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Telomeres and telomerase in cancer stem cells

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ABSTRACT

Alterations in telomere dynamics both suppress and facilitate malignant transformation by regulating genomic stability and cell lifespan. Checkpoints induced by telomere dysfunction play a major role in tumour suppression, whereas telomere shortening contributes to the initiation of cancer by inducing chromosomal instability. Since stem cells are exposed to various tumourigenic agents and stresses throughout their lifetime, the ageing stem cell is a major target of malignant transformation. This review summarises our knowledge of telomere length and telomerase activity in stem cells during ageing and carcinogenesis.

1. Telomere shortening: a dual role in cancer

Telomeres are nucleo-protein complexes composed of tandem DNA repeats and associated telomere binding proteins.[1] The main function of telomeres is to cap eukaryotic chromosome ends and protect chromosomes from degradation, fusions and instability.[2–5] Due to the end-replication problem of DNA polymerase and processing of telomere ends during the S-phase of the cell cycle, telomeres shorten during each round of cell division.[6,7] Telomere shortening limits the proliferative capacity of primary somatic human cells to 50–80 cell divisions.[8] Upon reaching a critically short length, dysfunctional telomeres induce replicative senescence, characterised by permanent cell cycle arrest and activation of DNA damage signalling pathways.[9] Telomere shortening is prevalent in most human organs and tissues during ageing.[10] There is growing evidence that telomere shortening contributes to limit the regenerative capacity of organs during ageing and chronic high-turnover diseases.[11] Originally it was proposed that telomere shortening and senescence represent a tumour suppressor mechanism limiting the growth of transformed cells.[12] According to this hypothesis cancer cells would have to re-activate telomerase – the enzyme that can synthesize telomere sequence de novo – in order to grow tumours.[13] Telomerase consists of two essential components: the telomerase reverse transcriptase (TERT) – catalytic subunit of the enzyme,[14,15] the telomerase RNA component – functional RNA serving as a template for telomere sequence synthesis.[16] In humans, telomerase activity is tightly regulated, displaying strict developmental and tissue specificity. It is readily detectable during embryogenesis and is suppressed postnatally in most tissues. In adults, telomerase remains active only in immature germ cells, certain stem and progenitor cell compartments. In line with the hypothesis that telomerase is

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required for cancer formation,[12] over 80% of human cancers show a re-activation of telomerase catalytic activity.[17]

In contrast to the model that telomerase repression and telomere shortening suppresses tumour formation, cancer risk sharply increases in response to telomere shortening during ageing and chronic disease.[10] Moreover, telomeres are significantly shortened in cancer cells compared with non-transformed surrounding cells.[18,19] Studies in telomerase deficient (mTERC$^{-/-}$) mice found an explanation for this apparent paradox: In mTERC$^{-/-}$ mice, telomere shortening had a dual role in tumourigenesis, enhancing the initiation of early tumours,[20] but at the same time inhibiting tumour progression and the development of macroscopic advanced tumours.[20–23]

2. Telomere shortening, chromosomal instability and cancer initiation

The normal ends of linear chromosomes and internal DNA double strand breaks (DSBs) are both potential substrates for DSB repair enzymes. Telomeres serve a critical function to differentiate chromosome termini from internal DSBs, thus avoiding chromosome instability due to inappropriate activation of DNA-repair reactions. However, critically short telomeres lose chromosome capping function, resulting in an activation of the DNA DSB repair machinery and chromosome fusions.[2,25] When cells with fused chromosomes enter the cell cycle the fusion has to be disrupted during the anaphase of mitosis, resulting in chromatin bridges spanning between the moving plates of sister chromosomes ('anaphase bridges').[20,26] Chromosomes lacking one telomere remain unstable until they are capped. The breakage of chromosomal fusion newly generates telomere-free chromosomal ends, which will fuse and break again during the next round of cell division. This cascade of fusion-bridge-breakage cycles results in chromosomal gains and losses and translocation of chromosome fragments.[24] Chromosomal instability is a hallmark of carcinogenesis in humans.[27] Findings in mTERC$^{-/-}$-deficient mice gave the first experimental evidence that telomere dysfunction can be a pro-tumourigenic event by causing chromosomal instability. In mTERC$^{-/-}$ mice telomere shortening increased the rate of tumour initiation by inducing chromosomal instability.[20,24] In human cancer, telomere shortening has been observed in very early tumour stages,[19] indicating that tumours arise from cells with telomere dysfunction. In line with this hypothesis a sharp increase in anaphase bridges was observed at the adenoma-carcinoma-transition in human colorectal carcinogenesis.[20] In addition, development of chromosomal instability and increasing cancer risk were associated with telomere shortening at the end stage of chronic diseases such as ulcerative colitis.[28] In human hepatocellular carcinoma (HCC) telomere shortening correlated with the grade of tumour cell aneuploidy[18] and the development of specific chromosomal gains that are characteristic for HCC.[29]

3. Telomere dysfunction, DNA-damage response and cancer suppression

Studies in mTERC$^{-/-}$ mice have shown that, in addition to increasing tumour initiation, telomere shortening also suppressed tumour progression.[22,23] Telomere dysfunction induces DNA-damage pathways leading to senescence. The DNA-damage pathways that are induced by telomere dysfunction are similar to those induced by irradiation, including the activation of ATM/ATR/p53/Chk2/p21/p16.[25,30,31] In agreement with the role of telomere dysfunction during cancer initiation, evidence for an up-regulation of DNA-damage response occurs in early stage tumours in humans[32] and telomerase knockout mice.[20] Activation of DNA-damage responses suppresses cell proliferation and induces apoptosis thereby inhibiting the progression of tumours harbouring dysfunctional telomeres.[20] At this stage telomere dysfunction and genomic instability select for loss of DNA-damage checkpoint function. In line with this hypothesis, mTERC$^{-/-}$ mice carrying a deletion of one p53 allele developed high rates of epithelial cancers showing loss of heterozygosity (LOH) of the wild-type p53 allele.[24] Cell culture studies have shown that deletion of p53 abrogates the senescence checkpoint in response to telomere shortening, thus extending the lifespan of primary human cells beyond the senescence checkpoint.[12] However, further telomere shortening occurs in cells that bypass the senescence checkpoint and eventually leads to the activation of a second p53-independent checkpoint called 'crisis'.[12] Crisis is characterised by extremely short telomeres, rampant chromosomal instability and high rates of cell death.[12] Similar to the cell culture-based data, p53 deletion rescued phenotypes of impaired organ homeostasis induced by telomere shortening in mTERC$^{-/-}$ mice.[33] However, studies in mTERC$^{-/-}$ p53$^{+/-}$ compound mutant mice revealed that loss of p53 co-operates with telomere dysfunction to induce chromosomal instability and cancer initiation.[24] These data provide a good explanation why in human cancer a combination of telomere shortening and loss of p53 function occurs frequently. For example, transition through the chromosome instability associated telomere crisis appears to be a crucial event in the progression of most breast carcinomas from hyperplasia stage.[34] However, recent experimental data have revealed that p53-independent induction of cell death occurs in vivo in response to high cellular levels of telomere dysfunction.[35] According to these findings human tumours need to stabilize telomeres in order to achieve tumour progression. In most human cancers telomere stabilization is achieved by re-activation of telomerase.[13,36] However, 10–20% of human tumours maintain telomeres by alternative lengthening of telomeres (ALT).[37] Together the above data suggest a two-stage model of telomere shortening followed by telomerase activation and telomere stabilization during human carcinogenesis (Fig. 1).

The role of telomerase in cancer progression has been assumed to be restricted to telomere stabilization, thereby preventing telomere exhaustion. However, recent evidence points to a novel function of TERT contributing to tumourigenesis in a manner independent of telomere-stabilization. Transgenic mice that expressed telomerase reverse transcriptase in skin keratinocytes were more susceptible to the development of skin tumours upon chemical carcinogenesis in a manner independent of net telomere lengthening.[38] Similarly, constitutive TERT-expression in a broad variety of tissues in another transgenic mouse strain promoted spontaneous development of mammary intraepithelial neoplasia and invasive mammary carcinomas independent of telomere length.[39]

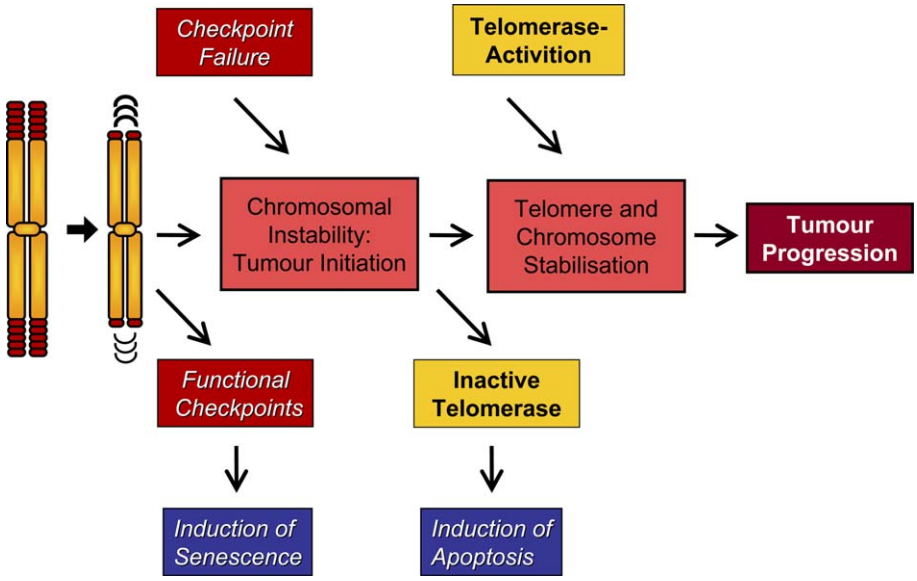

Fig. 1 – Telomere shortening and telomerase activation have a dual role in cancer. Telomere shortening during ageing and chronic disease lead to telomere dysfunction and induction of senescence checkpoints. Checkpoint failure co-operates with telomere dysfunction to induce chromosomal instability and cancer initiation. Initiated cancer cells have to stabilize telomeres and ongoing instability in order to survive and progress. Most human cancers achieve telomere stabilization by activating telomerase.

In addition, a hairpin siRNA specifically targeting human telomerase RNA rapidly inhibited growth of human cancer cells independently of p53 or telomere length.[40] One explanation is that telomerase may affect proliferation of cells not only by stabilizing telomeres, but also by affecting the expression of growth-controlling genes.[41] Telomerase RNA knock-down showed rapid growth inhibition in cancer cells involving multiple pathways that have been implicated in angiogenesis and metastasis.[42] In human cancer, telomerase activity is associated with a more aggressive tumour phenotype.[17,43,44] It remains to be analysed whether telomere-independent effects of telomerase play a functional role during carcinogenesis in humans. The fact that most human stem cells show a constitutive expression of telomerase yet retain normal cell proliferation control (see below) argue against telomere independent functions of telomerase during carcinogenesis.[45]

4. Telomeres and telomerase in stem cell cancer

The first direct evidence that stem cells are the origin of human cancer came from studies on haematopoietic malignancies.[46] Leukaemia has been shown to originate from leukaemic stem cell (LSC) and these LSCs were required to transplant leukaemia from patients into xenotranplant mouse models.[47] However, it has also been shown, that committed haematopoietic progenitors can transform into mixed lineage leukaemia (MLL), indicating that cancer stem cells do not necessarily derive from multipotent stem cells.[48,49] Some authors suggest that retention of self-renewal capacity in transformed stem cells is a more likely event leading to cancer formation than reactivation of an immortality program in transforming differentiated cells.[50] Nevertheless, it is still a matter of debate whether solid cancers derive from single stem cells, committed progenitor cells, or differentiated cells, although, the identification of tumour-initiating cells in human breast and brain cancers provides growing evidence supporting a stem cell origin of solid cancer.[51,52] In light of these findings it appears to be of great importance to elucidate the role of telomeres and telomerase in cancer stem cells. There is some evidence from haematological malignancies that the above model of a dual role of telomere shortening and telomerase re-activation during cancer initiation and progression (Fig. 1) also applies to cancer stem cells. On one hand, telomere shortening might be involved in the development of chromosomal instability and transformation of stem cells. On the other hand, tumour stem cells might require telomerase activation to achieve immortal self-renewal capacity.

5. Telomeres and telomerase in stem cells

In contrast to somatic tissues, most human stem cell compartments possess low levels of telomerase activity.[53] Despite these levels of telomerase activity, haematopoietic stem cells (HSC) show telomere shortening during in vitro culture[54,55] and in vivo ageing.[56] Since stem cell compartments undergo low but constant rates of cell turnover during their lifetime, telomerase expression in stem cells might be required to fulfil this challenge. However, the level of telomerase activity in stem cells apparently is not sufficient to maintain telomere length during stem cell ageing. Thus the proliferative capacity of stem cells is limited by telomere shortening. In addition, there is growing evidence that telomere shortening of stem cell compartments plays a causative role in ageing. Several gene mutations linked to different forms of the rare genetic disease Dyskeratosis Congenita have been identified.[57] These gene mutations led to a reduced telomerase expression and

activity in these patients, which was associated with telomere shortening, stem cell exhaustion, bone marrow failure and premature death.[57,58] These findings indicate that telomerase activity is in fact necessary for normal stem cell function in humans and that telomerase dysfunction leads to rapid telomere shortening, stem cell exhaustion and premature ageing. In addition, studies in mTERC$^{-/-}$ mice have provided experimental evidence that telomere shortening impacts on stem cell function in different organ systems.[11] In this experimental system reduced stem cell function was associated with impaired organ homeostasis of high-turnover organs, reduced longevity, impaired organ regeneration, and impaired stress responses during ageing.[11,21,59,60] The innate response pathways of stem cells to telomere dysfunction remain to be investigated since they might be distinct from what occurs in somatic cells (see above). It is not yet clear whether stem cells enter senescence or undergo apoptosis in response to telomere dysfunction. Studies on embryonic stem cells have revealed significant differences in DNA-damage checkpoints activated by irradiation in comparison with somatic cells.[61,62] Whether these findings apply to the DNA damage response induced by telomere dysfunction in embryonic or adult stem cells remains to be analysed.

6. Telomeres and telomerase in cancer stem cells

Most direct evidence for the hypothesis of cancer stem cells has come from study on haematological neoplasia (see above). Similar to the findings in solid organ cancer (see above) and in agreement with the hypothesis that telomeres have a dual role in cancer (Fig. 1), a combination of shortened telomeres and increased telomerase activity is seen in most haematological neoplasia.[63,64] Haematological neoplasia can be divided into pre-malignant, chronic and acute stages, the last being the most advanced and most malignant stage of the disease. It is known that pre-malignant disease stages, such as myelodysplasia (MDS), and chronic malignant disease stages, such as chronic myeloid or lymphoid leukaemia (CML or CLL), can progress into more malignant and acute stages of disease, e.g. acute lymphoid or myeloid leukaemia (AML or ALL).[65]

In general, telomere shortening appears to correlate with disease history,[66] evolution of cytogenetic abnormalities,[67] and the risk of disease progression towards acute disease stages.[68] Telomere loss is rapid during the progression of CML. Patients in late chronic phase (CP) had significantly shorter telomeres than those assessed earlier in CP.[68] Cells from patients in accelerated phase or blast phase (AP/BP) showed significantly shorter average telomere length than cells from patients in CP or cytogenetic remission. Patients in CP who subsequently developed blast phase (BP) within 2 years had significantly shorter telomeres than those who did not develop BP for at least 2 years.[69] CML is characterised by a marked expansion of myeloid Philadelphia chromosome positive (Ph+) cells. Ph+ peripheral blood leukocytes from patients with CML showed accelerated telomere shortening compared with blood leukocytes from normal individuals or normal Ph(−) T lymphocytes from the same individuals.[69] MDS patients with shortened telomeres showed a high incidence of cytogenetic abnormalities[70] and a poor prognosis with high rates of leukaemic transformation.[71] Together, these data indicate that telomere shortening is a risk factor that contributes to the development of chromosomal instability and malignant transformation in HSCs. In agreement with this hypothesis telomere shortening in patients with Dyskeratosis Congenita correlates with an increased risk of haematological neoplasia.[72]

In contrast to the apparent role of telomere shortening in cancer stem cell initiation, telomerase activation occurs in most human haematological neoplasia, indicating that telomerase activation and telomere stabilization are necessary for cancer stem cell progression. Telomerase activation in haematological neoplasia correlates with disease progression, poor prognosis and the risk of recurrence in treated patients.[63,64] Compared with haematopoietic progenitor cells from normal donors, telomerase activity was increased 2- to 5-fold in CP-AML, CML, CLL, polycythemia vera (PV), or MDS. In AML, AP and BP-CML, telomerase activity was 10- to 50-fold higher than normal.[73] Similarly progression of MDS into AML correlated with a striking increase in telomerase activity.[74] Together these data indicate that telomerase activation is necessary for progression in haematopoietic malignancies; possibly by stabilizing telomeres and chromosomes in the setting of massive chromosomal instability at the transition from early (pre-) malignant disease stage toward acute and fully malignant leukaemia.

7. Outlook

Telomere shortening and telomerase activation appear to have a dual role in cancer formation. On one hand, telomere shortening is associated with the development of chromosomal instability and cancer initiation. On the other hand, tumour progression requires telomerase activation to stabilize telomere attrition, thus preventing rampant chromosomal instability associated with telomere-induced crisis. This hypothesis explains the coexistence of telomere shortening and telomerase activation in the vast majority of human cancers, including haematological malignancies. Since haematological malignancies derive from transformed HSCs the hypothesis of a dual role of telomere shortening and telomerase activation during carcinogenesis appears to apply to stem cell cancer.

To better understand the role of telomeres and telomerase in stem cell cancer it is important to analyze the consequences of telomere dysfunction and telomerase activation in stem cells. The identification of better surface markers for HSC[75] and organ stem cells could help to characterise telomerase activity and telomere shortening in stem cell compartments in vivo during ageing and disease. It is not yet clear whether stem cells show the same two-mortality-stage checkpoint response to different levels of telomere dysfunction that is seen in somatic cells.[12] In somatic cells a p53 dependent senescence checkpoint is induced in response to low levels of telomere dysfunction, but high levels of telomere dysfunction induce p53-independent apoptosis.[12,35] Differences might occur since embryonic stem cells activate different checkpoints in response to DNA damage as compared with somatic cells (see above). However, in mTERC$^{-/-}$

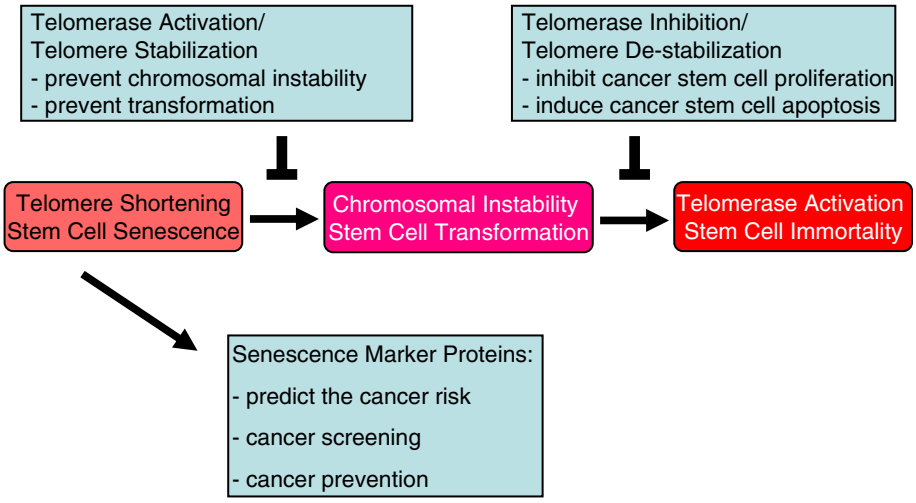

Fig. 2 – The telomere model of cancer stem cell initiation and progression predicts several therapeutic targets to improve cancer screening, prevention and treatment.

mice p53 deletion rescued apoptosis of germ cells, indicating that p53 plays an important role in stem cell checkpoints induced by telomere shortening.[33] It seems possible that checkpoints induced by telomere dysfunction and regulation of telomerase activity differ in different types of stem cells. For example it has been shown that human mesenchymal stem cells are telomerase-negative, which is different from intestinal stem cells and haematopoietic stem cells.[76] Combining fluorescence *in situ* hybridisation (FISH) for telomere length measurement and analysis of chromosome copy numbers has revealed a striking correlation between telomere shortening and chromosomal instability at the single cell level of human cancer.[29] It will be interesting to analyze whether the same holds true for cancer stem cells and whether this would fit with the clonality of tumours. If the mass of tumour cells comes from a single cancer stem cell, the cytogenetic profile of the tumour cells should be monoclonal. In contrast, tumours derived from multiple stem cells should exhibit multiclonality. Since the telomere dynamics represent the mitotic history of highly proliferating organs, analysing the telomere length, telomerase activity and cytogenetic profiles in defined cancer stem cell population could be helpful to exploit the origin of tumour-initiating cells and trace the development history of tumour formation. Finally, from a clinical standpoint, it is important to analyze whether telomeres and telomerase represent promising targets to attack cancer stem cells. In general three concepts seem to be promising (Fig. 2): (i) inhibition of telomerase to limit proliferation and induce apoptosis of cancer stem cells; (ii) stabilization of telomeres in pre-malignant diseases to inhibit the evolution of chromosomal instability and transformation of cancer stem cells; and (iii) identification of senescence marker proteins in stem cells to predict the cancer risk and to optimise screening and therapeutic interventions for cancer prevention.

A good deal of progress has been made in many fields of cancer biology. The cancer stem cell concept is one of the most intriguing areas of future cancer research. To extend our understanding of different areas of cancer biology to the stem cell compartment will surely disclose new avenues for the understanding of cancer pathology and treatment.

Conflict of interest statement

None declared.

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