Histol Histopath (1993) 8: 425-428

Histology and Histopathology

Laminin matrix formation and S-100 protein and/or desmin-positive cells in malignant fibrous histiocytoma (MFH)

H. Kosmehl[1], L. Langbein[2], D. Katenkamp[1], W. Vogel[1] and A. Berndt[1]

[1]Institute of Pathology, Friedrich Schiller University, Jena and [2]Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, FRG

Summary. 16/30 human storiform-pleomorphic malignant fibrous histiocytomas (MFH) showed a focal pericellular immunostaining for laminin. 14/16 of these laminin-positive tumours additionally revealed an atypical cellular differentiation (desmin- and/or S-100 protein-positive cells). The significance of focal laminin positivity along with atypical, non-entity specific differentiated cells is discussed before the background of laminin as being an indicator and promoter of differentiation in MFH. The limited value of a laminin detection in MFH for solving differential diagnostic questions (MFH versus other poorly differentiated sarcomas with basement membrane formation) has been pointed out.

Key words: Laminin, Malignant fibrous histiocytoma, Cellular differentiation

Introduction

Laminin is a major glycoprotein of the extracellular matrix and closely related to processes of cellular adhesion and tissue development (Martin and Timpl, 1987; Campbell and Terranova, 1988).

Previous immunohistochemical studies have failed to show laminin staining in malignant fibrous histiocytoma (MFH) (Miettinen et al., 1983; Beham et al., 1986; d'Ardenne, 1989).

Otherwise, differentiated sarcomas; e.g. leiomyosarcomas, rhabdomyosarcomas, or malignant schwannomas exhibited a prominent extracellular laminin matrix (Miettinen et al., 1984; Reibel et al., 1985; Autio-Harmainen et al., 1986; Leivo et al., 1989).

Taking into account the influence of laminin on processes of cellular differentiation and occurrence of atypical differentiated tumor cells (e.g. desmin, muscle-specific actin, cytokeratins, S-100 protein-positive cells) in MFH (Donhuijsen et al., 1988; Hirose et al., 1989; Miettinen and Soini, 1989; Mentzel et al., 1991) it seems to be justified to look for laminin occurrence and to examine possible relations between atypically differentiated tumor cells and laminin matrix formation in this sarcoma type.

Offprint requests to: Dr. H. Kosmehl, Institute of Pathology, Friedrich Schiller University, Ziegelmühlenweg 1, D-6900 Jena, FRG

Materials and methods

30 storiform-pleomorphic human MFH (buffered formalin-fixed and paraffin-embedded tissue, 1 paraffin block per 1 cm tumor diameter) diagnosed according to the criteria of Enzinger and Weiss (1988) were subjected to immunohistochemistry.

Immunohistochemical staining was performed using the APAAP (alkaline phosphatase monoclonal anti-alkaline phosphatase) method (Gustmann et al., 1991). To enhance tissue antigenicity a pronase E (Sigma, USA) predigestion (0.05% in TRIS buffer) at 37 °C was carried out for 10 to 20 minutes. The primary antibody was incubated for 12 hours at 4 °C.

Primary antibodies: α_1-antichymotrypsin (AAC, polyclonal, Behring, FRG); α_1-antitrypsin (AT, polyclonal, Behring, FRG); CD 68 (clone KP-1, Dako, Denmark); S-100 protein (polyclonal, Dako, Denmark); laminin (polyclonal, Radiochem. Lab. Behring, FRG; clone 2G6-A2, Boehringer, FRG; clone 4C7, Dako, Denmark; clone 4C12.8, Dianova, FRG); fibronectin (polyclonal, Langbein et al., 1990); desmin (clone D-33, Dako, Denmark); pan-cytokeratin (clone KL-1, Dianova, FRG and clone Lu-5, Hoffmann-La Roche, Switzerland); neurofilament (clone 2F11, Dako, Denmark); GFAP (polyclonal, Dako, Denmark); and neuron-specific enolase (NSE, polyclonal, Dako, Denmark).

After incubation with primary antibody and washing with TRIS buffer, sections were treated with rabbit anti-mouse γIG immunoglobulin, and then with the mouse APAAP-complex (both Dako, Denmark). In case of polyclonal primary antibody additionally a secondary mouse anti-rabbit immunoglobulin (Dako, Denmark) was introduced. These incubations were made for 30 minutes at room temperature. To

increase the staining intensity, the incubation with the rabbit anti-mouse immunoglobulin and with the APAAP-complex was repeated twice. Naphtol-AS-bi-phosphate (Sigma, USA) and new fuchsin (Merck, FRG) were used as substrate and developer, respectively. To inhibit endogenous tissue enzyme activity, the developing solution was supplemented with 0.25 mmol/l levamisole (Sigma, USA).

In one case with a strong laminin staining the APAAP-technique was replaced by indirect fluorescence technique for better photographic documentation; FITC-labelled anti-mouse antibodies (IFIN, FRG; Kosmehl et al., 1990a).

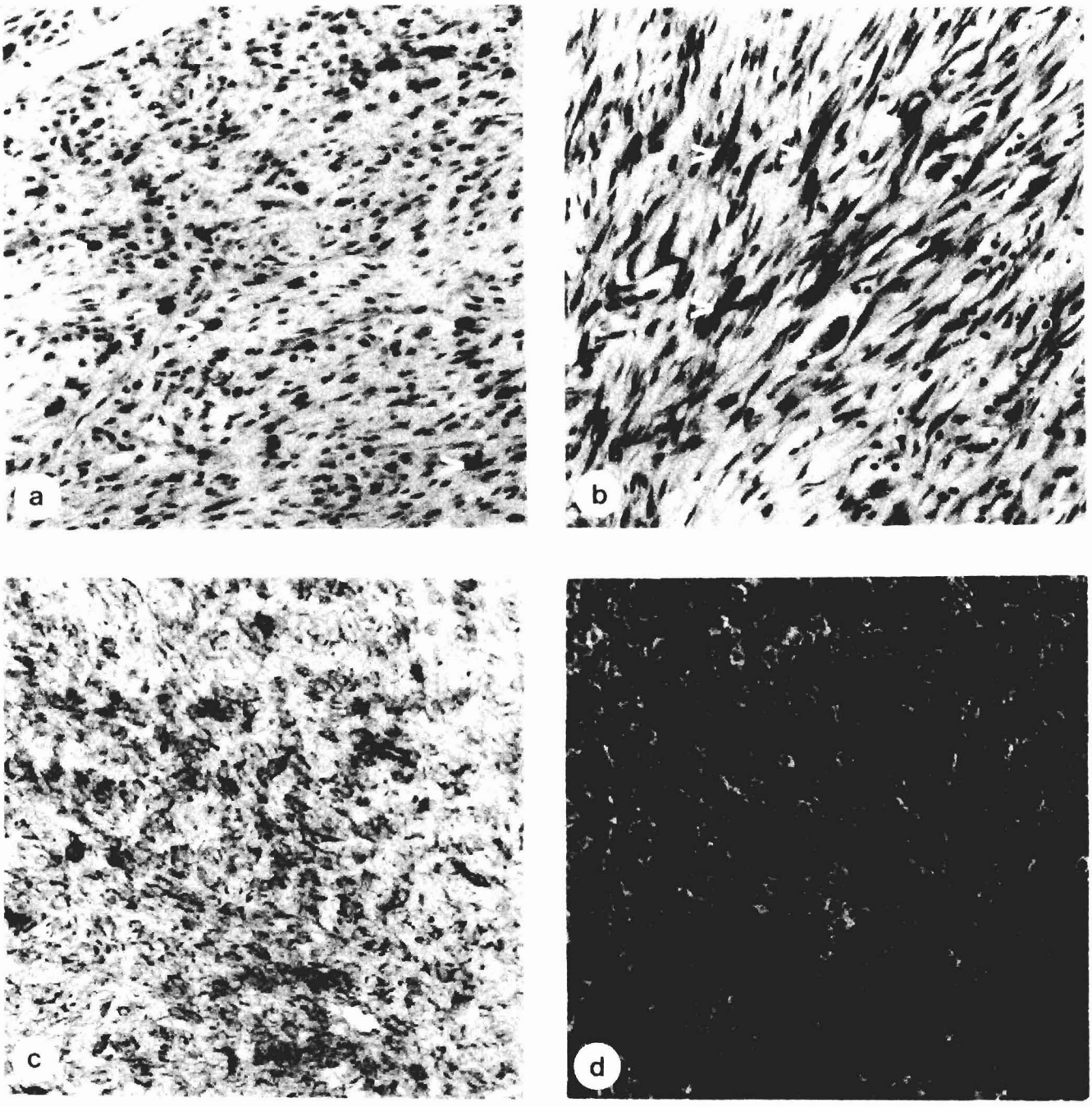

Fig. 1. Immunohistochemical demonstration of single epitopes reacting with antibodies to S-100 protein **(a)** and desmin **(b)** in MFH (arrowheads; APAAP-technique, haematoxylin counter staining; x 250). Visualization of focal pericellular laminin matrix expression in MFH (**c**, APAAP-technique, haematoxylin counter staining; **d,** indirect immunofluorescence technique, same case, x 250).

Results

All 30 MFH showed a strong reaction to the so called histiocytic markers (antibodies to AT, AAC, CD 68; c.p. Binder et al., 1992) in most giant cells as well as in some polygonal cells. In addition, CD 68 was expressed in few spindle cells.

A positivity for S-100 protein (12 cases) and desmin (4 cases) was restricted to few single cells that were haphazardly distributed within the tumour (Fig. 1a,b). In two of these cases S-100 protein- and desmin-positive cells were seen within the same tumour. In 16 cases a focal pericellular laminin staining was detected independent of vascular stromal formations. Laminin was distributed in the pericellular space in the form of small strands (Fig. 1c,d). Moreover, some of the pleomorphic tumour giant cells exhibited a cytoplasmic laminin positivity. Local laminin staining could be seen in all tumours showing S-100 protein- or desmin-positive cells, but not in every case laminin-positive foci did show S-100- or desmin-positive cells. Only in 2/16 cases of a laminin positivity in MFH desmin- and/or S-100 protein-positive cells were completely absent. The laminin staining with the polyclonal antibodies was more extensive, but also by the used monoclonals a reproducible immunostaining of pericellular laminin depositions was achieved.

In MFH areas with only a clear storiform growth pattern and without giant cells, laminin was regularly lacking.

Fibronectin could be visualized in all MFH. The staining was seen throughout the whole tumours, but with quantitatively local variations.

Staining for cytokeratins, neurofilaments, glia-filaments, or NSE was absent in all cases.

Discussion

Although in earlier investigations laminin could not be detected in MFH, our immunohistochemical demonstration of laminin is in line with recent results in literature (Soini et al., 1989; Soini and Autio-Harmainen, 1991).

In all cases of our MFH showing desmin- or S-100 protein-positive cells laminin-positive tumour areas were constantly seen. But it should be emphasized that not all of these regions also contained atypically differentiated sarcoma cells. The obvious correlation between the occurrence of laminin positivity and atypically differentiated tumour cells in MFH could be explained by the known association of muscle and Schwann cell differentiation with laminin expression. Moreover, laminin itself promotes differentiation of these cell types; e.g., extracellular laminin matrix is a prerequisite for terminal differentiation and myotube formation in normal and neoplastic skeletal muscle development (Leivo et al., 1989; von der Mark and Öcalan, 1989; Langbein et al., 1990).

Considering the possible interpretations of MFH as a specific tumour entity, a final common pathway of sarcoma progression or a histogenetic progenitor of otherwise differentiated sarcomas (Brooks, 1986; Dehner, 1988; Roholl et al., 1988), the local expression of laminin in MFH may be taken as an indicator of the start or endpoint of such modulation processes. In an experimental rhabdomyosarcoma model the modulation of phenotype from dedifferentiated (MFH-like) tumours to sarcomas, showing a rhabdomyosarcoma phenotype, was also associated with the formation of extracellular laminin matrix (Kosmehl et al., 1990b; Vogel et al., 1991).

Because laminin affects growth and differentiation of mesenchymal cells (so called structural growth factor), modulates the cellular susceptibility to growth factors and moreover shows structural homology to epidermal growth factor (Bissell et al., 1982; Bissell and Ram, 1989; Engel, 1989; Anderson et al., 1990), investigations concerning the relation of extracellular matrix and growth factors for phenotype expression may contribute to understanding differentiation processes in sarcomas.

From the diagnostic point of view a focal laminin positivity in MFH should initiate the search for atypical cellular differentiation. Furthermore, the question arises whether or not MFH has been mimicked by an otherwise differentiated sarcoma (so called secondary MFH). Because the definition «MFH» allows single cells to occur with other antigenic sites than usual in fibrohistiocytic cells (Dehner, 1988) focal laminin staining per se is not sufficient to exclude a MFH diagnosis.

Acknowledgements. The work was generous supported by Dako Diagnostika GmbH Hamburg.

References

Anderson T.L., Gorstein F. and Osteen K.G. (1990). Stromal-epithelial cell communication, growth factors, and tissue regulation. Lab. Invest. 62, 519-521.

Autio-Harmainen H., Apaja-Sarkkinen M., Martikainen J., Taipale A. and Rapola J. (1986). Production of basement membrane laminin and type IV collagen by tumors of striated muscle: An immunohistochemical study of rhabdomyosarcomas of different histiologic types and benign vaginal rhabdomyoma. Hum. Pathol. 17, 1218-1224.

Beham A., Wirnsberger G. and Schmid Ch. (1986). Immunhistochemische Untersuchungen zur Differentialdiagnose des malignen fibrösen Histiocytoms. Wien. klin. Wschr. 98, 617-622.

Binder S.W., Said J.W., Shinkatu P. and Pinkus G. (1992). A histiocyte-specific marker in diagnosis of malignant fibrous histiocytoma. Use of monoclonal antibody KP-1 (CD-68). Am. J. Clin. Pathol. 97, 759-763.

Bissell M.J., Hall H.G. and Parry G. (1982). How does the extracellular matrix direct gene expression? J. Theoret. Biol. 99, 31-68.

Bissell M.J. and Ram T.G. (1989). Regulation of functional cytodifferentiation and histogenesis in mammary epithelial cells. Role of extracellular matrix. Environm. Health Persp. 80, 61-70.

Brooks J.J. (1986). The significance of double phenotypic patterns and markers in human sarcomas. A new model of mesenchymal differentiation. Am. J. Pathol. 125, 113-123.

Campbell J.H. and Terranova V.P. (1988). Molecular organization and biological function. J. Oral. Pathol. 17, 309-323.

d'Ardenne A.J. (1989). Use of basement membrane markers in tumour diagnosis. J. Clin. Pathol. 42, 449-457.

Dehner L.P. (1988). Malignant fibrous histiocytoma. Nonspecific morphologic pattern, specific pathologic entity, or both? Arch. Pathol. Lab. Med. 112, 236-237.

Donhuijsen K., Metz K. and Leder L.D. (1988). Desmin- und S-100. Protein-positive maligne fibröse Histiocytome: praktische und theoretische Implikationen. Pathologe 9, 261-267.

Engel J. (1989). EGF-like domains in extracellular matrix proteins localized signals for growth and differentiation. FEBS Lett. 25, 1-4.

Enzinger F.M. and Weiss S.W. (1988). Soft tissue tumors. Mosby. St. Louis.

Gustmann Ch., Altmannsberger M., Osborn M., Griesser H. and Feller A.C. (1991). Cytokeratin expression and vimentin content in large cell anaplastic lymphomas and other non-Hodgkin lymphomas. Am. J. Pathol. 138, 1413-1422.

Hirose T., Kudo E., Hasegawa T., Abe J.-I. and Hizawa K. (1989). Expression of intermediate filaments in malignant fibrous histiocytomas. Hum. Pathol. 20, 871-877.

Kosmehl H., Langbein L. and Katenkamp D. (1990a). Lectin histochemistry of testicular germ cell tumors. Neoplasma 38, 29-39.

Kosmehl H., Langbein L. and Katenkamp D. (1990b). Experimental rhabdomyosarcoma with regions like malignant fibrous histiocytoma (MHF)-a true double phenotypic pattern? J. Pathol. 160, 135-140.

Langbein L., Kosmehl H., Katenkamp D., Neupert G. and Stiller K.J. (1990). Experimentally induced murine rhabdomyosarcomas-correlations between cellular contacts, matrix formation and cellular differentiation. Differentiation 44, 185-196.

Leivo I., Engvall E., Laurilla P. and Miettinen M. (1989). Distribution of merosin, a laminin-related tissue specific basement membrane protein, in human Schwann cell neoplasms. Lab. Invest. 61, 426-432.

Martin G.R. and Timpl R. (1987). Laminin and other basement membrane components. Annu. Rev. Cell Biol. 3, 57-85.

Mentzel Th., Kosmehl H. and Katenkamp D. (1991). Metastasizing phyllodes tumour with malignant fibrous histiocytoma-like areas. Histopathology 19, 557-560.

Miettinen M. and Soini Y. (1989). Malignant fibrous histocytoma. Heterogeneous patterns of intermediate filament protein immunohistochemistry. Arch. Pathol. Lab. Med. 113, 1363-1366.

Miettinen M., Foidart J.-M. and Ekblom P. (1983). Immunohistochemical demonstration of laminin, the major glycoprotein of basement membranes, as an aid in the diagnosis of soft tissue tumors. Am. J. Clin. Pathol. 79, 306-311.

Miettinen M., Lehto V.-P., Ekblom P., Tasanen A. and Virtanen I. (1984). Leiomyosarcoma of the mandible: diagnosis as made aided by immunohistochemical demonstration of desmin and laminin. J. Oral Pathol. 13, 373-381.

Reibel J., Wewer U. and Albrechtsen R. (1985). The pattern of distribution of laminin in neurogenic tumors, granular cell tumors, and nevi of the oral mucosa. Acta Pathol. Microbiol. Immunol. Scand. Sect. A 93, 41-47.

Roholl P.J.M., Rutgers D.H., Rademakers L.H.P.M., DeWeger R.A., Elbers J.R.J. and Van Unnik J.A.M. (1988). Characterization of human soft tissue sarcomas in nude mice. Evidence for histogenetic properties of malignant fibrous histiocytomas. Am. J. Pathol. 131, 559-568.

Soini Y. and Autio-Harmainen H. (1991). Tumor cells of malignant fibrous histiocytomas express mRNA for laminin. Am. J. Pathol. 139, 1061-1068.

Soini Y., Autio-Harmainen H. and Miettinen M. (1989). Immunoreactivity for laminin and type IV collagen in malignant and benign fibrous histiocytoma. J. Pathol. 158, 223-228.

Vogel W., Kosmehl H., Katenkamp D. and Langbein L. (1991). Differentiation dependent matrix formation (fibronectin and laminin) in an experimental rhabdomyosarcoma model. Acta Histochem. 90, 181-188.

von der Mark K. and Öcalan M. (1989). Antagonistic effects of laminin and fibronectin on expression of the myogenic phenotype. Differentiation 40, 150-157.

Accepted February 4, 1993