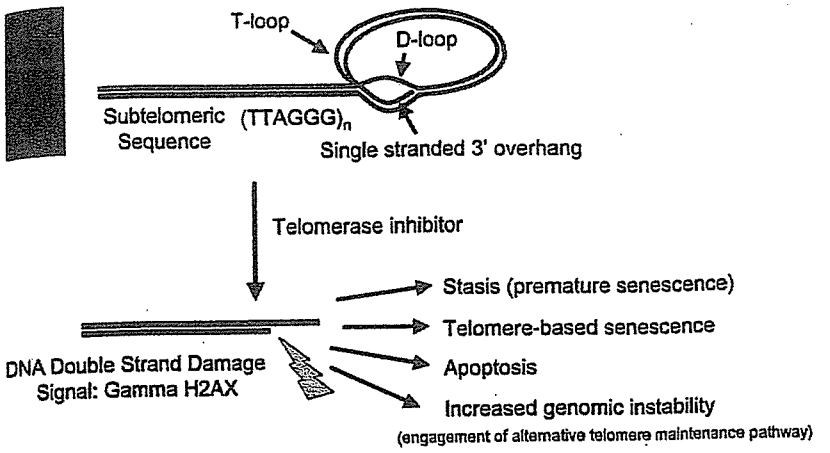

FIGURE 12.1. A model supported by electron microscopic observations demonstrates a lariat structure at each telomere end. The telomeres are protected by telomere binding proteins. In the presence of inhibitors that rapidly uncap telomeres in normal or cancer cells, there is a rapid growth arrest leading to apoptosis. When telomerase is inhibited in cancer cells there can be a period of cell growth with progressive telomere shortening eventually leading to a growth arrest or apoptosis. While it has not been demonstrated experimentally in human telomerase expressing cancer cells, inhibiting telomerase as part of cancer therapy could lead to an increased genomic instability and engagement of an alternative telomere maintenance mechanism.

GENETIC AND ENVIRONMENTAL EFFECTS ON TELOMERES

To investigate telomere dynamics and its role in cancer risk variations, telomere length in peripheral blood lymphocytes (PBLs) of monozygotic (n = 52) versus dizygotic (n = 26) twins was determined.[43] The heritability estimated as measured by the maximum likelihood estimates is approximately 90% for the monozygotic twins but is not significant for the dizygotic twins. These results indicate that telomere length is in part a heritable trait. Thus, while there is genetic heterogeneity of telomere length within matched age groups, there is a progressive loss of telomeres throughout life (Figure 12.2).

There are important connections between chronic inflammation and progressive carcinogenesis leading to neoplastic transformation. During inflammation (which can be caused by tobacco smoke and other pathological conditions), infiltrating neutrophils and macrophages can elicit a repertoire of cytokine signals that are critical for the repair and proper functioning of tissues. While the immediate targets of this signaling are likely to be the epithelial cells lining the nasal and bronchial passageways, the inflammation that occurs may also affect immune cell telomeres. Chronic exposure to tobacco smoke may result in increased cell divisions and turnover of immune cells resulting in enhanced telomere shortening. Since telomeres progressively shorten with each cell division and with increased age (Fig. 12.2), even in immune cells, a smoke-associated field effect can occur outside the airway passages. Because DNA damage caused by smoking is reflected in immune cells, such as lymphocytes, a molecular epidemiological approach using quantitative fluorescent *in situ* hybridization (Q-FISH) was undertaken (Figure 12.3). This study showed that individuals with short telomeres had a 6-fold significantly elevated risk for bladder cancer and similar findings were observed for lung, renal and head and neck cancers in ongoing case-control studies.[43]

These findings illustrate the genetic contribution of telomere variations and provide suggestive evidence that telomere dysfunction may be a predisposing factor for cancer. Telomere dysfunction associated with cancer predisposition is evident with advanced age. The ability to demonstrate that shortened telomeres in immune cells can be predictive for the onset of cancer should permit better identification of those at risk for smoke-related cancers, improve early

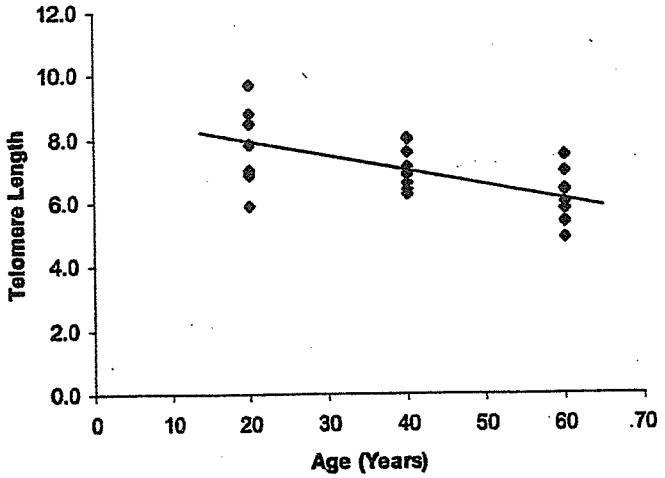

FIGURE 12.2. Telomere length measurement of peripheral blood mononuclear cells of normal volunteers of various ages. The measurement of telomere length relies on the absence of restriction enzyme recognition sites within the TTAGGG tandem repeat sequences. The length of telomeric repeats can be measured by analyzing the length of the "terminal restriction fragment" (TRF), the large DNA fragments remaining after the rest of the genomic DNA has been reduced to small sizes by digestion with frequent cutters (restriction enzymes with 4-base recognition sequences such as *Hinf*1). There is also a region of mostly repetitive subtelomeric DNA, probably ranging from 2–5 kb in length in humans, that lacks functional restriction sites and which contributes to the measured size of telomeres on gels. The DNA is probed with a labeled oligonucleotide containing TTAGGG repeats. The measured size of the TRF corresponds to the sum of the sizes of the subtelomeric DNA that lacks functional restriction sites and the length of the telomere repeats. Since small oligonucleotides easily penetrate dried agarose gels, it is more rapid and convenient to perform TRFs using "in gel hybridization" rather than Southern transfers.

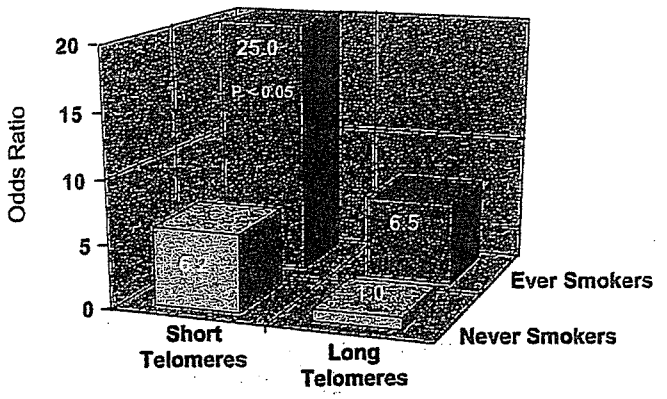

FIGURE 12.3. Smokers with short telomeres are at increased risk for smoke-associated cancer development compared to non smokers. In this study, lymphocyte telomere length was measured using quantitative fluorescent *in situ* hybridization laser scanning cytometry (Q-FISHLSC) using a fluorescent-labeled peptide nucleic acid (PNA) probe.[43] The fluorescent signal was measured by the laser scanning cytometry (LSC, CompuCyte, Cambridge, MA)™. When telomere length was analyzed as a categorical variable using the 25th percentile in the controls as the cutoff, individuals with shorter telomeres had a significantly increased risk of bladder cancer with an OR of 6.20 (95% CI, 2.40,16.05) in unconditional logistic regression analysis with multiple covariates to remove the confounding effects of age, sex, and smoking status. When subjects were categorized into quartiles of telomere length based on the distribution in the controls (with 3rd and 4th quartiles as the referent category), there was a dose-response relationship between bladder cancer risk and the degree of telomere shortening.[43] Similar results are noted in other smoke-associated cancers such as head and neck, lung, and renal cancer.

detection methods, and perhaps even impact the radiotherapeutic and chemotherapeutic treatments of airway passage disease.

CELL CULTURE (*IN VITRO*) MODELS

Telomere shortening in the absence of telomerase has been shown to be the fundamental mechanism by which cultured human cells "count" cell divisions and ultimately growth arrest.[8] There is compelling evidence that telomere shortening occurs *in vivo*, that telomere shortening is the mechanism by which cells "count" the number of times they have divided, and that too-short telomeres ultimately induce cellular senescence. A large number of strains of human skin fibroblasts from both normal donors and those with various genetic syndromes have all been immortalized following the introduction of telomerase. WI-38 fetal human lung fibroblasts have been a key reference cell strain for the study of cellular aging since Hayflick used them to demonstrate the limited proliferative capacity of normal diploid cells almost 40 years ago. Initial attempts to immortalize WI-38 cells using human telomerase reverse transcriptase (hTERT) to prevent telomere shortening failed.[44] However, WI-38 fibroblasts were successfully immortalized when grown in low (3%–5%) oxygen in medium supplemented with several micronutrients and antioxidants.

The role of ambient (21%) and physiological oxygen (2%–5%) on the ability to immortalize fetal versus adult human lung fibroblasts reveal that growth in low oxygen and antioxidants extends the lifespan of both fetal and adult strains. Since the ectopic expression of telomerase immortalizes adult lung fibroblasts cultured in normal atmospheric oxygen, the effects of oxygen must be largely limited to telomeres. In contrast, fetal lung fibroblasts (WI-38 and IMR-90) do not immortalize in ambient oxygen in spite of telomere elongation by telomerase, suggesting more widespread oxidative damage. The long-term culture requirements for the immortalization of WI-38 fetal lung fibroblasts included supplementation with N-(*tert*)butyl hydroxylamine, dexamethasone, zinc, and B12 in addition to growth in physiologic oxygen. These results suggest that both end-replication and oxidative damage events contribute to lung fibroblast telomere shortening *in vitro*. These observations emphasize the need for better analytic techniques to distinguish whether the correlation of short telomeres with disease and mortality in humans reflects the consequences of increased proliferation, telomere shortening due to oxidative damage, or some combination of these processes. These results show that WI-38 cells grown under conventional cell culture conditions do not reach telomere-based replicative senescence but prematurely growth arrest with increasing levels of p16ink4a due to the chronic stress of exposure to an inadequate culture environment (stress or aberrant signaling senescent-like arrest; "stasis"[1]), primarily due to oxygen toxicity.

Knowledge of the DNA damage signaling pathways in cell culture has recently permitted the immortalization of human bronchial epithelial cells using viral oncoproteins,[45] hTERT and Ras (Figure 12.4). By over-expressing the cell cycle gene, cdk4, to overcome the p16 stress response of growing human epithelial cells in plastic dishes, in combination with hTERT, we have recently determined that human bronchial epithelial cells can immortalize with a high frequency (unpublished results). This is particularly useful since it avoids the use of SV40 early genes that cause many unknown effects including genomic instability. The cells expressing CDK4 and hTERT retain a normal diploid karyotype and undergo normal differentiation into goblet and ciliated cells when place in organotypic culture conditions (unpublished results). This provides, for the first time, a method for examining in living cells early events in that may occur as part of lung cancer progression. In addition, these cells should have utility in developing *in vitro* models for lung carcinogenesis, to provide markers of smoking and carcinogen exposure (risk assessment), as well as for monitoring smoking cessation and chemoprevention efficacy. These cells may also provide makers for diagnostic classification and as well as a prognostic indicator and signature for directing therapy selection.

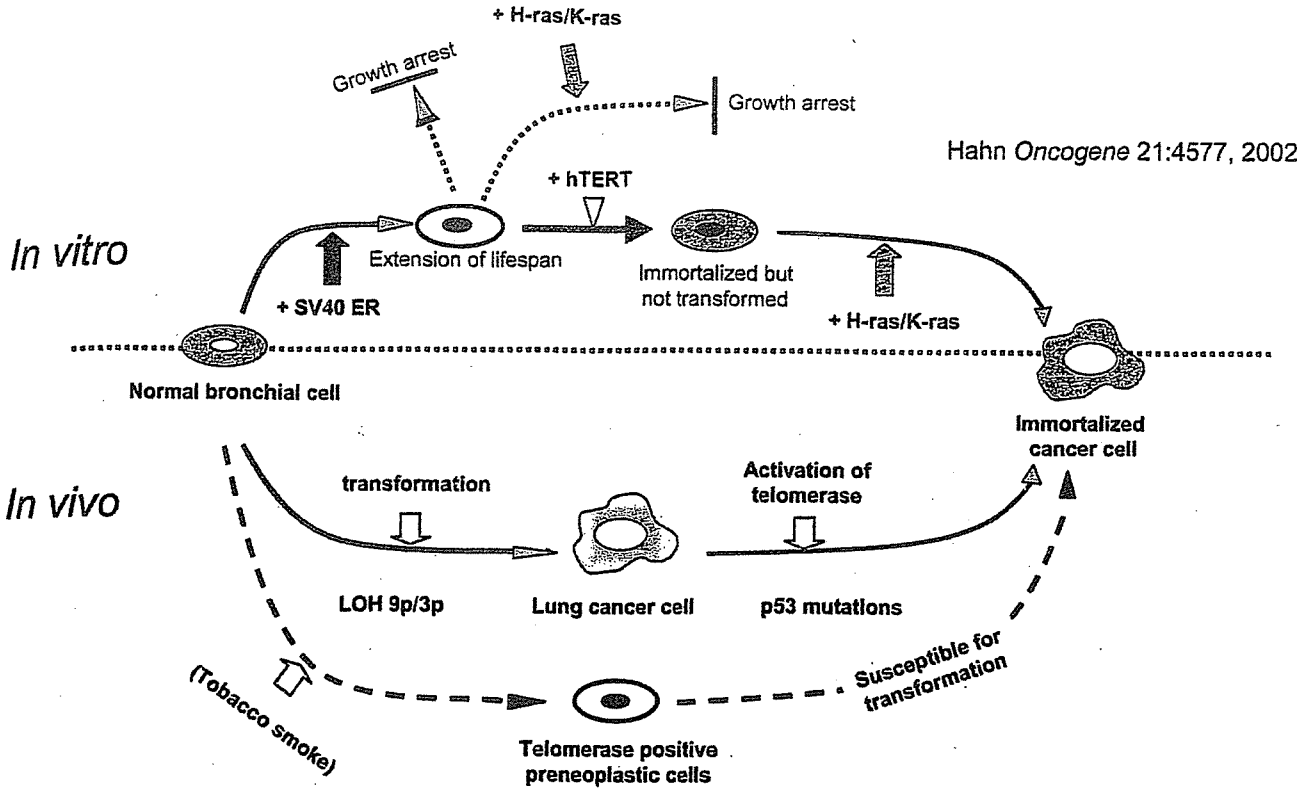

FIGURE 12.4. Cancer progression models of bronchial epithelial cells *in vitro* and *in vivo* **Upper half:** illustrates the critical steps in lung carcinogenesis *in vitro* using the early region (ER) of the SV40 genome, the hTERT gene, and an oncogenic allele of H-ras/K-ras gene.[45] Expression of SV40 ER alone extended the lifespan of the bronchial cell by eight additional population doublings and induction of H-ras/K-ras (without hTERT) did not affect replicative lifespan. In contrast, both expression of SV40 ER and hTERT lead to the immortalization of cells but the morphological findings were unchanged. Additional expression of H-ras/K-ras morphologically changed the cells to squamous cell carcinomas. **Lower half:** models of lung carcinogenesis *in vivo*. Many lung cancer cells have overcome the first stage of senescence but are still mortal and then and gradually acquire immortality concomitant with activation of telomerase during multi-step progression. In some instances, immortalization (i.e., activation of telomerase) may precede morphological changes in bronchial cells. The activation of telomerase in a field without morphological changes, which we propose to term "field immortalization", is considered to be susceptible to subsequent morphological changes. This condition is likely to occur in heavy smokers and thus telomerase expression in bronchial epithelia may become a useful marker for high-risk patients of developing lung cancer.

ROLE OF TELOMERASE IN CANCER

Diagnostic Potential

Lung cancer remains the leading cause of cancer related death in men and women. Even with modern diagnostic and therapeutic advances, the 5-year survival rate following surgery and combinatorial adjuvant therapy has only been improved in patients with early stages of disease. Significant efforts have focused on early detection and intervention at an earlier stage to help decrease the high mortality of lung cancer. The development, evaluation, and validation of biomarkers for earlier cancer detection and risk assessment are needed for both current and former smokers. Biomarkers such as telomerase[46–63] are alterations that permit one to recognize or monitor a biologic process. Biomarkers are measurable in biological media, such as in tissues, cells, or fluids. With improved methods of bronchoscopy, and the use of bronchial brushings and bronchial lavage, specimens from current and former smokers can be obtained for either histological or cytological analysis. To elucidate the role of telomere and telomerase in human lung cancer biogenesis,[64–111] various molecular changes including telomere length and telomerase activity in lung cancer tissues have been analyzed and the relationship between them and patient prognosis conducted (see Tables 12.1–12.3). In general, most small cell lung cancer cells uniformly express telomerase activity and have shortened telomeres. In these cases detection of telomerase activity is more useful for cancer diagnosis rather than as a marker for prognosis. In contrast, telomerase activity levels in non-small cell lung cancer varies from none to high and correlates with various biological or clinical features, such as pathological stage, lymph

TABLE 12.1. CHARACTERISTICS OF SMALL CELL (SCLC) AND NON-SMALL CELL LUNG CANCER (NSCLC)

	SCLC	NSCLC
Telomere length[106,110]	Most cases shortened	70% no alteration from noncancerous tissue 25% shortened, < 5% elongated
Primary lesion		
Metastatic lesion	Most cases shortened	Alteration correlates with *TP53/RB1* LOH
	A few cases elongated	Alteration correlates with poor prognosis
		Increase of alteration
Telomerase activity*		
Activity level	Most cases high[70,71]	30% high,[70] SqCC > adenocarcinoma[72, 84]
	LD < ED[71]	Correlates with TNM stage, LN metastasis, Ki-67 staining, grade of differentiation, mutant p53, bcl2 expression; inversely with age[72,74,75,77,79,81,84,89,90]
	SCLC > Large cell[72]	No/low activity correlates with good prognosis in stage I adenocarcinoma, stage I-III NSCLC, stage I NSCLC[72,77,82,83,86,89,95]
Prognosis		Correlates with recurrence[76]
		Increase of positive cases[70]
Metastatic lesion		Metaplasia < Dysplasia < SqCC[64,66]
Precancerous lesion	Most cases high[70]	Low-grade AAH < High-grade AAH < NMBAC[96]
	No precancerous lesion	

* Including reports on hTERT mRNA expression representing telomerase activity.
ED, extensive disease; LD, limited disease; SqCC, squamous cell carcinoma; LN, lymph node; AAH, atypical adenomatous hyperplasia; NMBAC, nonmucinous bronchioloalveolar carcinomas.

node metastasis, and grade of differentiation (Table 12.1), providing clinically useful information on malignant potentials. Among the other genetic aberrations or biological characteristics, loss of heterozygosity (LOH) of *RB1* and high telomerase activity are associated with poor prognosis in lung cancer patients.[106] Aberrant length of telomeres, aneuploidy, LOH at *RB1* or 1p34 locus, and a high percent S-phase fraction are associated with high telomerase activity. Accumulation of genetic aberrations, especially allelic loss of *RB1* and/or *TP53*, resulting in the activation of telomerase seems to be associated with poor prognosis in lung cancer patients. High telomerase activity and LOH of *RB1* and/or *TP53* may thus be clinically useful indicators for prognosis and biologically malignant potential in lung cancer.[106]

Telomerase activity or hTERT mRNA has been compared in noncancerous lung tissue, precancerous lesions and in both non small cell and small cell lung cancer specimens. In what appears to be histologically normal bronchial epithelial tissues, telomerase activity is detected into approximately 15% of specimens, suggesting upregulation or reactivation of telomerase might be a very early event. In some cases associated with smoke-related lung diseases, there may be false-positives with telomerase activity due to infiltrated lymphocytes in the inflammatory lesions. This suggests that hTERT protein distribution by immunohistochemistry may have some advantages (see next section). Interestingly, approximately 50% of preneoplastic lesions (dysplasia and carcinoma *in situ*) are telomerase positive (Table 12.2). In malignant disease telomerase is almost universally detected

TABLE 12.2. TELOMERASE ACTIVITY AND HTERT MRNA EXPRESSION IN LUNG TISSUES

	Telomerase Activity	hTERT mRNA
Noncancerous lung tissue	11/72 (15.3%)[64–68]	1/15 (6.7%)[69]
Precancerous lesion*	37/71 (52.1%)[64,66,68]	13/35 (37.1%)[64] [47/57 (82.5%)[69†]]
Lung cancer		
Small cell	48/52 (92.3%)[64,70–72]	3 3/ 4 (75.0%)[65]
Non-small cell	1,265/1,724 (73.4%)[65–67,70–92]	632 / 880 (51.8%)[64,65,69,77,78,83,88,93–98‡]

* Including bronchial metaplasia and dysplasia tissues.[64,66,68]
† Bronchial biopsy tissues derived from heavy smokers.[69]
‡ Including 187 samples analyzed by hTERT mRNA *in situ* hybridization.[94,96]

TABLE 12.3. TELOMERASE ACTIVITY IN CELLS DERIVED FROM LUNG

	Telomerase Activity
Noncancerous lung disease	9/148 (6.1%)[99–101]
Lung cancer	114 / 164 (69.5%)[54,68,99–103]

in small cell carcinoma but is only detected in about 75% of non-small cell lung tumors. This suggests that a substantial portion of non-small cell lung cancers have either engaged an alterative telomere maintenance pathway or that a subset may tumors that have not immortalized. However, since no cases without detectable telomerase activity show elongated telomeres, the latter possibility seems more plausible. In general, telomerase activity is a more accurate marker of cancer cells than hTERT mRNA, which has several, splice variants that can lead to inaccurate diagnoses. Even though pleural fluid cells are almost always telomerase positive,[104] other cells obtained from lung (bronchial washings, sputum) are less frequently telomerase positive[101] (Table 12.3).

We have also examined hTERT protein distribution by immunohistochemistry in tissue sections (Figure 12.5). In most normal epithelial tissues, hTERT expression is not detected but in both squamous cell carcinoma and adenocarcinoma hTERT protein was prominent in the nucleus of almost all neoplastic cells and correlated with telomerase activity levels in tissue extracts. While there is still a shortage

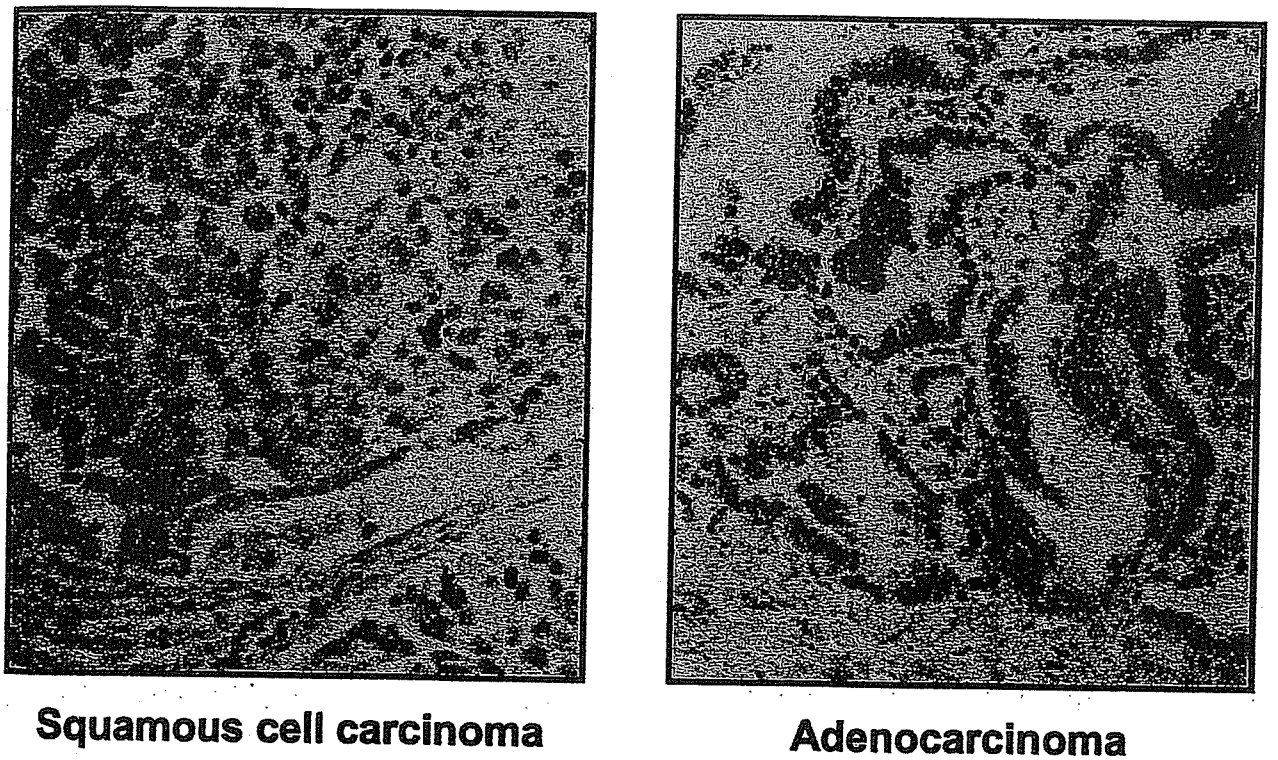

FIGURE 12.5. hTERT immunohistochemisty demonstrating nuclear localization of telomerase in squamous cell lung carcinoma and lung adenocarcinoma but not in adjacent non cancerous cells. *Antibodies.* An affinity-purified polyclonal rabbit antibody against hTERT (EST21A™) was raised against a 16 amino acids peptide sequence mapping in the middle of hTERT (Alpha Diagnostic International Inc., San Antonio, TX)™ and used at 5–10 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 min. in 0.01 M citric acid buffer, pH 6.0 in 2 atmosphere 120°C using an autoclave. After decreasing the pressure, sections were removed and permitted to cool for approximately 30 min. before being washed in 3 times for 5 min. in TBS. *Immunohistochemistry.* Endogenous peroxidase was quenched in 3% H2O2. After washing 3 times for 5 min. in TBS, nonspecific antibody binding was blocked by incubating the sections in Protein Blocking Solution (DAKO Corp., Carpinteria, CA)™ for 30 min. Sections were then transferred to a humidified chamber and incubated in antibody solution overnight. Following this and subsequent incubations, sections were thoroughly washed in 3 changes of TBS for 5 min each. For hTERT immunohistochemical staining, sections were incubated in the labeled streptavidin biotin polymer (Envision Plus, DAKO Corp.)™, followed by 0.05% 3,3'-diaminnobenzidine (DAB) in TBS with H2O2 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)™. (See color plate.)

of good hTERT antibodies for immunohistochemistry, and the techniques for detecting low abundance proteins such as hTERT are challenging, these initial results indicate that detection of telomerase at the cellular level is achievable and may have utility in cancer diagnostics.[105]

Most patients with centrally located early-stage lung cancer have been exposed to tobacco smoke carcinogens, and some of them develop subsequent primary and/or multicentric tumors. The use of autofluorescence bronchoscopy[112] has increased the detection rate of superficial early-stage lung cancers that have only subtle changes in mucosa, and provide an opportunity for endobronchial therapy such as photodynamic therapy (PDT). PDT is potentially a curative treatment in properly selected patients who have centrally located early-stage lung cancer.[112–114] Most patients treated with PDT demonstrate good prognosis, but some cases show recurrence shortly after successful PDT. Telomerase activation in lung cancer is generally thought to occur after many cell divisions through several rounds of clonal selections. However, in a recent study, three patients with early-stage lung cancer who had expression of telomerase protein in some areas of pathologically noncancerous bronchial epithelium, subsequently developed second primary squamous cell carcinomas shortly after the PDT, indicating the clinical utility of detecting hTERT expression as an indicator of high risk patients for lung cancer development (unpublished results).

Telomerase Therapeutic Opportunities

Telomerase is somewhat unusual among cancer targets because there has been an enormous amount of basic science in telomere and telomerase biology that has preceded development of effective lead compounds. This allows many potential problems to be anticipated before evidence of efficacy in clinical trials are conducted, exactly the opposite of the situation faced today during most drug development. Even though telomerase by itself does not cause cancer, and its role in cancer is most probably permissive, cancer therapy directed at telomerase has advanced in some instances to phase I clinical trials.[115–117] In this section, we will consider some of the most promising telomerase therapeutic areas; a gene therapy approach that uses the proximal hTERT promoter to make a general cancer-specific replication competent virus; a telomerase-specific immunotherapy; and the use of telomerase enzyme antagonists.

Before describing these most promising telomerase therapies, it is important to point out that there are many other approaches for telomerase inhibition being tested, such as down regulating the human telomerase (hTR) and hTERT genes at the promoter level, the use of a dominant negative hTERT gene delivery,[118,119] inhibition of telomerase assembly (e.g., interfering with p23 hsp90[120]), telomerase-specific phosphorylation inhibitors, blocking telomerase accessibility (G-quadraplex stabilizers[121–124]), hammerhead ribozymes directed against hTR,[125,126] mutant template RNA gene therapy,[24] and reverse transcriptase inhibitor approaches.[127,128] Due to space limitation these will not be covered and the reader is referred to the original reports or other recent reviews.[27,33,49,52,106,129]

As way of introduction into recent advances in telomerase therapeutics, it is important to point out that those agents that target only telomeres are likely to have more non-specific toxicities on normal cells while those that target telomeres though inhibition of telomerase are predicted to have fewer side effects. The concept of tumor cell senescence in cancer treatment was also reviewed recently[130] and there are many cancer approaches that target the induction of senescence-associated regulatory pathways including conventional chemotherapy, radiation, and hormone ablation. While there is some confusion about the differences between telomere-based replicative senescence and premature senescence, both may be thought of as evolutionarily conserved defense mechanisms through which cells *in vivo* are guarded against potentially oncogenic insults. Therefore, in some instances senescence may be due to a change in telomere state and not to progressive telomere erosion.

A working paradigm is that the telomere end structure, the T-loop may hide the chromosomal "ends" preventing them from resembling DNA double-strand breaks. Disruption of even a single T-loop via chemotherapy or telomerase inhibitors may potentially signal a cellular response that resembles a double-strand break leading to cell senescence, cell death or even cancer progression. It is generally thought that uncapped chromosome ends are at great risk for degradation, recombination, or fusion by cellular DNA repair systems leading to the loss of genetic information, rearrangement of chromosomes, and increased genomic instability. In normal cells without other alterations this most likely leads to replicative senescence that may have evolved as an anticancer protection mechanism acting as a failsafe mechanism to prevent the proliferation of cells at risk for neoplastic transformation but that has not yet accumulated all the necessary alterations.[14] In the presence of other cancer predisposing alterations (such as those caused by tobacco smoke damage), uncapped telomeres could lead to increased genomic instability and an increased probability of cancer formation including telomerase reactivation.

Thus, telomeres can be lost or rendered dysfunctional by DNA damage, repeated cell divisions in the absence of telomerase, or changes in telomere-associated proteins. In response to dysfunctional or damaged telomeres, cells can undergo apoptosis and die, continue to divide until a replicative senescence-induced growth arrest occurs, or develop genomic instability leading to a mutant phenotype (Fig. 12.1). The logic for developing inhibitors that are specific for telomerase is that this approach would have the potential to be more cancer-specific and perhaps with fewer cytotoxic side effects compared to currently used therapies. The hope

is that telomerase inhibitors might work as single agents and directly stop the growth and kill cancer cells, but may be even more effective in combination with conventional cancer treatments such as surgery, chemotherapy and radiation therapy to delay or prevent tumor regrowth.

Telomerase Specific Oncolytic Virus

Since almost all advanced human cancer cells express telomerase and most normal cells do not, the hTERT proximal promoter (from −1 to about −200–400) has been used to produce a more universal gene therapy approach. The logic is that only tumor cells expressing telomerase would activate the promoter. The approaches using "suicide gene strategies" described so far appear promising and include gene transfer via direct intratumor injections of plasmids or adenoviral vectors containing the human telomerase promoter upstream from pro-apoptotic genes such as the *FADD* gene,[131] *Caspase 6* and *8* genes,[132,133] and the *Bax* gene.[134,135] There is progress using the telomerase hTERT promoter (hTERTp) to drive a *T*umor-specific *R*eplication competent *AD*enoviral (hTERTp-TRAD) gene therapy approach. In this approach an introduced adenoviral vector could infect both normal and tumor cells, but the virus would only replicate in those cells that have robust telomerase activity. Thus, the virus would replicate and eventually kill the telomerase-expressing tumor cells and then spread to adjacent cells over the few weeks that adenovirus is active. There are limited normal stem-like cells that express telomerase in the brain[132] and thus hTERTp-TRAD therapy for gliomas may have few serious side effects when targeted appropriately. Immune cells that express telomerase are not easily infected by adenovirus so hematopoietic cells less likely to be affected. Systemic hTERTp-TRAD might be expected to have some immediate side effects on transient amplifying stem cells such as proliferating spermatocytes in the testes, cells in the crypts of the intestine, and a subset of cells in the basal and suprabasal layer of the epidermis.[4,63,136] However, it is not expected that this would be any more detrimental than conventional cytotoxic drugs that affect all proliferating cells.

In studies comparing a cytomegalovirus (CMV)-lacZ to hTERT-lacZ adenoviral vector by direct injections into the liver and spleen of mice (tissues which are telomerase-positive in mice), there was essentially no reporter activity (as measured by beta-galactosidase activity) with the hTERT vector but high levels with the constitutive CMV promoter.[134,135] Thus, it may be that normal cells in most organs do not express telomerase at sufficiently high levels to produce functional levels of downstream effector genes.

hTERT Immunotherapy

The catalytic protein component of telomerase (hTERT) may be an attractive candidate as a tumor associated antigen. hTERT protein is naturally processed and hTERT peptides are presented as epitopes, eliciting cytotoxic T lymphocyte (CTL) responses and protective immunity against tumors.[115,116,137,138] The *in vitro* studies on the immunogenicity of hTERT peptides suggest and provide a good rationale for using this approach for telomerase inhibition.[115,116,137,138] One advantage is that there would be no lag period required for telomere shortening prior to observation of cell growth arrest and death. A major disadvantage would be that normal cells expressing high levels of telomerase might also be affected. However, while investigators were able to elicit a specific CTL killing of tumor cells of prostate, lung, breast, colon and melanoma, they did not observe a CTL effect on telomerase positive CD34+ hematopoetic cells, suggesting that hTERT is a poor autoantigen in stem cells.[116] This may be due to the relatively low level of telomerase expression in these cells as compared with tumor cells, and that these types of cells do not continuously express telomerase. In ongoing phase I clinical trials to establish safety of the approach, measurable immune reactivity without any high grade toxic side effects was observed.[117] While vaccinations in patients with high-grade tumors are unlikely to be clinically effective, preventative immunotherapy could be an option (if there are minimal toxicities) in patients with minimal residual disease or in patients with a high risk for cancer development or recurrence.

Targeting the RNA Component of Telomerase (Telomerase Template Antagonists)

Oligonucleotides complementary to the template region of the RNA component of telomerase (hTR) offer certain advantages as well as disadvantages as a cancer therapeutic.[139–144] The major disadvantage is that, compared to telomerase promoter oncolytic viruses or the hTERT immunotherapy, inhibiting telomerase by targeting progressive telomere shortening would be predicted to take a period of treatment before telomere shortening affected tumor cell survival. However, telomerase is an ideal target since the template region of hTR is likely to be exposed in order for new telomeric repeats to be added onto the chromosomes. This makes the template region of hTR an accessible target for oligonucleotides. Rather than acting by "antisense" mechanisms to degrade mRNA or inhibit translation, oligonucleotide targeting the hTR template region functions as classical enzymatic inhibitors of telomerase activity.

Experiments have been reported demonstrating that telomerase template antagonists administered to intact cancer cells reduce telomerase activity, lead to progressive shortening of telomeres, and cause cell proliferation to decrease and apoptosis to increase in a time period proportional to initial telomere length.[145–148] Importantly, chemically related molecules that did not inhibit telomerase did not cause de-

creased cell proliferation or telomere shortening. When the telomerase template antagonist was removed from the cells in culture, the surviving cells regained baseline telomerase activity and their telomeres grew back to their original lengths, supporting the assumption that the mechanism of action was through a competitive inhibition of the telomerase enzyme,[145] and that the agents will most likely have to be administered to cancer patients for an extended period. During this study,[145] there was no evidence of any emergence of an alternative pathway for telomere elongation.[149]

FUTURE DIRECTIONS AND FINAL COMMENTS

It is becoming more persuasive that targeting telomere maintenance mechanisms will be important in our repertoire of future cancer strategies. Preclinical experimental evidence and in some cases phase I clinical trial results are providing hope that telomerase inhibitors may lead to effective interventions for the treatment of patients with cancer. In addition, recent advances in molecular diagnostics indicate that telomerase may also be a useful biomarker for cancer detection and as an early indicator of cancer relapse. In summary, both diagnostic and therapeutic approaches are currently being translated into hopefully well-designed clinical trials and this will ultimately determine the utility of this novel approach.

ACKNOWLEDGEMENT

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REFERENCES

1. Blackburn EH. Telomere states and cell fates. *Nature* 2000; 408:53–56.
2. Collins K. Mammalian telomeres and telomerase. *Curr Opin Cell Biol* 2000;12:378–383.
3. Fajkus J, Simickova M, Malaska J. Tiptoeing to chromosome tips: facts, promises and perils of today's human telomere biology. *Philos Trans R Soc Lond B Biol Sci* 2002;357:545–562.
4. Forsyth NR, Wright WE, Shay JW. Telomerase and differentiation in multicellular organisms: Turn it off, turn it on, and turn it off again. *Differentiation* 2002;69:188–197.
5. Lingner J, Cech TR. Telomerase and chromosome end maintenance. *Curr Opin Genet Dev* 1998;8:226–232.
6. de Lange T. Protection of mammalian telomeres. *Oncogene* 2002;21:532–540.
7. Aisner DL, Wright WE, Shay JW. Telomerase regulation: not just flipping the switch. *Curr Opin Genet Dev* 2002;12:80–85.
8. Harley CB. Telomere loss: mitotic clock or genetic time bomb? *Mutat Res* 1991;256:271–282.
9. Kim SH, Kaminker P, Campisi J. Telomeres, aging and cancer: in search of a happy ending. *Oncogene* 2002;21:503–511.
10. McEachern MJ, Krauskopf A, Blackburn EH. Telomeres and their control. *Annu Rev Genet* 2000;34:331–358.
11. Shay JW, Pereira-Smith OM, Wright WE. A role for both Rb and p53 in the regulation of human cellular senescence. *Exp Cell Res* 1991a;196:33–39.
12. Shay JW, Wright WE, Werbin H. Defining the molecular mechanisms of human cell immortalization. *Biochim Biophys Acta* 1991b;107:1–7.
13. Wright WE, Pereira-Smith OM, Shay JW. Reversible cellular senescence: A two-stage model for the immortalization of normal human diploid fibroblasts. *Mol Cell Biol* 1989;9: 3088–3092.
14. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Cur Opin Gen Dev* 2001;11:98–103.
15. Wright WE, Shay JW. Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. *Nat Med* 2000;6:849–851.
16. Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. *Cell* 199;97:503–514.
17. Smogorzewska A, van Steensel B, Bianchi A, et al. Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* 2000;20:1659–1668.
18. Van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. *Cell* 1998;92: 401–413.
19. Goytisolo FA, Blasco MA. Many ways to telomere dysfunction: *in vivo* studies using mouse models. *Oncogene* 2002;21: 584–591.
20. Hanahan D. Benefits of bad telomeres. *Nature* 2002;406: 573–574.
21. Greenberg RA, Chin L, Femino A, et al. Short dysfunctional telomeres impair tumorigenesis in the INK4a cancer-prone mouse. *Cell* 1999;97:515–525.
22. Harrington L, Robinson MO. Telomere dysfunction: multiple paths to the same end. *Oncogene* 2002;21:592–597.
23. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. *Science* 2002;295: 2446–2449.
24. Kim MM, Rivera MA, Botchkina IL, et al. A low threshold level of expression of mutant-template telomerase RNA inhibits human tumor cell proliferation. *Proc Natl Acad Sci U S A* 2001; 98:7982–7987.
25. Smogorzewska A, de Lange T. Different telomere damage signaling pathways in human and mouse cells. *EMBO J* 2002;21: 4338–4348.
26. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002;27:339–344.
27. Neidle S, Parkinson G. Telomere maintenance as a target for anticancer drug discovery. *Nat Rev Drug Discov* 2002;1: 383–393.
28. Kraemer K, Fussel S, Schimdt U, et al. Antisense-mediated hTERT inhibition specifically reduces the growth of human bladder cancer cells. *Clin Can Res* 2003;9:3794–3800.
29. Zhang X, Multani AS, Zhou JH, et al. Ad-RB94 produces rapid telomere erosion, chromosomal crisis and caspase dependent apoptosis in bladder cancer and immortalized human urothelial cells, but not in normal urothelial cells. *Cancer Res* 2003;63: 760–765.
30. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Curr Biol* 2003;13:1549–1556.
31. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated cellular senescence. *Nature* 2003;426:194–198. Epub 2003 Nov 05.
32. Bodnar AG, Ouellette M, Frolkis M, et al. Extension of life-

span by introduction of telomerase into normal human cells. *Science* 1998;279:349–352.
33. Granger MP, Wright WE, Shay JW. Telomerase in cancer and aging. *Crit Rev Oncol Hematol* 2002;4:29–40.
34. Harley CB. Telomerase is not an oncogene. *Oncogene* 2002;21: 494–502.
35. Sharma GG, Gupta A, Wang H, et al. hTERT associates with human telomeres and enhances genomic stability and DNA repair. *Oncogene* 2003;22:131–146.
36. Shay JW, Zou Y, Hiyama E, et al. Telomerase and cancer. *Hum Mol Genet* 2001;10:677–685.
37. Counter CM, Avilion AA, LeFeuvre CE, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 1992; 11:1921–1919.
38. de Lange T, Jacks T. For better or worse? Telomerase inhibition and cancer. *Cell* 1999;98:273–275.
39. Hackett JA, Greider CW. Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. *Oncogene* 2002;21:619–626.
40. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100:57–70.
41. Holt SE, Shay JW. Role of telomerase in cellular proliferation and cancer. *J Cell Physiol* 1999;180:10–18.
42. Wong K-K, DePinho RA. Walking the telomere plank into cancer. *J Natl Cancer Inst* 2003;95:1184–1186.
43. Wu X, Amos CI, Zhug Y, et al. Telomere dysfunction: a potential cancer predisposition factor *J Natl Cancer Inst* 2003;95: 1211–1218.
44. Forsyth NR, Evan AP, Shay JW, et al. Developmental differences in the immortalization of lung fibroblasts by telomerase. *Aging Cell* 2003;10:1–7.
45. Lundberg AS, Randell SH, Stewart SA, et al. Immortalization and transformation of primary airway epithelial cells by gene transfer. *Oncogene* 2002;21:4577–4586.
46. Collins K, Mitchell JR. Telomerase in the human organism. *Oncogene* 2002;21:564–579.
47. Feng J, Funk WD, Wang SS, et al. The RNA component of human telomerase. *Science* 1995;269:1236–1241.
48. Shay JW. Telomerase therapeutics: telomeres recognized as a DNA damage signal. *Clin Cancer Res* 2003;9:2321–2325.
49. Helder MN, Wisman GB, van der Zee GJ. Telomerase and telomeres: from basic biology to cancer treatment. *Cancer Invest* 2002;20:82–101.
50. Hiyama E, Hiyama K, Yokoyama T, et al. Correlating telomerase activity levels with human neuroblastoma outcomes. *Nat Med* 1995;1:249–257.
51. Hiyama K, Hirai Y, Kyoizumi S, et al. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol* 1995;155:3711–3715.
52. Kelland LR. Telomerase: biology and phase I trials. *Lancet Oncol* 2001;2:95–102.
53. Keith WK, Evans TRJ, Glasspool RM. Telomerase and cancer: time to move from a promising target to a clinical reality. *J Pathol* 2001;195:404–414.
54. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011–2015.
55. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* 1985;43:405–413.
56. Morin GB. The human telomerase terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989;59:521–529.
57. Nakamura TM, Morin GB, Chapman KB, et al. Telomerase catalytic subunit homologs from fission yeast and humans. *Science* 1997;277:955–959.
58. Nugent CI, Lundblad V. The telomerase reverse transcriptase: components and regulation. *Genes Dev* 1998;12:1073–1085.
59. Piatyszek MA, Kim NW, Weinrich SL, et al. Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). *Methods Cell Sci* 1995; 17:1–15.
60. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997:5;787–791.
61. Shay JW, Wright WE. Telomerase: a target for cancer therapy. *Cancer Cell* 2002;2:257–265.
62. Shay JW. Telomerase in cancer: Diagnostic, prognostic and therapeutic implications. *Cancer J Sci Am* 1998;4:26–33.
63. Wright WE, Piatyszek MA, Rainey WE, et al. Telomerase activity in human germline and embryonic tissues. *Dev Genet* 1996; 18:173–179.
64. Shibuya K, Fujisawa T, Hoshino H, et al. Increased telomerase activity and elevated hTERT mRNA expression during multistage carcinogenesis of squamous cell carcinoma of the lung. *Cancer* 2001;92:849–855.
65. Arinaga M, Shimizu S, Gotoh K, et al. Expression of human telomerase subunit genes in primary lung cancer and its clinical significance. *Ann Thorac Surg* 2000;70:401–405; discussion 2000;70:405–406.
66. Yashima K, Litzky LA, Kaiser L, et al. Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. *Cancer Res* 1997;57:2373–2377.
67. Gauthier LR, Granotier C, Soria JC, et al. Detection of circulating carcinoma cells by telomerase activity. *Br J Cancer* 2001; 84:631–635.
68. Kakihana M, Yahata N, Hirano T, et al. Telomerase activity during carcinogenesis in the bronchus. *Oncol Rep* 2002;9: 43–49.
69. Soria JC, Xu X, Liu DD, et al. Retinoic acid receptor beta and telomerase catalytic subunit expression in bronchial epithelium of heavy smokers. *J Natl Cancer Inst* 2003;5:165–168.
70. Hiyama K, Hiyama E, Ishioka S, et al. Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995;87:895–902.
71. Hirashima T, Yoshitaka O, Nitta T, et al. Telomerase activity in endoscopically visible lung cancer. *Anticancer Res* 2001;21: 3685–3689.
72. Kumaki F, Kawai T, Hiroi S, et al. Telomerase activity and expression of human telomerase RNA component and human telomerase reverse transcriptase in lung carcinomas. *Hum Pathol* 2001;32:188–195.
73. Ahrendt SA, Yang SC, Wu L, et al. Comparison of oncogene mutation detection and telomerase activity for the molecular staging of non-small cell lung cancer. *Clin Cancer Res* 1997;3: 1207–1214.
74. Albanell J, Lonardo F, Rusch V, et al. High telomerase activity in primary lung cancers: association with increased cell proliferation rates and advanced pathologic stage. *J Natl Cancer Inst* 1997;89:1609–1615.
75. Fujiwara M, Okayasu I, Takemura T, et al. Telomerase activity significantly correlates with chromosome alterations, cell differentiation, and proliferation in lung adenocarcinomas. *Mod Pathol* 2000;13:723–729.
76. Gonzalez-Quevedo R, Iniesta P, Moran A, et al. Cooperative role of telomerase activity and p16 expression in the prognosis of non-small-cell lung cancer. *J Clin Oncol* 2002;20:254–262.
77. Hara H, Yamashita K, Shinada J, et al. Clinicopathologic significance of telomerase activity and hTERT mRNA expression in non-small cell lung cancer. *Lung Cancer* 2001;34:219–226.
78. Hsu CP, Miaw J, Hsia JY, et al. Concordant expression of the

telomerase-associated genes in non-small cell lung cancer. *Eur J Surg Oncol* 2003;29:594–599.
Kiyooka K, Maniwa Y, Okada M. Analysis of mutant p53 and telomerase activity in non-small cell lung cancer. *Ann Thorac Cardiovasc Surg* 1999;5:293–299.
Lee JC, Jong HS, Yoo CG, et al. Telomerase activity in lung cancer cell lines and tissues. *Lung Cancer* 1998;21:99–103.
Maniwa Y, Yoshimura M, Obayashi C, et al. Association of p53 gene mutation and telomerase activity in resectable non-small cell lung cancer. *Chest* 2001;120:589–5941.
Marchetti A, Bertacca G, Buttitta F, et al. Telomerase activity as a prognostic indicator in stage I non-small cell lung cancer [In Process Citation]. *Clin Cancer Res* 1999:5:2077–2081.
Marchetti A, Pellegrini C, Buttitta F, et al. Prediction of survival in stage I lung carcinoma patients by telomerase function evaluation. *Lab Invest* 2002;82:729–736.
Ohmura Y, Aoe M, Andou A, et al. Telomerase activity and Bcl-2 expression in non-small cell lung cancer. *Clin Cancer Res* 2000;6:2980–298.
Osaki T, Oyama T, Inoue M, et al. Molecular biological markers and micrometastasis in resected non-small-cell lung cancer. Prognostic implications. *Jpn J Thorac Cardiovasc Surg* 2001;49: 545–551.
Taga S, Osaki T, Ohgami A, et al. Prognostic impact of telomerase activity in non-small cell lung cancers. *Ann Surg* 1999; 230:715–720.
Volm M, Mattern J, Koomagi R. Association of telomerase expression with successful heterotransplantation of lung cancer. *Int J Oncol* 2000;16:31–35.
Wang J, Liu X, Jiang W, et al. Telomerase activity and expression of the telomerase catalytic subunit gene in non-small cell lung cancer: correlation with decreased apoptosis and clinical prognosis. *Chin Med J (Engl)* 2000;113:985–990.
Wang L, Soria JC, Kemp BL, et al. hTERT expression is a prognostic factor of survival in patients with stage I non-small cell lung cancer. *Clin Cancer Res* 2002;8:2883–2889.
Wu X, Kemp B, Amos CI, et al. Associations among telomerase activity, p53 protein overexpression, and genetic instability in lung cancer. *Br J Cancer* 1999;80:453–457.
Xinarianos G, Scott FM, Liloglou T, et al. Telomerase activity in non-small cell lung carcinomas correlates with smoking status. *Int J Oncol* 1999;15:961–965.
Yahata N, Ohyashiki K, Ohyashiki JH, et al. Telomerase activity in lung cancer cells obtained from bronchial washings. *J Natl Cancer Inst* 1998;90:684–690.
Soria JC, Moon C, Wang L, et al. Effects of N-(4-hydroxyphenyl)retinamide on hTERT expression in the bronchial epithelium of cigarette smokers. *J Natl Cancer Inst* 2001;93:1257–1263.
Fujita Y, Fujikane T, Fujiuchi S, et al. The diagnostic and prognostic relevance of human telomerase reverse transcriptase mRNA expression detected in situ in patients with nonsmall cell lung carcinoma. *Cancer* 2003;98:1008–1013.
Komiya T, Kawase I, Nitta T, et al. Prognostic significance of hTERT expression in non-small cell lung cancer. *Int J Oncol* 2000;16:1173–1177.
Nakanishi K, Kawai T, Kumaki F, et al. Expression of human telomerase RNA component and telomerase reverse transcriptase mRNA in atypical adenomatous hyperplasia of the lung. *Hum Pathol* 2002;3:697–702.
Toomey D, Smyth G, Condron C, et al. Immune function, telomerase, and angiogenesis in patients with primary, operable nonsmall cell lung carcinoma: tumor size and lymph node status remain the most important prognostic features. *Cancer* 2001; 92:2648–2657.
Zhang A, Zheng C, Lindvall C, et al. Frequent amplification of the telomerase reverse transcriptase gene in human tumors. *Cancer Res* 2000;60:6230–6235.
Hiyama K, Ishioka S, Shay JW, et al. Telomerase activity as a novel marker of lung cancer and immune-associated lung diseases. *Int J Mol Med* 1998;1:545–549.
Xinarianos G, Scott FM, Liloglou T, et al. Evaluation of telomerase activity in bronchial lavage as a potential diagnostic marker for malignant lung disease. *Lung Cancer* 2000;28: 37–42.
Dikmen E, Kara M, Dikmen G, et al. Detection of telomerase activity in bronchial lavage as an adjunct to cytological diagnosis in lung cancer. *Eur J Cardiothorac Surg* 2003;23:194–199; discussion 2003;23:199–120.
Dejmek A, Yahata N, Ohyashiki K, et al. Correlation between morphology and telomerase activity in cells from exfoliative lung cytologic specimens. *Cancer* 2000;90:117–125.
Sen S, Reddy VG, Khanna N, et al. A comparative study of telomerase activity in sputum, bronchial washing and biopsy specimens of lung cancer. *Lung Cancer* 2001;33:41–49, 2001. Ahrendt SA, Yang SC, Wu L, et al. Comparison of oncogene mutation detection and telomerase activity for the molecular staging of non-small cell lung cancer. *Clin Cancer Res* 1997;3: 1207–1214.
Dikmen G, Dikemn E, Kara M, et al. Diagnostic implications of telomerase activity in pleural effusions. *Eur Respir J* 2003; 22:1–5.
Hiyama E, Hiyama K, Yokoyama T, et al. Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. *Neoplasia* 2001; 3:17–26.
Hiyama K, Ishioka S, Shirotani Y, et al. Alterations in telomeric repeat length in lung cancer are associated with loss of heterozygosity in p53 and Rb. *Oncogene* 1995;10:937–944.
Kolquist KA, Ellisen LW, Counter CM, et al. Expression of TERT in early premalignant lesions and a subset of cells in normal tissues. *Nat Genet* 1998;19:182–186.
Lonardo F, Albanell J. Telomerase in lung cancer. In: Pass HI, Mitchell JB, Johnson DN, et al., eds. *Lung Cancer: Principals and Practice*, 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2002:170–177.
McKenzie KE, Umbricht CB, Sukumar S. Applications of telomerase research in the fight against cancer. *Mol Med Today* 1999;5:114–122.
Shirotani Y, Hiyama K, Ishioka S, et al. Alteration in length of telomeric repeats in lung cancer. *Lung Cancer* 1994;11:29–41.
Soria JC, Moon C, Wang L, et al. Effects of N-(4-hydroxyphenyl)retinamide on hTERT expression in the bronchial epithelium of cigarette smokers. *J Natl Cancer Inst* 2001;93:1257–1263.
Lam S, Kennedy T, Unger M, et al. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. *Chest* 1998;113:696–702.
Miyazu Y, Miyazawa T, Kurimoto N, et al. Endobronchial ultrasonography in the assessment of centrally located early-stage lung cancer before photodynamic therapy. *Am J Respir Crit Care Med* 2002;165:832–837.
Saito Y, Nagamoto N, Ota S, et al. Results of surgical treatment for roentgenographically occult bronchogenic squamous cell carcinoma. *J Thorac Cardiovasc Surg* 1992;104:401–407.
Su Z, Dannull J, Heiser A, et al. Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res* 2003;63: 2127–2133.
Vonderheide RH, Schultze JL, Anderson KS, et al. Equivalent induction of telomerase-specific cytotoxic T lymphocytes from tumor-bearing patients and healthy individuals. *Cancer Res* 2001;61:8366–8370.

117. Vonderheide RH. Telomerase as a universal tumor-associated antigen for cancer immunotherapy. *Oncogene* 2002;21: 674–679.
118. Hahn WC, Stewart SA, Brooks MW, et al. Inhibition of telomerase limits the growth of human cancer cells. *Nat Med* 1999;5:1164–1170.
119. Zhang X, Mar V, Zhou W, et al. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. *Genes Dev* 1999;13:2388–2399.
120. Holt SE, Aisner DL, Baur J, et al. Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev* 1999;13: 817–826.
121. Riou JF, Guittat L, Mailliet P, et al. Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. *Proc Natl Acad Sci U S A* 2002;99: 2672–2677.
122. Gowan SM, Harrison JR, Patterson L, et al. A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting *in vitro* and *in vivo* antitumor activity. *Mol Pharmacol* 2002;61:1154–1162.
123. Sun D, Thompson B, Cathers BE, et al. Inhibition of human telomerase by a G-quadruplex-interactive compound. *J Med Chem* 1997;40:2113–2116.
124. Read MA, Wood AA, Harrison JR, et al. Molecular modeling studies on G-quadruplex complexes of telomerase inhibitors: structure-activity relationships. *J Med Chem* 1999;42: 4538–4546.
125. Yokoyama Y, Takahashi Y, Shinohara A, et al. Attenuation of telomerase activity by a hammerhead ribozyme targeting the template region of telomerase RNA in endometrial carcinoma cells. *Cancer Res* 1998;58:5406–5410.
126. Folini M, Colella G, Villa R, et al. Inhibition of telomerase activity by a hammerhead ribozyme targeting the RNA component of telomerase in human melanoma cells. *J Invest Dermatol* 2000;114:259–267.
127. Strahl C, Blackburn EH. Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortal human cell lines. *Mol Cell Biol* 1996;16: 53–56.
128. Gomez DE, Tejera AM, Olivera OA. Irreversible telomere shortening by 3′azido-2′, 3′dideoxythymidine (AZT) treatment. *Biochem Biophys Res Commun* 1998;246:107–110.
129. Hodes R. Molecular targeting of cancer: telomeres as targets. *Proc Natl Acad Sci U S A* 2001;98:7649–7651.
130. Ronison, IB. Tumor cell senescence in cancer treatment. *Cancer Res* 2002;63:2705–2715.
131. Koga S, Hirohata S, Kondo Y, et al. FADD gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter to restrict induction of apoptosis to tumors *in vitro* and *in vivo*. *Anticancer Res* 2001;21:1937–1943.
132. Komata T, Kondo Y, Kanzawa T, et al. Treatment of malignant glioma cells with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. *Cancer Res* 2001; 61:5796–5802.
133. Koga S, Hirohata S, Kondo Y, et al. A novel telomerase-specific gene therapy: gene transfer of caspase-8 utilizing the human telomerase catalytic subunit gene promoter. *Hum Gene Ther* 2001;11:1397–1406.
134. Gu J, Kagawa S, Takakura M, et al. Tumor-specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the Bax gene to cancers. *Cancer Res* 2001;60:5359–5364.
135. Gu J, Andreff M, Roth JA, et al. hTERT promoter induces tumor-specific Bax gene expression and cell killing in syngenic mouse tumor model and prevents systemic toxicity. *Gene Ther* 2002;9:30–37.
136. White L, Wright WE, Shay JW. Telomerase inhibitors. *Trends Biotech* 2000;3:146–149.
137. Nair SK, Heiser A, Boczkowski D, et al. Induction of cytotoxic T lymphocyte responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. *Nat Med* 2000;6:1011–1017.
138. Minev B, Hipp J, Firat H, et al. T cell immunity against telomerase reverse transcriptase in humans. *Proc Natl Acad Sci U S A* 2000;97:4796–4801.
139. Corey DR. Telomerase: an unusual target for cytotoxic agents. *Chem Res Toxicol* 2000;13:957–960.
140. Corey DR. Telomerase inhibition, oligonucleotides, and clinical trials. *Oncogene* 2002;21:31–637.
141. Elayadi AN, Corey DR. Applications of LNA and PNA oligomers to chemotherapy. *Curr Opin Invest New Drugs* 2001;2: 558–561.
142. Hamilton SE, Simmons CG, Kathriya I, et al. Cellular delivery of peptide nucleic acids and inhibition of human telomerase. *Chem Biol* 1999;6:343–351.
143. Norton JC, Piatyszek MA, Wright WE, et al. Inhibition of human telomerase activity by peptide nucleic acids. *Nat Biotech* 1996;14:615–619.
144. Pitts AE, Corey DR. Inhibition of human telomerase by 2′-O-Methyl RNA oligonucleotides. *Proc Natl Acad Sci U S A* 1998; 5:11549–11554.
145. Herbert BS, Pitts AE, Baker SI, et al. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc Nat Acad Sci U S A* 1999;96:14276–14281.
146. Herbert BS, Wright AC, Passons CM, et al. Inhibition of the spontaneous immortalization of breast epithelial cells from individuals predisposed to breast cancer: effects of chemopreventive and anti-telomerase agents. *J Nat Can Inst* 2001;93:39–45.
147. Herbert BS, Pongracz K, Shay JW, et al. Oligonucleotide N3′-P5′ phosphoramidates as efficient telomerase inhibitors. *Oncogene* 2002;21:638–642.
148. Gryaznov S, Pongracz K, Matray T, et al. Telomerase inhibitors—oligonucleotide phosphoramidates as potential therapeutic agents. *Nucleosides Nucleotides Nucleic Acids* 2001;20: 401–410.
149. Bryan TM, Engelzou A, Gupta J, et al. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J* 1995;14:4240–4248.

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診療を深めるための最新の知識とケア

編集

別所文雄　横森欣司

CANCER IN CHILDREN

永井書店

▶CHAPTER 5 神経芽腫

はじめに

神経芽腫は、胎生期の神経冠に由来する体幹の交感神経節、副腎髄質から発生する(図1)。このため腫瘍の発生部位は副腎、後腹膜部、後縦隔部が多い。神経芽腫は、次の特徴をもつ。①わが国の小児悪性腫瘍の中で、神経芽腫の発生頻度は白血病に次いで多く、また小児固形悪性腫瘍としては最も頻度が高い。②臨床経過中に腫瘍細胞の分化・成熟が起こり、また腫瘍の自然褪縮がみられることがある。③予後が発症年齢により異なる。1.5歳未満(乳児)の神経芽腫の予後はよい。しかし1.5歳以上(幼児)の神経芽腫の予後は悪い。④治療面では、最近の化学療法の進歩にかかわらず、なお1.5歳以上の神経芽腫の予後は不良である。⑤形態学的に一見同じにみえる神経芽腫は、実は多様な腫瘍群から成り立っていることが、最近の細胞・分子生物学的研究の進歩により、明らかとなってきた。

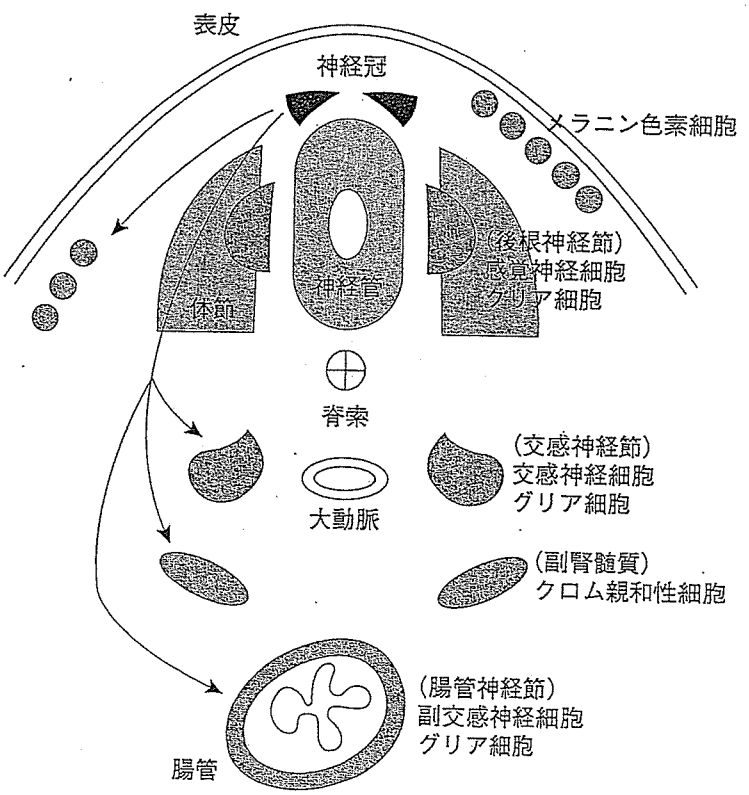


図1 神経冠の発生

神経管の背外側面にある神経冠細胞は、胎生初期に腹側に遊走し、頸部から骨盤内までの交感神経を形成する。一部の細胞は副腎に遊走し、先に形成されつつある髄質部に達し、髄質を形成する。また神経冠細胞は、末梢神経(知覚神経、Schwann細胞)、皮膚メラノサイト、平滑筋細胞などに分化する。神経芽腫は、発生学的に神経冠由来の腫瘍である。

1 発生頻度と年齢分布

わが国での新規の年間の神経芽腫の発生数は、小児慢性特定疾患研究事業の登録によると約320例で、その頻度は15歳未満の小児10万人あたり約1.6例と推定される[1]。発症の年齢分布は1歳未満に多く、1歳を過ぎると減少し、3歳で第二のピークを示す。以降、年齢とともに減少し、10歳以降の発症は稀となる(図2)。1.5歳未満の神経芽腫は低リスク腫瘍(1型)、1.5歳以上の神経芽腫は中間リスク腫瘍(2型)、1.5～5歳の神経芽腫は、高リスク腫瘍(3型)からなると考えられる。小児慢性特定疾患研究事業の登録によると、1歳未満の神経芽腫は51%、1～3歳が28%、4歳以上は21%を占める。

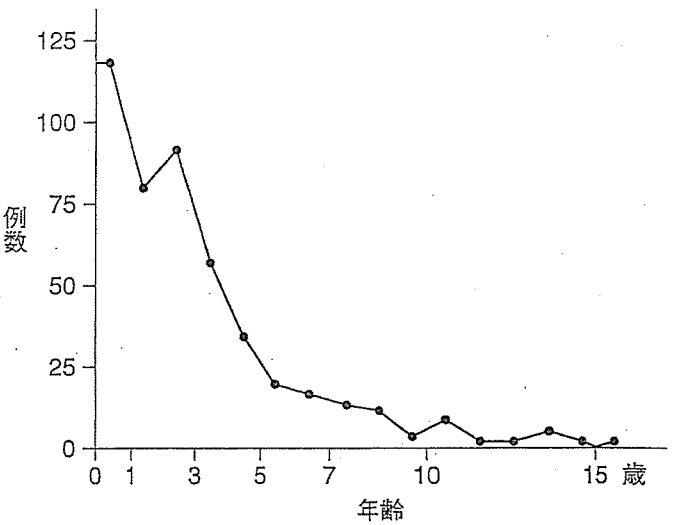


図2 日本での神経芽腫の発症年齢分布
発生数は1歳未満に多く、1歳を過ぎると減少し、3歳で2番目のピークを有し、以降年齢とともに減少する。1.5歳未満は低リスク腫瘍(1型)、1.5歳以上は中間リスク腫瘍(2型)、1.5～5歳は高リスク腫瘍(3型)からなると考えられる。

注意点 【神経芽腫の多様性】

神経芽腫は、一見、形態学的には同じ腫瘍にみえるが、予後の異なる多様な腫瘍群からなる。年齢、病期、*MYCN* がん遺伝子増幅などが、予後を左右する(図3)。

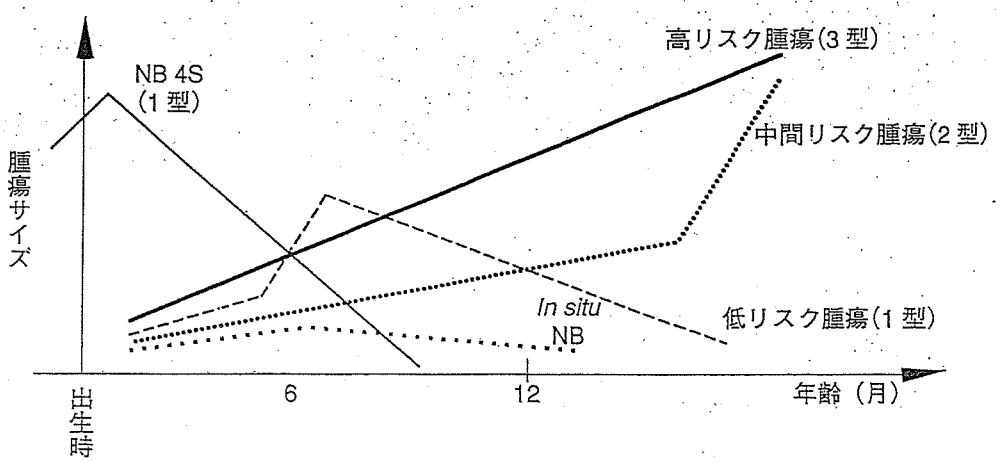


図3 神経芽腫の多様性
神経芽腫は、一見、形態学的には同じ腫瘍にみえるが、予後の異なる多様な腫瘍群からなる。低リスク腫瘍(1型)[4S、*in situ*(もともとの部位に限局)の神経芽腫などの褪縮する腫瘍を含む]、中間リスク腫瘍(2型)、高リスク腫瘍(3型)からなると考えられる。

2 病理組織分類

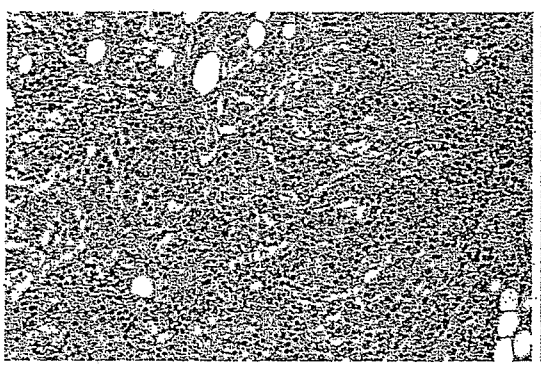
病理組織分類を正確に診断するためには、腫瘍摘出術や生検術による十分な腫瘍組織の採取が必要である。針穿刺による生検では、採取腫瘍組織が挫滅したり、組織検査と遺伝子検査に必要な腫瘍組織が採取できないことがある。

1 わが国での組織分類

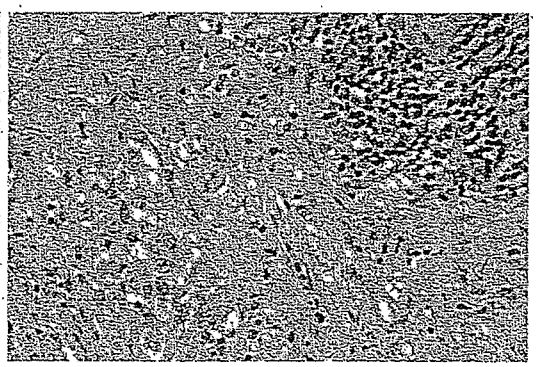
組織学的には腫瘍細胞の分化度により、①神経芽腫(neuroblastoma、図4-a)、②神経節芽腫(ganglioneuroblastoma、図4-b)、③神経節腫(ganglioneuroma、図4-c)、に分類される。さらに神経芽腫は、①花冠細線維型と②円形細胞型に、神経節芽腫は、①高分化型、②混成型と③低分化型、に亜分類される。神経節腫は良性腫瘍である。

2 国際神経芽腫病理分類

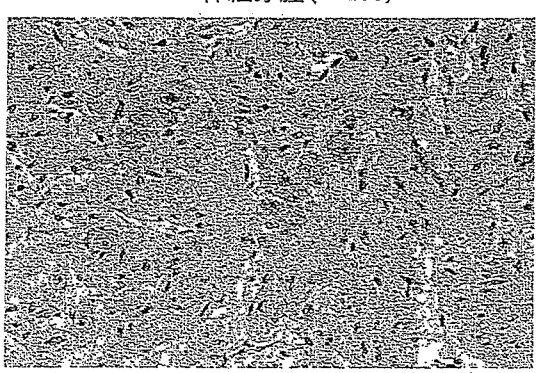
Shimadaらは、①腫瘍組織内の間質(Schwann細胞)の発達の程度、②神経芽腫細胞の成熟度、核分裂・核崩壊像、③年齢要因、を組み合わせた組織分類を提唱した。この分類は、予後良好群と不良群の分類に有効で、Shimada分類として世界中で用いられてきた。また1999年になって、従来のShimada分類を改訂した国際神経芽腫病理分類(International Neurob-



a：神経芽腫(×200)



b：神経節芽腫(×400)



c：神経節腫(×400)

図 4■神経芽腫の組織像
a：4歳の男児。右副腎原発の神経芽腫円形細胞型腫瘍。
b：生後8ヵ月の男児。マススクリーニング発見例の後腹膜腔原発腫瘍。腫瘍は神経芽腫の部分(右上)と神経節様の組織からなった。
c：5歳の女児。左骨盤交感神経節原発。組織は神経節とSchwann細胞組織からなった。

lastoma Pathology Classification；INPC)が発表され、現在用いられている[2]。

3 臨床症状

臨床症状は、発症年齢、原発腫瘍部位と病期で異なる。

①新生児期では病期4Sを示す。分娩時に巨大な胎盤、浮腫、貧血、黄疸、多発性肝転移による肝腫大により、著明な腹部膨満と呼吸困難を認める(図5)。

②乳児期では、新生児期と同様に病期4Sの症状、または限局腫瘍例が多い。限局腫瘍例は臨床的に無症状で、乳児健診または他の疾患で医療機関を受診したときに偶然に腫瘍が発見されることが多い。

③幼児期では大きな原発腫瘍と多彩な転移症状(発熱、貧血、骨・関節痛、歩行障害、眼球突出、リンパ節腫大、体重増加不良など)で発見されることが多い(図6)。

頸部～上部胸髄(C8～Th2)に発生する神経芽腫は、星状神経節を圧迫し、Horner症候群(眼瞼下垂、縮瞳、眼球陥凹、発汗低下)を起こす(図7)。胸部原発の神経芽腫は、下大静脈の機械的閉塞により下大静脈症候群を起こす。また交感神経節原発の神経芽腫は、椎間孔を遡上して脊柱内に腫瘍が浸潤し、脊髄圧迫または後根刺激症状を起こし、腰痛、下肢痛、下肢麻痺をきたすことがある。腫瘍は椎間孔の部分で細く(亜鈴型)なるので、dumb bell型の神経芽腫と

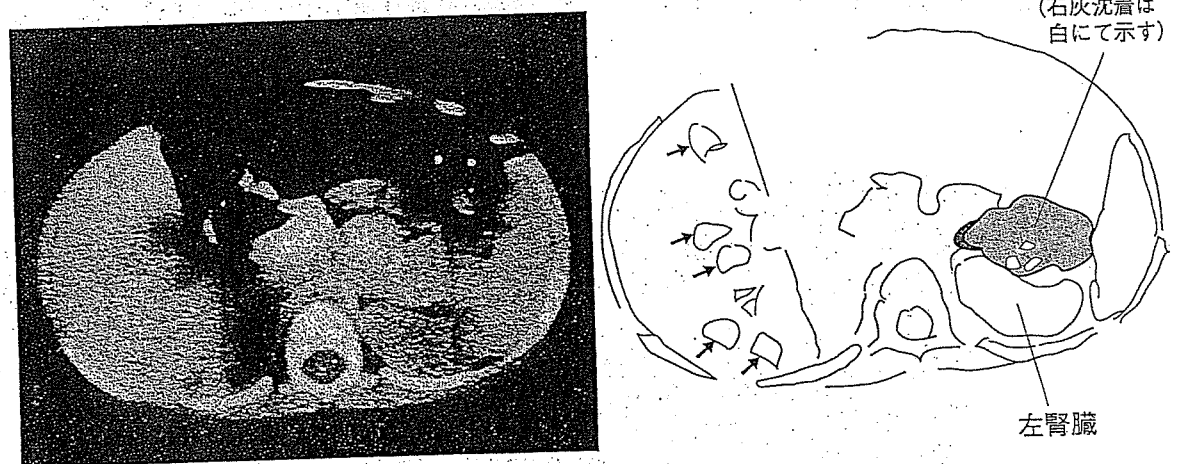


図5 神経芽腫4S
7ヵ月の女児。左副腎原発腫瘍と肝臓に多発性転移巣(CTにて低周域、右図の矢印)、また骨髄に少数の転移細胞を認めた。

MEMO 【神経芽腫4Sとは】

病期4(進行例で転移巣がみられる)であるが、特別に(special)治る。原発腫瘍は病期1または2の限局腫瘍で、肝・皮膚・骨髄などに転移がみられる。発症年齢は1歳未満で、自然退縮の可能性大である。予後はよい。*MYCN*増幅は通常みられない。

呼ばれる(図8)。稀ではあるが、血管作動性腸管由来ペプチド(vasoactive intestinal peptide；VIP)を産生する神経芽腫は、難治性下痢、低カリウム血症と脱水を起こす。また神経芽腫の臨床症状として、オプソミオクローヌス(opsomyoclonus、眼球運動異常と小脳失調)がみられる症例がある。

用語解説 【オプソミオクローヌス】

1962年Kinsbourneが初めて記載したため、Kinsbourne症候群ともいう。神経芽腫の4%以下にみられる。眼球の付随運動と全身性多発性ミオクローヌスを起こす。臨床経過が長引き再発増悪し、精神発達遅延、言語障害を残すことが多い。原因は不明であるが、自己免疫機序によると考えられる。

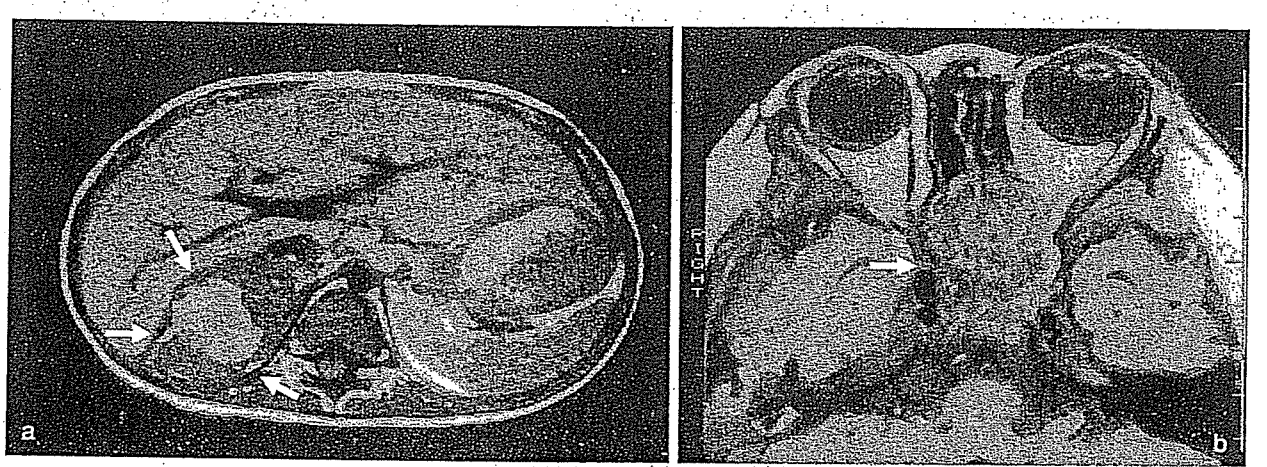


図 6 神経芽腫進行例の原発腫瘍と転移巣 MRI
2歳2ヵ月の男児。MRI水平断にて右副腎腫瘍(白矢印)(a)と左視神経・眼窩後部、篩骨洞、蝶形骨洞への骨転移(白矢印)を認めた(b)。

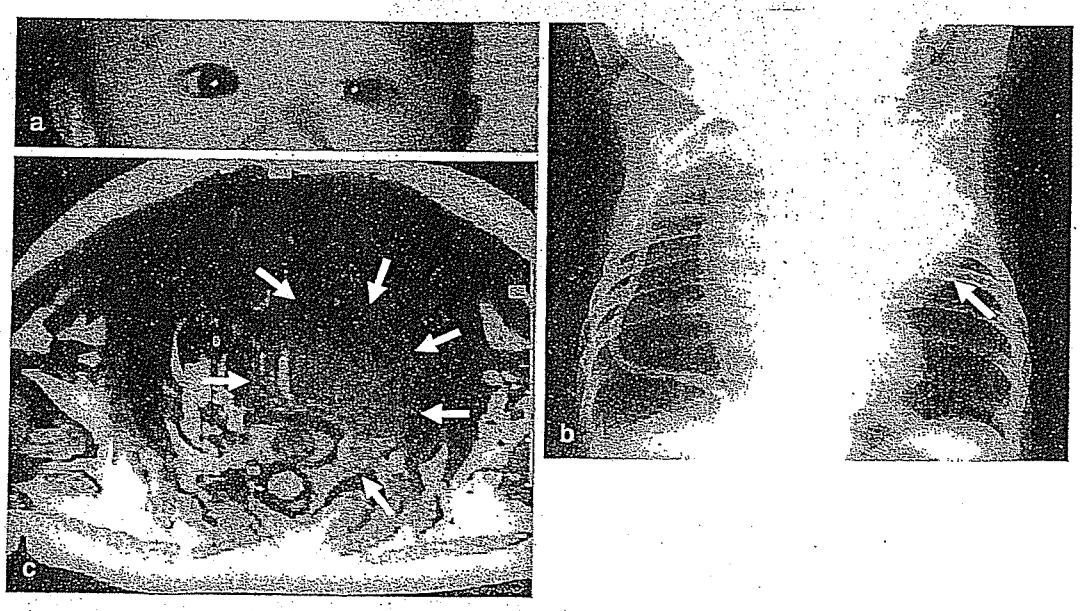


図 7 Horner症候群の神経芽腫
10ヵ月の男児。左眼瞼下垂、縮瞳、眼球陥凹を認める(a)。胸部単純X線にて左上縦隔腫瘍陰影(白矢印)(b)、胸部CTにて後縦隔に腫瘍(白矢印)(c)を認めた。

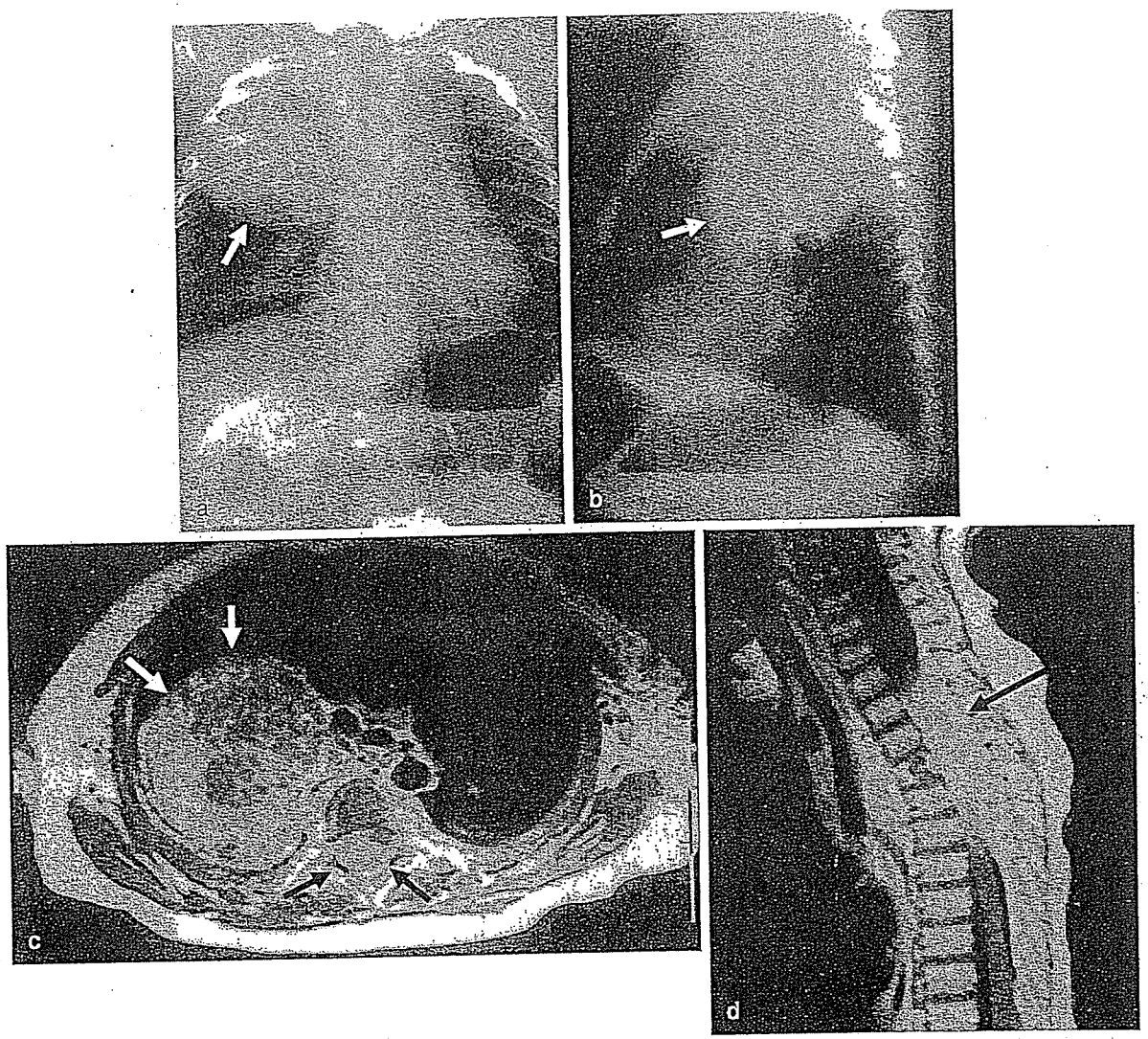


図 8 Dumb bell 型神経芽腫
5ヵ月の男児。胸部X線正面にて右縦隔に腫瘍陰影、第3肋間腔の拡大と腫瘍浸潤による背側肋骨の萎縮(a)、胸部X線側面にて縦隔中～後部に腫瘍陰影を認めた(b)。MRI水平断にて右上後縦隔腫瘍(白矢印)と脊柱内への腫瘍浸潤(黒矢印)(c)、矢状断にて脊髄への腫瘍浸潤(黒矢印)(d)を認めた。

4 診 断

1 腫瘍の原発と転移部位の診断

触診、腹部エコー、CT、MRI、meta-iodobenzylguanide(MIBG)シンチ、Gaシンチ、タリウムシンチにより腫瘍の原発と転移部位を診断する。特にMIBGシンチは、放射性カテコラミン前駆物質のMIBGの同位元素が、カテコラミン産生細胞に取り込まれるため、カテコラミンを産生する神経芽腫に特異的に集積し、原発腫瘍と転移腫瘍部位の診断に有用である(図9)。

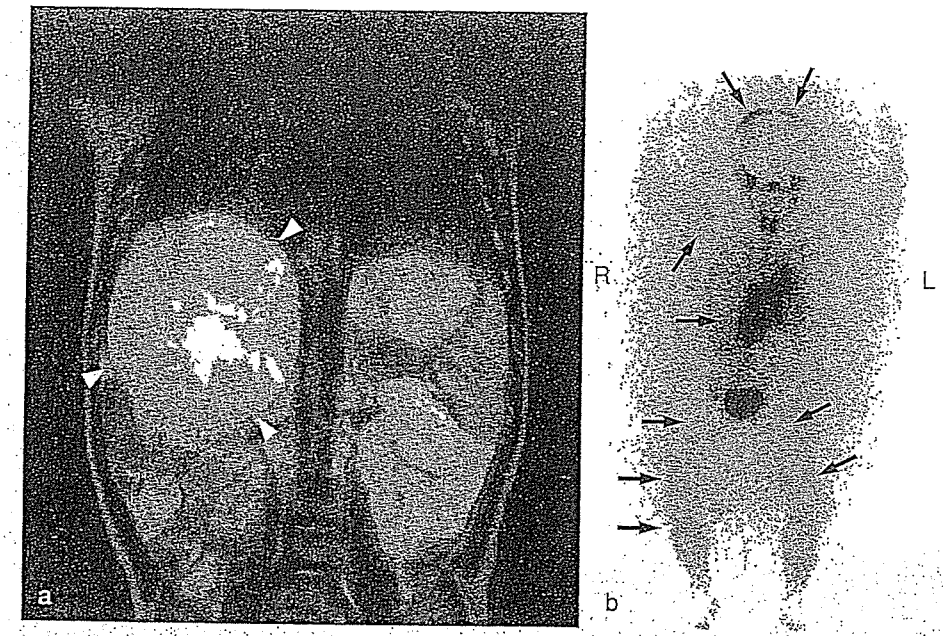


図 9 MIBG シンチ
2歳の女児。MRI 矢状断にて右上腹部に後腹膜原発腫瘍(白矢頭)(a)を認めた。MIBG シンチにて頭蓋、両側眼窩、両側上腕骨近位部、右上腹部の原発腫瘍、両側大腿近位部と遠位部、右脛骨近位部などに同位元素の集積を認めた(黒矢印)(b)。

5 尿と血液の生化学的診断

腫瘍細胞が産生するカテコラミンが体内で代謝され、尿中にバニリルマンデル酸(vanilly mandelic acid；VMA)、ホモバニリン酸(homovanillic acid；HVA)などが尿中に排泄される。神経芽腫例の 95%では尿中 VMA または HVA が高値を示し、診断と臨床経過の評価に役立つ。

血清中の神経特異性エノラーゼ(neuron specific enolase；NSE)、LDH、フェリチンが高値を示すこともある。

6 腫瘍の確定診断

神経芽腫の確定診断は、病理組織診断と電子顕微鏡学的診断(カテコラミン分泌顆粒または神経線維の検出)に基づき行われる。またモノクローナル抗体(神経芽腫に比較的特異性の高い細胞表面膜抗原または細胞内蛋白に対する)を用いた腫瘍組織と骨髄転移細胞の免疫組織診断、*MYCN* 増幅、DMS(double minute stain)、HSR(homogeneously staining regions)(い

用語解説 【カテコラミン】
交感神経興奮作用のある類似した化合物で、ドパミン、ノルエピネフリン、エピネフリンが含まれる。

ずれも *MYCN* 遺伝子の集塊)の検出、染色体検査での1pの欠失が神経芽腫の診断に役立つ。

超音波検査や単純X線検査、MRI、CTは腫瘍の原発部位、浸潤の程度、転移の有無の診断に役立つ。またMIBGシンチは神経芽腫に比較的特異的で、原発部位と転移部位の診断に有用である。

4 骨髄検査

初診時の病期の決定には、少なくとも左右2ヵ所ずつの骨髄穿刺と骨髄生検を行い、塗抹標本、穿刺生検標本での顕微鏡学的検査が必要とされる。近年は、治療効果判定、骨髄または自家造血幹細胞移植細胞中の微小残存腫瘍の検出の目的のために、モノクローナル抗体を用いた免疫組織学法またはPCR(polymerase chain reaction)法による高感度の検査が可能である。

用語解説 【PCR(polymerase chain reaction)法】

特異的DNAセグメントの大量合成技術。神経芽腫細胞では普遍的な染色体上にかつ神経細胞に特異的な ①PGP 9.5、②神経中間フィラメント、または③カテコラミン代謝の酵素であるチロシン水酸化酵素、をPCRで増幅し、高感度に残存腫瘍(10万～1千万個に1個の腫瘍細胞)を検出する。

5 病期分類

1 日本の病期分類

病期分類は神経芽腫の治療方針の決定と予後の把握に重要である。わが国では、Evansらによって1971年に提唱された病期分類を基礎に、日本小児外科学会悪性腫瘍委員会の分類が用いられてきた。病期IVS(4S)は、新生児または乳児期に発症し、肝、皮膚、骨髄への遠隔転移がみられるが予後はよい。またEvans分類との相違点は、病期IVを骨転移があるIVAと骨髄転移を示すIVBの2つに分類する点である。

2 神経芽腫国際病期分類

最近、米国、欧州と日本の間でまとめられた神経芽腫国際病期分類(International neuroblastoma staging system；INSS)が、神経芽腫の病期分類に用いられている(表1)[3]。INSSは腫瘍の外科的切除の可能性の有無、リンパ節転移および遠隔転移の有無により病期1～4と4Sに分類される。現在、世界中で広く用いられている。従来の病期分類に比較して、予後、予後因子との相関に優れ、適切な治療の判断基準になるか否かは、今後のさらなる国際的なデー