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Monoclonal Antibodies to a Rat Nestin Fusion Protein Recognize a 220-kDa Polypeptide in Subsets of Fetal and Adult Human Central Nervous System Neurons and in Primitive Neuroectodermal Tumor Cells

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Nestin is the major intermediate filament protein of embryonic central nervous system (CNS) progenitor cells. To identify proteins involved in early stages of lineage commitment in the developing human CNS we generated monoclonal antibodies to a TrpE-rat nestin fusion protein. This resulted in a monoclonal antibody (designated NST11) that did not recognize authentic human nestin, but did recognize a novel neuron-specific human polypeptide expressed in a subset of embryonic and adult CNS neurons as well as in medulloblastomas. NST11 immunoreactivity was abundant in developing spinal cord motor neurons, but was extinguished in these neurons by 17 weeks gestation. NST11 also labeled Purkinje cells at 17 weeks gestation, but Purkinje cells continued to express the NST11 antigen throughout gestation as well as in the adult cerebellum, and NST11 immunoreactivity was more abundant in Purkinje cells than in any other human CNS neurons. No NST11 immunoreactivity was detected in cells of the adult human peripheral nervous system or in a variety of adult non-neural human tissues. Further, NST11 almost exclusively stained cerebellar medulloblastomas. In Western blots of immature and mature human cerebral and cerebellar extracts, NST11 did not bind human nestin, but did detect an immunoband with a molecular weight of 220 kd. A similar immunoband was detected in medulloblastoma-derived cell lines with a neuron-like phenotype. These findings suggest that the NST11 monoclonal antibody recognizes a novel protein expressed by a subpopulation of immature and mature human CNS neurons, medulloblastomas, and medulloblastoma-derived cell lines. (Am J Pathol 1993, 143:258–268)

Intermediate filaments (IFs), the 10- to 12-nm diameter filaments found in the cytoplasm and nucleus of most mammalian cells, assemble from proteins that belong to a large family of related polypeptides.[1–3] Based on the number and position of the exons and introns in their genes, six different classes of IF subunits are recognized. The precise biological functions of each IF class is uncertain, but the IFs of keratinocytes (heterodimers of class I and II IF proteins) are essential for the integrity of the epidermis,[4] and neurofilament (NF) subunits (a triplet of class IV IF proteins in neurons) regulate axon diameter.[2,5]

Nestin is a newly described IF protein that appears briefly in developing central nervous system (CNS) progenitors and astrocytes.[6] Based on a rat complementary DNA, nestin was designated a unique class VI IF protein.[6–12] To characterize human nestin, we used an antiserum (i.e., anti-nestin 129) raised to a TrpE-rat nestin fusion protein to probe the expression of nestin in the developing human CNS and in human brain tumors.[11] Here we describe the generation of

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monoclonal antibodies (MAbs) to a TrpE-rat nestin fusion protein to identify IF proteins expressed early in the embryonic human CNS. We obtained a new MAb (designated NST11) that did not bind human nestin, but recognized a novel, developmentally regulated, 220-kd protein expressed in a subset of human CNS neurons, cerebellar medulloblastomas, and medulloblastoma-derived cell lines.

Materials and Methods

Generation and Screening of MAbs Raised to a TrpE-Rat Nestin Fusion Protein

The TrpE-rat nestin fusion protein was generated and expressed in *Escheria coli* using a λgt10 401:16 clone and the pATH1 vector[13] as described.[11,12] The TrpE protein was fused to the last 1,197 amino acids of rat nestin and had an apparent molecular weight (M_r) of about 240 kd in sodium dodecyl sulfate (SDS) polyacrylamide (6%) gel electrophoresis (PAGE) gels. The fusion protein was purified by electro-elution, solubilized in phosphate-buffered saline, and used to immunize BALB/c mice and produce hybridomas as reported.[14–16] Hybridoma supernatants were screened by enzyme-linked immunosorbent assay (ELISA) using the nestin fusion protein and then characterized further by Western blots using the same fusion protein as well as homogenates of rat and human CNS tissues. Additionally, lysates of *E. coli* that contained the pATH1 vector alone were used as negative control samples for ELISA and Western blots. One MAb (designated NST11) was produced and characterized in detail because it recognized a novel neuron-specific polypeptide in human CNS neurons and brain tumors.

Human CNS Tissues and Neural Cell Lines

Twelve samples of fetal human brain and spinal cord without evidence of abnormalities were used in these studies.[17] The gestational age (GA) of these samples was 6, 7, 8, 11, 17, 19, 20, 23, 30, and 39 weeks, and another sample came from a term fetus.[17] The tissue were obtained following a postmortem interval (PMI) of 1 to 11 hours. Seven of these cases were used for biochemical and immunochemical studies, and five were used for immunohistochemical analysis. Samples from the latter cases were fixed in 70% ethanol containing 150 mmol/L NaCl for 24 to 36 hours and then embedded in paraffin as described.[16] Paraffin sections (6 μ thick) cut from the cervical spinal cord of 5 of these cases (i.e., those with a GA of 6, 11, 17, and 20 weeks as well as from the term fetus) were affixed to glass slides with poly-L-lysine for immunohistochemical analysis.[11,17,18] Adult human postmortem tissue samples of the CNS, muscle, esophagus, stomach, intestine, lung, liver, kidney, spleen adrenal gland, pituitary gland, thyroid gland, and trigeminal ganglia were obtained, fixed, and prepared in the same manner for immunohistochemistry. The PMI of these cases ranged from 5 to 12 hours. Additionally, samples of developing rat cerebellum at postnatal day 6 were obtained and processed in the same manner. These studies were approved by the University of Pennsylvania Committee for the Study of Human Beings and the Committee on the Use of Experimental Animals.

Six well-characterized human medulloblastoma cell lines (i.e., D283 Med, D341 Med, D384 Med, D425 Med, D458 Med, and DAOY), a human cerebral neuroblastoma cell line (CHP707m), and a human malignant glioma cell line (U251 MG) were studied.[19–28] Further, neurons (NT2N cells) induced to differentiate in culture from a human teratocarcinoma cell line (Ntera2) with retinoic acid also were examined.[29] The cell lines were maintained as described,[11,23,25,29] and cell pellets (about 10^8 cells) were fixed, embedded in paraffin, and sectioned for immunocytochemistry as described.[11,23] Alternatively, some of the cell lines were grown on coverslips and examined by immunocytochemistry (see below). Finally, primary cultures of neonatal rat Scwhann cells were prepared as described[30] for immunocytochemical analysis.

Immunohistochemical studies also were performed on 33 samples of human CNS tumors including 15 primitive neuroectodermal tumors, 14 of which were cerebellar medulloblastomas. Other tumors included six astrocytomas, six ependymomas, and six other neuroepithelial tumors. Most of this tissue was obtained at the time of surgical excision, but three samples were harvested at autopsy. The assignment of these tumors to specific diagnostic categories was based on criteria outlined earlier.[11,31–33] These samples were snap-frozen at −20 C in OCT and 6- to 8-μ thick frozen sections were cut, attached to poly-L-lysine-coated slides, and fixed in cold acetone at −20 C for 10 minutes as described.[11,31] Further, closely adjacent tissue from 10 cases were fixed in ethanol, paraffin-embedded, and sectioned for immunohistochemical analysis.

Immunohistochemical and Immunocytochemical Procedures

Paraffin and frozen sections of tissues and cells as well as cells grown on coverslips were probed with MAb supernatants using previously described methods.[11,14–19,29,31,34] Spent supernatant from unfused, nonsecreting mouse myeloma cells (designated SP2) was used as a negative control. Also, other MAbs produced to the same nestin fusion protein that recognized this immunogen in Western blots and ELISA, but failed to stain tissues or cells by immunohistochemistry, were used as negative controls. Positive control tissues included frozen and paraffin-embedded sections of rat cerebellum and human fetal and adult cerebellum, and positive control MAbs included several antibodies in our library of anti-NF protein MAbs.[14,15]

SDS-PAGE and Western Blot Analysis

To identify proteins recognized by NST11, samples of the cell lines as well as of the postnatal day 6 rat cerebellum and human postmortem cerebrum, cerebellum, and spinal cord from adult (n = 5) and fetal (n = 7) cases were subjected to SDS-PAGE and Western blot analysis.[11,14–17,25–27,29] The PMI of the adult human cases ranged from 5 to 11.5 hours, whereas the PMI of the human fetal cases ranged from 1 to 11 hours. Fresh or frozen (stored at −70 C until analyzed) rat tissues and human postmortem samples were used for biochemical and immunochemical studies. Embryos at 6, 7, and 8 GA weeks were homogenized in 2 to 5 times volume of hot Laemmli sample buffer, whereas cells (approximately 3 to 5 × 10^6) and other tissue samples (approximately 50 mg wet weight) were homogenized in 5 to 10 times volume of hot Laemmli sample buffer. To enrich for cytoskeletal proteins, cells or tissue were homogenized in 10 times volume of cold cytoskeletal extraction buffer (0.1 mol/L MES, 1% Triton X-100, 170 mmol/L NaCl, 0.5 mmol/L, EGTA, 1 mmol/L dithiotreitol 2 mol/L sucrose, 50 units/ml of DNAse I at pH 6.8) including protease inhibitors (i.e., 0.5 mmol/L PMSF, TPCK, TLCK, soybean trypsin inhibitor, pepstatin A, leupeptin) and the homogenates were incubated on ice for 15 minutes. The insoluble cytoskeletal proteins were pelleted at 100,000g for 30 minutes at 4 C, and the pellet was solubilized in 2 times volume of hot Laemmli sample buffer. Proteins in the soluble fraction were precipitated with cold methanol at −20 C for 2 hours, centrifuged at 100,000g for 30 minutes, and the precipitated pellet was solubilized in hot Laemmli sample buffer. SDS-PAGE (6% polyacrylamide) and Western blot analyses were performed as described.[11,14,15,25–27,29]

Results

Recognition of the TrpE-Rat Nestin Fusion Protein by the NST11 MAb

The NST11 MAb and three other MAbs (NST2, NST4, NST6) generated with the nestin fusion protein recognized this fusion protein in ELISA and Western blots (Figure 1), but none reacted with *E. coli* proteins from bacteria containing only the pATH1 vector (data not shown). These MAbs showed the same reaction pattern for the nestin fusion protein as the Rat-401 MAb (which was used to identify rat nestin)[6] and the nestin-129 antiserum.[11,12] However, none of the MAbs recognized recently characterized human nestin[11] (data not shown), and only NST11 recognized a human protein and stained human CNS neurons (see below). Because the NST6 MAb resembled the Rat-401 MAb,[7,8,35] whereas neither NST2 or NST4 recognized proteins in homogenates of rat or human cells and tissues (data not shown), we characterized the protein recognized by NST11.

Developmentally Regulated Expression of the NST11 Antigen in Human Spinal Cord

The NST11 antigen was abundant already at a GA of 6 weeks in the developing cervical spinal cord

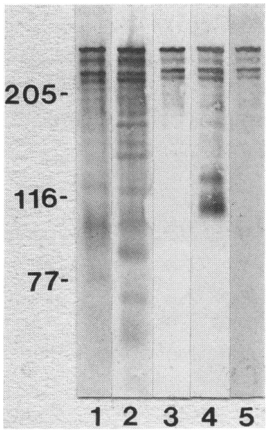

Figure 1. *Western blots of the E. coli-derived TrpE-rat nestin fusion protein probed with Rat-401* (lane 1), *the anti←nestin-129 antiserum* (lane 2), *and three of the MAbs described here, i.e., NST4* (lane 3), *NST6* (lane 4), *and NST11* (lane 5). *The high M_r immunobands (i.e., those >200 kd) recognized by these antibodies are the same, and the immunobands below 240 kd probably represent breakdown products of the TrpE-rat nestin fusion protein because none of these antibodies recognized proteins in extracts of* E. coli *containing only the pATH1 vector (data not shown). The position of M_r markers is shown to the left of* lane 1. *See text for additional details.*

where the NST11 MAb intensely stained immature neurons in the mantle layer (Figure 2A), but did not stain the neuroepithelial layer containing mitotically active multipotential progenitor cells. This contrasts with our previous studies of these same tissue samples using the nestin-129 antiserum, which demonstrated abundant nestin immunoreactivity throughout the neuroepithelial layer.[11] Also, NST11 did not stain radial glial in the mantle and marginal layers like the nestin-129 antiserum. At 11 weeks GA, spinal cord motor neurons were weakly immunoreactive with NST11 compared to the 6-week GA spinal cord (Figure 2B). At 17 weeks GA and beyond, there was no evidence of NST11 immunoreactivity in the spinal cord (data not shown).

NST11 Immunoreactivity in Human Brain and Peripheral Tissues

In the developing 17-week GA human cerebellum, NST11 stained immature neurons in the nascent Purkinje cell layer before Purkinje cell dendrites were evident (Figure 2C). In the term cerebellum, NST11 staining was seen in Purkinje cell bodies as well as in their simplified apical dendrites (Figure 2D). In adult human cerebellum, the NST11 MAb labeled Purkinje cells and their dendrites very intensively (Figure 2, E and F). The primary dendrites and the secondary and tertiary branches of adult Purkinje cells were especially prominent in NST11 stained preparations, but NST11-positive Purkinje cell axons were also noted. Aside from glomerular structures and a small subset of white matter axons, no other structures were NST11-positive in the adult cerebellum. An immunohistochemical survey of normal adult human cerebral cortex, basal ganglia, amygdala, hippocampus, and brain stem with the NST11 MAb documented the weak expression of the NST11 antigen in a subset of neurons in these regions. For example, neocortical neurons were weakly labeled by NST11 (Figure 2G), and in the hippocampus, NST11 stained the neuropil in the inner third of the dentate molecular layer (Figure 2H). Among the normal adult peripheral organs examined with NST11, all (i.e., muscle, stomach, intestine, lung, liver, kidney, spleen, adrenal gland, thyroid gland) were negative with the exception of the pituitary gland, which contained NST11-positive endocrine cells in the anterior lobe (data not shown). Thus, we conclude that the NST11 antigen is restricted in fetal and adult human tissues almost exclusively to neurons of the CNS. Further, the NST11 antigen seemed to be expressed by only a subset of large pyramidal neurons of the adult human telencephalon and cerebellum.

NST11 Recognizes a 220-kd Protein in the Fetal and Adult Human CNS

The identity of the NST11 antigen in the adult and fetal human CNS was characterized further by using the NST11 MAb to probe homogenates of various tissues. In immunoblots of homogenates of embryos with a GA of 6, 7, and 8 weeks, NST11 labeled an immunoband with an M_r of about 220 kd (see lanes 2, 3, and 4 of Figure 3A) that is distinct from human nestin.[11] Presumably, the lower M_r immunobands represent breakdowns products of the NST11 antigen. In immunoblots of cytoskeletal extracts of adult human cerebellum, NST11 identified an immunoband (Figure 3, B and C, lanes 1 and 2) with an M_r of 220 kd, but no immunobands were detected with NST11 in similar extracts of adult human spinal cord (Figure 3, B and C, lanes 3 and 4). Although the cytoskeletal extracts contained a number of other cytoskeletal proteins such as NF, vimentin, and glial fibrillary acidic proteins GFAPs; (see Coomassie blue stained gel in Figure 3B), the NST11 MAb did not cross react with these or other polypeptides in extracts. When nitrocellulose replicas of SDS-PAGE gels containing both Triton X-100 soluble and insoluble adult cerebellar extracts were probed with the NST11 MAb, the 220-kd immunoband described above partitioned with the insoluble material, although a similar 220-kd immunoband was detectable in the soluble fraction (data not shown). Cytoskeletal extracts of developing human cerebrum and cerebellum from four cases with GAs of 19, 20, 30, and 39 weeks revealed a single NST11-labeled immunoband with a M_r of 220 kd. Figure 3 (see D, lanes 1 to 4) shows representative immunoblots from two different samples from cases with a GA of 19 and 30 weeks. Because NST11 immunoreactivity remained robust in Purkinje cells of the fully mature cerebellum (see Figure 2, C and D), the NST11 immunobands detected throughout gestation reflect the continued expression of the NST11 antigen by this class of cerebellar neurons. Although the protein recognized by NST11 seemed to be similar to nestin with respect to its immunological properties and its high M_r, this protein was relatively stable at PMIs of approximately 5 hours, but, like nestin,[6,11,12] it was more labile than vimentin and the NF triplet proteins. For example, the NST11-reactive immunoband degraded rapidly (see Figure 3A), even when solubilized in SDS, if the samples

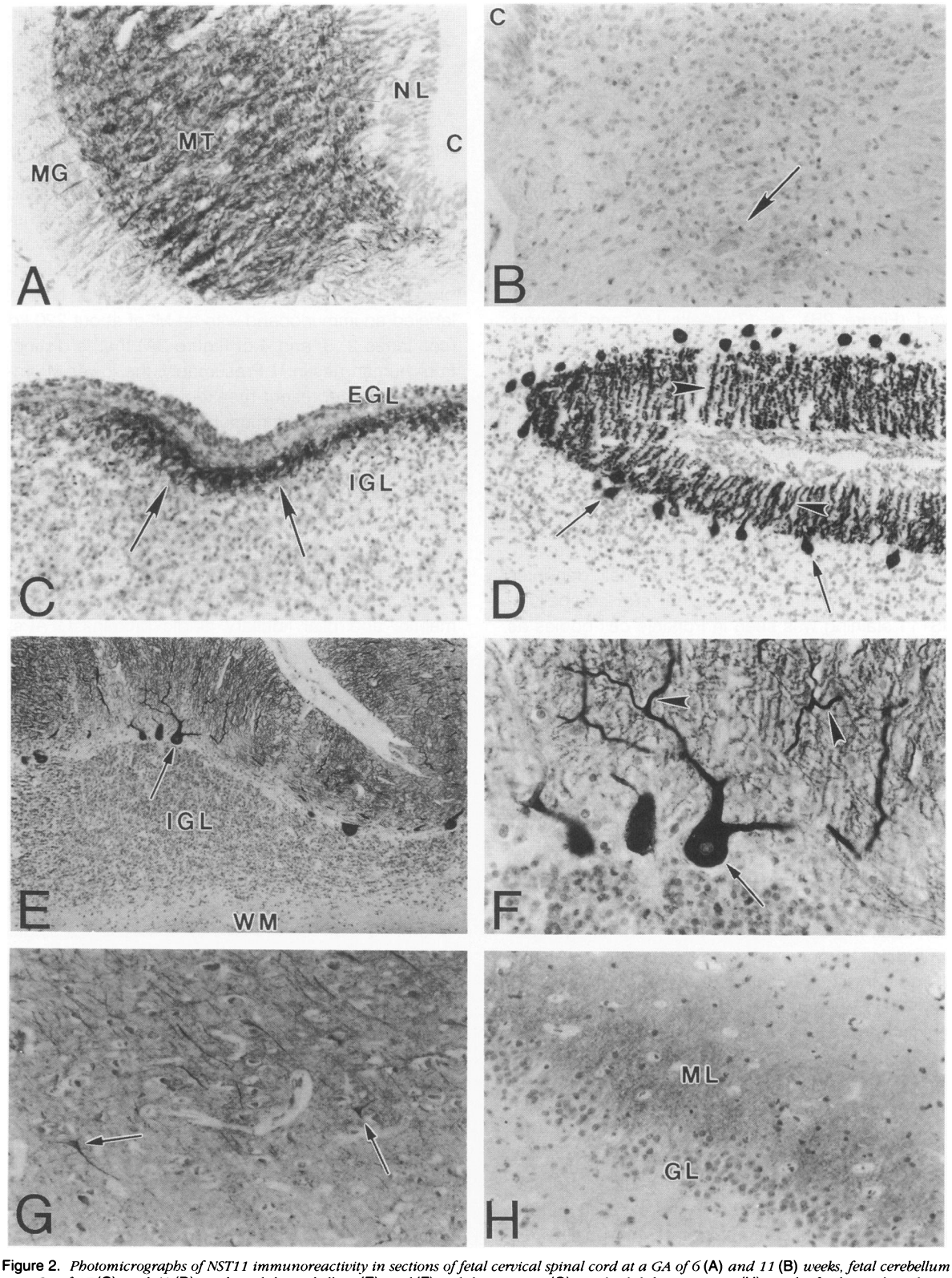

Figure 2. *Photomicrographs of NST11 immunoreactivity in sections of fetal cervical spinal cord at a GA of 6* (A) *and 11* (B) *weeks, fetal cerebellum at a GA of 17* (C) *and 40* (D) *weeks, adult cerebellum* (E) *and* (F), *adult neocortex* (G), *and adult hippocampus* (H). *In the fetal spinal cord at a GA of 6 weeks* (A), *NST11 intensely stains the cell bodies of immature neurons in the mantle layer (MT) and neuronal processes in the marginal layer (MG). No other cells, including those in the neuroepithelial layer (NL) lining the central canal (C), are NST11-positive. By 11 weeks GA* (B), *only weak NST11 immunoreactivity is seen in neurons of the mantle layer (arrow). In the fetal cerebellum at a GA of 17 weeks* (C), *NST11 immunoreactivity is intense in the nascent Purkinje cell layer (arrows) before Purkinje cells have extended apical dendrites or assumed their distinctive*

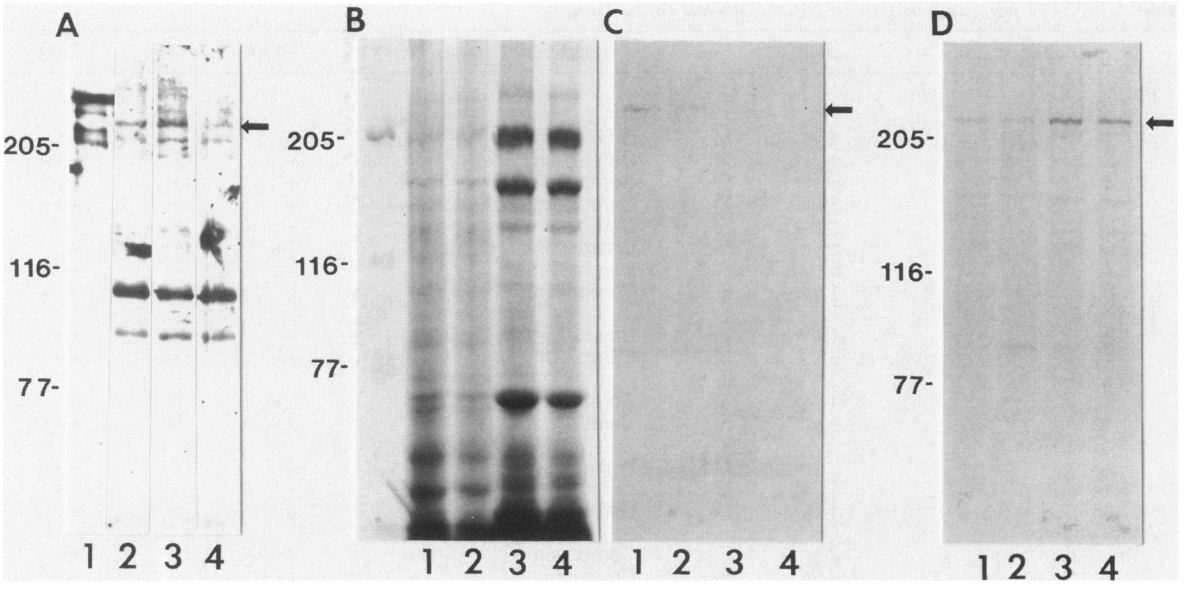

Figure 3. *Biochemical and immunological properties of the NST11 antigen. In* A, lanes 2, 3, and 4 *show the 220-kd immunoband stained by NST11 in homogenates of human embryos at a GA of 6, 7, and 8 weeks, respectively, whereas* lane 1 *is a control lane containing the TrpE-rat nestin fusion protein. The nitrocellulose replicas probed with NST11 in* A *were generated from 6% SDS polyacrylamide gels.* Lanes 2 *to* 4 *were loaded with approximately 200 μg of protein.* B *shows a representative Coomassie blue stained 6% SDS polyacrylamide gel similar to those used to analyze proteins in homogenates of normal human CNS tissues. Cytoskeletal proteins (approximately 200 μg in* lanes 1 *and* 3 *or about 100 μg in* (lanes 2 *and* 4) *extracted from adult cerebellum* (lanes 1 *and* 2) *or from adult spinal cord* (lanes 3 *and* 4) *were loaded in the gel shown in* B. C *shows a representative nitrocellulose replica of a gel like that shown in* B *that was probed with the NST11 MAb.* Lanes 1 *to* 4 *in* C *correspond to* lanes 1 *to* 4 *in the gel in* B. *NST11 immunobands are present in adult cerebellum* (lanes 1 *and* 2), *but not in the adult spinal cord* (lanes 3 *and* 4). D *illustrates a representative Western blot of cytoskeletal protein extracts from fetal human cerebrum* (lane 1 *at a GA of 30 weeks;* lane 3 *at a GA of 19 weeks) and cerebellum* (lane 2, *which is from the same case shown in* lane 1; lane 4, *which is from the same case shown in* lane 3). *NST11 immunobands are present in each lane. Approximately 100 μg of protein was loaded in* lanes 1 *to* 4 *in* D. *The position of the molecular weight markers run with each gel is shown to the left of* B *and* D. *The arrows mark the position of the human NST11 protein in the blots illustrated in* A, C, *and* D. *The immunobands in* C *and* D *were visualized using diaminobenzidine as the chromogen, whereas an enhanced chemiluminescence detection kit (Amersham) was used to visualize the immunobands in* A.

were not stored immediately at −70 C or if they were frozen and thawed more than twice.

NST11 Stains a Subset of Primitive Neuroectodermal Tumors

As summarized in Table 1, primitive neuroectodermal tumors (as exemplified by posterior fossa medulloblastomas) were the major category of brain tumors stained by NST11. As shown in Figure 4, NST11 immunoreactivity was cytoplasmic and perinuclear. NST11 staining was seen in 7 of 15 primitive neuroectodermal tumors (PNETs; Figure 4, A and B), especially those that contained a high proportion of tumor cells with an immature neuronal phenotype (as monitored by the expression of NF proteins). Brain tumors that exhibited a glial lineage were NST11-negative as were meningiomas. Although NST11 immunoreactivity was not detected in a choroid plexus papilloma, focal NST11-positive cells were seen in one choroid plexus carcinoma (data not shown).

NST11 Recognizes a 220-kd Protein in Neuronlike Primitive Neuroectodermal Tumor Cell Lines

Immunohistochemical studies of ethanol-fixed, paraffin-embedded cell pellets from seven human brain tumor-derived cell lines showed NST11 immunoreactivity in four (i.e., D283 Med, D341 Med, D425 Med, and D458 Med) cell lines, all of which were derived from cerebellar medulloblastomas (re-

morphology in Nissl or hematoxylin and eosin-stained sections (data not shown). At a GA of 40 weeks (D). *Purkinje cell bodies (arrows) and dendrites (arrowheads) are intensely stained by NST11. The low and high power photomicrographs* (E *and* F, *respectively) of the adult cerebellum illustrate the intense NST11 immunoreactivity found in mature Purkinje cells (arrows) and their dendrites (arrowheads). The external (EGL) and internal (IGL) granule cell layers and white matter (WM) contain no NST11-positive elements except for glomerular structures in the internal granular layer and a few axons in the white matter. Neurons in the adult neocortex (arrows) also exhibit NST11 positivity* (G), *but they are less intensely stained than Purkinje cells (compare* G *with* C *to* F). *In the adult hippocampus (H), the perikarya of the dentate granule cells are negative, but neurites in the inner third of the molecular layer (ML) of the dentate granule cell layer (GL) are NST11-positive. The magnification is* ×*125 in* A *to* D, G, *and* H, ×*60 in* E *and* ×*250 in* F. *The sections were lightly counterstained with hematoxylin.*

Table 1. *NST11 Immunoreactivity in Neuroepithelial Brain Tumors*

Case	Age	Sex	Location	NST11	GFAP	NF	SYN
PNETs							
PNET(N+G)							
1	1 yr	M	Cerebellum	–	+	+	–
2	1 yr	M	Cerebellum	–	+	+	+++
3	5 yr	F	Cerebellum	++	+	+	++
4	1 yr	M	Cerebellum	+++	+	+	+++
5	1 mo	F	Cerebellum	–	+	+++	+
6	5 yr	M	Cerebellum	–	++	+	+
7	8 mo	F	Cerebellum	–	++	+	+
8	1 yr	F	Cerebellum	+	+	+	+++
PNET(N)							
9	13 yr	F	Cerebellum	+++	–	++	++
10	6 yr	M	Cerebellum	+++	+/–	++	++
PNET(G)							
11	11 yr	M	Cerebellum	–	++	N	+
12	4 yr	F	Cerebellum	–	++	N	+++
PNET(U)							
13	2 yr	M	Pineal gland	++	–	–	++
14	11 yr	M	Cerebellum	++	–	–	++
15	14 yr	M	Cerebellum	–	–	–	+
Astrocytomas							
16	2 yr	F	Cerebellum	–	++++	–	–
17	6 yr	F	Cerebellum	–	+++	–	–
18	6 mo	M	Hypothalamus	–	+++	–	–
19	17 yr	M	Cerebellum	–	+++	–	–
20	6 yr	M	Occipital lobe	–	+++	–	–
21	13 yr	M	Cerebellum	–	+++	–	–
Ependymomas							
22	3 yr	F	Parietal lobe	–	+	–	–
23	2 yr	M	Cerebellum	–	+	–	–
24	3 yr	M	Cerebellum	–	+++	–	–
25	5 yr	M	Cerebellum	–	++	–	–
26	15 yr	F	Cerebrum	–	+	–	–
27	8 yr	F	Parietal lobe	–	++	–	–
Choroid Plexus Papilloma							
28	1 yr	M	Fourth ventricle	–	+	–	–
Choroid Plexus Carcinoma							
29	2 yr	M	Lateral ventricle	++	+	–	–
Gangliogliomas							
30	2 yr	F	Temporal lobe	–	+++	+	–
31	1 yr	F	Occipital lobe	–	+++	+	–
Meningiomas							
32	8 yr	M	Posterior fossa	–	–	–	–
33	27 yr	M	Anterior fossa	–	–	–	–

Summary of NST11 immunoperoxidase data from each of the 33 neuroectodermal brain tumors studied here. Each tumor (identified as cases 1 to 33) is listed with the age and sex of the patient and the location of the tumor. To place the NST11 data (5th column from the left) in perspective, the last three columns of this table incorporate information from previous studies[32,33] of the expression of GFAP, the NF triplet proteins (i.e., NF-L, NF-M, and NF-H), and synaptophysin (SYN) in these same tumor samples. Immunoreactivity for the NST11 protein, GFAP, NF protein, and synaptophysin is recorded according to the approximate percentage of cells in each tumor that were positive, i.e., + = <5%, ++ = 5–50%, +++ = 50–95%, ++++ = >95%. Some tumors were negative (–) or showed equivocal (+/–) staining. The cerebellar PNETs were medulloblastomas. The presence of neoplastic cells with evidence of neuronal (N), glial (G), or neuronal and glial (N+G) differentiation (based on the detection of GFAP or NF protein immunoreactivity in these cells) is recorded as PNET(N), PNET(G), and PNET(N+G), respectively. PNET(U) refers to PNETs without neuronal or glial differentiation. Case 8 was a recurrence of case 7, 1 year after the first surgical removal of this tumor.

viewed in refs. 11 and 28). Examples of NST11-labeled cells in D283 Med and D341 Med are shown in Figure 4, C and D, respectively. Notably, all four of these cell lines express two or more NF proteins and synaptophysin, but none of them express GFAP[11,23,26] The most intense NST11 immunoreactivity was seen in the D283 Med cell line (Figure 4C), which exhibits the most mature neuronal phenotype of all of these cell lines.[11,25,27] The human glioma-derived cell line (U251 MG), which has been shown to contain abundant GFAP-positive cells[20,26] and low levels of the smallest NF protein,[26] did not contain any NST11-positive tumor cells (data not shown). Finally, NST11 immunoreactivity was detected in a human teratocarcinoma cell line (Ntera2 cells) that resembles neuronal progenitor cells (data not shown) as well as in neuronlike cells (NT2N cells) that differentiate from the Ntera2 cells[30] following retinoic acid treatment (Figure 4, E and F). The NST11 data on these cell lines are summarized in Table 2. Western blots performed on cytoskeletal extracts from each of these cell lines re-

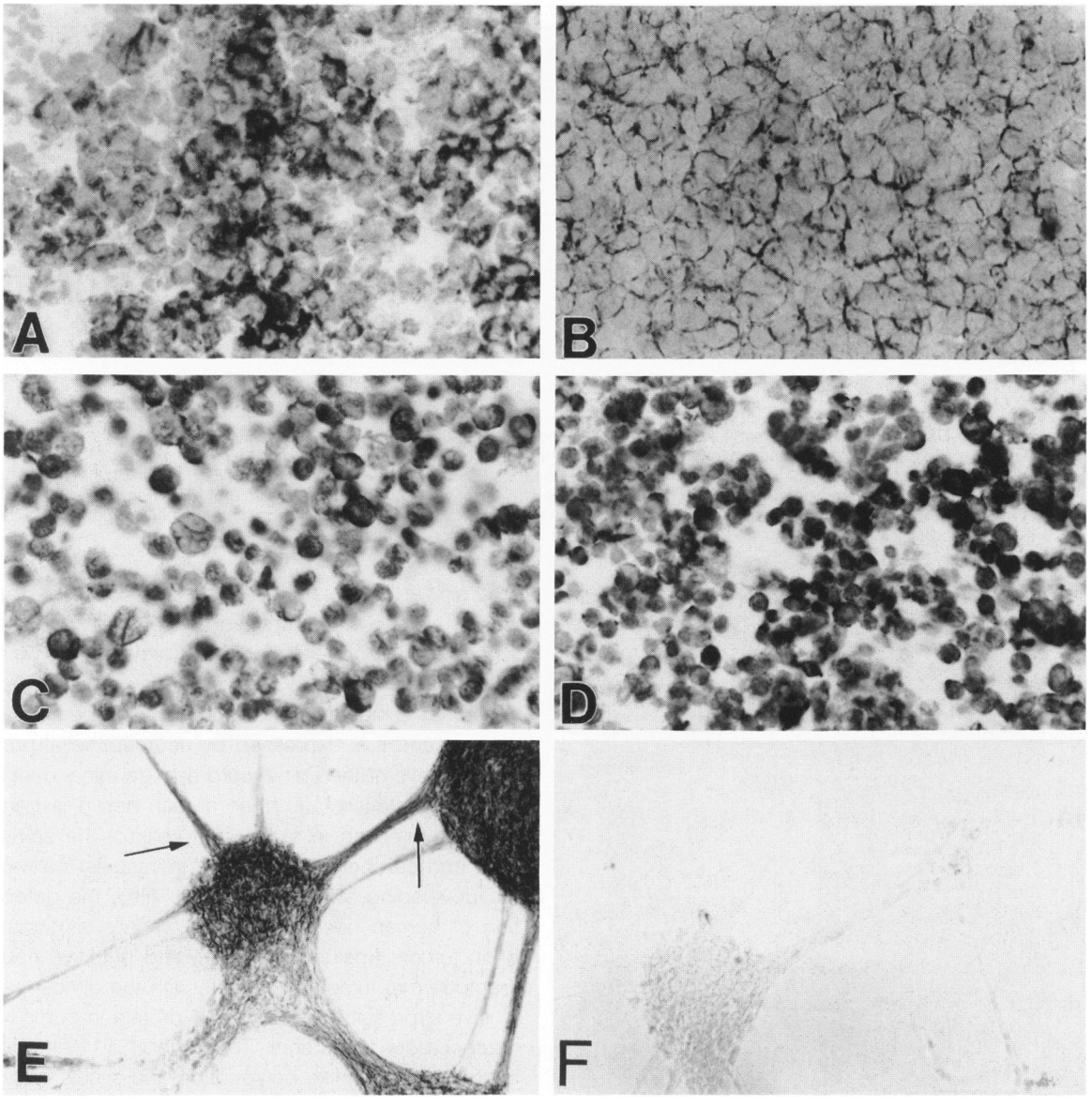

Figure 4. *NST11 immunoreactivity in medulloblastoma biopsies* (A *and* B), *continuous medulloblastoma-derived cell lines* (*D283 Med in* C *and D341 Med in* D), *and in NT2N cells* (E *and* F) *induced to differentiate into neurons from Ntera2 cells following treatment with retinoic acid. Numerous NST11-positive tumor cells are demonstrated by immunohistochemistry in frozen sections of two different PNETs* (A, *case 9;* B, *case 10*). *Immunocytochemical studies of ethanol-fixed paraffin sections of cell pellets from two PNET cell lines* (*D283 Med in* C, *D341 Med in* D) *reveal multiple NST11-positive tumor cells. In* E, *multiple NT2N cells (aggregated into a ganglionlike mass in the center and upper right corner of the photomicrograph) and their processes (arrows) show NST11 immunoreactivity* (E), *whereas an identical sample probed with supernatant (SP2) from unfused mouse myeloma cells is negative* (F). *The magnification in* A *to* D *is* × *500, and each of the sections were lightly counterstained with hematoxylin. No counterstain was used in* E *and* F *where the magnification is* × *150.*

vealed the 220-kd NST11 protein only in the D283 Med cells (which showed the most intense NST11 staining by immunohistochemistry), the Ntera2 cells and in NT2N cells (Figure 5, A and B). This 220-kd, NST11-positive immunoband partitioned mostly with the Triton X-100 insoluble fraction, although a similar NST11-positive immunoband also was detected in the Triton X-100 soluble material (Figure 5A).

Discussion

This report characterizes a novel, developmentally regulated 220-kd polypeptide (the NST11 antigen) expressed in subsets of neurons in the developing and mature human CNS. The developmentally regulated and highly restricted pattern of expression of the NST11 antigen is exemplified by the staining of

Table 2. *NST11 Expression in Cell Lines Derived from Human Neuroepithelial Tumors*

PNET cell lines	NST11	Nestin 129	NFH	NFM	NFL	GFAP	VIM	SYN
PNET(N)								
D283 Med	+	+	+	+	+	–	+	+
D341 Med	+	+	+	+	–	–	+	+
D384 Med	–	+	+	+	+	–	+	+
D425 Med	+	+	+	+	+	–	+	+
D458 Med	+	+	+	+	+	–	+	+
CHP707m	–	+	–	–	+	–	+	+
PNET(U)								
DAOY	–	–	–	–	+	–	+	–
Astrocytoma cell line								
U251 MG	–	+	–	–	+	+	+	–
Neuronal progenitor line								
Ntera2	+	+	–	+	+	–	+	–
Postmitotic Ntera2-derived neurons								
NT2N cells	+	–	+	+	+	–	+	+

The data on the NST11 protein in 10 different neuroepithelial cell lines (listed in the column on the far left) are summarized here under the NST11 column. The data summarized in the subsequent columns for the NF triplet proteins (NFH, NFM, NFL), GFAP, vimentin (VIM), and synaptophysin (SYN) were reported earlier.[11,19,23,25] Nestin 129 refers to a rabbit antiserum raised to the TrpE-rat nestin fusion protein used in a previous study.[11] These data are included here for comparison with the NST11 data. The presence or absence of NST11 immunoreactivity in each cell line is indicated by a + (present) or – (absent) sign. The same scheme is used to summarize data on the other polypeptides in the table. The NST11 positivity in these cell lines reflects the results of the immunocytochemical studies.

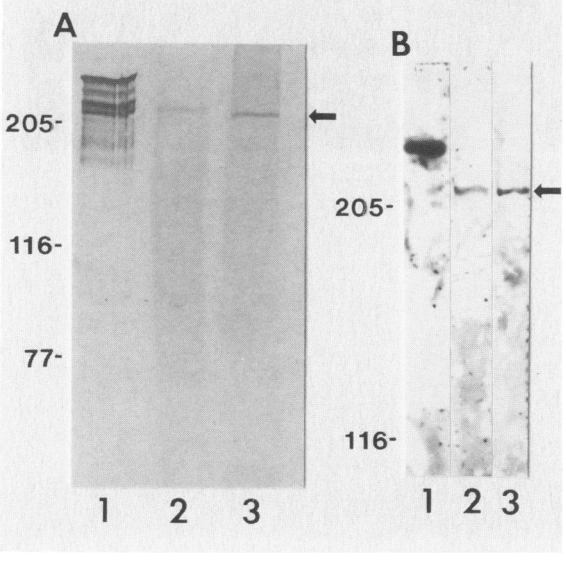

Figure 5. **A** *and* **B** *show representative immunoblots of the TrpE-rat nestin fusion protein* (**lane 1** *in* **A** *and* **B**), *extracts of the D283 MED PNET cell line* (**lanes 2** *and* **3** *in* **A**), *the Ntera2 cells* (**lane 2** *in* **B**), *and the NT2N cells* (**lane 3** *in* **B**) *that were produced using the NST11 MAb. Approximately 40 μg of Triton X-100 insoluble and soluble proteins were loaded in* **lanes 2** *and* **3**, *respectively, of* **A** *whereas approximately 100 μg of total homogenate from the Ntera2 and NT2N cells were loaded in* **lanes 2** *and* **3**, *respectively, of* **B**. *NST11 recognizes the TrpE-rat nestin fusion protein and a series of breakdown products derived therefrom* (**lane 1** *in* **A** *and* **B**). *The D283 MED cell line, the Ntera2 cell, and the NT2N neuronlike derivatives of the latter cell line contain a single 220-kd, NST11-positive immunoband (arrows). The position of the molecular weight markers run as standards is shown on the left of each panel. Bound antibody was visualized using diaminobenzidine in* **A** *and with the enhanced chemiluminescence detection kit in* **B**.

nascent and mature cerebellar Purkinje cells with the NST11 MAb. Further, this protein was detected almost exclusively in a medulloblastomas comprised of neoplastic cells with a molecular phenotype similar to immature neurons.[11,31] Because the NST11 antigen was identified with a MAb raised to a nestin fusion protein, the NST11 antigen may be a polypeptide related to nestin.[6,35] However, human nestin has a higher M_r (i.e., 240 kd) than the NST11 protein, and it is expressed by neuroepithelial progenitor cells of the spinal cord and germinal matrix early in gestation.[11] Further, human nestin is transiently expressed in radial glial cells of the spinal cord and cerebellum as well as in vascular cells of the developing and mature brain. Also, the detection of human nestin in a wide variety of human brain tumors (including gliomas and primitive neuroectodermal tumors) contrasts with the almost exclusive expression of the NST11 protein in primitive neuroectodermal tumors. Thus, the NST11 antigen might be a nestinlike polypeptide and a new member of the family of IF proteins, or it might be unrelated to nestin except for the presence of immunologically similar epitopes in both proteins.

This notwithstanding, the NST11 polypeptide is a novel early molecular marker of the commitment of CNS progenitors to the neuronal lineage. However, this protein is expressed only in a subset of CNS neurons such as Purkinje cells of the cerebellum. This suggests that the NST11 polypeptide may play a role in the emergence of these neurons in development. Notably, most antigens detected in Purkinje cells are expressed later in development (e.g., cerebellin,[36] precerebellin),[37] in subsets of Purkinje cells (i.e., B1 antigen,[38] zebrin),[39] or in several different cerebellar neurons (e.g., NF proteins).[34] Because the NST11 MAb identified Purkinje cells at very early stages in embryogenesis, the NST11

MAb could be exploited to analyze the developmental neurobiology of Purkinje cells. Further, the NST11 antigen could be used with other markers of the stepwise maturation of neuronal progenitors and neurons to stage the progression or monitor the malignant potential of medulloblastomas as has been described recently for neuroblastomas.[40] Finally, neuronal markers such as NST11 could also facilitate efforts to define effective animal models of medulloblastomas for the laboratory investigation of these tumors.[28]

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