading to telomere shortening is associated with the end-replication problem intrinsic to linear chromosomes (i.e. the inability of the DNA polymerase to replicate a linear DNA to its very end) (42). During DNA replication, both DNA strands serve as templates for the synthesis of two complementary strands

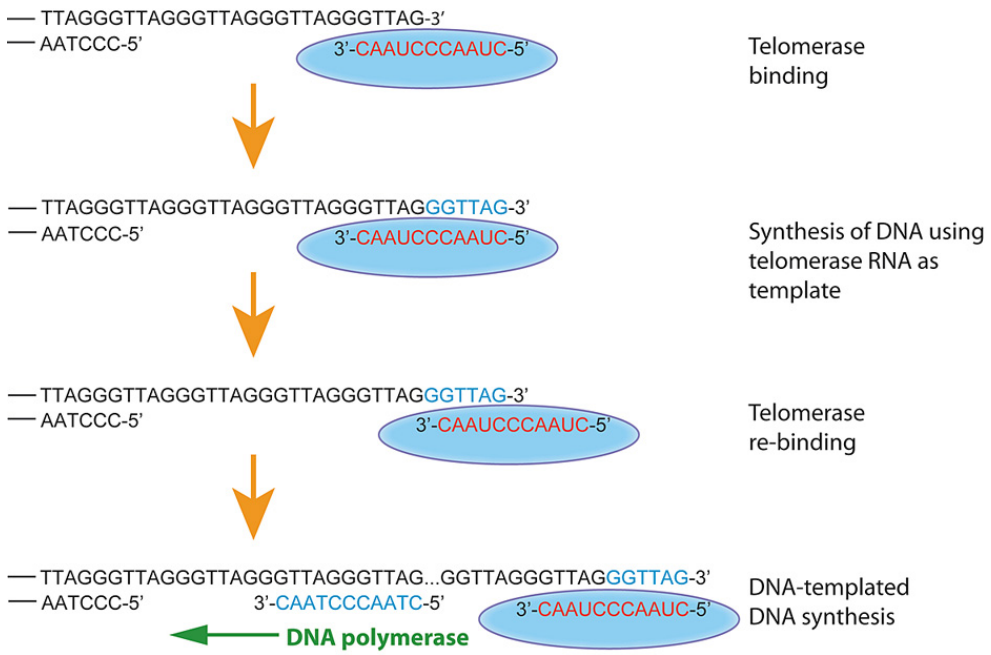

Figure 2. Telomerase adds telomeres by using its internal RNA template.

of DNA. Since DNA polymerase catalyzes DNA synthesis in a 5' to 3' direction, DNA is synthesized continuously along the 3'-5' template strand. On the 5'-3' template strand, however, DNA replication proceeds in segments according to the RNA primers that are attached to the template at different points. In eukaryotes, these RNA primers have a length of about 10 nucleotides and are made at intervals of 100-200 nucleotides on the lagging strands. Subsequent removal of the RNA primers leaves "gaps" between fragments, called Okazaki fragments, but the gaps are filled by the action of DNA ligase which joins the 5' end of the previous fragments to the 3' end of the new ones to make a continuous DNA strand. However, a special problem is encountered at the ends of a linear chromosome: either there is no place to produce the RNA primer at the end of a linear DNA molecule, or the DNA sequence at the terminal Okazaki fragment cannot be filled after the terminal RNA primer is removed because DNA ligase needs two ends to act upon. Therefore, the DNA at the terminal end of a linear DNA cannot be fully replicated. As a result, telomeres shorten progressively with cell divisions. To date, numerous experiments have demonstrated that telomeric DNA is indeed progressively lost with cell divisions in most human somatic cells that lack telomerase activity.

In addition, recent studies also suggest that oxidative stress contributes to telomere shortening (43-47). Ambient oxygen (21%) in ordinary cell culture conditions, for example, causes more severe oxidative stress to cells than that under physiological condition (2-5% oxygen) (44,48). It has been demonstrated that oxidative stress efficiently induces DNA damage at the 5' sites of 5'-GGG-3' in the telomere sequence, indicating that oxidative stress can specifically cause cleavage at polyguanosine sequences in the telomere sequence (49,50). Under conditions of additional oxidative stress induced by hydrogen peroxide treatment, human cells show preferential accumulation of single-strand breaks within the telomeres (51,52). It has been proposed that a low efficiency in telomeric single-strand break repair may further exacerbate telomere shortening. During DNA replication, damaged sites stop polymerases transiently, which may lead to premature termination of telomere replication (52). Therefore, it seems that multiple mechanisms contribute to telomere shortening. The effects of oxidative stress on telomere erosion has important significance in the study of telomere dynamics in aging and cancer since numerous oxidants are produced *in vivo* due to normal metabolism and extracellular stresses, and oxidative stress increases with the aging process *in vivo* (53).

5. TELOMERASE ADDS TELOMERES TO THE END OF LINEAR CHROMOSOMES

Telomerase, a ribonucleoprotein, was first identified and characterized in the ciliate *Tetrahymena* (54). It is now known that telomerase is a special enzyme that adds telomeres onto the existing telomeres and maintains the 3' overhang (55). Interestingly, telomerase contains an essential RNA component, a portion of which serves as a template for telomere DNA repeat sequence synthesis (54). The rule of the synthesis is Watson-Crick base-pairing: C's and A's in the RNA template specify G's and T's, respectively (Figure 2).

Telomerase activity in human cells was first reported in 1989 (56). Subsequently, the RNA component of telomerase, termed hTR, in human cells was cloned and sequenced (57). The template region of hTR encompasses 11 nucleotides (5'-CUAACCCUAAC-3') that are complementary to the human telomere sequence

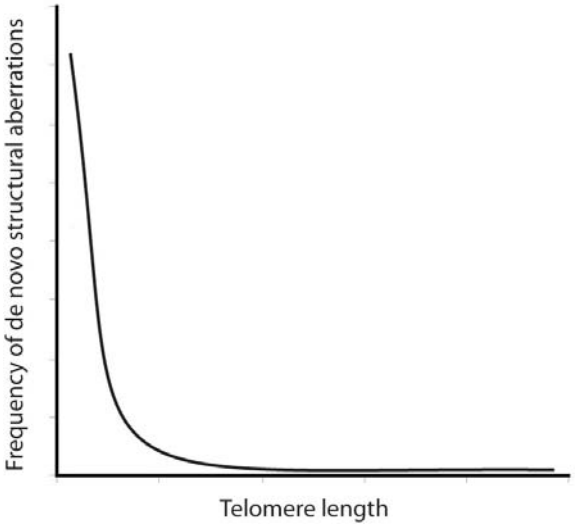

Figure 3. Non-linear relationship between telomere length and frequencies of chromosomal structural aberrations.

(TTAGGG)n. Telomerase recognizes the tip of the G-rich strand of an existing telomere DNA repeat sequence and uses its own RNA template to synthesize TTAGGG sequences. Shortly after the discovery of hTR, an important protein component of human telomerase, now termed human telomerase reverse transcriptase (hTERT), was discovered (58,59). The hTERT provides the catalytic action of telomere synthesis by telomerase. While there is abundant hTR expression in all human tissues regardless of telomerase activity, hTERT mRNA expression is only expressed in embryonic stem cells and germ cells, and is undetectable or detected at very low levels in most somatic cells. The hTERT mRNA expression correlates with telomerase activity in normal, immortal or cancer cells, indicating that hTERT is a key determinant of telomerase activity (60-62). In addition to hTERT, a number of proteins have been reported to be associated with human telomerase, but a recent study indicates that the active human telomerase enzyme is a complex of the RNA component hTR (153 kD), and two protein components, hTERT (127 kD) and dyskerin (57 kD) (63).

Although telomerase can add telomeres onto the ends of linear chromosomes, telomerase activity is very weak or undetectable in most normal human somatic cells. Therefore the intrinsic end replication problem cannot be solved and telomere shortening ensues.

6. TELOMERE DYSFUNCTION: CAUSES AND CONSEQUENCES

For many years, telomeric function was thought to depend on telomere length but the current concept is that it depends on telomere structure rather than the length *per se* (64,65). Telomere shortening can contribute to the collapse of telomere loop structures either in two ways. First, the double-stranded TTAGGG repeats may become too short to bind enough telomere binding proteins for T-loop formation. Second, the single-stranded 3' overhang may become too short to form a D-loop for the appropriate sealing of the overhang. In addition, deficiency of the telomere associated proteins (31,66) also compromise telomeric stability. Based on our observation of a non-linear relationship between telomere lengths and the probabilities of chromosome structural instability in telomerase-negative human cells undergoing immortalization (Figure 3) (67), we believe that as long as the telomeric T-loop and D-loop structures or other capping structures can be formed, a short telomere has the same protective efficiency as a longer one.

6.1. Short telomeres trigger DNA damage signals that lead to cell cycle arrest in normal human cells

Telomere shortening induces replicative senescence, which is a form of cellular senescence. Normal human somatic cells have a limited lifespan *in vitro*. This was first demonstrated in human fibroblasts by Hayflick and Moorhead in 1961 (68-70). Since then, it has been repeatedly proven that cultured normal human fibroblasts go through finite numbers of population doublings. Toward the end of a cell's lifespan, cell proliferation slows down and finally stops and the cell enters a state of irreversible growth arrest (the first mortality barrier or mortality stage 1). Importantly, the timing of growth arrest is determined by the number of population doublings the cells have undergone, not by the calendar time they have stayed in culture. In addition to fibroblasts, other somatic cells including epithelial cells, endothelial cells, lymphocytes, smooth muscle cells, and astrocytes all show replicative senescence (71-77), which serves as an intrinsic mechanism to prevent normal somatic cells from replicating indefinitely.

Senescent human cells have increased p53 activity (78) and nuclear foci where several DNA double-strand break repair and checkpoint factors, such as ATM, γ-H2AX, 53BP1, MDC1, NBS1, phospho-CHK1 and phospho-CHK2 are located. Specifically, these multiple DNA damage response factors are assembled at those exceptionally short telomeres in senescent cells (79-81), indicating that dysfunctional short telomeres trigger the response. The sustained DNA damage response, signaling through p53, can induce both G1 and G2 phase arrest (82,83). Numerous studies have also demonstrated that human fibroblasts or epithelial cells undergoing natural replicative senescence have elevated protein levels of hypo-phosphorylated Rb, $p16^{INK4a}$, as well as $p21^{CIP1}$, or decreased hyper-phosphorylated Rb compared with early and proliferating cells (78,84-88). Interestingly, $p16^{INK4a}$ rarely co-localizes with the biomarkers of DNA double-strand breaks mentioned above (82). Another study has also shown the induction of $p16^{INK4a}$ is a delayed response after p53 and $p21^{CIP1}$ induction in cells exposed to DNA damage agents (89). Telomere-mediated senescence is therefore induced by activation of the multiple DNA damage responses, which then leads to cell cycle arrest in normal human cells. However, the activation of DNA damage response factors is not due to the complete loss of telomere sequence at chromosome ends since DNA damage foci are found co-localized with detectable (though relatively weak) telomere signals (83).

Several lines of evidence have shown that replicative senescence also occurs *in vivo* and is thought to be associated with cellular aging. First, many investigations demonstrated an inverse correlation between donor age and the number of population doublings at which human cells senesce (48,90,91). Second, inter-species comparisons showed that cells from species with longer lifespan *in vivo* achieve more population doublings *in vitro* (92). Third, cells from humans with premature aging syndromes have shorter lifespan in culture than those from age-matched controls (93-95). Fourth, high proportions of CD8 (+) T cells with characteristics of replicative senescence accumulate during normal aging (96). These data suggest that there might be commonality in genetic basis that controls replicative lifespan of cells *in vitro* and lifespan *in vivo*. However, although telomere-associated senescence is supposedly an intrinsic barrier to cellular immortalization, which represents an early first step in the multi-stage process of cancer development, accumulation of senescent cells (especially senescent fibroblasts) during aging *in vivo* seems to create a microenvironment conductive to cancer (97,98). Telomere shortening or dysfunction, therefore, has a close association with cancer.

6.2. Telomere dysfunction triggers genomic instability in checkpoint defective cells

As early as 1941, Barbara McClintock reported that, in maize, broken chromosome ends tend to fuse with their sister chromatids or other broken chromosomes, leading to formation of anaphase bridges, and cycles of chromosome breaks and further fusions during subsequent cell divisions (10). Telomere dysfunction initiates chromosomal instability through such breakage-fusion-bridge (BFB) cycles. This relationship is best appreciated by tracking the changes in telomere lengths and chromosomal aberrations in cells undergoing cellular immortalization *in vitro*. As mentioned above, the p53 and pRb/$p16^{INK4a}$ pathways are vital for DNA damage responses, cell cycle regulation as well as apoptosis, which are indispensable for maintaining genomic stability. Most cancers have defects in either or both of these pathways (99-101). Inactivation of the p53 and $p16^{INK4a}$/Rb pathways can be accomplished *in vitro* by expression of viral oncogenes such as HPV (human papillomavirus) E6 and E7 (102,103), SV40 large-T (104) or adenovirus E1B and E1A (105,106) to simulate the sustained inactivation of p53 and Rb genes observed in cancers. Such defects extend the lifespan and allow the cells to override cell cycle checkpoints and continue to proliferate. However, as telomeres continue to shorten with further cell divisions after the cells bypass senescence, the chromosome ends are no longer protected and become unstable. At this stage, the cells eventually succumb to a second mortality barrier (mortality stage 2) termed crisis (107), which is characterized by wide-spread cell death and extensive chromosomal instability (108). A very low percentage of cells (10^{-9}–10^{-5}), however, may survive the crisis period by a mutation or an epigenetic event that activates a telomere maintenance mechanism. This may involve activation of telomerase, which "rejuvenates" the telomeres by synthesizing telomeric DNA and maintaining pre-existing telomeres, or an alternative telomere lengthening (ALT) mechanism (109-111) which involves copying of DNA sequences from telomere to telomere by means of homologous recombination (112). The resulting telomere preservation allows the cells to continue their long-term proliferation to become immortalized.

Critically short telomeres are hotspots for illegitimate recombination. Whereas very few chromosome end-to-end fusions are observed in near-senescent or senescent normal human cells (80,113), the frequencies of chromosome end-to-end fusions, forming dicentrics, reach a peak during crisis and decrease after crisis due to telomere preservation (108,114). Dicentrics are usually lethal. This is because the two centromeres are pulled in opposite directions during mitotic anaphase, forming a bridge between the daughter cells (115-117). The bridge can cause cell death independent of p53 and Rb genes partly because the bridge compromises the integrity of cellular and nuclear membranes (two intact cells cannot be formed). In addition, the breakage of the anaphase bridge due to the pulling force between the two daughter cells during cell division may be an important source of new chromosome aberrations.

It was hypothesized that the breakage of a dicentric chromosome could occur either in a chromatid or microtubule, resulting in the deletion of a chromosome-element or loss of a whole-chromosome, respectively, in one daughter cell and a corresponding gain in the other (118) (Figure 4). The broken ends trigger DNA recombination by rejoining to other broken or unprotected chromosome ends. This can produce additional aberrations, such as structurally stable translocations, and unstable dicentrics which can undergo another round of BFB. Since broken or unprotected chromatid ends can also fuse with their sister-chromatids after DNA synthesis, another hypothesis was proposed to explain how gene deletion and amplification may be generated through breakage of sister-chromatid fusions. As shown in Figure 5, a bridge is formed as the two centromeres on the end-fused sister chromatids go to different daughter cells during cell division. Repeated cycles of sister-chromatid fusion-bridge-breakage may therefore generate multiple copies of the same gene on the same chromosome in the same cell while causing the loss of the genes in its sister cells after several rounds of cell divisions.

In summary, regardless of how telomeres become disrupted, the key message is that when telomeres become dysfunctional or "uncapped" and can no longer protect the chromosome ends, they elicit a DNA damage response to activate the p53 and $p16^{INK4a}$/Rb pathways which prompt the cell to undergoing either irreversible cell cycle arrest (if both p53 and pRb checkpoints are intact) or p53-mediated apoptosis (if only the p53 checkpoint is intact) (79). In cells defective in both p53 and $p16^{INK4a}$/Rb pathways, excessive shortening of telomeres coupled with the freedom to continue proliferation promote aberrant fusions of unprotected chromosome ends and trigger massive chromosomal instability (8), which puts the cell at risk for malignant transformation (Figure 6). Thus, telomere shortening can be both a barrier and a facilitator for cancer

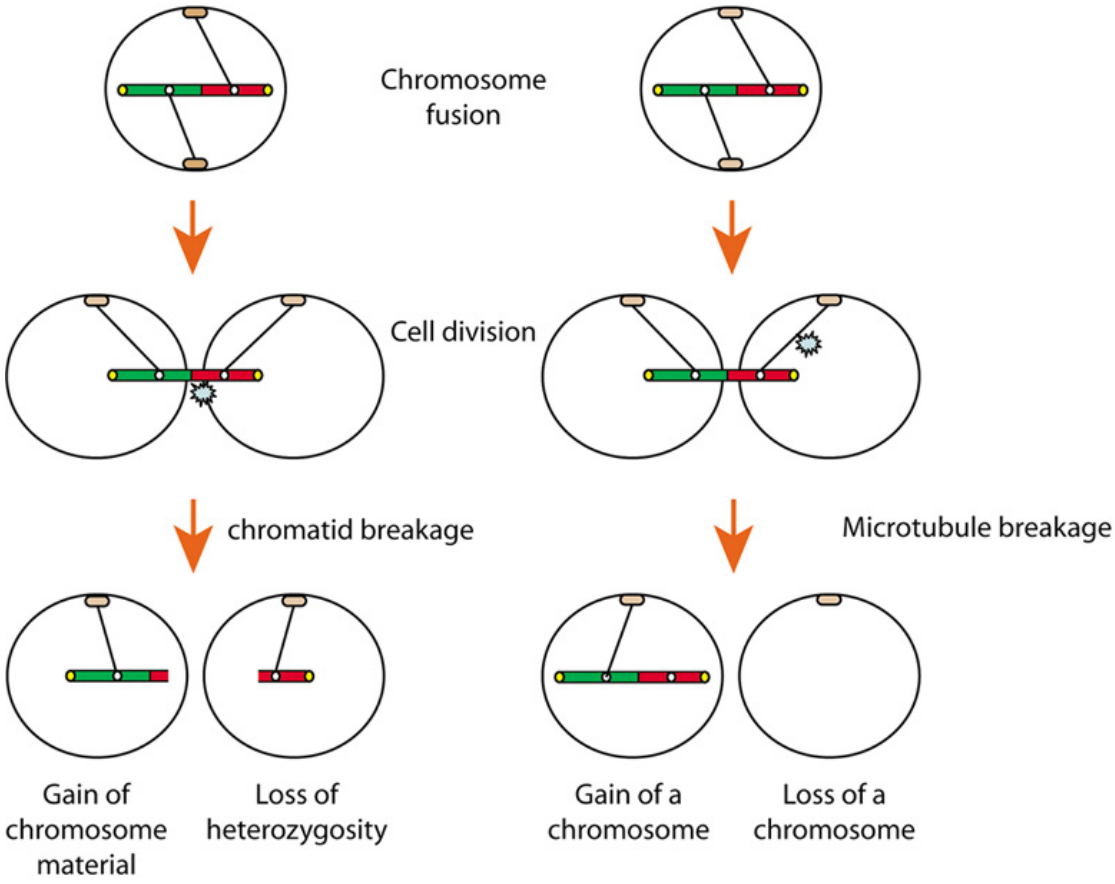

Figure 4. Proposed mechanism of chromosome imbalances through chromosome end-to-end fusion. Modified with permission from 27.

development depending on the integrity of checkpoint response.

7. ROLE OF TELOMERE-MEDIATED GENOMIC INSTABILITY IN CARCINOGENESIS

Carcinogenesis is regarded as a multi-step process in which a normal cell undergoes immortalization and then oncogenesis to become a fully transformed malignant cell. It is well documented that even after cells are immortalized, they are not yet oncogenic, and that additional genetic alterations are required for malignant transformation (119-123).

One of the core concepts in cancer research is that genomic instability helps drive development of human cancer (124). Rapid evolution of genomic alterations in genetically unstable cells makes them advantageous in natural selection by acquiring new features. Of the two categories of genomic instability, microsatellite instability exists only in a small subset of solid tumors, whereas chromosomal instability is present in most cancers. Chromosomal instability includes numerical instability reflected by alterations in chromosome numbers, and structural instability which is characterized by continuous generation of new structural chromosome aberrations. Figures 4 and 5 illustrate that the genetic aberrations characteristic of cancers, including aneuploidy, loss of heterozygosity (LOH), gene amplification and gene loss can be explained by the BFB events initiated by telomere dysfunction (115,118).

Regarding the role of chromosome aberrations in cancer development, it is well recognized that chromosome aberrations have oncogenic potential in at least two ways. First, chromosome translocations can result in the formation of fusion genes or dys-regulation of gene transcription at or near the translocation points as clearly demonstrated in most leukemia and many soft-tissue tumors, and some of these aberrations are disease-specific (125-127). Second, gains or losses of chromosome elements or whole chromosomes can lead to large scale genomic imbalances or alterations in gene dosage in virtually all types of human cancer (128). Molecular cytogenetic studies have shown that multiple chromosome abnormalities can be observed in carcinoma-*in-situ* stage. These abnormalities persist along with continued acquisition of additional abnormalities with tumor progression towards late stage malignancies (3,129-131). It is understood that chromosomal instability is an important mechanism leading to genomic rearrangement and imbalances that provides a platform for continuous

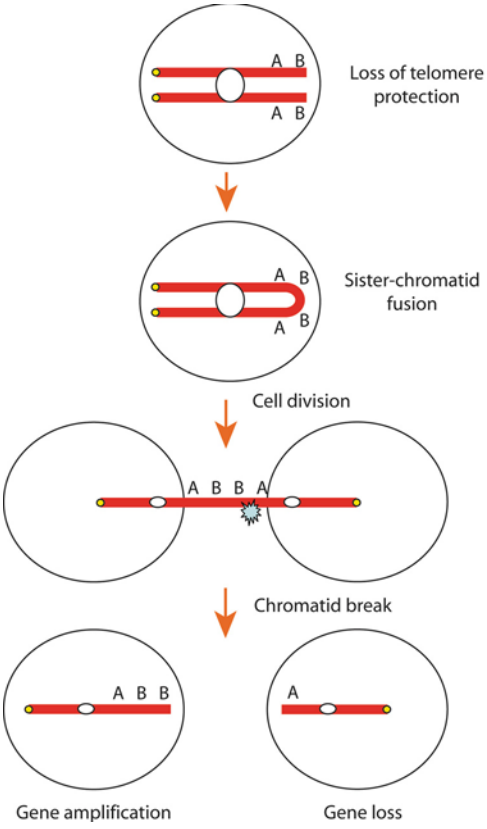

Figure 5. Proposed mechanism of gene amplification and loss through breakage of sister-chromatid fusion. Modified with permission from 27.

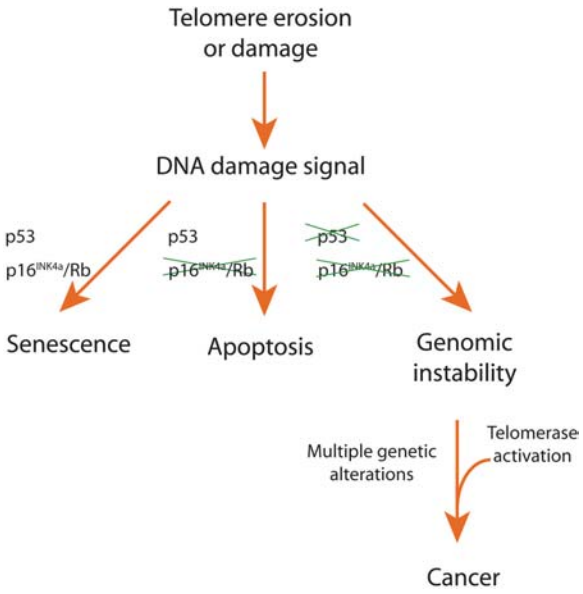

Figure 6. Consequences of telomere dysfunction

selection of aberrant cells for cellular immortalization and cancer development (124,132). In support of this, the mouse model with the depletion of telomerase RNA component (mTerc) shows increased incidence of spontaneous malignancies (mainly lymphoma, teratocarcinoma) in late generation (G4-G6) animals (133). Combination of telomerase depletion and p53 double mutations ($mTerc^{-/-}$ $p53^{-/-}$) leads to significantly shorter tumor latency and promotes the development of epithelial cancers (9%), which do not commonly occur in wild-type and $p53^{-/-}$ mice. Surprisingly, in $mTerc^{-/-}$ $p53^{+/-}$ mice, carcinomas become the largest class of clinically apparent tumors (131). It is reasoned that the relatively longer tumor latency in $mTerc^{-/-}$ $p53^{+/-}$ mice might better reveal the role of age-dependent telomere attrition on carcinogenesis than the $mTerc^{-/-}$ $p53^{-/-}$ mice because the latter rapidly succumb to lymphoid and mesenchymal cancers. Chromosome end-to-end fusions and non-reciprocal translocations, which are typical chromosomal abnormalities in human solid tumors, are frequently observed in the cells cultured from the mTerc and p53 deficient mouse tumors. Since epithelial cancer is the dominant cancer type in aged humans, and p53 pathway deficiency is common in human malignancies, these mouse models are particularly relevant to the development of epithelial cancers in humans.

Interestingly, severe telomere dysfunction in late generation $mTerc^{-/-}$ mice lacking tumor suppressor genes other than p53, such as INK4a/Arf, Apc, and Atm (134-136), or overexpressing the onocgene c-myc (137), significantly suppresses tumor formation. Telomere dysfunction-mediated chromosomal instability in telomerase deficient mice also suppresses carcinogen-induced skin carcinogenesis in $Terc^{-/-}$ mice (138). The cancer suppression by telomere dysfunction is thought to be related to intolerable levels of massive chromosomal instability which impairs cell survival and growth. In particular, in the $mTerc^{-/-}$ $Apc^{+/-}$ mouse model, although progressive telomere shortening increases the initiation of microscopic adenomas, it significantly reduces the multiplicity and the sizes of macroscopic adenomas. In sporadic human colorectal tumors, anaphase bridges (associated with, but not specific for, telomere dysfunction) sharply increase at the transitional stage from adenoma to early carcinomas, but decline at metastasis stage (135). Taken together, the accumulating evidence indicates that whereas early, transient and moderate telomere dysfunction may be an important driving force for cancer development, severe telomere dysfunction causing intolerable massive chromosomal instability may actually inhibit cancer development. This particular property of telomere dysfunction may be exploited for cancer therapy.

8. HETEROGENITY IN TELOMERE LENGTHS ON INDIVIDUAL HUMAN CHROMOSOMES AND ITS IMPACT ON CANCER DEVELOPMENT

Before the use of fluorescein-labeled PNA (peptide nucleic acids) probes, the most commonly used tool to estimate telomere length was Southern analysis of genomic DNA digested with selected restriction enzymes. Such analysis requires thousands of cells and provides only a crude estimation of the average length of all chromosome ends in the cells. The use of fluorescein-labeled telomeric PNA probes in fluorescence *in situ* hybridization (FISH)

has made it possible to quantify telomere lengths on individual chromosome ends (termed Q-FISH) (17). This is because the neutral backbone of PNA allows the molecule to hybridize to negatively charged DNA or RNA with much higher affinity and specificity than conventional DNA probes. Investigations have shown that the telomere fluorescence intensities using PNA probes are directly related to the amount of available target sequences. To date, the major conclusions on human telomere lengths based on Q-FISH studies are: (a) there are large differences in telomere lengths between p- and q-arms of the same chromosomes, as well as between the same arms of homologous chromosomes in the cells of the same individual; (b) different individuals have different profiles of telomere length heterogeneity which are largely inherited; and (c) different tissues of the same individual have similar telomere length heterogeneity (17-19,139,140).

If telomere erosion induces replicative senescence and promotes genomic instability, then is it the shortest telomere or the average telomere length in a cell that triggers these events? Are chromosomes with shorter telomeres more susceptible to chromosomal instability? Two hypotheses regarding this issue were proposed by the pioneers of telomere biology: (a) Critically short telomeres are the primary determinant of chromosome structural instability (118); (b) There is an inverse linear relationship between telomere lengths and the probabilities of stochastic telomere dysfunction, suggesting that cellular senescence kinetics is associated with stochastic uncapping of telomeres, rather than telomere length alone (141). The latter hypothesis seems to be experimentally supported by a study showing that the onset of replicative senescence in cell lines derived from human fetal fibroblasts was not associated with the shortest telomere lengths (142), although the authors did not examine the relationship between the shortest telomere lengths and chromosome instability.

However, an extensive study using telomerase knock-out mice demonstrated that cellular senescence as well as the frequencies of chromosome end-to-end fusions were determined exclusively by the shortest telomeres (143). More recently, it was shown that a specific group of chromosomes with the shortest telomeres, rather than one or two sentinel telomeres, is responsible for inducing replicative senescence in human foreskin fibroblasts (80). We used a human ovarian surface epithelial cell line (HOSE 6-3) immortalized by HPV-16 E6/E7 expression as an *in vitro* model to study the relationship between telomere shortening and chromosomal instability in the early process of cancer. We showed that a subset of chromosomes with the shortest telomeres (lacking detectable telomere FISH signals) in pre-immortal (or pre-crisis) cells were most frequently involved in various types of chromosomal structural aberrations, including dicentrics, translocations, losses or gains of chromosome elements, thus supporting the hypothesis that chromosomes with the shortest telomeres are most susceptible to chromosomal instability (144). Our findings were supported by a later report of similar observations in SV40-ER-transformed embryonic kidney epithelial cells (145).

Chromosomal aberrations are common in cancers. A number of cytogenetic studies have revealed that cancers have recurrent and non-random chromosome imbalances (gains or losses of chromosome elements) and that some of the chromosomal regions involved in gains or losses are shared by many types of tumors and immortalized cell lines (146-152). However, in addition to the gross overlapping chromosome imbalances, the vast majority of human solid tumors have complex karyotypes that differ within the same types of tumors. For example, a number of widely used classical cell lines from the same types of tumors have dramatically different karyotypes, such as cervical cancer cell lines: HeLa, CaSki, SiHa, HT-3 (153,154); prostate cancer cell lines: androgen-dependent LNCaP, MDA-PCa-2b, androgen-independent DU145, PC-3 (155,156); and breast cancer cell lines: estrogen-dependent MCF-7, T47D, ZR-75-1, and estrogen-independent SK-BR-3 and MDA-MB-231 (157,158). The same is true in a number of newly established cancer cell lines, including those derived from esophageal cancers (159-161) and nasopharyngeal cancers (151). The non-random pattern of chromosome aberrations has also been observed in pre-immortal transformed cells *in vitro*, with independent cell lines of the same cell types having different profiles of aberrant chromosomes (113,162).

The next question that comes to mind is whether specific chromosomal aberrations associated with specific types of solid tumors or specific cell types of immortalized cell lines can be explained by the inherent differences in distributions of the shortest telomeres. In our study of multiple cell lines derived from human ovarian surface epithelial and esophageal epithelial cells expressing HPV-16 E6/E7 (67), we found that these cell lines derived from unrelated donors had different profiles of critically short telomeres, and that as the cells underwent immortalization *in vitro*, their distinct profiles of critically short telomeres were a key determinant of the early events of chromosomal structural instability before immortalization. These early events were mainly chromosome end-fusions. With further passaging, the original "sub-short" telomeres also shortened to dysfunctional threshold and the cells entered cellular crisis due to massive chromosome instability. This process generated increasingly wide-spread chromosome aberrations including end-fusions, translocations, insertions, losses or gains of chromosome elements. In other words, most of the structural aberrations did not occur randomly throughout the genome during the process of continued cell proliferation, but rather occurred on those specific chromosomes that had critically short telomeres until rare cells emerged from crisis. In post-crisis cells, the stabilized structural aberrations were again associated with the specific distribution of critically short telomeres before and during crisis in whole-genome. Thus, the individual-specific profiles of critically short telomeres throughout the entire process of immortalization (not just the shortest telomeres at the beginning of the process) have a profound long-term impact on the karyotypes of the immortalized epithelial cells. Since chromosomal instability may help drive the multiple genetic changes that are required for

cancer development, these data provide a plausible explanation for the long-standing mystery of why human solid tumors of the same type have distinct structural chromosomal aberrations.

In addition to structural chromosome aberrations, aneuploidy (numerical chromosome abnormalities) is also almost ubiquitous in cancers (2) and has been proposed to be one of the mechanisms for cancer development (163-167). Chromosome end-to-end fusions mediated by telomere dysfunction can theoretically contribute to gains or losses of whole-chromosomes (see Figure 4). Graakjaer *et al.* found a negative correlation between telomere lengths from their data set and chromosome-specific aneuploidy in human B lymphocytes reported by another group (139). A similar study by Leach *et al.* (168) detected such correlation for telomere lengths on short arm only (and not long arm or average chromosome-specific telomere length) in normal cells. Since we found a non-linear relationship between telomere lengths and the probabilities of chromosome structural instability in human cells undergoing immortalization (Figure 3), we sought to find out whether there is any correlation between the distributions of the shortest telomeres and the frequencies of whole-chromosome losses or gains. Our whole-genome data obtained from five human epithelial cells expressing HPV-16 E6 and E7 (including two human ovarian surface epithelial, two esophageal epithelial and one nasopharyngeal epithelial cell lines) showed that despite dynamic gains or losses of whole-chromosomes from pre-immortal stage to crisis, there was no consistent correlation between the distributions of the shortest telomeres and the frequencies of whole-chromosome losses or gains (169). Therefore, in contrast to its role in structural chromosomal instability, telomere dysfunction does not seem to play a significant role in inducing nonrandom numerical chromosome instability in human cells undergoing immortalization. An interesting implication of this finding is that microtubule-kinetochore attachment may be stronger than chromosome structure.

9. TELOMERASE ACTIVATION ALLEVIATES CHROMOSOME INSTABILITY DURING IMMORTALIZATION AND TUMORIGENESIS

Short telomeres are present in premalignant lesions in many cancer types (170-173). Since the BFB cycles initiated by telomere dysfunction lead to extensive genomic instability which is detrimental to cell survival, telomere length must be stabilized to alleviate chromosomal instability to a level permitting tumor progression. This is mainly accomplished by telomerase activation (174). In immortalized cells and tumor cells that do not have detectable telomerase activity (such as sarcomas and astrocytomas), telomere lengthening is achieved by ALT (175,176). Extensive studies have demonstrated that over 90% of cancers and immortalized cell lines do have activated telomerase, as detected by a highly sensitive PCR-based telomere repeat amplification protocol (TRAP) (174). Ducray *et al. (*108) reported that telomerase activation leads to alleviation of structural chromosome instability in post-crisis SV40 transformed human fibroblasts, as indicated by the dramatic decreases in the frequencies of chromosome end-to-end fusions compared with cells in crisis. Our study of post-crisis human epithelial cells yielded similar results, with whole-genome alleviation of structural chromosomal instability being accompanied by a significant decrease in overall frequency of telomere signal-free ends (representing critically short telomeres) in post-crisis cells (67). In addition, as in the case of human fibroblasts (108), the average telomere length in the post-crisis epithelial cells was shorter compared with that of cells in crisis. This can be explained by the suggestion that telomerase elongates telomeres most efficiently on chromosomal ends with the critically short telomeres, which leads to telomere length homogenization but does not necessarily an increase in average telomere length (108,143). It is possible that the decrease in the frequencies of telomere signal-free ends, rather than the overall elongation of telomeres, is responsible for alleviation of structural chromosomal instability during cancer progression. The underlying mechanism for the preferential telomere elongation of short telomeres by telomerase may be related to easier accessibility of telomerase to short telomeres, because critically short telomeres affect the epigenetic status of telomeric and subtelomeric chromatin, making telomeric heterochromatin switch to a relatively more "open" state which allows for telomerase action (37,177).

10. CONCLUSIONS AND FUTURE PERSPECTIVE

It is well established that loss of checkpoint function is insufficient for immortalization and cancer development. Since cancer development involves accumulation of multiple genetic alterations, what we have reviewed above provides a brief account of how telomere-driven chromosomal instability may pave the way for the initiation and continuous evolution of genetic alterations needed for cancer development. Through continuous generation of new structural and numerical chromosome aberrations, genomic instability provides cells with selective advantages during cancer development.

Most cancer cells have strong telomerase activity to maintain telomere lengths for long-term cell proliferation. It has been proposed that cancer cells can be killed by inducing critical shortening of telomeres, and hence senescence or apoptosis, through inhibition of telomerase. A telomerase inhibitor, GRN163L, is now in Phase I and II clinical trials. In this regard, the non-linear relationship between specific telomere length and the probability of structural chromosomal instability we observed in human epithelial cells undergoing immortalization (Figure 3) has important implications -- that telomerase inhibition may not be immediately effective until telomeres shorten to a critical length, thereby casting some doubt on the efficacy of short-term telomerase inhibition treatment. However, this problem may be overcome by lentiviral delivery and over-expression of mutant-template human telomerase RNA (MT-hTer) to add mutant DNA to telomeres in cancer cells. This results in rapid inhibition of cell growth and induction of apoptosis in telomerase-positive precancerous or cancer cells but not in

telomerase-negative cells, with the effects occurring independent of telomere length. Co-expression of MT-hTer with a hairpin short-interfering RNA that specifically targets the endogenous wild-type human telomerase RNA has been shown to synergistically induce rapid DNA damage response, telomere uncapping, and inhibition of cell proliferation in a variety of human cancer cell lines (178,179), thus providing a promising approach for cancer therapy.

Recently, new discoveries in telomere structure and the functions of telomere associated proteins have led to an increasing interest in targeting telomeres instead of telomerase in anti-cancer therapy. One strategy is to target telomere associated proteins that regulate telomere function, e.g. triggering telomere loss and inducing apoptosis or senescence through inhibition of the telomeric DNA-binding protein TRF2 (180-182). Another strategy is to stabilize the G-quadruplexes, which are formed in the G-rich single-stranded 3' overhang of telomeres, so as to render telomeres inaccessible to telomerase action. In particular, the G-quadruplex ligand telomestatin not only binds to telomeric overhang and impairs its single-stranded conformation, but also induces dissociation of TRF2 and POT1 from telomeric sequences in human cancer cells (183-185). More importantly, it was shown that short term treatment with telomestatin at an appropriate dose can selectively kill cancer cells but not normal cells (185).

Another category of interesting finding in recent years is that the average telomere lengths in non-transformed cells are significantly shorter in patients with head and neck, renal, and bladder cancer (186-189) as well as non-Hodgkin's lymphoma than in normal individuals (189). However, as we have reviewed above, detailed studies on dynamic chromosomal abnormalities in animal models and human cells in culture have consistently revealed that it is not the average telomere but rather the shortest telomere length that determines the profiles of chromosomal instability. It is well known that chromosomal aberrations in human cancers are not randomly distributed throughout the whole-genome. Therefore, it would be interesting in the future to decipher further whether the chromosomal aberrations detected in human cancers are associated with the specific profiles of the shortest telomeres in normal cells from cancer patients, and whether cancer patients or cancer-prone patient have some specific profiles of the shortest telomere (s) as compared with the healthy individuals. In this regard, we expect that the profiles of the shortest telomeres to be more significantly associated with cancer risk than average telomere lengths. The information might lead to identification of the shortest telomere (s) as a risk factor for cancer.

From the time Herman J Muller coined the term "telomere" (from the Greek *telos* meaning end, and *meros* meaning part) in the early 1930s to now, telomere research has come a long way but has not lost its momentum. New discoveries pertaining to telomere structure and telomere homeostasis that shed light on the complex relationship between telomeres, telomerase, chromosomal instability and cancer will undoubtedly have an important impact in cancer therapeutics.

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12. REFERENCES

1. R. D. Klausner: The fabric of cancer cell biology-Weaving together the strands. *Cancer Cell* 1 (1), 3-10 (2002)
2. C. Lengauer, K. W. Kinzler and B. Vogelstein: Genetic instabilities in human cancers. *Nature* 396 (6712), 643-649 (1998)
3. T. Ried, K. Heselmeyer-Haddad, H. Blegen, E. Schrock and G. Auer: Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. *Genes Chromosomes Cancer* 25 (3), 195-204 (1999)
4. M. M. Weiss, E. J. Kuipers, C. Postma, A. M. Snijders, M. Stolte, M. Vieth, D. Pinkel, S. G. Meuwissen, D. Albertson and G. A. Meijer: Genome wide array comparative genomic hybridisation analysis of premalignant lesions of the stomach. *Mol Pathol* 56 (5), 293-298 (2003)
5. A. H. Moskovitz, N. J. Linford, T. A. Brentnall, M. P. Bronner, B. E. Storer, J. D. Potter, R. H. Bell, Jr. and P. S. Rabinovitch: Chromosomal instability in pancreatic ductal cells from patients with chronic pancreatitis and pancreatic adenocarcinoma. *Genes Chromosomes Cancer* 37 (2), 201-206 (2003)
6. G. A. Pihan and S. J. Doxsey: The mitotic machinery as a source of genetic instability in cancer. *Semin Cancer Biol* 9 (4), 289-302 (1999)
7. R. S. Maser and R. A. DePinho: Take care of your chromosomes lest cancer take care of you. *Cancer Cell* 3 (1), 4-6 (2003)
8. D. M. Feldser, J. A. Hackett and C. W. Greider: Telomere dysfunction and the initiation of genome instability. *Nat Rev Cancer* 3 (8), 623-627 (2003)
9. H. J. Muller: The remaking of chromosomes. *Collecting Net* 13, ←181-195 (1938)
10. B. McClintock: The stability of broken ends of chromosomes in Zea mays. *Genetics* 26 (2), 234-282 (1941)
11. E. H. Blackburn: Switching and signaling at the telomere. *Cell* 106 (6), 661-673 (2001)
12. R. W. Levis: Viable deletions of a telomere from a Drosophila chromosome. *Cell* 58 (4), 791-801 (1989)
13. T. de Lange: T-loops and the origin of telomeres. *Nat Rev Mol Cell Biol* 5 (4), 323-329 (2004)
14. T. de Lange: Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 19 (18), 2100-2110 (2005)
15. T. de Lange, L. Shiue, R. M. Myers, D. R. Cox, S. L. Naylor, A. M. Killery and H. E. Varmus: Structure and

variability of human chromosome ends. *Mol Cell Biol* 10 (2), 518-527 (1990)
16. R. C. Allsopp, H. Vaziri, C. Patterson, S. Goldstein, E. V. Younglai, A. B. Futcher, C. W. Greider and C. B. Harley: Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A* 89 (21), 10114-10118 (1992)
17. P. M. Lansdorp, N. P. Verwoerd, F. M. van de Rijke, V. Dragowska, M. T. Little, R. W. Dirks, A. K. Raap and H. J. Tanke: Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet* 5 (5), 685-691 (1996)
18. U. M. Martens, J. M. Zijlmans, S. S. Poon, W. Dragowska, J. Yui, E. A. Chavez, R. K. Ward and P. M. Lansdorp: Short telomeres on human chromosome 17p. *Nat Genet* 18 (1), 76-80 (1998)
19. J. A. Londono-Vallejo, H. DerSarkissian, L. Cazes and G. Thomas: Differences in telomere length between homologous chromosomes in humans. *Nucleic Acids Res* 29 (15), 3164-3171 (2001)
20. V. L. Makarov, Y. Hirose and J. P. Langmore: Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell* 88 (5), 657-666 (1997)
21. J. D. Griffith, L. Comeau, S. Rosenfield, R. M. Stansel, A. Bianchi, H. Moss and T. de Lange: Mammalian telomeres end in a large duplex loop. *Cell* 97 (4), 503-514 (1999)
22. R. M. Stansel, T. de Lange and J. D. Griffith: T-loop assembly *in vitro* involves binding of TRF2 near the 3' telomeric overhang. *EMBO J* 20 (19), 5532-5540 (2001)
23. G. N. Parkinson, M. P. Lee and S. Neidle: Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature* 417 (6891), 876-880 (2002)
24. N. Zhang, A. T. Phan and D. J. Patel: (3 + 1) Assembly of three human telomeric repeats into an asymmetric dimeric G-quadruplex. *J Am Chem Soc* 127 (49), 17277-17285 (2005)
25. C. C. Chang, I. C. Kuo, I. F. Ling, C. T. Chen, H. C. Chen, P. J. Lou, J. J. Lin and T. C. Chang: Detection of quadruplex DNA structures in human telomeres by a fluorescent carbazole derivative. *Anal Chem* 76 (15), 4490-4494 (2004)
26. C. Granotier, G. Pennarun, L. Riou, F. Hoffschir, L. R. Gauthier, A. De Cian, D. Gomez, E. Mandine, J. F. Riou, J. L. Mergny, P. Mailliet, B. Dutrillaux and F. D. Boussin: Preferential binding of a G-quadruplex ligand to human chromosome ends. *Nucleic Acids Res* 33 (13), 4182-4190 (2005)
27. L. Chong, B. van Steensel, D. Broccoli, H. Erdjument-Bromage, J. Hanish, P. Tempst and T. de Lange: A human telomeric protein. *Science* 270 (5242), 1663-1667 (1995)
28. T. Bilaud, C. Brun, K. Ancelin, C. E. Koering, T. Laroche and E. Gilson: Telomeric localization of TRF2, a novel human telobox protein. *Nat Genet* 17 (2), 236-239 (1997)
29. D. Broccoli, A. Smogorzewska, L. Chong and T. de Lange: Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Genet* 17 (2), 231-235 (1997)
30. D. Loayza, H. Parsons, J. Donigian, K. Hoke and T. de Lange: DNA binding features of human POT1: a nonamer 5'-TAGGGTTAG-3' minimal binding site, sequence specificity, and internal binding to multimeric sites. *J Biol Chem* 279 (13), 13241-13248 (2004)
31. Q. Yang, Y. L. Zheng and C. C. Harris: POT1 and TRF2 cooperate to maintain telomeric integrity. *Mol Cell Biol* 25 (3), 1070-1080 (2005)
32. A. Smogorzewska and T. de Lange: Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 73, 177-208 (2004)
33. F. Rodier, S. H. Kim, T. Nijjar, P. Yaswen and J. Campisi: Cancer and aging: the importance of telomeres in genome maintenance. *Int J Biochem Cell Biol* 37 (5), 977-990 (2005)
34. N. Hug and J. Lingner: Telomere length homeostasis. *Chromosoma* 115 (6), 413-425 (2006)
35. M. S. O'Connor, A. Safari, H. Xin, D. Liu and Z. Songyang: A critical role for TPP1 and TIN2 interaction in high-order telomeric complex assembly. *Proc Natl Acad Sci U S A* 103 (32), 11874-11879 (2006)
36. M. Garcia-Cao, R. O'Sullivan, A. H. Peters, T. Jenuwein and M. A. Blasco: Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. *Nat Genet* 36 (1), 94-99 (2004)
37. M. A. Blasco: The epigenetic regulation of mammalian telomeres. *Nat Rev Genet* 8 (4), 299-309 (2007)
38. C. B. Harley, A. B. Futcher and C. W. Greider: Telomeres shorten during ageing of human fibroblasts. *Nature* 345 (6274), 458-460 (1990)
39. R. C. Allsopp, E. Chang, M. Kashefi-Aazam, E. I. Rogaev, M. A. Piatyszek, J. W. Shay and C. B. Harley: Telomere shortening is associated with cell division *in vitro* and *in vivo*. *Exp Cell Res* 220 (1), 194-200 (1995)
40. N. D. Hastie, M. Dempster, M. G. Dunlop, A. M. Thompson, D. K. Green and R. C. Allshire: Telomere reduction in human colorectal carcinoma and with ageing. *Nature* 346 (6287), 866-868 (1990)
41. B. M. Unryn, L. S. Cook and K. T. Riabowol: Paternal age is positively linked to telomere length of children. *Aging Cell* 4 (2), 97-101 (2005)
42. A. M. Olovnikov: A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol* 41 (1), 181-190 (1973)
43. T. Kurz, A. Leake, T. Von Zglinicki and U. T. Brunk: Lysosomal redox-active iron is important for oxidative stress-induced DNA damage. *Ann N Y Acad Sci* 1019, (285-288 (2004)
44. N. R. Forsyth, A. P. Evans, J. W. Shay and W. E. Wright: Developmental differences in the immortalization of lung fibroblasts by telomerase. *Aging Cell* 2 (5), 235-243 (2003)
45. T. Von Zglinicki: Oxidative stress shortens telomeres. *Trends Biochem Sci* 27 (7), 339-344 (2002)
46. V. Serra, T. Von Zglinicki, M. Lorenz and G. Saretzki: Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. *J Biol Chem* 278 (9), 6824-6830 (2003)
47. A. Tchirkov and P. M. Lansdorp: Role of oxidative stress in telomere shortening in cultured fibroblasts from normal individuals and patients with ataxia-telangiectasia. *Hum Mol Genet* 12 (3), 227-232 (2003)

48. J. Campisi: From cells to organisms: can we learn about aging from cells in culture? *Exp Gerontol* 36 (4-6), 607-618 (2001)
49. S. Kawanishi and S. Oikawa: Mechanism of telomere shortening by oxidative stress. *Ann N Y Acad Sci* 1019, 278-284 (2004)
50. S. Oikawa and S. Kawanishi: Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Lett* 453 (3), 365-368 (1999)
51. S. Petersen, G. Saretzki and T. Von Zglinicki: Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp Cell Res* 239 (1), 152-160 (1998)
52. T. von Zglinicki, R. Pilger and N. Sitte: Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radic Biol Med* 28 (1), 64-74 (2000)
53. A. Bokov, A. Chaudhuri and A. Richardson: The role of oxidative damage and stress in aging. *Mech Ageing Dev* 125 (10-11), 811-826 (2004)
54. C. W. Greider and E. H. Blackburn: A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. *Nature* 337 (6205), 331-337 (1989)
55. S. A. Stewart, I. Ben Porath, V. J. Carey, B. F. O'Connor, W. C. Hahn and R. A. Weinberg: Erosion of the telomeric single-strand overhang at replicative senescence. *Nature Genetics* 33 (4), 492-496 (2003)
56. G. B. Morin: The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 59 (3), 521-529 (1989)
57. J. Feng, W. D. Funk, S. S. Wang, S. L. Weinrich, A. A. Avilion, C. P. Chiu, R. R. Adams, E. Chang, R. C. Allsopp, J. Yu, S. Le, M. D. West, C. B. Harley, W. H. Andrews, C. W. Greider and B. Villepponteau: The RNA component of human telomerase. *Science* 269 (5228), 1236-1241 (1995) Le,S.; West,M.D.; Harley,C.B.; Andrews,W.H.; Greider,C.W.; Villepponteau,B.
58. M. Meyerson, C. M. Counter, E. N. Eaton, L. W. Ellisen, P. Steiner, S. D. Caddle, L. Ziaugra, R. L. Beijersbergen, M. J. Davidoff, Q. Liu, S. Bacchetti, D. A. Haber and R. A. Weinberg: hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90 (4), 785-795 (1997)
59. T. M. Nakamura, G. B. Morin, K. B. Chapman, S. L. Weinrich, W. H. Andrews, J. Lingner, C. B. Harley and T. R. Cech: Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277 (5328), 955-959 (1997)
60. J. W. Shay and S. Bacchetti: A survey of telomerase activity in human cancer. *Eur J Cancer* 33 (5), 787-791 (1997)
61. J. C. Poole, L. G. Andrews and T. O. Tollefsbol: Activity, function, and gene regulation of the catalytic subunit of telomerase (hTERT). *Gene* 269 (1-2), 1-12 (2001)
62. Y. S. Cong, W. E. Wright and J. W. Shay: Human telomerase and its regulation. *Microbiol Mol Biol Rev* 66 (3), 407-425, 2002)
63. S. B. Cohen, M. E. Graham, G. O. Lovrecz, N. Bache, P. J. Robinson and R. R. Reddel: Protein composition of catalytically active human telomerase from immortal cells. *Science* 315 (5820), 1850-1853 (2007)
64. J. Karlseder, A. Smogorzewska and T. de Lange: Senescence induced by altered telomere state, not telomere loss. *Science* 295 (5564), 2446-2449 (2002)
65. L. Crabbe and J. Karlseder: In the end, it's all structure. *Curr Mol Med* 5 (2), 135-143 (2005)
66. B. van Steensel, A. Smogorzewska and T. de Lange: TRF2 protects human telomeres from end-to-end fusions. *Cell* 92 (3), 401-413 (1998)
67. W. Deng, S. W. Tsao, X. Y. Guan, J. N. Lucas, H. X. Si, C. S. Leung, P. Mak, L. D. Wang and A. L. M. Cheung: Distinct profiles of critically short telomeres are a key determinant of different chromosome aberrations in immortalized human cells: whole-genome evidence from multiple cell lines. *Oncogene* 23 (56), 9090-9101 (2004)
68. L. Hayflick and P. S. Moorhead: The serial cultivation of human diploid cell strains. *Exp Cell Res* 25, 585-621 (1961)
69. L. Hayflick: Aging under glass. *Mutat Res* 256 (2-6), 69-80 (1991)
70. J. W. Shay and W. E. Wright: Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol* 1 (1), 72-76 (2000)
71. J. G. Rheinwald and H. Green: Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 6 (3), 331-343 (1975)
72. J. Tassin, E. Malaise and Y. Courtois: Human lens cells have an *in vitro* proliferative capacity inversely proportional to the donor age. *Exp Cell Res* 123 (2), 388-392 (1979)
73. E. Blomquist, B. Westermark and J. Ponten: Ageing of human glial cells in culture: increase in the fraction of non-dividers as demonstrated by a minicloning technique. *Mech Ageing Dev* 12 (2), 173-182 (1980)
74. E. M. Levine and S. N. Mueller: Cultured vascular endothelial cells as a model system for the study of cellular senescence. *Int Rev Cytol Suppl* 10), 67-76 (1979)
75. R. B. Effros: Replicative senescence of CD8 T cells: potential effects on cancer immune surveillance and immunotherapy. *Cancer Immunol Immunother* 53 (10), 925-933 (2004)
76. E. L. Bierman: The effect of donor age on the *in vitro* life span of cultured human arterial smooth-muscle cells. *In vitro* 14 (11), 951-955 (1978)
77. R. J. Evans, F. S. Wyllie, D. Wynford-Thomas, D. Kipling and C. J. Jones: A P53-dependent, telomere-independent proliferative life span barrier in human astrocytes consistent with the molecular genetics of glioma development. *Cancer Res* 63 (16), 4854-4861 (2003)
78. P. Atadja, H. Wong, I. Garkavtsev, C. Veillette and K. Riabowol: Increased activity of p53 in senescing fibroblasts. *Proc Natl Acad Sci U S A* 92 (18), 8348-8352 (1995)
79. F. d'Adda di Fagagna, P. M. Reaper, L. Clay-Farrace, H. Fiegler, P. Carr, T. Von Zglinicki, G. Saretzki, N. P. Carter and S. P. Jackson: A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426 (6963), 194-198 (2003)

80. Y. Zou, A. Sfeir, S. M. Gryaznov, J. W. Shay and W. E. Wright: Does a sentinel or a subset of short telomeres determine replicative senescence? *Mol Biol Cell* 15 (8), 3709-3718 (2004)
81. d. F. d'Adda, S. H. Teo and S. P. Jackson: Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev* 18 (15), 1781-1799 (2004)
82. U. Herbig, W. A. Jobling, B. P. Chen, D. J. Chen and J. M. Sedivy: Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21 (CIP1), but not p16 (INK4a). *Mol Cell* 14 (4), 501-513 (2004)
83. O. A. Sedelnikova, I. Horikawa, D. B. Zimonjic, N. C. Popescu, W. M. Bonner and J. C. Barrett: Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. *Nat Cell Biol* 6 (2), 168-170 (2004)
84. D. A. Alcorta, Y. Xiong, D. Phelps, G. Hannon, D. Beach and J. C. Barrett: Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci U S A* 93 (24), 13742-13747 (1996)
85. D. G. Farwell, K. A. Shera, J. I. Koop, G. A. Bonnet, C. P. Matthews, G. W. Reuther, M. D. Coltrera, J. K. McDougall and A. J. Klingelhutz: Genetic and epigenetic changes in human epithelial cells immortalized by telomerase. *Am J Pathol* 156 (5), 1537-1547 (2000)
86. R. Marcotte and E. Wang: Replicative senescence revisited. *J Gerontol A Biol Sci Med Sci* 57 (7), B257-B269 (2002)
87. H. Kim, J. Farris, S. A. Christman, B. W. Kong, L. K. Foster, S. M. O'Grady and D. N. Foster: Events in the immortalizing process of primary human mammary epithelial cells by the catalytic subunit of human telomerase. *Biochem J* 365 (Pt 3), 765-772 (2002)
88. C. M. Beausejour, A. Krtolica, F. Galimi, M. Narita, S. W. Lowe, P. Yaswen and J. Campisi: Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J* 22 (16), 4212-4222 (2003)
89. S. J. Robles and G. R. Adami: Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. *Oncogene* 16 (9), 1113-1123 (1998)
90. G. M. Martin, C. A. Sprague and C. J. Epstein: Replicative life-span of cultivated human cells. Effects of donor's age, tissue, and genotype. *Lab Invest* 23 (1), 86-92 (1970)
91. B. M. Stanulis-Praeger: Cellular senescence revisited: a review. *Mech Ageing Dev* 38 (1), 1-48 (1987)
92. D. Rohme: Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts *in vitro* and erythrocytes *in vivo*. *Proc Natl Acad Sci U S A* 78 (8), 5009-5013 (1981)
93. S. Goldstein, C. B. Harley and E. J. Moerman: Some aspects of cellular aging. *J Chronic Dis* 36 (1), 103-116 (1983)
94. L. Hayflick: The cell biology of aging. *Clin Geriatr Med* 1 (1), 15-27 (1985)
95. J. R. Smith and O. M. Pereira-Smith: Replicative senescence: implications for *in vivo* aging and tumor suppression. *Science* 273 (5271), 63-67 (1996)
96. R. B. Effros, M. Dagarag, C. Spaulding and J. Man: The role of CD8+ T-cell replicative senescence in human aging. *Immunol Rev* 205, (-)147-157 (2005)
97. A. Krtolica, S. Parrinello, S. Lockett, P. Y. Desprez and J. Campisi: Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci U S A* 98 (21), 12072-12077 (2001)
98. J. Campisi: Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 120 (4), 513-522 (2005)
99. C. J. Sherr: Cancer cell cycles. *Science* 274 (5293), 1672-1677 (1996)
100. D. Hanahan and R. A. Weinberg: The hallmarks of cancer. *Cell* 100 (1), 57-70 (2000)
101. C. J. Sherr and F. McCormick: The RB and p53 pathways in cancer. *Cancer Cell* 2 (2), 103-112 (2002)
102. Y. Katakura, S. Alam and S. Shirahata: Immortalization by gene transfection. *Methods Cell Biol* 57, (-)69-91 (1998)
103. K. Munger and P. M. Howley: Human papillomavirus immortalization and transformation functions. *Virus Res* 89 (2), 213-228 (2002)
104. M. T. Saenz-Robles, C. S. Sullivan and J. M. Pipas: Transforming functions of Simian Virus 40. *Oncogene* 20 (54), 7899-7907 (2001)
105. E. Moran: Interaction of adenoviral proteins with pRB and p53. *FASEB J* 7 (10), 880-885 (1993)
106. N. Sang, J. Caro and A. Giordano: Adenoviral E1A: everlasting tool, versatile applications, continuous contributions and new hypotheses. *Front Biosci* 7, (-)d407-d413 (2002)
107. W. E. Wright and J. W. Shay: The two-stage mechanism controlling cellular senescence and immortalization. *Exp Gerontol* 27 (4), 383-389 (1992)
108. C. Ducray, J. P. Pommier, L. Martins, F. D. Boussin and L. Sabatier: Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process. *Oncogene* 18 (29), 4211-4223 (1999)
109. T. M. Bryan, L. Marusic, S. Bacchetti, M. Namba and R. R. Reddel: The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. *Hum Mol Genet* 6 (6), 921-926 (1997)
110. R. R. Reddel, T. M. Bryan and J. P. Murnane: Immortalized cells with no detectable telomerase activity. A review. *Biochemistry (Mosc)* 62 (11), 1254-1262 (1997)
111. A. Muntoni and R. R. Reddel: The first molecular details of ALT in human tumor cells. *Hum Mol Genet* 14 (Spec No. 2), R191-R196 (2005)
112. M. A. Dunham, A. A. Neumann, C. L. Fasching and R. R. Reddel: Telomere maintenance by recombination in human cells. *Nat Genet* 26 (4), 447-450 (2000)
113. P. A. Benn: Specific chromosome aberrations in senescent fibroblast cell lines derived from human embryos. *Am J Hum Genet* 28 (5), 465-473 (1976)
114. C. M. Counter, A. A. Avilion, C. E. LeFeuvre, N. G. Stewart, C. W. Greider, C. B. Harley and S. Bacchetti: Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 11 (5), 1921-1929 (1992)

115. D. Gisselsson, T. Jonson, A. Petersen, B. Strombeck, P. Dal Cin, M. Hoglund, F. Mitelman, F. Mertens and N. Mandahl: Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. *Proc Natl Acad Sci U S A* 98 (22), 12683-12688 (2001)
116. A. W. Plug-DeMaggio, T. Sundsvold, M. A. Wurscher, J. I. Koop, A. J. Klingelhutz and J. K. McDougall: Telomere erosion and chromosomal instability in cells expressing the HPV oncogene 16E6. *Oncogene* 23 (20), 3561-3571 (2004)
117. D. Gisselsson, M. Lv, S. W. Tsao, C. Man, C. Jin, M. Hoglund, Y. L. Kwong and Y. Jin: Telomere-mediated mitotic disturbances in immortalized ovarian epithelial cells reproduce chromosomal losses and breakpoints from ovarian carcinoma. *Genes Chromosomes Cancer* 42 (1), 22-33 (2005)
118. T. de Lange, *Telomere dynamics and genome instability in human cancer*. In *Telomeres*. (Ed. E. H. Blackburn and C. W. Greider) pp. 265-293, Cold Spring Harbor Laboratory Press, New York 1995.
119. J. S. Rhim: Current state-of-the-art in human cell transformation in culture. *Yonsei Med J* 32 (3), 195-206 (1991)
120. J. L. Cook, B. A. Routes and L. Sompayrac: Experimental tumour induction by SV40 transformed cells. *Dev Biol Stand* 94, (-303-309 (1998)
121. N. E. Fusenig and P. Boukamp: Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. *Mol Carcinog* 23 (3), 144-158 (1998)
122. W. C. Hahn: Immortalization and transformation of human cells. *Mol Cells* 13 (3), 351-361 (2002)
123. J. S. Rhim: Development of human cell lines from multiple organs. *Ann N Y Acad Sci* 919, (-16-25 (2000)
124. R. S. Maser and R. A. DePinho: Connecting chromosomes, crisis, and cancer. *Science* 297 (5581), 565-569 (2002)
125. P. C. Nowell: Genetic alterations in leukemias and lymphomas: impressive progress and continuing complexity. *Cancer Genet Cytogenet* 94 (1), 13-19 (1997)
126. J. D. Rowley: The role of chromosome translocations in leukemogenesis. *Semin Hematol* 36 (4 Suppl 7), 59-72 (1999)
127. M. Nilbert, L. A. Meza-Zepeda, P. Francis, J. M. Berner, H. M. Namlos, J. Fernebro and O. Myklebost: Lessons from genetic profiling in soft tissue sarcomas. *Acta Orthop Scand Suppl* 75 (311), 35-50 (2004)
128. D. G. Albertson, C. Collins, F. McCormick and J. W. Gray: Chromosome aberrations in solid tumors. *Nat Genet* 34 (4), 369-376 (2003)
129. T. Nishizaki, S. DeVries, K. Chew, W. H. Goodson, III, B. M. Ljung, A. Thor and F. M. Waldman: Genetic alterations in primary breast cancers and their metastases: direct comparison using modified comparative genomic hybridization. *Genes Chromosomes Cancer* 19 (4), 267-272 (1997)
130. D. L. Stoler, N. Chen, M. Basik, M. S. Kahlenberg, M. A. Rodriguez-Bigas, N. J. Petrelli and G. R. Anderson: The onset and extent of genomic instability in sporadic colorectal tumor progression. *Proc Natl Acad Sci U S A* 96 (26), 15121-15126 (1999)
131. S. E. Artandi, S. Chang, S. L. Lee, S. Alson, G. J. Gottlieb, L. Chin and R. A. DePinho: Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* 406 (6796), 641-645 (2000)
132. D. Gisselsson: Chromosome instability in cancer: how, when, and why? *Adv Cancer Res* 87, (1-29 (2003)
133. K. L. Rudolph, S. Chang, H. W. Lee, M. Blasco, G. J. Gottlieb, C. Greider and R. A. DePinho: Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* 96 (5), 701-712 (1999)
134. R. A. Greenberg, L. Chin, A. Femino, K. H. Lee, G. J. Gottlieb, R. H. Singer, C. W. Greider and R. A. DePinho: Short dysfunctional telomeres impair tumorigenesis in the INK4a (delta2/3) cancer-prone mouse. *Cell* 97 (4), 515-525 (1999)
135. K. L. Rudolph, M. Millard, M. W. Bosenberg and R. A. DePinho: Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. *Nat Genet* 28 (2), 155-159 (2001)
136. L. Qi, M. A. Strong, B. O. Karim, D. L. Huso and C. W. Greider: Telomere fusion to chromosome breaks reduces oncogenic translocations and tumour formation. *Nat Cell Biol* 7 (7), 706-711 (2005)
137. D. M. Feldser and C. W. Greider: Short telomeres limit tumor progression *in vivo* by inducing senescence. *Cancer Cell* 11 (5), 461-469 (2007)
138. E. Gonzalez-Suarez, E. Samper, J. M. Flores and M. A. Blasco: Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat Genet* 26 (1), 114-117 (2000)
139. J. Graakjaer, C. Bischoff, L. Korsholm, S. Holstebroe, W. Vach, V. A. Bohr, K. Christensen and S. Kolvraa: The pattern of chromosome-specific variations in telomere length in humans is determined by inherited, telomere-near factors and is maintained throughout life. *Mech Ageing Dev* 124 (5), 629-640 (2003)
140. K. Nordfjall, A. Larefalk, P. Lindgren, D. Holmberg and G. Roos: Telomere length and heredity: Indications of paternal inheritance. *Proc Natl Acad Sci U S A* 102 (45), 16374-16378 (2005)
141. E. H. Blackburn: Telomere states and cell fates. *Nature* 408 (6808), 53-56 (2000)
142. U. M. Martens, E. A. Chavez, S. S. Poon, C. Schmoor and P. M. Lansdorp: Accumulation of short telomeres in human fibroblasts prior to replicative senescence. *Exp Cell Res* 256 (1), 291-299 (2000)
143. M. T. Hemann, M. A. Strong, L. Y. Hao and C. W. Greider: The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107 (1), 67-77 (2001)
144. W. Deng, S. W. Tsao, X. Y. Guan, J. N. Lucas and A. L. M. Cheung: Role of short telomeres in inducing preferential chromosomal aberrations in human ovarian surface epithelial cells: A combined telomere quantitative fluorescence *in situ* hybridization and whole-chromosome painting study. *Genes Chromosomes Cancer* 37 (1), 92-97 (2003)
145. H. Der-Sarkissian, S. Bacchetti, L. Cazes and J. A. Londono-Vallejo: The shortest telomeres drive karyotype evolution in transformed cells. *Oncogene* 23 (6), 1221-1228 (2004)

146. F. Mertens, B. Johansson, M. Hoglund and F. Mitelman: Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. *Cancer Res* 57 (13), 2765-2780 (1997)
147. N. Wong, P. Lai, S. W. Lee, S. Fan, E. Pang, C. T. Liew, Z. Sheng, J. W. Lau and P. J. Johnson: Assessment of genetic changes in hepatocellular carcinoma by comparative genomic hybridization analysis: relationship to disease stage, tumor size, and cirrhosis. *Am J Pathol* 154 (1), 37-43 (1999)
148. A. Pimkhaokham, Y. Shimada, Y. Fukuda, N. Kurihara, I. Imoto, Z. Q. Yang, M. Imamura, Y. Nakamura, T. Amagasa and J. Inazawa: Nonrandom chromosomal imbalances in esophageal squamous cell carcinoma cell lines: possible involvement of the ATF3 and CENPF genes in the 1q32 amplicon. *Jpn J Cancer Res* 91 (11), 1126-1133 (2000)
149. S. W. Tsao, N. Wong, X. Wang, Y. Liu, T. S. Wan, L. F. Fung, W. D. Lancaster, L. Gregoire and Y. C. Wong: Nonrandom chromosomal imbalances in human ovarian surface epithelial cells immortalized by HPV16-E6E7 viral oncogenes. *Cancer Genet Cytogenet* 130 (2), 141-149 (2001)
150. S. Struski, M. Doco-Fenzy and P. Cornillet-Lefebvre: Compilation of published comparative genomic hybridization studies. *Cancer Genet Cytogenet* 135 (1), 63-90 (2002)
151. N. Wong, A. B. Hui, B. Fan, K. W. Lo, E. Pang, S. F. Leung, D. P. Huang and P. J. Johnson: Molecular cytogenetic characterization of nasopharyngeal carcinoma cell lines and xenografts by comparative genomic hybridization and spectral karyotyping. *Cancer Genet Cytogenet* 140 (2), 124-132 (2003)
152. M. Hoglund, C. Jin, D. Gisselsson, G. B. Hansen, F. Mitelman and F. Mertens: Statistical analyses of karyotypic complexity in head and neck squamous cell carcinoma. *Cancer Genet Cytogenet* 150 (1), 1-8 (2004)
153. M. Macville, E. Schrock, H. Padilla-Nash, C. Keck, B. M. Ghadimi, D. Zimonjic, N. Popescu and T. Ried: Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. *Cancer Res* 59 (1), 141-150 (1999)
154. C. P. Harris, X. Y. Lu, G. Narayan, B. Singh, V. V. Murty and P. H. Rao: Comprehensive molecular cytogenetic characterization of cervical cancer cell lines. *Genes Chromosomes Cancer* 36 (3), 233-241 (2003)
155. Y. Pan, S. Kytola, F. Farnebo, N. Wang, W. O. Lui, N. Nupponen, J. Isola, T. Visakorpi, U. S. Bergerheim and C. Larsson: Characterization of chromosomal abnormalities in prostate cancer cell lines by spectral karyotyping. *Cytogenet Cell Genet* 87 (3-4), 225-232 (1999)
156. A. van Bokhoven, A. Caires, M. D. Maria, A. P. Schulte, M. S. Lucia, S. K. Nordeen, G. J. Miller and M. Varella-Garcia: Spectral karyotype (SKY) analysis of human prostate carcinoma cell lines. *Prostate* 57 (3), 226-244 (2003)
157. J. M. Davidson, K. L. Gorringe, S. F. Chin, B. Orsetti, C. Besret, C. Courtay-Cahen, I. Roberts, C. Theillet, C. Caldas and P. A. Edwards: Molecular cytogenetic analysis of breast cancer cell lines. *Br J Cancer* 83 (10), 1309-1317 (2000)
158. D. Xie, A. Jauch, C. W. Miller, C. R. Bartram and H. P. Koeffler: Discovery of over-expressed genes and genetic alterations in breast cancer cells using a combination of suppression subtractive hybridization, multiplex FISH and comparative genomic hybridization. *Int J Oncol* 21 (3), 499-507 (2002)
159. Y. Hu, K. Y. Lam, T. S. Wan, W. Fang, E. S. Ma, L. C. Chan and G. Srivastava: Establishment and characterization of HKESC-1, a new cancer cell line from human esophageal squamous cell carcinoma. *Cancer Genet Cytogenet* 118 (2), 112-120 (2000)
160. J. C. Tang, T. S. Wan, N. Wong, E. Pang, K. Y. Lam, S. Y. Law, L. M. Chow, E. S. Ma, L. C. Chan, J. Wong and G. Srivastava: Establishment and characterization of a new xenograft-derived human esophageal squamous cell carcinoma cell line SLMT-1 of Chinese origin. *Cancer Genet Cytogenet* 124 (1), 36-41 (2001)
161. Y. C. Hu, K. Y. Lam, S. Y. Law, T. S. Wan, E. S. Ma, Y. L. Kwong, L. C. Chan, J. Wong and G. Srivastava: Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human esophageal squamous cell carcinoma cell lines. *Cancer Genet Cytogenet* 135 (2), 120-127 (2002)
162. A. Cottage, S. Dowen, I. Roberts, M. Pett, N. Coleman and M. Stanley: Early genetic events in HPV immortalised keratinocytes. *Genes Chromosomes Cancer* 30 (1), 72-79 (2001)
163. R. Holliday: Chromosome error propagation and cancer. *Trends Genet* 5 (2), 42-45 (1989)
164. P. Duesberg, C. Rausch, D. Rasnick and R. Hehlmann: Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc Natl Acad Sci U S A* 95 (23), 13692-13697 (1998)
165. R. Li, A. Sonik, R. Stindl, D. Rasnick and P. Duesberg: Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. *Proc Natl Acad Sci U S A* 97 (7), 3236-3241 (2000)
166. P. Duesberg and R. Li: Multistep carcinogenesis: a chain reaction of aneuploidizations. *Cell Cycle* 2 (3), 202-210 (2003)
167. P. Duesberg: Does aneuploidy or mutation start cancer? *Science* 307 (5706), 41 (2005)
168. N. T. Leach, C. Rehder, K. Jensen, S. Holt and C. Jackson-Cook: Human chromosomes with shorter telomeres and large heterochromatin regions have a higher frequency of acquired somatic cell aneuploidy. *Mech Ageing Dev* 125 (8), 563-573 (2004)
169. W. Deng, S. W. Tsao, X. Y. Guan and A. L. M. Cheung: Microtubule breakage is not a major mechanism for resolving end-to-end chromosome fusions generated by telomere dysfunction during the early process of immortalization. *Chromosoma (*in press)
170. N. T. van Heek, A. K. Meeker, S. E. Kern, C. J. Yeo, K. D. Lillemoe, J. L. Cameron, G. J. Offerhaus, J. L. Hicks, R. E. Wilentz, M. G. Goggins, A. M. De Marzo, R. H. Hruban and A. Maitra: Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol* 161 (5), 1541-1547 (2002)
171. A. K. Meeker, J. L. Hicks, E. A. Platz, G. E. March, C. J. Bennett, M. J. Delannoy and A. M. De Marzo:

Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* 62 (22), 6405-6409 (2002)
172. A. K. Meeker, J. L. Hicks, C. A. Iacobuzio-Donahue, E. A. Montgomery, W. H. Westra, T. Y. Chan, B. M. Ronnett and A. M. De Marzo: Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin Cancer Res* 10 (10), 3317-3326 (2004)
173. A. Zhang, J. Wang, B. Zheng, X. Fang, T. Angstrom, C. Liu, X. Li, F. Erlandsson, M. Bjorkholm, M. Nordenskjord, A. Gruber, K. L. Wallin and D. Xu: Telomere attrition predominantly occurs in precursor lesions during *in vivo* carcinogenic process of the uterine cervix. *Oncogene* 23 (44), 7441-7447 (2004)
174. N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich and J. W. Shay: Specific association of human telomerase activity with immortal cells and cancer. *Science* 266 (5193), 2011-2015 (1994)
175. R. R. Reddel: Alternative lengthening of telomeres, telomerase, and cancer. *Cancer Lett* 194 (2), 155-162 (2003)
176. J. D. Henson, J. A. Hannay, S. W. McCarthy, J. A. Royds, T. R. Yeager, R. A. Robinson, S. B. Wharton, D. A. Jellinek, S. M. Arbuckle, J. Yoo, B. G. Robinson, D. L. Learoyd, P. D. Stalley, S. F. Bonar, D. Yu, R. E. Pollock and R. R. Reddel: A robust assay for alternative lengthening of telomeres in tumors shows the significance of alternative lengthening of telomeres in sarcomas and astrocytomas. *Clin Cancer Res* 11 (1), 217-225 (2005)
177. R. Benetti, M. Garcia-Cao and M. A. Blasco: Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. *Nat Genet* 39 (2), 243-250 (2007)
178. S. Li, J. E. Rosenberg, A. A. Donjacour, I. L. Botchkina, Y. K. Hom, G. R. Cunha and E. H. Blackburn: Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. *Cancer Res* 64 (14), 4833-4840 (2004)
179. A. Goldkorn and E. H. Blackburn: Assembly of mutant-template telomerase RNA into catalytically active telomerase ribonucleoprotein that can act on telomeres is required for apoptosis and cell cycle arrest in human cancer cells. *Cancer Res* 66 (11), 5763-5771 (2006)
180. J. Karlseder, D. Broccoli, Y. Dai, S. Hardy and T. de Lange: p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science* 283 (5406), 1321-1325 (1999)
181. J. Karlseder, A. Smogorzewska and T. de Lange: Senescence induced by altered telomere state, not telomere loss. *Science* 295 (5564), 2446-2449 (2002)
182. A. Biroccio, A. Rizzo, R. Elli, C. E. Koering, A. Belleville, B. Benassi, C. Leonetti, M. F. Stevens, M. D'Incalci, G. Zupi and E. Gilson: TRF2 inhibition triggers apoptosis and reduces tumourigenicity of human melanoma cells. *Eur J Cancer* 42 (12), 1881-1888 (2006)
183. D. Gomez, M. F. O'Donohue, T. Wenner, C. Douarre, J. Macadre, P. Koebel, M. J. Giraud-Panis, H. Kaplan, A. Kolkes, K. Shin-ya and J. F. Riou: The G-quadruplex ligand telomestatin inhibits POT1 binding to telomeric sequences *in vitro* and induces GFP-POT1 dissociation from telomeres in human cells. *Cancer Res* 66 (14), 6908-6912 (2006)
184. D. Gomez, T. Wenner, B. Brassart, C. Douarre, M. F. O'Donohue, K. El, V, K. Shin-ya, H. Morjani, C. Trentesaux and J. F. Riou: Telomestatin-induced telomere uncapping is modulated by POT1 through G-overhang extension in HT1080 human tumor cells. *J Biol Chem* 281 (50), 38721-38729 (2006)
185. H. Tahara, K. Shin-ya, H. Seimiya, H. Yamada, T. Tsuruo and T. Ide: G-Quadruplex stabilization by telomestatin induces TRF2 protein dissociation from telomeres and anaphase bridge formation accompanied by loss of the 3' telomeric overhang in cancer cells. *Oncogene* 25 (13), 1955-1966 (2006)
186. X. Wu, C. I. Amos, Y. Zhu, H. Zhao, B. H. Grossman, J. W. Shay, S. Luo, W. K. Hong and M. R. Spitz: Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst* 95 (16), 1211-1218 (2003)
187. K. Broberg, J. Bjork, K. Paulsson, M. Hoglund and M. Albin: Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis* 26 (7), 1263-1271 (2005)
188. M. McGrath, J. Y. Wong, D. Michaud, D. J. Hunter and I. De Vivo: Telomere length, cigarette smoking, and bladder cancer risk in men and women. *Cancer Epidemiol Biomarkers Prev* 16 (4), 815-819 (2007)
189. T. A. Widmann, M. Herrmann, N. Taha, J. Konig and M. Pfreundschuh: Short telomeres in aggressive non-Hodgkin's lymphoma as a risk factor in lymphomagenesis. *Exp Hematol* 35 (6), 939-946 (2007)

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