Cytotechnology **45:** 61–74, 2004.

Telomerase detection in the diagnosis and prognosis of cancer

Eiso Hiyama[1,*] and Keiko Hiyama[2]
[1]*Natural Science Center for Basic Research and Development, RIRBM, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734–8551, Hiroshima, Japan;* [2]*Department of Translational Cancer Research, RIRBM, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734–8551, Hiroshima, Japan;* **Author for correspondence (e-mail: eiso@hiroshima-u.ac.jp; phone: +81-82-255-5951; fax: +81-82-257-5909)*

Received 15 June 2004; accepted in revised form 21 September 2004

Key words: Cytology, Diagnosis, Fine needle aspiration, hTERT, Human telomerase reverse transcriptase, Immunohistochemistry, *in situ* hybridization, Prognosis, Telomerase, Telomeric repeat amplification protocol, TRAP

Abstract

Telomerase, a critical enzyme responsible 'for cellular immortality, is usually repressed in somatic cells except for lymphocytes and self-renewal cells, but is activated in approximately 85% of human cancer tissues. The human telomerase reverse transcriptase (hTERT) is the catalytic component of human telomerase. In cancers in which telomerase activation occurs at the early stages of the disease, telomerase activity and hTERT expression are useful markers for the detection of cancer cells. In other cancers in which telomerase becomes upregulated upon tumor progression, they are useful as prognostic indicators. However, careful attention should be paid to false–negative results caused by the instability of telomerase and of the *hTERT* mRNA and the presence of PCR inhibitors, as well as to false–positive results caused by the presence of alternatively spliced *hTERT* mRNA and normal cells with telomerase activity. Recently, methods for the *in situ* detection of the *hTERT* mRNA and protein have been developed. These methods should facilitate the unequivocal detection of cancer cells, even in tissues containing a background of normal telomerase-positive cells.

Abbreviations: BAL – bronchoalveolar lavage; FNA – fine needle aspiration; IHC – immunohistochemistry; ISH – in situ hybridization; hTERT – human telomerase reverse transcriptase; hTR – human telomerase RNA; TRAP – telomeric repeat amplification protocol.

Introduction

Mammalian telomeres are made of many hundreds to thousands units of the simple DNA repeat TTAGGG. Telomeres form a nucleoprotein that cap and protect the ends of linear chromosomes. Because the DNA replication machinery cannot fully replicate the ends of linear DNA molecules, telomeres progressively shorten with each cell division (Watson 1972). Eventually, when a critically short telomere length is reached, cells stop dividing and senesce (Greider 1990; Wright and Shay 1992). This phenomenon is thought to function as a 'mitotic clock' that limits the lifespan of individual cells. Telomerase is a specialized reverse transcriptase that synthesizes telomeric

repeats onto chromosomal ends and thus compensates for the progressive shortening of the telomeres caused by the end-replication problem (Lingner et al. 1995). This enzyme lacks from most somatic human cells and is typically restricted to certain specialized cell types, such as germ cells and stem/progenitor cells of self-renewal tissues, which must perform unusually large numbers of cell divisions (Hiyama et al. 1995c; Wright et al. 1996; Sakabe et al. 1998).

Cancer is a disease characterized by uncontrolled proliferation and invasion into surrounding tissues or distant organs. Most somatic human cells lack telomerase activity, have a limited life span, and require the activation of telomerase for unlimited proliferation. Although telomerase activation is not always concomitant with carcinogenesis, its presence in 85% of more than 3000 tumor samples tested makes telomerase activity the most universal maker of human cancers (Kim 1997; Shay and Gazdar 1997; Dhaene et al. 2000). Recent reports revealed that telomerase activity is upregulated during mouse tumorigenesis in spite of the fact that mice have very long telomeres (Blasco et al. 1996; Broccoli et al. 1996). This observation and others have suggested that telomerase may promote tumorigenesis independently of telomere length. By stabilizing telomeres and supporting the indefinite growth of most cancer cells, telomerase most certainly plays a crucial role in the progression and maintenance of tumors.

An important question, is when telomerase is activated during the multi-step process of carcinogenesis. In some instances, telomerase may already be ubiquitously expressed at the preneoplastic or *in situ* stage; while in other instances, the enzyme may be activated gradually with cancer progression (Shay and Bacchetti 1997). These differences are crucial in dictating whether telomerase might be clinically useful for either diagnostic or prognostic purposes. Although most somatic human cells lack telomerase activity, some tissues contain specialized cells, including germ cells, lymphocytes, stem cells, or certain epithelial cells, that display weak levels of telomerase activity, which can be upregulated concomitantly with growth signals. In such tissues, *in situ* immunohistochemical detection of telomerase may be necessary to determine whether telomerase expression is derived from normal telomerase-positive cells or from cancer cells. Taking these key points into account, telomerase is now being explored as a novel marker for early detection and/or the grading of malignant tumors. The present article reviews the use of human telomerase as a cancer diagnostic marker and as a prognostic tool for predicting the outcome of individual patients.

Detecting human telomerase in clinical materials

Telomerase can be measured by a PCR-based assay called telomeric repeat amplification protocol (TRAP) (Kim et al. 1994). The assay is quite sensitive and can detect as few as 10 telomerase positive cells (Wright et al. 1995). With this high sensitivity, telomerase activity can also be detected in certain normal somatic tissues, especially in proliferative and/or stem cells of self-renewing tissues (such as intestinal epithelium) and activated lymphocytes (Hiyama et al. 1995c, 1996b; Wright et al. 1996). Moreover, this activity is also detectable, albeit at low levels, in some benign tumors such as fibroadenomas of the breast (Hiyama et al. 1996a), hyperplastic nodule/adenomas of the thyroid (Matthews et al. 2001), and colon adenomas (Hiyama et al. 1996b). As a general rule, telomerase activity in normal somatic cells tends to be much lower in comparison to that detected in cancer cells. In clinical samples from tissues containing normal telomerase-positive cells, evidence of cancer cells requires levels of telomerase activity that are significantly higher than those of matched control tissues (Shay and Bacchetti 1997). As the TRAP assay is based on semi-quantitative PCR, a more precise method of quantification might be needed for such samples. To overcome this limitation, a real-time PCR assay (RTQ-TRAP) has been developed that allows quantitative measurements of telomerase activity in tissue samples (Hou et al. 2001). To avoid false–positive results due to contamination of cell samples with lymphocytes, we recommended using a thousand cell equivalents of cell lysate per assay, as proteins extracted from a thousand adult lymphocytes do not produce detectable telomerase activity (Hiyama et al. 1995c; Iwao et al. 1997). To avoid false–negative results, careful attention should be paid to the stability of telomerase and the presence of PCR inhibitors when examining clinical specimens.

Human telomerase activity is associated with the expression of two major components: human

telomerase RNA (*hTR*) (Feng et al. 1995) and human telomerase reverse transcriptase (hTERT) (Nakamura et al. 1997). Recent studies have targeted the expression of these two components as surrogates for telomerase activity and discussed their value as tumor markers. Since hTR is expressed at low levels in all cells, including cells that lack telomerase activity (Koyanagi et al. 2000), detection of the *hTERT* mRNA is believed to be a more reliable marker of the presence of cancer cells in clinical samples. However, the existence of splicing variants of the *hTERT* mRNA that fail to produce telomerase activity (Ulaner et al. 1998) can also be problematic for the use of the *hTERT* mRNA as surrogate for telomerase activity.

Telomerase as a diagnostic marker

Recently, there has been an increasing amount of experimental data on the detection of telomerase activity and/or hTERT expression in clinical materials as a diagnostic tool for various cancers (Table 1).

Head and neck tumors

Most cancer tissues in head and neck lesions show high levels of telomerase expression. Although the viability of cancer cells in these specimens is not particularly high, telomerase activity is often detected in oral washings of patients with oral malignancy (Califano et al. 1996; Sumida et al. 1998). In such specimens, it is difficult to avoid contamination by substances that interfere with PCR, such as necrotic tissue, leukocytes, erythrocytes, dental plaque, and bacteria. The presence of these substances in cancer samples can lead to false–negative results. Although currently limited in its sensitivity, the detection of telomerase activity or *hTERT* mRNA in oral washings is a novel marker indicating the presence of cancer cells shed from the upper aerodigestive tract.

In tumor biopsies, telomerase activity and the *hTERT* mRNA are almost always detected at high levels. Since low levels of telomerase activity are detected in the normal buccal epithelia and in approximately 20% of non-cancerous biopsy samples, a quantitative TRAP assay may be required for cancer diagnosis.

Thyroid and breast tumors

Because thyroid and breast lesions are easily palpable, fine needle aspiration (FNA) is widely used as a diagnostic tool for cancer detection in these lesions. For tumors of the thyroid gland, differential diagnosis between follicular adenoma and adenocarcinoma is difficult by FNA cytology alone. The detection of telomerase activity and/or *hTERT* mRNA has been found to be a useful tool for this differential diagnosis; as cancers gave positive signals while adenomas were negative for telomerase expression (Umbricht et al. 1997; Zeiger et al. 1999). However, thyroid tissues often contain lymphocytes, so that telomerase activity and *hTERT* mRNA derived from these inflammatory cells may also be detectable in certain benign diseases, such as Hashimoto thyroiditis (Haugen et al. 1997).

In the breast, normal mammary tissue lacks detectable telomerase activity, while the activity is expressed in 80–90% of ductal carcinoma *in situ* (DCIS) lesions and 90% of invasive breast cancers (Hiyama et al. 1996a; Umbricht et al. 1999). *hTERT* mRNA is detected at high frequency in breast cancers, where its levels are relatively high (Bieche et al. 2000). One of the most common problems in using telomerase for breast cancer diagnosis is the presence of telomerase activity in benign fibroadenomas. Approximately 40% of fibroadenoma tissues display low-level telomerase activity (Hiyama et al. 1996a; Pearson et al. 1998). In combination with cytology and with careful attention to benign diseases, the screening of FNA samples for telomerase expression is likely to become a powerful tool for the detection of breast and thyroid cancers (Pearson et al. 1998; Mokbel et al. 1999; Poremba et al. 1999a; Hiyama et al. 2000).

Lung and mediastinum

Sputum, bronchoalveolar lavage (BAL), bronchial brushing, and bronchial washing samples have all been tested in the TRAP assay for the detection of lung cancer cells (Sen et al. 2001). Use of the TRAP assay on sputum samples might hold potential for the early and non-invasive diagnosis of lung cancer. However, since sputum contains an abundance of mucus, which interferes with PCR

Table 1. Telomerase/*hTERT* mRNA as a diagnostic marker.

Organs/samples	Telomerase activity		*hTERT* mRNA	
	Cancer positive (%)	Non-cancerous positive (%)	Cancer positive (%)	Non-cancerous positive (%)
Head & neck				
Oral/Washing	110/195 (56)	70/321 (22)	21/26 (81)	9/39 (23)
Oral/Biopsy	25/26 (96)	9/41 (22)	47/58 (81)	11/13 (85)
Thyroid & breast				
Thyroid/FNA	64/96 (67)	23/155 (15)	44/57 (77)	15/52 (29)
Breast/FNA	210/265 (79)	40/355 (11)		
Chest				
Lung/Sputum	15/42 (36)	0/10 (0)		
Lung/Brushing, BAL	123/188 (65)	16/211 (8)		
Lung/Biopsy	86/128 (67)	0/10 (0)		
Mediastinal LN/FNA			10/16 (63)	18/71 (25)
Pleural effusion	175/205 (85)	20/155 (13)	14/15 (93)	6/15 (40)
Digestive organs				
Esophagus/Biopsy	52/54 (96)	33/48 (69)		
Stomach/Biopsy	23/29 (79)	10/28 (36)	88/101 (87)	62/192 (32)
Colon/Washing	20/34 (59)	0/20 (0)		
Colon/Biopsy	110/126 (87)	57/148 (39)	32/32 (100)	17/49 (35)
Liver/Biopsy	53/86 (62)	17/58 (29)	21/23 (91)	17/63 (27)
Biliary duct/Bile	4/37 (11)	0/25 (0)	10/20 (50)	0/14 (0)
Biliary duct/Biopsy	20/26 (77)	0/10 (0)	6/10 (60)	0/6 (0)
Pancreas/Pancreatic juice	59/72 (82)	2/51 (4)	15/17 (88)	2/19 (11)
Pancreas/FNA	18/18 (100)			
Peritoneal Lavage	102/141 (72)	5/117 (4)		
Genitourinary organs				
Bladder/Voiding urine	374/637 (59)	44/488 (9)	159/179 (89)	6/169 (4)
Bladder/Washing urine	229/302 (76)	6/153 (4)	125/168 (74)	19/165 (12)
Bladder/Biopsy	46/54 (85)	30/56 (54)		
Prostate/Voiding urine[a]	21/33 (64)	1/21 (5)		
Prostate/Biopsy	130/166 (78)	19/136 (14)		
Uterus/Cervical scraping	105/273 (38)	37/233 (16)	14/17 (82)	11/44 (25)
Uterus/Biopsy	138/164 (84)	58/158 (37)	83/104 (80)	1/8 (13)
Others				
Skin/Biopsy	130/159 (82)	11/109 (10)		
Blood/Serum	59/95 (62)	0/80 (0)	4/16 (25)	0/23 (0)

These percentages were calculated from the review papers Dhaene et al. (2000), Hiyama and Hiyama (2002, 2003) and Orlando et al. (2001) and recent numerous reports in addition to our unpublished data.
Abbreviations: BAL, bronchoalveolar lavage; FNA, fine needle aspirates.
[a]Voiding urine after massage.

and other enzyme reactions, the sensitivity of the telomerase assay in sputum is unsatisfactory for the detection of cancer (Sen et al. 2001). In brushing or BAL samples, on the other hands, telomerase activity showed a relatively high sensitivity for the detection of lung cancer cells, but more so for squamous cell carcinoma than for adenocarcinoma. However, BAL samples can contain activated lymphocytes, which can give false–positive results in benign diseases. The clonal expansion of lymphocytes, in particular, can produce strong telomerase activity in BAL samples (Haruta et al. 1999), which may instead reflect the aggressiveness of autoimmunity in certain benign diseases (Hiyama et al. 1998).

Several attempts to detect telomerase activity or *hTERT* mRNA have been reported using pleural effusion and mediastinal lymph node aspiration samples (Yang et al. 1998; Braunschweig et al. 2001; Dejmek et al. 2001; Wallace et al. 2003). Because carcinomas from almost any tumor sites can metastasize to the pleura, pleural effusions

may contain cancer cells originating from other organs such as the breast, ovary, or gastrointestinal tract. In malignant pleural effusions diagnosed by either fluid cytology or pleural biopsy, Yang et al. (1998) detected telomerase activity in 91% of cases with a specificity of 94%, indicating that the measurement of telomerase activity is a useful adjunct to cytology for detecting cancer cells. In this study, the only false positives were three samples from patients with tuberculosis. Thus, in pleural effusions as well as BAL samples, the sensitivity of the telomerase assay for detecting cancer cells is relatively high, with most of the false–positive signals being caused by lymphocytes contamination of non-cancerous lesions.

Digestive organs and peritoneum

Cancers of the digestive system are frequently diagnosed by endoscopic examination. In biopsies of the esophagus, telomerase activity was present in almost all esophageal cancers but was also detected in more than half of non-cancerous tissues, where the activity can be found in the normal epithelial basal cells. Similar results were obtained for biopsies of the stomach and colon. The sensitivity of telomerase activity or *hTERT* mRNA expression for detecting cancerous lesions was high, but telomerase was also present, albeit at lower levels, in non-cancerous tissues, where it localizes to the basal cells of crypts (Hiyama et al. 1996b). Hence, a more precise measurement of the level of telomerase activity in biopsy samples of the gut may be necessary for the diagnosis of cancers. Cells derived from colon luminal washing can also be applied to the TRAP assay for cancer diagnosis. Because washing samples rarely contain basal crypt cells, the specificity of the TRAP assay for colon washing samples was remarkable but at the expense of sensitivity, which was found to be relatively low (Yoshida et al. 1997).

In liver biopsies, detection of telomerase activity or *hTERT* mRNA shows promises for cancer diagnosis, albeit low-level expression of these markers was also reported in non-cancerous tissues (Nagao et al. 1999). In pancreatico-biliary cancers, detection of telomerase activity or *hTERT* mRNA in biopsy samples displayed a high sensitivity for cancer diagnosis. With the exception of pancreatic juice, the assay had a sensitivity that was low for excretion and secretion samples, such as bile (Itoi et al. 1999, 2001). In patients with pancreatic ductal adenocarcinomas, the pancreatic juice contains freshly exfoliated ductal cells that carry very high levels of telomerase activity. Because of its high sensitivity and specificity, the detection of telomerase activity and/or *hTERT* mRNA in pancreatic juice has become a promising new application of cancer diagnosis (Hiyama et al. 1997b; Iwao et al. 1997; Suehara et al. 1997; Morales et al. 1998). Moreover, detection of telomerase activity in pancreatic juice was additionally useful for the differential diagnosis of benign and malignant intraductal papillary mucinous tumors (IPMT) of the pancreas, which can be difficult to distinguish preoperatively (Inoue et al. 2001; Uemura et al. 2003).

Because peritoneal dissemination usually occurs in advanced stages of digestive cancers, telomerase activity in peritoneal lavage samples also showed a high specificity for cancer cell detection. Tangkijvanich et al. (1999) measured telomerase activity in nonmalignant and malignancy-related ascites associated with hepatocellular carcinoma and peritoneal carcinomatosis. Both the sensitivity and specificity of the telomerase assay were higher than those of cytology for diagnosis of the malignancy. The incidence of false–positive for telomerase activity was only of 4%, and all of these false positives showed evidence of lymphocytic contamination. Duggan et al. (1998) also found telomerase activity to be more sensitive than cytology in ascitic samples obtained from patients with ovarian cancer.

Genitourinary organs

Among exfoliating materials, voiding urine is easiest to examine. For the detection of bladder cancer using voided urine samples obtained from bladder cancer patients and controls, the TRAP assay showed the highest sensitivity (67%) and specificity (99%) Ramakumar et al. (1999). Since the viability of cells in voided urine samples varies, the sensitivity of telomerase activity for cancer diagnosis was lower than specificity. As an alternative, high sensitivity could be obtained in urine samples by detection of the *hTERT* mRNA by RT-PCR (Ito et al. 1998; Fukui et al. 2001). While telomerase activity and *hTERT* mRNA might both be useful for the detection of cancer cells in

bladder washings, detection of the *hTERT* mRNA may be preferable for the screening of voided urine (Lee et al. 1998; Fukui et al. 2001).

In voided urine samples obtained after prostate massage, the sensitivity of telomerase activity was higher than that of cytologic examination for the detection of prostate cancer (Meid et al. 2001). As a surrogate for unstable telomerase, *hTERT* mRNA was an even more reliable marker. They reported that the addition of EDTA to a final concentration of 20 mmol/l stabilized the RNA for up to 2 h at 4 °C.

As a potential biomarker of cervical dysplasia, telomerase has also been the focus of intense investigations. In cervical cancers, whether telomerase is activated in pre-malignant lesions remains controversial. According to several studies published on cervical biopsies (Wisman et al. 1998; Zheng et al. 2000; Jarboe et al. 2002), telomerase activity is abnormally present in a remarkably high proportion of high-grade squamous intraepithelial lesions (HSILs), indicating that the activation of telomerase is an early event in the malignant progression of cervical lesions. Still, a more complex situation has been suggested by histochemical studies, which revealed that the hTERT protein was present in the lower suprabasal levels of the normal cervical mucosa (Frost et al. 2000). In cancers, the hTERT protein was relocalized to virtually all levels of the lesional epithelia, concomitantly with the aberrant reexpression of telomerase activity. Thus, for the diagnosis of cervical cancers, a more precise measurement of telomerase activity might be needed, which should be confirmed by the histochemical staining of mirror image specimens. Thus, the detection of *in situ* carcinomas and precancerous lesions in cervical biopsies would likely require the application of methodologies for the *in situ* detection of the *hTERT* mRNA or protein, as described later in this review.

Skin

Skin is a surface organ from which biopsy specimens are easily prepared. Investigations of telomerase activity as a marker of skin cancer showed that epidermal basal cells had low levels of telomerase activity; that telomerase was not activated in the vast majority of squamous cell carcinoma; but that most cutaneous malignant melanoma displayed high-levels of telomerase activity (Parris et al. 1999). To elucidate the correlation between carcinogenesis and telomerase activation in the skin, further studies on skin cancers and related lesions are necessary.

Circulating cancer cells

Irrespective of the tumor type, the blood of cancer patients is likely to contain circulating cancer cells that could potentially be detected using the telomerase assay (Gauthier et al. 2001). The detection of these rare cancer cells in whole blood samples would be predicted to be masked by the potential presence of activated lymphocytes expressing high levels of telomerase activity (Hiyama et al. 1995c; Haruta et al. 1999). To detect circulating carcinoma cells using the telomerase assay, immunomagnetic separation can first be used to isolate epithelial cells from peripheral blood mononuclear cells, after which point the harvested cells can be tested for telomerase activity. In one report, the harvested circulating epithelial cells showed telomerase activity in 70–80% of patients with advanced lung, colon, and breast cancers, suggesting that telomerase activity may become a useful clinical marker of circulating epithelial cancer cells (Gauthier et al. 2001).

One of the most routinely collected bodily fluids is blood plasma, which can easily be prepared by centrifugation of whole blood. If tumor cells undergo necrosis and release their contents, some tumor-specific molecules might be present in plasma that could be detected. Although several studies have addressed the detection of tumor-specific mRNA in plasma, this broader topic is beyond the scope of this review. While it is unlikely that intact telomerase might be detected in plasma, a recent report has detected the *hTERT* mRNA and hTR in plasma (Chen et al. 2000).

Hematopoietic malignancies

The presence of endogenous telomerase in normal hematopoietic stem cells and activated lymphocytes is an important confounding factor that can limit the value of the telomerase assay in the detection of hematopoietic malignancies. Blood samples from patients at the early stages of chronic

lymphoid leukemia (CLL) display low-level telomerase activity, which progressively increases over the course of the disease to reach levels that are much higher than those detected in normal blood samples. Moreover, this increase is accompanied by a net decrease in telomere length (Shay et al. 1996). A series of 58 patients with CLL showed that higher telomerase activity and shorter telomeres were associated with an adverse prognosis (Bechter et al. 1998). In chronic myeloid leukemia, telomerase activity is not increased over that of normal peripheral mononuclear cells, but decreases in telomere lengths are observed, which correlate with shorter intervals to the blast crisis phase (Iwama et al. 1997). In acute lymphoblastic leukemia, smaller studies have found telomerase activity to be variable (Shay et al. 1996). With the exceptions of Hodgkin's lymphoma and chronic myeloid leukemia, all of acute myelogenous leukemia, multiple myeloma, plasma cell leukemia and non-Hodgkin's lymphoma exhibited marked increases in telomerase activity that were well above that of normal peripheral mononuclear cells (Norrback et al. 1998; Xu et al. 2001).

In situ detection of the hTERT mRNA and protein

When using RT-PCR or the TRAP assay to detect the *hTERT* mRNA or telomerase activity, the presence of normal telomerase-positive cells, such as lymphocytes or basal epithelial cells, can cause false–positive results. Methodology for the *in situ* detection of telomerase in individual cells would be expected to solve this problem. An *in situ* TRAP assay had previously been developed to detect the telomerase activity, but this methodology could only be used on fresh viable cells (Ohyashiki et al. 1997b). The use of *in situ* hybridization (ISH) to detect components of the telomerase complex (hTR and *hTERT* mRNA), on the other hand, would be applicable to fixed tissues. However, hTR is also present at low levels in most cells lacking telomerase activity, and its level does not always correlate with telomerase activity. A better target for ISH detection would be the *hTERT* mRNA, whose levels appear to closely parallel those of telomerase activity.

For evaluating hTERT expression, several studies have reported the successful use of *hTERT* mRNA ISH (Chou et al. 2001; Kumaki et al. 2001). While it is necessary to target sequences specific to the full-length mRNA to avoid false positives resulting from splice variants of *hTERT* mRNA, the ISH detection of the *hTERT* mRNA is predicted to become a powerful tool of cancer detection. For detecting hTERT expression, immunohistochemistry (IHC) can also be used to reveal the presence of the hTERT protein in a wide variety of clinical samples, including archival paraffin-embedded specimens (Hiyama et al. 2001; Kumaki et al. 2001, 2002). In spite of its very low abundance, the hTERT protein can now be detected in paraffin-embedded samples and core biopsies with the use of polyclonal or monoclonal antibodies in conjunction with appropriate antigen retrieval (Figure 1) and/or the highly sensitive tyramide-based method of signal amplification (Frost et al. 2000). Since IHC does not require specialized equipment for detection, hTERT IHC is predicted to become a powerful new technology for cancer detection. In most cancer tissues, the hTERT protein is heterogeneously distributed and, in some cases, can display regional variability. In most cancer specimens, the signal intensity of individual hTERT-positive cells did not differ substantially between tumors with high and low telomerase activity, and the level of telomerase activity was mainly dependent on the percentage of cells displaying hTERT expression (Hiyama et al. 2001). This heterogeneity in telomerase expression appears to be an important factor dictating the overall levels of telomerase activity in tumors. With the availability of hTERT IHC, telomerase-positive cancer cells can now be detected in tissues containing a background of normal telomerase-positive cells. Likewise, Frost et al. (2000) have observed changes in the tissue distribution of the hTERT protein in cervical cancers: while the hTERT protein was limited to the lower suprabasal cells of the normal cervix, it was present at all levels of the lesional epithelium in moderate to severe dysplasia.

Telomerase in cancer prognosis and the grading of malignant tumors

In certain types of cancers, telomerase activity is upregulated during tumor progression, so that the level of telomerase activity can be used to evaluate the malignant grade of tumors and predict patient

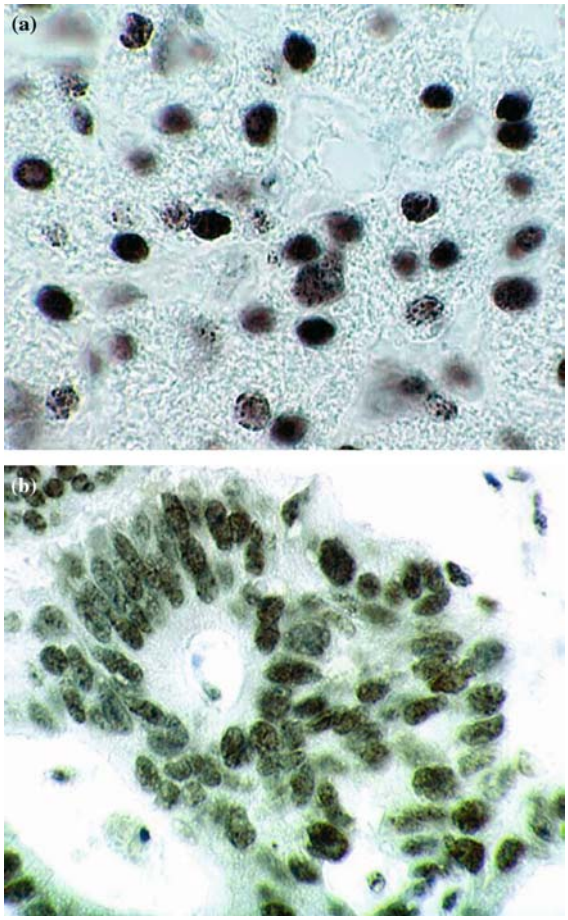

Figure 1. Immunohistochemical detection of hTERT in cancer samples. An anti-hTERT sera (EST21-A™, Alpha Diagnostic Int. Co., San Antonio, TX) was employed to reveal the presence of the hTERT protein in a FNA sample of a duct cell adenocarcinoma of the breast (a) and in a biopsy derived from an adenocarcinoma of the sigmoid colon (b). Tumor cells are revealed by the presence of brown pigments in the nucleus of hTERT-positive cells. Staining with 3,3′-diaminobenzidine (DAB) was preformed as described previously (Hiyama et al. 2001). The cells obtained from FNA sample were formalin-fixed and paraffin-embedded. For both samples, heat-based antigen retrieval was performed using a citrate buffer.

prognosis (Table 2). In certain cancers of the adults, the activation of telomerase correlates with advanced disease and poor prognosis, as in the cases of non-small cell lung cancer, gastric cancer, colorectal cancer, soft tissue tumors, and myelodysplastic disease (Chadeneau et al. 1995; Hiyama et al. 1995b; Marchetti et al. 1999; Tahara et al. 1999; Tatsumoto et al. 2000; Tomoda et al. 2002; Kido et al. 2003). In patients with colorectal cancer (Tatsumoto et al. 2000), including those undergoing curative resection of liver metastases (Smith et al. 2004), both telomerase activity and *hTERT* mRNA expression could be used as independent prognostic factors. In a retrospective study of a large number of breast cancer patients, telomerase activity correlated with a more aggressive tumor phenotype and its level was highly predictive of clinical outcomes (Clark et al. 1997). These findings suggest that telomerase activity is an useful indicator for identifying patients that would benefit from postoperative adjuvant chemotherapy.

Neuroblastomas are pediatric tumors that display a well-documented relationship between tumor biology and patient outcome. In these tumors, poor prognosis is associated with high levels of telomerase activity and full-length *hTERT* mRNA expression (Hiyama et al. 1995a; Hiyama et al. 1997a; Poremba et al. 1999b; Streutker et al. 2001; Krams et al. 2003). Interestingly, in stage 4S neuroblastoma, which represents a unique entity characterized by a high frequency of spontaneous regression, telomeres were shortened and telomerase activity was undetectable (Hiyama et al. 1995a, 1997a). Telomerase activity can also predict the outcomes of patients with gliomas, brain tumors that are consistently difficult to assign as either benign or malignant. Studies have shown that telomerase activity is present in most cases of malignant gliomas but is undetectable in grade I gliomas, making it a useful indicator of the malignant grade of gliomas (Nakatani et al. 1997; Huang et al. 1999). In tumors of the thyroid gland, telomerase activity may be useful to distinguish benign from malignant tumors and might provide a useful indicator of prognosis (Haugen et al. 1997; Saji et al. 1997). In pituitary adenoma, detection of telomerase expression may also correlate with biological aggressiveness and potential for regrowth (Yoshino et al. 2003). Telomerase activity in bone marrow has recently been reported to be a highly significant prognostic factor in pediatric patients with acute myeloid leukemia (Verstovsek et al. 2003). Collectively, these findings demonstrate that telomerase activity and hTERT expression are markers that can be used successfully to predict the outcome of cancer patients and take decisions on the appropriate treatments.

Table 2. Telomerase activity/*hTERT* mRNA as a prognostic marker.

Site	Tumor type	Correlated with prognosis	Correlated with other markers
Brain	Central nervous system malignant lymphoma; Pituitary tumor	Harada et al. (1999)[a,b] and Yoshino et al. (2003)[a]	
Head & Neck	Head & neck cancer; Oral cavity and oropharynx postchemotherapeutic tumors	Patel et al. (2002) and Ogawa et al. (1998)[a]	
Lung	Non-small cell lung cancer	Gonzalez-Quevedo et al. (2002), Hirashima et al. (2000), Marchetti et al. (1999), Taga et al. (1999)[a] and Hara et al. (2001)[b]	Dysplasia in smokers Soria et al. (2001)[b]
Breast	Invasive duct cell carcinoma		Proliferative index Carey et al. (1999)[a]; Relapse-free period Bieche et al. (2000)[a]
	Node-positive breast cancer	Clark et al. (1997)[a,c]	
Thyroid	Papillary carcinoma		Extrathyroidal extension Okayasu et al. (1997)[a]
Stomach	Adenocarcinoma	Hiyama et al. (1995b), Kakeji et al. (2001) and Usselmann et al. (2001)[a]	
Colon	Adenocarcinoma	Tatsumoto et al. (2000)[a]	Advanced stages Okayasu et al. (1998), Yoshida et al. (1999)[a], Boldrini et al. (2002), Naito et al. (2001) and Niiyama et al. (2001)[b]; Risk for metastasis Shoji et al. (2000)[a]
	Hepatic metastasis of colorectal cancer	Smith et al. (2004)	
Liver	Hepatocellular carcinoma	Kishimoto et al. (1998), Hisatomi et al. (1999) and Shimada et al. (2000)[a]	Recurrence risk (Suda et al. 1998)[a]
Pancreas	Endocrine tumors	Pearson et al. (2000)[a]	
Urogenital	Renal cell carcinoma		Tumor grade Hara et al. (2001)[a]; Advanced stage Paradis et al. (2001)[a]
	Transitional cell carcinoma	De Kok et al. (2000) and Nakanishi et al. (1999)[b]	Tumor relapse Lancelin et al. (2000)[a]
Prostate	Prostate cancer		Advanced stage Engelhardt et al. (1997)[a, b]
Uterus	Endometrial carcinoma		Recurrence risk Bonatz et al. (2001)[a]
Soft tissue	Osteosarcoma	Sangiorgi et al. (2001)[a]	Response to chemotherapy Kido et al. (2003)[a]
	Soft tissue sarcoma	Schneider-Stock et al. (2000) and Wurl et al. (2002)[a]	Recurrence and metastasis Tomoda et al. (2002)[a, b]
	Liposarcomas	Schneider-Stock et al. (2000)[b]	
Blood	Acute leukemia, B-cell Lymphocytic leukemia, Adult T-cell leukemia, Acute myelogenous leukaemia (AML)	Shay et al. (1996), Ohyashiki et al. (1997a), Uchida et al. (1999), Verstovsek et al. (2003)[a] and Xu et al. (1998)[a,b]	Recurrence in MDS Ohshima et al. (2003)[a]
	Pediatric AML	Verstovsek et al. (2003)	
	Multiple myeloma	Wu et al. (2003)	
Childhood tumor	Neuroblastoma	Hiyama et al. (1997a), Hiyama et al. (1995a), Poremba et al. (1999b)[a] and Krams et al. (2003)[b]	Cytogenetic abnormalities Brinkschmidt et al. (1998), Hiyama et al. (1997a), Hiyama et al. (1995a) and Wu et al. (2003)[a]
	Wilms tumor		Recurrent risk Dome et al. (1999)[b]
	Hepatoblastoma	Hiyama et al. (2004)[a,b]	

[a]Telomerase activity.
[b]*hTERT* mRNA.
[c]Conflicting findings have been reported.

Conclusion

In conclusion, measurement of telomerase activity and/or hTERT expression has several clinical utilities: for the early detection of cancer cells (in tumors that acquire telomerase activity at the early stages); as a prognostic indicator (in tumors that acquire telomerase activity upon progression); a marker that can distinguish malignancies from benign tumors; and a marker for detecting circulating cancer cells in the blood. *In situ* hybridization and the immunohistochemical detection of hTERT can now be used to identify telomerase-positive cancer cells in a background of non-cancerous cells. In the near future, methods for the *in situ* detection of hTERT are likely to become of common use in the clinics for both the diagnosis of cancers and the grading of malignancies.

References

Bechter O.E., Eisterer W., Pall G., Hilbe W., Kuhr T. and Thaler J. 1998. Telomere length and telomerase activity predict survival in patients with B cell chronic lymphocytic leukemia. Cancer Res. 58: 4918–4922.

Bieche I., Nogues C., Paradis V., Olivi M., Bedossa P., Lidereau R. and Vidaud M. 2000. Quantitation of hTERT gene expression in sporadic breast tumors with a real-time reverse transcription-polymerase chain reaction assay. Clin. Cancer Res. 6: 452–459.

Blasco M.A., Rizen M., Greider C.W. and Hanahan D. 1996. Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. Nature Genet. 12: 200–204.

Boldrini L., Faviana P., Gisfredi S., Zucconi Y., Di Quirico D., Donati V., Berti P., Spisni R., Galleri D., Materazzi G., Basolo F., Miccoli P., Pingitore R. and Fontanini G. 2002. Evaluation of telomerase mRNA (hTERT) in colon cancer. Intl. J. Oncol. 21: 493–497.

Bonatz G., Frahm S.O., Klapper W., Helfenstein A., Heidorn K., Jonat W., Krupp G., Parwaresch R. and Rudolph P. 2001. High telomerase activity is associated with cell cycle deregulation and rapid progression in endometrioid adenocarcinoma of the uterus. Hum. Pathol. 32: 605–614.

Braunschweig R., Yan P., Guilleret I., Delacretaz F., Bosman F.T., Mihaescu A. and Benhattar J. 2001. Detection of malignant effusions: comparison of a telomerase assay and cytologic examination. Diagn. Cytopathol. 24: 174–180.

Brinkschmidt C., Poremba C., Christiansen H., Simon R., Schafer K.L., Terpe H.J., Lampert F., Boecker W. and Dockhorn-Dworniczak B. 1998. Comparative genomic hybridization and telomerase activity analysis identify two biologically different groups of 4S neuroblastomas. Brit. J. Cancer 77: 2223–2229.

Broccoli D., Godley L.A., Donehower L.A., Varmus H.E. and de Lange T. 1996. Telomerase activation in mouse mammary tumors: lack of detectable telomere shortening and evidence for regulation of telomerase RNA with cell proliferation. Mol. Cell Biol. 16: 3765–3772.

Califano J., Ahrendt S.A., Meininger G., Westra W.H., Koch W.H. and Sidransky G. 1996. Detection of telomerase activity in oral rinses from head and neck squamous cell carcinoma patients. Cancer Res. 56: 5720–5722.

Carey L.A., Kim N.W., Goodman S., Marks J., Henderson G., Umbricht C.B., Dome J.S., Dooley W., Amshey S.R. and Sukumar S. 1999. Telomerase activity and prognosis in primary breast cancers. J. Clin. Oncol. 17: 3075–3081.

Chadeneau C., Hay K., Hirte H.W., Gallinger S. and Bacchetti S. 1995. Telomerase activity associated with acquisition of malignancy in human colorectal cancer. Cancer Res. 55: 2533–2536.

Chen X.Q., Bonnefoi H., Pelte M.F., Lyautey J., Lederrey C., Movarekhi S., Schaeffer P., Mulcahy H.E., Meyer P., Stroun M. and Anker P. 2000. Telomerase RNA as a detection marker in the serum of breast cancer patients. Clin. Cancer Res. 6: 3823–3826.

Chou S.J., Chen C.M., Harn H.J., Chen C.J. and Liu Y.C. 2001. In situ detection of hTERT mRNA relates to Ki-67 labeling index in papillary thyroid carcinoma. J. Surg. Res. 99: 75–83.

Clark G.M., Osborne C.K., Levitt D., Wu F. and Kim N.W. 1997. Telomerase activity and survival of patients with node-positive breast cancer. J. Natl. Cancer Inst. 89: 1874–1881.

Dejmek A., Yahata N., Ohyashiki K., Ebihara Y., Kakihana M., Hirano T., Kawate N. and Kato H. 2001. In situ telomerase activity in pleural effusions: a promising marker for malignancy. Diagn. Cytopathol. 24: 11–15.

De Kok J.B., Schalken J.A., Aalders T.W., Ruers T.J., Willems H.L. and Swinkels D.W. 2000. Quantitative measurement of telomerase reverse transcriptase (hTERT) mRNA in urothelial cell carcinomas. Intl. J. Cancer 87: 217–220.

Dhaene K., Van Marck E. and Parwaresch R. 2000. Telomeres, telomerase and cancer: an up-date. Virchows Arch. 437: 1–16.

Dome J.S., Chung S., Bergemann T., Umbricht C.B., Saji M., Carey L.A., Grundy P.E., Perlman E.J., Breslow N.E. and Sukumar S. 1999. High telomerase reverse transcriptase (hTERT) messenger RNA level correlates with tumor recurrence in patients with favorable histology Wilms' tumor. Cancer Res. 59: 4301–4307.

Duggan B.D., Wan M., Yu M.C., Roman L.D., Muderspach L.I., Delgadillo E., Li W.Z., Martin S.E. and Dubeau L. 1998. Detection of ovarian cancer cells: comparison of a telomerase assay and cytologic examination. J. Natl. Cancer Inst. 90: 238–242.

Engelhardt M., Albanell J., Drullinsky P., Han W., Guillem J., Scher H.I., Reuter V. and Moore M.A. 1997. Relative contribution of normal and neoplastic cells determines telomerase activity and telomere length in primary cancers of the prostate, colon, and sarcoma. Clin. Cancer Res. 3: 1849–1857.

Feng J., Funk W.D., Wang S.-S., Weinrich S.L., Avilion A.A., Chiu C.-P., Adams R.R., Chang E., Allsopp R.C., Yu J., Le S., West M.D., Harley C.B., Andrews W.H., Greider C.W.

and Villeponteau B. 1995. The RNA component of human telomerase. Science 269: 1236–1241.

Frost M., Bobak J.B., Gianani R., Kim N., Weinrich S., Spalding D.C., Cass L.G., Thompson L.C., Enomoto T., Uribe-Lopez D. and Shroyer K.R. 2000. Localization of telomerase hTERT protein and hTR in benign mucosa, dysplasia, and squamous cell carcinoma of the cervix. Am. J. Clin. Pathol. 114: 726–734.

Fukui T., Nonomura N., Tokizane T., Sato E., Ono Y., Harada Y., Nishimura K., Takahara S. and Okuyama A. 2001. Clinical evaluation of human telomerase catalytic subunit in bladder washings from patients with bladder cancer. Mol. Urol. 5: 19–23.

Gauthier L.R., Granotier C., Soria J.C., Faivre S., Boige V., Raymond E. and Boussin F.D. 2001. Detection of circulating carcinoma cells by telomerase activity. Brit. J. Cancer 84: 631–635.

Gonzalez-Quevedo R., Iniesta P., Moran A., de Juan C., Sanchez-Pernaute A., Fernandez C., Torres A., Diaz-Rubio E., Balibrea J.L. and Benito M. 2002. Cooperative role of telomerase activity and p16 expression in the prognosis of non-small-cell lung cancer. J. Clin. Oncol. 20: 254–262.

Greider C.W. 1990. Telomere, telomerase, and senescence. Bioassays 12: 363–369.

Hara T., Noma T., Yamashiro Y., Naito K. and Nakazawa A. 2001. Quantitative analysis of telomerase activity and telomerase reverse transcriptase expression in renal cell carcinoma. Urol. Res. 29: 1–6.

Harada K., Kurisu K., Arita K., Sadatomo T., Tahara H., Tahara E., Ide T. and Uozumi T. 1999. Telomerase activity in central nervous system malignant lymphoma. Cancer 86: 1050–1055.

Haruta Y., Hiyama K., Ishioka S., Hozawa S., Maeda H. and Yamakido M. 1999. Activation of telomerase is induced by a natural antigen in allergen-specific memory T lymphocytes in broncheal asthma. Biochem. Biophys. Res. Commun. 259: 617–623.

Haugen B.R., Nawaz S., Markham, N., Hashizumi T., Shroyer A.L., Werness B. and Shroyer K.R. 1997. Telomerase activity in benign and malignant thyroid tumors. Thyroid 7: 337–342.

Hirashima T., Komiya T., Nitta T., Takada Y., Kobayashi M., Masuda N., Matui K., Takada M., Kikui M., Yasumitu T., Ohno A., Nakagawa K., Fukuoka M. and Kawase I. 2000. Prognostic significance of telomeric repeat length alterations in pathological stage I–IIIA non-small cell lung cancer. Anticancer Res. 20: 2181–2187.

Hisatomi H., Nagao K., Kanamaru T., Endo H., Tomimatsu M. and Hikiji K. 1999. Levels of telomerase catalytic subunit mRNA as a predictor of potential malignancy. Intl. J. Oncol. 14: 727–732.

Hiyama E., Gollahan L., Kataoka T., Kuroi K., Yokoyama, T., Gazdar A.F., Hiyama K., Piatyszek M.A. and Shay J.W. 1996a. Telomerase activity in human breast tumors. J. Natl. Cancer Inst. 88: 116–122.

Hiyama E. and Hiyama K. 2002. Clinical utility of telomerase in cancer. Oncogene 21: 643–649.

Hiyama E. and Hiyama K. 2003. Telomerase as tumor marker. Cancer Lett. 194: 221–233.

Hiyama K., Hirai Y., Kyoizumi S., Akiyama M., Hiyama E., Piatyszek M.A. and Shay J.W. 1995c. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J. Immunol. 155: 3711–3715.

Hiyama E., Hiyama K., Ohtsu K., Yamaoka H., Ichikawa T., Shay J.W. and Yokoyama T. 1997a. Telomerase activity in neuroblastoma: is it a prognostic indicator of clinical behavior? Eur. J. Cancer 33: 1932–1936.

Hiyama E., Hiyama K., Shay J.W. and Yokoyama T. 2001. Immunhistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia 3: 17–26.

Hiyama E., Hiyama K., Tatsumoto N., Kodama T., Shay J.W. and Yokoyama T. 1996b. Telomerase activity in human intestine. Int. J. Oncol. 9: 453–458.

Hiyama E., Hiyama K., Yokoyama T., Matsuura Y., Piatyszek M.A. and Shay J.W. 1995a. Correlating telomerase activity levels with human neuroblastoma outcomes. Nature Med. 1: 249–255.

Hiyama K., Ishioka S., Shay J.W., Taooka Y., Maeda A., Isobe T., Hiyama E., Maeda H. and Yamakido M. 1998. Telomerase activity as a novel marker of lung cancer and immune-associated lung diseases. Intl. J. Mol. Med. 1: 545–549.

Hiyama E., Kodama T., Sinbara K., Iwao T., Itoh M., Hiyama K., Shay J.W., Matsuura Y. and Yokoyama T. 1997b. Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res. 57: 326–331.

Hiyama E., Saeki T., Hiyama K., Takashima S., Shay J.W., Matsuura Y. and Yokoyama T. 2000. Telomerase activity as a marker of breast cancer in fine needle aspirated samples. Cancer Cytopathol. 90: 235–238.

Hiyama E., Yamaoka H., Matsunaga T., Hayashi Y., Ando H., Suita S., Horie H., Kaneko M., Sasaki F., Hashizume K., Nakagawara A., Ohnuma N. and Yokoyama T. 2004. High expression of telomerase is an independent prognostic indicator of poor outcome in hepatoblastoma. Brit. J. Cancer 91: 972–979.

Hiyama E., Yokoyama T., Tatsumoto N., Hiyama K., Imamura Y., Murakami Y., Kodama T., Piatyszek M.A., Shay J.W. and Matsuura Y. 1995b. Telomerase activity in gastric cancer. Cancer Res. 55: 3258–3262.

Hou M., Xu D., Bjorkholm M. and Gruber A. 2001. Real-time quantitative telomeric repeat amplification protocol assay for the detection of telomerase activity. Clin. Chem. 47: 519–524.

Huang F., Kanno H., Yamamoto I., Lin Y. and Kubota Y. 1999. Correlation of clinical features and telomerase activity in human gliomas. J. Neuro-oncol. 43: 137–142.

Inoue H., Tsuchida A., Kawasaki Y., Fujimoto Y., Yamasaki S. and Kajiyama G. 2001. Preoperative diagnosis of intraductal papillary-mucinous tumors of the pancreas with attention to telomerase activity. Cancer 91: 35–41.

Ito H., Kyo S., Kanaya T., Takakura M., Koshida K., Namiki M. and Inoue M. 1998. Detection of human telomerase reverse transcriptase messenger RNA in voided urine samples as a useful diagnostic tool for bladder cancer. Clin. Cancer Res. 4: 2807–2810.

Itoi T., Ohyashiki K., Yahata N., Shinohara Y., Takei K., Takeda K., Nagao K., Hisatomi H., Ebihara Y., Shay J.W. and Saito T. 1999. Detection of telomerase activity in exfoliated cancer cells obtained from bile. Intl. J. Oncol. 15: 1061–1067.

Itoi T., Shinohara Y., Takeda K., Nakamura K., Shimizu M., Ohyashiki K., Hisatomi H., Nakano H. and Moriyasu F.

2001. Detection of telomerase reverse transcriptase mRNA in biopsy specimens and bile for diagnosis of biliary tract cancers. Intl. J. Mol. Med. 7: 281–287.

Iwama H., Ohyashiki K., Ohyashiki J.H., Hayashi S., Kawakubo K., Shay J.W. and Toyama K. 1997. The relationship between telomere length and therapy-associated cytogenetic responses in patients with chronic myeloid leukemia. Cancer 79: 1552–1560.

Iwao T., Hiyama E., Yokoyama T., Tsuchida A., Hiyama K., Murakami Y., Shimamoto F., Shay J.W. and Kajiyama G. 1997. Telomerase activity for the preoperative diagnosis of pancreatic cancer. J. Natl. Cancer Inst. 89: 1621–1623.

Jarboe E.A., Liaw K.L., Thompson L.C., Heinz D.E., Baker P.L., McGregor J.A., Dunn T., Woods J.E. and Shroyer K.R. 2002. Analysis of telomerase as a diagnostic biomarker of cervical dysplasia and carcinoma. Oncogene 21: 664–673.

Kakeji Y., Maehara Y., Koga T., Shibahara K., Kabashima A., Tokunaga E. and Sugimachi K. 2001. Gastric cancer with high telomerase activity shows rapid development and invasiveness. Oncol. Rep. 8: 107–110.

Kido A., Schneider-Stock R., Hauptmann K. and Roessner A. 2003. Telomerase activity in juxtacortical and conventional high-grade osteosarcomas: correlation with grade, proliferative activity and clinical response to chemotherapy. Cancer Lett. 196: 109–115.

Kim N.W. 1997. Clinical implications of telomerase in cancer. Eur. J. Cancer 33: 781–786.

Kim N.W., Piatyszek M.A., Prowse K.R., Harley C.B., West M.D., Ho, P.L.C., Coviello G.M., Wright W.E., Weinrich S.L. and Shay J.W. 1994. Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011–2015.

Kishimoto K., Fujimoto J., Takeuchi M., Yamamoto H., Ueki T. and Okamoto E. 1998. Telomerase activity in hepatocellular carcinoma and adjacent liver tissues. J. Surg. Oncol. 69: 119–124.

Koyanagi Y., Kobayashi D., Yajima T., Asanuma K., Kimura T., Sato T., Kida T., Yagihashi A., Kameshima H. and Watanabe N. 2000. Telomerase activity is down regulated via decreases in hTERT mRNA but not TEP1 mRNA or hTERC during the differentiation of leukemic cells. Anticancer Res. 20: 773–778.

Krams M., Hero B., Berthold F., Parwaresch R., Harms D. and Rudolph P. 2003. Full-length telomerase reverse transcriptase messenger RNA is an independent prognostic factor in neuroblastoma. Am. J. Pathol. 162: 1019–1026.

Kumaki F., Kawai T., Hiroi S., Shinomiya N., Ozeki Y., Ferrans V.J. and Torikata C. 2001. Telomerase activity and expression of human telomerase RNA component and human telomerase reverse transcriptase in lung carcinomas. Hum. Pathol. 32: 188–195.

Kumaki F., Takeda K., Yu Z.X., Moss J. and Ferrans V.J. 2002. Expression of human telomerase reverse transcriptase in lymphangioleiomyomatosis. Am. J. Resp. Crit. Care Med. 166: 187–191.

Lancelin F., Anidjar M., Villette J.M., Soliman A., Teillac P., Le Duc A., Fiet J. and Cussenot O. 2000. Telomerase activity as a potential marker in preneoplastic bladder lesions. BJU Intl. 85: 526–531.

Lee D.H., Yang S.C., Hong S.J., Chung B.H. and Kim I.Y. 1998. Telomerase: a potential marker of bladder transitional cell carcinoma in bladder washes. Clin. Cancer Res. 4: 535–538.

Lingner J., Cooper J.P. and Cech T.R. 1995. Telomerase and DNA end replication: no longer a lagging strand problem? Science 269: 1533–1534.

Marchetti A., Bertacca G., Buttitta F., Chella A., Quattrocolo G., Angeletti C.A. and Bevilacqua G. 1999. Telomerase activity as a prognostic indicator in stage I non-small cell lung cancer. Clin. Cancer Res. 5: 2077–2081.

Matthews P., Jones C.J., Skinner J., Haughton M., de Micco C. and Wynford-Thomas D. 2001. Telomerase activity and telomere length in thyroid neoplasia: biological and clinical implications. J. Pathol. 194: 183–193.

Meid F.H., Gygi C.M., Leisinger H.J., Bosman F.T. and Benhattar J. 2001. The use of telomerase activity for the detection of prostatic cancer cells after prostatic massage. J. Urol. 165: 1802–1805.

Mokbel K., Williams N.J., Leris A.C. and Kouriefs C. 1999. Telomerase activity in fine-needle aspirates of breast lesions. J. Clin. Oncol. 17: 3856–3860.

Morales C.P., Burdick J.S., Saboorian M.H., Wright W.E. and Shay J.W. 1998. In situ hybridization for telomerase RNA in routine cytologic brushings for the diagnosis of pancreaticobiliary malignancies. Gastrointest. Endosc. 48: 402–405.

Naito Y., Takagi T., Handa O., Ishikawa T., Matsumoto N., Yoshida N., Kato H., Ando T., Takemura T., Itani K., Hisatomi H., Tsuchihashi Y. and Yoshikawa T. 2001. Telomerase activity and expression of telomerase RNA component and catalytic subunits in precancerous and cancerous colorectal lesions. Tumour Biol. 22: 374–382.

Nakamura T.M., Morin G.B., Chapman K.B., Weinrich S.L., Andrews W.H., Lingner J., Harley C.B. and Cech T.R. 1997. Telomerase catalytic subunit homologs from fission yeast and human. Science 277: 955–959.

Nakanishi K., Kawai T., Hiroi S., Kumaki F., Torikata C., Aurues T. and Ikeda T. 1999. Expression of telomerase mRNA component (hTR) in transitional cell carcinoma of the upper urinary tract. Cancer 86: 2109–2116.

Nakatani K., Yoshim N., Mori H., Yoshimura S., Sakai H., Shinoda J. and Sakai N. 1997. The significant role of telomerase activity in human brain tumors. Cancer 80: 471–476.

Niiyama H., Mizumoto K., Sato N., Nagai E., Mibu R., Fukui T., Kinoshita M. and Tanaka M. 2001. Quantitative analysis of hTERT mRNA expression in colorectal cancer. Am. J. Gastroenterol. 96: 1895–1900.

Norrback K.F., Enblad G., Erlanson M., Sundstrom C. and Roos G. 1998. Telomerase activity in Hodgkin's disease. Blood 92: 567–573.

Ogawa Y., Nishioka A., Hamada N., Terashima M., Inomata T., Yoshida S., Seguchi H. and Kishimoto S. 1998. Changes in telomerase activity of advanced cancers of oral cavity and oropharynx during radiation therapy: correlation with clinical outcome. Intl. J. Mol. Med. 2: 301–307.

Ohshima K., Karube K., Shimazaki K., Kamma H., Suzumiya J., Hamasaki M. and Kikuchi M. 2003. Imbalance between apoptosis and telomerase activity in myelodysplastic syndromes: possible role in ineffective hemopoiesis. Leukemia Lymphoma 44: 1339–1346.

Ohyashiki J.H., Ohyashiki K., Iwama H., Hayashi S., Toyama K. and Shay J.W. 1997a. Clinical implications of telomerase

activity levels in acute leukemia. Clin. Cancer Res. 3: 619–625.

Ohyashiki K., Ohyashiki J.H., Nishimaki J., Toyama K., Ebihara Y., Kato H., Wright W.E. and Shay J.W. 1997b. Cytological detection of telomerase activity using an in situ telomeric repeat amplification protocol assay. Cancer Res. 57: 2100–2103.

Okayasu I., Mitomi H., Yamashita K., Mikami T., Fujiwara M., Kato M. and Oshimura M. 1998. Telomerase activity significantly correlates with cell differentiation, proliferation and lymph node metastasis in colorectal carcinomas. J. Cancer Res. Clin. Oncol. 124: 444–449.

Okayasu I., Osakabe T., Fujiwara M., Fukuda H., Kato M. and Oshimura M. 1997. Significant correlation of telomerase activity in thyroid papillary carcinomas with cell differentiation, proliferation and extrathyroidal extension. Jpn. J. Cancer Res. 88: 965–970.

Orlando C., Gelmini S., Selli C. and Pazzagli M. 2001. Telomerase in urological malignancy. J. Urol. 166: 666–673.

Paradis V., Bieche I., Dargere D., Bonvoust F., Ferlicot S., Olivi M., Lagha N.B., Blanchet P., Benoit G., Vidaud M. and Bedossa P. 2001. hTERT expression in sporadic renal cell carcinomas. J. Pathol. 195: 209–217.

Parris C.N., Jezzard S., Silver A., MacKie R., McGregor J.M. and Newbold R.F. 1999. Telomerase activity in melanoma and non-melanoma skin cancer. Brit. J. Cancer 79: 47–53.

Patel M.M., Parekh L.J., Jha F.P., Sainger R.N., Patel J.B., Patel D.D., Shah P.M. and Patel P.S. 2002. Clinical usefulness of telomerase activation and telomere length in head and neck cancer. Head Neck 24: 1060–1067.

Pearson A.S., Chiao P., Zhang L., Zhang W., Larry L., Katz R.L., Evans D.B. and Abbruzzese J.L. 2000. The detection of telomerase activity in patients with adenocarcinoma of the pancreas by fine needle aspiration. Int. J. Oncol. 17: 381–385.

Pearson A.S., Gollahon L.S., O'Neal N.C., Saboorian H., Shay J.W. and Fahey T.R. 1998. Detection of telomerase activity in breast masses by fine-needle aspiration. Ann. Surg. Oncol. 5: 186–193.

Poremba C., Shroyer K.R., Frost M., Diallo R., Fogt F., Schafer K.L., Burger H., Shroyer A.L., Dockhom-Dwomiczak B. and Bocker W. 1999a. Telomerase is a highly sensitive and specific molecular marker in fine-needle aspirates in breast lesions. J. Clin. Oncol. 17: 2020–2026.

Poremba C., Willenbring H., Hero B., Christiansen H., Schafer K.L., Brinkschmidt C., Jurgens H., Bocker W. and Dockhorn-Dworniczak B. 1999b. Telomerase activity distinguishes between neuroblastomas with good and poor prognosis. Ann. Oncol. 10: 715–721.

Ramakumar S., Bhuiyan J., Besse J.A., Roberts S.G., Wollan P.C., Blute M.L. and O'Kane D.J. 1999. Comparison of screening methods in the detection of bladder cancer. J. Urol. 161: 388–394.

Saji M., Westra W.H., Chen H., Umbricht C.B., Tuttle R.M., Box M.F., Udelsman R., Sukumar S. and Zeiger M.A. 1997. Telomerase activity in the differential diagnosis of papillary carcinoma of the thyroid. Surgery 122: 1137–1140.

Sakabe H., Yahata N., Kimura T., Zeng Z.Z., Minamiguchi H., Kaneko H., Mori K.J., Ohyashiki K., Ohyashiki J.H., Toyama K., Abe T. and Sonoda Y. 1998. Human cord blood-derived primitive progenitors are enriched in CD34 + c-kit-cells: correlation between long-term culture-initiating cells and telomerase expression. Leukemia 12: 728–734.

Sangiorgi L., Gobbi G.A., Lucarelli E., Sartorio S.M., Mordenti M., Ghedini I., Maini V., Scrimieri F., Reggiani M., Bertoja A.Z., Benassi M.S. and Picci P. 2001. Presence of telomerase activity in different musculoskeletal tumor histotypes and correlation with aggressiveness. Intl. J. Cancer 95: 156–161.

Schneider-Stock R., Jaeger V., Rys J., Epplen J.T. and Roessner A. 2000. High telomerase activity and high HTRT mRNA expression differentiate pure myxoid and myxoid/round-cell liposarcomas. Intl. J. Cancer 89: 63–68.

Sen S., Reddy V.G., Khanna N., Guleria R., Kapila K. and Singh N. 2001. A comparative study of telomerase activity in sputum, bronchial washing and biopsy specimens of lung cancer. Lung Cancer 33: 41–49.

Shay J.W. and Bacchetti S. 1997. A survey of telomerase activity in human cancer. Eur. J. Cancer 33: 787–791.

Shay J.W. and Gazdar A.F. 1997. Telomerase in the early detection of cancer. J. Clin. Pathol. 50: 106–109.

Shay J.W., Werbin H. and Wright W.E. 1996. Telomeres and telomerase in human leukemias. Leukemia 10: 1255–1261.

Shimada M., Hasegawa H., Gion T., Utsunomiya T., Shirabe K., Takenaka K., Otsuka T., Maehara Y. and Sugimachi K. 2000. The role of telomerase activity in hepatocellular carcinoma. Am. J. Gastroenterol. 95: 748–752.

Shoji Y., Yoshinaga K., Inoue A., Iwasaki A. and Sugihara K. 2000. Quantification of telomerase activity in sporadic colorectal carcinoma: association with tumor growth and venous invasion. Cancer 88: 1304–1309.

Smith D.L., Soria J.C., Morat L., Yang Q., Sabatier L., Liu D.D., Nemr R.A., Rashid A. and Vauthey J.N. 2004. Human telomerase reverse transcriptase (hTERT) and Ki-67 are better predictors of survival than established clinical indicators in patients undergoing curative hepatic resection for colorectal metastases. Ann. Surg. Oncol. 11: 45–51.

Soria J.C., Moon C., Wang L., Hittelman W.N., Jang S.J., Sun S.Y., Lee J.J., Liu D., Kurie J.M., Morice R.C., Lee J.S., Hong W.K. and Mao L. 2001. Effects of *N*-(4-hydroxyphenyl)retinamide on hTERT expression in the bronchial epithelium of cigarette smokers. J. Natl. Cancer Inst. 93: 1257–1263.

Streutker C.J., Thorner P., Fabricius N., Weitzman S. and Zielenska M. 2001. Telomerase activity as a prognostic factor in neuroblastomas. Pediatr. Dev. Pathol. 4: 62–67.

Suda T., Isokawa O., Aoyagi Y., Nomoto M., Tsukada K., Shimizu T., Suzuki Y., Naito A., Igarashi H., Yanagi M., Takahashi T. and Asakura H. 1998. Quantitation of telomerase activity in hepatocellular carcinoma: a possible aid for a prediction of recurrent diseases in the remnant liver. Hepatology 27: 402–406.

Suehara N., Mizumoto K., Tanaka M., Niiyama H., Yokohata K., Yominaga Y., Shimura H., Muta T. and Hamasaki N. 1997. Telomerase activity in pancreatic juice differentiates ductal carcinoma from adenoma and pancreatitis. Clin. Cancer Res. 3: 2479–2483.

Sumida T., Sogawa K., Hamakawa H., Sugita A., Tanioka H. and Ueda N. 1998. Detection of telomerase activity in oral lesions. J. Oral Pathol. Med. 27: 111–115.

Taga S., Osaki T., Ohgami A., Imoto H. and Yasumoto K. 1999. Prognostic impact of telomerase activity in non-small cell lung cancers. Ann. Surg. 230: 715–720.

Tahara H., Yasui W., Tahara E., Fujimoto J., Ito K., Tamai K., Nakayama J., Ishikawa F., Tahara E. and 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.

Tangkijvanich P., Tresukosol D., Sampatanukul P., Sakdikul S., Voravud N., Mahachai V. and Mutirangura A. 1999. Telomerase assay for differentiating between malignancy-related and nonmalignant ascites. Clin. Cancer Res. 5: 2470–2475.

Tatsumoto N., Hiyama E., Murakami Y., Imamura Y., Shay J.W., Matsuura Y. and Yokoyama T. 2000. High telomerase activity is an independent prognostic indicator of poor outcome in colorectal cancer. Clin. Cancer Res. 6: 2696–2701.

Tomoda R., Seto M., Tsumuki H., Iida K., Yamazaki T., Sonoda J., Matsumine A. and Uchida A. 2002. Telomerase activity and human telomerase reverse transcriptase mRNA expression are correlated with clinical aggressiveness in soft tissue tumors. Cancer 95: 1127–1133.

Uchida N., Otsuka T., Arima F., Shigematsu H., Fukuyama T., Maeda M., Sugio Y., Itoh Y. and Niho Y. 1999. Correlation of telomerase activity with development and progression of adult T-cell leukemia. Leukemia Res. 23: 311–316.

Uemura K., Hiyama E., Murakami Y., Kanehiro T., Ohge H., Sueda T. and Yokoyama T. 2003. Comparative analysis of K-ras point mutation, telomerase activity, and p53 overexpression in pancreatic tumours. Oncol. Rep. 10: 227–283.

Ulaner G.A., Hu J.F., Vu T.H., Giudice L.C. and Hoffman A.R. 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.

Umbricht C.B., Saji M., Westra W.H., Udelsman R., Zeiger M.A. and Sukumar S. 1997. Telomerase activity: a marker to distinguish follicular thyroid adenoma from carcinoma. Cancer Res. 57: 2144–2147.

Umbricht C.B., Sherman M.E., Dome J., Carey L.A., Marks J., Kim N. and Sukumar S. 1999. Telomerase activity in ductal carcinoma in situ and invasive breast cancer. Oncogene 18: 3407–3414.

Usselmann B., Newbold M., Morris A.G. and Nwokolo C.U. 2001. Telomerase activity and patient survival after surgery for gastric and oesophageal cancer. Eur. J. Gastroenterol. Hepatol. 13: 903–908.

Verstovsek S., Manshouri T., Smith F.O., Giles F.J., Cortes J., Estey E., Kantarjian H., Keating M., Jeha S. and Albitar M. 2003. Telomerase activity is prognostic in pediatric patients with acute myeloid leukemia: comparison with adult acute myeloid leukemia. Cancer 97: 2212–2217.

Wallace M.B., Block M., Hoffman B.J., Hawes R.H., Silvestri G., Reed C.E., Mitas M., Ravenel J., Fraig M., Miller S., Jones E.T. and Boylan A. 2003. Detection of telomerase expression in mediastinal lymph nodes of patients with lung cancer. Am. J. Resp. Crit. Care Med. 167: 1670–1675.

Watson J.D. 1972. Origin of concatemeric T7 DNA. Nature 239: 197–201.

Wisman G.B., Hollema H., de Jong S., ter Schegget J., Tjong-A-Hung S.P., Ruiters M.H., Krans M., de Vries E.G. and der Zee A.G. 1998. Telomerase activity as a biomarker for (pre)neoplastic cervical disease in scrapings and frozen sections from patients with abnormal cervical smear. J. Clin. Oncol. 16: 2238–2245.

Wright W.E., Piatyszek M.A., Rainey W.E., Byrd W. and Shay J.W. 1996. Telomerase activity in human germline and embryonic tissues and cells. Dev. Genet. 18: 173–179.

Wright W.E. and Shay J.W. 1992. The two-stage mechanism controlling cellular senescence and immortalization. Exp. Gerontol. 27: 383–389.

Wright W.E., Shay J.W. and Piatyszek M.A. 1995. Modification of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. Nucl. Acid Res. 23: 3794–3795.

Wu K.D., Orme L.M., Shaughnessy J. Jr., Jacobson J., Barlogie B. and Moore M. 2003. Telomerase and telomere length in multiple myeloma: correlations with disease heterogeneity, cytogenetic status, and overall survival. Blood 101: 4982–4989.

Wurl P., Kappler M., Meye A., Bartel F., Kohler T., Lautenschlager C., Bache M., Schmidt H. and Taubert H. 2002. Co-expression of survivin and TERT and risk of tumour-related death in patients with soft-tissue sarcoma. Lancet 359: 943–945.

Xu D., Gruber A., Peterson C. and Pisa P. 1998. Telomerase activity and the expression of telomerase components in acute myelogenous leukaemia. Br. J. Haematol. 102: 1367–1375.

Xu D., Zheng C., Bergenbrant S., Holm G., Bjorkholm M., Yi Q. and Gruber A. 2001. Telomerase activity in plasma cell dyscrasias. Brit. J. Cancer 84: 621–625.

Yang C.T., Lee M.H., Lan R.S. and Chen J.K. 1998. Telomerase activity in pleural effusions: diagnostic significance. J. Clin. Oncol. 16: 567–573.

Yoshida R., Kiyozuka Y., Ichiyoshi H., Senzaki H., Takada H., Hioki K. and Tsubura A. 1999. Change in telomerase activity during human colorectal carcinogenesis. Anticancer Res. 19: 2167–2172.

Yoshida K., Sugino T., Goodison S., Warren B.F., Nolan D., Wadsworth S., Mortensen N.J., Toge T., Tahara E. and Tarin D. 1997. Detection of telomerase activity in exfoliated cancer cells in colonic luminal washings and its related clinical implications. Brit. J. Cancer 75: 548–553.

Yoshino A., Katayama Y., Fukushima T., Watanabe T., Komine C., Yokoyama T., Kusama K. and Moro I. 2003. Telomerase activity in pituitary adenomas: significance of telomerase expression in predicting pituitary adenoma recurrence. J. Neuro-oncol. 63: 155–162.

Zeiger M.A., Smallridge R.C., Clark D.P., Liang C.K., Carty S.E., Watson C.G., Udelsman R. and Saji M. 1999. Human telomerase reverse transcriptase (hTERT) gene expression in FNA samples from thyroid neoplasms. Surgery 126: 1195–1199.

Zheng P.S., Iwasaka T., Zhang Z.M., Pater A. and Sugimori H. 2000. Telomerase activity in Papanicolaou smear-negative exfoliated cervical cells and its association with lesions and oncogenic human papillomaviruses. Gynecol. Oncol. 77: 394–398.