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BRIEF REPORT

SOX10 Distinguishes Pilocytic and Pilomyxoid Astrocytomas From Ependymomas but Shows No Differences in Expression Level in Ependymomas From Infants Versus Older Children or Among Molecular Subgroups

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Abstract

SOX10 is important in nonneoplastic oligodendroglial development, but mRNA transcripts and protein expression are identified in a wider variety of CNS glial neoplasms than oligodendrogliomas. We previously demonstrated high levels of SOX10 mRNA and protein in pilocytic astrocytomas (PAs) but not ependymomas (EPNs). We now extend these studies to investigate subsets of these 2 tumors that affect infants, pilomyxoid astrocytomas (PMAs) and infant (<1 year) ependymomas (iEPNs). By gene expression microarray analysis, we found that iEPNs and all EPNs in older children showed very low SOX10 expression levels, on average 7.1-fold below normal control tissues. EPN groups showed no significant difference in SOX10 expression between iEPN and EPN. PAs/PMAs had 24.1/29.4-fold higher transcript levels, respectively, than those in normal tissues. Using immunohistochemical analysis of adult, pediatric, and infantile EPNs and of PAs/PMAs, we found that EPNs from multiple anatomical locations and both age groups (n = 228) never showed 3+ diffuse nuclear immunostaining for SOX10; the majority were scored at 0 or 1+. Conversely, almost all pediatric and adult PAs and PMAs (n = 47) were scored as 3+. These results suggest that in select settings, SOX10 immunohistochemistry can supplement the diagnosis of PMA and PA and aid in distinguishing them from EPNs.

Key Words: Developmental, Gene expression microarray, Infant ependymoma, Myxopapillary ependymoma, Pilocytic astrocytoma, Pilomyxoid astrocytoma, SOX10.

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INTRODUCTION

SOX10, a developmentally regulated transcription factor that interacts with Olig 2, is important in nonneoplastic oligodendroglial development (1). mRNA transcripts and protein expression are, however, identified in a wider variety of CNS glial neoplasms than oligodendrogliomas (2–6). We have previously shown by gene expression microarray and Western blot analyses that high levels of SOX10 mRNA and protein exist in pilocytic astrocytomas (PAs), but not ependymomas (EPNs) (6). We now extend our studies to investigate subsets of PAs and EPNs known to affect infants, specifically, pilomyxoid astrocytomas (PMAs) and ependymomas in infants (iEPNs) who are <1 year of age. All iEPNs were posterior fossa group A (7). Group A EPN patients in general are younger, have tumors more frequently located in the cerebellopontine angle (67% vs 5%) and they experience a higher recurrence rate (56% vs 25%), metastasis at recurrence, and death (35% vs 5%) compared to group B patients (7, 8). In particular, iEPNs appear to have a worse prognosis than EPNs in older children, with 26% survival vs 63% in older children (2–3 years) over the same follow-up time period (9). Thus, specific attention to the biological factors important in the iEPN subset may be warranted. We hypothesized that SOX10 transcripts might be lower in these generally more aggressive tumors that affect infants.

MATERIALS AND METHODS

Gene Expression Microarray

Ten PMAs and 6 iEPNs were compared with 21 pediatric PAs and 52 pediatric EPNs including both groups A and B, supratentorial and myxopapillary ependymomas (MPE) in children (>1 year) and 27 normal control tissue samples using gene expression microarray analysis (Affymetrix HG-U133plus2, Santa Clara, CA), as described (6).

Immunohistochemistry

Recognizing the diagnostic potential for distinguishing PA/PMA from EPN in clinical practice (particularly in small

biopsies from eloquent areas such as the brainstem or spinal cord), we applied SOX10 immunohistochemistry (IHC) to PMAs, PAs, iEPNs, and EPNs from various anatomical sites. We included adult EPNs, subependymomas, MPEs, and PAs to broaden the application beyond pediatric EPNs; we did not have sufficient numbers of adult (>18 years) PMAs to provide meaningful data. SOX10 IHC (Cell Marque, Rocklin, CA, Catalog # 383A-78, predilute, multimer staining kit from Ventana, amplification but no blocker) was performed on Ventana Benchmark equipment.

We first utilized whole slide sections so that distribution of immunoreactivity could be better assessed; specifically, this also allowed for better appreciation of the edges of EPNs where normal nonneoplastic cells might be included in the otherwise usually sharply demarcated tumors. It also made available adjacent nervous system tissues that served, in many cases, as an internal control of antigen fidelity.

For whole slide assessment, 23 adult EPNs of different types, grades, and anatomical locations and 14 pediatric EPNs of different grades and from different ages were assessed. Cerebral or cerebellar cortex, where included, showed a moderate number of cells with immunoreactivity, with IHC (+) cells corresponding in size and location to oligodendroglial cells in the tissue (Fig. 1b). Neurons, endothelial cells, and cells within choroid plexus and leptomeninges were negative, as expected. Nerve, when rarely included in spinal cord specimens, was positive, as previously described (4). Control tissues from other organs were also assessed, including lymph node, liver, gastrointestinal tract, and muscle, and were all completely devoid of cells with nuclear immunoreactivity.

IHC Scoring

Only nuclear immunoreactivity was scored. Scoring incorporated counts in several microscopic fields, that is, was not confined to a single high-power microscopic field. IHC was scored as zero (0) if no cells were positive (Fig. 1b), or if <1% of cells located within the tumor showed nuclear immunoreactivity.

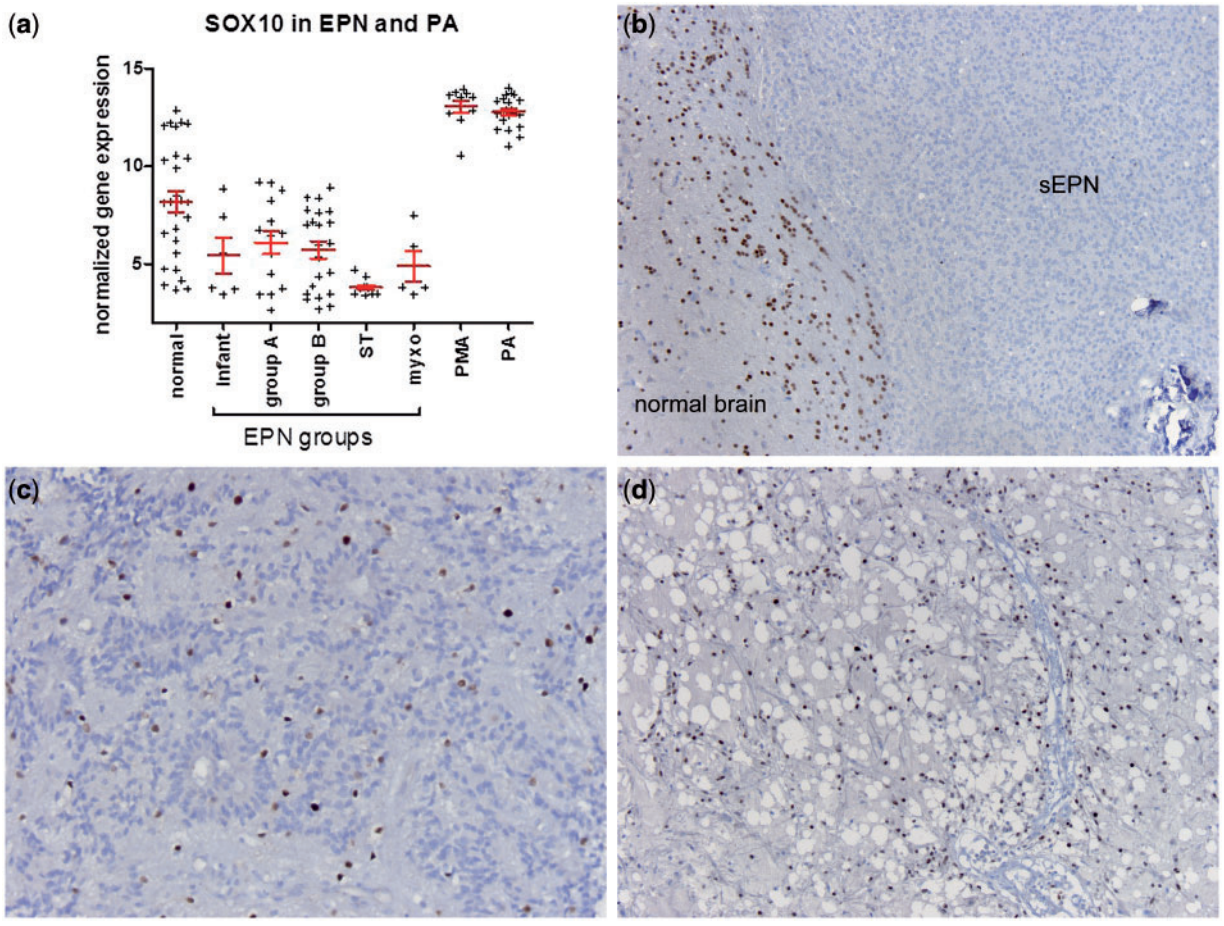

FIGURE 1. (a) Gene expression levels for SOX10 comparing pediatric ependymomas (EPN) and pilocytic astrocytomas (PA) (group A and B, ependymoma subtypes; ST, supratentorial; myxo, myxopapillary ependymoma; PMA, pilomyxoid astrocytoma). **(b)** SOX10 in normal brain (cerebral cortex, left) compared to a completely immunonegative (score 0) supratentorial ependymoma; note the sharp demarcation of the tumor and the focal calcification (lower right). **(c)** Infant ependymoma with 1+ nuclear SOX10 immunoreactivity; positive cells in 1+ tumors were inevitably individual, scattered cells. **(d)** Pilomyxoid astrocytoma with 3+, diffuse strong nuclear SOX10 immunoreactivity. **b–d**, SOX10 immunohistochemistry with light hematoxylin and eosin counterstain. Magnifications: **b**, 40X; **c**, 200X; **d**, 100X.

TABLE. Summary of SOX10 Immunohistochemistry Results

Score	0	1+	2+	3+
Pediatric ependymomas (n = 50)	35	14	1	0
Infant ependymomas (n = 6)	1	3	2	0
Adult ependymomas (supratentorial + spinal cord, including myxopapillary, subependymoma) (n = 172)	159	13	0	0
Pediatric pilocytic astrocytoma (n = 33)	2	0	0	31
Pediatric pilomyxoid astrocytoma (n = 7)	0	0	0	7
Adult pilocytic astrocytoma (n = 7)	1 (focally 3+)	0	0	6

Data are from combined whole slide and tissue microarray results.

Very rare (<1% overall) IHC-positive cells confined to the extreme edge/perimeter of tumor were not counted, especially if adjacent normal nontumorous CNS tissue was present in the specimen because these cells might represent entrapped normal SOX10 IHC-positive cells. Tumors were scored as 1+ if 1% to 15% of cells were positive within tumor; these were inevitably individual scattered cells (Fig. 1c). Tumors with 16% to 75% IHC-positive cells were scored as 2+, including those cases with weak IHC positivity. Those with >75% IHC-positive cells were scored as 3+ and additionally had to show strong IHC signal and diffuse distribution (Fig. 1d).

To expand our numbers, tissue microarrays (TMAs) of pediatric intracranial EPNs (n = 42), prepared at the University of California San Francisco (UCSF), were immunostained.

RESULTS AND DISCUSSION

The iEPNs and all EPNs in older children (supratentorial, MPEs, posterior fossa EPNs) showed very low SOX10 expression levels on average 7.1-fold below normal control tissues ($p = 8.0 \times 10^{-7}$). EPN groups showed no significant difference in SOX10 expression between iEPNs and older pediatric EPNs. PAs/PMAs had 24.1-fold ($p = 1.4 \times 10^{-5}$) and 29.4-fold ($p = 6.1 \times 10^{-9}$) higher transcript levels, respectively, than normal tissues. Gene expression level results are diagrammatically shown in Fig. 1a.

SOX10 IHC has shown utility in diagnosis of soft tissue and melanocytic neoplasms (10–12), as well as in diagnosis of granular cell tumors (13) (albeit not of utility in CNS sites (14)). Thus, the widespread application of this antibody and use in general surgical pathology laboratories, and not simply neuropathology laboratories, made this a particularly attractive antibody to investigate. We are aware of only 1 other study that investigated SOX10 IHC in EPNs and then only in conjunction with Olig 2 and without specific details as to the skew of ages and anatomical locations of the cohort (15).

Six of 7 anaplastic EPNs (ages 2–16 years, WHO grade III) were scored as 0, with 1 as 1+. One grade II EPN (spinal cord) was scored as 1+. Six iEPNs (ie, 6 of the same iEPNs used the gene expression assay) on whole slide assessment showed a wider range of scores, with 2 at 2+, 3 at 1+, and 1 at 0. However, both 2+ tumors reached this score only focally within definite tumor; both reached the 20% level of scoring in these highest scoring areas, that is, they did not approach the upper limits of the 2+ bracket of 50% to 75% in any area.

Five of 6 spinal cord EPNs (ages 36–71 years, WHO grade II) were scored as 0, with 1 scored at 1+. Five of 6 adult MPEs (ages 33–70 years, WHO grade I) were scored as 0, with 1 scored as 1+. Four of 5 adult subependymomas (ages 22–52 years, WHO grade I) were scored as 0, with 1 as 1+. Three of 3 adult 4th ventricle EPNs (ages 54–65 years, WHO grade II), 1 adult anaplastic 4th ventricle EPN (age 22, WHO grade III), 1 adult left frontal lobe EPN (age 39, WHO grade III), and 1 adult temporal lobe EPN (WHO grade II) were all scored as 0.

TMAs of adult intracranial EPNs (n = 42), also prepared at UCSF, were immunostained. All 33 EPNs and 8 of 9 subependymomas were scored at 0, with 1 subependymoma at 1+. TMAs of adult spinal EPNs (n = 107) containing a mixture of EPNs, MPEs, and subependymomas were immunostained, with 98 scored at 0 and 9 at 1+.

For pediatric PAs (ages 2–12 years, 8 of 11 showing *KIAA1549: BRAF* fusion; 1 noninformative, 1 spinal cord example negative), 11 of 11 whole slide sections were scored at 3+. For PMAs, 7 of 7 whole slide sections were scored at 3+; these were all well characterized, previously published, and illustrated examples (16). For adult PAs (ages 18–33 years), 6 of 7 whole slide sections were scored at 3+, with 1 showing smaller tissue fragments at 3+ but a larger fragment of tumor at 0; tissue fixation issues were suspected.

Twenty-nine of 42 EPNs were scored at 0, 12 at 1+, and 1 at 2+. TMAs of pediatric PAs from UCSF (all <age 18 years, most posterior fossa) were also utilized and 20/22 scored at 3+, with 2 at score 0.

In summary, 56 total pediatric EPNs (combining pediatric and infant, whole slide and TMA data), and 172 adult EPNs (whole slide + TMA) from multiple anatomical locations (n = 228) never showed 3+ diffuse nuclear IHC positivity for SOX10; the overwhelming majority scored at 0 or 1+ (Table). Although a small percentage of EPNs, particularly pediatric and infant EPNs, showed 2+ scores, the IHC positivity always consisted of individual scattered immunopositive cells. Conversely, 11 whole slide pediatric PAs, 22 pediatric PAs on TMA, 7 pediatric PAs, and 7 whole slide adult PAs (n = 47) overwhelmingly were scored at 3+.

We conclude that diffuse strong 3+ nuclear SOX10 IHC + distinguishes PA and PMA from EPN but does not differentiate infantile subtypes (PMA, iEPN) from related tumors (PA, EPNs) in older children.

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