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Expression of Transforming Growth Factor-β Isoforms in Small Round Cell Tumors of Childhood

An Immunohistochemical Study

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The transforming growth factor (TGF)-βs are a highly conserved group of potent multifunctional cell regulatory proteins with variable effects on cell growth and differentiation. Most of the small round cell group of childhood tumors are thought to arise from either primitive mesenchyme or neuroectoderm and show evidence of skeletal muscle or neural differentiation, and rarely both. To investigate the possibility that the TGF-βs have a role in the growth or differentiation of these neoplasms, we used antibodies specific for peptide sequences of the three known mammalian TGF-β isoforms (TGF-βs 1, 2, and 3) to probe for TGF-β protein expression in a total of 49 cases. TGF-β1 immunoreactivity was present in 16/17 (94%) of rhabdomyosarcomas, and the staining intensity was usually strong. TGF-β1 was also present in three of three ectomesenchymomas. In contrast, TGF-β1 was absent in all but one out of nine poorly differentiated neuroblastomas. Differentiating neuronal cells of ganglioneuroblastomas, however, were strongly positive for TGF-β1. Ewing's sarcomas and peripheral primitive neuroectodermal tumors had a less consistent, but usually positive, staining pattern. TGF-β3 staining patterns were very similar to those of TGF-β1. TGF-β2 immunoreactivity was only rarely detected in this group of tumors. The results suggest different roles for TGF-βs 1 and 3 in neuroblastoma and rhabdomyosarcoma. Expression of TGF-βs 1 and 3 is associated with neuronal differentiation of neuroblastoma. In contrast, these proteins may promote the growth of rhabdomyosarcoma by suppresing differentiation. (Am J Pathol 1993, 142:49–59)

The small round cell tumors of childhood are a heterogeneous group of malignant solid neoplasms often sharing a similar appearance at the level of light microscopy.[1,2] Included in this grouping are neuroblastoma and rhabdomyosarcoma, which are among the most common solid tumors of childhood. Other important small round cell tumors are Ewing's sarcoma, peripheral primitive neuroectodermal tumor (PNET), ectomesenchymoma, and lymphoma. Certain light microscopic, ultrastructural, immunohistochemical, and/or genetic features may suggest the primitive tissues of origin of these neoplasms, or at least may demonstrate partial differentiation toward a certain tissue type. For example, rhabdomyosarcoma shares a variable number of markers with skeletal muscle, such as myoglobin, MyoD1, and cytoplasmic muscle filaments. The presence of neurofibrillary material and nonspecific enolase in neuroblastoma suggests a neural crest origin. Ectomesenchymoma may show evidence of differentiation toward multiple tissues. The molecular mechanisms involved in the establishment of cell phenotype are of obvious importance both in tumor biology and developmental biology.

The transforming growth factor-βs (TGF-βs) are a group of multifunctional cell regulatory proteins capable of modulating cell growth and differentia-

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tion, and enhancing extracellular matrix accumulation.[3,4] In culture, their constellation of effects depend on cell type, other cytokines present, and culture conditions.[3–5] To date, three TGF-β isoforms have been described in mammalian cells (TGF-βs 1, 2, and 3).[6–8] Since the different isoforms bind to the same receptor and, with few exceptions, exert similar biological effects, the significance of multiple isoforms may lie in differential regulation of expression. Indeed, differences in the promoters for TGF-βs 1, 2, and 3 have been described.[9–11] There are growing numbers of reports suggesting a role for the TGF-βs in embryonic development.[12–15] TGF-β has also been implicated in a number of pathological conditions including neoplasia. Its role in neoplasia may depend on the cell type involved. For example, TGF-β inhibits the proliferation and induces differentiation of many epithelial cell types,[3,4] suggesting that underexpression or escape from the inhibitory effects of TGF-β may contribute to the development of carcinomas. On the other hand TGF-β stimulates proliferation and inhibits differentiation of other cell types,[3,4] raising the possibility that overexpression or inappropriate expression of TGF-β may contribute to neoplasia.

Using antibodies raised against peptide sequences specific for TGF-βs 1, 2, and 3, we evaluated a variety of small round cell tumors of childhood for the presence of TGF-β isoforms. We found that TGF-βs 1 and 3 were, with rare exceptions, consistently detected in rhabdomyosarcomas but not in poorly differentiated neuroblastomas. The differentiating neuronal-type cells of ganglioneuroblastoma, however, were positive for TGF-βs 1 and 3. These findings may indicate different roles for TGF-β in tumors of neural crest origin versus mesenchymal origin.

Materials and Methods

Tissue

A total of 49 cases diagnosed between 1976 and 1991 were selected from the surgical pathology files of the National Cancer Institute Laboratory of Pathology and the Childrens Hospital Medical Center, Washington, DC. Sections were cut from paraffin-embedded blocks of routinely processed formalin-fixed tissue. The diagnoses were based on a combination of currently accepted light microscopic, ultrastructural, and immunohistochemical features. The diagnostic breakdown is shown in Table 1. A range of clinical characteristics was represented for each tumor classification. Cases of rhabdomyosarcoma included stages II, III, and IV; primary tumor locations included the extremities, trunk, and head. The patients ranged from 1 to 21 years of age. The neuroblastomas included multiple cases of both adrenal and extraadrenal tumors and the patients age ranged from 12 days to 6 years. The majority of peripheral PNETs were chest wall tumors, but cases arising in the extremities and pelvis were also included; these patients were 16 to 32 years of age. The Ewing's sarcomas included six osseous and one soft-tissue tumor; the patients ranged from 9 to 37 years of age. The cases of ectomesenchymoma included lesions arising in the thigh, arm, and abdominal cavity; the patients ranged from 4 to 20 years of age.

Table 1. *Small Round Cell Tumors Evaluated for TGF-β Expression*

Neuroblastoma
Poorly differentiated (9)
Ganglioneuroblastoma (3)
Ewing's sarcoma (7)
Primitive neuroectodermal tumor (10)
Rhabdomyosarcoma
Alveolar (8)
Embryonal (6)
Mixed (3)
Ectomesenchymoma (3)

Antibodies

Affinity-purified rabbit polyclonal antibodies raised to peptide sequences unique to TGF-βs 1, 2, and 3 were employed. TGF-β1 antibodies were raised against the peptide sequence of amino acids 266–278 in the pro region of the precursor (also referred to as the latency associated peptide or LAP). TGF-β2 antibodies were raised to amino acids 50–75 in the mature region. Two different TGF-β3 antibodies were available, one raised to a sequence in the mature region (amino acids 50–60) and one to the pro region or LAP (amino acids 81–100). The TGF-β1 and TGF-β3 antibodies used in this study react specifically with the isoform to which they were raised on Western blots[16] (K. C. Flanders and B. K. McCune, unpublished results). The TGF-β2 antibodies do not cross-react with TGF-β1, but show some cross-reactivity with TGF-β3 on Western blots[16]; however, previous studies as well as the present one indicate that significant cross-reactivity does not commonly occur under the conditions used for immunohistochemistry.[16,17] Details of antibody preparation and tissue staining characteristics have been described.[16–19]

Immunohistochemical Staining

TGF-β isotypes were localized in 5-μm sections using an avidin-biotin-peroxidase kit (Vector Laboratories Inc., Burlingame, CA). After deparaffinization, blocking endogenous peroxidase with 0.6% hydrogen peroxide in methanol, and permeabilization with hyaluronidase (1 mg/ml, Calbiochem, La Jolla, CA), the sections were blocked with 5% normal goat serum, 1% bovine serum albumin fraction V (Miles, Kankakee, IL), and 1% ovalbumin (Fluka, Ronkonkoma, NY) for 1 hour at room temperature and then incubated with 4 to 10 μg/ml primary antibodies in Tris-buffered saline (10 mmol/L Tris, pH 7.4, 0.85% NaCl) with 1% bovine serum albumin. The sections were then washed extensively in Tris-buffered saline/0.1% bovine serum albumin, and incubated with biotinylated goat anti-rabbit immunoglobulin G followed by avidin-enzyme complex according to the manufacturers instructions. The sections were then incubated in 0.05% 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) in 0.1% hydrogen peroxide for 3 to 5 minutes to produce the peroxidase reaction product. The sections were counterstained with Mayer's hematoxylin.

Controls included one or both of the following: 1) replacing the primary antibody with 10 μg/ml of nonimmune rabbit immunoglobulin G, 2) using primary antibody that had been preincubated with a 20-fold molar excess of the immunizing peptide for 2 hours at room temperature. Sections of human breast tissue were included as positive controls; the ductal epithelium typically shows positive immunoreactivity for all three TGF-β isoforms.[17]

Staining reactions were judged as either negative (–), weakly positive (+), or moderately to strongly positive (++) relative to the reaction with nonimmune rabbit serum (Tables 2–6). In most cases, the reaction with nonimmune rabbit serum was negligible, and thus any cytoplasmic reaction product was considered positive. Positive staining limited to tissue section edges was disregarded. Staining was repeated with each antibody on one or more cases from each tumor category with consistent results.

Table 2. *Reactivity of Rhabdomyosarcomas with TGF-β Antibodies*

Case	Histology	Antibody TGF-β1	TGF-β2	TGF-β3
1	Alveolar	+	NT	NT
2	Alveolar	++	NT	NT
3	Alveolar	++	–	+
4	Alveolar	++	+	+
5	Alveolar	++	–	++
6	Alveolar	+	NT	+
7	Alveolar	++	–	++
8	Alveolar	++	–	+
9	Embryonal	++	–	++
10	Embryonal	–	NT	–
11	Embryonal	++	NT	NT
12	Embryonal	++	NT	+
13	Embryonal	++	NT	NT
14	Embryonal	++	NT	NT
15	Mixed	++	–	–
16	Mixed	++	–	+
17	Mixed	++	–	++
Positives/total		16/17	1/9	10/12

Staining intensity: – = no staining, + = weakly positive staining, ++ = moderately to strongly positive staining; NT, not tested.

Table 3. *Reactivity of Neuroblastomas with TGF-β Antibodies*

Case	Histology	Antibody TGF-β1	TGF-β2	TGF-β3
1	PD	–	NT	NT
2	PD	–	–	–
3*	PD	++	–	+
4	PD	–	NT	NT
5	PD	–	–	–
6	PD	–	–	–
7	PD	–	NT	NT
8	PD	–	–	–
9	PD	–	NT	NT
Positives/total		1/9	0/5	1/5
1†	GNB	+	NT	+
2†	GNB	++	–	+
3†	GNB	++	–	+
Positives/total		3/3	0/2	3/3

PD, poorly differentiated neuroblastoma; GNB, ganglioneuroblastoma; NT, not tested.
* Primitive histology, see text.
† Positivity limited to cells showing neuronal differentiation. See legend to Table 2.

Results

We initially focused on TGF-β1, and thus all cases were probed with anti-TGF-β1 antibodies. A subset of each tumor type was evaluated for TGF-β2 and TGF-β3 reactivity because of limited availability of unstained sections from some cases. Tables 2 to 6 detail the staining reactions with TGF-β antibodies. With the exception of one case of rhabodmyosarcoma and one case of ectomesenchymoma, all tumors that were probed with anti-TGF-β2 at a concentration that gives a strong reaction in control tissues, failed to stain significantly above background (Figures 1b, 2b). A more heterogeneous pattern of reactivity was observed with anti-TGF-β1 and anti-TGF-β3, as detailed below for each tumor type.

Table 4. *Reactivity of PNET with TGF-β Antibodies*

Case	Antibody		
	TGF-β1	TGF-β2	TGF-β3
1*	++	NT	NT
2†	–	–	+
3*	++	–	+
4	++	NT	NT
5	+	NT	NT
6‡	–	–	–
7*	+	–	+
8	++	NT	NT
9	++	NT	NT
10	+	–	+
Positives/total	8/10	0/5	4/5

NT, not tested.
* Nuclear staining present.
† Rare cells ++ for TGF-β1.
‡ Rare areas + for TGF-β1 and -β3. See legend to Table 2.

Table 5. *Reactivity of Ewing's Sarcoma with TGF-β Antibodies*

Case	Antibody		
	TGF-β1	TGF-β2	TGF-β3
1	++	NT	NT
2	++	–	+
3	–	–	+
4	–	–	–
5	–	–	–
6	++	–	++
7	++	–	–
Positives/total	4/7	0/6	3/6

NT, not tested. See legend to Table 2.

Table 6. *Reactivity of Ectomesenchymoma with TGF-β Antibodies*

Case	Antibody		
	TGF-β1	TGF-β2	TGF-β3
1*	++	+	+
2*	++	NT	NT
3†	+	NT	–

NT, not tested.
* Primitive neuroectodermal tumor and rhabdomyosarcoma elements present.
† Neuroblastoma and rhabdomyosarcoma elements present. See legend to Table 2.

Rhabdomyosarcoma

The great majority (94%) of alveolar, embryonal, and mixed rhabdomyosarcomas showed cytoplasmic positivity with TGF-β1 antibodies (Table 2, Figures 1a, d). In some cases the positivity was homogeneous and in other cases an admixture of positive and negative tumor cells was observed. No morphological differences between positive and negative cells within a given tumor were appreciated. The "strap" cells observed in one embryonal rhabdomyosarcoma showed strong cytoplasmic reactivity (Figure 1d). The normal skeletal muscle also present in this section showed much less intense staining (Figure 1d, inset). However, the higher intensity of staining in the tumor cells may reflect a higher TGF-β concentration because of a lower cell volume and not increased absolute quantities. TGF-β3 antibodies reacted with a similarly positive pattern, albeit generally weaker, in 10 of 12 rhabdomyosarcomas tested (Figure 1c). One lesion diagnosed as embryonal rhabdomyosarcoma failed to stain for TGF-β1 and TGF-β3 (Table 2, case 10). This tumor was unusual in that it had a more primitive histological appearance and had only occasional cells reactive with desmin and actin antibodies. Only one of the nine cases tested for TGF-β2 reactivity showed positivity (case 4). In this case, the positivity for TGF-β2 was limited to rhabdomyoblasts, whereas TGF-βs 1 and 3 were present in undifferentiated cells as well.

Neuroblastoma and Ganglioneuroblastoma

Eight of nine poorly differentiated neuroblastomas were either completely devoid of reactivity with any TGF-β antibody or showed weak positivity only in very rare cells and thus were considered essentially negative (Figure 2a). One unusual case of neuroblastoma was positive for TGF-β1 (Table 3, case 3). This tumor had a more primitive histological appearance (ie, no rosettes, little neurofibrillary material) than the others in this group and had a high mitosis/karyorrhexis index. Also, the primary site of this tumor was uncertain.

The poorly differentiated component of ganglioneuroblastomas was similarly nonreactive with all TGF-β antibodies. Interestingly, the differentiating ganglion-like cells of ganglioneuroblastoma showed strong reactivity with TGF-β1 antibodies (Figure 2b). The spindle cell component showed variable reactivity with these antibodies. Fortuitously, a normal ganglion was identified in one section of ganglioneuroblastoma. The ganglion cell bodies stained intensely with antibodies to TGF-β1 (Figure 2c). Normal ganglion cells as well as ganglion cells of ganglioneuroblastoma also reacted with TGF-β3 antibodies, albeit somewhat less intensely.

Peripheral Primitive Neuroectodermal Tumor, Ewing's Sarcoma, and Ectomesenchymoma

The TGF-β1 staining intensity and distribution was variable in peripheral PNETs. Staining was predominantly negative in two cases and ranged from weak nuclear positivity to strong cytoplasmic positivity in

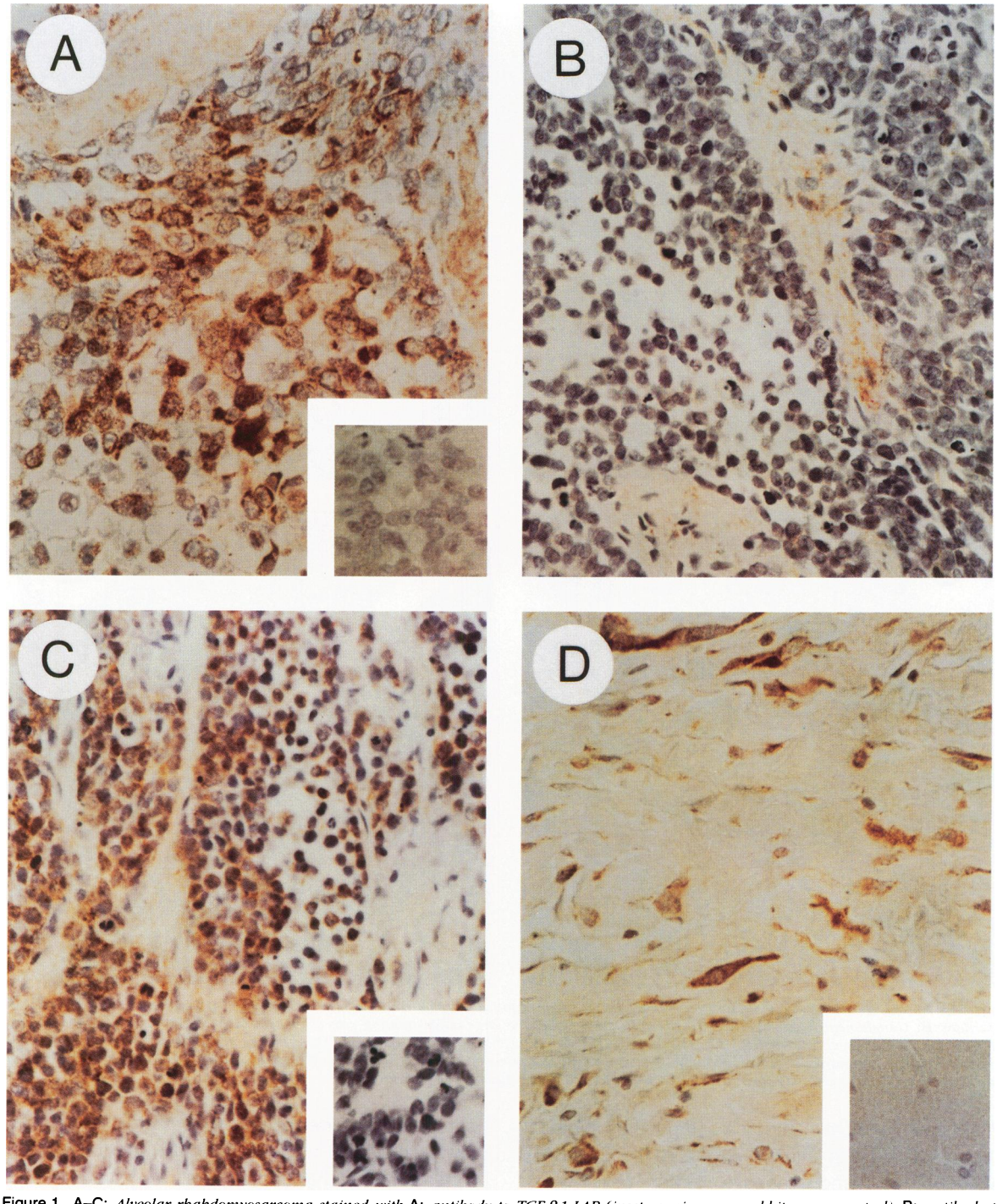

Figure 1. **A–C:** *Alveolar rhabdomyosarcoma stained with* **A:** *antibody to TGF-β1-LAP (inset: nonimmune rabbit serum control);* **B:** *antibody to TGF-β2;* **C:** *antibody to TGF-β3-LAP (inset: antibody preincubated with immunizing peptide). TGF-β3-mature antibodies showed reactivity similar to that shown in* **(A)** *and* **(C)**. *TGF-β2 positivity is seen in the stroma in* **(B)**, *providing a positive internal control.* **D:** *Embryonal rhabdomyosarcoma with strap cells stained with TGF-β1 antibodies (inset: an area of normal skeletal muscle in this section shows weak staining). (Original magnification all panels ×320.)*

the other eight cases (Table 4, Figure 3, a and b). TGF-β3 generally colocalized with TGF-β1, except in case 2, which showed weak reactivity with TGF-β3 antibodies in areas showing no TGF-β1 reactivity. Ewing's sarcoma also had a variable reaction with anti-TGF-β1, reacting with four of seven tumors (Table 5, Figure 3c). TGF-β3 was detected in three of the six cases tested and did not consistently colocalize with TGF-β1 (Table 5).

Two cases of ectomesenchymoma containing

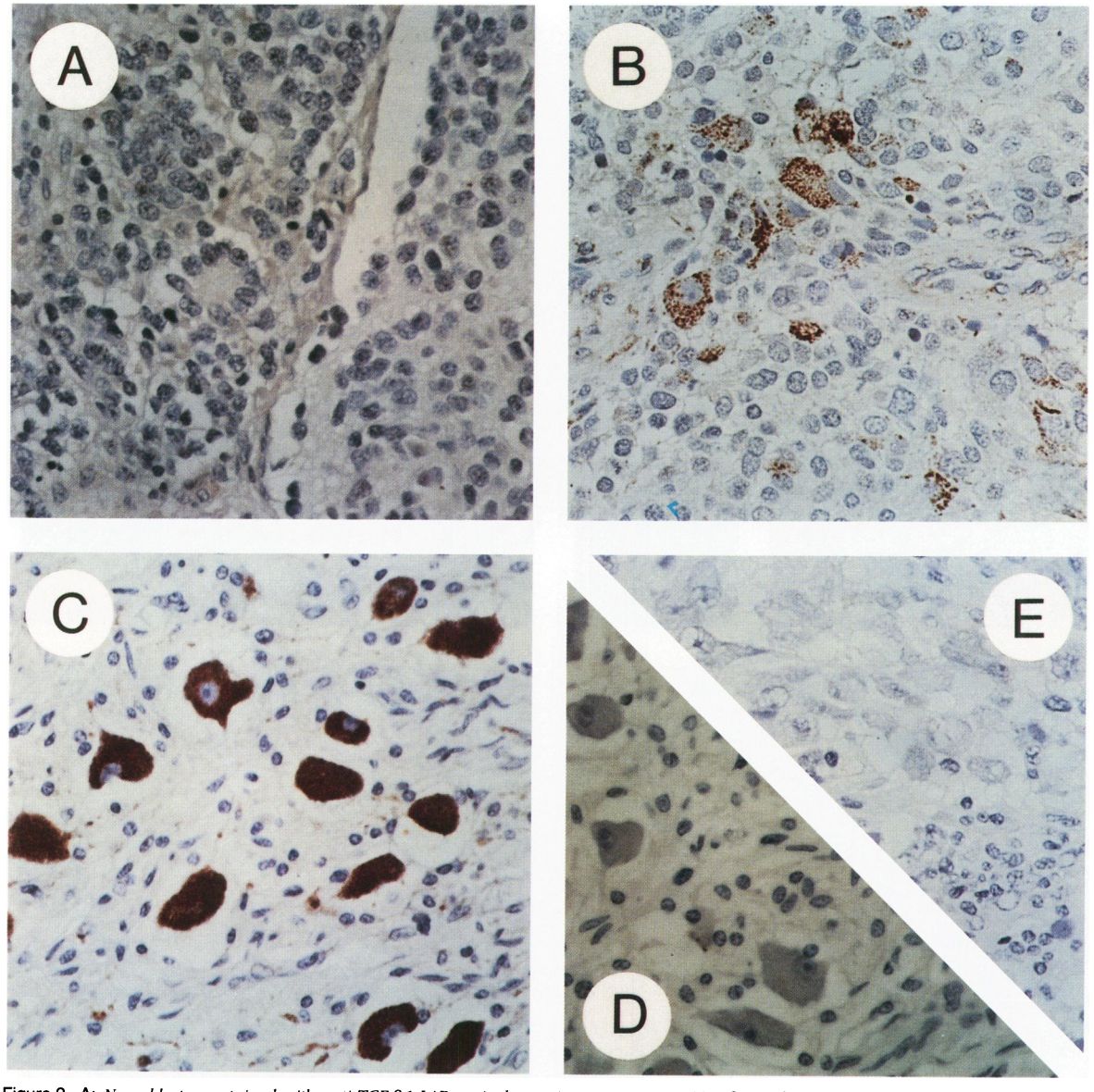

Figure 2. **A:** *Neuroblastoma stained with anti-TGF-β1-LAP: typical negative reaction. Anti-TGF-β2 and anti-TGF-β3-LAP gave similarly negative reactions.* **B:** *Ganglioneuroblastoma stained with anti-TGF-β1-LAP: the large differentiating cells show cytoplasmic positivity. A similar staining pattern was observed with anti-TGF-β3.* **C:** *Normal ganglion stained with anti-TGF-β1-LAP. The neurons are strongly positive. Anti-TGF-β3-LAP antibodies reacted similarly.* **D:** *Serial section of* (C) *stained with nonimmune rabbit serum.* **E:** *Serial section of* (B) *stained with anti-TGF-β1-LAP that had been preincubated with immunizing peptide. (Original magnification all panels, ×320).*

PNET and rhabdomyosarcoma components showed strong staining with anti-TGF-β1 (Table 6, Figure 3d). Although scattered negative tumor cells were present in areas, strong positive staining was seen in small poorly differentiated cells, as well as rhabdomyoblasts of these ectomesenchymomas. TGF-β3 colocalized with TGF-β1 in the one of these two lesions which was tested (case 1). Positive staining for TGF-β2 was also observed in this case. However, the reaction was seen only in the rhabdomyoblast component, similar to that observed in rhabdomyosarcoma case 4. The third case, which was composed of neuroblastoma and rhabdomyosarcoma, contained relatively weak cytoplasmic positivity for TGF-β1 in occasional cells. This tumor failed to react with TGF-β3 antibodies.

Discussion

The "small round cell" tumors of childhood may contain certain diagnostic features that are apparent on routine histological examination. However, they often exhibit a histological appearance so similar that a diagnosis can only be established with a battery of ultrastructural and molecular marker studies. Based on features shared with normal differentiated tissues,

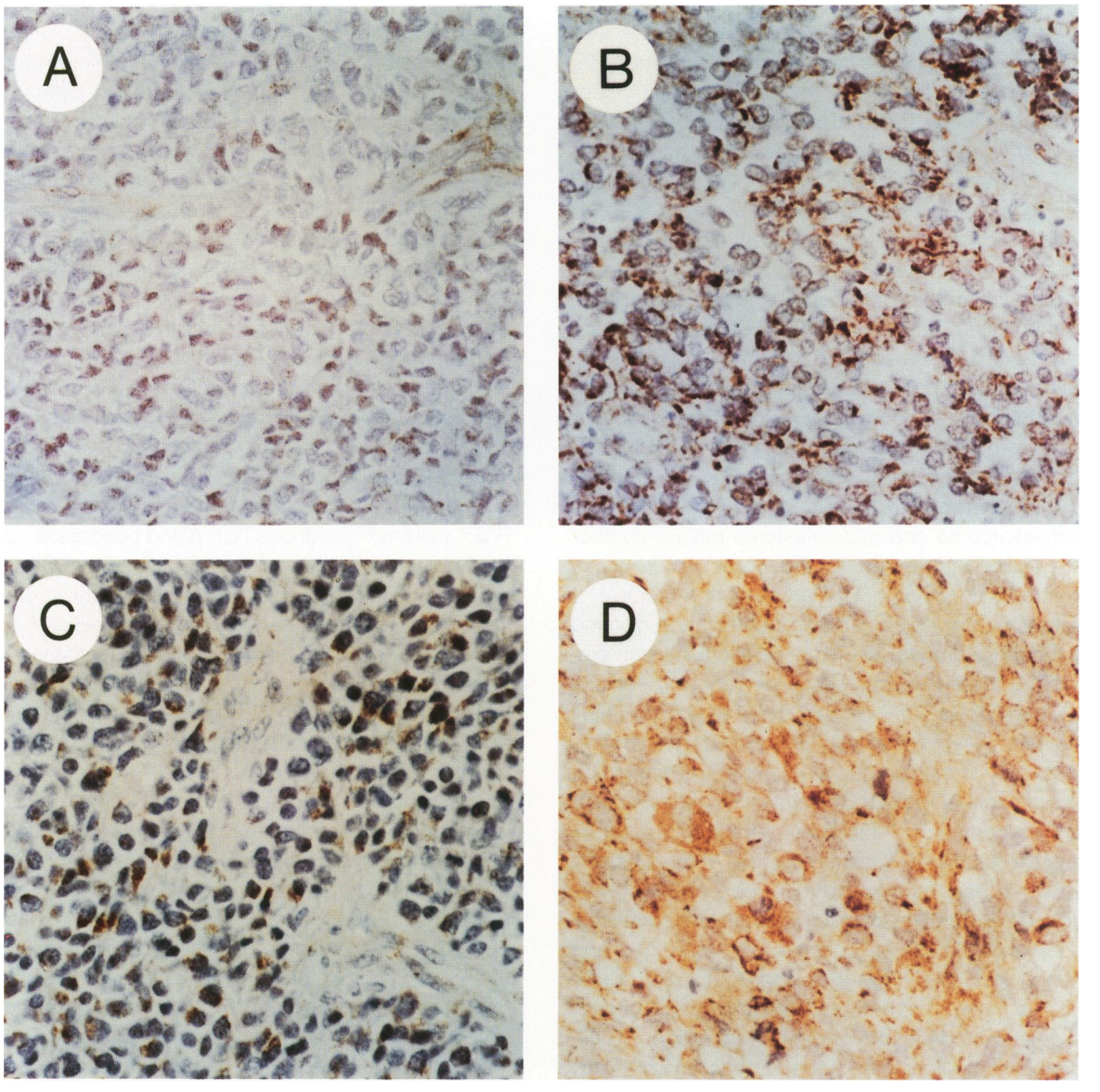

Figure 3. *TGF-β1 immunostaining in peripheral PNET* (A, B), *Ewing's sarcoma* (C), *and ectomesenchymoma* (D). A: *A weak nuclear staining pattern is present in this case.* B: *Strong cytoplasmic staining is present in this case. In addition, some cases were negative.* C: *An admixture of positive and negative tumor cells is present. Some cases were negative.* D: *Both small and large (rhabdomyoblast) cell components are positive in this PNET- and rhabdomyosarcoma-containing ectomesenchymoma. (Original magnification all panels, ×320.)*

such studies have provided clues as to the origins of these neoplasms. Neuroblastoma, primitive neuroectodermal tumor, and arguably Ewing's sarcoma are thought to arise from neural crest cells normally destined to become part of the peripheral nervous or neuroendocrine system. Rhabdomyosarcoma presumably arises from mesenchymal tissues thought to give rise to skeletal muscle. Ectomesenchymoma may show evidence of both neural and skeletal muscle differentiation, suggesting a multipotent primitive cell of origin or reflecting instability in the differentiation pathways. Because a greater degree of differentiation in these tumors is generally associated with a better prognosis, studies on factors that may affect growth and differentiation are of obvious relevance.

Although the present study does not demonstrate an effect of TGF-β on phenotype in these neoplasms, the results do show some distinct differences in expression at the protein level pointing to potential roles for this multifunctional cytokine. With rare exceptions, rhabdomyosarcomas and ectomesenchyomas were positive, and usually strongly so, for TGF-βs 1 and 3. In contrast, poorly differentiated neuroblastomas were negative. Immunoreactive TGF-β2 was detected only in two cases: one rhabdomyosarcoma and one ectomesenchymoma, and its distribution was limited to rhabdomyoblasts in these cases. While the general lack of TGF-β2 immu-

noreactivity does not necessarily indicate absence of the protein, it indicates at least that there is less TGF-β2 than in positive control tissues and suggests that expression of this isoform may be relatively suppressed in these tumors. The differentiating ganglion cells of ganglioneuroblastomas were positive for TGF-βs 1 and 3. Although the sample size was small (n = 3), all ectomesenchymomas contained TGF-β1-positive cells admixed with negative ones; this observation may have some significance with regard to the presence of both neural and skeletal muscle differentiation in these tumors. The staining pattern may be a function of the tumor cell types present, inasmuch as the neuroblastoma-containing lesion was predominantly negative and the PNET-containing ectomesenchymomas were predominantly, and strongly, positive for TGF-β1.

The patterns of expression of the TGF-βs in rhabdomyosarcoma and neuroblastoma raise a number of possibilities regarding their function in these tumors. TGF-β may act as an autocrine factor promoting the growth of rhabdomyosarcoma. Assuming rhabdomyosarcoma arises from skeletal muscle precursors, there is experimental evidence to support this hypothesis. TGF-β1 has been shown to inhibit the differentiation of myoblasts in culture.[20,21] This effect may in part be due to its demonstrated inhibition of skeletal muscle differentiation genes MyoD1 and myogenin.[22,23] Since most rhabdomyosarcomas express MyoD1 and myogenin,[2,24] this pathway would appear not to be operative. TGF-β1 has indeed been shown to inhibit myoblast differentiation independent of its effects on MyoD1 and myogenin.[23] Thus TGF-βs 1 and 3 may play a permissive role in rhabdomyosarcoma tumor growth through autocrine or paracrine inhibition of differentiation. Alternatively, rhabdomyosarcoma may arise from cells ordinarily inhibited by TGF-β; the neoplastic counterpart may have become refractory to its inhibitory effects.

TGF-β1 and 3 accumulation clearly is associated with differentiation in neuroblastoma. The functional significance of this accumulation is uncertain. Increased TGF-β expression may induce neuronal differentiation in neuroblastoma. Alternatively, increased TGF-β may accumulate as a consequence of neuronal differentiation. In this case, it may serve as an autocrine inhibitor of proliferation of the differentiated cells. The staining pattern observed in the normal ganglion suggests that accumulation of TGF-βs 1 and 3 is also associated with normal ganglion cell differentiation. Preliminary data indicate that the relationship between differentiation and TGF-β1 expression may be maintained *in vitro*: retinoic acid and phorbol esters, which induce differentiation in some cultured neuroblastoma cell lines,[25,26] induced TGF-β1 mRNA in the one such cell line tested (McCune BK, Tsokos M, unpublished data). The role of TGF-β in neuroblastoma differentiation is currently under investigation.

The TGF-β staining patterns were less consistent in PNET and Ewing's sarcoma. Although most PNETs were positive for TGF-β1 (80%), the staining intensity and distribution was highly variable. A lower proportion of Ewing's sarcoma (58%) stained positive for TGF-β1. No morphological or clinical differences were appreciated between positive and negative tumors. It seems paradoxical that PNET and Ewing's sarcoma, which rarely show differentiation, should frequently express TGF-β, when TGF-β expression in neuroblastoma is strongly associated with differentiation. Studies *in vitro* are very limited but a few relevant observations have been made. A673, a PNET cell line[27] produces TGF-β and possesses surface receptors but is not growth inhibited by TGF-β.[5] We have observed that the PNET cell line SK-N-MC, in contrast to neuroblastoma lines, expresses high basal levels of TGF-β1 mRNA; retinoic acid failed to induce differentiation in this line (McCune BK, unpublished data). TGF-β expression and differentiation may thus be uncoupled in the more primitive neural crest neoplasms.

The consistent colocalization of TGF-βs 1 and 3, but not TGF-β2, immunoreactivity in rhabdomyosarcomas and some of the other tumors suggests that control of TGF-β1 and 3 expression may be linked in these neoplasms. Coexpression of TGF-β1 and TGF-β3 mRNA, with much smaller amounts of TGF-β2 mRNA, has been observed in mesenchymal cell lines, indicating that this may be a common pattern.[8]

There have been a number of studies on other growth factors with direct or indirect relevance to small round cell tumors, particularly rhabdomyosarcoma and neuroblastoma. Several observations have suggested a role for basic fibroblast growth factor (bFGF) in regulation of myoblast differentiation. Like TGF-β, bFGF has been shown to inhibit myoblast differentiation and suppress myogenin and MyoD1 expression.[22] Furthermore, expression of acidic and basic FGF and FGF receptor decreases during differentiation of myoblasts.[28] Schweigerer et al demonstrated that bFGF produced by cultured rhabdomyosarcoma cell lines could stimulate proliferation of these cells and vascular endothelial cells, suggesting a role for basic FGF in the proliferation and neovascularization of rhabdomyosarcoma.[29] There are growing numbers of reports describing modulation of FGF activity by TGF-β and vice versa in a number of cell systems.[30–35] Insulin-

like growth factor (IGF)-II promotes growth and differentiation of myoblasts.[36] A study by El-Badry et al suggested that IGF-II could function as an autocrine growth and motility factor in rhabdomyosarcoma.[37] The net growth characteristics of rhabdomyosarcoma is thus likely to reflect in part the complex autocrine and paracrine interactions of FGF, TGF-β, and IGF-II and probably other cytokines. For example, it is conceivable that FGF and TGF-β could suppress the differentiation function of IGF-II, allowing its effect on proliferation to dominate.

Function of the nerve growth factor (NGF)/NGF-receptor system may be important in neuroblastoma. NGF induces the differentiation of neuronal stem cells and certain neuroblastoma cell lines *in vitro*.[38,39] In a recent study, transfection of NGF-receptor cDNA into a neuroblastoma cell line that did not previously respond to NGF, resulted in the establishment of a functional NGF/NGF-receptor system capable of inducing differentiation.[40] An inverse relationship has been observed between NGF receptor and *N-myc* expression in neuroblastoma cell lines and tumor tissues.[41,42] The association of *N-myc* amplification with poor prognosis, although not exclusive, has been well established.[1] It has been speculated that the presence of NGF receptors in neuroblastoma may be a favorable prognostic factor, reflecting a tumor derived from a later stage of neuroblast development than those without NGF receptors.[41–43] There is evidence that IGF-II may play a role in autocrine stimulation of cultured neuroblastoma cell growth.[44] In light of the increased TGF-βs 1 and 3 we have observed in ganglioneuroblastomas, it will be of great interest to determine the relationship between the TGF-βs, the NGF/NGF-receptor system and other ligand/receptor systems, and *N-myc* expression in these neoplasms.

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