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REVIEW ARTICLE

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Telomeres, telomerase and cancer: an up-date

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Abstract In the mid 1990s, the hypothesis emerged that the upregulation or re-expression of a telomere-synthesising ribonucleoprotein, called telomerase, is a critical event responsible for continuous tumour cell growth. In contrast to normal cells, in which gradual mitosis-related erosion of telomeres eventually limits replicative life span, tumour cells have telomerase and show no loss of these chromosomal ends. These data suggest that telomere stabilisation may be required for cells to escape replicative senescence and to proliferate indefinitely. Because of the close association between telomerase and malignancy, both pathologists and clinicians expect this molecule to be a useful malignancy-marker and a new therapeutic target. This review focuses on the components of the human telomere and of the human telomerase enzyme. A synopsis of reports studying the clinical–diagnostic value of telomere length measurements, of telomerase activity analyses and of the in situ telomerase detection is given. Finally, a summary of recent experimental work that sheds new light on the biological role of this fascinating molecule is presented.

Key words Telomeres · Telomerase · Cancer

The telomere–telomerase hypothesis of ageing and cancer

The concept established in 1908 by Alexis Carrel, that vertebrate cells are immortal, was challenged in 1961 by Hayflick and Moorehead, who showed that normal somatic cells have a limited proliferative life span, related not to elapsed time but to the increasing number of population doublings, eventually terminating in a quiescent but viable state, 'replicative senescence' or 'mortality' stage 1 (M1). In 1973, Olovnikov hypothesised that this 'Hayflick limit' results from the exhaustion of chromosomal end-genes or telogenes. Because DNA polymerases can synthesise DNA only in the 5' to 3' direction and need a short RNA primer to initiate DNA synthesis, lagging-strand synthesis remains incomplete. This leads to a gradual loss of chromosomal termini with each new mitosis in the somatic cells and has been referred to as the 'end-replication problem' (Fig. 1) [30]. After the identification of the human telomeric repeat sequence in 1988,

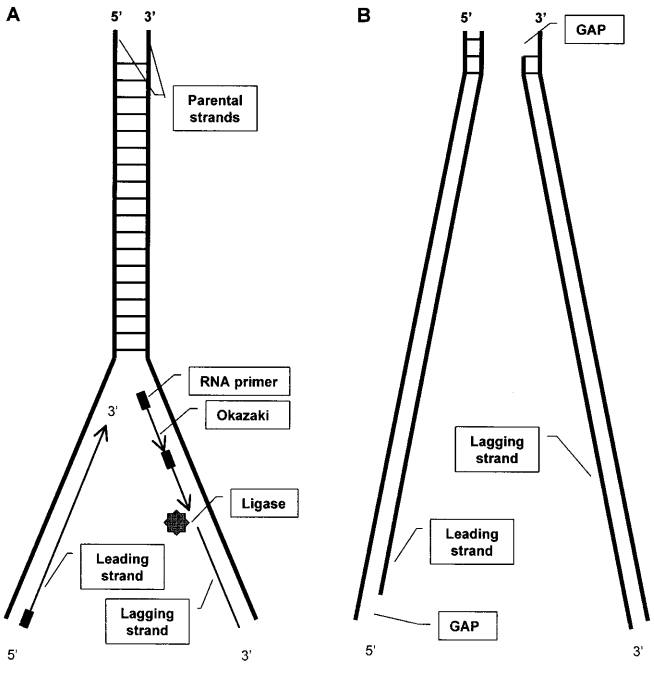

Fig. 1 Scheme depicting the 'end-replication problem'. **A** As the replication fork opens, lagging strand synthesis proceeds from 3' to 5' as the overall result of removal of RNA primers and ligation of the individual 5' to 3' synthesised Okazaki fragments. **B** After removal of the terminal RNA primers, gaps remain at the 5' end of the lagging (and leading) strand which cannot be filled

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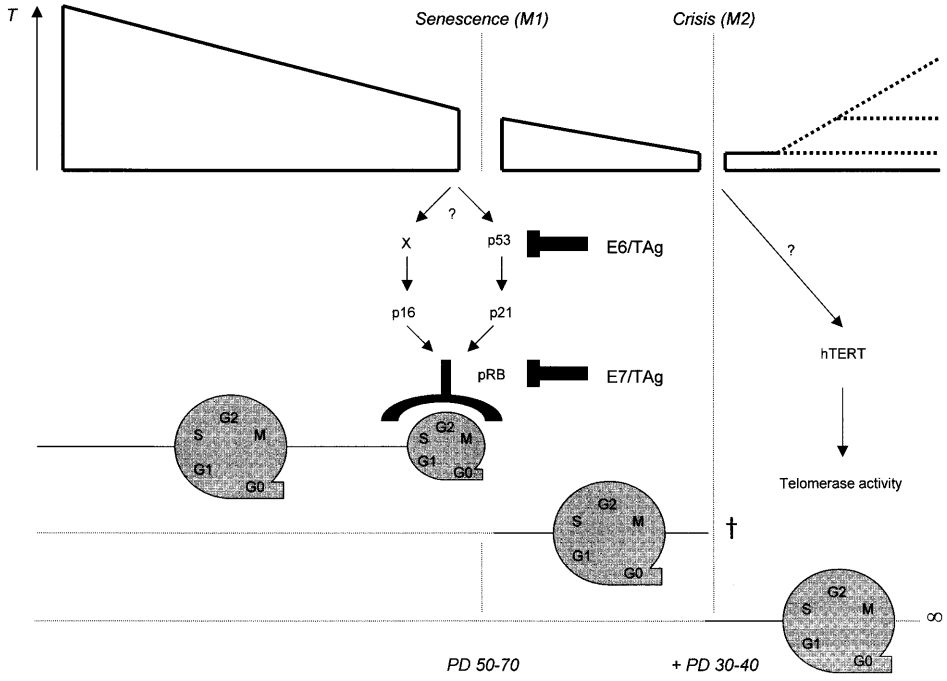

Fig. 2 The two stages of the immortalisation hypothesis as observed for fibroblasts. Via unknown mechanisms, mitosis-dependent shortening of telomeres (*T*) activates at least two growth inhibitory pathways: one acting via p53 and p21, and the other (less well characterised) acting through p16, resulting in pRB-mediated growth arrest at M1. Inhibition of p53 and/or pRB by viral oncoproteins, such as HPV-E6/E7 or SV40-TAg, results in a life-span extension of approximately 30–40 population doublings (*PD*) until cell death at M2. At crisis, telomerase (or ALT) is activated and telomeres stabilised, giving rise to rare cell clones with the immortal phenotype. Note that telomeres can be stabilised at virtually any length. Remember that cell-type diversity exists with regard to the nature and timing of proliferative life-span barriers [113]

Table 1 Head and neck. A complete list of all the references cited in tables 1–13 is available upon request. *OSCC* oral squamous cell carcinoma

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal oral mucosa	0/14	0	1
Mucosa adjacent to OSCC	7/20	35	1
Pre-malignant lesions[a]	14/20	70	1
Oral squamous cell carcinoma	81/86	94	4
Oral rinses from OSCC	4/15	27	1

[a] Oral lichen planus

Olovnikov's telogenes or telomeres were indeed found to shorten by 25–200 bp per division [33]. Since the early 1990s, this progressive erosion of telomeres is regarded as a 'mitotic clock' [2]. Apart from being a molecular indicator of the proliferative potential of cells, interesting evidence for a causal role of telomere shortening in ageing has been obtained. A reduction in telomere length and replicative capacity in fibroblasts from patients with the accelerated ageing syndrome, Hutchison-Gilford progeria, relative to age-matched normal individuals has been noted [2]. Similarly, lymphocytes from Down's patients, which show many of the features of premature ageing, lost telomeres at three times the rate of age-matched controls [104].

At present, it is unclear how telomere shortening directly activates the M1 antiproliferative machinery. It has been suggested that the telomere on chromosome 17p, harbouring the p53 gene, represents a 'clock' telomere, which is activated at a critical threshold [67]. p53 and/or pRb gene products are essential parts of the M1 downstream pathway, as documented via in vitro transformation experiments with oncogenic DNA viruses [11, 110]. M1 can be bypassed by sequestration of p53 and pRb by viral oncogenes, such as SV40 large T-antigen (TAg) or HPV E6/E7, conferring an extended life span of around 30–40 population doublings, whereupon net growth again ceases in a state variously termed 'crisis' or 'mortality' stage 2 (M2) [109]. M2 cells are characterised by ultra-short telomeres and chromosomal instability, as evidenced by the occurrence of end-to-end chromosome fusions. To prevent lethal loss of genetic information, cells are forced to stabilise their telomeres. As a result, rare cells escape crisis by reactivation of telomerase and divide indefinitely (Fig. 2) [16].

Telomerase is capable of synthesising new telomeres, thereby providing the cells a mechanism to circumvent gradual telomere erosion. The finding of high telomerase levels in immortal cell lines and the assumption that most malignant tumours are composed of at least some immortal cells prompted the search for telomerase in a variety of normal, benign, pre-malignant and malignant tissue specimens (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, and Table 13) [51]. Normal human somatic cells, with the exception of germ cells and stem cells of renewable tissues, do not have detectable telomerase activity. In contrast, nearly all malignant tumours express telomerase activity. As a result, it has been hypothesised that telomere stability may be required for cells to escape the restraint of their mortal phenotype, and that telomerase action may be a rate-limiting, if not the critical step required for the development and/or con-

Table 2 Gastrointestinal tract and pancreas

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Gastric metaplasia	2/9	22	1
Gastric carcinoma	133/153	87 (61–89)	3
Adjacent tissue	0/99	0	2
Inflammatory bowel disease[a]	10/22	45 (40–50)	2
Colorectal adenoma (all sizes)	39/108	36 (15–60)	6
Colorectal carcinoma[b] (all stages)	395/473	83 (75–100)	11
Luminal washing			
Benign	1/10	10	1
Colorectal cancer	18/31	58 (56–60)	2
Adjacent and normal tissue	15/203	7 (0–14)	7
Benign pancreatic lesion[c]	0/40	0	3
Pancreatitis	1/8	12 (0–33)	2
Pancreatic carcinoma	106/116	91 (84–100)	4
Normal and adjacent tissue	5/70	7 (0–11)	3
Pancreatic brushing/juice			
Benign	1/28	3 (0–4)	3
Carcinoma	23/31	74 (50–100)	4

[a] Ulcerative colitis, Crohn's disease
[b] Hereditary non-polyposis cases, sporadic cases with and without microsatellite instability
[c] Serous/mucinous cystadenoma, intraductal papillary adenoma, solid-cystic tumour

Table 3 Lung and thoracic/peritoneal cavity

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal lung tissue	7/190	4 (0–8)	2
Non-small cell lung cancer	203/240	85 (80–100)	4
Non-small cell lung cancer (broncho-alveolar lavage)	47/59	79 (78–82)	2
Small cell lung carcinoma	3/3	100	1
Malignant mesothelioma	20/22	91	1
Benign solitary fibrous tumour	2/2	100	1
Non-cancerous cavity fluid[a,b]	3/52	6	1
Malignant body cavity fluid[b]	94/119	79 (52–91)	4

[a] Positive samples from patients with tuberculosis
[b] Fluids of lung and/or peritoneal cavity

Table 4 Hepatic/biliary tissue

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal liver	0/6	0	2
Non-malignant liver disease[a]	29/214	13 (0–50)	5
Adenomatous hyperplasia	2/2	100	1
Hepatocellular carcinoma	221/248	89 (61–100)	8
Adjacent liver tissue	17/77	22 (15–37)	3
Normal gallbladder/bile duct	0/7	0	1
Benign biliary tract disease[b]			
Tissue/biopsy	1/44	2	1
Bile sample	0/11	0	1
Biliary tract neoplasm[c]			
Tissue/biopsy	10/16	62	1
Bile sample	1/19	5	1

[a] Chronic hepatitis, cirrhosis
[b] Gallbladder and bile duct adenoma, cholecystitis, cholesterol polyp, cholecystolithiasis
[c] Gallbladder and bile duct carcinoma

Table 5 Breast

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal breast[a]	4/36	11 (0–17)	2
Fibroadenoma	23/51	45 (11–67)	4
Ductal carcinoma in situ	56/73	77 (70–100)	4
Breast carcinoma	363/417	87 (44–100)	9
Fine-needle aspirate breast carcinoma	17/19	90	1
Adjacent benign tissue	0/10	0	1

[a] Activity in breast tissue during luteal phase and pregnancy

Table 6 Neural tissue. *PNET* primitive neuroectodermal tumour

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal brain	0/10	0	2
Astrocytoma			
Grade I	0/19	0	2
Grade II	12/66	18 (0–33)	6
Anaplastic astrocytoma	23/63	36 (0–45)	6
Glioblastoma multiforme	146/227	64 (26–89)	8
Oligodendroglioma[a]	21/40	52 (24–100)	5
Miscellaneous[b]	8/29	27	4
PNET/Ewing's sarcoma	15/16	94	3
Neuroblastoma[c] (all stages)			3
With N-myc amplification	15/15	100	
No N-myc amplification	24/39	61 (0–88)	
Ganglioneuroma	0/1	0	1
Meningioma			
Ordinary	5/108	5 (0–17)	6
Atypical/malignant	25/31	81 (16–100)	4
Pituitary adenoma[d]	0/36	0	3

[a] Also oligoastrocytoma and anaplastic variant
[b] Dysembryoplastic neuroepithelial tumour, xanthoastrocytoma, ependymoma, hemangiopericytoma
[c] Low activity in N-myc single copy tumours relative to multicopy tumours, and lowest activity in non-progressing stage IVs
[d] Including six invasive variants

Table 7 Female reproductive tract. For cervical samples no classification according to human papilloma virus (HPV) status has been made

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal endometrium[a]			
Proliferative phase	77/85	91 (73–100)	5
Secretory phase	24/73	33 (12–58)	4
Atrophic endometrium	5/22	23 (0–55)	3
Hyperplastic endometrium	29/33	88 (76–100)	2
Endometrial carcinoma[b]	93/104	89 (83–100)	4
Normal cervix (tissue/smear)	39/356	11 (0–19)	9
Preneoplastic lesion (tissue)			
LSIL[c]	32/93	34 (0–56)	5
HSIL[d]	90/186	48 (26–96)	7
Cervical carcinoma[e] (tissue)	198/213	93 (86–100)	7
Cervical smear			
LSIL[c]	18/66	27 (11–63)	3
HSIL[d]	59/147	40 (27–73)	3
Carcinoma	26/38	68 (31–88)	3
Normal ovary	0/8	0	2
Benign ovarian tumour[f]	7/38	18 (0–21)	3
Borderline ovarian tumour	21/28	75 (17–100)	3
Ovarian carcinoma	88/99	88 (80–100)	4
Peritoneal fluid/washing			
Benign	2/43	5	1
Ovarian carcinoma	44/52	85 (70–88)	2
Placenta[g]	59/149	40 (0–76)	4
Hydatidiform/invasive mole[h]	44/68	65 (12–100)	3
Choriocarcinoma	7/7	100	2

[a] Highest activity in the period between mid proliferative and early secretory phase
[b] Endometrial, endometrioid adenocarcinoma and papillary serous carcinoma
[c] Low-grade squamous intraepithelial lesion
[d] High-grade squamous intraepithelial lesion
[e] Micro-invasive and invasive carcinoma
[f] Cystadenoma, clear activity was found in all papillary variants
[g] Activity is largely absent in late chorions (34–41 weeks) relative to normal early chorions (5–9 weeks)
[h] Activity is more frequent in persistent moles relative to regressing moles

tinuing proliferation of cancers. In this conceptual framework, telomere erosion and the associated limitations in replicative life span have been proposed as a potent tumour suppressor mechanism that can be subverted by the action of the telomerase oncogene, providing cells with unlimited growth capabilities. Immortality and telomere stabilisation may also be maintained by telomerase-independent mechanisms, the nature of which has not been elucidated yet. This alternative lengthening of telomeres (ALT), possibly by recombination, has been found in a minority of human tumours and tumour-derived cell lines that have been claimed to lack telomerase activity [12]. This review focuses on the components of the human telomere and of the human telomerase enzyme complex. A synopsis of reports studying the clinical value of telomere length measurements, telomerase activity analyses and detection of telomerase at the cellular level is also provided. Finally, recent experimental work has been summarised that contributes to the understanding of the biological role of telomerase.

Table 8 Male reproductive tract. *PIN* prostatic intra-epithelial neoplasia; *BPH* benign prostatic hyperplasia; *Pc* prostate carcinoma

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal prostate	2/38	5 (0–13)	3
Prostate adjacent to Pc	9/39	23 (11–36)	3
Atrophy adjacent to carcinoma	1/6	16	1
BPH without carcinoma	8/40	20 (0–37)	3
BPH adjacent to carcinoma	30/63	48 (46–50)	2
High grade PIN without Pc	4/25	16	1
High grade PIN adjacent to Pc	11/15	73	1
Prostate carcinoma	170/207	82 (47–92)	5
Prostate carcinoma (fine-needle biopsy)	25/38	66	1

Table 9 Kidney/urinary tract. *TCC* transitional cell carcinoma

[a] Bladder calculi, urethral stricture, chronic cystitis, inverted papilloma, cystitis cystica, cystitis glandularis
[b] Oncocytoma, angiomyolipoma
[c] Significantly lower frequency of telomerase activity in chromophobe renal carcinoma (17%) compared with renal cell carcinomas of clear cell type [53]

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal urothelium/urine	9/183	5 (0–21)	6
Benign bladder disease[a]	26/74	35 (14–61)	2
Dysplastic urothelium	22/35	63 (0–69)	3
TCC of the bladder (all stages)	311/348	89 (80–100)	10
TCC of the bladder (washing)	128/178	72 (50–96)	5
TCC of the bladder (urine)	128/242	53 (0–81)	7
TCC of the renal pelvis	1/2	50	1
Normal renal tissue	0/114	0	4
Renal tissue adjacent to renal cell carcinoma	6/35	17	1
Benign renal tumour[b]	0/10	0	2
Renal cell carcinoma[c]	150/229	65 (56–63)	5

Table 10 Endocrine system

[a] Diffuse and Hashimoto
[b] Nodular hyperplasia, adenoma, Graves' disease and colloid goiter
[c] Adrenocortical carcinoma, malignant pheochromocytoma

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal thyroid tissue	2/64	3 (0–17)	4
Thyroiditis[a]	19/29	65 (54–100)	3
Benign thyroid disease[b]	25/135	18 (0–39)	9
Thyroid carcinoma			
Papillary	70/124	56 (20–100)	7
Follicular	31/40	77 (0–100)	7
Medullary	2/3	66 (0–100)	2
Anaplastic	3/5	60 (0–66)	3
Hürthle	1/2	50 (0–100)	2
Thyroid fine-needle aspirate			
Benign	4/48	8	1
Malignant	7/8	87	1
Adjacent tissue	6/36	17 (14–21)	2
Normal adrenal tissue	0/43	0	2
Adrenal tumour			
Benign	9/101	9 (0–15)	4
Malignant[c]	9/9	100	3

Table 11 Bone/soft tissue

[a] (Neuro)fibrosarcoma, liposarcoma, synovial sarcoma

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Osteosarcoma	22/26	85	1
Chondrosarcoma	3/5	60	1
Rhabdo/leiomyosarcoma	4/4	100	2
Malignant fibrous histiocytoma	2/2	100	1
Other[a]	8/8	100	1
Rheumatoid arthritis synovial tissue	14/25	56	1
Osteoarthritis synovial tissue	0/15	0	1

Table 12 Skin

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Normal epidermis	30/115	26 (12–37)	3
Benign proliferative lesion[a]	4/21	19 (6–60)	2
Pre-malignant lesion[b]	27/44	61 (42–89)	2
Squamous cell carcinoma	9/18	50 (25–100)	2
Basal cell carcinoma	21/26	81 (77–85)	2
Normal melanocyte[c]	0/10	0	1
Benign naevi	10/36	28	1
Atypical/Spitz naevi	6/8	75	1
Melanoma	54/68	79 (69–90)	3

[a] Viral/seborrhoeic wart, eccrine poroma
[b] Bowen's disease, actinic keratosis, melanoma in situ, Paget's disease and sebaceous epithelioma
[c] Post-primary cultures

Table 13 Haematological tissue. *ATL* adult T-cell leukaemia; *AML* acute myelocytic leukaemia; *ALL* acute lymphocytic leukaemia; *CLL* chronic lymphocytic leukaemia; *CML* chronic myelocytic leukaemia; *MDS* myelodysplastic syndromes

Pathology	No. positive/no. tested	% Positive (range)	No. of studies
Myeloma	0/6	0	1
Hodgkin's disease[a]	33/97	34 (10–40)	2
MDS	17/51	33	1
CML			
Chronic phase	33/43	77 (0–100)	2
Blast crisis	25/25	100	2
CLL	1/10	10	1
ALL	20/29	69 (66–70)	2
AML	62/76	81	2
ATL (HTLV-I$^+$)			
Asymptomatic	7/24	29	1
Smouldering	7/10	70	1
Chronic	5/5	100	1
Acute	18/18	100	1

[a] Low frequency (particularly when accompanied with eosinophilia) is possibly due to telomerase degradative activity of eosinophils

Structure and function of human telomeres

Telomeres have several functions, ranging from the protection of chromosomal ends from exonucleolytic degradation, the prevention of aberrant chromosomal recombination, and the segregation of chromosomes during mitosis and (pre)-meiosis to the attachment of chromosomes to the nuclear matrix. Importantly, telomeres provide the structural basis for solving the 'end-replication problem' and allow cells to distinguish random DNA breaks and natural chromosome ends. In 1978, Blackburn reported the first description of the repeated DNA sequence in *Tetrahymena* telomeres. In the simplest mammalian model, telomeres consist of 2–30 kb of non-coding double-stranded TTAGGG repeats extended by 50- to 150-nucleotide overhangs of single-stranded TTAGGG repeats [54].

Recent work substantiated that the chromosome stabilising capacity depends on the non-covalent binding of telomeric proteins to the single-stranded and double-stranded regions. Mammalian homologues of the single-stranded telomeric DNA-binding proteins of *Saccharomyces cerevisiae* (Cdc13p) and of *Oxytricha nova* have not yet been identified [40, 78]. Two human double-stranded telomeric DNA-binding proteins are currently known. TRF1 (telomeric repeat binding factor 1), the gene of this Myb-like protein localising to chromosome 8q13, acts as a telomerase repressor and has not been implicated in cancer or chromosome instability syndromes [92]. TRF2 localises to chromosome 16q22.1, and initial reports suggested that this second Myb-like protein prevents chromosome fusions by maintaining the single-stranded G tails [102]. Recent work revealed the existence of two possible mediators of TRF1 function. In vitro, TRF1 can be detached from its telomere by poly(ADP-ribosyl)ation by tankyrase (TRF1-interacting, ANKYrin-related ADP-ribose polymerase), thereby making telomeric DNA accessible to telomerase [94, 125]. Co-localisation studies recently detected this telomeric poly(ADP-ribose) polymerase (PARP) at nuclear pore complexes and around centrosomes, which could represent pools of tankyrase waiting for the signal to go to the telomeres [93]. On the contrary, to stick to the telomere and to exert its negative regulation of telomere length, TRF1 may need to bind to TIN2 (TRF1-interacting nuclear protein 2) [52]. Finally, the Ku heterodimer – a subunit of the DNA-dependent protein kinase – may be another important telomere-associated protein, the role of which needs further elucidation [41]. Importantly, specialised electron microscopy recently revealed that human telomeric DNA loops back on itself forming a t-loop, with the G tail invading the duplex telomeric repeats and forming a D (displacement) loop (Fig. 3A,B) [27]. In this constellation, TRF2 and TFR1 (perhaps bound to TIN2) may play a role in stabilising or allowing formation of the D and t-loops, respectively. Inappropriate DNA damage responses being cleverly prevented, telomerologists now face the problem of how telomerase accesses the hidden 3' terminus.

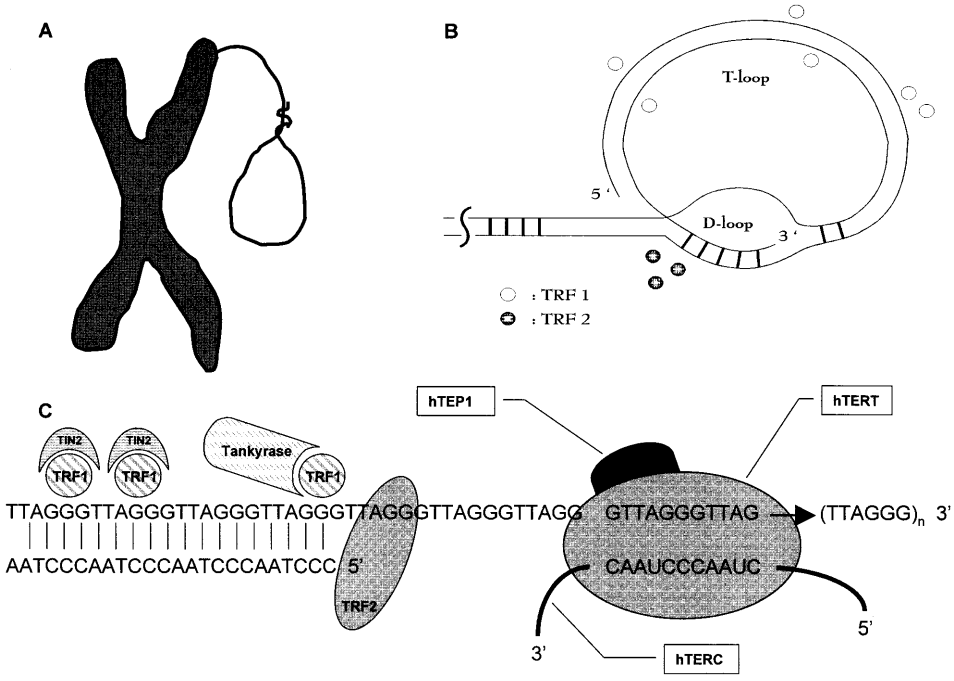

Fig. 3 Schemes depicting our current knowledge of the human telomere structure and the various components of the human telomerase complex. **A** Telomeric DNA forms lasso-like structures, referred to as t-loops, providing a solution to mask natural chromosome ends. **B** Telomeres end in a duplex loop. The free 3' end is tucked back inside the double-stranded DNA at the loop junction, forming a D (displacement) loop, with TRF2 bound at the base of the top. **C** The G-rich strand is elongated at its 3' end by the combined action of the telomerase subunits hTERT and hTERC. The C-rich strand is elongated at its 5' end by conventional DNA polymerases. Access of the telomerase complex to the 3' telomere end may be mediated by several telomere binding proteins

Structure and function of the human telomerase complex

The telomerase complex represents a specialised terminal reverse transcriptase with an estimated molecular mass of ~1000 kDa. The active ribonucleoprotein is made up of an RNA strand and at least one catalytic protein component (Fig. 3C). The holoenzyme synthesises telomeric repeats to the 3' ends of human chromosomes, utilising its integrated RNA molecule as an internal template.

The telomerase RNA component

The RNA component was first cloned in *Tetrahymena thermophila*. Later, the homologous genes were identified in ciliates such as *Oxytricha* and *Euplotes*, in the yeast *S. cerevisiae* (TLC1) and *Kluyveromyces lactis* (TER1), and in mammals such as mouse (mTR) and human (hTR, currently referred to as hTERC for human telomerase RNA component) [23]. hTERC is a single-copy gene localised to the distal quarter of the long arm of chromosome 3 (3q26.3), and hTERC amplification by gene amplification, isochromosome 3q formation or polysomy 3 has been described in cervix, lung and squamous cell carcinoma of the head and neck, indicating that hTERC could act as an oncogene [95]. The occurrence of CpG islands in the promoter region of the hTERC gene may indicate that the expression is regulated by DNA methylation [124]. The promoter regions have consensus sites for the binding of glucocorticoid/progesterone/androgen receptors and of transcription factors implicated in haematopoiesis and leukaemogenesis [124]. In humans, the length of the mature hTERC gene transcript is 451 nucleotides and lacks polyadenylation. In all organisms analysed to date, a 'template' region complementary to the sequence of the telomere repeats is embedded in the integrated telomerase RNA sequence. For humans, the hTERT (human telomerase reverse transcriptase) template element encompasses 11 nucleotides: 5' CUAACCCUAAC 3'. Finally, mammalian telomerase RNAs resemble small nucleolar RNAs (snoRNAs) – an RNA family required for pseudouridine modification and precursor processing of rRNA – because of the presence of an H/ACA box in their 3' domain [70].

Telomerase reverse transcriptase

A special class of related reverse transcriptases that functions as the rate-limiting step in telomerase activity has been identified in the yeast *S. cerevisiae* (Sc-Est2p) and the ciliate *E. aediculatus* (Ea-p123), in the fission yeast *Schizosaccharomyces pombe* (Sp-Trt1p), in *Oxytricha trifallax* (Ot-TERT), in *Tetrahymena thermophila* (Tt-TERT/p133), and in mammals such as mouse (mTERT) and human (hTRT/hEst2/hTCS1/TP2, currently referred to as hTERT) [32, 49, 69, 73]. hTERT contains a telomerase-specific amino acid motif (T motif) and seven conserved reverse transcriptase motifs (RT motifs), making it phylogenetically related to RTs coded for by a subclass of retrotransposable elements rather than to RTs from retroviruses like human immunodeficiency virus (HIV) [22]. This implicates that drugs that inhibit the HIV enzyme may not affect telomerase. However, this has been questioned [84]. The 40-kb single-copy hTERT gene, mapping to chromosome 5p15.33, codes for a 127-kDa protein of 1132 amino acids con-

tained in 16 exons [69]. Although 5p is one of the most common targets for amplification, e.g. in non-small cell lung cancers, information on in vivo hTERT gene amplification in primary tumours is currently lacking. How telomerase is regulated in cancer is not yet established, but the recent cloning and characterisation of the hTERT promoter will definitely provide useful information [15, 39, 99, 108]. The promoter contains binding sites for several transcription factors. At least oestrogen and the the c-myc protein have been found to positively regulate hTERT expression [62, 112], whereas methylation of the GC-rich hTERT promoter and associated histone deacetylation are suggested to induce transcriptional repression of the hTERT gene. Alternative splicing of the hTERT transcript [49, 69, 101] and phosphorylation/de-phosphorylation could be other important regulatory mechanisms [65]. There is support for the presence of a telomerase repressor on chromosome 3p and efforts to map the putative gene are currently under way [88]. Finally, telomerase activity is downregulated by a high calcium concentration [6].

Other telomerase-associated proteins

Prior to the discovery of the TERTs, the first proteins shown to be associated with telomerase activity were two proteins of 80 kDa and 95 kDa from *Tetrahymena thermophila*, which were initially mistaken for the telomerase catalytic subunits. The mammalian p80 homologue was identified in rat, mouse and human (TP1/TLP1, currently referred to as hTEP1 for human telomerase-associated protein 1), but, similar to hTERC, the expression of this protein does not correlate with telomerase activity in cells and tissues [31, 73, 75]. Recently, hTEP1 was found to be identical to the 240-kDa subunit of vaults – large cytoplasmic ribonucleoprotein complexes of undetermined function [48]. The presence of multiple WD40 repeat motifs additionally suggests that hTEP1 may play a common role in some aspect of ribonucleoprotein structure, function or assembly. The mammalian homologue of p95 has not been identified yet.

Clinical value of telomere length

The measurement of telomeric length has traditionally been accomplished through Southern blotting [89]. Genomic DNA is purified from the test sample, and digested with a mixture of frequently cutting restriction enzymes, e.g. *Hin*fI or RSA, which do not have a target sequence within the telomeric repeat. The chromosomal DNA will be cut into small fragments, except for the telomeres and subtelomeric regions (DNA adjacent to the telomere) which will be left intact and which together comprise the terminal restriction fragment (RF). The cleaved DNA is separated on a low-percentage agarose gel and transferred to a membrane for Southern-blot analysis. The Southern blot can then be hybridised with a $(TTAGGG)_n$ or $(CCCTAA)_n$ probe, which is either radioactively labelled or biotinylated (TeloQuant, PharMingen), and detected using an isotopic or a chemiluminescent detection system. The average terminal RF length is then calculated from the position of the detected signal relative to the position of known size standards. Because accurate Southern-blot analysis is not possible when DNA is broken or scant, a slot-blot assay can be used to determine the relative contents of telomere DNA in microdissected archival (paraffin-embedded) specimens [13]. To obtain some quantitative information on telomere length, fluorescence in situ hybridisation (FISH) techniques have been developed. In quantitative (Q)-FISH, the signal intensity of telomeric DNA visualised by FISH using, for example, telomeric peptide nucleic acid (PNA) probes (Dako, Denmark), can be related to the approximate number of kilobase pairs of TTAGGG repeats present on a given chromosome end. Conventionally, one telomere fluorescence unit (TFU) is defined as the signal that represents 1 kb of TTAGGG repeats [67]. A newly developed flow cytometry FISH (flow-FISH) method yields similar results and can be performed on primary cells, e.g. derived from peripheral bleeds [42].

Most malignant tumours have short TRFs, since telomerase activation is a late event in carcinogenesis and is usually preceded by M2-associated pre-cancerous proliferative events (Fig. 2). However, telomeres can be stabilised at virtually any length. Indeed, some cancers, such as basal cell carcinomas and renal cell carcinomas, may have unchanged or elongated TRFs [18, 105]. This could explain why, in contrast to enzyme activity, relatively few studies regard TRF measurements as a useful prognosticator. Most conclusive studies have been performed for the leukaemias (for a review see [90]). In chronic myelocytic leukaemia (CML), short TRFs may predict early progression to blast crisis, and mean TRF may be a marker of disease severity. Furthermore, CML patients with normal TRFs may be at an early stage of the disease and respond better to therapy [44]. For chronic B-cell lymphocytic leukaemia (B-CLL), short telomere length was significantly associated with a shorter median survival [4]. Recently, in patients with myelodysplastic syndromes (MDS), shortened TRFs were significantly correlated with cytogenetic abnormalities and with leukemic transformation, and it was concluded that changes in TRF length rather than telomerase activity may be useful in the future to stratify MDS patients according to risk scoring systems [81]. For solid tumours, with the exception of a study that found association of poor prognosis and telomere elongation in lung cancer [91], shorter telomere lengths reflected growth advantage and poor prognosis in neuroblastoma [37], breast cancer [79] and brain tumours [36].

Clinical value of telomerase activity

In 1994, the TRAP assay (for telomeric repeat amplification protocol) was published, allowing biochemical detection of telomerase activity in fresh or frozen cell or tissue lysates [51]. In this assay, an aliquot of the test lysate is incubated with a mixture containing a synthetic telomere-like oligonucleotide, which, in the presence of telomerase activity, will be elongated by a number of hexameric repeats. In a conventional polymerase chain reaction (PCR), the mixture of telomerase products, which differ in size by a multiplicand of 6 bases, is amplified and separated on polyacrylamide gels. In case of telomerase activity, a typical 6-bp incremental ladder is visualised. Numerous modifications of the original protocol have been made in order to improve reliability, linearity and sensitivity, to exclude false-positive results due to primer–dimer formation, to quantify enzyme levels and to exclude false negativity due to the presence of *Taq* polymerase inhibitors [50, 58, 100, 111]. Whilst in the original assay radiolabelled nucleotides are incorporated, a variety of non-isotopic variants have been elaborated, e.g. using fluorescein-labelled primers in combination with detection on an automated DNA sequencer or by capillary electrophoresis [1, 56]. We and others described quick, safe and effective detection of non-labelled amplicons using a silver staining method, the fluorescent dye SYBR Green I or ethidium bromide (Fig. 4) [19, 24, 107]. Commercial kits, e.g. 'TRAPeze' (Appligene-Oncor) and 'Telomerase PCR Elisa' (Roche), are currently available. Histochemical visualisation using 5-bromo-2-desoxy uridine (BRDU) as a marker or 'in situ TRAP' by in situ PCR of FITC-labelled telomerase products are other techniques that have been proved efficient for the in situ detection of telomerase activity in cryostat sections or cytospins, respectively [46, 82].

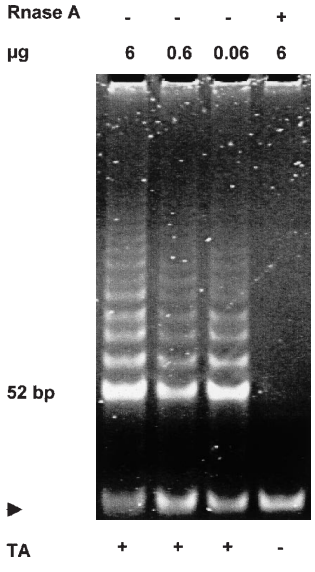

Fig. 4 Example of a non-isotopic telomeric repeat amplification protocol (TRAP) assay for HeLa cells. Telomerase activity (TA) is semi-quantitatively graded using 1/10 (0.6 µg) and 1/100 (0.06 µg) dilutions of total protein. Assay specificity is checked by RNase A-mediated degradation of hTERC, resulting in disappearance of the 6-bp ladder. False-negative results are excluded by inclusion of a 36-bp internal control (*arrowhead*). Amplicons are separated on a 12.5% non-denaturing polyacrylamide gel, ethidium bromide stained, and visualised by the CCD camera coupled Gel Doc 1000 Molecular Analyst Software package (Bio-Rad Laboratories GmbH, Germany)

Since the development of the TRAP assay, the number of reports investigating the association of telomerase activity with human cancer has increased considerably. Nearly all cancer types have been screened, and a strong association between the presence of telomerase and malignancy has been established. Importantly, high telomerase activity generally indicates high aggressive potential and, usually (but not always [20]), high proliferation rates [10, 59]. Such observations inspired many researchers to regard telomerase as a new proliferation marker for in vitro and in vivo conditions [5]. Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, and Table 13 survey the majority of recent studies (1997–1999) on the telomerase activity of different cancer types. Cancers have been combined in tables according to tissue of origin or body site, and, for most cancer types, results from several studies have been pooled. No attempts, however, have been made to critically evaluate the study results. Nevertheless, it should be cautioned that due to the susceptibility of the TRAP assay, false-negative and false-positive results are not infrequent. The technical reasons have been variously detailed elsewhere [58].

Clinical value of the telomerase components

Expression of hTERC RNA

hTERC expression does not parallel telomerase activity. Using RT-PCR or Northern-blot assays, hTERC was found to be present in mortal primary cells and in a variety of normal human tissues that lacked enzyme activity [3, 23]. These results have been confirmed by several groups showing hTERC expression in both normal and neoplastic tissues and in cell lines of diverse organs such as uterine cervix [98], urinary bladder [43], kidney [47], mesothelium [21], ovary [61], endometrium [60], oral mucosa [96], stomach [121] and the myeloid lineage [115]. Quantitative analyses have provided further insight into the role of hTERC expression during carcinogenesis. Maria Blasco and co-workers examined telomerase activity and mTERC expression during the development of HPV16-induced pancreatic islet cell carcinomas and of SV40-induced squamous cell skin carcinomas over distinct histological stages of islet and skin hyperplasia and dysplasia [7]. Telomerase activity was detected only in late-stage tumours, whereas mTERC levels were upregulated in the early pre-neoplastic stages and further increased during progression. These results suggest that, in mice, telomerase is activated in the late stages of tumour progression and showed that the initial upregulation of telomerase RNA seems an early event. Their data further suggested that mTERC might be either directly regulated by viral oncogenes or mTERC transcription might be responsive to deregulated cell proliferation, which can be the result of inhibitory binding of viral oncogenes to p53 and pRb.

In order to investigate hTERC expression at the cellular level during human carcinogenesis, an in situ hybridisation method (ISH) has been developed to detect hTERC in archival paraffin sections [118]. In the positive control, hTERC ISH signals – predominantly or entirely nuclear – are limited to primary spermatocytes and, possibly, spermatogonia in the seminiferous tubules, and to germinal centres of secondary follicles in lymph node. ISH has been applied to characterise hTERC expression in the histological progression of metaplastic over dysplastic and carcinoma in situ lesions (CIS) to carcinomatous lesions of the oesophagus [71], stomach [34, 38], lung [117], cervix [119] and breast [120]. In most cases, a marked rise in hTERC expression occurs during the transition from low-grade to high-grade dysplasia. Interestingly, in the Barrett's metaplasia–dysplasia carcinoma, sequence signals were found of stronger intensity in low-grade dysplasia associated with cancer than in foci of dysplasia not associated with cancer. The authors questioned whether this difference in signal intensity could be of prognostic value, identifying a subset of patients with low-grade dysplasia who were at increased risk for progression to malignancy. Studies of lung, cervix and breast lesions showed intense focal upregulation in CIS adjacent to or continuous with foci of microinvasive or invasive cancer. This finding was interpreted by the authors as a predictor of imminent invasion.

ISH detection of hTERC, in combination with immunohistochemistry for proliferative activity, has been used to clarify the debated relationship between telomerase activity and tumour proliferation activity [26]. In a variety of cancers such as astrocytomas [87], basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) [80], the distribution patterns of hTERC and the proliferation marker Ki-67 were found to be similar. However, in the normal skin, hTERC is preferentially localised in basal keratinocytes, whereas Ki-67 positive cells are predominantly in the suprabasal layers, suggesting that proliferating but also resting epidermal cells, likely including stem cells, express hTERC [80]. Similarly, Bickenbach and co-workers found that cells expressing histon-3, a marker for S-phase cells, were distributed evenly within the basal layer of the epidermis as single cells, unlike the hTERC expressing cells that clustered around the lower ends of the rete ridges, and concluded that upregulation of hTERC does not correlate with cells entering proliferation [6]. Another example of dissociation between Ki-67 and hTERC expression are stage-IV neuroblastomas, a widely metastatic variant associated with excellent clinical prognosis. These tumours were found to have high Ki-67 but weak hTERC expression, indicating that the latter may be a better discriminator of true biological potential and that hTERC levels do not always correlate with cell proliferation [66]. However, this statement should critically be reconsidered since Rudolph and co-workers previously found low proliferative activity in stage-IV neuroblastomas [86]. It is obvious that the debate did not yet come to an end. In this respect, it may be better to compare the staining patterns of proliferation markers with those of hTERT, which correlates better with telomerase activity than hTERC.

Expression of hTERT mRNA

The close correlation in cell lines between the presence of hTERT expression, determined by either RNase protection or RT-PCR techniques, and that of the telomerase assay, combined with evidence that ectopic hTERT expression is sufficient to confer enzymatic activity to telomerase-negative cells (see below), suggests that hTERT is the catalytic sub-unit of telomerase [9, 69, 73]. Therefore, strategies detecting hTERT mRNA and protein by RT-PCR or ISH and immunohistochemistry, respectively, are under development to find out whether hTERT mRNA or protein detection is useful as a surrogate marker for telomerase activity or transformed status of tissues.

Ramakrishnan and co-workers performed an extensive and detailed expression profiling of the hTERT gene in diverse tumour-derived cell lines, tumours, several normal human tissues and cultured normal cells [83]. Although generally corroborating the studies of both Nakamura et al. [73] and Meyerson et al. [69] with reference to the tumour cell lines, some normal tissues, such as heart, brain, liver, prostate and ovary, were found to be weakly positive for hTERT using RT-PCR. Surprisingly, telomerase-negative early passage cultures of renal epithelial cells, prostate epithelial cells and WI38 fibroblasts also had detectable hTERT, which cannot be ascribed to contamination by activated telomerase-positive lymphocytes. Therefore, he concluded that, in analogy to hTERC expression, the differences in expression of hTERT in tumour versus normal cells are quantitative rather than qualitative. In contrast, differences in hTERT mRNA expression were found qualitative in benign versus malignant cervical tissues [98], urothelium and urinary sediment [43], renal tissues [47], ovaries [61] and in human oral lesions [96]. In human endometrium, the expression of hTERT mRNA was observed in most endometrial cancers. In normal endometrium, it depended on the phases of the menstrual cycle, as reported for telomerase activity (Table 7) [60].

To date, only two reports described the expression pattern of hTERT mRNA at the single cell level in normal tissues and in various stages of tumour progression. Kolquist et al. found hTERT mRNA to be detectable in cells thought to have long-term proliferative capacity, like haematopoietic precursor cells, germinal centre lymphocytes, spermatogonia and in some actively regenerating cells, like the stratum basale of the skin, which is largely consistent with previous reports of the presence of telomerase activity in somatic stem cells [57]. The temporal relationship of hTERT expression to tumour progression was investigated by analysing the levels of hTERT mRNA in colon and breast lesions [57], and in cervical lesions [74]. Surprisingly, high levels of hTERT

mRNA, similar to levels detected in higher degrees of epithelial dysplasia and invasive and metastatic carcinomas, were already locally observed in adenomatous colonic epithelium and in early stage breast and cervical lesions. These results led to a model of in vivo telomerase activation distinct from that based on the results of experiments examining in vitro cell immortalisation. According to the in vitro model, which is based on the apparently exclusive appearance of telomerase activity and hTERT mRNA in post-crisis, telomere-deficient cells, the activation of telomerase is thought to occur late in the progression of tumours. The aforementioned observations led the authors to conclude that hTERT expression is already apparent in early precursor lesions, suggesting that cells may be continuously selected for incrementally higher levels of telomerase activity as they proliferate and acquire genetic changes associated with invasive cancer.

Expression of hTERT protein

Further information about the function and diagnostic value of telomerase will emerge as monoclonal anti-hTERT antibodies, suitable for immunohistochemical procedures on frozen and archived samples, become commercially available. At present, few groups reported hTERT staining using polyclonal antibodies raised against synthetic oligopeptides or recombinant protein. The K-370 antibody, specific for mouse and human hTERT, stains nuclei but not the cytoplasm of HeLa cells in both immunofluorescence and Western-blot analysis [68]. The epitope appeared to be concentrated in nuclear speckles reminiscent of those formed by many proteins involved in DNA replication and transcription. Alternatively, this hTERT dot-like expression pattern could correspond to the telomeres, which, in interphase nuclei, are similarly located. An identical nuclear staining pattern was previously described by the use of an anti-peptide antiserum to a different region of the hTERT [32].

Tahara et al. reported the first success in in situ immunohistochemical detection of anti-hTERT antibody-reactive proteins and compared the expression pattern with telomerase activity in human gastric carcinoma and colorectal tissue sections [97, 121]. In contrast to non-neoplastic mucosal cells and stromal cells, carcinoma cell nuclei were moderately positive. Curiously, in normal colonic glandular epithelium, dissociation was observed between expression of the hTERT protein and the telomerase activity, with protein expression extending from the bottom of the crypts to nearly the tip of the gland, whereas telomerase activity was only found at the Ki-67-rich bottom half of the crypt. The authors could not determine whether the upper part of the gland contains degraded proteins that retain antigenicity of hTERT or contain de-phophorylated, dormant hTERT protein. Some anti-hTERT polyclonal antibodies are currently commercially available (Santa Cruz Biotechnology, Calif. and Biotrend Chemikalien, Germany), but more studies are necessary to examine their specificity to the p127 protein [21].

New insights in the telomerase–tumorigenesis connection

Based on the results of the aforementioned studies, a variety of questions have emerged. Is telomerase capable of providing the immortal phenotype? Should telomerase be regarded as an oncogene? Is telomerase essential for carcinogenesis? Recent experiments provide, at least partially, answers to some of these questions.

Lessons from hTERT transfection experiments

In contrast to hTERC and hTEP1, expression of the hTERT gene parallels telomerase activity, and exciting experiments convincingly showed that hTERT is rate limiting for telomere elongation. Human telomerase activity could be reconstituted both in vitro, after co-synthesis or mixing of hTERC and the in vitro transcription/translation product of the hTERT gene, and in vivo, after transfection of the gene encoding hTERT into telomerase-negative human normal fibroblasts [76, 106]. Transfection of pre-senescent cultures of telomerase-negative retinal pigment epithelial cells, human vascular endothelial cells and young/midlife and old fibroblasts [9, 103, 116], as well as of pre-crisis cells [17, 28] resulted in telomerase activity, elongation of telomeres and indefinite replicative growth, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. Interestingly, while sufficient for immortalisation, ectopic expression of telomerase did not result in changes typically associated with malignant transformation, such as increased growth rate, loss of contact inhibition and acquisition of serum-independent growth, disturbances in the pRB and p53-mediated cell cycle checkpoints, and cytogenetic abnormalities, indicating that telomerase expression per se is not oncogenic [45, 72].

Lessons from the mTERC knock-out mouse

To explore the impact of telomere loss in vivo, telomerase-deficient mice were generated by deletion of the mTERC gene ($mTR^{-/-}$ mice). For the sake of clarity, the effect of telomere loss on physiological processes and on neoplastic growth is best discussed separately. As expected, the $mTR^{-/-}$ mice lacked detectable telomerase activity, and, per $mTR^{-/-}$ generation, telomeres shortened. Interestingly, from the fourth $mTR^{-/-}$ generation onwards, complete absence of telomeric repeats, aneuploidy and chromosomal abnormalities, like end-to-end associations including Robertsonian fusions, were seen. Surprisingly, mTERC-deficient mice were viable for the six generations analysed [8]. Similarly, first-generation

mice knocked-out for TERT (mTERT$^{-/-}$ mice) were recently found fertile too, and did not show any noticeable macroscopic or microscopic phenotypic change [122]. From the mTR$^{-/-}$ results, it was initially concluded that telomerase is essential for telomere length maintenance but not for mouse viability or fertility. However, fundamental differences exist between the genomic organisation of mouse and human telomeres, meaning that results on murine sources cannot be extrapolated to humans [55, 63, 114, 123]. Indeed, continuing phenotypic analysis of the mTR$^{-/-}$ mice showed that the initial findings were inherently misleading due to the use of the laboratory mouse strain Mus musculus, which possesses telomeres that are very much longer than in humans or in Mus spretus. Considerable time (and cell doublings) passed until the adverse effects of telomerase deficiency appeared in late-generation animals. They exhibited defective spermatogenesis and haematopoiesis, coupled with telomere-length reductions and abnormal cytogenetic profiles, indicating an essential role for telomerase and telomeres in the maintenance of genomic integrity and in the long-term viability of high-renewal tissues [29, 64]. In addition, it was shown that telomere loss in late-generation mTR$^{-/-}$ embryos was associated with a developmental defect that consisted of a failure in the closure of the neural tube, suggesting that telomeres may have an important role during the formation of the neural tube [35]. It is presently unknown whether some cases of infant neural tube defects are associated with telomere shortening.

mTR$^{-/-}$ mice provide an opportunity to study the effect of telomere dysfunction on important biological processes, such as those associated with ageing. As predicted from in vitro experiments [77], it was recently shown that age-dependent telomere shortening associates with shortened life span and reduced capacity to respond to stresses such as wound healing and haematopoietic ablation, demonstrating a critical role for telomere length in the overall fitness, reserve and well being of the ageing organism [85]. Thus, in contrast to what has been concluded from the first studies, most highly proliferative organs appear to be affected by mediators that impede telomere synthesis, indicating that, although diverse stem-cell compartments may sustain organ function after systemic administration of telomerase inhibitors during normal life expectancy, telomerase inhibition has to be monitored carefully in clinical settings. The impact of telomere dysfunction on immortalisation and tumorigenesis is far more complex. Indeed, telomerase null embryonic fibroblast cultures could be unexpectedly immortalised in culture, transformed by the Ras and SV40 TAg oncogenes, and were found to generate tumours in nude mice [8]. Furthermore, a progressive increase in the rate of cytogenetic abnormalities and of spontaneous tumour formation was found in aged late-generation mTR$^{-/-}$ mice [85]. These data, suggesting that in tumorigenesis telomerase expression might be an innocent bystander, are regarded as a serious challenge to the original model in which telomerase activation is a key step in cancer. Here too, it has been stressed that mice and humans are very different when it comes to cancer, since murine cells both in vitro and in vivo more efficiently immortalise and form tumours than human cells. It is possible that, in mice, the natural overlength of telomeres renders telomerase of minor importance for the longevity of cells compared with human cells. However, this does not explain why telomerase activity is upregulated in murine cancers relative to normal tissues. Most recently, studies conducted with mice doubly null for mTR and p53 (mTR$^{-/-}$ p53$^{-/-}$ mice) or INK4a/ARF (mTR$^{-/-}$ INK4a$^{-/-}$ mice) showed that telomerase may play a paradoxical role, either promoting or inhibiting tumour formation depending on the genetic context of the would-be cancer cell [14, 25].

Perspectives

The presence of telomerase in nearly all cancers offers important therapeutic possibilities. Numerous novel anticancer strategies, based on the use of telomerase inhibitors, are currently under investigation. However, although ALT has been discussed in only a minority of tumours, it is possible that in tumours subjected to effective telomerase inhibition, there would be a strong selection pressure for the emergence of treatment-resistant cells via the activation of ALT. Moreover, research groups have just started to unravel the relationship between telomeres, telomerase and key tumour suppressor pathways, such as the pRB/p16^{INK4a} and the p53/p19^{ARF} pathways. It is not unimaginable that only a genotypic subset of tumours will be sensible to telomerase inhibitors. Therefore, the human telomere itself could turn out to be a better therapeutic target, e.g. via tankyrase inhibitors. The finding that transfection of primary human cells with hTERT can confer endless growth capability in culture, without leading to a cancerous phenotype, currently receives much attention. The repair of human tissues, e.g. the enhanced ex vivo growth of skin cells for burn victims, and rejuvenation of stem cell populations based on telomerase therapy could be within reach. Apart from its therapeutic applications, our ability to extend cellular life span, whilst maintaining the diploid status, growth characteristics and gene expression pattern typical of young normal cells, has important implications for the development of in vitro models of carcinogenesis.

Conclusions

Over the last years, exciting reports have disclosed the enormous complexity of telomerase biology, and some authors already regard the enzyme as the molecule of the 1990s. A tally of studies screening most types of human cancers has established a strong association between the presence of telomerase activity and malignancy. Particularly the relationship between ‘high telomerase activity and poor prognosis’ makes the enzyme, rather than its

substrate, an interesting tumour marker worthwhile being studied further. However, the existence of telomerase-competent normal cells, the presence of ALT mechanisms and the results of telomerase knockouts have underscored the unique role of telomeres and telomerase in tumorigenesis, and the initial enthusiasm for this putative malignancy marker has dwindled to some extent. Pathologists in particular hopefully await the advent of reliable telomerase antibodies, which will allow a better in situ evaluation of telomerase, particularly in proliferating versus non-proliferating and in reactive versus pre-malignant lesions. Based on ISH experiments, hTERT expression, like that of hTERC, appears to occur early during carcinogenesis, either as a gain of function or by quantitative upregulation, whereas telomerase activity is a rather late event. Therefore, it is likely that still unknown factors that modulate the enzyme activity are important players in this complex biological system. Recent knowledge of the hTERT promoter possibly leads to a breakthrough in our understanding of cancer-associated activation. Finally, telomerase has rendered to represent neither a tumour suppressor nor an oncogenic agent. Since, in most cancers and in stem cells of renewal tissues, telomerase activity levels generally (but not always) correlate with the proliferation state of the cells, it seems more correct to regard the enzyme activity as an indication for indefinite cellular replicative capacity.

References

1. Aldous WK, Grabill NR (1997) A fluorescent method for detection of telomerase activity. Diagn Mol Pathol 6:102–110
2. Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider C, Harley CB (1992) Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci U S A 89:10114–10118
3. Avilion AA, Piatyszek MA, Gupta J, Shay JW, Bacchetti S, Greider CW (1996) Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res 56:645–650
4. Bechter OE, Eisterer W, Pall G, Hilbe W, Kühr T, Thaler J (1998) Telomere length and telomerase activity predict survival in patients with B cell chronic lymphocytic leukemia. Cancer Res 58:4918–4922
5. Belair CD, Yeager TR, Lopez PM, Reznikoff CA (1997) Telomerase activity: a biomarker of cell proliferation, not malignant transformation. Proc Natl Acad Sci U S A 94: 13677–13682
6. Bickenbach JR, Vornwald D, V, Bachor C, Bleuel K, Schnapp G, Boukamp P (1998) Telomerase is not an epidermal stem cell marker and is downregulated by calcium. J Invest Dermatol 111:1045–1052
7. Blasco MA, Rizen M, Greider CW, Hanahan D (1996) Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. Nat Genet 12:200–204
8. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25–34
9. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279:349–352
10. Bonatz G, Klapper W, Barthe A, Heidorn K, Jonat W, Krupp G, Parwaresch R (1998) Analysis of telomerase expression and proliferative activity in the different layers of cyclic endometrium. Biochem Biophys Res Commun 253:214–221
11. Bond JA, Wyllie FS, Wynford-Thomas D (1994) Escape from senescence in human diploid fibroblasts induced directly by mutant p53. Oncogene 9:1885–1889
12. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR (1997) Evidence for an alternative mechanism for maintaining telomere length in human tumours and tumour-derived cell lines. Nat Med 3:1271–1274
13. Bryant JE, Hutchings KG, Moyzis RK, Griffith JK (1997) Measurement of telomeric DNA content in human tissues. Biotechniques 23:476–483
14. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider C, DePinho R (1999) p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 97:527–538
15. Cong YS, Wen JP, Bacchetti S (1999) The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum Mol Genet 8:137–142
16. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 11:1921–1929
17. Counter CM, Hahn WC, Wei W, Caddle SD, Beijersbergen RL, Lansdorp PM, Sedivy JM, Weinberg RA (1998) Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalisation. Proc Natl Acad Sci U S A 95: 14723–14728
18. Dahse R, Fiedler F, Ernst G, Kosmehl H, Schlichter A, Schubert J, Claussen U (1996) Changes in telomere lengths in renal cell carcinomas. Cell Mol Biol 42:477–485
19. Dhaene K, Hübner R, Kumar-Singh S, Weyn B, Van Marck E (1998) Telomerase activity in human pleural mesothelioma. Thorax 53:915–918
20. Dhaene K, Vancoillie G, Lambert J, Naeyaert JM, Van Marck E (2000) Absence of telomerase activity and telomerase catalytic subunit mRNA in melanocyte cultures. Br J Cancer 82:1051–1057
21. Dhaene K, Wauters J, Weyn B, Timmermans JP, Van Marck E (2000) Expression profile of telomerase subunits in human pleural mesothelioma. J Pathol 190:80–85
22. Eickbush TH (1997) Telomerase and retrotransposons: which came first? Science 277:911–912
23. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, Le S, West MD, Harley CB, Andrews WH, Greider C, Villeponteau B (1995) The RNA component of human telomerase. Science 269: 1236–1241
24. Fong D, Burke JP, Chan MM (1997) Nonradioactive, agarose minigel procedure for telomeric amplification protocol. Biotechniques 23:1029–1032
25. Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider C, DePinho RA (1999) Short dysfunctional telomeres impair tumorigenesis in the INK4a$^{\Delta 2/3}$ cancer-prone mouse. Cell 97:515–525
26. Greider CW (1998) Telomerase activity, cell proliferation, and cancer. Proc Natl Acad Sci U S A 95:90–92
27. Griffith JK, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, De Lange T (1999) Mamalian telomeres end in a large duplex loop. Cell 97:503–514
28. Halvorsen TL, Leibowitz G, Levine F (1999) Telomerase activity is sufficient to allow transformed cells to escape from crisis. Mol Cell Biol 19:1864–1870
29. Hande MP, Samper E, Lansdorp PM, Blasco MA (1999) Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. J Cell Biol 144:589–601
30. Harley CB, Kim NW, Prowse KR, Weinrich SL, Hirsch KS, West MD, Bacchetti S, Hirte HW, Counter CM, Greider CW (1994) Telomerase, cell immortality, and cancer. Cold Spring Harb Symp Quant Biol 59:307–315
31. Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Amgen EST Program, Bass MB, Arruda I, Robinson MO (1997) A

mammalian telomerase-associated protein. Science 275:973–977
32. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DS, Mar V, Bass MB, Robinson MO (1997) Human telomerase contains evolutionarily conserved catalytic and structural subunits. Genes Dev 11:3109–3115
33. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC (1990) Telomere reduction in human colorectal carcinoma and with ageing. Nature 346:866–868
34. Heine B, Hummel M, Demel G, Stein H (1998) Demonstration of constant upregulation of the telomerase RNA component in human gastric carcinomas using in situ hybridization. J Pathol 185:139–144
35. Herrera E, Samper E, Blasco MA (1999) Telomere shortening in mTR–/– embryos is associated with failure to close the neural tube. EMBO J 18:1172–1181
36. Hiraga S, Ohnishi T, Izumoto S, Miyahara E, Kanemura Y, Matsumura H, Arita N (1998) Telomerase activity and alterations in telomere length in human brain tumours. Cancer Res 58:2117–2125
37. Hiyama E, Hiyama K, Yokoyama Y, Ichikawa T, Matsuura Y (1992) Length of telomeric repeats in neuroblastoma: correlation with prognosis and other biological characteristics. Jpn J Cancer Res 83:159–164
38. Hiyama T, Yokozaki H, Kitadai Y, Tahara E, Tahara H, Ide T, Haruma K, Yasui W, Kajiyama G (1998) In situ mRNA hybridization technique for analysis of human telomerase RNA in gastric precancerous and cancerous lesions. Jpn J Cancer Res 89:1187–1194
39. Horikawa I, Cable PL, Afshari C, Barrett JC (1999) Cloning and characterisation of the promoter region of human telomerase reverse transcriptase gene. Cancer Res 59:826–830
40. Horvath MP, Schweiker VL, Bevilacqua JM, Ruggles JA, Schultz SC (1998) Crystal structure of the Oxytricha nova telomere end binding protein complexed with single strand DNA. Cell 95:963–974
41. Hsu H, Gilley D, Blackburn EH, Chen DJ (1999) Ku is associated with the telomere in mammals. Proc Natl Acad Sci U S A 96:12454–12458
42. Hultdin M, Gronlund E, Norrback KF, Eriksson-Lindstrom E, Just T, Roos G (1998) Telomere analysis by fluorescence in situ hybridisation and flow cytometry. Nucleic Acids Res 26:3651–3656
43. Ito H, Kyo S, Kanaya T, Takakura M, Inoue M, Namiki M (1998) Expression of human telomerase subunits and correlation with telomerase activity in urothelial cancer. Clin Cancer Res 4:1603–1608
44. Iwama H, Ohyashiki K, Ohyashiki JH, Hayashi S, Kawakubo K, Shay JW, Toyama K (1997) The relationship between telomere length and therapy-associated cytogenetic responses in patients with chronic myeloid leukemia. Cancer 79:1552–1560
45. Jiang XR, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, Beeche M, Bodnar AG, Wahl GM, Tlsty TD, Chiu CP (1999) Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nat Genet 21:111–114
46. Jirikowski GF (1999) Histochemical visualisation of telomerase activity (abstract). Proceedings of the 6th International Conference and Workshops on Molecular Morphology, 5–9 October, 1998, Salzburg, Austria. Appl Immunohistochem Mol Morphol 7:89–102
47. Kanaya T, Kyo S, Takakura M, Ito H, Namiki M, Inoue M (1998) hTERT is a critical determinant of telomerase activity in renal-cell carcinoma. Int J Cancer 78:539–543
48. Kickhoefer VA, Stephen AG, Harrington L, Robinson MO, Rome LH (1999) Vaults and telomerase share a common subunit, TEP1. J Biol Chem 274:32712–32717
49. Kilian A, Bowtell DD, Abud HE, Hime GR, Venter DJ, Keese PK, Duncan EL, Reddel RR, Jefferson RA (1997) Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types. Hum Mol Genet 6:2011–2019
50. Kim NW, Wu F (1997) Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). Nucleic Acids Res 25:2595–2597
51. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015
52. Kim S, Kaminker P, Campisi J (1999) TIN2, a new regulator of telomere length in human cells. Nat Genet 23:405–412
53. Kinoshita H, Ogawa O, Mitsumori K, Kakehi Y, Terachi T, Yoshida O (1998) Low frequency of positive telomerase activity in a chromophobe subtype of renal cell carcinoma. J Urol 159:245–251
54. Kipling D (ed) (1995) The telomere. Oxford University Press, Oxford
55. Kipling D (1997) Mammalian telomerase: catalytic subunit and knockout mice. Hum Mol Genet 6:1999–2004
56. Klapper W, Singh KK, Heidorn K, Parwaresch R, Krupp G (1998) Regulation of telomerase activity in quiescent immortalized human cells. Biochem Biophys Acta 1442:120–126
57. Kolquist KA, Ellisen LW, Counter CM, Meyerson M, Tan LK, Weinberg RA, Haber DA, Gerald WL (1998) Expression of TERT in early premalignant lesions and a subset of cells in normal tissues. Nat Genet 19:182–186
58. Krupp G, Kühne K, Tamm S, Klapper W, Heidorn K, Rott A, Parwaresch R (1997) Molecular basis of artifacts in the detection of telomerase activity and a modified primer for a more robust 'TRAP' assay. Nucleic Acids Res 25:919–921
59. Kubota Y, Nakada T, Sasagawa I, Yanai H, Itoh K (1998) Elevated levels of telomerase activity in malignant pheochromocytoma. Cancer 82:176–179
60. Kyo S, Kanaya T, Takakura M, Tanaka M, Inoue M (1999) Human telomerase reverse transcriptase as a critical determinant of telomerase activity in normal and malignant endometrial tissues. Int J Cancer 80:60–63
61. Kyo S, Kanaya T, Takakura M, Tanaka M, Yamashita A, Inoue H, Inoue M (1999) Expression of human telomerase subunits in ovarian malignant borderline and benign tumors. Int J Cancer 80:804–809
62. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A, Inoue M (1999) Estrogen activates telomerase. Cancer Res 59:5917–5921
63. Lansdorp PM (1997) Lessons from mice without telomerase. J Cell Biol 139:309–312
64. Lee HW, Blasco MA, Gottlieb GJ, Horner JW, Greider CW, DePinho RA (1998) Essential role of mouse telomerase in highly proliferative organs. Nature 392:569–574
65. Li H, Zhao LL, Yang ZY, Funder JW, Liu JP (1998) Telomerase is controlled by protein kinase C alpha in human breast cancer cells. J Biol Chem 273:33436–33442
66. Maitra A, Yashima K, Rathi A, Timmons CF, Rogers BB, Shay JW, Gazdar AF (1999) The RNA component of telomerase as a marker of biologic potential and clinical outcome in childhood neuroblastic tumors. Cancer 85:741–749
67. Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, Lansdorp PM (1998) Short telomeres on human chromosome 17p. Nat Genet 18:76–80
68. Martin RL, Herrera E, Albar JP, Blasco MA (1998) Expression of mouse telomerase catalytic subunit in embryos and adult tissues. Proc Natl Acad Sci U S A 95:10471–10476
69. Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu QY, Bacchetti S, Haber DA, Weinberg RA (1997) hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 90: 785–795
70. Mitchell JR, Cheng J, Collins K (1999) A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. Mol Cell Biol 19:567–576
71. Morales CP, Lee EL, Shay JW (1998) In situ hybridization for the detection of telomerase RNA in the progression from Bar-

rett's esophagus to esophageal adenocarcinoma. Cancer 83: 652–659
72. Morales CP, Holt SE, Ouellette M, Kaur KJ, Yan Y, Wilson KS, White MA, Wright WE, Shay JW (1999) Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 21:115–118
73. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR (1997) Telomerase catalytic subunit homologs from fission yeast and human. Science 277:955–959
74. Nakano K, Watney E, McDougall JK (1998) Telomerase activity and expression of telomerase RNA component and telomerase catalytic subunit gene in cervical cancer. Am J Pathol 153:857–864
75. Nakayama J, Saito M, Nakamura H, Matsuura A, Ishikawa F (1997) TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Cell 88:875–884
76. Nakayama J, Tahara H, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Ide T, Ishikawa F (1998) Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. Nat Genet 18:65–68
77. Niida H, Matsumoto T, Satoh H, Shiwa M, Tokutake Y, Furuichi Y, Shinkai Y (1998) Severe growth defect in mouse cells lacking the telomerase RNA component. Nat Genet 19:203–206
78. Nugent CI, Hughes TR, Lue NF, Lundblad V (1996) Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. Science 274:249–252
79. Odagiri E, Kanada N, Jibiki K, Demura R, Aikawa E, Demura H (1994) Reduction of telomeric length and c-erbB-2 gene amplification in human breast cancer, fibroadenoma, and gynecomastia. Relationship to histologic grade and clinical parameters. Cancer 73:2978–2984
80. Ogoshi M, Le T, Shay JW, Taylor RS (1998) In situ hybridization analysis of the expression of human telomerase RNA in normal and pathologic conditions of the skin. J Invest Dermatol 110:818–823
81. Ohyashiki JH, Iwama H, Yahata N, Ando K, Hayashi S, Shay JW, Ohyashiki K (1999) Telomere stability is frequently impaired in high-risk groups of patients with myelodysplastic syndromes. Clin Cancer Res 5:1155–1160
82. Ohyashiki K, Ohyashiki JH, Nishimaki J, Toyama K, Ebihara Y, Kato H, Wright WE, Shay JW (1997) Cytological detection of telomerase activity using an in situ telomeric repeat amplification protocol assay. Cancer Res 57:2100–2103
83. Ramakrishnan S, Eppenberger U, Mueller H, Shinkai Y, Narayanan R (1998) Expression profile of the putative catalytic subunit of the telomerase gene. Cancer Res 58:622–625
84. Ricchetti M, Buc H (1997) Telomerase activity of reverse transcriptase. Science 277:887
85. Rudolph KL, Chang S, Lee HW, Blasco MA, Gottlieb GJ, Greider C, DePinho R (1999) Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96:701–712
86. Rudolph P, Lappe T, Hero B, Parwaresch R, Harms D, Schmidt D (1997) Prognostic significance of the proliferative activity in neuroblastoma. Am J Pathol 150:133–145
87. Sallinen P, Miettinen H, Sallinen SL, Haapasalo H, Helin H, Kononen J (1997) Increased expression of telomerase RNA component is associated with increased cell proliferation in human astrocytomas. Am J Pathol 150:1159–1164
88. Shay JW (1999) Toward identifying a cellular determinant of telomerase repression. J Natl Cancer Inst 91:4–6
89. Shay JW, Brasiskyte D, Quelette M (1994) Analysis of telomerase and telomeres. Methods Mol Genet 5:263–268
90. Shay JW, Werbin H, Wright WE (1996) Telomeres and telomerase in human leukemias. Leukemia 10:1255–1261
91. Shirotani Y, Hiyama K, Ishioka S, Inyaku K, Awaya Y, Yonehara S, Yoshida Y, Inai K, Hiyama E, Hasegawa K (1994) Alteration in length of telomeric repeats in lung cancer. Lung Cancer 11:29–41
92. Smith S, De Lange T (1997) TRF1, a mammalian telomeric protein. Trends Genet 13:21–26
93. Smith S, De Lange T (1999) Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes. J Cell Science 112:3649–3656
94. Smith S, Giriat I, Schmitt A, De Lange T (1998) Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 282:1484–1487
95. Soder AI, Hoare SF, Muir S, Going JJ, Parkinson EK, Keith WN (1997) Amplification, increased dosage and in situ expression of the telomerase RNA gene in human cancer. Oncogene 14:1013–1021
96. Sumida T, Hamakawa H, Sogawa K, Sugita A, Tanioka H, Ueda N (1999) Telomerase components as a diagnostic tool in human oral lesions. Int J Cancer 80:1–4
97. Tahara H, Yasui W, Tahara E, Fujimoto J, Ito K, Tamai K, Nakayama J, Ishikawa F, Ide T (1999) Immuno-histochemical detection of human telomerase catalytic component, hTERT, in human colorectal tumor and non-tumor tissue sections. Oncogene 18:1561–1567
98. Takakura M, Kyo S, Kanaya T, Tanaka M, Inoue M (1998) Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. Cancer Res 58:1558–1561
99. Takakura M, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M, Inoue M (1999) Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res 59: 551–557
100. Tatematsu K, Nakayama J, Danbara M, Shionoya S, Sato H, Omine M, Ishikawa F (1996) A novel quantitative 'stretch PCR assay', that detects a dramatic increase in telomerase activity during the progression of myeloid leukemias. Oncogene 13:2265–2274
101. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR (1998) Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. Cancer Res 58:4168–4172
102. Van Steensel B, Smogorzewska A, De Lange T (1998) TRF2 protects human telomeres from end-to-end fusions. Cell 92:401–413
103. Vaziri H, Benchimol S (1998) Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr Biol 8:279–282
104. Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effros RB, Cohen D, Harley CB (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am J Hum Genet 52:661–667
105. Wainwright J, Middleton PG, Rees JL (1995) Changes in mean telomere length in basal cell carcinomas of the skin. Genes Chromosomes Cancer 12:45–49
106. Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB (1997) Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. Nat Genet 17:498–502
107. Wen JM, Sun LB, Zhang M, Zheng MH (1998) A non-isotopic method for the detection of telomerase activity in tumour tissues: TRAP-silver staining assay. J Clin Pathol Mol Pathol 51:110–112
108. Wick M, Zubov D, Hagen G (1999) Genomic organisation and promoter characterisation of the gene encoding the human telomerase reverse transcriptase (hTERT). Gene 232: 97–106
109. Wright WE, Shay JW (1992) The two-stage mechanism controlling cellular senescence and immortalization. Exp Gerontol 27:383–389

110. Wright WE, Pereira SO, Shay JW (1989) Reversible cellular senescence: implications for immortalization of normal human diploid fibroblasts. Mol Cell Biol 9:3088–3092
111. Wright WE, Shay JW, Piatyszek MA (1995) Modifications of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. Nucleic Acids Res 23:3794–3795
112. Wu KJ, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J, Dalla-Favera R (1999) Direct activation of TERT transcription by c-Myc. Nat Genet 21:220–224
113. Wynford-Thomas D (1999) Cellular senescence and cancer. J Pathol 187:100–111
114. Wynford-Thomas D, Kipling D (1997) Telomerase. Cancer and the knockout mouse. Nature 389:551–552
115. Xu DW, Gruber A, Peterson C, Pisa P (1998) Telomerase activity and the expression of telomerase components in acute myelogenous leukaemia. Br J Haematol 102:1367–1375
116. Yang JY, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar AG, Bronstein A, Chiu CP, Herron GS (1999) Human endothelial cell life extension by telomerase expression. J Biol Chem 274:26141–26148
117. Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, Milchgrub S, Srivastava S, Piatyszek MA, Shay JW, Gazdar AF (1997) Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. Cancer Res 57:2373–2377
118. Yashima K, Piatyszek MA, Saboorian HM, Virmani AK, Brown D, Shay JW, Gazdar AF (1997) Telomerase activity and in situ telomerase RNA expression in malignant and non-malignant lymph nodes. J Clin Pathol 50:110–117
119. Yashima K, Ashfaq R, Nowak J, Von Gruenigen V, Milchgrub S, Rathi A, Albores Saavedra J, Shay JW, Gazdar AF (1998) Telomerase activity and expression of its RNA component in cervical lesions. Cancer 82:1319–1327
120. Yashima K, Milchgrub S, Gollahon LS, Maitra A, Saboorian MH, Shay JW, Gazdar AF (1998) Telomerase enzyme activity and RNA expression during the multistage pathogenesis of breast carcinoma. Clin Cancer Res 4:229–234
121. Yasui W, Tahara H, Tahara E, Fujimoto J, Nakayama J, Ishikawa F, Ide T (1998) Expression of telomerase catalytic component, telomerase reverse transcriptase, in human gastric carcinomas. Jpn J Cancer Res 89:1099–1103
122. Yuan X, Ishibashi S, Hatakeyama S, Saito M, Nakayama J, Nikaido R, Haruyama T, Watanabe Y, Iwata H, Iida H, Sugimura H, Yamada N, Ishikawa F (1999) Presence of telomeric G-strand tails in the telomerase catalytic subunit TERT knockout mice. Genes Cells 4:563–572
123. Zakian VA (1997) Life and cancer without telomerase. Cell 91:1–3
124. Zhao JQ, Hoare SF, McFarlane R, Muir S, Parkinson EK, Black DM, Keith WN (1998) Cloning and characterization of human and mouse telomerase RNA gene promoter sequences. Oncogene 16:1345–1350
125. Zhu L, Smith S, De Lange T, Seldin MF (1999) Chromosomal mapping of the tankyrase gene in human and mouse. Genomics 57:320–321