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Rapid Communication

Expression of the Common Acute Lymphoblastic Leukemia Antigen (CD10) in Mesenchymal Tumors

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The expression of the CD10 antigen, formerly designated as common acute lymphoblastic leukemia antigen and recently identified as neutral endopeptidase, was examined immunohistochemically in 26 benign and in 55 malignant mesenchymal tumors. CD10 expression was found in 4 of 4 leiomyomas, 7 of 10 leiomyosarcomas, 1 of 6 rhabdomyosarcomas, 2 of 2 Triton tumors, 1 of 2 aggressive fibromatoses, 1 of 3 fibrosarcomas, 1 of 4 synovial sarcomas, 1 of 1 giant cell tumors of tendon sheath, 4 of 4 malignant fibrous histiocytomas, 3 of 3 Ewing's sarcomas, and 2 of 3 osteosarcomas. Furthermore, CD10 was expressed consistently in the myoepithelial compartment of 12 fibroadenomas and, in 7 of these cases, in a minor stromal cell population, probably of (myo-) fibroblastic origin. Tumors of adipose tissue (4 lipomas, 5 liposarcomas), tumors of autonomic ganglia (2 ganglioneuromas, 1 ganglioneuroblastoma, 2 neuroblastomas), tumors of peripheral nerves with purely schwannian differentiation (7 malignant schwannomas), and tumors of disputed origin were consistently CD10-negative, however, as were single cases of fibroma and chondrosarcoma. These findings indicate that the expression of CD10 is a frequent but not obligatory feature in some mesenchymal tumors. Therefore CD10 is of value in the differential diagnosis of mesenchymal tumors. (Am J Pathol 1989, 134:961–965)

The CD10 antigen, formerly known as common acute lymphoblastic leukemia antigen (CALLA),[1] is a 100-kd cell surface glycoprotein.[2] Its recently cloned DNA sequence predicts a 750-amino acid integral membrane protein with a single 24-amino acid hydrophobic segment that could function both as a transmembrane region and as a signal peptide.[3] Recently, the CD10 molecule has been identified to be identical to neutral endopeptidase.[4] CD10 has been detected on various nonlymphoid cell types, such as renal tubular and glomerular cells, epithelial cells of small intestine, myoepithelial cells of the breast,[5–7] neutrophils,[8,9] cultured fibroblasts,[8] cultured bone marrow stromal cells,[9] some human tumor cell lines,[11,12] and on one case of mediastinal germ cell tumor.[13] Although it has been argued that several types of CD10 proteins may exist, monoclonal antibody (MAb) J5,[14] a well-known CD10 reference antibody, has been shown to recognize the authentic CALLA on granulocytes and on other nonlymphoid cell lines and not another protein with a common J5 epitope.[3] We therefore investigated a comprehensive series of mesenchymal tumors for CALLA expression by application of CD10 (J5).

Materials and Methods

Tissue

Representative frozen tissue samples from 26 benign and 55 malignant mesenchymal tumors collected at our institute over a period of 2.5 years were examined immunohistochemically for the expression of CD10 (CALLA). Diagnosis was based on standard histopathologic criteria as described by Enzinger and Weiss.[15] The tissue samples were quick-frozen in liquid nitrogen and stored at −70 C. Frozen sections of about 1 sq cm and a thickness of 4 to 6 μ were air-dried, acetone-fixed at room temperature for

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Table 1. *Expression of CD10(J5) in Mesenchymal Tumors*

Tumor type	Number		Staining +	±	−
Leiomyoma	4		4	–	–
Leiomyosarcoma	10		6	1	3
Rhabdomyosarcoma	6		–	1	5
Triton tumor	2		–	2	–
Fibroadenoma	12	ME	12	–	–
		SC	–	7	5
Fibroma	1		–	–	1
Aggr. fibromatosis	2		–	1	1
Fibrosarcoma	3		1	–	2
Synovial sarcoma	4		1	–	3
Giant cell tumor of tendon sheath	1		1	–	–
Malignant fibrous histiocytoma	4		3	1	–
Ewing's sarcoma	3		3	–	–
Osteosarcoma	3		2	–	1
Chondrosarcoma	1		–	–	1
Malignant schwannoma	7		–	–	7
Lipoma	4		–	–	4
Liposarcoma	5		–	–	5
Ganglioneuroma	2		–	–	2
Ganglioneuroblastoma	1		–	–	1
Neuroblastoma	2		–	–	2
Alveolar soft-part sarcoma	1		–	–	1
Clear cell sarcoma	1		–	–	1
Epithelioid sarcoma	2		–	–	2

ME, myoepithelial cells; SC, stromal cells.

10 minutes, and immediately stained or stored at −20 C for a short period.

Reagents

Monoclonal antibody (MAb) J5 (isotype IgG2a) recognizing the CD10 antigen (CALLA) was obtained from Coulter Immunology, Hialeah, FL. MAb W6/32.HK (isotype IgG2a), which is a nonbinding variant of the anti-HLA-A,B,C MAb W6/32,[16] was provided by Dr. A. Ziegler, Marburg, FRG. A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes) and a streptavidin-biotinylated peroxidase complex, serving as detection system for the monoclonal primary antibody, were provided by Amersham, High Wycombe, UK. 3-amino-9-ethyl-carbazole (AEC) and N'N-dimethylformamide (DMF) were obtained from Sigma, St. Louis, MO.

Staining Procedure

Immunohistochemical staining was performed essentially as described previously.[17] After rehydration with phosphate-buffered saline solution (PBS; pH 7.4), the frozen sections were incubated for 1 hour with MAb J5 or MAb W6/32.HK (final dilution of both MAbs, 1:100). The sections were then incubated with biotinylated sheep anti-mouse Ig (1:50) and streptavidin-biotinylated peroxidase complex (1:100) for 30 minutes each. All incubation steps were carried out in a humid chamber at room temperature and followed by double-rinsing with PBS. The binding of antibody was visualized by incubating with 0.4 mg/ml AEC, 5% DMF, and 0.015% H_2O_2 in 0.1 M acetate buffer (pH 5.2) for 10 minutes. The sections were counterstained with Mayer's hemalaun and mounted with glycerol gelatin.

Controls

Negative controls in each case were performed by omitting the primary antibody: no staining was observed except for scattered granulocytes. This staining was due to endogenous peroxidase, which was not blocked for the benefit of optimal antigenicity. The positive results were confirmed by a control using the irrelevant, isotype-matched MAb W6/32.HK, which gave negative results in each case, ruling out nonspecific antibody binding.

Evaluation

The staining of tumor cells was evaluated semi-quantitatively as follows: +, positivity of the entire population: ±, positive and negative tumor cells in various amounts (for details see results); −, negative staining of the entire tumor cell population.

Results

Some types of neoplasms (tumors of adipose tissue, tumors of peripheral nerves with purely schwannian differentiation, tumors of autonomic ganglia) were uniformly CD10-negative (Table 1). The tumor entities exhibiting positive results for CD10 will be analyzed in detail.

Tumors of Muscle Tissue

Consistent staining for CD10 was observed in 4 of 4 leiomyomas (LM) and in 6 of 10 leiomyosarcomas (LMS; Figure 1a). CD10-positive and negative tumor cells were detectable in about equal amounts in another LMS as well as in one rhabdomyosarcoma (RMS; Figure 1b). Three LMS and five RMS (two of them composed of undifferentiated small round cells) were CD10-negative throughout.

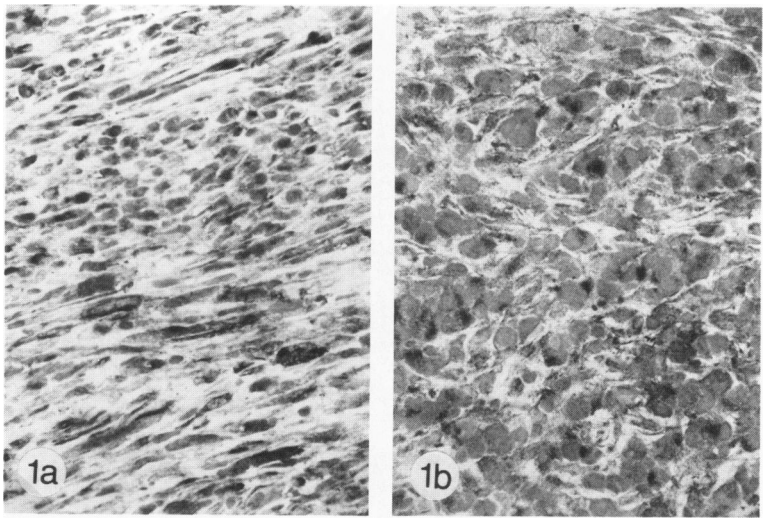

Figure 1a. *Pleomorphic leiomyosarcoma (× 280). The tumor cells exhibit positive staining for CD10 molecules on the cell membrane as well as intracytoplasmatically (immunoperoxidase staining of frozen sections, faint hematoxilin counterstain; same technique for all photomicrographs).* **b:** *Undifferentiated rhabdomyosarcoma (× 220). Tumor cells with a dotlike intracytoplasmatic positivity for CD10 antigens and CD10-negative tumor cells in about equal proportions.*

Triton Tumors

A subpopulation of tumor cells was CD10-positive in the two cases of malignant schwannoma with rhabdomyoblastic differentiation.

Fibroadenomas

In 12 fibroadenomas investigated the entire myoepithelial compartment exhibited positive staining for CD10. In addition, in 7 of 12 cases a minor stromal cell population, probably of (myo-)fibroblastic origin, expressed the CD10 antigen.

Tumors of Fibrous Tissue

In one fibrosarcoma (FS) the entire tumor cell population, and in one aggressive fibromatosis (AFI), a tumor cell subpopulation expressed the CD10 antigen. Two FS, one AFI, and one fibroma were CD10-negative.

Tumors of Synovial Tissue

In one giant cell tumor of tendon sheath and in one monophasic fibrous synovial sarcoma (SS) the entire tumor cell population was CD10-positive. Three cases of SS, also of monophasic fibrous type, lacked any detectable CD10.

Fibrohistiocytic Tumors

In three malignant fibrous histiocytomas (MFH) the entire tumor cell population expressed the CD10 antigen (Figure 2); one case contained CD10-positive and negative tumor cells in about equal proportions.

Tumors of Cartilage and Bone-Forming Tissues

One chondrosarcoma was CD10-negative, as was one osteosarcoma (OS). In contrast, two OS expressed CD10 on the entire tumor cell population (Figure 3).

Miscellaneous

Three Ewing's sarcomas (ES) composed of undifferentiated small round cells expressed CD10 in virtually every tumor cell. Two epithelioid sarcomas, one alveolar soft part sarcoma, and one clear cell sarcoma of tendons and aponeuroses were CD10-negative.

Discussion

Previous studies demonstrated that CD10 is not specific for either leukemic or normal hematopoietic cells and suggested the expression of this antigen on cells widely distributed in tissues of mesenchymal and ectodermal origin. The molecule is identical to neutral endopeptidase (NEP), also known as enkephalinase.[4] This enzyme inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin.[4] In this context it is noteworthy that CD10 has been observed in myoepithelial cells of the mammary gland[7] and in some glioma cell lines.[18] This study was aimed at determining the distribution pattern of CD10 in mesenchymal tumors and its possible value for histopathologic diagno-

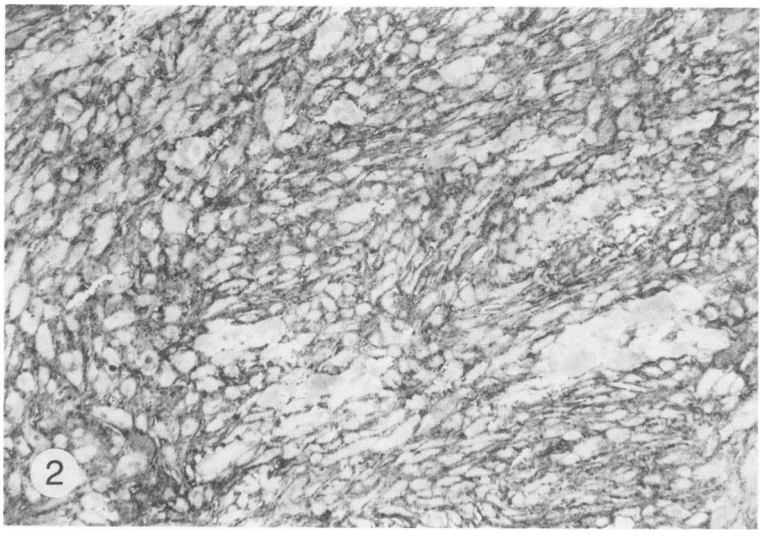

Figure 2. *Storiform-pleomorphic malignant fibrous histiocytoma (× 175). Virtually every tumor cell exhibits strong cell membrane staining for CD10 antigens.*

sis. It supplies the first *in situ* expression of CD10 in a large series of mesenchymal tumors of various origin. In agreement with Wood et al,[19] we found CD10 to be expressed in malignant fibrous histiocytomas. This was a consistent feature in the series of four cases. As a histogenetic marker, however, CD10 is only valuable in comparison with other tissue or cell type-specific antibodies because it also has been found in other mesenchymally-derived tumors—we found CD10 to be expressed frequently in leiomyomas as well as in leiomyosarcomas. Furthermore, its consistent expression in myoepithelial cells in mammary tissue[5,7] could be confirmed in 12 fibroadenomas studied. The expression of CD10 in myogenous tissue, however, is not restricted to smooth muscle derived cells but also occurs in tissues of striated muscle cell differentiation. This could be demonstrated by the CD10 positivity of a tumor cell subpopulation in one RMS and in two malignant schwannomas with rhabdomyoblastic differentiation (so-called Triton tumors). Malignant schwannomas with purely schwannian cell differentiation, however, were consistently CD10-negative, as were tumors of adipose tissue. From these data we conclude that the expression of CD10 might be a complementary immunohistochemical tool in the differential diagnosis of LMS *vs.* malignant schwannoma and liposarcoma, which may share similar morphologic appearances. Furthermore, it is well known that some LMS are desmin-negative.[15] In such cases the expression of CD10 might be an argument in favor of a LMS.

Braun et al[8] and Pesando et al[12] succeeded in demonstrating the expression of CD10 in cultured fibroblasts whereas Metzgar et al,[5] in an *in situ* study of fetal fibroblasts, did not find any CD10-positivity. Gusterson et al,[7] however, detected some CD10-positive stormal cells in mammary tissue and considered them to be of fibroblastic origin. We found a CD10-positive stromal subpopula-

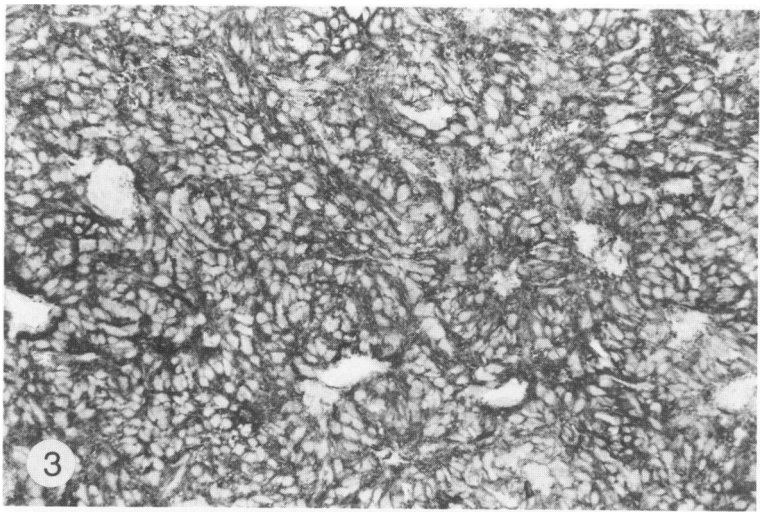

Figure 3. *Spindle-cell osteosarcoma (× 220). CD10 molecules are expressed by the entire tumor cell population. Endothelial cells of small blood vessels are unstained.*

tion, probably of (myo-)fibroblastic origin, in 7 of 12 fibroadenomas. Furthermore, the *in situ* demonstration of this antigen in one fibrosarcoma and in one aggressive fibromatosis underlines its possible presence in fibrous tissue components.

A striking feature was the consistent expression of CD10 in the three cases of Ewing's sarcoma under study. In contrast, two undifferentiated small round-cell neuroblastomas lacked any detectable CD10. This indicates that CD10 might be of help in the differential diagnosis of these two tumors.

In summary, we found CD10 to be expressed in a considerable number of mesenchymal tumors. These included tumors of myogenous, fibrous, and fibrohistiocytic origin as well as Ewing's sarcomas and osteosarcomas. In contrast, the series of tumors of adipose tissue and of peripheral nerves with purely schwannian differentiation completely lacked this antigen. We therefore consider CD10, in comparison with other tissue or cell type-specific antigens, to be of value in the differential diagnosis of mesenchymal tumors. These findings should be confirmed in additional studies that increase the total number of mesenchymal tumors examined for CD10.

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