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Immunohistochemical Detection of Telomerase (hTERT) Protein in Human Cancer Tissues and a Subset of Cells in Normal Tissues[1]

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Abstract

We examined human telomerase reverse transcriptase (hTERT) protein distribution by immunohistochemistry in cultured cells and tissue sections. Cells with telomerase activity had nuclear positive signals whereas cells without telomerase activity did not. In most normal epithelial tissues, hTERT expression was prominent in the early proliferative descendent progenitor cells. In cancers with high telomerase activity, hTERT expression was detected in almost all neoplastic cells and correlated with telomerase activity levels, whereas cancers with low telomerase activity had fewer hTERT-positive cancer cells. In pediatric neuroblastomas with a favorable outcome, both the percentage of positive cells and the signal intensity of each hTERT-expressing cell decreased. These studies indicate that detection of telomerase at the cellular level is achievable and may have utility in cancer diagnostics. *Neoplasia* (2001) **3,** 17–26.

Keywords: telomerase, immunohistochemistry, hTERT, cancer, human.

Introduction

Telomerase, a highly conserved enzyme, is a specialized cellular reverse transcriptase catalyzing the synthesis and extension of telomeric DNA with its own RNA template [1,2]. Telomerase compensates for telomere loss due to the end-replication problem [3], and is expressed in germ cells, proliferative cells of renewal tissues, and immortal cells [4]. Activation of telomerase and stabilization of telomeres are consistent with the hypothesis that telomere maintenance is essential for attainment of immortality in cancer cells and may be a rate-limiting step in cancer progression [4,5]. Using a highly sensitive PCR-based assay, telomeric repeat amplification protocol (TRAP), telomerase activity is detected in approximately 85% to 90% of human cancer specimens [5] and the levels of telomerase activity estimated by the TRAP assay vary among cancer tissues [6–8]. These results suggest that human cancer cells *in vivo* do not always have, or absolutely require, telomerase reactivation [4,6,7]. Our previous studies of neuroblastoma, lung, breast, gastric, colorectal, and pancreatic cancers revealed that telomerase activity may be useful for the detection of cancer cells [6–12]. In some instances high telomerase activity in tumor specimens statistically correlates with poor prognosis of the patients. One central issue that remains unanswered is what high and low telomerase activities represent at the cellular level in clinical cancer specimens. At least, two explanations can be considered. First, the variation in telomerase activity among cancer tissues might be due to different levels of telomerase expression in each tumor cell. Second, the difference might be due to the absolute number of tumor cells within a tumor that have telomerase activity. To determine which explanation or both are correct, techniques for examining human telomerase reverse transcriptase (hTERT) protein distribution at the cellular levels in clinical specimens are needed.

Human telomerase consists of two major components: human telomerase RNA (hTR), which consists of a 451-base integral RNA providing the template for the synthesis of the human telomeric repeat (TTAGGG)n [13], and human telomerase reverse transcriptase, hTERT, which is a 127-kDa protein providing catalytic function to replicate the ends of linear DNA [14,15]. Although other components such as hTEP1 (telomerase associated protein 1) [16], hsp90, and p23 [17] are present in the human telomerase complex *in vivo*, their role in telomerase function is less clear. *In vitro* studies using rabbit reticulolysate extracts containing transcribed hTR and hTERT protein show that these two components are sufficient to reconstitute telomerase activity [18]. Although hTR expression can be detected by Northern blot analysis, the very low expression of hTERT

Abbreviations: hTERT, human telomerase reverse transcriptase; TRAP, telomeric repeat amplification protocol; IHC, immunohistochemistry; ISH, *in situ* hybridization; TBS, Tris-buffered saline.

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mRNA in cells and tissues with telomerase activity is difficult to detect using Northern analysis or *in situ* hybridization (ISH). Using RT-PCR, hTERT mRNA expression appears to correlate with the levels of telomerase activity *in vitro* and *in vivo* [19,20], but alternative splice variants of hTERT could cause misleading interpretation of hTERT expression [21]. Northern or RT-PCR procedures are able to estimate the total amount of telomerase in whole tissues but are unable to estimate the level of telomerase activation in each cell.

Telomerase activity is repressed during embryonic development in most tissues [19,22], but remains detectable in proliferative germ-line cells, proliferating hematopoietic stem-like cells, activated lymphocytes, proliferative premenopausal endometrial cells, a subset of cells in the epidermis, and in the crypts of the intestine [23–26]. These cells may become the origin of telomerase activity in the tumor tissues with detectable telomerase activity. Thus, detecting telomerase activation at the cellular level in tumor specimens would facilitate evaluating the origin of telomerase activity. In the present study, we examined hTERT expression at the cellular level in sectioned clinical materials using immunohistochemistry (IHC), and then compared these results to the same specimens in which the levels of telomerase activity had also been analyzed.

Materials and Methods

Cell Samples

hTERT transfected BJ fibroblasts were used as positive controls whereas SW13 fibroblasts immortalized with SV40 large T-antigen were used as a telomerase negative cell line, often referred to as alternative lengthening of telomeres (ALT) pathway. HT1080 (human fibrosarcoma) cells growing with and without serum were used as positive and negative controls for the IHC. Telomerase activity is dramatically reduced in HT1080 cells when maintained in culture without serum [27].

Tissues

Human normal tissue specimens were obtained at autopsy of four adults (aged 27, 30, 32, and 39 years old) who died of injury or arrythmia. For further analysis of normal epithelial cells, human normal tissues including skin and digestive organ mucosa specimens were obtained with informed consent from patients undergoing surgery. Tumor tissues in breast, lung, stomach, colon, liver, pancreas, neuroblastoma, Wilms tumor, and hepatoblastoma were obtained at the time of surgery. In the lung cancer cases with multiple metastases, primary and metastatic tumor specimens were obtained at autopsy.

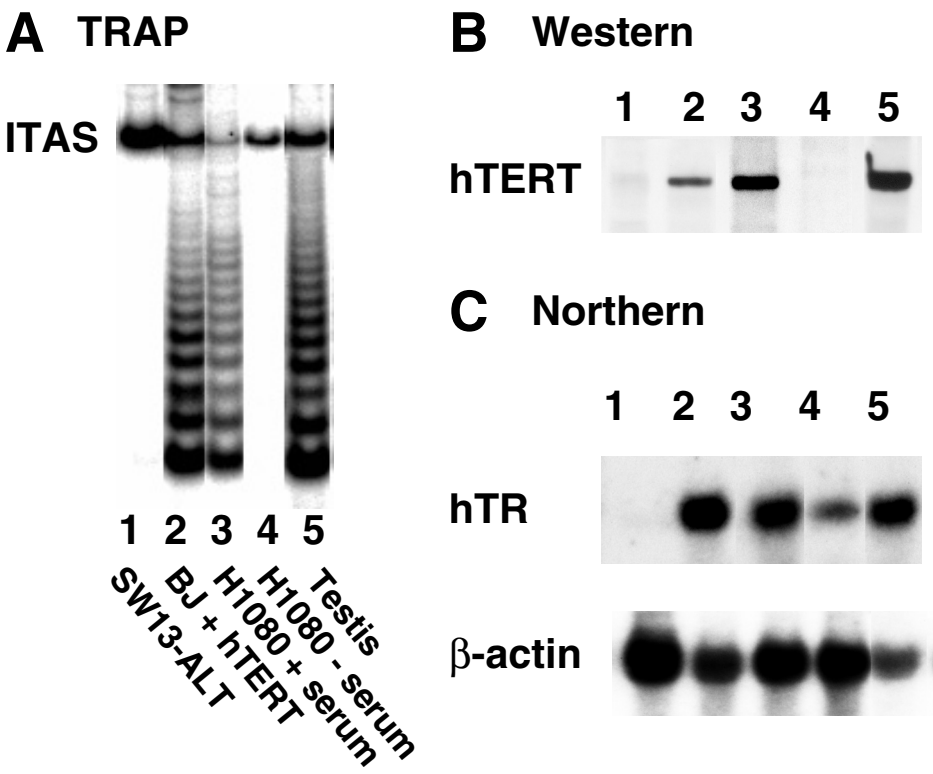

Figure 1. *Correlation between telomerase activity, hTR, and hTERT expression in cell lines and tissue specimens. (A) TRAP assay using the extracts derived from 1000 cells or containing 6 μg of protein. Lane 1, SW13-ALT (telomerase negative immortal fibroblast) cells; lane 2, BJ+hTERT (normal fibroblasts transfected with hTERT) cells; lane 3, HT1080 cells growing in serum containing medium; lane 4, HT1080 cells cultured serum-free for 1 week, and lane 5, normal human testis. (B) Western blot assay for hTERT. As previously reported [27], in the absence of serum, the HT1080 cells become quiescent and telomerase activity is dramatically reduced. In the HT1080 cells cultured without serum over a week, telomerase activity and hTERT was undetectable. (C) Northern blot assay for hTR expression. Loading is standardized using β-actin mRNA.*

Informed written consent was obtained for all samples. Of each tissue specimen obtained, two portions were flash frozen, one for the telomerase (TRAP) assay and the other for RNA isolation. The remaining sample was fixed in 10% formalin and embedded into paraffin for IHC as described below.

Telomerase Assay

Telomerase extracts from 50 to 100 mg and cell samples of 10^6 cells were prepared and assays of its activity were done as described earlier [28,29]. An aliquot of tissue extract containing 6 μg of protein or an aliquot of cell extract containing 10^3 cells was used for each TRAP assay. Each reaction mixture contained 5 attograms of an internal telomerase assay control (ITAS), a 150-bp DNA standard, for the identification of false-negative tumor samples that contain telomerase assay inhibitors [30]. For the modified telomerase assay, which removes tissue extract inhibitors, the mixture after the step of telomerase mediated extension was treated with phenol/chloroform followed by ethanol precipitation, the precipitate was resuspended, and then subjected to PCR similarly to the standard TRAP assay. The PCR product was subjected to electrophoresis on a 10% polyacrylamide gel and then autoradiographed.

Immunohistochemistry

Antibodies An affinity-purified polyclonal rabbit antibody against hTERT (EST21A) was raised against a 16 amino acid peptide sequence mapping in the middle of hTERT (Alpha Diagnostic International, San Antonio, TX) and used at 5 to 10 μg/ml.

Tissue preparation The tissues were cut at 6-μm serial sections. Sections were deparaffinized and rehydrated through ascending grades of alcohol to Tris-buffered saline (TBS), pH 7.4. Heat-based antigen retrieval was performed as follows: sections were treated for 15 minutes in 0.01 M citric acid buffer, pH 6.0 in 2 atm and 120°C using an autoclave. After decreasing the pressure, sections were removed and permitted to cool for approximately 30 minutes before being washed three times for 5 minutes in TBS.

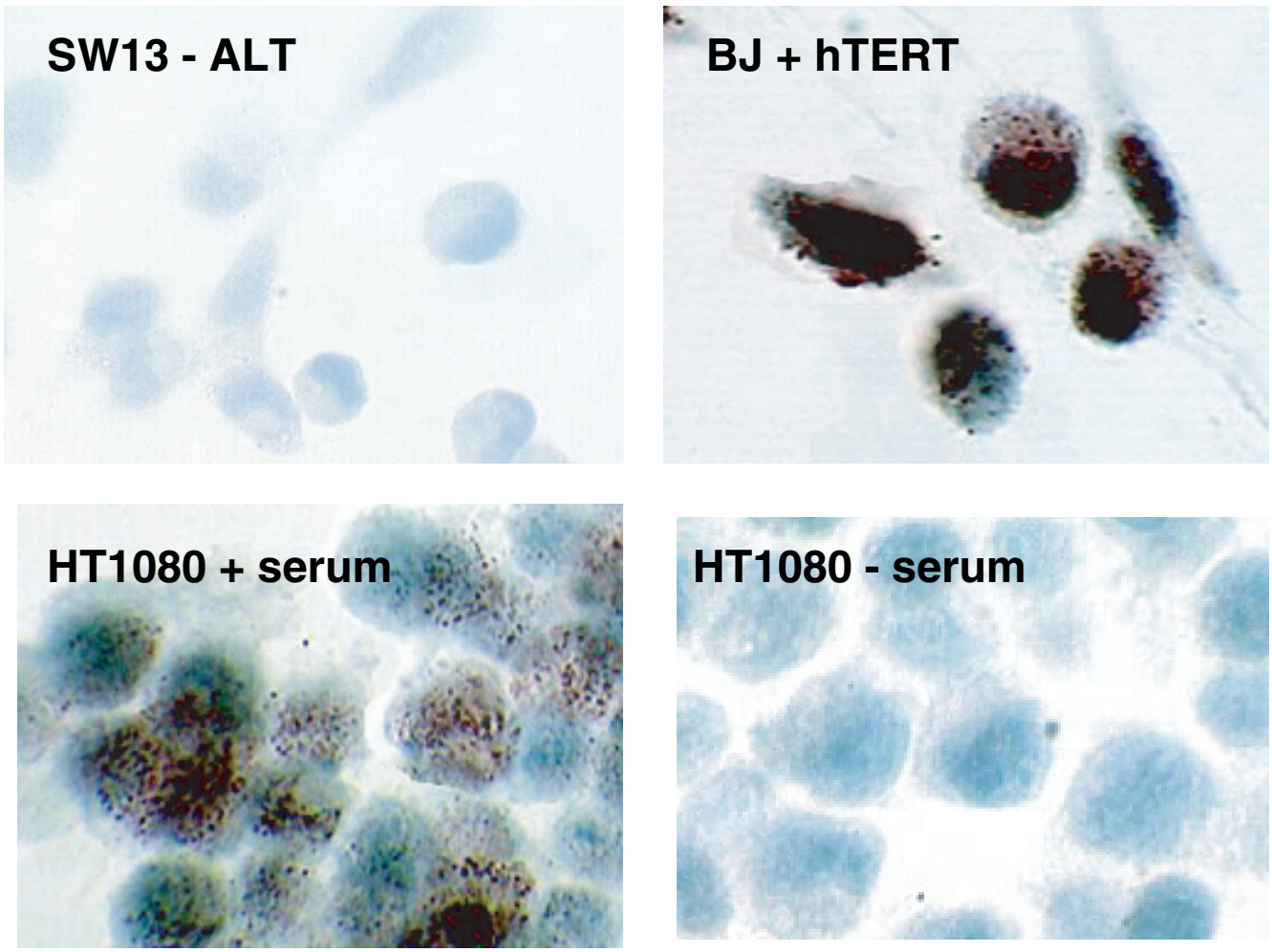

Figure 2. *hTERT expression in cell lines. The expression of hTERT was undetectable in SW13-ALT (telomerase negative fibroblast) cells, whereas BJ+hTERT cells and HT1080 cells (+serum) showed nuclear staining by hTERT IHC. The HT1080 cells cultured in the absence of serum (− serum), hTERT staining signals were dramatically reduced.*

Immunohistochemistry

Endogenous peroxidase was quenched in 3% H_2O_2. After washing three times for 5 minutes in TBS, nonspecific antibody binding was blocked by incubating the sections in protein blocking solution (Dako, Carpinteria, CA) for 30 minutes. Sections were then transferred to a humidified chamber and incubated in antibody solution overnight. Following this and subsequent incubations, sections were thoroughly washed in three changes of TBS for 5 minutes each. For hTERT immunohistochemical staining, sections were incubated in the labeled streptavidin biotin polymer (Envision Plus, Dako), followed by 0.05% 3,3′-diaminobenzidine (DAB) or 3% 3-amino-9-ethyl carbazole (AEC) in TBS with H_2O_2 as a substrate. Sections were lightly counterstained with Mayer's hematoxylin, and then mounted.

The intensity of the immunostaining was evaluated by light microscopy and the software Image-Pro Plus ver. 4 (Media Cybernetics, Silver Spring, MD). The individual signals were classified into one of three categories: ++ (strong, IHC signals were strongly detected throughout the nucleus not the nucleolus), + (weak, IHC signals were weakly detected or appeared as a speckled/dotted pattern in the nucleus), and − (no staining).

In situ hybridization A fluorescyl tyramide ISH hTR detection system was supplied from Dako. Sections were deparaffinized and rehydrated through ascending grades of alcohol to TBS, pH 7.4. After heat-based antigen retrieval, sections were treated with 0.005% pepsin in 0.2 N HCl for 20 minutes and then hybridized overnight with Fluorescein isothiocyanate (FITC)-labeled probe. The sections were washed stringently and immersed in 3% H_2O_2, and then, sections were treated with anti-FITC antibody horseradish peroxidase (HRP) conjugate for 30 minutes and applied with the fluorescyl tyramide reagent for 15 minutes.

For double staining, hTR ISH was performed first and then hTERT IHC was developed on the same slides. The intensity of the signal was evaluated by fluorescent microscopy and quantitated using the software Leica ver. 4 (Leica Microsystem Imaging Solutions, Cambridge, UK).

RNA Isolation and Northern Blot Analysis

Using the acid-guanidium–phenol–chloroform method [31], total cellular RNA was extracted from the frozen tissue samples. To detect hTR expression by Northern blot analysis, 10 μg of total RNA was subjected to electrophoresis on 1.5% agarose gels, transferred onto a nitrocellulose filter in 20$\times$ standard saline citrate, and hybridized with ^{32}P-labelled hTR probe. The hybridized filter was subjected into autoradiography on an imaging plate and the intensities of these signals were estimated using a phosphor imaging analyzer (BAS 2000, Fuji, Tokyo, Japan) and evaluated using the ratio to β-actin signals.

Western Blot Analysis

Tissue or cell extracts were prepared by lysis in 10% sucrose and 20 mM Tris–HCl (pH 7.5). Protein extracts of 20 μg were subjected to electrophoresis on a sodium dodecyl sulfate (SDS)–7.5% polyacrylamide gel and

Table 1. hTERT Expression in Normal Tissues.

Tissue Type	Telomerase Activity*	Location Cell Type with Expression	Level of Expression†
Lung	−		−
Skin	low	Basal keratinocytes	+
Tonsil	low	lymphocytes	++
Spleen	low	Lymphocytes	++
Thyroid	low	lymphocytes	++
Breast	−	Epithelial cells	+
Kidney	−		−
Prostate	−		−
Esophagus	low	Basal cells/lymphocytes	+
Stomach	low	Mucosal basal cells/lymphocytes	+
Small intestine	low	Basal cells of villi/lymphocytes	+
Colon	low	Basal cells of crypt/lymphocytes	+
Liver	−	Infiltrating lymphocytes	+
Pancreas	−		−
Brain	−		−
Skeletal	−		−
Muscle	−		−
Cardiac muscle	−		−
Placenta	low	Trophoblasts in chorionic villi	+
Endometrium	high	Basal cells of Endometrial glands	++
Testis	high	Spermatocytes/maturing spermatids	++

*Telomerase activity detected by TRAP assay was divided into three groups: high (positive using a tissue extract containing 0.06 μg of protein), low (positive using a tissue extract containing 6 μg of protein), and − (negative).

†hTERT IHC signals were divided into three groups: ++ (strong, IHC signals were strongly detected throughout the nucleus not the nucleolus), + (weak, IHC signals were weakly detected or appeared as a speckled/dotted pattern in the nucleus (Figure 6*C*), and − (no staining).

transferred to nitrocellulose filters (Amersham Pharmacia Biotech, Sunnyvale, CA). The nitrocellulose filter was blocked in TBS and 5% nonfat dried milk and then subjected to immunoblotting for detection of hTERT with the polyclonal antibody washed with TBS plus 0.1% Tween 20 incubated with HRP-conjugated goat anti-rabbit antiserum (Amersham Pharmacia Biotech), washed with TBS plus Tween 20. The specific bands of hTERT were detected by using enhanced chemiluminescence system as recommended by the manufacturer (ECL-Plus, Amersham Pharmacia Biotech).

Results

Cells

Telomerase activity was detected in BJ fibroblasts transfected with hTERT and rapidly proliferating HT1080 fibrosarcoma cells using the TRAP assay (Figure 1*A*). Western blot analysis using an anti-hTERT rabbit polyclonal antibody detects an estimated molecular mass of 127 kDa (Figure 1*B*), close to that expected from the amino acid sequence in the extracts from telomerase positive cultured cells. However, the 127 kDa band was undetectable in telomerase negative fibroblasts (SW13-ALT, T-antigen immortalized cells) and in quiescent (serum-starved) and telomerase negative HT1080 cancer cells (Figure 1*A* and *B*). By Northern blot analysis (Figure 1*C*), hTR was detectable, at differing levels, in the cell lines with telomerase activity and also in the cells without telomerase activity.

Figure 2 illustrates hTERT protein in cells by IHC. Signals of hTERT-IHC were detected in hTERT-transfected fibroblast (BJ+hTERT) cells but not in SW13-ALT immortal fibroblast cells without telomerase activity (Figure 2). HT1080 cells, which have high telomerase activity when actively growing, can be induced to become quiescent upon serum deprivation. Telomerase activity was dramatically reduced after 1 week of cell culture without serum, as previously reported [27].

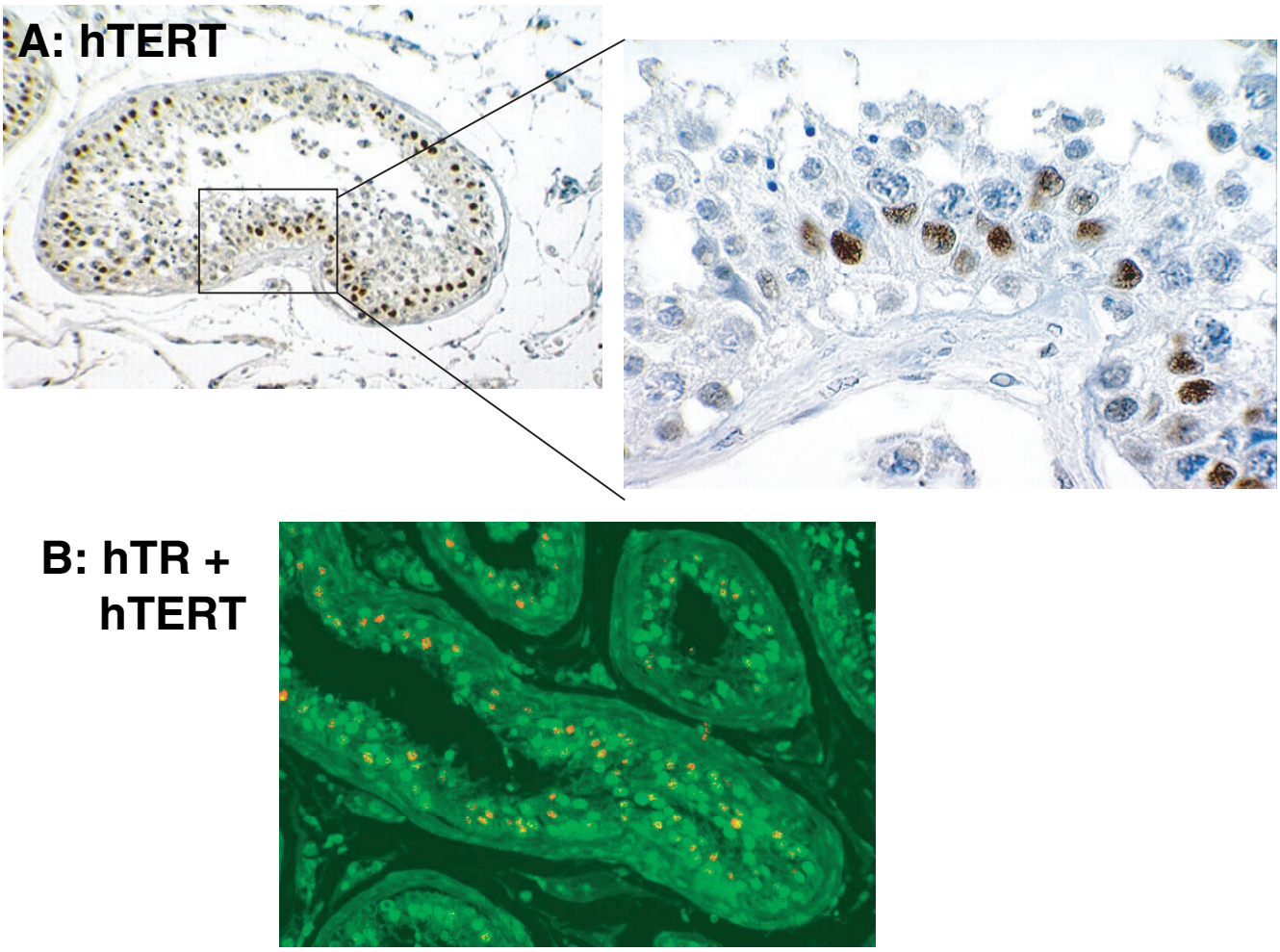

Figure 3. *hTR and hTERT expression in normal testis. (A) In the section of human testis, hTERT-IHC revealed that the expression of hTERT was detected in spermatocytes but not in quiescent spermatogonia and mature spermatozoa. (B) Double labeling revealed that the expression of hTR was detected in most cells of the seminiferous tubules whereas hTERT signals were detected in only a subset of spermatocytes and maturing spermatids. There were no cells with hTERT signals without hTR expression.*

Tissues

Telomerase activity and hTERT mRNA were not detected in the majority of normal adult tissues, including cardiac/skeletal muscle, liver, prostate, breast, pancreas, brain, lung, and kidney (Table 1). Low to high levels of telomerase activity was detected in germ-line tissues, proliferative premenopausal endometrium, tissues containing activated lymphocytes, and at lower levels in some epithelial tissues with high rates of cell proliferation.

Testis

In homogenates of adult human testis, high telomerase activity, high levels of hTR (Northern analysis), and hTERT protein were detected (Figure 1). In tissue sections of this testis sample, hTERT signals were strongly expressed in spermatocytes/maturing spermatids but not in quiescent spermatogonial cells and mature spermatozoa (Figure 3). Double labeling with hTR-ISH and hTERT IHC revealed many cells with only hTR signals, some cells with both hTERT protein and hTR, but no cells with hTERT signals without hTR expression. This indicates that the functional RNA component of telomerase (hTR) is detected in most cells of the seminiferous tubules, whereas hTERT protein is expressed only in restricted subsets of activated spermatogonial cells, spermatocytes, and some maturing spermatids. The positive staining of hTERT was detected almost exclusively in the nucleus. No staining was detected in Leydig cells, Sertoli cells, or the blood vasculature.

Normal Epithelium

Esophageal and colon mucosal tissues have low levels of telomerase activity and hTERT protein (Figure 4). By IHC in normal esophageal mucosa (Figure 4*A*), hTERT expression was detected in a small number of basal and suprabasal cells (Figure 4*A*, insert). Previously there have been conflicting results as to whether telomerase activity is present in colon adenomas. In the normal colon mucosa, the hTERT expressing cells were also located at the bottom of crypts (Figure 4*B*). Moreover, there were many positive intestinal lymphocytes that contribute to telomerase activity as identified by antibody staining (data not shown) in the intestinal mucosa and submucosal lymphatic nodules. Thus,

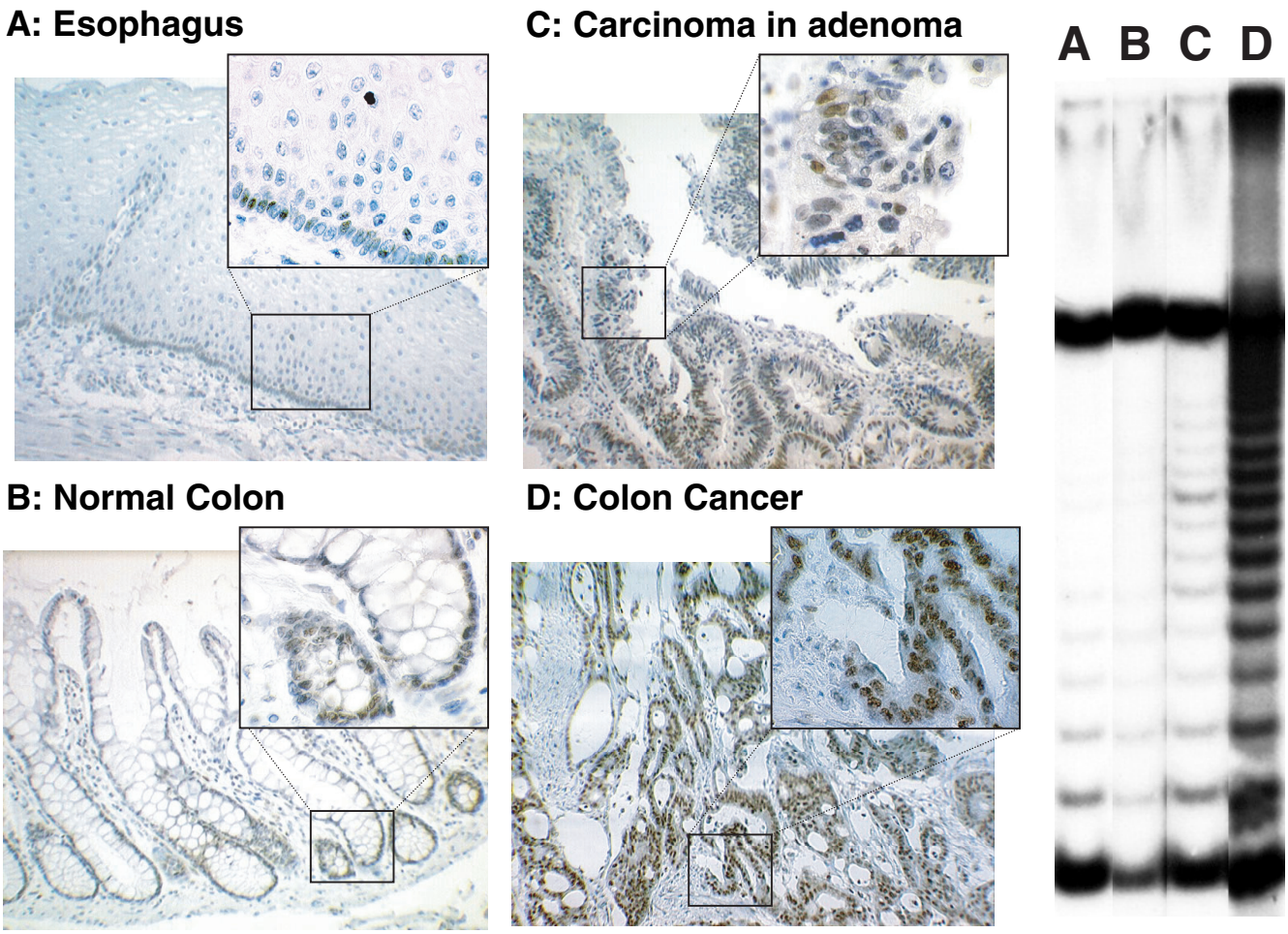

Figure 4. *hTERT expression in normal epithelium and colon cancer. hTERT expression was detected in epithelial cells located in the lower portion of esophageal mucosa (A) and in the lower crypt cells of the normal colonic mucosa (B). In the specimen with intramucosal carcinoma of the colon (C), the hTERT strong signals were detected in some but not all cancer cells. In the advanced colon cancer specimens with high telomerase activity, almost all tumor cells showed strong hTERT immunoreactivity (D).*

variable amounts of telomerase positive lymphoid cells in combination with the telomerase activity in the crypt areas could lead to the false impression that colonic polyps without carcinoma have telomerase activity.

Carcinoma In Situ

In the colon adenoma specimens with intramucosal carcinoma, telomerase activity was detected, but the level of telomerase was not significantly higher than that of normal colonic mucosa. Although hTERT signals were detected in some of the cancer cells located in the restricted areas of the small lesion, this did not contribute much to the overall level of telomerase activity in the whole tissue specimen (Figure 4*C*).

In three of the four breast ductal cell carcinoma *in situ* (DCIS) specimens, and two of two breast lobular cell carcinoma *in situ* (LCIS) specimens, telomerase activity was detected using whole tissue but the overall levels were low. By Northern or RT-PCR analyses, the expression of hTR and hTERT mRNA was also detected in most of these samples with detectable telomerase activity (data not shown). IHC analysis revealed that hTERT was strongly detected in the DCIS lesions (Figure 5*A*). Thus, the low telomerase activity of breast tissue in such patients was likely derived from the DCIS cells in admixture with massive normal telomerase negative cells. In addition, the hTERT signals were also detected in lobular CIS cells (data not shown). Even though the number of positive tumor cells was relatively low in the whole tissues, the intensities of the hTERT signals per cell were as strong as those detected in invasive carcinoma cells (Figure 5*B*).

Cancers

In the tissues examined, telomerase activity was detected in 85% of cancers that were enrolled in this study (Table 2), and the levels of telomerase activity varied. For quantitative analysis of telomerase activity levels, the intensity of the TRAP ladder was estimated by comparing the ratio of the entire TRAP ladder to the signal of the amplified internal control [8]. In general, the relative telomerase activity value is about 50 for telomerase positive cancer cell lines. Only a subset of cancer tissues with high telomerase activity had a relative telomerase activity level above 50 [276/475 (58%)]. In breast invasive ductal cell carcinoma tissue (Figure 5*B*), hTERT expression was detected in the cancer cells with strong signals and the intensity of the telomerase-mediated

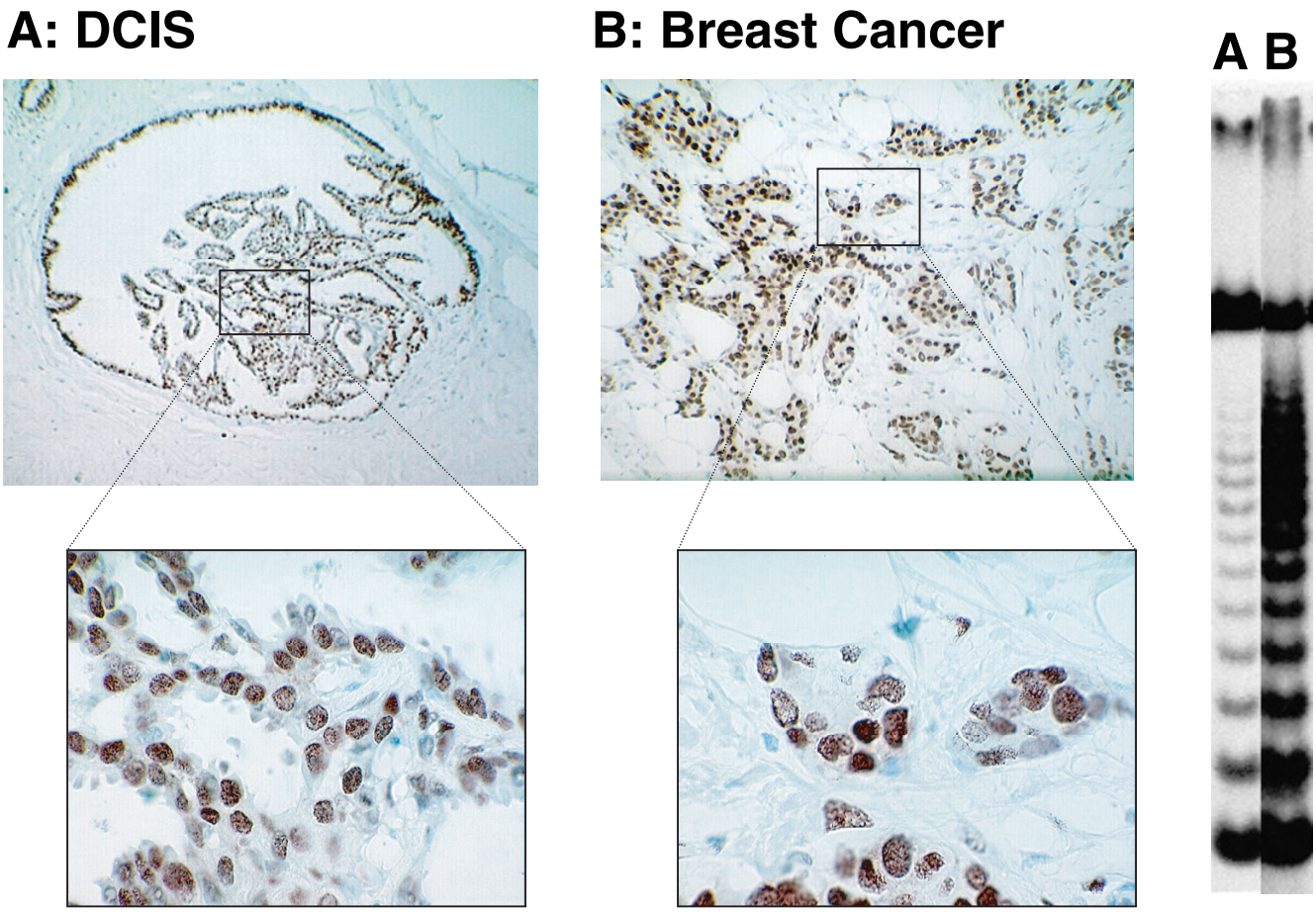

Figure 5. *hTERT expression in breast tumors. The signals of hTERT were detected in the tumor cells of ductal cell carcinoma in situ (DCIS) (A) and of invasive ductal carcinoma (B). Telomerase activity was detected in both of these tissues but there were considerably more cancer cells relative to stromal cells per specimen analyzed by TRAP in the advanced cancer compared to the DCIS. This is likely to account for the differing levels of telomerase activity in tissue specimens.*

Table 2. hTERT Expression in Human Tumors.

Tissues/Tumors	Telomerase Activity		Location Cell Type with Expression	Level of Expression*
Breast				
FC†	0/4	–	Epithelial component	+
Fibroadenoma	5/12	low	Epithelial component	+
DCIS‡	3/3	low	Cancer cells	+ +
LCIS§	2/2	low	Cancer cells	+ +
Invasive carcinoma	84/86	low–high	Cancer cells	+ +
Lung				
SCLC	9/9	high	Cancer cells	+ +
NSCLC	23/26	low–high	Cancer cells	+ +
Stomach				
Adenoma	2/3	low	Mucosal basal cells/lymphocytes	+
CIS	4/4	low	Cancer cells	+ +
Invasive carcinoma	50/52	low–high	Cancer cells	+ +
Colon				
Adenoma	14/14	low–high	Basal cells of crypt/lymphocytes	+ (+ +)
CIS	3/3	low–high	Cancer cells	+/+ +
Invasive carcinoma	96/100	low–high	Cancer cells	+ +
Liver				
HCC	15/16	high	Cancer cells/lymphocytes	+ +
Pancreas				
Duct cell carcinoma	41/42	high	Cancer cells	+ +
Islet cell carcinoma	3/3	low–high	Cancer cells	+
Childhood				
Neuroblastoma	102/108	low–high	Cancer cells	–/+ +
Wilms	12/12	high	Cancer cells	+/+ +
Hepatoblastoma	15/15	low–high	Cancer cells	+/+ +

*Telomerase activity detected by TRAP assay was divided into three groups: high, low, and – (negative). hTERT IHC signals were divided into three groups: + + (strong), + (weak), and – (no staining).
†Fibrocystic disease.
‡Ductal carcinoma *in situ.*
§Lobular carcinoma *in situ.*

(TRAP) ladder was often stronger than those of DCIS. Because the hTERT signal in each cancer cell was not different between DCIS and invasive cancer, the relative telomerase activity level mainly reflect the number of telomerase positive cancer cells in the tissue specimens. The signals of hTERT in colon cancer cells in the tissues with high telomerase activity were stronger than those in normal basal cells in the colonic crypt. The IHC of hTERT revealed that hTERT-positive cancer cells were dominant in the cancer tissues with high telomerase activity (Figure 4*D*). In contrast, tumors with low telomerase activity (e.g., carcinomas in adenoma), cancer cells with telomerase activity were a minority (Figure 4*C*). The intensities of hTERT signals of each cancer cell in these tumors were not weaker than those of the tumors with high telomerase activity.

We also examined a lung squamous cell carcinoma case with multiple metastases (Figure 6*A* and *B*). This case had a subclavicular lymph node metastasis, a hilar lymph node metastasis, and a liver metastasis as previously described [7]. In the primary tumor and liver metastasis, there was no detectable telomerase activity using the whole tissue extract, but the hilar lymph node metastasis showed high telomerase activity. IHC of hTERT staining revealed that the hilar lymph node specimen showed upregulated hTERT expression in almost all cells (Figure 6*B*), whereas the primary tumor (Figure 6*A*) only had a rare cell with a positive hTERT signal. This indicates, in some instances, telomerase reactivation occurs at a later stage in cancer progression. Interestingly, only the hilar lymph node metastasis had TP53 gene aberrations in both alleles, whereas other lesions did not [7].

In childhood neuroblastoma, we previously reported that high telomerase activity correlated with the malignant potential of tumor cells [6,12]. In neuroblastoma tissues with favorable outcomes (neuroblastoma stages 1 and 2), the tissues contained only low telomerase activity (data not shown), hTERT was detected in only a small number of cells, and the hTERT signals were often weaker (Figure 6*C*) when compared to those of the tumors with high telomerase activity (Figure 6*D*). In neuroblastoma tissues with high telomerase activity, almost all tumor cells expressed strong hTERT signals (Figure 6*D*). However, in the regressing cases (neuroblastoma 4S) that did not have detectable telomerase activity, hTERT was not detected in any cells, even though the proliferation index was high (data not shown).

Discussion

The catalytic protein of telomerase, hTERT, is believed to be a critical if not rate-limiting step in the production of telomerase activity. Even though hTERT protein is of very

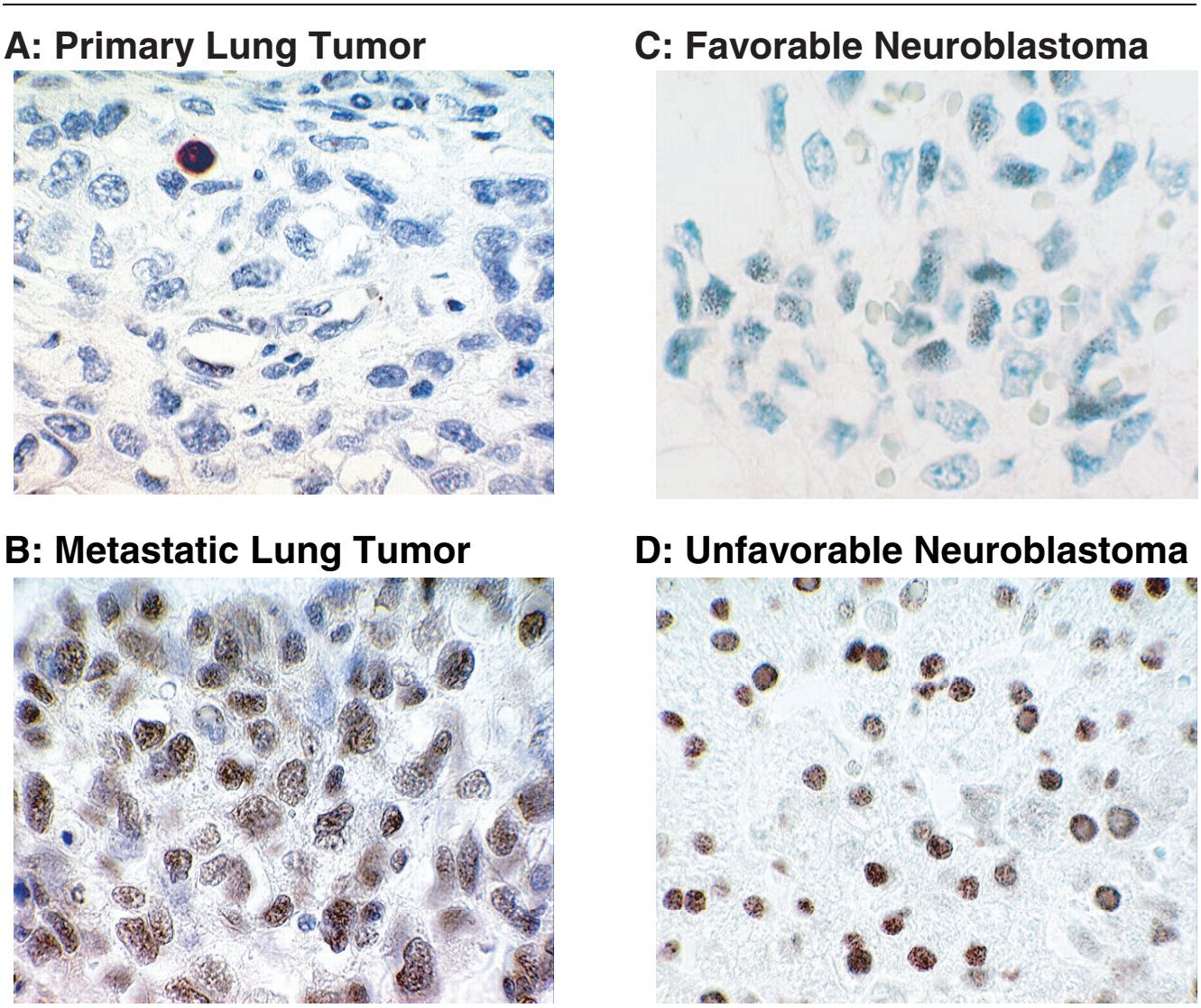

Figure 6. *hTERT expression in a primary lung squamous cell carcinoma, its metastases and neuroblastoma specimens. In the cases with lung cancer, the primary tumor showed no telomerase activity, whereas only a hilar lymph node metastasis, among the multiple metastases, showed high telomerase activity. The IHC of hTERT staining revealed that only the hilar lymph node specimen showed intense upregulated hTERT expression in most cells (B), whereas other lesions, including the primary tumor, showed only a rare cell with positive hTERT signals (A). The intensities of hTERT signal of each cancer cell in these two tumors were equivalent. Among childhood malignancies, the favorable outcome (stage 1) neuroblastoma tissue usually shows low telomerase activity, whereas the unfavorable outcome (stage 4) neuroblastoma tissue shows high telomerase activity. In the favorable neuroblastoma (C), hTERT was detected in only small number of cells and the hTERT signals were generally weaker than those of tumors with high telomerase activity. The unfavorable neuroblastoma tissue (D) expressed hTERT intensely in almost all tumor cells.*

low abundance, this report illustrates that IHC can be used to detect hTERT protein at the cellular level in paraffin-embedded tissues. hTERT was detected in cell types of tissues that had previously been reported to have telomerase activity based on microdissected frozen sections [25,32]. Importantly, there was little background nonspecific staining of cell types believed to be telomerase activity negative. This indicates that archival clinical materials may be used to detect hTERT protein and this is likely to correlate with telomerase activity.

The staining pattern of hTERT was restricted to the nucleus in both normal telomerase positive cells as well as cancer cells. The positive hTERT signals were detected as dotted/speckled patterns distributed throughout the nucleus, but were consistently absent from nucleoli. It is well established that there is considerable variation in the intensity of the TRAP signal using whole tissues of human cancer specimens [6–8,11], but in some instances relative telomerase activity levels correlate with disease progression and can be a prognostic indicator of outcomes. The immunolocalization of hTERT in specimens of adult cancers revealed that the levels of telomerase activity mainly depended on the number of tumor cells with telomerase activity. The signal intensity of hTERT-positive cells did not differ substantially between tumors with various levels of telomerase activity suggesting that relative telomerase activity of tissue specimens from cancer patients may be a surrogate indicator of overall tumor burden. Although telomerase activity in most cancers depends on the absolute number of cells containing hTERT protein, at least in some

types of tumors such as in neuroblastoma, there appears to be differing levels of telomerase in each cell.

In summary, the present study indicates that IHC for the detection of the telomerase catalytic protein (hTERT) is feasible and may have important implications in future cancer diagnostics, prognostics, and as an indicator of residual disease.

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