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Immunohistochemical Analysis of Ewing's Sarcoma Cell Surface Antigen p30/32^{MIC2}

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Monoclonal antibody (MAb) HBA71, which was raised against Ewing's sarcoma cells, recognizes a cell-surface glycoprotein, p30/32^{MIC2}, that is encoded by the* MIC2 *gene in the pseudoautosomal region of human chromosomes X and Y. This immunohistochemical study evaluates the specificity and sensitivity of MAb HBA71 for tumor diagnosis. Frozen and paraffin-embedded tissues of more than 300 tumors of diverse histologic type, including more than 100 small round cell tumors of childhood and adolescence, were tested with this MAb by the avidin-biotin immunoperoxidase procedure. The authors found HBA71 immunoreactivity in 61 of 63 Ewing's sarcomas studied and 9 of 11 primitive neuroectodermal tumors and peripheral neuroepitheliomas. HBA71-negative tumors included neuroblastomas (0 of 24), melanomas (0 of 13), an esthesioneuroblastoma, small cell osteosarcomas (0 of 2), a malignant ectomesenchymoma, desmoplastic SRCT (0 of 5), and medulloblastomas (0 of 5). Heterogeneous expression of HBA71 immunostaining was found in some embryonal rhabdomyosarcomas (3 of 14) and astrocytomas (4 of 7), and in a few neuroendocrine tumors (4 of 26), carcinomas (3 of 94), and lymphomas (6 of 30). Because Ewing's sarcomas are consistently HBA71 positive, the authors searched for antigen-positive normal cells that may represent precursors for these tumors; however, no obvious candidate for the elusive cell of origin for Ewing's sarcoma was identified in the normal fetal tissues tested. Their findings indicate that HBA71 is a highly restricted cell-surface antigen of Ewing's sarcomas and primitive neuroectodermal tumors, and immunohistochemistry employing this antibody may be of value in the differential diagnosis of selected small round cell tumors in childhood and adolescence. (Am J Pathol 1991, 139:317–325)

Small round cell tumors (SRCT) of childhood and adolescence represent a diverse group of tumors of neural, mesenchymal, and lymphoid derivation that may share a similar histologic appearance, characterized by the presence of 'small blue round cells,' ie, poorly differentiated cells with uniform nuclei and scanty cytoplasm.[1,2] The differential diagnosis of SRCT poses a number of problems, but based on histology, electronmicroscopy, immunohistochemistry, and clinical findings, three major types of SRCT have been established as distinct clinicopathologic and histogenetic entities: 1) neuroblastomas, which show sympathetic neuroblast differentiation; 2) embryonal (ERMS) and alveolar rhabdomyosarcomas, which show skeletal muscle differentiation; and 3) various lymphomas. In contrast, a fourth group of SRCT is characterized primarily by the lack of overt differentiation along a single pathway; these cases may show virtually no specific histotypic markers or they may show complex patterns of multilineage differentiation with coexpression of neural, mesenchymal and epithelial traits.[3] Because of the phenotypic heterogeneity of this last group of SRCT, attempts have been made to define subsets within this group that represent clinically and histogenetically more uniform tumor entities. In some diagnostic schemes, the term Ewing's sarcoma (ES) is applied to a large proportion of these tumors,[4–6] with some additional tumors being referred to as primitive neuroectodermal tumor (PNET),[7,8] atypical ES,[9,10] or unclassified SRCT. In other schemes, ES is less commonly diagnosed, and the diagnoses of PNET and peripheral neuroepithelioma (PN) are more frequent.[11,12] Still other schemes use designations such as malignant peripheral neuroectodermal tumor (MPNT),[13,14] small cell osteogenic sarcoma,[15,16] desmoplastic SRCT,[17] and mesenchymal chondrosarcoma[18,19] to denote distinct tumor entities, or designations such as Askin's tumor[20,21] and paravertebral SRCT[22] to distinguish site variants of PN/PNET. These

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varied schemes do not yet provide a satisfactory histogenetic or etiologic classification or uniform diagnostic criteria for SRCT of childhood and adolescence.[1,2]

With advanced cytogenetic and molecular genetic techniques and the advent of hybridoma technology, probes have become available to examine in more detail whether ES, PNET, PN, MPNT, or small cell osteogenic sarcoma represent clinically, etiologically, or histogenetically distinct tumor types or simply variants of the same tumor. One indication that ES and PNET/PN-related SRCT, or at least a proportion of tumors within this group, are pathogenetically related and are distinct from other types of SRCT has come from cytogenetic studies. A common chromosomal abnormality, a reciprocal translocation between chromosomes 11 and 22, t(11;22) (q24;q12), has been identified in ES, PN, Askin tumor, PNET, esthesioneuroblastoma, and small cell osteogenic sarcoma.[23–28] This chromosomal abnormality is not found in neuroblastomas, ERMS, or lymphomas. Another diagnostic approach has been the immunohistochemical analysis of SRCT with antibodies against cell-surface and intracellular antigens such as HNK1, vimentin, neuron-specific enolase (NSE), or cytokeratins.[3,28–37] In general, these studies have shown complex patterns of antigen expression in SRCT that do not yet permit the positive identification of tumors such as ES, PNET, or PN. The monoclonal antibody (MAb) HBA71 of interest in these studies defines an M_r 30,000/32,000 glycoprotein, referred to as p30/32^{MIC2}.[38,39] In the present study, we used tumor tissues obtained and diagnosed at Memorial Hospital with additional cases studied at Childrens Hospital and Yale University to evaluate the specificity and sensitivity of p30/32^{MIC2} immunohistochemistry for tumor diagnosis.

Materials and Methods

Tissues

Fresh tissues were obtained from autopsy or surgical specimens received in the Department of Pathology at Memorial Hospital, New York, New York, frozen in isopentane precooled in liquid nitrogen, and stored at −70°C. Additional cases of SRCT were obtained from the frozen tissue bank of Childrens Hospital, Los Angeles, California. Five-micron–thick frozen sections were cut, mounted on gelatin-coated slides, air dried, and fixed in cold acetone for 10 minutes before immunoperoxidase staining. Paraffin-embedded and paraffin-embedded decalcified tissues were obtained from the files of the Department of Pathology at Memorial Sloan-Kettering Cancer Center. The desmoplastic SRCT were provided by Dr. J. Rosai (Yale University, New Haven, CT) and have been described previously,[17] and four cases of medulloblastoma were provided by Dr. G. Budzilovich (Bellevue Hospital, New York University, New York, NY). Five-micron–thick sections were cut and deparaffinized for immunoperoxidase staining.

Antibodies

The generation and initial characterization of mouse MAb HBA71 (immunoglobulin subclass: IgG1) has been described.[38,39] A single batch of HBA71 hybridoma tissue culture supernatant, which showed a reciprocal titration end point of 1:30,000 in mixed hemadsorption assays with Ewing's sarcoma cell line 6647,[38] was used throughout the present study. Unrelated mouse immunoglobulins of the IgG1 subclass (Becton-Dickinson; Mountain View, CA) were used as negative controls.

Immunohistochemical Procedures

The avidin-biotin-immunoperoxidase procedure was used as described.[27,34] Briefly, frozen sections were treated with 0.3% H_2O_2 for 5 to 10 minutes to block endogenous peroxidase activity and incubated with normal horse serum for 30 minutes at room temperature (RT). Subsequently sections were incubated with MAbs for 12 to 18 hours at 4°C, followed by biotinylated horse anti-mouse IgG (0.015 mg/ml; Vector Laboratories, Burlingame, CA) for 30 minutes at RT, and avidin-biotin-horseradish peroxidase complex (1:100 dilution at a 1:1 ratio; Vector) for 30 minutes at RT. The final reaction product was visualized by the H_2O_2-diaminobenzidine reaction. The sections were counterstained with Harris' hematoxylin. Paraffin-embedded tissue sections were tested as described for frozen sections, except that they were deparaffinized and pretreated with 0.05% saponin (Sigma Chemical Co., St. Louis, MO) in H_2O for 30 minutes at RT as previously described,[40] and blocking of endogenous peroxidase was carried out for 30 minutes. Saponin pretreatment was found to produce optimal staining results when compared with untreated sections or sections pretreated with pronase, pepsin, or trypsin.

Results

HBA71 Expression in Tumors

A panel of more than 300 tumors of diverse histologic type were tested with MAb HBA71 by the avidin-biotin

immunoperoxidase procedure. The tumor panel included frozen tissues and formalin-fixed, paraffin-embedded tissues, and a summary of the immunohistochemical results obtained with both types of tissues is presented in Table 1.

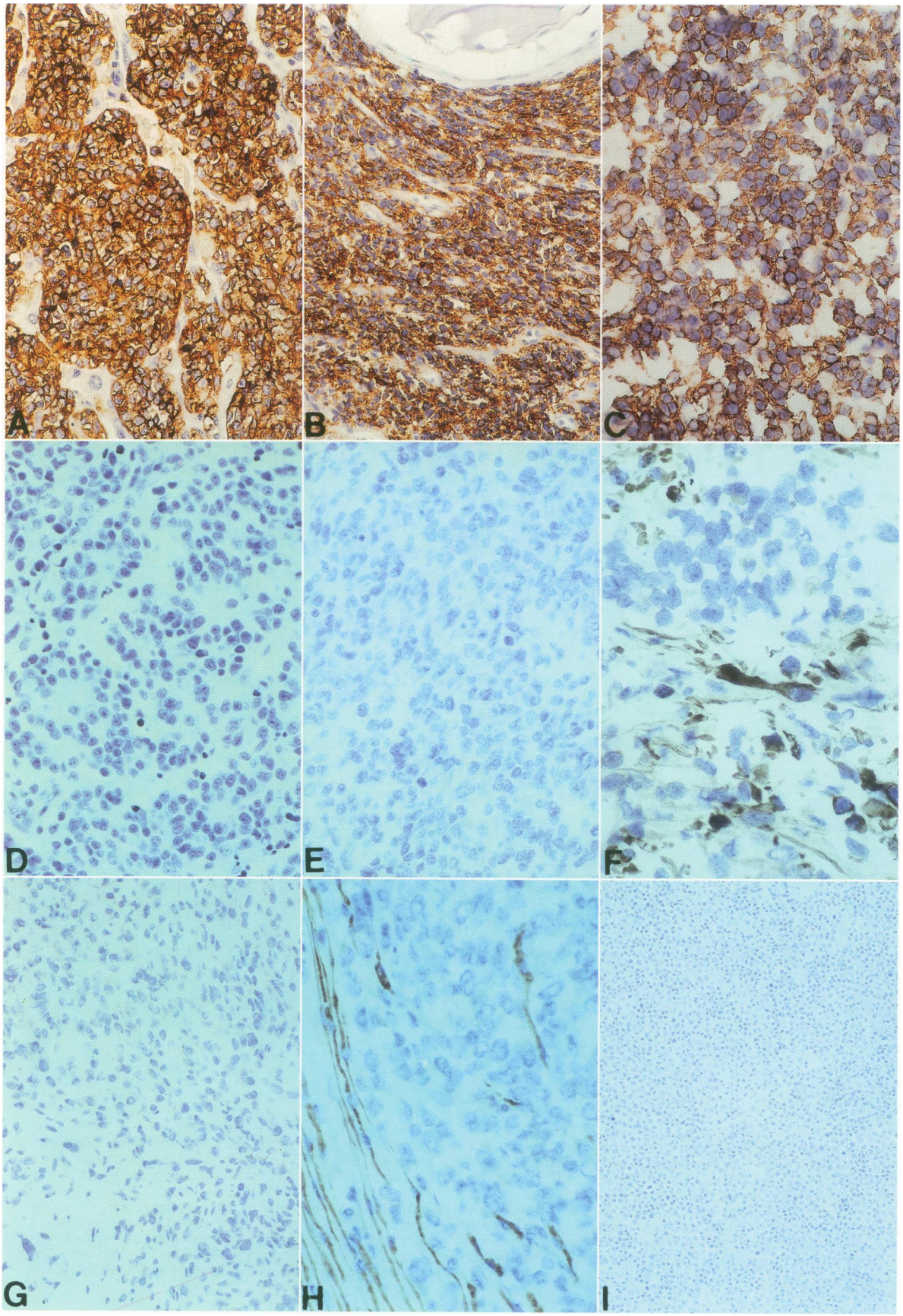
Tumor tissues from 63 patients with ES (including frozen tissues for 23 cases and paraffin-embedded tissues for 40 cases) were tested, and 61 of these tumors were HBA71$^+$ (Table 1), showing strong and homogeneous immunoreactivity as illustrated in Figure 1A and B. In addition, three cases of PNET diagnosed at Memorial Hospital and eight cases of PN and PNET diagnosed at Childrens Hospital were included in this study. All three tumors of the former group and six tumors of the latter

Table 1. *p30/32^{MIC2} Expression in Human Tumors*

Tumor type	n	p30/32^{MIC2} Expression
SRCT (Nonlymphoid)*		
Ewing's sarcoma	63	●′ ●′●′●′●′●′●′●′●′●′●′●′●′●′●′●′●●●●●●●● ●●●●●●●●●●●●●●●○′○′
PNET/PN	11	●′●′●′●●●●●●○○
Neuroblastoma	24	○′○′○′○′○′○′○′○′○′○′○○○○○○○○○○○○○ ○
Esthesioneuroblastoma	1	○
Malignant ectomesenchymoma	1	○
Small-cell osteosarcoma	2	○○
Medulloblastoma	5	○′○′○′○′○
Desmoplastic SRCT	5	○′○′○′○′○′
ERMS	15	◒◒◒○○○○○○○′○′○′○′○′○′
Sarcomas		
Leiomyosarcoma	11	●●●●◒○○○○○○
MFH	10	●●●◒○○○○○○
Liposarcoma	7	○′○′○′○○○○
Chondrosarcoma	8	●○′○○○○○○
Fibrosarcoma	6	●●○○○○
Osteogenic sarcoma	4	○○○○
Synovial sarcoma	3	○○○
Lymphomas		
Non-Hodgkin's lymphoma	19	●′●′◒′◒○′○′○′○′○′○′○○○○○○○○○
Hodgkin's disease	9	○′○′○′○′○′○○○○
Thymoma	2	●●
Neuroectodermal tumors		
Melanoma	13	○○○○○○○○○○○○○
Malignant schwannoma	8	●○○○○○○○
Astrocytoma	7	●◒◒◒○○○
Ganglioneuroma	4	○○○○
Neuroendocrine tumors	26	●●●●○′○′○′○′○′○′○′○′○′○′○○○○○○○○○ ○○○
Carcinomas		
Kidney	13	○○○○○○○○○○○○○
Breast	12	○○○○○○○○○○○○
Colon	10	○○○○○○○○○○
Lung	9	○○○○○○○○○
Testis	8	◒○○○○○○○
Duodenum	1	○
Stomach	6	○○○○○○
Pancreas	6	○○○○○○
Bladder	7	◒○○○○○○
Thyroid	4	○○○○
Ovary	10	○○○○○○○○○○
Uterus	4	○○○○
Esophagus	4	◒○○○
Thymus	1	○

HBA71 expression was determined by the avidin-biotin immunoperoxidase method. Symbols indicate: ●′, antigen-positive; ○′, antigen-negative; ◒′, heterogeneous immunoreactivity with 10%–40% of tumor cells; all results determined through tests with routine formalin-fixed, paraffin-embedded tissues. ●,○,◒: results indicated as above but obtained with acetone-fixed frozen tissue sections. n, number of tumors tested for each category shown on the left.

* Abbreviations: SRCT, small round cell tumor; PNET, primitive neuroectodermal tumor; PN, peripheral neuroepithelioma; ERMS, embryonal rhabdomyosarcoma; MFH, malignant fibrous histiocytoma. The neuroendocrine and endocrine tumors tested included 4 islet cell tumors, 9 gastric and bronchial carcinoid tumors, 1 Merkel cell tumor, 3 small-cell lung carcinomas, 2 medullary thyroid carcinomas, 2 pheochromocytomas, 1 large-cell neuroendocrine carcinoma of the larynx, 1 paraganglioma, 1 Leydig cell tumor, and 1 granulosa cell tumor of the ovary. The testicular tumors tested included 5 seminomas, 2 teratomas (1 showing HBA71 immunoreactivity in areas of skeletal muscle differentiation), and 1 embryonal carcinoma.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group were HBA71$^+$ (Table 1), showing strong and homogeneous immunoreactivity comparable to that seen in ES (Figure 1C).

Unlike ES and PN/PNET, other SRCT of childhood and adolescence showed no or less consistent HBA71 immunoreactivity. For example, all 24 neuroblastomas (Figure 1D), four ganglioneuromas, and five medulloblastomas tested were HBA71$^-$ (Table 1). Five desmoplastic SRCT were also HBA71$^-$, as were two small cell osteosarcomas, one malignant ectomesenchymoma, and one esthesioneuroblastoma. Of 15 cases of ERMS tested, 12 were HBA71$^-$ (Figure 1E). The remaining three cases showed immunostaining in a discrete subset of tumor cells (10% to 30%), which were scattered throughout the tumors and, in some instances, showed morphologic evidence of myoblast differentiation with cross-striations (Figure 1F).

In addition to ES and ERMS, 49 sarcomas of diverse histologic type were examined. Twelve cases were found to be HBA71$^+$, including 4 of 10 MFH, 2 of 6 fibrosarcomas, 1 of 8 chondrosarcomas, and 5 of 11 leiomyosarcomas. Liposarcomas, osteogenic sarcomas (Figure 1G), and synovial sarcomas were HBA71$^-$. Among neuroectodermal tumors, HBA71 was detected in astrocytomas, but immunoreactivity in most of these tumors was heterogeneous (Table 1). Most malignant schwannomas (eight cases tested; Figure 1H) and melanomas (13 cases tested) were found to be HBA71$^-$.

Among the 26 neuroendocrine and endocrine tumors tested, HBA71 immunoreactivity was observed in only four cases, including one islet cell tumor, one carcinoid, one small cell lung cancer, and one Leydig cell tumor.

Ninety-four carcinomas of diverse histologic type were included in the present analysis (Table 1). Only three of these tumors were found to express the HBA71 antigen, namely, one case each of esophagus (1 of 4 cases studied), bladder (1 of 7), and colon carcinoma (1 of 10). The intensity of HBA71 immunostaining in these tumors was weak to moderate, and antigen expression was limited to subsets (10% to 40%) of tumor cells. Most of the lymphomas tested were HBA71$^-$ (Figure 1I). For example, of 28 cases of Hodgkin's and non-Hodgkin's lymphomas tested, four showed HBA71 immunoreactivity. In one T-cell lymphoma and in one gastric B-cell lymphoma, we found moderate HBA71 immunoreactivity and two T-cell lymphomas showed weak staining in 10% to 40% of the tumor cells. In contrast, two cases of thymoma were found to be strongly and homogeneously HBA71$^+$.

HBA71 Expression in Normal Tissues

A number of normal fetal and adult tissues were included in the present immunohistochemical analysis to provide a reference system for the interpretation of HBA71 tumor phenotypes. Among the large number of tissues tested (Table 2), only few HBA71$^+$ cell types were detected. Both fetal and newborn thymocytes showed strong HBA71 immunoreactivity, with immunostaining being restricted to cortical thymocytes (Figure 2A). In addition, a subset of pancreatic islet cells were strongly HBA71$^+$. Weak or variable HBA71 immunoreactivity was observed in fibroblasts in some organs, such as ovary and uterus, in perineural cells, a subset of glial cells, some epithelial cells of the rete testis, and fetal pancreatic ductal cells. Developing fetal bone (Figure 2B) and the fetal adrenal gland (Figure 2C) are HBA71$^-$. The vascular endothelial cells in some but not all fetal organs, for example, umbilical cord, adrenal gland, and brain, also show HBA71 immunoreactivity (Figure 2D).

Finally we found that normal fetal and adult skeletal muscle are HBA71$^-$ or HBA71^{weak} but that reactive skeletal muscle fibers adjacent to some tumors, eg, ERMS and malignant schwannomas, show strong HBA71 staining (Figure 1H), consistent with localized antigen induction in these cells.

Discussion

The present immunohistochemical study employs an MAb, HBA71, that recognizes the p30/32^{MIC2} antigen[38] in frozen, paraffin-embedded, and decalcified paraffin-embedded tissues, to examine *MIC2* expression in over 100 cases of SRCT, and to address some of the unresolved questions concerning the differential diagnosis and histogenetic classification of SRCT of childhood and adolescence. We show that p30/32^{MIC2} immunoreactivity is present in the great majority of ES and PN/PNET tissues included in this study, but not in neuroblastomas, desmoplastic SRCT, most ERMS, medulloblastomas, and other types of SRCT, eg, esthesioneuroblastoma, small cell osteosarcoma, mesenchymal chondrosarcoma, and malignant ectomesenchymoma. For some of these rare tumor types, only one or two cases were available for study, and additional cases need to be tested to confirm their *MIC2* phenotype. The finding that ES and PN/PNET consistently express p30/32^{MIC2}, whereas that classical neuroblastomas are antigen negative is consistent with some

Figure 1. *Immunohistochemical detection of p30/32^{MIC2} expression in tumor tissues.* **A**: *Ewing's sarcoma.* **B**: *Ewing's sarcoma.* **C**: *PNET.* **D**: *Neuroblastoma.* **E**: *ERMS, no immunoreactivity.* **F**: *ERMS, note small subset of p30/32^{MIC2+} tumor cells.* **G**: *Osteogenic sarcoma.* **H**: *Malignant schwannoma.* **I**: *Non-Hodgkin lymphoma. Paraffin-embedded tissues* (**A, D–I**), *decalcified paraffin-embedded tissues* (**B**), *or frozen tissues* (**C**) *were stained with MAb HBA71 by the avidin-biotin immunoperoxidase procedure and counterstained with hematoxylin. Original magnification, ×100* (**A–F, H**); *×50* (**G,I**).

Table 2. *p30/32^{MIC2} Immunoreactivity in Normal Tissues*

Strong p30/32^{MIC2} immunoreactivity
Thymocytes (cortical)*
Pancreatic islet cells (subset)
Weak or moderate p30/32^{MIC2} immunoreactivity
Perineural and glial cells (subset)
Smooth muscle and skeletal muscle cells (variable)
Fibroblasts in some organs (eg, ovary and uterus)
Fetal vascular endothelial cells (variable)
Fetal exocrine pancreas
Seminiferous tubules (subset)

Acetone-fixed frozen sections of normal fetal and adult tissues were tested with MAb HBA71 by the avidin-biotin immunoperoxidase procedure. Adult tissues tested: testis, ovary, uterus, cervix, prostate, urinary bladder, kidney, stomach, esophagus, colon, liver, pancreas, bronchus, lung, skin, mammary gland, adrenal gland, thyroid gland, parathyroid gland, spleen, lymph node, skeletal muscle, heart, peripheral nerves, brain, and spinal cord. Newborn tissue tested: thymus. Fetal tissues (12 to 20 weeks of gestation) tested: thymus, umbilical cord, ovary, kidney, stomach, lung, skin and connective tissue of hand, adrenal gland, spleen, lymph node, smooth muscle, skeletal muscle, heart, cartilage, brain. In addition, a coronal section of a 7-week-old fetus was tested.

* For a detailed description of staining patterns see the Results section.

previous reports suggesting that PN/PNET are phenotypically and genetically more closely related to ES than to neuroblastomas.[25,26,28,41–43] However ES and PN/PNET are not invariably p30/32^{MIC2+}, as illustrated by two ES and two PN/PNET in our extensive analysis. For two of these cases, frozen tissue sections were available for testing with a MAb specific for human neural cell adhesion molecule (NCAM), MAb 5.1H11, and both cases were NCAM$^+$; this finding is noteworthy because NCAM shows a reciprocal pattern of expression to p30/32^{MIC2} among SRCT, being found in neuroblastomas but not in the large majority of ES and PN/PNET.[44] Conceivably both an p30/32^{MIC2+}/NCAM$^-$ phenotype and less common phenotypes, eg, p30/32^{MIC2-}/NCAM$^+$, may exist for PN/PNET, and the clinical and diagnostic significance of this finding is under investigation. *MIC2* expression clearly parallels the presence of the t(11;22) marker chromosome in some SRCT, for example, ES and PN/PNET, in contrast to neuroblastoma. It may not follow the distribution of t(11;22) in other SRCT, for example, esthesioneuroblastomas,[43] small cell osteosarcomas,[27] and some PN and PNET. Instead *MIC2* expression and the presence of t(11;22) may be independent markers in clinical and diagnostic studies and may play distinct biologic roles in SRCT of childhood and adolescence.

With regard to the use of p30/32^{MIC2} immunohistochemistry in the differential diagnosis of SRCT, it is of interest to compare this antigen with previously described ones. In a separate immunohistochemical study, we have carried out a side-by-side analysis of 40 cases of ES for the expression of p30/32^{MIC2}, NSE, muscle-specific actin, leukocyte common antigen, and intermediate filament proteins, including vimentin, desmin, keratins, glial fibrillary acid protein, and neurofilament proteins. The results of this study, to be described in detail elsewhere (Fellinger et al, manuscript in preparation), show that p30/32^{MIC2} is expressed independently of the previously defined antigens and, except for p30/32^{MIC2}, only NSE and vimentin are expressed in a significant proportion of the ES tested. Thus, 38 of 40 ES were p30/32^{MIC2+} and of these, 19 were NSE$^+$, 27 were vimentin$^+$, and 14 were NSE$^+$/vimentin$^+$. Two tumors were p30/32^{MIC2-}/NSE$^-$/vimentin$^-$. These results suggest that p30/32^{MIC2} is the most consistently expressed antigen of ES and PN/PNET described to date. In our analysis, neither p30/32^{MIC2} nor NSE or vimentin serve to distinguish between ES and PN/PNET.

To study the possible role of *MIC2* in the histogenesis of ES, we have begun to examine p30/32^{MIC2} expression in normal adult and fetal tissues. We found strong p30/32^{MIC2} immunostaining in a small range of normal cell types, most notably cortical thymocytes and pancreatic islet cells, and weak staining in some mesenchymal tissues. In the fetal and newborn thymus, p30/32^{MIC2} immunostaining was restricted to cortical thymocytes. Medullary thymocytes, lymphocytes in spleen and lymph nodes, and peripheral blood lymphocytes[38] are antigen negative, suggesting that *MIC2* expression in the thymocyte/T-cell lineage is stage specific. In addition, we found that reactive skeletal muscle fibers adjacent to some tumors, like ERMS or malignant Schwannomas, show strong p30/32^{MIC2} immunoreactivity, whereas normal adult and fetal skeletal muscle fibers are unreactive or only weakly reactive with MAb HBA71. Embryonal rhabdomyosarcomas are generally p30/32^{MIC2-}, except for a heterogeneous expression in a small proportion of tumor cells in some cases. This pattern distinguishes p30/32^{MIC2} and NCAM expression in skeletal muscle tissues, as NCAM is expressed in fetal skeletal muscle, in several types of non-neoplastic muscle disorders,[45] and in ERMS.[44]

We also searched for a normal p30/32^{MIC2+} fetal cell type that may represent the elusive target for transformation in ES. Although we found weak or moderate p30/32^{MIC2} expression in certain fetal mesenchymal cells, there was no apparent correlation with early stages of neuroectodermal or mesenchymal differentiation. For example, no p30/32^{MIC2+} cells were found in fetal mesenchyme undergoing early stages of bone formation, or in the fetal adrenal gland. At least two explanations could account for these observations. First p30/32^{MIC2+} tumors may arise from p30/32^{MIC2-} normal cells that express antigen only after malignant transformation. Alternatively ES may arise from certain p30/32^{MIC2+} normal cells that

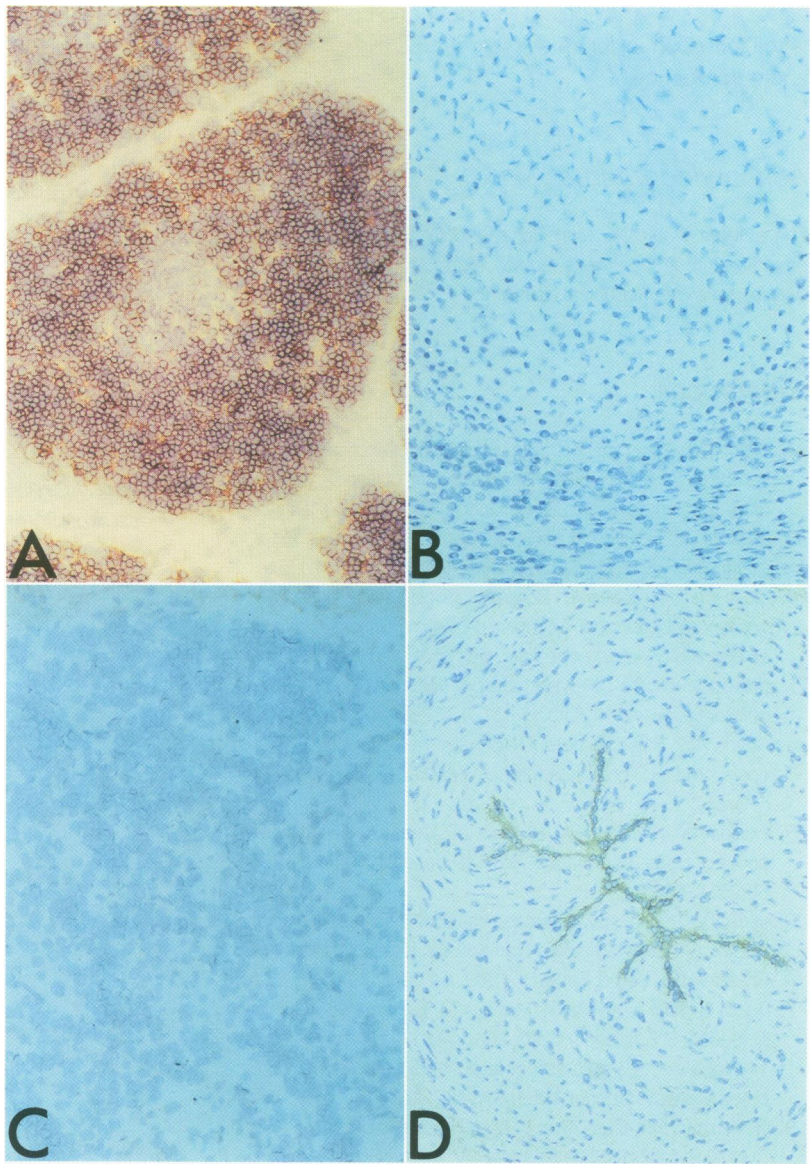

Figure 2. *Immunohistochemical detection of p30/32^{MIC2} expression in normal fetal tissues.* **A**: *Thymus.* **B**: *Developing bone (precartilage blastema) of the hand.* **C**: *Adrenal gland.* **D**: *Endothelium of umbilical blood vessel. Frozen tissues were stained with MAb HBA71 by the avidin-biotin immunoperoxidase procedure and counterstained with hematoxylin. Original magnification, ×50.*

were not represented in our test samples, either because of their low frequency or because of the specific stages of development tested. In particular, human embryonic tissues were not available for our studies, preventing analysis of very earliest steps of development, including early stages of neuroectodermal differentiation.

A broad range of tumors of diverse histologic types were tested for p30/32^{MIC2} expression to determine the specificity of this antigen for ES and PN/PNET. We found that many tumor types tested, including most carcinomas and lymphomas, were antigen negative or showed antigen expression in only a small proportion of cases. Nevertheless our findings caution that p30/32^{MIC2} immunohistochemistry by itself is not an all-encompassing diagnostic test that distinguishes ES and PN/PNET from all other tumors. Instead immunohistochemical results for *MIC2* expression need to be interpreted in view of histologic appearance and other diagnostic parameters.

The biologic role of p30/32^{MIC2} in ES and PN/PNET or in normal thymic development is unknown so far, and structural analysis of the *MIC2* gene and protein have yet to provide any clues as to its molecular function.[46] It is already known, however, that *MIC2* is located in the pseudoautosomal region of human chromosomes X and Y.[38,47] This finding suggests that there is no direct link between high-level expression of *MIC2* in ES and PN/PNET and the presence of the t(11;22)(q24;q12) marker chromosome in these tumors. Instead it is likely that cytogenetic analysis and immunohistochemical analysis with MAbs against p30/32^{MIC2} and other antigens, such

as NCAM,[44] will provide independent and complementary information in future studies of SRCT in childhood and adolescence.

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