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The potential utility of telomere-related markers for cancer diagnosis

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Abstract

The role telomeres and telomerase play in the initiation and progression of human cancers has been extensively evaluated. Telomeres are nucleoprotein complexes comprising the hexanucleotide DNA repeat sequence, TTAGGG and numerous telomere-associated proteins, including the six member Shelterin complex. The main function of the telomere is to stabilize the ends of the chromosomes. However, through multiple mechanisms, telomeres can become dysfunctional, which may drive genomic instability leading to the development of cancer. The majority of human cancers maintain, or actively lengthen, telomeres through up-regulation of the reverse transcriptase telomerase. Because there are significant differences in telomere length and telomerase activity between malignant and non-malignant tissues, many investigations have assessed the potential to utilize these molecular markers for cancer diagnosis. Here, we critically evaluate whether measurements of telomere lengths and telomerase levels may be clinically utilized as diagnostic markers in solid tumours, with emphasis on breast and prostate cancer as representative examples. Future directions focusing on the direct detection of dysfunctional telomeres are explored. New markers for telomere dysfunction may eventually prove clinically useful.

Keywords: breast cancer • cancer • detection • diagnosis • prostate cancer • telomerase • telomere • telomere dysfunction

Introduction

Telomeres are nucleoprotein complexes located at the extreme ends of eukaryotic chromosomes [1]. In normal human somatic cells, telomeres comprise 5–12 kb of the repeating hexanucleotide DNA sequence, TTAGGG [2, 3]. Numerous proteins are associated with these repetitive regions. The Shelterin complex, a core set of six proteins integral for telomere function, is composed of telomeric repeat binding factor (TRF)1, TRF2, protection of telomeres 1 (POT1), TRF interacting protein 1 (TIN2), repressor/activator protein 1 (RAP1) and TPP1 [4, 5]. The telomere complex primarily functions to mask double strand break DNA damage signals at telomeres, inhibit exonucleolytic degradation and prevent chromosomal fusions [6, 7].

Telomeres can be critically shortened by incomplete replication of the lagging strand during DNA synthesis, known as the 'end-replication problem' [8, 9]. Through this process, each telomeric end shortens by approximately 50–100 base pairs during each successive cell division. Other known mechanisms leading to telomere loss include oxidative DNA damage [10, 11] and alterations of Shelterin proteins [12]. In normal somatic cells, significant telomere shortening leads to p53-dependent senescence

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or apoptosis [13, 14]. As a result, there is a limited number of population doublings a somatic cell may undergo before entering a senescent state. In cancer cells, these cell cycle checkpoints are abrogated, for example through mutations in tumour suppressor proteins. Consequently, unchecked cellular proliferation continues and genomic instability may ensue *via* chromosomal breakage–fusion–bridge cycles [15].

In the vast majority (85–90%) of human cancers, telomere length appears to be maintained, or actively lengthened, through up-regulation of the enzyme telomerase. Telomerase is a reverse transcriptase that has the ability to synthesize new telomere DNA using an internal RNA template [1, 16, 17]. Telomerase is minimally composed of two components, the telomerase reverse transcriptase (TERT) protein [human telomerase reverse transcriptase (hTERT)] and the telomerase RNA template component [human telomerase RNA (hTR)] [18–22]. Because hTR is ubiquitously expressed, hTERT is considered the rate-limiting component that determines telomerase activity. Telomere loss may also be compensated in some cancers, by the telomerase-independent alternative lengthening of telomeres (ALT) pathway [23].

The basic biology of telomeres and telomerase has been a focus of research for decades and mounting evidence demonstrates the crucial role telomere biology plays in the initiation and progression of carcinogenesis. Previous reviews have discussed the potential prognostic significance of telomere and telomerase measurements in solid tumours [24, 25] and haematological malignancies [26, 27]. Here, we critically assess whether measurements of telomere lengths and/or telomerase levels will be useful as diagnostic markers for solid tumours. Due to space limitations, we focus predominantly on two common malignancies, breast and prostate cancer, and provide specific examples for other cancer types.

Methods for telomere length and telomerase detection

Numerous methods have been developed to measure either actual telomere length or total relative telomere content, a proxy for mean length. These methods include terminal restriction fragment (TRF) Southern blot analysis [28, 29], quantitative fluorescence *in situ* hybridization (Q-FISH) [30–32], Flow-FISH [33], slot blot assay [34, 35], quantitative telomere-specific PCR (Q-PCR) [36, 37] and single telomere length analysis (STELA) [38]. Likewise, measurement of telomerase enzymatic activity or telomerase gene expression in human biological samples, either in tissue or other bodily fluids, can be performed by different methods. These methods include telomere repeat amplification protocol (TRAP) [39] or detection of transcript levels of hTERT or hTR, either by RT-PCR or *in situ* hybridization. The potential strengths and limitations of each assay are summarized in Table 1.

Telomere length as a potential diagnostic marker in cancer

Breast cancer

Mirroring similar observations in most other cancers, initial studies measuring bulk telomere lengths by TRF analysis [40–42] or the slot blot assay [43] demonstrated that the majority of invasive mammary carcinomas had shorter telomeres than adjacent, benign breast tissues. Telomere lengths in cancer cells were shorter in high-grade tumours [40] and short telomeres correlated with aneuploidy and the development of lymph node metastases [43]. Subsequently, high resolution *in situ* telomere length assessment combined with immunostaining to differentiate specific cell types [32], confirmed that significant telomere shortening is prevalent in ~70% of invasive mammary carcinomas [44]. Interestingly, ~25% of invasive breast carcinomas contain telomeres that are either similar or longer than the adjacent stromal fibroblasts [44]. Additionally, two studies have identified breast tumours displaying the ALT phenotype, a telomerase-independent telomere length maintenance mechanism characterized by remarkable telomere length heterogeneity, ranging from ultra-short to ultra-long telomeres [45, 46]. The ALT phenotype [45] has been primarily observed in sarcomas, but is relatively rare in most carcinomas [47]. Similar telomere length distributions seen in cancer cells have been observed in the preneoplastic lesions, ductal carcinoma *in situ* and lobular carcinoma *in situ* [44], although the ALT phenotype has not been reported.

Surprisingly, not only does telomere shortening occur in the majority of *in situ* and invasive breast carcinomas [48], but telomere length alterations also occur in seemingly histologically normal breast tissues. These alterations have been observed in normal terminal ductal lobular units adjacent to a tumour and even in disease-free breast tissues obtained from reduction mammoplasties [44, 49, 50]. Using Q-FISH, telomere lengths were assessed in normal lobules and normal lactiferous ducts. Strikingly, telomere shortening was observed in the majority of normal lobules, but not in normal lactiferous ducts. Notably, short telomeres were only seen in the luminal cells and not in the myoepithelial cells. This finding was confirmed in a recent study that observed telomere shortening in normal luminal and tumour cells, but not shortening in the myoepithelial or fibroblast cell populations [49]. Although interesting biologically that telomere shortening may contribute to breast cancer promoting genomic alterations, these observations most likely preclude the use of telomere length measurements, particularly bulk measurements, as a diagnostic marker in breast tissues (*e.g.* needle core biopsy specimens). Telomere length measurements in cell preparations, for example fine-needle aspirates (FNAs), may be difficult because the histological information is lost. However, telomere length measurements in breast tissues adjacent to tumours may still have clinical utility. In particular, telomere DNA content measured by the slot blot assay was observed to be decreased in benign tissues 1 cm

Table 1 Telomere length and telomerase detection methods: strengths and limitations

Method	Strengths	Limitations	References
Telomere length methods			
TRF Southern blot analysis	Widely used; provides telomere length distributions	Requires relatively large amounts of DNA (μg range); inclusion of sub-telomeric regions in the telomere length estimation; variability in interpretation; relatively laborious; cannot be used on fixed tissues	[28, 29]
Q-FISH	Allows telomere length assessment in fixed material; provides single cell resolution while maintaining tissue architecture; allows identification of telomere lengths in specific cell types	Although quantitative analysis may be performed, values are relative telomere measurements, not actual length	[30–32]
Flow-FISH	Average telomere lengths can be quantitated; provides a distribution of telomere lengths	Relatively laborious; can only be used on single cell suspensions, such as blood leucocyte samples; cannot be used on fixed tissues	[33]
Slot blot assay	Low DNA input requirement (ηg range); can used on fixed tissues	Provides mean telomere content (not length); does not provide a distribution of telomere lengths; no identification of telomere lengths from specific cell types	[34, 35]
Quantitative telomere-specific PCR (Q-PCR)	High-throughput analysis allows for assessment of large sample sets; requires low DNA input (ηg range)	Provides mean relative telomere content (not length); no distribution of telomere lengths within a particular sample; not easily performed on fixed tissues	[36, 37]
STELA	Can be used to detect telomere lengths from specific, individual chromosomes; can identity extremely short telomeres	Relatively laborious; primers for all chromosome arms have not been developed; may not detect extremely long telomeres	[38]
Telomerase detection methods			
TRAP	'Gold-standard' for telomerase activity measurements; need for only small amount of cells	Relatively laborious; hard to quantify; need for appropriate controls	[39]
Detection of transcript levels of hTERT or hTR by RT-PCR	Allows for quantitative measurement of the specific subunits; fairly high throughput; can detect slice variants	mRNA expression levels of hTERT may not directly correlate with telomerase activity due to post-transcriptional processes	
Detection of transcript levels of hTERT or hTR by *in situ* hybridization	Allows for detection and visualization of transcripts at single cell level; allows for detection in specific cell types	Detection of transcripts does not always correlate with telomerase activity; decreased sensitivity due to low abundance of hTERT may be problematic	

away from the visible tumour margin, but not to the same extent as in tissue 5 cm from the tumour margin, suggesting a possible cancer field effect [50]. The concept of cancer field effect, or field cancerization, refers to the occurrence of molecular alterations in histologically normal tissues surrounding tumours [51]. Although still preliminary, the identification of molecular alterations, such as telomere shortening, in histologically normal cells may have clinical implications for breast-sparing surgery by defining appropriate molecular tumour margins and assessing risk factors for the development of recurrent disease [52].

A newer area of investigation has focused on measuring telomere lengths in peripheral blood lymphocytes, either by TRF analysis or Q-PCR, to assess potential links between constitutive telomere length and risk of breast cancer development. However, to date, the results have been largely conflicting. When comparing telomere lengths to healthy controls, three studies have observed shorter telomeres in cancer patients [53–55], one study did not observe any significant difference [56], and two studies observed longer telomeres in cancer patients [57, 58]. Recently, a prospective cohort of postmenopausal women did not show a significant association between increased risk of developing breast cancer and telomere length in peripheral blood lymphocytes [59]. Pooley *et al.* showed that decreased mean telomere length in peripheral blood lymphocytes was associated with a significant, yet modest increased risk of developing breast cancer in a retrospective study; however, the association was not replicated in a prospectively collected cohort [60]. The authors postulated that the observed telomere shortening predominantly occurs after diagnosis, thus diminishing its potential value as a predictive risk marker for breast cancer. More research is warranted to clarify these issues.

Whereas, the above studies have investigated mean telomere length, Zheng and colleagues have focused on assessing telomere alterations on individual chromosome arms [61, 62]. Suggesting that critically short telomeres on specific chromosome arms may be an underlying mechanism for chromosome specific instability, chromosome arm-specific telomere lengths were measured by Q-FISH in short-term cultured blood lymphocytes. In the first case–control study, short telomere lengths on chromosome 9p were associated with an increased risk of breast cancer [61]. Interestingly, the *CDKN2A* gene which encodes for $p16^{INK4}$ and $p14^{ARF}$, tumour suppressor proteins that regulate the Rb and p53 pathways, is located on chromosome 9p. In premenopausal women, these findings where confirmed and extended; in addition to 9p, short telomere lengths on 15p, 15q and Xp were also associated with an increased risk of the development of breast cancer [62]. Chromosomal arm-specific telomere length analysis could be incorporated in a panel of biomarkers used for risk assessment of breast cancer.

Prostate cancer

A major characteristic of prostate cancer is prominent chromosomal instability. Prostate cancer is thought to develop from benign epithelium through high-grade prostatic intraepithelial neoplasia (PIN), the earliest precursor lesion, to invasive adenocarcinoma. Because telomere dysfunction causes chromosomal instability, Sommerfeld and colleagues studied telomere dynamics in the prostate by measuring telomere lengths in matched samples of benign prostatic hyperplasia (BPH), benign nodules composed of stromal components and epithelial cells and invasive prostatic adenocarcinomas obtained by radical prostatectomy [63]. Prostate cancer tissue telomere lengths were significantly shorter than the telomeres from cells in BPH tissues and from adjacent normal tissues. These results were confirmed [64, 65], and extended to demonstrate an association between reduced telomere lengths in prostate tumours and disease recurrence [66].

Using high-resolution *in situ* methods, telomere shortening was observed in tumour epithelial cells compared to normal prostatic epithelial cells in the vast majority of prostate tumours [32], as well as in high-grade PIN lesions [67, 68]. Within these PIN lesions, telomere shortening only occurred in the luminal epithelial cells and not in the basal epithelial cells or the surrounding stromal cells. The high prevalence of short telomeres in high-grade PIN lesions, of which only a small fraction progress to invasive carcinomas, would preclude the use of this molecular marker for prostate cancer detection. However, telomere length measurements in tumour specimens obtained at time of surgery or taken at time of biopsy do seem promising as a potential prognostic marker in prostate cancer [66, 69, 70].

To date, only one study has assessed the relationship between constitutive telomere length in peripheral blood lymphocytes and prostate cancer. Using Q-PCR, this nested case–control study showed no association between mean telomere length and risk of aggressive prostate cancer development [71]. More interestingly, recent studies have reported telomere shortening in histologically normal prostate tissues from diseased prostates. In one study, telomere lengths were assessed by Q-FISH in biopsies from a cohort of men diagnosed with high-grade PIN, but without evidence of prostate cancer [72]. The degree of telomere shortening in the surrounding stromal cells and within cells from the high-grade PIN lesions were associated with the eventual diagnosis of prostate cancer [72]. In another report, mapping of the spatial distributions of telomere DNA content, measured by the slot blot assay, revealed telomere length variations in fields of histologically normal tissues surrounding tumours in a small set of radical prostatectomies specimens [73]. Expanding on their previous work, Joshua *et al.* assessed telomere lengths topographically by Q-FISH in normal epithelium, adjacent stroma, BPH, high-grade PIN and cancer in whole mount tissue sections [74]. Here, the presence of short telomeres in different prostatic histologies correlated with telomere lengths within adjacent stromal cells, suggesting microenvironmental effects within the prostate gland, such as increased oxidative stress [74].

The lack of association between constitutive telomere length, as measured in peripheral blood lymphocytes, and increased prostate cancer risk and the existence of telomere shortening in seemingly histologically normal prostate tissues suggests measurement of telomere lengths will not be useful as a direct diagnostic marker of prostate cancer. However, it is possible that telomere length analysis could nonetheless be potentially useful. For example, in settings where there is suspicion of cancer, *i.e.* in men with persistently elevated serum prostate specific antigen (PSA) but negative biopsy results. If abnormal telomeres in the stromal or epithelial cell populations are present on the biopsy, then it may suggest an underlying defect, thus triggering a repeat biopsy.

Other cancer types

As illustrated with breast and prostate cancers, telomere lengths have been extensively studied in most cancer types [25]. In general, the majority of studies have compared tumour samples to either histologically normal, tumour-adjacent tissues or truly disease-free tissues. Although telomere alterations are found in the majority of cases, the direction of the alteration, either shortening or lengthening, appears tissue dependent and may vary within a particular tumour type. For example, a fraction of colorectal carcinomas have cancer cells with telomere lengths longer than adjacent normal cells and these cases tend to have a poor survival [75, 76]. Additionally, telomere alterations frequently occur in precursor lesions of most human epithelial cancers [77, 78]; therefore, telomere length analysis alone cannot differentiate between the presence of a precursor lesion or invasive cancer. Because most precursor lesions are not treated and will subsequently never progress to invasive carcinomas, the direct measurement of telomere length in tissue, or even in cytological preparations, is not a suitable molecular marker for the diagnosis of cancer. However, an area of investigation that may be promising is *in situ*

telomere length analysis to identify patients that are good, or poor, candidates for a particular therapy. For example, recently developed telomerase inhibitors work most effectively on cells with short telomeres; therefore, assessment of the telomere lengths, along with telomerase activity, prior to treatment would be necessary to differentiate the patients most likely to benefit from the treatment [79].

Another avenue of investigation has concentrated on telomere length measurement in peripheral blood lymphocytes which can be easily obtained. Taking advantage of the high-throughput Q-PCR assay, numerous groups have assessed mean telomere length in peripheral blood as a possible marker for the risk of development of different cancer types, including lung, bladder, oesophagus, skin, head and neck and kidney; the field has been recently reviewed by Svenson and Roos [25]. Since that comprehensive review, additional population-based investigations correlated shorter mean leucocyte telomere length with an increased risk of gastric cancer [80] and serous ovarian adenocarcinoma [81]. Conversely, longer leucocyte telomere length was associated with an increased risk of developing non-Hodgkin lymphoma [82], whereas no association between telomere length and risk of incident colorectal carcinoma was found in two recent prospective studies [83, 84]. One particularly intriguing study performed by Willeit and colleagues, analysed mean leucocyte telomere length in 787 participants free of cancer at baseline and prospectively followed for 10 years [85]. In this cohort, short telomeres were associated with subsequent cancer development independent of other cancer risk factors. Although associations could not be assessed between telomere length and each specific cancer type due to the relatively small number of cases, this inverse association is of particular interest and warrants further investigation. As with the tissue-based findings, the correlations with mean leucocyte telomere length and the risk of the development of cancer depends on the cancer type. Although, larger, prospective studies are needed, these preliminary findings suggest that telomere length analysis may provide some screening diagnostic benefit, most likely in conjunction with other molecular markers, to identify a subset of patients at risk for development of a particular cancer.

Telomerase activity as a potential diagnostic marker in cancer

Breast cancer

The development of the PCR-based TRAP assay by Kim *et al.* [39] greatly improved our ability to assess the levels of telomerase activity. Initial results demonstrated that 93% of breast cancers and only 4% of histologically normal adjacent tissues were telomerase positive [86]. Follow-up studies confirmed the presence of telomerase activity in the vast majority of invasive breast carcinomas (range 73–95%), ductal carcinoma *in situ* lesions (range 59–100%), but only in a small fraction of benign breast tissues [87–91]. However, as an ideal diagnostic marker would be available prior to surgery, the TRAP assay was slightly modified to increase sensitivity for use on FNAs [92]. In comparison to cytological preparations, >90% of breast cancers were telomerase positive, whereas, only a small fraction of benign breast lesions were positive for telomerase activity [93, 94]. Taken together, these studies suggest that detection of telomerase activity may be a useful breast cancer marker in FNAs; however, as alluded to by Mokbel and colleagues, the role of telomerase detection may only be useful as a complementary marker to a traditional cytopathological diagnosis [95].

In agreement with hTERT as the catalytic and rate-limiting telomerase component, mRNA expression levels of hTERT have been shown to roughly correlate with telomerase activity in breast cancer [96]. Several studies have measured mRNA expression levels of hTERT, and occasionally also hTR, using quantitative RT-PCR in a variety of samples. One investigation found that hTERT mRNA expression was significantly higher in breast cancer tissues compared to adjacent normal breast tissues, suggesting a possible role for the measurement of hTERT mRNA levels in breast cancer diagnosis [97]. Ultimately, a panel of markers that assesses hTERT expression levels in combination with mRNA expression profiling of other key telomere-related genes may prove beneficial for breast cancer detection [98].

More recent developments have focused on the detection of hTERT in peripheral blood from breast cancer patients, with the idea of detecting circulating tumour cells (CTC). Shen and colleagues measured mRNA levels of hTERT, survivin and mammaglobin in peripheral blood samples from breast cancer patients and healthy individuals. Individually, the sensitivity of the three markers was extremely low (33–60%), with hTERT being the highest, but the combination of the three markers increased the sensitivity to 70% and an overall specificity of 100% [99]. Another study assessed hTERT mRNA in plasma from breast cancer patients, women diagnosed with fibroadenomas and healthy controls. hTERT levels in the plasma showed a sensitivity of 50% and specificity of 90% in the ability to detect malignancy [100]. These interesting findings suggest a possible role for assaying hTERT in the detection of CTCs. In the future, telomerase measurements, in conjunction with other molecular markers, may have utility in the early diagnosis of breast cancer.

Prostate cancer

While investigating prostate telomere biology, Sommerfeld and colleagues demonstrated the presence of telomerase activity in 84% of prostatic adenocarcinomas, 12% in matched adjacent normal tissues and 0% in adjacent BPH tissues [63]. A follow-up study observed that 90% of prostate cancers were telomerase positive, whereas, normal prostate tissues were all telomerase negative [101]. As well as confirming telomerase activity in prostate cancers, Koeneman *et al.* observed telomerase in 16% of

samples of high-grade PIN [65]. Extensive evaluation of telomerase activity in prostate needle biopsies shows similar results to the observations in the radical prostatectomy tissues (reviewed in [102]). Additionally, analysis of hTR and hTERT, either by RT-PCR or by *in situ* hybridization, has shown similar trends to the earlier telomerase activity studies, whereby most invasive cancers as well as PIN lesions are positive, BPH lesions are intermediate and normal adjacent areas show low to no levels of expression (reviewed in [102]).

Telomerase activity can also be detected in bodily fluids, such as expressed prostatic secretions and urine [103–107]. Telomerase activity was detected in prostatic fluids in 83% of prostate cancer patients compared to only 11% of patients without clinical evidence of prostate cancer [103]. Attempting to improve sensitivity for detecting cancer, hTERT expression was measured in conjunction with hypermethylation of the glutathione S-transferase P1 promoter, another common molecular alteration in prostate cancer [107]. The sensitivity for this combined assay was 73%, but the specificity was only 43%. Across studies using freshly voided urine samples after prostatic massage, telomerase activity has been detected in men diagnosed with prostate cancer (range 58–100%), but also in a subset of men (range 13–30%) with BPH and no evidence of concurrent adenocarcinoma [104–106].

The most promising studies have come in the last several years. Pfitzenmaier *et al.* analysed telomerase activity in bone marrow aspirates from men with localized prostate cancer to detect disseminated prostate cancer cells [108]. Although 49% of the men had detectable telomerase activity demonstrating the feasibility of the approach, the procedure work in only half of the patients due to technical difficulties, highlighting the need for improved technology before the approach can be implemented in a clinical setting. In a different approach, Dasi and colleagues evaluated plasma hTERT mRNA levels in patients with elevated PSA levels and healthy men. Using a cut-off value (the highest value observed in the control group), the authors reported a 81% sensitivity and a 60% specificity, suggesting that hTERT mRNA levels may be able to differentiate between patients with prostate cancer and patients without evidence of disease [109]. Because free plasma DNA had been suggested to be a diagnostic marker for cancer, Altimari *et al.* assessed hTERT mRNA levels from blood samples in patients diagnosed with localized prostate cancer and determined an 80% sensitivity and 82% specificity, implying its potential use as an early diagnostic and monitoring marker for prostate cancer [110]. Finally, Fizazi and colleagues developed a method using telomerase activity to specifically detect CTCs in patients with prostate cancer. Epithelial cells from peripheral blood mononuclear cells were harvested and telomerase activity measured; CTCs were detected in 79% of patients with localized prostate cancer prior to radical prostatectomy or brachytherapy, in 79% of patients with advanced metastatic disease and in 0% of healthy patients [111]. Other capture strategies, for example the use of microfilter-based platforms to determine telomerase activity from live-captured CTCs, are currently being developed and validated [112].

Other cancer types

As with breast and prostate cancer, telomerase activity or hTERT expression is present in the vast majority of solid tumours and has proved to be a marker of malignancy [113]. However, telomerase activity or hTERT expression has been shown to be present in some normal tissues and benign conditions. Due to the size of the field, the reader is directed to other comprehensive reviews discussing the potential clinical utility of using telomerase as a diagnostic marker in cancer in general [76] or within specific tumour types [114, 115].

To provide a specific example, the detection of telomerase by non-invasive means such as analysing urine or other bodily secretions seems to be potentially useful some cancer types, in particular for bladder cancer [116, 117]. A case–control study comparing patients with confirmed bladder cancer and healthy controls analysed telomerase activity from urine samples. Using an arbitrary cut-off value, Sanchini and colleagues reported a 90% sensitivity and a 88% specificity with similar patterns observed in low-grade tumours [118]. These preliminary results seem promising, but caution must be taken because the presence of acute or chronic inflammation may affect the telomerase activity measurements. Nevertheless, the non-invasive evaluation of telomerase activity in urine may provide additional diagnostic information, independent of routine cytology and most importantly may identify low-grade tumours, which are difficult to identify by cytological examination alone.

Although measurement of telomerase activity or hTERT expression in blood may provide some diagnostic utility, concerns still remain about the specificity of the telomerase activity measurements because activated lymphocytes display telomerase activity and any lymphocyte contamination is a possible confounder. Although, hTR and hTERT have been analysed at the RNA transcript level, it is still a major limitation that there are no reliable hTERT antibodies for use in immunohistochemistry. Although several antibodies are claimed to be specific for hTERT, none have been adequately validated in tissues. For example, it was even shown that a widely used antibody recognized nucleolin, not telomerase [119]. Telomerase antibodies are still being generated and evaluated; however, the detection in tissue has been problematic, likely due to the relatively low abundance of the telomerase protein. Newer antibody amplification techniques may prove beneficial and establishment of immunohistochemical protocols would open new research avenues for cancer diagnostics.

Interestingly, recent large, high-resolution analyses of somatic DNA copy-number alterations revealed that the *TERT* gene is located in one of the most significant focal amplifications in lung adenocarcinoma [120] and across multiple other cancer types [121]. Likewise, genomic amplification of the *TERC* gene, which codes for hTR, has been associated with the development of invasive carcinomas, for example in lung cancer [122], but most notably has been associated in the development of cervical cancer and may aid in the diagnosis of low-grade lesions when combined with cytology [123–125]. Additionally, studies stemming from the large-scale genome-wide association studies efforts have

discovered sequence variants in the TERT-CLPTM1L locus on chromosome 5p15.33 that associated with increased risk of cancer development [126–129]. Although these associations are modest, this TERT polymorphism has been statistically associated with increased risk of basal cell carcinoma and cancers of the lung, bladder, prostate, cervix and pancreas. However, it must be noted, that this association was not confirmed by another group analysing the polymorphism and risk of breast cancer, colorectal cancer and melanoma [130]. Nevertheless, these genome-wide association studies still may provide useful information implicating variation in the *TERT* gene as a cancer risk factor [131]. Ultimately, a unique single nucleotide polymorphism or a panel of single nucleotide polymorphisms, may provide useful diagnostic information and may identify a subset of people at an increased risk for the development of cancer.

Future directions

An exciting new area of ongoing research focuses on the assessment of telomere dysfunction, rather than telomere length alone, as a potential marker in cancer. In general, the telomere is 'capped', or functional, when the Shelterin complex is bound to the telomere, protecting the end of the chromosome from being recognized as a double-strand break. However, when the telomere is no longer protected, then the telomere is considered 'uncapped', or dysfunctional [4]. At this point, the telomere no longer inhibits the DNA damage response pathway and is prone to telomere fusions *via* the non-homologous end joining DNA repair pathway [7]. Such chromosome fusions can initiate genomic instability because the resulting dicentric chromosomes can be pulled to opposite poles during mitosis, eventually breaking, thus creating a cycle of breakage, fusion and bridging. This carcinogenesis-promoting genomic instability occurs when telomeres become critically short in the presence of abrogated tumour-suppressive checkpoint pathways, such as p53 and pRb [132]. Consistent with the notion that critical telomere shortening causes telomere dysfunction, Hemann *et al.* demonstrated that the shortest telomere within a cellular population can generate telomere dysfunction [133]. More recently, a large-scale genome sequencing study revealed that pancreatic cancer acquires genomic rearrangements consistent with telomere dysfunction [134]. Additionally, telomere dysfunction resulting from telomere shortening can induce tetraploidization that drives tumorigenesis [135].

Although telomere shortening can eventually lead to telomere dysfunction, recent investigations have shown that there are multiple mechanisms that may lead to telomere dysfunction. For example, alterations in TRF2, a major component of the Shelterin complex, lead to uncapping of the telomere even in the presence of adequate telomeric repeat sequences [136, 137]. Another investigation demonstrated that telomere dysfunction can increase telomeric homologous recombination in cancer cells, as monitored by telomere sister chromatid exchanges, even in the presence of telomerase [138]. Additionally, recent observations have demonstrated the critical role of the Shelterin component Rap1 for repressing homology-directed repair at telomeres [139]. New investigations on the cellular consequences of short telomeres have highlighted the importance of the induction of the DNA damage response pathway. Telomere-dysfunction induced foci can be visualized by the accumulation of γH2AX at the telomere [140]. Ultimately, it is hoped that further insights into the induction of the DNA damage response at the telomere will provide future avenues of study that may provide translational utility.

Although previous investigations have assessed telomere shortening in cancer cells, these length measurements have been considered a proxy for telomere dysfunction. The schematic in Figure 1 highlights the finding that the majority of tumours have telomere length alterations, either shortening or lengthening, that can eventually lead to telomere dysfunction. Although telomere length changes *per se* are unlikely to be useful diagnostic biomarkers, identification of the fundamental underlying molecular changes that cause telomere destabilization may unmask new markers that can aid in diagnosis of cancer.

Conclusions

Telomeres and telomerase have been focal points of cancer research for several decades. The dynamic interplay between telomeres and telomerase is critical in the development and progression of human cancer. The diagnostic utility of measurements of telomere length, or content, in solid tumours has been assessed. Early studies highlighted significant shortening in telomere lengths in cancer cells compared with normal adjacent cells from a variety of tissue types. However, more refined techniques, such as Q-FISH, have revealed more complex telomere phenotypes, including the presence of telomere alterations in premalignant and even normal-appearing cells, thus impacting the cancer specificity of telomere length changes. In addition, cancer cell telomere lengths vary considerably among different cancers. In some tumour types (*e.g.* breast cancer), there can be extreme heterogeneity within a particular cancer type and even within an individual tumour. Therefore, the use of telomere length measurements alone may not be suitable as a cancer diagnostic marker. However, telomere length measurements in tumour tissue, either from surgical specimens or specimens taken at the time of biopsy, or from other bodily fluids may serve as a molecular marker for risk assessment, prediction for response to therapy (*e.g.* setting of telomerase inhibitors) or prognosis.

Significant differences in telomerase activity and expression levels of hTERT have been observed between cancerous and benign tissues and assessed for possible diagnostic utility. However, it has been shown that telomerase may also be present in a small fraction of some benign lesions (*e.g.* fibroadenomas of the breast), pre-invasive lesions (*e.g.* high-grade PIN), as well as inflammatory cells. Therefore, the diagnostic use of the detection

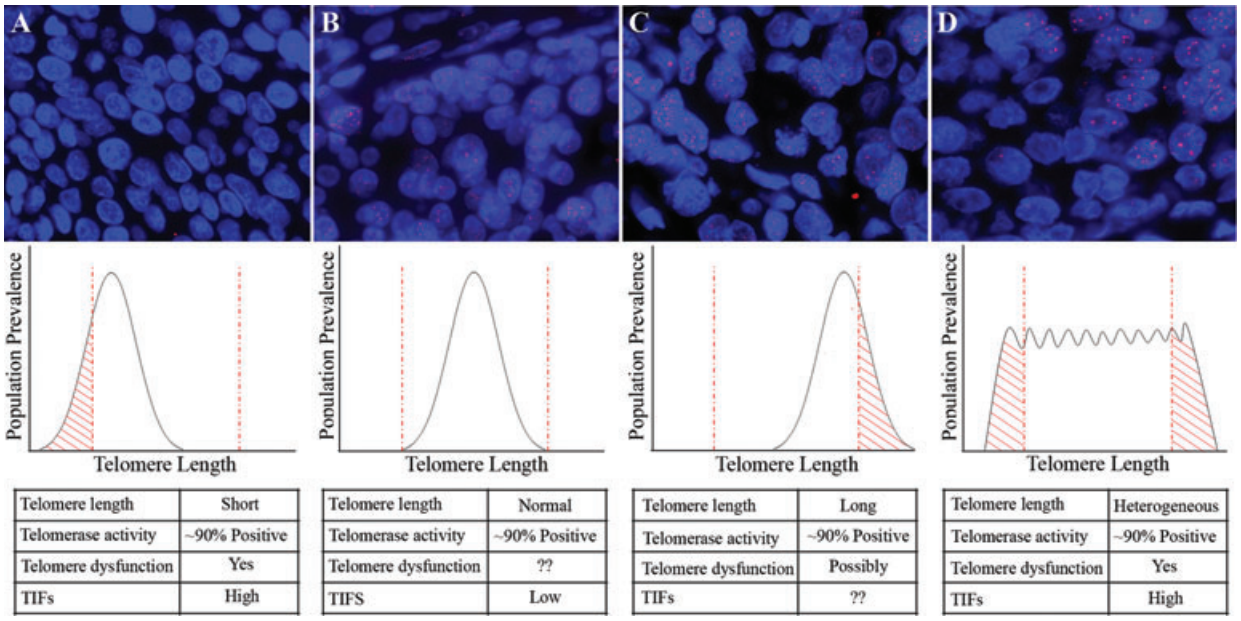

Fig. 1 Model depicting the possible relationships between telomere lengths, telomerase activity and telomere dysfunction in human carcinomas. Telomere length analysis by FISH from representative examples of tumours displaying (**A**) extremely diminished telomere signals in cancer cells, (**B**) comparable telomere intensities in cancer cells and benign stromal cells, (**C**) extremely bright telomere signals in cancer cells compared to benign stromal cells and (**D**) heterogeneous cancer cell telomere lengths varying from extremely short to relatively long. For the images (original magnification ×400), the DNA is stained with DAPI (blue) and telomeric DNA is stained with a Cy3-labeled telomere-specific peptide nucleic acid probe (red). Below each panel is a proposed model depicting the telomere length distributions in each tumour and the relationship to telomere dysfunction. The critical threshold levels for telomere function for critically short and abnormally long telomeres are shown (dashed red lines). Although ~90% of tumours display telomerase activity, the cancer cell telomere lengths may vary drastically. Thus, ongoing investigations into the cause of telomere dysfunction may unravel new molecular markers with potential translational utility.

of telomerase activity in tissues seems limited except in specific circumstances, such as detection of CTCs or in urine to detect bladder cancer.

In summary, although initial expectations for the use of telomere shortening or telomerase activity as highly specific markers of cancer have since been tempered, there are many avenues for research being investigated that may provide new molecular markers related to telomere biology. In particular, the area of telomere dysfunction, rather than telomere length alone, may yield new insights not only into the pathogenesis of a particular cancer, but could also have major implications in the diagnosis of cancer.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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