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Immunohistochemical Analysis of Neural Cell Adhesion Molecules

Differential Expression in Small Round Cell Tumors of Childhood and Adolescence

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The neural cell adhesion molecule (NCAM) was discovered in a search for cell surface antigens of chicken neurons that contribute to cell adhesion and pattern formation during development. Homologous adhesion molecules have been identified in several species, including humans. In this immunohistochemical study, the authors examine the role of human NCAM in tumor diagnosis. The authors used a monoclonal antibody (MAb), 5.1H11, to examine NCAM immunoreactivity in frozen sections of more than 450 tumors, including more than 80 small round cell tumors (SRCT) of childhood and adolescence (neuroblastomas, Ewing's sarcomas [ES], peripheral neuroepitheliomas [PN], primitive neuroectodermal tumors [PNET], esthesioneuroblastomas, malignant ectomesenchymoma, medulloblastomas, small cell osteosarcomas, mesenchymal chondrosarcomas, embryonal rhabdomyosarcomas, and lymphomas). The authors show that 1) neuroblastomas and primary brain tumors are NCAM$^+$; 2) ES, most PN/PNETs, and melanomas are NCAM$^-$; 3) embryonal rhabdomyosarcomas and various other sarcomas are NCAM$^+$; 4) neuroendocrine tumors are NCAM$^+$; 5) subsets of carcinomas of kidney, ovary, lung, and other organs are NCAM$^+$; and 6) lymphoid tumors are NCAM$^-$. Tests with normal fetal and adult tissues indicate that these findings reflect only in part the NCAM phenotypes of corresponding normal tissues. Notably the NCAM$^-$ phenotype of ES and PN/PNET is not explained by current histogenetic models for these tumors, which suggest a primitive neuroectodermal origin. Finally the authors show that NCAM expression among SRCT has an inverse relationship with the expression of p30/32^{MIC2}, a cell surface antigen of ES and PN/PNET detected with MAb HBA71. These results suggest that immunohistochemical assays for NCAM and p30/32^{MIC2} expression may aid in the further characterization of SRCT of childhood and adolescence. (Am J Pathol 1991, 139:275–286)

The neural cell adhesion molecule (NCAM) was discovered in a search for cell-surface molecules that contribute to cell–cell interactions during neural development, using antibodies that inhibit the aggregation of dissociated chicken neurons in tissue culture.[1] Subsequent immunohistochemical studies have shown that NCAM is widely expressed in neural tissues in the chicken and that certain non-neural tissues in the chicken are also NCAM$^+$.[1] Homologous adhesion molecules have been identified in several other species, including humans.[2] Biochemical and molecular genetic studies have disclosed considerable diversity among NCAM molecules expressed in different tissues or at distinct stages of development. This diversity is due to alternative splicing of mRNAs transcribed from the single NCAM gene, and to distinct patterns of glycosylation of the NCAM polypeptides.[1] The analysis of temporospatial expression patterns for NCAM during embryonic and fetal development and functional studies *in vitro* and *in vivo* have implicated NCAM in diverse developmental processes, ranging from neural pattern formation to myogenesis, nerve and muscle regeneration, and feather formation.[3]

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The study of NCAM expression in human tumors has not evolved directly from the developmental studies in animals. Instead several laboratories unwittingly generated monoclonal antibodies (MAbs) against human NCAM in their search for cell-surface antigens restricted to leukemias and lymphocytes with natural killer cell phenotype,[4] neuroblastoma,[5] small cell lung cancer,[6–8] and skeletal muscle and muscle tumors.[9,10] Only recent serologic, biochemical, and genetic studies have shown that these MAbs recognize a common antigen, NCAM.[11–16] In this immunohistochemical study, we have used one of these MAbs, 5.1H11,[9,11,15] to determine the distribution of NCAM in more than 450 tumors of diverse histologic types. To be able to interpret the observed tumor phenotypes with regard to cell lineage, degree of differentiation, and malignant transformation, we also identified the pattern of 5.1H11 immunoreactivity with normal fetal and adult human tissues. As a key finding of this analysis, we report that NCAM is differentially expressed among distinct groups of small round cell tumors (SRCT) of childhood and adolescence and that among these tumors, NCAM expression is inversely related to the expression of a second antigen, the p30/32^{MIC2} cell-surface glycoprotein defined by MAb HBA71.[17] Thus immunohistochemical analysis of NCAM and p30/32^{MIC2} is recommended in the differential diagnosis of selected SRCT of childhood and adolescence.

Materials and Methods

Monoclonal Antibodies

The generation of the mouse MAb 5.1H11 and its specificity for human NCAM have been described.[9,11,15] 5.1H11 was obtained from Drs. Frank Walsh (Guy's Hospital, London, UK) and Orest Hurko (Johns Hopkins University, Baltimore, MD). Unrelated negative control MAbs were purchased from Becton & Dickinson (Mountain View, CA). Monoclonal antibody 5.1H11 detects NCAM in acetone-fixed frozen tissues but not in formalin-fixed, paraffin-embedded tissues.

Tissues and Immunohistochemical Procedures

Normal and tumor tissues were obtained at autopsy or from surgical specimens in the Department of Pathology at Memorial Hospital. Tissues were quick-frozen in isopentane precooled in liquid N_2 and stored at −70°C. Additional cases of SRCT of childhood and adolescence were obtained from the frozen tissue bank at Childrens Hospital. Sections of 5-μ thickness were cut, mounted on gelatin-coated slides, air-dried for 30 minutes at room temperature (RT), and fixed with acetone for 10 minutes at 4°C. The avidin-biotin complex immunoperoxidase procedure was used as described.[18,19] Briefly, sections were treated with 0.3% H_2O_2 for 5 minutes to block endogenous peroxidase, and incubated with normal horse serum for 30 minutes at RT. Subsequently sections were incubated with MAb 5.1H11 (ascites fluid diluted 1:500, or hybridoma culture supernatant diluted 1:2) or unrelated negative control antibodies for 12 to 18 hours at 4°C. Sections were washed, incubated with biotinylated horse anti-mouse immunoglobulin for 30 minutes at RT, followed by avidin-biotin horseradish peroxidase complex (Vector Laboratories, Burlingame, CA). The final reaction product was visualized with diaminobenzidine. Sections were counterstained with Harris' hematoxylin.

Results

NCAM Expression in Tumor Tissues

The tumor panel used in the present study was designed to accomplish two major objectives: 1) to establish the general distribution of NCAM immunoreactivity among the common histologic types of human tumors; and 2) to explore in detail the NCAM immunoreactivity in distinct subtypes of SRCT of childhood and adolescence. To accomplish these two goals, frozen sections of more than 450 tumors of diverse histologic types were tested (Table 1). Among the nervous system tumors available for this study, all ganglioneuromas (6 of 6 cases studied; Figure 1A), ganglioneuroblastomas (5 of 5), neuroblastomas (16 of 16; Figure 1B), and an esthesioneuroblastoma were NCAM$^+$. Therefore it was of interest to determine whether other SRCT of childhood and adolescence that are commonly included in the differential diagnosis of neuroblastoma do express NCAM. We found that Ewing's sarcomas (ES) are generally NCAM$^-$ (19 of 20 cases tested), as illustrated in Figure 1C. Similarly three cases of primitive neuroectodermal tumors (PNET) diagnosed at Memorial Sloan-Kettering Cancer Center (Figure 1D), a majority of PNETs and peripheral neuroepitheliomas (PN) obtained from the frozen tissue bank at Childrens Hospital (6 of 8), mesenchymal chondrosarcomas (3 cases), and a malignant ectomesenchymoma were found to be NCAM$^-$. In contrast, embryonal rhabdomyosarcomas (ERMS; 10 of 10; Figure 1E), small cell osteogenic sarcomas (2 of 2), and medulloblastomas (2 of 2; Figure 2F) were NCAM$^+$. Among the 56 lymphomas (ie, 14 Hodgkin's lymphomas and 42 non-Hodgkin's lymphomas, including four cutaneous T-cell lymphomas) and four thymomas tested, we found only a single

Table 1. *NCAM Expression in Human Tumors*

Tumor type	n	NCAM expression
SRCT (nonlymphoid)		
Neuroblastoma	16	●●●●●●●●●●●●●●●●
Ganglioneuroblastoma	5	●●●●●
Ganglioneuroma	6	●●●●●●
Esthesioneuroblastoma	1	●
Medulloblastoma	2	●●
PN/PNET (non-CNS)	11	●●○○○○○○○○○
Ewing's sarcoma	20	●○○○○○○○○○○○○○○○○○○○
Malignant ectomesenchymoma	1	○
ERMS	10	●●●●●●●●●●
Mesenchymal chondrosarcoma	3	○○○
Small-cell osteosarcoma	2	●●
Unclassified SRCT	1	●
Neuroectodermal tumors		
Schwannoma	6	●●●●◒◒
Glioma	9	●●●●●●●●●
Meningioma	4	●◒◒○
Melanoma	34	◒◒◒◒◒○○○○○○○○○○○○○○○○ ○○○○○○○○○○○○○
Nevus	4	●●○○
Mesenchymal tumors		
Leiomyosarcoma	8	●●●◒○○○○
Leiomyoma, uterus	5	●●●●●
Synovial sarcoma	2	●●
Chondrosarcoma	6	●●●○○○
Osteogenic sarcoma	4	●●○○
MFH	4	○○○○
Liposarcoma	7	○○○○○○○
Fibrosarcoma	3	○○○
Fibroma, ovary	2	○○
Giant cell tumor	1	○
Undifferentiated	1	○
Lymphoid tumors		
Hodgkin's lymphoma	14	○○○○○○○○○○○○○○
Non-Hodgkin's lymphoma	38	○○○○○○○○○○○○○○○○○○○○○ ○○○○○○○○○○○○○○○○○
Cutaneous T-cell lymphoma	4	●○○○
Thymoma	4	○○○○
Neuroendocrine tumors	23	●●●●●●●●●●●●●●●●●●●●●●○
Carcinomas		
Skin	8	○○○○○○○○
Breast	17	◒○○○○○○○○○○○○○○○○
Lung (non-SCLC)	13	◒◒◒◒○○○○○○○○○
Mesothelioma	7	◒◒○○○○○
Esophagus	4	○○○○
Stomach	6	○○○○○○
Duodenum	1	○
Colon	25	○○○○○○○○○○○○○○○○○○○○○ ○○○○
Pancreas	5	○○○○○
Kidney	12	◒○○○○○○○○○○○
Bladder	13	◒◒◒◒◒◒◒○○○○○○
Prostate	2	○○
Testis	5	◒◒○○○
Ovary		
Adenocarcinoma	37	◒◒◒◒◒◒◒◒◒◒◒◒◒○○○○○○○○ ○○○○○○○○○○○○○○○○
Brenner tumor	1	○
Granulosa cell tumor	2	●◒
Mixed mesodermal tumor	1	◒
Immature teratoma	1	○
Dermoid cyst	1	◒
Endometrium	7	◒○○○○○○
Cervix	2	○○
Thyroid, Hürthle cell tumor	1	●
Thymus	1	○
Undifferentiated	3	○○○

Table 1. (*Continued*).

Tumor type	n	NCAM expression
Benign lesions		
Adrenal cortical adenoma	1	●
Colonic polyp	4	○○○○
Fibroadenoma, breast	3	◒○○
Fibrocystic disease, breast	14	◒◒◒◒◒◒◒◒○○○○○○○
Dermal scars	3	○○○

Results are indicated as follows: ●, NCAM$^+$ with homogeneous antigen expression; ◒, NCAM$^+$ with heterogeneous antigen expression in 10% to 50% of tumor cells; ○, NCAM$^-$; n, number of tumors examined for tumor types listed to the left. For epithelial tumors, staining results refer to the epithelial tumor cells; for NCAM expression in the stroma of epithelial tumors, refer to the results section. Testicular tumors tested included two seminomas (NCAM$^-$), one embryonal carcinoma (NCAM$^-$), and two teratomas (NCAM$^{+/-}$; antigen expression mainly in mesenchymal components).

ERMS, embryonal rhabdomyosarcoma; MFH, malignant fibrous histiocytoma; non-SCLC, non–small-cell lung cancers.

NCAM$^+$ tumor, namely, a cutaneous T-cell lymphoma. Most of the gliomas, malignant Schwannomas, and meningiomas tested were NCAM$^+$. Melanomas were generally NCAM$^-$, with patchy immunostaining (10% to 30% NCAM$^+$ tumor cells) seen in only 5 of 34 cases. Two of four compound nevi were NCAM$^+$. Among the additional mesenchymal tumors tested, NCAM was found in half of the leiomyosarcomas (4 of 8), five uterine leiomyomas, two benign ovarian fibromas, chondrosarcomas (3 of 6; Figure 2A) synovial sarcomas (2 of 2; Figure 2B), and osteogenic sarcomas (2 of 4).

Among carcinomas, neuroendocrine carcinomas showed consistent and homogeneous NCAM immunoreactivity (22 of 23). This group included pancreatic endocrine tumors (5 of 5 cases NCAM$^+$; Figure 2C), carcinoid tumors of the bronchus (4 of 4), stomach (4 of /4), and ileum (1 of 1), medullary thyroid carcinomas (3 of 4), Merkel cell tumors (2 of 2; Figure 2D), small cell lung carcinomas (2 of 2), and a pheochromocytoma. Neural cell adhesion molecule immunostaining was found in a proportion of non-small cell lung cancers (4 of 13), mesotheliomas (2 of 7), renal cell carcinomas (7 of 12), ovarian adenocarcinomas (13 of 37), a breast carcinoma (1 of 16), and an endometrial carcinoma (1 of 7). As indicated in Table 1, NCAM immunostaining commonly produces a heterogeneous pattern in this last group of epithelial tumors, which contrasts with the homogeneous staining seen in tumors such as neuroblastomas, ERMS, and neuroendocrine carcinomas. With regard to NCAM expression in the breast epithelium, we found that NCAM is expressed in the epithelial cells of benign breast lesions, such as fibrocystic disease, fibroadenoma, and papillomatosis of the breast, showing heterogeneous expression (Table 1). Among ovarian tumors, NCAM is found in adenocarcinomas, showing a heterogeneous pattern with less than 10% to 30% of NCAM$^+$ tumor cells in different cases. In addition, NCAM was found in two granulosa cell tumors but not in the epithelial component of a Brenner tumor. Finally NCAM was detected in two benign ovarian fibromas and in the stroma of several ovarian adenocarcinomas and the single case of Brenner tumor tested, reminiscent of the strong NCAM expression seen in the stroma of the normal ovary.

Reciprocal Expression of NCAM and p30/32^{MIC2} in SRCT

The highly characteristic pattern of NCAM expression in subsets of SRCT of childhood and adolescence prompted us to compare it with the distribution of the p30/32^{MIC2} cell-surface antigen of ES and PN/PNET that is recognized by MAb HBA71.[17] As summarized in Table 2, we found that these two antigenic systems show reciprocal patterns of expression in 37 of the 39 cases of SRCT included in this side-by-side analysis. In this group, 17 tumors (including 9 neuroblastomas, 1 esthesioneuroblastoma, 1 PNET of bone, 1 PN, 1 small cell osteosarcoma, and 4 ERMS) were NCAM$^+$ and p30/32^{MIC2-}, and 20 tumors (including 11 ES and 9 PN/PNETs) were NCAM$^-$ and p30/32^{MIC2+}. A malignant ectomesenchymoma was negative for both antigens and one ES was positive for both antigens. Furthermore this analysis showed that the two cases of PN/PNET that are listed as being NCAM$^+$ lack p30/32^{MIC2} immunoreactivity, whereas all NCAM$^-$ cases of PN/PNET are p30/32^{MIC2+}.

NCAM Expression in Normal Tissues

Immunohistochemical analysis of normal adult tissues with MAb 5.1H11 showed NCAM expression in a diverse range of cell types. Neural cell adhesion molecule immunoreactivity was detected throughout the central and peripheral nervous system (Table 3; Figure 3A). In the adrenal gland, both the cortex (Figure 3B) and the medulla were found to be NCAM$^+$. Neural cell adhesion molecule immunoreactivity was found in a number of mesenchymal tissues, including reactive skeletal muscle fibers (Figure 3C), cardiac muscle (Figure 3D), and visceral smooth muscle (Figure 3E). In the heart, NCAM immuno-

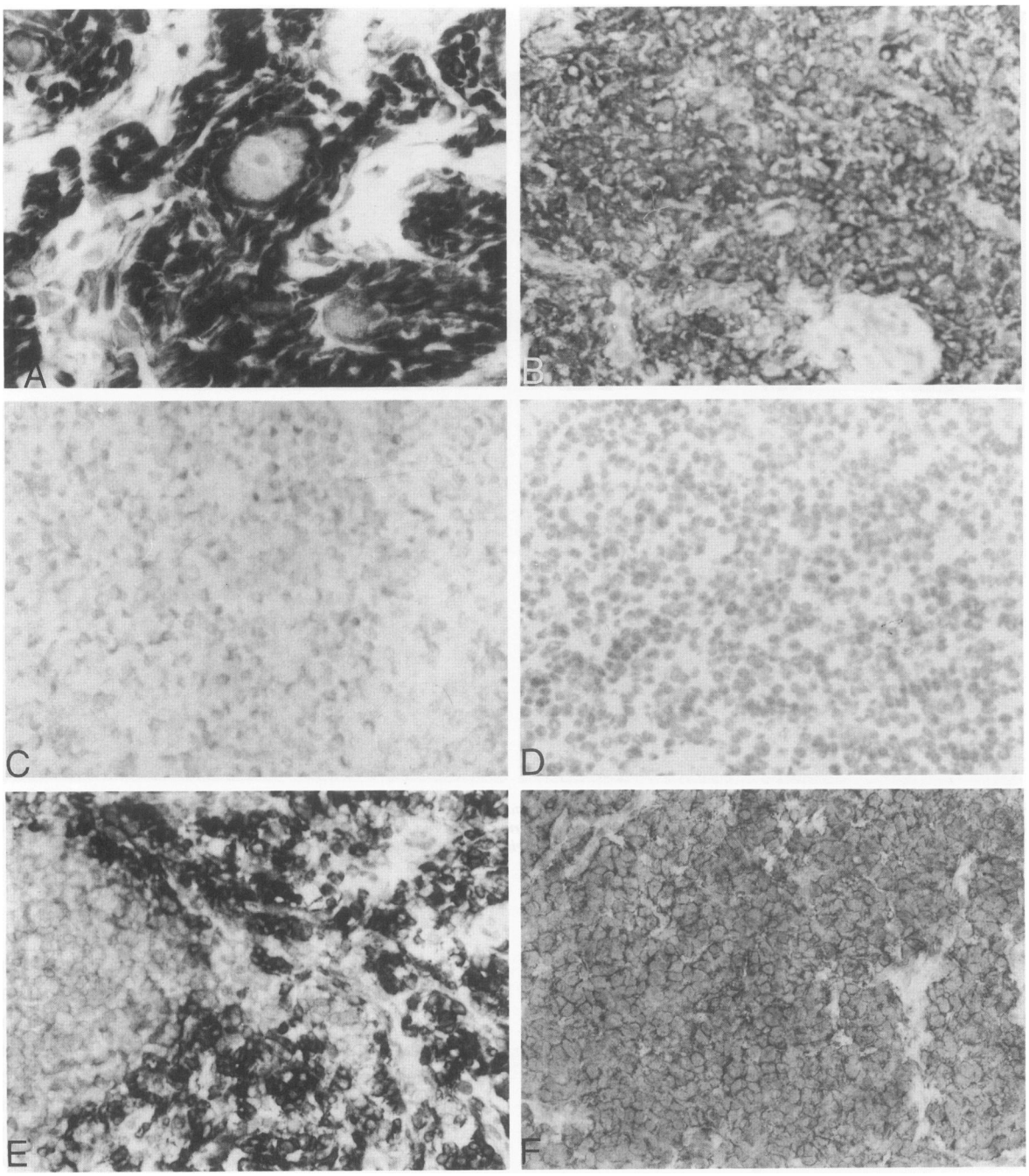

Figure 1. *Immunohistochemical analysis of solid tumors of childhood and adolescence with MAb 5.1H11.* **A:** *Ganglioneuroma, NCAM$^+$ ganglionic and Schwann-like tumor cells.* **B:** *Neuroblastoma, NCAM$^+$* **C:** *Ewing's sarcoma, NCAM$^-$.* **D:** *PNET, NCAM$^-$.* **E:** *Lymph node metastasis of embryonal rhabdomyosarcoma, NCAM$^+$ tumor cells and antigen-negative lymphoid and stromal cells.* **F:** *Medulloblastoma, NCAM$^+$. Original magnification, ×50.*

staining was most prominent along the intercalated discs, but was found also along the remainder of the muscle fibers. The stromal fibroblasts of the ovary, especially in the ovarian cortex, and the fibromuscular stroma of the prostate and cervix uteri were strongly NCAM$^+$. Among epithelial tissues, NCAM was detected in the epithelium and in parafollicular cells of the thyroid gland. In the stomach, NCAM was present in the epithelial cells of the lower portions of the gastric glands. In the small and large intestine, the mucosa was NCAM$^-$ but the lamina propria was NCAM$^+$. Finally NCAM immunoreactivity was found in Leydig cells of the testis and in the islet cells of the pancreas.

In this study, the distribution of NCAM immunoreactivity in the fetal and newborn tissues included in this study largely follows its distribution in adult tissues, but

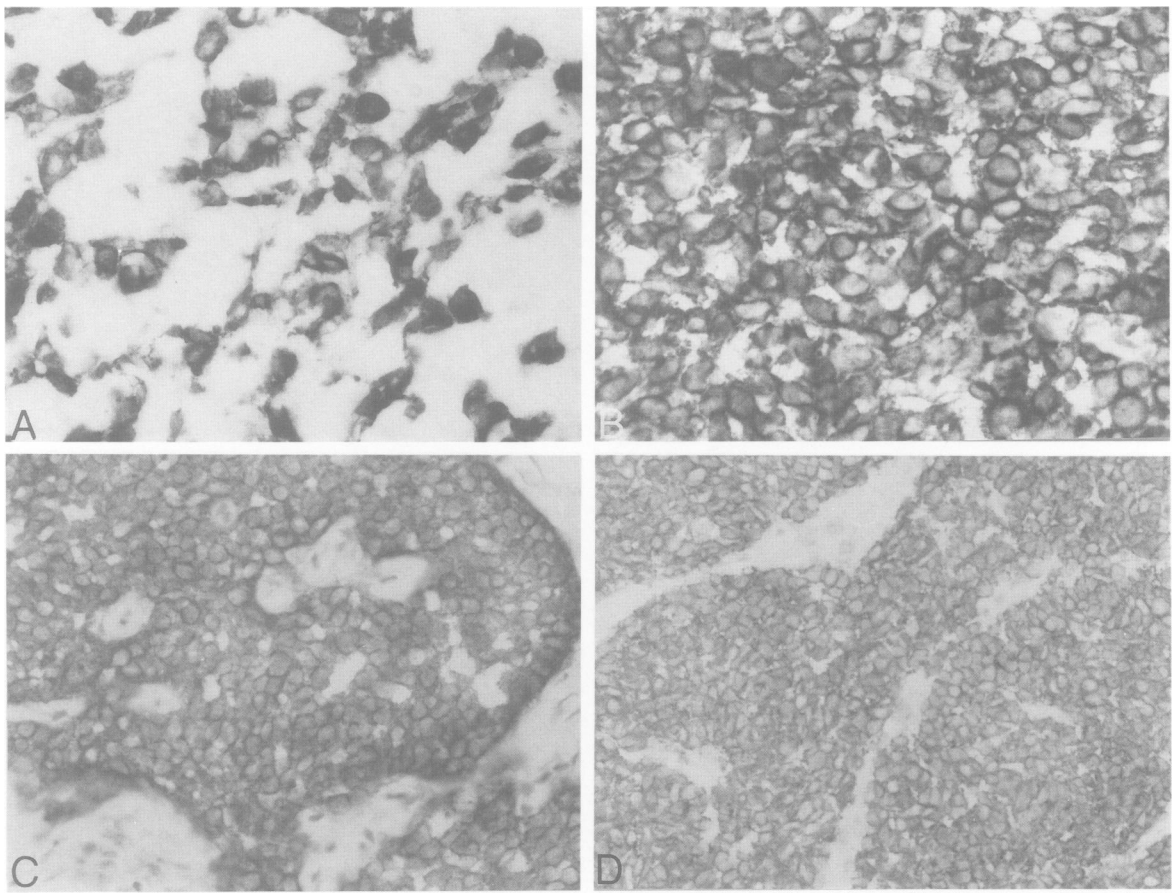

Figure 2. *Immunohistochemical analysis of sarcomas and neuroendocrine carcinomas with MAb 5.1H11.* **A:** *Myxoid chondrosarcoma, NCAM$^+$.* **B:** *Synovial sarcoma, NCAM$^+$.* **C:** *Pancreatic neuroendocrine carcinoma (insulinoma), NCAM$^+$ tumor cells and NCAM$^-$ stroma.* **D:** *Merkel cell tumor, NCAM$^+$ tumor cells and NCAM$^-$ stroma. Original magnification,* **A, B:** *×100;* **C, D:** *× 50.*

differences were noted in some organs, such as skeletal muscle, kidney, lung, and skin (Table 3). In the fetal adrenal gland, NCAM is present in the endocrine cells of the permanent cortex as well as medullary chromaffin cells. In fetal kidney, the epithelial cells of primitive nephrons are NCAM$^+$, and in fetal lung, NCAM is found in a subset of bronchial epithelial cells. Finally in fetal skin, NCAM is present in the papillae of developing hair follicles (Figure 3F), in some of the dermal fibroblasts, the paraderm, and the ductal epithelium of sweat glands.

Because the histogenesis of ES and PN/PNET to date remains uncertain, we searched for normal tissues that express the NCAM$^-$/p30/32^{MIC2+} surface antigenic phenotype common to most cases of ES and PN/PNET. No such cells were found among the fetal and adult neural and mesenchymal tissues tested, however, including fetal central and peripheral nervous system, fetal adrenal gland, fetal bone, and other primitive mesenchymal tissues. In contrast, the NCAM$^+$/p30/32^{MIC2-} phenotype of neuroblastomas was readily detected in fetal adrenal chromaffin cells (Table 2), the presumed targets for transformation in neuroblastoma.

Discussion

The present study examines differential expression of NCAM among SRCT of childhood and adolescence; NCAM expression in other tumor types; and the relationship between tumor phenotypes and NCAM expression in normal fetal and adult human tissues. A number of MAbs that react with human NCAM have been identified,[11–16] and we selected MAb 5.1H11 for the present study because many details of its specificity and reactivity with different isoforms of NCAM, including transmembrane, glycosyl-phosphatidylinositol-linked, and secreted forms, are known.[9,11,15] For example, MAb 5.1H11 recognizes all four brain and muscle isoforms of NCAM studied in gene transfer experiments,[11,14,15] and the 5.1H11-reactive epitope is unrelated[14] to the α2,8-linked polysialic acid moieties[20] and the HNK-1 carbohydrate epitope,[21] which are present on some NCAM species but are also found on unrelated molecules.[3]

Small round cell tumors of childhood and adolescence represent a diverse group of tumors of neural, mesenchymal, and lymphoid derivation that share a sim-

Table 2. *Comparative Immunohistochemical Analysis of NCAM and p30/32^{MIC2} Expression in SRCT of Childhood and Adolescence and in Fetal Adrenal Neuroblasts*

Patient			Antigen phenotype	
No.	Diagnosis	Cytogenetics	NCAM	p30/32^{MIC2}
Tumors				
1	Neuroblastoma	NA	+ + +	−
2	Neuroblastoma	NA	+ + +	−
3	Neuroblastoma	NA	+ + +	−
4	Neuroblastoma	NA	+ + +	−
5	Neuroblastoma	NA	+ + +	−
6	Neuroblastoma	NA	+ + +	−
7	Neuroblastoma	NA	+ + +	−
8	Neuroblastoma	NA	+ + +	−
9	Neuroblastoma	NA	+ + +	−
10	Esthesioneuroblastoma	NA	+ + +	−
11	PNET of bone	NA	+ + +	−
12	PN	NA	+ + +	−
13	PNET	t(11;22)(q24;q12)	−	+ +
14	PNET	NA	−	+ +
15	PNET	NA	−	+ +
16	PNET of bone	NA	−	+ + +
17	PNET, lung metastasis	22q$^-$; 1p$^-$	−	+ +
18	Askin tumor	t(11;22)(q24;q12)	−	+ +
19	Askin tumor	NA	-	+ +
20	Askin tumor	t(11;22)(q24;q12)	−	+ + +
21	PN	t(11;22)(q24;q12)	−	+ + +
22	Ewing's sarcoma	t(11;22)(q24;q12)	−	+ + +
23	Ewing's sarcoma	t(11;22)(q24;q12)	−	+ + +
24	Ewing's sarcoma	t(11;22)(q24;q12)	−	+ + +
25	Ewing's sarcoma	t(11;22)(q24;q12)	−	+ + +
26	Ewing's sarcoma	t(11;22)(q24;q12)	−	+ + +
27	Ewing's sarcoma	NA	−	+ + +
28	Ewing's sarcoma	NA	−	+ + +
29	Ewing's sarcoma	NA	−	+ + +
30	Ewing's sarcoma	NA	−	+
31	Ewing's sarcoma	NA	−	+ +
32	Ewing's sarcoma	NA	−	+ +
33	Ewing's sarcoma	NA	+ +	+ +
34	Malignant ectomesenchymoma	NA	−	−
35	Small cell osteosarcoma	NA	+ + +	−
36	ERMS	NA	+ + +	−
37	ERMS	NA	+ + +	−
38	ERMS	NA	+ + +	−
39	ERMS	NA	+ + +	−
Fetal adrenal neuroblasts			+ + +	−

Antigen expression was determined by immunohistochemical analysis of parallel sections of acetone-fixed frozen tissues with MAbs 5.1H11 and HBA71,[17,32] respectively. (In addition, three cases of ES, numbers 22 to 24, were also tested with a polyclonal rabbit antibody against NCAM, provided by Dr. K. Crossin, Edelman Laboratory, Rockefeller University, New York, and all three tumors were antigen negative.) Immunohistochemical staining results are indicated as follows: + + + strong, + + intermediate, + weak immunoreactivity; −, antigen negative. Negative control experiments with unrelated mouse MAbs showed no immunoreactivity. The cytogenetic data indicate the presence or absence of the t(11;22)(q24;q12) found in ES and PN/PNET.[36] Case 17 shows chromosome 1 and chromosome 22 abnormalities similar to those previously described in esthesioneuroblastoma.[36] A detailed description of the cytogenetic analysis of cases 14 and 23 to 27 has been reported by Ladanyi et al.[39] NA, data not available.

ilar histologic appearance, characterized by the presence of 'small blue round cells,' ie, poorly differentiated cells with uniform nuclei and scanty cytoplasm.[22] Three types of SRCT have been identified as distinct histogenetic and clinicopathologic entities: neuroblastomas, which show signs of neuroblastic differentiation; rhabdomyosarcomas, with skeletal muscle differentiation; and lymphomas. A fourth group of SRCT is defined mostly by absence of clear differentiation along a single cellular pathway; these tumors may show virtually no histotypic markers or display complex patterns of multilineage differentiation with coexpression of neural, mesenchymal, and epithelial traits.[23] In some classification schemes, this last group of SRCT comprises several partially overlapping entities such as ES, PNET, PN, Askin tumor, esthesioneuroblastoma, mesenchymal chondrosarcoma, desmoplastic SRCT, and small cell osteosarcoma.[22,24] Past attempts to distinguish these tumor entities from each other and from neuroblastoma, rhabdomyosarcomas, and lymphomas with MAbs against cell-surface or intracellular antigens have yielded complex antigenic patterns.[19,23,25–31] In contrast, the present study and the

Table 3. *NCAM Expression in Normal Human Tissues*

Tissue/organ	NCAM expression	
	Staining level	Antigen-expressing cells/tissues
Adult tissues		
Neural tissues		
Cerebral cortex	+ + +	Gray, white matter
Diencephalon	+ + +	Gray, white matter
Pons	+ + +	Gray, white matter
Cerebellum	+ + +	Gray, white matter
Medulla oblongata	+ + +	Gray, white matter
Spinal cord	+ + +	Gray, white matter
Autonomic ganglia	+ + +	Ganglion, satellite cells
Peripheral nerves	+ + +	Axons; Schwann cells
Meninges	−	
Choroid plexus	−	
Skin	−	
Breast	−	
Larynx	−	
Bronchus	−	
Lung	−	
Tongue	−	
Esophagus	−	
Stomach	+ +	Mucosa (bottom of crypts)
Jejunum	+ +	Lamina propria
Colon	+ +	Lamina propria
Liver	−	
Pancreas	+ +	Islets, ducts (subset)
Thyroid gland	+ + +	Follicular epithelium; parafollicular cells
Adrenal gland	+ + +	Cortex, medulla
Kidney	−	(Rare collecting ducts +)
Bladder	−	
Prostate	−	Stroma (+)
Testis	+ +	Leydig cells
Ovary	+ + +	Stroma (cortex)
Uterus	−	Smooth muscle (+ +)
Cervix	−	Fibromuscular stroma (+ +)
Fallopian tube	−	Smooth muscle (+ +)
Visceral smooth muscle	+ +	eg, uterus, colon, bladder, Fallopian tube
Skeletal muscle	−	Reactive muscle fibers (+ +)
Cardiac muscle	+ + +	Intercalated discs (+ + +); myocytes (+)
Cartilage	−	
Adipose tissue	−	
Blood vessels	−	
Spleen	−	
Lymph node	−	
Tissues in newborn		
Skin	+ +	Fibroblasts (subset); sweat gland ducts
Fetal tissues (15 weeks)		
Neural tissues		
Brain		
Cortex	+ + +	Gray, white matter
Midbrain	+ + +	Gray, white matter
Pons	+ + +	Gray, white matter
Cerebellum	+ + +	Gray, white matter
Medulla	+ + +	Gray, white matter
Meninges	−	
Peripheral nerves	+ + +	Axons; Schwann cells
Autonomic ganglia	+ + +	Ganglion cells; nerve fibers
Thymus	−	
Skin	+ +	Fibroblasts (subset), dermal papillae
Kidney	+ + +	Immature nephron
Lung	+ +	Epithelial cells (subset)
Stomach	+ +	Mucosa (subset); smooth muscle
Peritoneum	−	
Liver	−	
Spleen	−	Capsule (+ +)
Pancreas	+	Islet cells
Ovary/uterus	+ + +	Stromal fibroblasts (coelomic epithelium negative)

Table 3. (*Continued*).

Tissue/organ	NCAM expression	
	Staining level	Antigen-expressing cells/tissues
Adrenal gland	+ + +	Permanent cortex, chromaffin cells
Cardiac muscle	+ + +	Myocytes
Skeletal muscle	+ +	Myocytes
Smooth muscle	+ +	Myocytes
Blood vessels	−	
Cartilage	+	Chondrocytes
Term placenta	−	

Level of immunostaining was scored as strong (+ + +), intermediate (+ +), weak (+), or negative (−).

accompanying report[32] suggest a relatively simple pattern of differential NCAM and p30/32^{MIC2} expression in distinct types of SRCT. Our results confirm[5,33] that neuroblastomas and rhabdomyosarcomas are NCAM$^+$ and show that a large number of lymphoid tumors are also NCAM$^-$. More importantly, however, they demonstrate that both ES and PN/PNET are generally NCAM$^-$.

Previous studies did not predict this difference between neuroblastoma and ES and PN/PNET with regard to NCAM expression; NCAM was regarded as a general marker for neuroectoderm-derived tumors,[5] and ES and PN/PNET are thought to be of primitive neuroectodermal origin.[22,24] Furthermore cell-surface expression of NCAM had been found in a ES-derived cell line, IARC-EW7.[34] The tumor from which IARC-EW7 originated was not tested for NCAM expression,[34] however, and it is conceivable that NCAM was induced after the tumor cells were cultured. This type of induction *in vitro* have been described for other antigens[35] and may explain why most melanoma cell lines are NCAM$^+$ (unpublished results),[6] whereas uncultured melanomas are NCAM$^-$. Alternatively IARC-EW7 may have been derived from an unusual ES, comparable to case 33 in the present study (Table 2), and notably several NCAM$^-$ cultured ES cell lines have been described recently.[16] It is also noteworthy that we found no NCAM immunostaining in our 5.1H11-negative ES tissues using a polyclonal rabbit antibody against NCAM, provided by Dr. K. Crossin, Edelman Laboratory, Rockefeller University, New York, New York. Together with the known wide reactivity of MAb 5.1H11 with different isoforms of human NCAM,[11,14,15] this result suggests that ES tissues do not simply express NCAM isoforms that lack the 5.1H11-reactive epitope. With regard to esthesioneuroblastomas, malignant ectomesenchymomas, small cell osteosarcomas, and mesenchymal chondrosarcomas, too few cases have been examined to date to draw any firm conclusions about their NCAM phenotypes. Testing additional tumors will be an important next step in determining whether immunohistochemical analysis of NCAM phenotypes, alone or in combination with p30/32^{MIC2} analysis,[32] can help to simplify the disparate diagnostic schemes currently in use for SRCT of childhood and adolescence.[22,24]

Detailed analysis of NCAM expression in normal tissues and a broad range of tumors of adults provides several types of information. First it addresses some unexpected results of a recent immunohistochemical study,[33] in which tissues that react with other anti-NCAM MAbs[5–7] were reportedly unreactive with MAb 5.1H11. We show that MAb 5.1H11 in fact reacts strongly with several of these tissues, including adrenal cortex and medulla, lamina propria of the colonic mucosa, visceral smooth muscle, gastric mucosa, Leydig cells in the testis, fibromuscular stroma of the prostate, and epithelial and parafollicular cells of the thyroid gland. Differences in immunohistochemical technique and tissue selection or handling probably explain why MAb NE-25 (another NCAM-specific antibody) was reported initially as being unreactive with some NCAM-expressing tumor types,[6] for example, subsets of renal cell carcinomas and non-small cell lung cancers.

Second our survey of epithelial, neural, mesenchymal, and lymphoid tumors demonstrates that NCAM is not restricted to tumors that share a common histogenesis. Thus NCAM is found in tumors of neural and non-neural origin, and among neuroectodermally derived lesions, some are NCAM$^+$ whereas others are NCAM$^-$, eg, melanomas. For some tumors, an NCAM$^+$ phenotype follows the phenotype of the corresponding normal adult tissue, as illustrated by gliomas and ovarian fibromas. For other tumors, NCAM expression is found in related fetal tissues: neuroblastomas and fetal adrenal chromaffin cells, subsets of renal cell carcinomas and fetal kidney tubules, and non-small cell lung carcinomas and fetal bronchial epithelium. Finally for some NCAM$^+$ tumors we did not find an NCAM$^+$ normal counterpart, as illustrated by the NCAM$^+$ subset of ovarian carcinomas. With regard to this last group of tumors, transient NCAM expression may occur during the earliest stages of fetal or embryonic development that were not represented in our tissue panel. Alternatively NCAM expression may be induced after the malignant transformation of certain NCAM$^-$ target cells. This might explain why we failed to detect the NCAM$^-$/p30/32^{MIC2+} phenotype typical of ES and PN/PNET in fetal neural or mesenchymal cells.

In summary, this study defines the pattern of NCAM

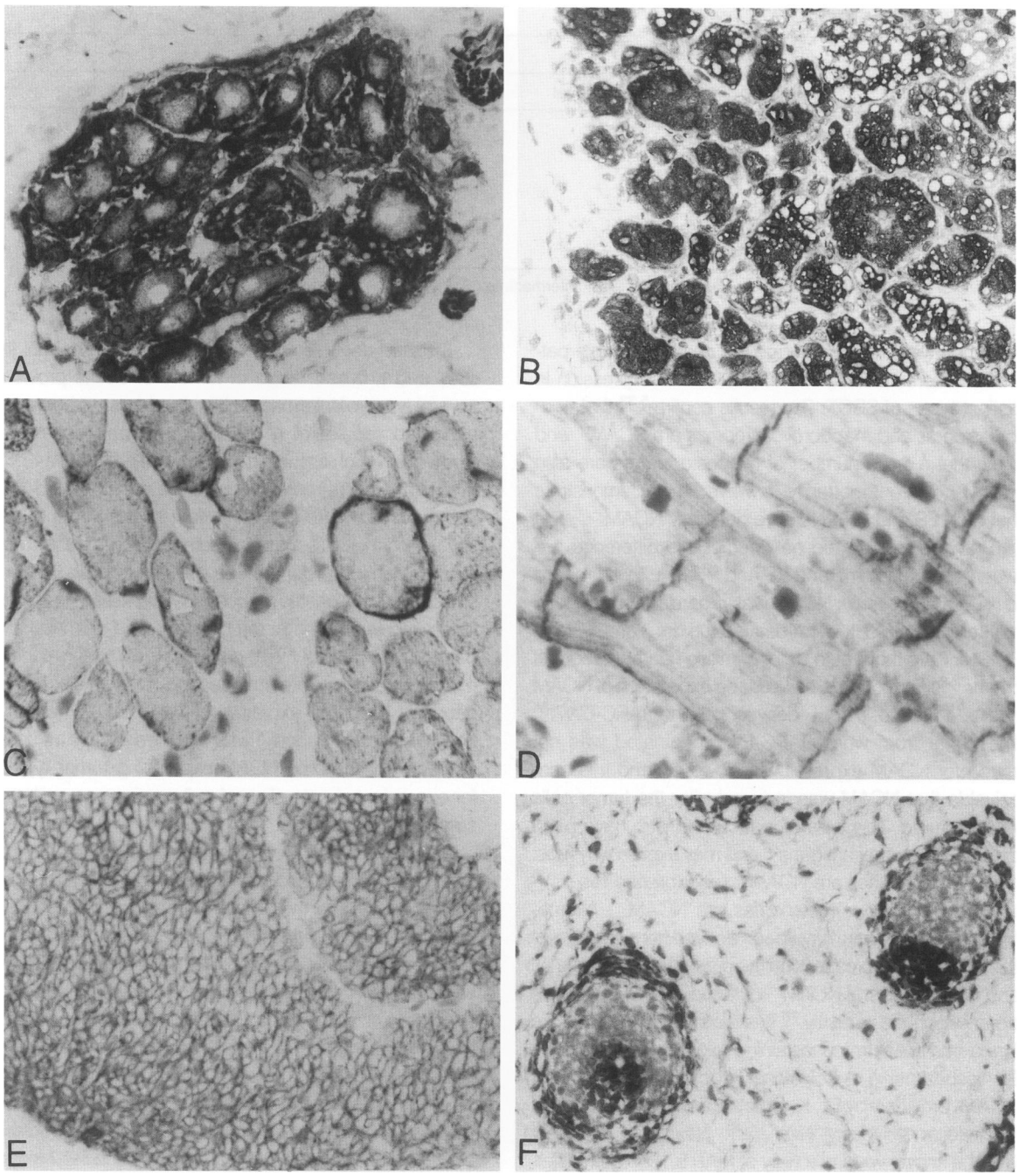

Figure 3. *Immunohistochemical analysis of NCAM expression in normal human tissues using MAb 5.1H11. Avidin-biotin immunoperoxidase method and hematoxylin counterstaining.* **A:** *Parasympathetic ganglion, NCAM$^+$ ganglion cells, satellite cells, and nerve fibers.* **B:** *Adult adrenal cortex, NCAM$^+$ endocrine cells.* **C:** *Reactive skeletal muscle (adjacent to a malignant Schwannoma), NCAM$^+$.* **D:** *Adult cardiac muscle, NCAM$^+$ with prominent staining of intercalated discs.* **E:** *Visceral smooth muscle of adult urinary bladder, NCAM$^+$.* **F:** *Fetal dermis, NCAM$^+$ papillae of hair follicles and scattered NCAM$^+$ fibroblasts. Original magnification,* **A, C, D:** *×100;* **B, E, F:** *×50.*

expression in a broad range of human tumors of diverse histologic type and suggests that NCAM immunohistochemistry may aid in the further characterization of SRCT of childhood and adolescence, especially if it is combined with immunohistochemical assays for other cell-surface antigens and selected intermediate filament proteins,[17,22,25–32] and genetic studies[36,37] of chromosomal abnormalities and specific patterns of oncogene expression and amplification.

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