Telomere-Dependent Chromosomal Instability

Petra Boukamp, Susanne Popp, and Damir Krunic
German Cancer Research Center, Division of Genetics of Skin Carcinogensis, Im Neuenheimer Feld, Heidelberg, Germany

Telomeres are specialized DNA-protein structures at the ends of the linear chromosomes. In mammalian cells, they are composed of multifold hexameric TTAGGG repeats and a number of associated proteins. The double-stranded telomeric DNA ends in a 3′ single stranded overhang of 150 to 300 base pair (bp) which is believed to be required for a higher order structure (reviewed in (Blackburn, 2001)). One important model is that the telomeres form loop structures, the T-loops, and by invasion of the 3′ overhang into the duplex region of the double stranded part protect the DNA against degradation and hinder the cellular machinery to recognize the ends as broken DNA, thus providing chromosomal integrity (Griffith *et al*, 1999). If telomeres become critically short they loose their capping function, become sticky, and are prone to illegitimate chromosome end-to-end fusions. The resulting dicentric chromosomes are highly unusable and because of bridge-fusion-breakage cycles they give rise to chromosomal translocations, deletions, and amplifications. Thus, critically short telomeres are thought to be responsible for the onset of genomic instability. In addition, we provide evidence that in a length-independent manner telomeres can confer to genomic instability by forming telomeric aggregates which through chromosomal dys-locations contribute to chromosomal aberrations.

Key words: anaphase bridges/Bridge-Fusion Breakage cycle/telomeric aggregates/chromosomal abberations/telomeric length
J Investig Dermatol Symp Proc 10:89–94, 2005

Telomere Shortening During Cellular Aging

During proliferation of normal human somatic cells, telomere length progressively shortens and the cells finally arrest in a stage called replicative senescence. This termination of growth, also defined as the Hayflick (1965) limit, is now believed to be a consequence of one or a few critically short telomeres resulting from the continuous replication-dependent telomere erosion and triggering a signal cascade that causes a damage-dependent irreversible growth arrest (for a review, see Wright and Shay, 2002). This telomere shortening is a consequence of the so-called end replication problem. As shown by Olovnikov (1973) and Watson (1972), DNA polymerase α is not able to replicate the lagging strand DNA up to the outermost end and therefore with each replication cycle, a number of bp of telomeric DNA are lost. In addition, oxidative damage adds to telomere shortening (Saretzki and von Zglinicki, 2002), causing a 50–200 bp loss per cell generation. This continuous telomere loss is now thought to be the "mitotic clock" of cellular aging (Harley, 1991).

It was found that the stage of critically short telomeres correlated with a great number of telomeric end-to-end fusions, thus giving rise to dicentric chromosomes (reviewed in Mathieu *et al*, 2004). Nevertheless, it is still a matter of debate as to whether the stage of instable chromosomes is related to the onset of senescence, also called mortality stage 1, or requires additionally forced proliferation leading to additional telomere erosion with a mean final length significantly shorter than at senescence. At this second state, mortality stage 2, the cells enter crisis and most cells die (reviewed in Shay and Wright, 2005). Our studies did not provide evidence for a senescence-dependent chromosomal instability, either in human aging fibroblasts (Figueroa *et al*, 2000) or in human skin keratinocytes.[1] But we observed an increased number of abnormal chromosomes shortly after trypsinization of the cells, which disappeared again few days after passaging. We presently cannot exclude that the fragility of the chromosomes increases with aging, and that therefore the high frequency of aberrations determined by Benn (1976) one day after plating of the senescent fibroblasts may be related to a similar trypsinization-dependent process.

Telomere Length-Dependent Chromosomal Instability

Ducray *et al* (1999), on the other hand, analyzed SV-40-transfected cells, thereby overcoming the normal process leading to cellular senescence (M1) and studying changes related to the phase of prolonged growth, i.e., occurring between the M1 and M2 phase. When the replicative potential is extended either spontaneously or due to introduction of viral oncogenes (e.g., SV-40 or HPV), telomeres

Abbreviations: BFB, Bridge-Fusion-Breakage; TA, telomeric aggregrates, TRF2, telomere repeat binding factor 2

[1]Peter and Boukamp, unpublished observations.

continue to shorten. In agreement with telomeres becoming critically short, a high frequency of chromosome abnormalities were detected (Ducray *et al*, 1999). Accordingly, studies with telomerase-deficient mice had shown that in late-generation mice where the originally long telomeres (mean telomere length of about 50 kb) had finally shortened to a length similar to human telomeres, end-to-end chromosomal fusions were the most prominent aberrations (Blasco *et al*, 1997). Since chromosomes with the shortest telomeres were frequently involved, this also argued for critically short and therefore unprotected telomeres to be causal for the end-to-end fusions (Hemann *et al*, 2001). Uncapping of the telomeres by overexpressing a dominant-negative version of the a protein known to be involved in protecting the T-loop structure, the telomere repeat binding factor 2 (TRF2), finally demonstrated that it is not necessarily the shortest telomere. Instead, loss of the capping function of the telomeres resulted in chromosomal end-to-end fusions and with that in the formation of dicentric chromosomes (van Steensel *et al*, 1998).

It is now commonly discussed that this scenario, critically short telomeres forming dicentric chromosomes, is a first step of telomere length-dependent genomic instability (reviewed in Murnane and Sabatier, 2004). Dicentric chromosomes are highly unstable. If both centromeres are active during mitosis, each centromere may attach to a different pole, thus forming an anaphase bridge when the chromosomes are torn apart. As a result of this bipolar tension, breakage can occur all along the fused chromosomes. Thus, anaphase bridges are often viewed as a sign of genomic instability. Depending on whether both parts contain a centromere, this can easily result in loss of part of the chromosomes. In addition, and as extensively discussed by Murnane and Sabatier (2004), this can also lead to amplification cycles of certain chromosome parts. On the other hand, the telomere-free and highly recombinant DNA ends may invade other chromosomes, perhaps based on areas of micro-homology, and thereby causing non-reciprocal translocations. All this led to the proposal of a fusion-bridge-breakage cycle, also known as Bridge-Fusion-Breakage (BFB) model, as a primary mechanism of genomic instability in cells that suffer from critically short telomeres (reviewed in Chang *et al*, 2001; Mathieu *et al*, 2004). This BFB, which was first described by McClintock (1941), for *Zea mays* is now extensively used to explain the various translocation chromosomes found in tumor cells.

Additional Models of Telomere-Dependent Genomic Instability

Alternatively, Greider and co-workers suggest that end-to-end chromosome fusions may not initiate rearrangements but may rather be a secondary effect of end resection and thus represent stable by-products. Studying chromosomal rearrangements in diploid yeast strains, they found that chromosomal rearrangements predominantly occurred in the terminal region of the chromosome where exonuclease (Exo1p) had eliminated the telomere and allowed end-to-end fusions to occur (Hackett and Greider, 2003). Genes inserted at the ends of the chromosomes were eliminated

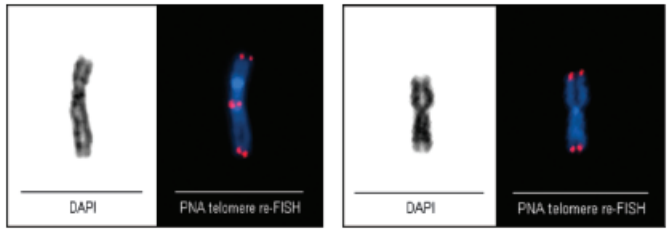

Figure 1
Translocation chromosomes with and without inter-chromosomal telomeres. As a result of the Fusion-Bridge-Breakage cycle, translocation chromosomes can establish which, on the one hand, are derived by telomeric fusion and still maintain telomeric sequences. On the other hand, the dicentric chromosomes can break and rejoin at non-telomeric sequences, giving rise to a translocation chromosome without intra-chromosomal telomeric sequences.

in a non-random fashion in Exo1p efficient yeast strains, whereas there were fewer chromosome rearrangements in yeast strains missing Exo1p. From this, they concluded that Exo1p plays a major role in the generation of these rearrangements and that end resection initiates genomic instability at dysfunctional telomeres (Hackett and Greider, 2003).

Along this line, Weinberg and co-workers propose that it is not the overall telomere length (critically short telomere) but loss of the single-strand overhang that is crucial, and that telomerase plays an important role in maintaining the 3′ overhang (Stewart *et al*, 2003). Thus, it still remains to be resolved as to which of these changes will be most important in causing genomic instability. It also cannot be excluded at present that different mechanisms may act together and thereby give rise to the different kinds of translocation chromosomes either maintaining or lacking intrachromosomal telomeres (Fig 1).

Telomere Length-Dependent Genomic Instability a Continuous Process?

Critically short and unprotected telomeres are supposed to be an initial event of genomic instability in cells lacking telomerase activity and, therefore, loss of capping function can be viewed as an early stage in the transformation process from normal to tumor cells. Furthermore, it is suggested that the fusions generated by the first BFB cycle could generate new unstable chromosomes, perpetuating the BFB cycle and chromosomal instability until no more open ends are available (extensively reviewed in Murnane and Sabatier, 2004). This implies that genomic instability induced by BFB is not a single event but may continue for quite some time.

Telomere-Dependent Changes in Telomerase-Positive Cells

Once telomerase is upregulated, the telomeres should be stabilized and tumor cells should be protected against "telomere length-dependent" genomic instability. Nevertheless, immortal and tumor cells are similarly prone to gen-

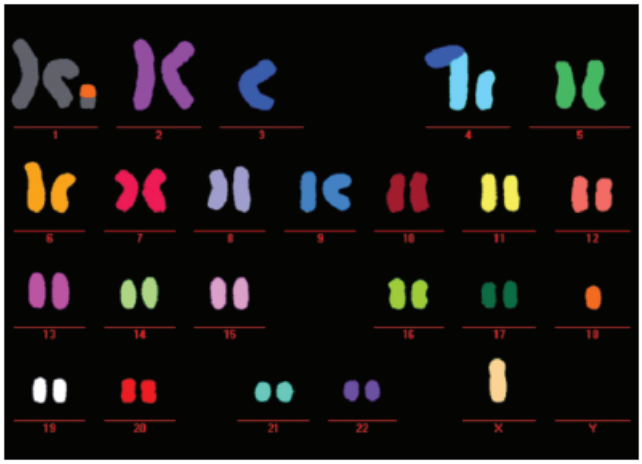

Figure 2
Pseudo-diploid karyotype of immortal human skin keratinocytes. Spontaneous immortalization of the HaCaT skin keratinocytes correlated with telomerase upregulation (Harle-Bachor and Boukamp, 1996) as well as expression of distinct chromosomal changes, t(1;18), t(3q;4q), del(4q), del(6p), and i(9q), as demonstrated by multicolor fluorescence *in situ* hybridization (m-FISH). These marker chromosomes were also maintained stable during continuous passaging of the cells.

omic instability. Interestingly, our observations indicate that immortal and tumor cells that exhibit a near-diploid karyotype are genetically rather stable. HaCaT skin keratinocytes immortalized spontaneously and selected for maintaining a pseudodiploid karyotype hardly showed any new chromosomal aberrations as measured by comparative genomic hybridization or multicolor fluorescence *in situ* hybridization (Boukamp *et al*, 1997[2]) (Fig 2). The primary karyotypic alterations established with immortalization were stablely maintained over many passages in culture. Once polyploidization had occurred, the cells were prone to a continuous, although low level of changes, thereby favoring the development of genetically divergent subpopulations (Boukamp *et al*, 1997).

These changes occurred in cells that were able to counteract telomere erosion by expressing the ribonucleoprotein complex telomerase (Greider and Blackburn, 1985). Telomerase is able to use its RNA component (in the human cells: hTR) to anchor to the outermost end of the telomere and to act as a template for the catalytic subunit hTERT, which synthesizes telomeric DNA, thus compensating for the replication-dependent telomere loss. In early studies, telomerase activity could only be detected in germ line cells and tumor cells, whereas it was absent in normal somatic cells (Kim *et al*, 1994). In the meantime, however, it is well established that normal regenerative tissues also express telomerase activity, although at a generally lower level than immortal cells or tumor cells (summarized in Bachor *et al*, 1999). Although it is still a matter of debate whether the level of telomerase activity is sufficient for maintaining telomere length or at least minimizing telomere erosion (Shay and Wright, 2005), our recent experiments suggest that in the epidermis, telomerase activity is not only present but may also be functionally important (Harle-Bachor and Boukamp, 1996).[3]

[2]Boukamp and Popp, unpublished observations.
[3]Moshir *et al*, in revision.

Telomerase expression by infection or transfection of hTERT, the limiting component of the telomerase complex, has been shown to prevent telomere shortening and to extend the life span of the normal cells for a variety of normal cells, thus being essential for indefinite cell growth (Bodnar *et al*, 1998; for a review, see Tominaga *et al*, 2002). hTERT-infected cells, however, often differ from those cells that have upregulated endogenous telomerase during tumor progression. Generally, telomerase causes a dynamic balance between telomere loss and telomere elongation, which results in a net telomere stabilization and length homogenization. hTERT infection or transfection often correlates with significant telomere elongation (Cerezo *et al*, 2003; Swiggers *et al*, 2004). In addition, evidence is increasing that upon exogeneous hTERT overexpression, telomerase exerts additional non-telomeric functions including changes in growth potential (Wang *et al*, 1998; Mondello *et al*, 2003), differentiation (Cerezo *et al*, 2003), induction of a premalignant phenotype (Milyavsky *et al*, 2003), and even increased tumorigenic potential in mice (Artandi *et al*, 2002; Gonzalez-Suarez *et al*, 2002). Different from early reports that highlighted the phenotypic and genotypic integrity of hTERT-dependent cell immortalization (Morales *et al*, 1999), the role of telomerase is now being explored more broadly. So far, it is still not known as to what causes the additional telomerase effect. It thus remains to be determined whether these extra activities are a consequence of high levels of hTERT itself, an unusually high level of telomerase activity, or due to the fact that hTERT is no longer under the control of cell growth and differentiation-specific regulations.

Insults Causing Genomic Instability in Telomerase-Positive Cells

Genomic instability cannot always be explained by the fact that once it occurs, it is transmitted to daughter cells and is self-perpetuating. Instead, other mechanisms are likely to contribute to genomic instability as well. One mechanism that is frequently discussed is centrosome duplication. Carroll *et al* (1999) suggested that p53 mutations as well as MDM2 overexpression, induced aneuploidy through centrosome amplification. Similarly, Munger and co-workers provided evidence that HPV-induced genomic instability is in part mediated through centrosome amplification (reviewed in Duensing and Munger, 2004). It has long been known that HPV-16 E6 can cause structural chromosomal changes (White *et al*, 1994), whereas E7 is responsible for aneuploidy (Hashida and Yasumoto, 1991; White *et al*, 1994). Correspondingly, they showed that E7 is responsible for abnormal centrosome synthesis (Duensing *et al*, 2001), and that E6 and E7 cooperated in inducing genomic instability by uncoupling centrosome duplication from cell division (Duensing *et al*, 2000).

Similar to HPV, c-Myc deregulation is also thought to induce genomic instability and centrosome duplication may be one potential mechanism (Mai and Mushinski, 2003). Evidence is increasing that an important consequence of deregulated *c-myc* expression is the induction of gene amplification and gene rearrangements as well as karyotypic instability leading to aneuploidy and structural chromosom-

al aberrations (for a review, see Mai and Mushinski, 2003). The most intriguing features of c-Myc-induced alterations are their reversibility. Using inducible systems, genomic instability proved to be transient upon a single induction of c-Myc *in vitro* and *in vivo* (Mai *et al*, 1996; Felsher and Bishop, 1999). Continued c-Myc activation, on the other hand, was described to be accompanied by aneuploidy and structural chromosomal changes such as extra-chromosomal elements and chromosomal breakage but also centromere–telomere fusions. (Kuschak *et al*, 1999).

Telomeric Aggregates an Additional Mechanism of Telomere-Dependent Genomic Instability

We have recently started to study the telomere distribution in the 3D nucleus by using 3D fixation, deconvolution microscopy, and reconstruction of the 3D structure by Amira software (Indeed-Visual Concepts GmbH, Berlin, Germany). This method allowed to determine that the telomere organization is cell cycle dependent, with assembly of the telomeres in a highly defined position during the G2 phase, termed a telomeric disc (Chuang *et al*, 2004). In some tumor cells, this organization was distorted due to the formation of telomeric aggregates (TA). These TA, which could be of different size and number, were found to be present in all tumor cells with known dysregulated c-Myc. In addition, when comparing HaCaT cells and HaCaT cells constitutively expressing the *c-myc* gene (HaCaT-myc), we could further show that TA were present in the HaCaT-myc cells also during the different phases of mitosis (Ermler *et al*, 2004). This suggests that these aggregated telomeres are now segregated as a group during mitosis (Fig 3). Depending on the chromosome distribution within the TA, they are likely to cause an uneven chromosome distribution during mitosis, leading to gain of chromosome(s) in one and loss in the other daughter cell. Thus, TA are likely to contribute to aneuploidy.

Most recent studies further suggest that in addition to numerical abnormalities, TA also contribute to structural chromosomal aberrations. In fact, we have evidence that TA formation is also of consequence for the chromosome involved in the TA. By causing an overlap of chromosomal territories upon TA induction, different chromosomes now come into such close vicinity that translocations between parts of different chromosomes can occur (Louis *et al*, 2005). Different from uncapping of the telomeres, which can also be experimentally induced in long telomeres by eliminating TRF2 (van Steensel *et al*, 1998), we have no evidence so far that TRF2 is involved in TA formation (Ermler *et al*, 2004). One major function of TRF2 is to stabilize the 3′ overhang, and with this, to protect the chromosomes from end-to-end fusions. Correspondingly, when TRF2 was inhibited, a high percentage of the telomeres were fused, resulting in dicentric chromosomes and chromatid dicentrics (van Steensel *et al*, 1998). Although the level of TRF2 was reduced to only 50% in HaCaT-myc cells that contained TA in $>60\%$ of the nuclei, double-labeling studies of telomeric DNA and TRF2 protein unequivocally demonstrate co-localization of TRF2 on all telomeres including the TA (Ermler *et al*, 2004). Taken together, our findings are in support of the hypothesis that TA formation is a length-independent mechanism of telomere-dependent chromosomal instability by which aneuploidy as well as structural chromosomal aberrations (translocation chromosomes) can be generated. Most importantly, TA formation may follow transfection of certain genes, such as the *c-myc* oncogene, suggesting that TA-dependent genomic instability can occur *de novo* in tumor cells at any stage of carcinogenesis and thereby allow these cells to develop genetically divergent subpopulations—a hallmark of tumor development and progression.

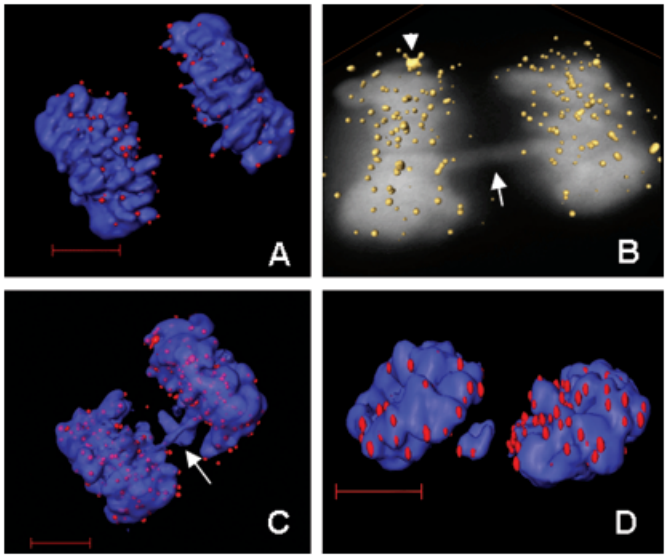

Figure 3
Telomere aggregates contribute to genomic instability. (*A*) Three-dimensional (3D) distribution of chromosomes and telomeres during anaphase of HaCaT cells showing a normal chromosome (in *blue*) and telomere (in *red*) distribution. (*B*) Telomeres in yellow (3D) are shown on the projected anaphase chromosomes (two-dimensional). Note the telomeric aggregate (marked by *arrowhead*) and the connected chromosomes forming an anaphase bridge (marked by *arrow*). (*C*) 3D distribution of chromosomes (in *blue*) and telomeres (in *red*) in a cell with an anaphase bridge. Also note the extra chromosome that will be lost during mitosis and is likely to give rise to extra-chromosomal DNA (micronucleus) in one of the daughter cells. (*D*) 3D distribution of telomeres and DNA in telophase. Note that a complete chromosome will be lost.

Conclusion

Telomeres are likely to be involved in genomic instability by a number of means. On the one hand, progressive telomere shortening and the eventual critical short length are implicated in uncapping of the telomeres. This is thought to be causal for end-to-end fusions, leading to BFB cycles, characterized by chromosome breakage, loss or amplification of parts of the chromosome, and rejoining with other chromosomes, i.e., generation of structural chromosomal aberrations. On the other hand, telomeres can also form aggregates that in turn cluster their respective chromosomes, and may thereby favor uneven chromosome distribution during mitosis with loss or gain of entire chromosomes as well as inappropriate proximity of the chromosome, allowing exchange of chromosomal material

(structural aberrations). A better understanding of both mechanisms of telomere-dependent genomic instability is essential for designing specific drugs that are able to interfere with these processes. At present, a first telomerase inhibitor is in clinical trial aiming to stop tumor growth by inhibiting telomerase and pushing the cells into a telomere loss-dependent senescence or apoptosis. Alternatively, or in addition, there is a need to fight telomere-dependent genomic instability in order to hinder the development of genetically divergent subpopulations, i.e., to hinder tumor heterogeneity.

The authors wish to thank Dr Jackie R. Bickenbach for her helpful comments and Angelika Lampe for her help in editing the manuscript. This work was in part supported by the European Union (TACIT and MOL CANCER MED), as well as the Deutsche Krebshilfe eV and Tumorzentrum Heidelberg/Mannheim.

DOI: 10.1111/j.1087-0024.2005.200401.x

Manuscript received December 30, 2004; revised February 15, 2004; accepted for publication March 9, 2005

Address correspondence to: Petra Boukamp, German Cancer Research Center, Division of Genetics of Skin Carcinogensis, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. Email: p.boukamp@dkfz-heidelberg.de

References

Artandi SE, Alson S, Tietze MK, *et al*: Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci USA 99:8191–8196, 2002

Bachor C, Bachor OA, Boukamp P: Telomerase is active in normal gastrointestinal mucosa and not up-regulated in precancerous lesions. J Cancer Res Clin Oncol 125:453–460, 1999

Benn PA: Specific chromosome aberrations in senescent fibroblast cell lines derived from human embryos. Am J Hum Genet 28:465–473, 1976

Blackburn EH: Switching and signaling at the telomere. Cell 106:661–673, 2001

Blasco MA, Lee HW, Rizen M, Hanahan D, DePinho R, Greider CW: Mouse models for the study of telomerase. Ciba Found Symp 211:160–170, 1997

Bodnar AG, Ouellette M, Frolkis M, *et al*: Extension of life-span by introduction of telomerase into normal human cells. Science 279:349–352, 1998

Boukamp P, Popp S, Altmeyer S, Hulsen A, Fasching C, Cremer T, Fusenig NE: Sustained nontumorigenic phenotype correlates with a largely stable chromosome content during long-term culture of the human keratinocyte line HaCaT. Genes Chromosomes Cancer 19:201–214, 1997

Carroll PE, Okuda M, Horn HF, *et al*: Centrosome hyperamplification in human cancer: Chromosome instability induced by p53 mutation and/or Mdm2 overexpression. Oncogene 18:1935–1944, 1999

Cerezo A, Stark HJ, Moshir S, Boukamp P: Constitutive overexpression of human telomerase reverse transcriptase but not c-myc blocks terminal differentiation in human HaCaT skin keratinocytes. J Invest Dermatol 121: 110–119, 2003

Chang S, Khoo C, DePinho RA: Modeling chromosomal instability and epithelial carcinogenesis in the telomerase-deficient mouse. Semin Cancer Biol 11:227–239, 2001

de Lange T: T-loops and the origin of telomeres. Nat Rev Mol Cell Biol 5:323–329, 2004

Ducray C, Pommier JP, Martins L, Boussin FD, Sabatier L: Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process. Oncogene 18:4211–4223, 1999

Duensing S, Duensing A, Crum CP, Munger K: Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. Cancer Res 61:2356–2360, 2001

Duensing S, Lee LY, Duensing A, *et al*: The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. Proc Natl Acad Sci USA 97:10002–10007, 2000

Duensing S, Munger K: Mechanisms of genomic instability in human cancer: Insights from studies with human papillomavirus oncoproteins. Int J Cancer 109:157–162, 2004

Ermler S, Krunic D, Knoch TA, Moshir S, Mai S, Greulich-Bode KM, Boukamp P: Cell cycle-dependent 3D distribution of telomeres and TRF2 in HaCaT and HaCaT-myc cells. Eur J Cell Biol 83:681–690, 2004

Felsher DW, Bishop JM: Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc Natl Acad Sci USA 96:3940–3944, 1999

Figueroa R, Lindenmaier H, Hergenhahn M, Nielsen KV, Boukamp P: Telomere erosion varies during *in vitro* aging of normal human fibroblasts from young and adult donors. Cancer Res 60:2770–2774, 2000

Gonzalez-Suarez E, Flores JM, Blasco MA: Cooperation between p53 mutation and high telomerase transgenic expression in spontaneous cancer development. Mol Cell Biol 22:7291–7301, 2002

Greider CW, Blackburn EH: Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43:405–413, 1985

Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T: Mammalian telomeres end in a large duplex loop. Cell 97:503–514, 1999

Hackett JA, Greider CW: End resection initiates genomic instability in the absence of telomerase. Mol Cell Biol 23:8450–8461, 2003

Harle-Bachor C, Boukamp P: Telomerase activity in the regenerative basal layer of the epidermis inhuman skin and in immortal and carcinoma-derived skin keratinocytes. Proc Natl Acad Sci USA 93:6476–6481, 1996

Harley CB: Telomere loss: Mitotic clock or genetic time bomb? Mutat Res 256:271–282, 1991

Hashida T, Yasumoto S: Induction of chromosome abnormalities in mouse and human epidermal keratinocytes by the human papillomavirus type 16 E7 oncogene. J Gen Virol 72:1569–1577, 1991

Hayflick L: The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res 37:614–636, 1965

Hemann MT, Strong MA, Hao LY, Greider CW: The shortest telomere, not average telomerelength, is critical for cell viability and chromosome stability. Cell 107:67–77, 2001

Kim NW, Piatyszek MA, Prowse KR, *et al*: Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015, 1994

Kuschak TI, Taylor C, McMillan-Ward E, *et al*: The ribonucleotide reductase R2 gene is a non-transcribed target of c-Mycinduced genomic instability. Gene 238:351–365, 1999

Louis SF, Vermolen BJ, Garini Y, *et al*: C-myc induces chromosomal rearrangements through telemere and chromosome remodelling in the interphase nucleus. Proc Nat Acad Sci, in press, 2005

Mai S, Fluri M, Siwarski D, Huppi K: Genomic instability in MycER-activated Rat1AMycER cells. Chromosome Res 4:365–371, 1996

Mai S, Mushinski JF: c-Myc-induced genomic instability. J Environ Pathol Toxicol Oncol 22:179–199, 2003

Mathieu N, Pirzio L, Freulet-Marriere MA, Desmaze C, Sabatier L: Telomeres and chromosomal instability. Cell Mol Life Sci 61:641–656, 2004

McClintock B: The stability of broken ends of chromosomes in Zea mays. Genetics 26:234–282, 1941

Milyavsky M, Shats I, Erez N, *et al*: Prolonged culture of telomerase-immortalized human fibroblasts leads to a premalignant phenotype. Cancer Res 63: 7147–7157, 2003

Mondello C, Chiesa M, Rebuzzini P, *et al*: Karyotype instability and anchorage-independent growth in telomerase-immortalized fibroblasts from two centenarian individuals. Biochem Biophys Res Commun 308:914–921, 2003

Morales CP, Holt SE, Ouellette M, *et al*: Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 21:115–118, 1999

Murnane JP, Sabatier L: Chromosome rearrangements resulting from telomere dysfunction and their role in cancer. Bioessays 26:1164–1174, 2004

Olovnikov AM: A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol 41:181–190, 1973

Saretzki G, von Zglinicki T: Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci 959:24–29, 2002

Shay JW, Wright WE: Senescence and immortalization: Role of telomeres and telomerase. Carcinogenesis 26:867–874, 2005

Stewart SA, Ben Porath I, Carey VJ, O'Connor BF, Hahn WC, Weinberg RA: Erosion of the telomeric single-strand overhang at replicative senescence. Nat Genet 33:492–496, 2003

Swiggers SJ, Nibbeling HA, Zeilemaker A, Kuijpers MA, Mattern KA, Zijlmans JM: Telomerase activity level, but not hTERT mRNA and hTR level, regulates telomere length in telomerase-reconstituted primary fibroblasts. Exp Cell Res 297:434–443, 2004

Tominaga K, Olgun A, Smith JR, Pereira-Smith OM: Genetics of cellular senescence. Mech Ageing Dev 123:927–936, 2002

van Steensel B, Smogorzewska A, de Lange T: TRF2 protects human telomeres from end-to-end fusions. Cell 92:401–413, 1998

Wang J, Xie LY, Allan S, Beach D, Hannon GJ: Myc activates telomerase. Genes Dev 12:1769–1774, 1998

Watson JD: Origin of concatemeric T7 DNA. Nat New Biol 239:197–201, 1972

White AE, Livanos EM, Tlsty TD: Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. Genes Dev 8:666–677, 1994

Wright WE, Shay JW: Historical claims and current interpretations of replicative aging. Nat Biotechnol 20:682–688, 2002