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ORIGINAL REPORT

Human Telomere Reverse Transcriptase Expression Predicts Progression and Survival in Pediatric Intracranial Ependymoma

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ABSTRACT

Purpose
Pediatric intracranial ependymomas are a heterogeneous group of neoplasms with unpredictable clinical and biologic behavior. As part of ongoing studies to identify potential biologic and therapeutic markers, we analyzed the role of human telomere reverse transcriptase (hTERT; the catalytic subunit of telomerase) expression as a prognostic marker for this disease.

Patients and Methods
Primary intracranial ependymomas that were resected at our institution between 1986 and 2004 were identified through the pathology and oncology databases. A tissue array was constructed from the patient samples and hTERT expression was evaluated by immunohistochemistry. Twenty-one samples were also analyzed for telomerase activity (telomerase repeat amplification protocol assay).

Results
Eighty-seven tumors from 65 patients were analyzed. Five-year progression-free survival was 57% (SEM, 12%) and 21% (SEM, 8%) for hTERT-negative and hTERT-positive tumors, respectively ($P = .002$). Five-year overall survival was 84% (SEM, 7%) and 41% (SEM, 7%) for hTERT-negative and hTERT-positive tumors, respectively ($P = .001$). There was good correlation between telomerase activity and hTERT expression ($\kappa = 0.637$). Multivariate analysis revealed hTERT expression to be the single most important predictor of survival of all known pathologic, clinical, and treatment factors (hazard ratio, 60.4; 95% CI, 6.4 to 561). All four patients with hTERT-negative tumors at relapse are still alive, with median follow-up of 11.2 years.

Conclusion
In this study, hTERT expression was the strongest predictor of outcome and was independent of other clinical and pathologic prognostic markers. It represents a simple and reliable biologic prognostic factor for intracranial ependymomas. These results should be confirmed in larger prospective trials.

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INTRODUCTION

Intracranial ependymomas are the third most frequent brain tumor in children, with a 5-year overall survival (OS) rate of approximately 50%.[1,2] Although ependymoma is estimated to account for 6% to 12% of pediatric brain tumors,[3] it is still rare and even large pediatric neuro-oncology centers see only about two to five affected patients per year on average.[4] This has resulted in a lack of large prospective clinical trials aimed at improving clinical outcome and better defining prognostic factors in pediatric ependymoma. Retrospective studies investigating histologic grade, metastatic status, age, and site as prognostic factors have yielded contradictory results.[3-6] At present, the only widely accepted important predictive factor is extent of resection[3,7-9]; in particular, gross total resection (GTR). Nevertheless, approximately 50% of children, even with extensive resections, will experience tumor recurrence. Another issue relevant to the treatment of these tumors is that half of childhood ependymomas occur in children younger than 5 years of age, raising concern about the use of radiation therapy. Furthermore, the clinical behavior of ependymomas is extremely variable, with some patients experiencing an aggressive and relentless course, and others experiencing late recurrences (which mainly are local) and prolonged remissions after second and even third debulking surgeries.[10] All of these factors, including the lack of reliable

prognostic markers, make treatment decisions difficult. In fact, a recent review of the literature failed to find a significant improvement in the prognosis for these patients during the last three decades.[11] These disturbing findings emphasize the need to identify biologic correlates of tumor behavior to improve outcome prediction and therapeutic decision making for affected patients.

One of the hallmarks of cancer is unlimited growth potential, which is associated with telomere maintenance.[12] Telomeres are nucleoprotein structures that cap chromosome ends protecting cells from inducing inappropriate DNA damage responses. With each cellular division, telomeres erode until senescence or apoptosis occurs. Most cancer cells maintain their telomeres by reactivating telomerase, a reverse transcriptase that elongates telomeres, or rarely by an alternative lengthening of telomeres (Alt) mechanism. Several studies have used telomere maintenance as a prognostic marker in various tumors.[13-16] The erratic clinical behavior of pediatric ependymomas suggests variable proliferative potentials and thus makes them an attractive candidate for studying telomere maintenance as a possible prognostic and therapeutic marker. The catalytic subunit of the human telomerase complex is a protein known as human telomere reverse transcriptase (hTERT). hTERT is tightly regulated both at the transcriptional and post-translational levels, and it is believed that regulation of telomerase activity occurs at the level of hTERT. Therefore, we hypothesized that measurement of the expression of hTERT in ependymomas may be a simple method to detect telomerase activity and thus be used as a potential prognostic marker.

PATIENTS AND METHODS

Patients operated on at the Hospital for Sick Children (HSC; Toronto, Canada) between 1986 and 2004 with a pathologic diagnosis of intracranial ependymoma were identified retrospectively through the pathology and neuro-oncology databases. Patients with spinal ependymomas were excluded. In total, 75 patients who had undergone a first-time surgical resection at HSC were identified and the pathology reviewed. Nine patients had only their surgery done at HSC and received their adjuvant therapy elsewhere, and one patient did not have ependymoma on review of pathology, leaving 65 patients who were included in the study. Of these, 38 survivors were actively observed; seven were lost to follow-up and, for this analysis, were censored at the time when they were last seen.

Clinical Data

Clinical data collected included age and metastatic disease status at presentation, sex, extent of surgical resection, chemotherapy use (including protocol and drug used), radiotherapy use, progression-free survival (PFS), and OS. The latter was the primary end point for this study. Metastatic disease was defined as either the presence of malignant cells on CSF cytology (obtained at least 14 days postsurgery) or definite radiographic evidence of spread before the onset of chemotherapy or radiotherapy. Complete metastatic work-up data were available for 53 patients. Extent of surgical resection was recorded as GTR if no tumor was apparent in the surgical report or postoperative magnetic resonance imaging. The latter imaging technique was used for all patients. Subtotal resection was defined as less than 50% and partial resection was defined as more than 50% residual tumor. Tumors that were only biopsied were recorded as such. Total radiation doses to the tumor bed and craniospinal axis were recorded separately. The study had prior approval from the Research Ethics Board at HSC. All data were made anonymous before publication.

Ependymoma Tissue Array Construction

Tissue arrays were prepared as previously described by our group.[17] Briefly, for each patient, all pathologic blocks and corresponding slides were obtained and reviewed by a neuropathologist (C.H.) for diagnostic accuracy and tissue adequacy. Ependymomas were graded based on WHO criteria.[18] Representative tumor areas were identified, and three 1-mm cores were obtained from each tumor, providing a sampling accuracy of at least 95%.[19,20] A variety of tissues including liver, ependyma, choroid-plexus, neuroblastoma, and breast cancer were included around the periphery of each array to serve as internal controls.

Immunohistochemistry for hTERT

Five-micrometer sections were cut from the tissue microarray and mounted on positively charged microscope slides. Tissue sections were then baked overnight at 60°C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. hTERT (clone 44F12; NovoCastra, Newcastle-upon-Tyne, United Kingdom)[21] immunohistochemistry was performed manually at a dilution of 1:25, incubated overnight at 4°C, and immunodetected using the Vector Elite avidin-biotin complex method detection system (Vector Laboratories, Burlingame, CA). All tissue sections were treated with heat-induced epitope retrieval and blocked for endogenous peroxidase and biotin. The counterstain of preference was hematoxylin. Appropriate positive and negative controls were also tested in parallel. Conventional sections from paraffin blocks were used for samples that were technically poor and tumors that were resected after the construction of the tissue array.

Immunohistochemical Grading

Immunohistochemical staining for hTERT (nuclear) was reviewed and graded for both strength (0, none; 1, weak; 2, strong) and distribution ($<$ 25%, 25% to 50%, $>$ 50% of tumor cells) as shown in Figure 1. The reviewers were blinded to clinical patient data at the time of grading. Only tumors with strong (grade 2) nuclear staining in more than 25% of tumor cells were considered to be positive for hTERT. Overall, 20 of the 27 tumors that were scored as negative stained 0 for intensity and only seven stained weakly at different distributions, so the results were easy to interpret in 90% of samples.

Telomerase Activity Assay

Tissue extraction and polymerase chain reaction (PCR) enzyme-linked immunosorbent assay were performed according to the Telomerase PCR-ELISA kit (Roche Diagnostics, Mannheim, Germany) as previously

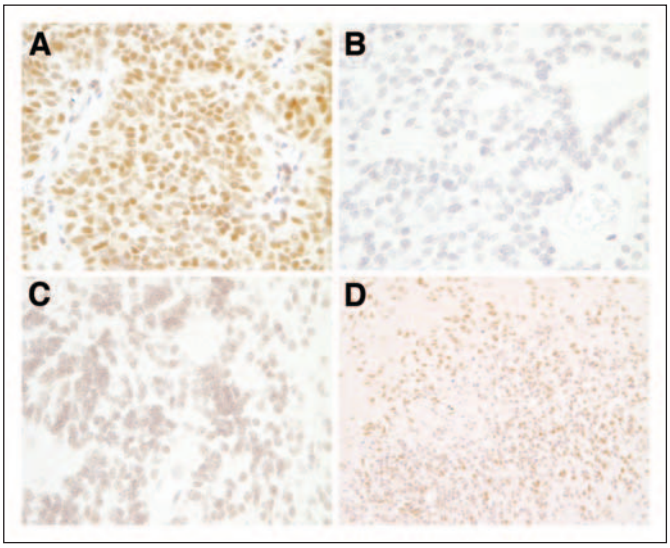

Fig 1. Human telomere reverse transcriptase expression by immunohistochemistry. (A) Negative staining; (B) positive staining; (C) weak staining, interpreted as negative; (D) mixed picture. Ninety percent of tumors were either (A) or (B). Analysis could be done on ×100 or ×200 magnification in most cases (A, B, C, ×400; D, ×200 magnification).

described by our group.[22] Briefly, a fragment of frozen tumor tissue was homogenized in lysis buffer, centrifuged, and the supernatant was removed. Protein concentration was determined using the Protein Assay ESL Kit (Roche Diagnostics). For each sample a negative control was created by incubation with RNase at 37°C for 20 minutes. To identify false-negative tumor samples, for each specimen a spiked sample containing both tumor lysate and positive control was also created. The test positive control consisted of 2 mL of the supplied positive control solution; the test negative control consisted of 5 mL of lysis buffer added to the reaction mixture. These samples were then incubated at 25°C. Next, telomerase was inactivated by heat treatment and the reaction mixtures then underwent 30 PCR cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 90 seconds. On completion of the PCR protocol, denaturation and hybridization were performed according to the kit protocol. The samples were read on a Multiskan MCC/340 microtiter plate reader (Titertek Instruments Inc, Huntsville, AL) at 450 nm. The test negative control was accepted if the maximum absorbance was 0.2 $A_{450nm} - A_{690nm}$ units. The test positive control was considered valid if absorbance was 1.5 $A_{450nm} - A_{690nm}$ units or greater. Samples were regarded as positive if the reading was greater than twice the negative control and greater than 0.5.

Statistical Analysis

For each biologic and clinical marker, PFS and OS were estimated using the Kaplan and Meier method, and significance testing ($\alpha = .05$) done on the basis of the log-rank test. Multivariate Cox proportional hazards models were used to estimate hazard ratio and 95% CIs, after controlling for the effects of other possible prognostic factors. Correlation between hTERT expression and clinical parameters was assessed using the Pearson χ^2, with $P < .05$ considered a significant correlation. Reliability of hTERT immunohistochemistry results versus telomerase repeat amplification protocol (TRAP) assay results was calculated using the κ statistic.

RESULTS

Clinical and Demographic Features

Sixty-five patients were included in the study (35 males and 30 females). The median age at presentation was 4.2 years (range, 4 months to 17.5 years). Mean age of presentation was 5.7 years. The median follow-up time for surviving patients was 4.4 years (range, 1 to 16.5 years). The clinical and treatment details of the patients are summarized in Table 1. Five patients deteriorated rapidly or died after initial diagnosis. Two of them had only biopsy for their tumors and none of these patients received medical or radiation therapy. Fifteen patients received craniospinal irradiation; 12 before 1990, when this was the protocol at HSC and three more recently due to metastatic disease at diagnosis. Thirty-two patients did not receive chemotherapy. For the others, chemotherapy protocols varied widely and included ifosfamide, carboplatin, etoposide in 13 patients[23]; baby POG (Pediatric Oncology Group; vincristine-cyclophosphamide-etoposide-cisplatin) in

Table 1. Patients Characteristics and Univariate Survival Comparisons for 65 Patients

Characteristic	No. of Patients	%	5-Year Survival					
			PFS (%)	SE	*P* (log rank)	OS (%)	SE	*P* (log rank)
Age > 3 years								
Yes	37	65	58	9	.004	69	9	.019
No	28	35	19	8		48	10	
Sex								
Male	35	54	39	10	.98	56	10	.78
Female	30	46	43	10		64	9	
Tumor location								
Supratentorial	18	28	52	13	.18	71	12	.185
Infratentorial	47	72	33	8		57	8	
Grade								
2	29	45	53	11	.049	80	8	.021
3	36	55	31	8		46	9	
Metastatic*								
Yes	8	15	25	15	.003	31	18	.04
No	45	85	42	9		60	9	
Resection								
GTR	30	46	54	10	.047	75	9	.032
Subtotal	33	51	31	10		51	10	
Partial	0	0	NA			NA		
Biopsy	2	3	0			0		
Radiation								
Yes	41	63	57	10	< .0001	74	8	.006
No	24	37	13	8		38	11	
Chemotherapy								
Yes	32	49	34	18	.378	69	9	.43
No	33	51	48	10		52	10	
hTERT								
Positive	38	58	21	8	.002	41	7	.001
Negative	27	42	57	12		84	7	

Abbreviations: PFS, progression-free survival; OS, overall survival; GTR, gross total resection; NA, not applicable; hTERT, human telomere reverse transcriptase.
*Only for patients who had complete metastatic work-up.

seven[24]; baby SFOP (French Society of Pediatric Oncology; procarbazine-carboplatin-etoposide-cisplatin-vincristine-cyclophosphamide) in two[25]; and a variety of chemotherapeutic regimens in 11 patients. Progression-free 5- and 10-year survivals were 41% (SEM, 7%) and 32% (SEM, 8%), respectively. Overall 5- and 10-year survivals were 60% (SEM, 8%) and 45% (SEM, 9%), respectively.

hTERT Immunostaining

In the vast majority of samples (90%), the tumors showed either no staining or staining in greater than 25% of cells (Fig 1B). hTERT was positive in 58% of tumor samples (Table 1). Immunostaining was concordant between triplicates in 89% of tumors. Positive control tissues including testis, breast cancer, and neuroblastoma expressed hTERT, whereas liver and brain (including ependyma, astrocytes, neurons, and choroid-plexus) were immunonegative.

Prognostic Features

Univariate analysis of clinical and pathologic prognostic factors and hTERT expression are listed in Table 1. hTERT positivity, age younger than 3 years, histologic grade 3, metastasis at diagnosis, less than GTR, and lack of radiotherapy were associated with a significantly worse OS and PFS. Kaplan and Meier analysis of the effect of hTERT expression on PFS and OS is shown in Figure 2. On multivariate analysis (Cox regression), only metastatic status, radiation therapy, and hTERT reached significance (Table 2). hTERT was the strongest predictive variable, with hazard ratio of 60 ($P < .0001$; Table 2).

Comparison of hTERT Expression and Telomerase Activity

To confirm our results and correlate expression of hTERT with telomerase activity, TRAP assays were performed on 21 frozen samples and compared with hTERT staining (Table 3). There was a good correlation between the two assays ($\kappa = 0.674$; $P = .003$). TRAP assay was more sensitive than hTERT immunohistochemistry for detecting telomerase, thus three tumors were positive for telomerase activity but negative for hTERT expression. The reverse was never the case.

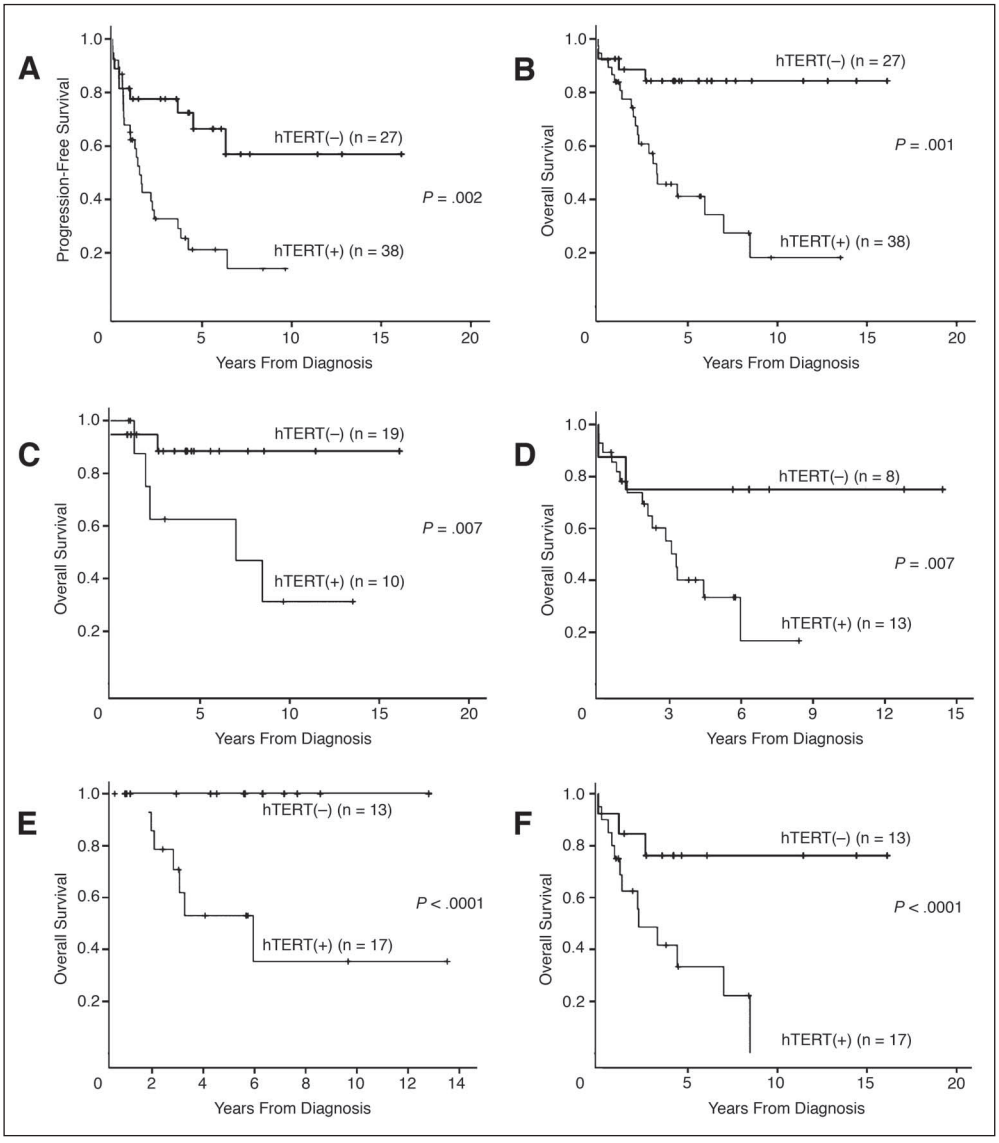

Fig 2. Kaplan-Meier estimates of survival for patients with human telomere reverse transcriptase (hTERT) positive (+) or negative (−) ependymomas. (A) Progression-free survival for all patients; (B) overall survival (OS) for all patients. OS for hTERT expression stratified by histologic grade: (C) grade 2 tumors; (D) grade 3 tumors. OS for hTERT expression stratified by extent of resection: (E) gross total resection (GTR); (F) subtotal resection.

Table 2. Cox Regression Model for Multivariate Analysis (n = 65)

Covariable	Hazard Ratio	95% CI	*P*
hTERT expression (positive *v* negative)	60.4	6.4 to 561	< .0001
Tumor grade (2 *v* 3)	0.87	0.3 to 2.7	.81
Age (< 3 *v* > 3 years)	1.0	0.36 to 2.75	.99
Metastasis (yes *v* no)	14.8	2.3 to 94	.004
Surgical resection (subtotal *v* GTR)	2.1	0.7 to 5.9	.17
Radiation (yes *v* no)	0.28	0.09 to 0.9	.033

Abbreviations: hTERT, human telomere reverse transcriptase; GTR, gross total resection.

Correlation of hTERT Expression With Tumor Grade and Extent of Resection

To determine whether hTERT expression is a marker of anaplasia, we correlated its expression with pathologic grade of the tumors. Although there was correlation between tumor grade and hTERT expression (Pearson χ^2; $P < .0001$), hTERT expression was still able to divide these patients into different prognostic groups. hTERT expression was associated with reduced OS regardless of tumor grade as shown by Kaplan and Meier analysis (Fig 2). Because extent of resection is believed to be the most reliable clinical prognostic marker in ependymoma, we studied hTERT status in GTR and subtotally resected tumors. Strikingly, all of the patients who underwent GTR and had hTERT-negative tumors are long-term survivors, whereas all patients with hTERT-positive tumors who had less than GTR eventually died as a result of their disease (Fig 2).

hTERT Expression in Recurrent Tumors

Thirty-two patients experienced disease progression or relapse during the follow-up period. Twenty-nine repeat surgical excisions were done and hTERT expression was analyzed in 22 patients. hTERT expression was positive in 18 (81%), of whom 15 are dead as a result of disease and three are alive with disease at different stages of treatment, with follow-up of 0.5, 2, and 4 years, respectively. Three of the 18 positive tumors were negative for hTERT expression at first biopsy. Two of them were grade 2 tumors that evolved to grade 3 at the time of relapse. Interestingly, four patients were negative for hTERT at both the first and second surgeries. All of these patients are still alive at a median follow-up of 11.2 years (range, 4.7 to 14.6 years).

DISCUSSION

The aim of this study was to investigate the hypothesis that telomerase expression has prognostic significance in childhood intracranial ependymomas. We found that hTERT expression was the single most important predictive factor in our group of 65 primary ependymomas (Tables 1 and 2). Furthermore, there was high correlation between hTERT expression and telomerase activity (Table 3). These results add to the growing body of evidence showing association between telomerase expression and adverse prognosis in pediatric cancers such as osteosarcomas,[16] neuroblastomas,[15] and acute myelogenous leukemias.[26] It is important, however, to note that in these tumors, other robust and reliable biologic and clinical prognostic factors exist that explain why this method is not widely accepted in clinical practice. In contrast, apart from extent of resection, such prognostic factors are lacking in ependymoma.

Table 3. Correlation Between Telomerase Activity and hTERT Expression in 21 Tumors

	Telomerase Activity	
hTERT Expression	Positive	Negative
Positive	13	0
Negative	3	5

NOTE. Direct correlation between telomerase activity (telomerase repeat amplification protocol assay) and hTERT expression by immunohistochemistry was shown with $\kappa = 0.674$ and $P = .001$.
Abbreviation: hTERT, human telomere reverse transcriptase.

In our study, several risk factors, namely age, pathologic grade, and extent of resection reached significance in univariate analysis but were not independent of other factors on multivariate analysis. Age is an inconsistent prognostic factor in ependymomas especially because younger children tend to have tumors in the posterior fossa where they are often less amenable to GTR, and radiation therapy is often delayed or not used for these patients because of concerns over long-term adverse effects. There was no association between age and metastatic disease status or hTERT expression; however, those younger than 3 years were less likely to have received radiation in our cohort. Thus, much of the effect of age found on univariate analysis was likely accounted for by the lack of radiation therapy in the younger children.

Histologic grading is extremely controversial in pediatric ependymomas[3,27] and the definition of anaplasia varies greatly between centers, which makes study interpretation challenging.[27-29] In our study, histologic grade was prognostic on univariate analysis but was not independently prognostic on multivariate analysis. This may be explained by our finding of a correlation between histologic grade and hTERT expression, with higher grade tumors more likely to be hTERT positive (Pearson χ^2; $P < .0001$). hTERT expression was not associated with tumor location (Pearson χ^2; $P = .392$). There was no correlation between extent of surgical resection and hTERT expression, metastatic status, or radiation therapy. As shown in Figure 2, hTERT expression can be used to divide both totally and subtotally resected tumors into good and bad prognostic groups. It is interesting to speculate that incompletely resected tumors may recur and require reresection but are not ultimately fatal in patients with hTERT-negative tumors, explaining why the extent of resection failed to predict survival independently.

Multivariate analysis revealed radiotherapy and metastatic status to be important risk factors in our group. However, radiation therapy is an evolving issue in pediatric ependymoma, and patients who were previously treated with craniospinal irradiation are treated differently

now. In the new Children's Oncology Group protocol, completely resected grade 2 supratentorial ependymomas do not receive radiotherapy. This favorable-risk group may change future use of this modality in risk stratification. Furthermore, Merchant et al[30] recently published their data on children younger than 3 years of age who were irradiated, indicating that radiation guidelines vary widely between groups. Interestingly, hTERT was able to stratify both radiated and nonradiated patients into good- and poor-outcome groups (data not shown). Of particular importance is the fact that even in nonirradiated patients, 5-year survival is 69% (SEM, 15%) for patients with hTERT-negative tumors versus 18% (SEM, 11%) for patients with hTERT-positive tumors. Thus, hTERT negativity may help to identify a subgroup of patients in whom radiation may be delayed or not used.

Metastases at diagnosis, although important, are relatively rare in childhood ependymomas. There was no direct correlation between hTERT expression and metastatic status (Pearson χ^2; $P = .437$). Interestingly, none of the 16 metastatic-negative/hTERT-negative patients have died as a result of their disease. Therefore, this may represent a particularly good prognostic group. In view of the inconsistency and changes in the clinical and pathological risk stratification in ependymomas, hTERT expression may be a reliable, objective, and much needed prognostic factor for this disease.

In recent years, other biologic risk factors for ependymoma have been pursued with variable success. Proliferative markers such as MIB1 (Ki-67) tend to show worse outcomes with higher proliferative rates, but the cutoff for what is considered high is extremely variable from study to study, ranging from more than 1% to more than 20%.[6] There seems to be a good correlation between MIB1 index and pathology grade; however, the importance of this marker needs additional validation.[27,31-33] Rushing et al[34] described a high correlation among telomerase RNA component, MIB1 index, and anaplasia in a small group of adult ependymomas. However, this study had only four anaplastic tumors and a large group of spinal grade 1 tumors, which are excluded in our study. Furthermore, telomerase RNA component currently is considered to be expressed ubiquitously and hTERT correlates better with telomerase activity.

Cytogenetic studies of ependymomas, especially by comparative genomic hybridization, have generated possibly the most promising results. Carter et al[35] reported the largest series of 86 pediatric and adult ependymomas. Significant differences were found between adult and childhood ependymomas with respect to balanced karyotype. In this study, gain of 1q was associated with poor clinical outcome, especially in anaplastic ependymomas. Dyer et al[36] studied 53 primary and recurrent pediatric ependymomas and found that tumors with structural chromosomal abnormalities had significantly worse outcomes when compared with numerical or balanced comparative genomic hybridization profile. Gilbertson et al[37] studied the role of ERBB family of receptor tyrosine kinases in 121 pediatric ependymomas. High expression of ERBB2 and ERBB4 did not reach significance in univariate analysis, but when combined with other prognostic factors, it enabled better resolution of patients' prognosis. Other oncogenes, tyrosine kinases, and tumor suppressors did not have a significant impact on survival in ependymomas. The results observed in this study may add a new dimension to the role of biology in the management of these tumors.

This study has the classical setbacks of a retrospective analysis, especially in an era when changes in treatment approaches, surgical techniques, and imaging capabilities occurred. Therefore, our results should be interpreted accordingly. We elected to start the analysis time from when magnetic resonance imaging was introduced as a routine diagnostic tool, and therefore, extent of resection and metastatic status were always analyzed using modern imaging techniques. Furthermore, as mentioned, the management and outcome of ependymomas has not changed dramatically during the last two decades.

Another obstacle in the use of biologic markers in brain tumors is the small biopsy sample. In contrast to other pediatric solid tumors such as Wilms' tumor and neuroblastoma, in which the whole tumor is available to the pathologist to find the most significant areas of interest, in brain tumors often only a small part of the tumor is available for pathology and biologic studies, leading to potential sample errors. Indeed, several of our patients who experienced relapse had different hTERT expression and, in some, different pathologic grade than that of the primary tumor. Although clonal evolution can be responsible for such changes, sample error cannot be ruled out.

We believe that hTERT immunostaining is a reproducible method with a strong ability to predict outcome in pediatric intracranial ependymoma. Ninety percent of the samples could be interpreted clearly as positive or negative. In contrast to other biologic markers, there is no need for frozen material or for sophisticated techniques. hTERT immunostaining can be performed on paraffin sections and should be reproducible simply in most pathology laboratories. The strong correlation between hTERT expression and telomerase activity manifested by the TRAP assay (Table 3) suggests that hTERT expression is also a reliable marker of telomerase activity. Furthermore, the striking correlation between negative hTERT expression and prolonged survival (even in patients who experienced relapse), and the observation that all of the patients with hTERT-negative tumors who had GTR survived, whereas none of the patients with hTERT-positive tumors who had subtotal resection are long-term survivors, suggest that telomere maintenance may have an important role in ependymoma progression and tumor viability. Alt is another mechanism by which tumor cells maintain their telomeres; it also plays a significant role in osteosarcomas and glioblastomas. Preliminary data from 31 ependymomas did not show evidence of Alt in our cohort (data not shown) and we are pursuing this question further.

Although it is retrospective, we believe our study contributes substantially to the body of existing knowledge of ependymoma prognosis, and future efforts should be directed toward validating hTERT as a marker in prospective studies. Confirmation of our results may have additional value because telomerase inhibition may have therapeutic implications in the near future.[38,39]

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