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Jacalin, another marker for histiocytes in paraffin-embedded tissues

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Summary. Jacalin is a lectin which reacts with D-galactose. We have tested jacalin on 75 samples of different formalin- and alcohol-fixed tissues. A consistent cytoplasmic stain of the histiocytes was observed in paraffin-embedded tissues in all cases studied of reactive sinus histiocytosis, macrophages in clear centres of follicular hyperplasia, in tuberculosis granulomas and in osteoclast-like giant cells in a breast carcinoma. We failed to find any clear binding of jacalin to the cells of eosinophilic granulomas, giant cell tumors of tendon sheath, pleomorphic malignant fibrous histiocytomas, Hodgkin's disease, melanomas, nevi or signet ring cell carcinomas of the breast and stomach. It seems that jacalin is a good marker for free histiocytes/macrophages, not for fixed histiocytes and tumors related to them. This lectin might play a role in differential diagnosis with histiocyte mimicking processes.

Key words: Histiocytes, Macrophages, Lectins, Jacalin

Introduction

Lectins are proteins and glycoproteins of plants or animal nature, of nonimmune origin, that bind specific carbohydrate side chains of glycoconjugates, with high specificity and affinity (Franklin, 1983; Alroy et al., 1988). It seems that carbohydrate structures are highly resistant to the process of paraffin embedding (Krogerus and Anderson, 1990). For this reason, lectins are useful tools for the study of carbohydrate structures in routine surgical pathology.

Jacalin is a lectin which was isolated from dried seeds of the jackfruit *Artocarpus intergrifolia*. This lectin reacts with D-galactose and interacts with human IgA (only with IgA_1 not with IgA_2 (Aucouturier et al., 1987; Alroy et al., 1988; Hagiwara et al., 1988).

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Using different lectins in metastased lymph nodes, we appreciated a consistent binding of jacalin to histiocytes. This finding prompted us to test jacalin in different processes trying to prove the possible value of jacalin as a histiocytic marker.

Materials and methods

For this study we have used 75 samples of different tissues, always formalin-fixed and paraffin-embedded, except two tonsils which were fixed in alcohol.

The cases herein studied include: ten lymph nodes with reactive sinus histiocytosis, belonging to axillary lymph node dissections in female breast cancers; ten lymph nodes with reactive follicular hyperplasia; six vermiform appendixes with follicular hyperplasia; two tonsils with follicular hyperplasia; one granulomatous lymphadenitis; three renal tuberculosis; three tuberculosis of the pleura; one tuberculosis of the fallopian tube; two eosinophilic granulomas (these cases were also decalcified with 5% nitric acid in 10% formalin); five giant cell tumors of tendon sheath; two pleomorphic malignant fibrous histiocytomas; two cases of Hodgkin's disease; one breast carcinoma with osteoclast-like giant cells; two intestinal polyps; ten melanomas; five nevi; five signet ring cell carcinomas of the breast; and five gastric signet ring cell carcinomas. Some of the cases here considered have been in paraffin for more than ten years.

One selected section from each one of the cases previously mentioned was deparaffinized in three 10-minute washes of xylene, rehydrated through graded alcohols, and treated with 0.1% trypsin (type II T-8128, Sigma) and 0.1% calcium chloride in phosphate buffer, pH 7.5 at 37 ºC for 10 minutes. Endogenous peroxidase activity was blocked with fresh 3% hydrogen peroxide in methanol for 30 minutes. Sections were incubated with biotinylated jacalin at 1/250 dilution for 24 hours (two hours at room temperature and 22 hours at 4 ºC) (Menarini, Biogenex, cat. 2530). Then, the avidin-biotin complex (Vector Laboratories, Inc.) was applied for 45

minutes at room temperature. Diaminobenzidine was used as chromogen (Taylor, 1978). Control slide staining was abolished by preabsorption of the jacalin with D-galactose.

Results

We found a clear cytoplasmic staining in histiocytes in all the reactive sinus histiocytosis, while the lymphoid cells were negative (Fig. 1). In all the cases of tuberculosis, the histiocytes were also positive, the reaction being more intense in multinucleated giant cells (Fig. 2). In all reactive follicular hyperplasia, both in lymph nodes and in vermiform appendixes, macrophages were positive for jacalin (Fig. 3). The osteoclast-like giant cells in a breast carcinoma were positive for jacalin and the carcinoma cells were negative (Fig. 4). Goblet cells in vermiform appendixes and intestinal polyps were also positive for jacalin (fig. 5). Finally, sebaceous glands in the skin were positive, but only the cytoplasmic membranes.

The two eosinophilic granulomas, two pleomorphic malignant fibrous histiocytomas, five giant cell tumors of tendon sheath, two cases of Hodgkin's disease, ten melanomas, five nevi and all the signet ring cell carcinomas tested, were negative to jacalin. Only one signet ring cell carcinoma of the breast gave a weak, focal and uncertain positivity limited to the cytoplasmic

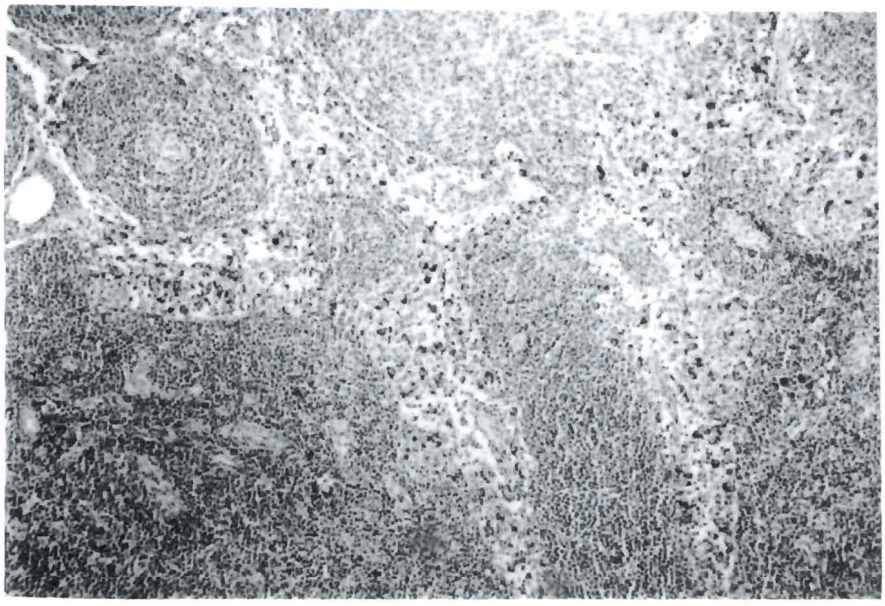

Fig. 1. Positive histiocyte in reactive sinus histiocytosis. Lymphoid cells are negative. ABC stain. x 100

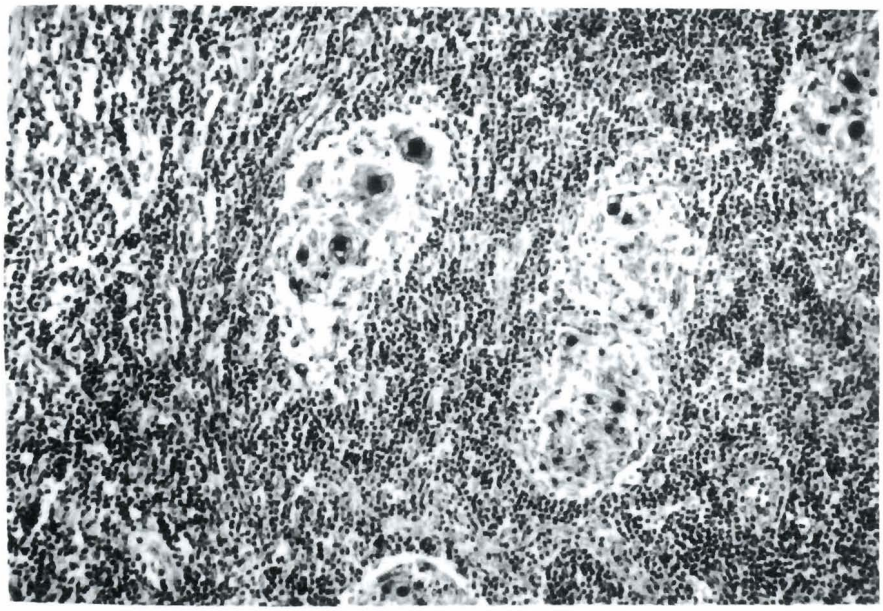

Fig. 2. Granulomas with positive cells for jacalin. ABC stain. x 250

membranes.

Cases with paraffin blocks older than 10 years provided good positive results. The two alcohol-fixed tonsils also gave good results. In formalin-fixed cases, some gave stronger positivity than others. As the cases were randomly collected from our files, we do not know for how long they had been fixed in formalin.

Discussion

The mononuclear phagocytic system is made up of a heterogeneous population (Roholl et al., 1988). The blood monocytes are not end cells, but develop further to become macrophages after diapedesis from blood vessels (Wickramasinghe, 1992). It is also interesting to note that interdigitating reticulum cells and Langerhans cells are considered histiocyte-derived cells (Alpers et al., 1984).

Besides the early use of peroxidase and nonspecific esterase cytochemistry for the detection of histiocytes (Elias, 1990), many antibodies have been used to identify histiocytic cells (Leu-M1, Leu-M3, Leu-M5, My4, My7, My8, My9, Mo1, Mo2, RFD-7, RFD-9, Mac 387, Cathepsin B, OKM1, VIM-D5, FMC17, anti HLA-DR, anti lysozyme, anti alpha-1-antitrypsin, anti alpha-1-antichymotripsin, MT1, 3MA134, LN2, Mb2) (Meister and Nathrath, 1980; Isaacson et al., 1981, Roholl et al., 1988; Norton and Isaacson, 1989; Elias,

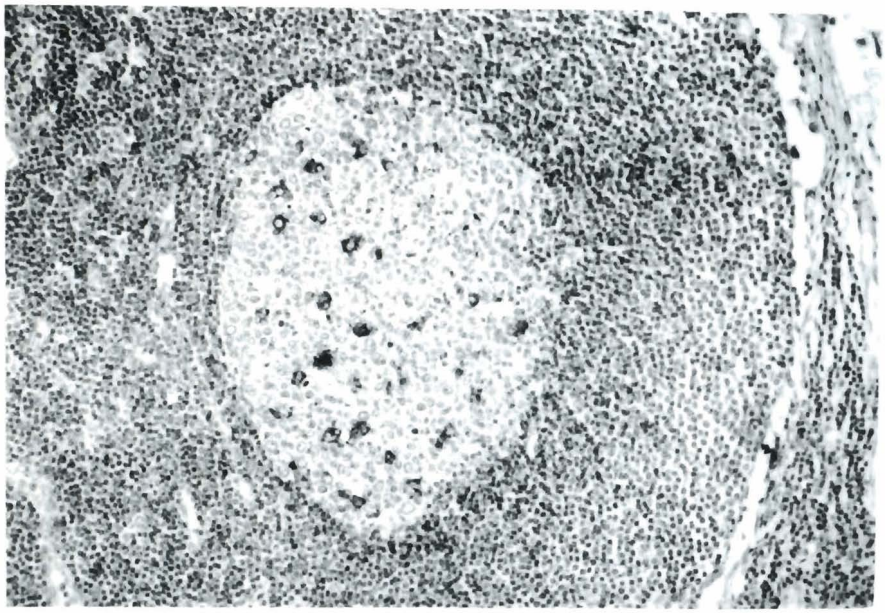

Fig. 3. Positive macrophages in lymph node follicles. ABC stain. x 250

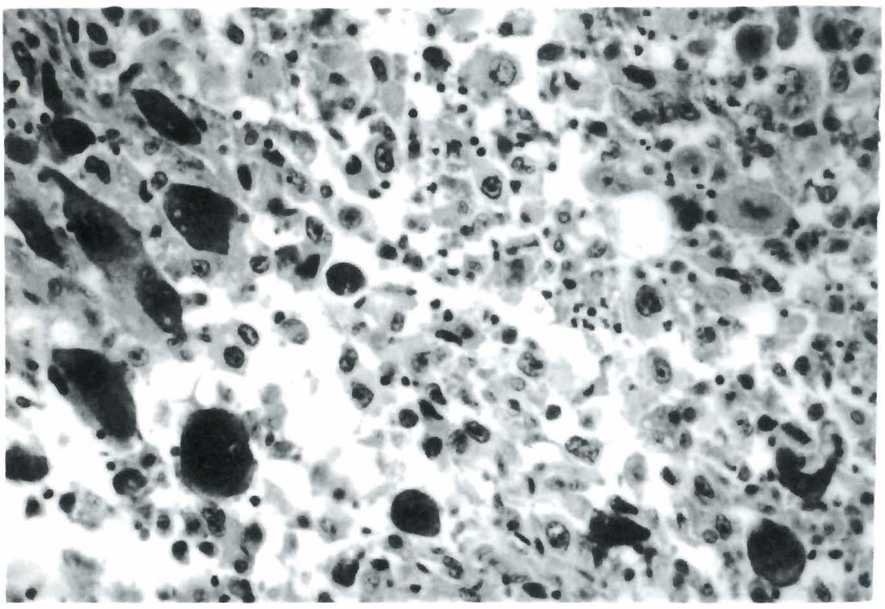

Fig. 4. Negative breast carcinoma and positive osteoclast-like giant cells. ABC stain. x 400

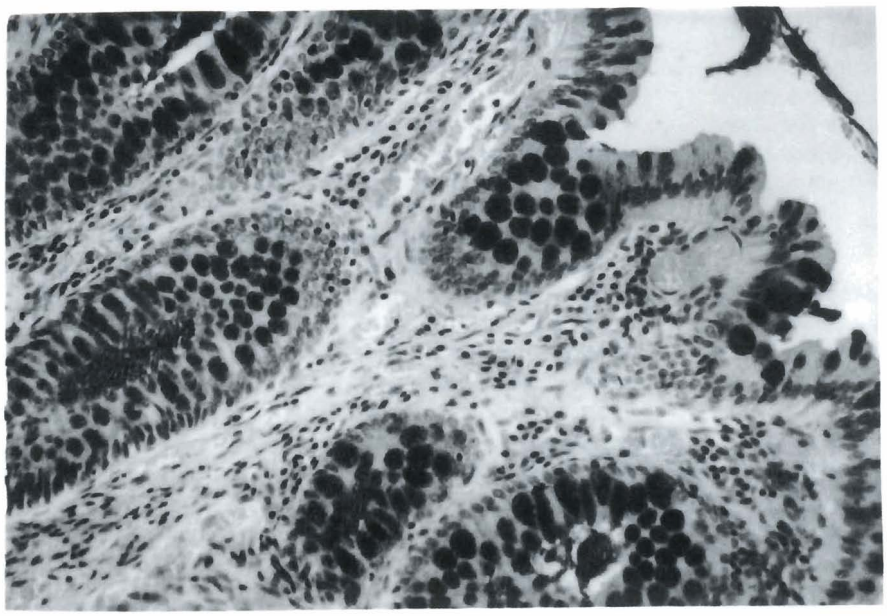

Fig. 5. Goblet cells positive for jacalin in an intestinal polyp. ABC stain. x 400

1990).

Many lectins (*Triticum vulgaris* agglutinin (WGA), Concanavalin ensiformis agglutinin (Con A), *Ricinus communis* agglutinin (RCA), *Phaseolus vulgaris* agglutinin (PHA), *Pisum sativum* agglutinin (PSA), *Lens culinaris* agglutinin (LCA), Peanut agglutinin (PNA), Soybean agglutinin (SBA)) have also been tested to demonstrate histiocytes in paraffin-embedded tissue (Howard and Batsakis, 1981; Ree and Hsu, 1983; Ree, 1983, 1986; Roholl et al., 1985a,b; Ueda et al., 1987). It seems that PNA and Con A are good markers, the latter being the most reliable one to demonstrate histiocytes in routine paraffin sections (Ree, 1983). Unfortunately, there is not an ideal histiocytic marker, and each one has a different staining pattern on the several types of histiocytes.

There are very few reports about the detection of the jackfruit lectin, jacalin, on tissue sections. Developing podocytes have a prominent jacalin reactivity (Laitinen et al., 1989). In ten-day-old rats, muscle fibres were stained with jacalin (Kirkeby et al., 1992). In formalin-fixed biopsies of conjunctival tissue it was observed that jacalin bound selectively to goblet cells (Prause et al., 1989). This last finding correlates with our observation of positivity of the goblet cells in the studied appendixes and intestinal polyps. We have not found any report related to histiocytes and jacalin.

We have observed that jacalin consistently stains histiocytes in paraffin-embedded tissues in all the cases studied of reactive sinus histiocytosis, macrophages in clear centres of follicular hyperplasia and in tuberculosis granulomas. On the contrary, we failed to find any binding of jacalin to the cells of eosinophilic granulomas, giant cell tumors of the tendon sheath, pleomorphic fibrous histiocytomas and Hodgkin's disease. All those mentioned processes that are negative to jacalin have been considered in the past of a histiocytic origin (Kadin, 1982; Roholl et al., 1985a,b; Elias, 1990).

Generally speaking, histiocytes can be separated into two groups with different phenotype: 1) free histiocytes or macrophages, which express monocyte markers; and 2) fixed histiocytes, where interdigitating reticulum cells, Langerhans cells and cells of the fibrous histiocytomas could be included (Roholl et al., 1988; Elias, 1990). Our finding seems to indicate that jacalin is a good marker for free histiocytes/macrophages. On the contrary, fixed histiocytes and tumors related to them (fibrous histiocytomas, histiocytosis X and Reed-Sternberg cells) are not marked with jacalin.

We observed a strong positivity of osteoclast-like giant cells in a breast carcinoma. This positivity of the multinucleated giant cells, along with the negativity of the neoplastic epithelial cells, might indicate a histiocytic origin of the osteoclast-like cells, although in a previous report we failed to detect lysozyme and alpha-I-antitrypsin in those cells (Caballero et al., 1984). In a recent publication about osteoclast-like giant cells in carcinomas of another location, the authors suggest that those cells have a monocytic/histiocytic origin, with positivity to 3MA134 (KP1) (Gaffey et al., 1991).

Various lymph node inclusions and metastatic tumors simulate histiocytes. The lesions that more frequently pose a diagnostic challenge in the differential diagnosis are: nevus cells (Subramony and Lewin, 1985); metastatic invasive breast lobullar carcinomas (Rosen and Oberman, 1992); and lymphomas, melanomas and metastatic carcinomas, mainly of the signet-ring cell variant (Grogan et al., 1985; Sheibani and Battifora, 1988; Brooks et al., 1990). None of the nevi, signet-ring cell carcinomas, melanomas and lymphocytes that we have tested, show a cytoplasmic

positivity to jacalin. Further studies would be interesting, testing jacalin on signet ring cell sinus histiocytosis (Gould et al., 1989), signet ring cell lymphomas and signet ring cell melanomas, although jacalin also binds epithelial cells (goblet cells). It is our belief that jacalin may be useful as a histochemical tool in detecting and differentiating, in routinely processed pathological specimens, histiocytes/macrophages from histiocyte-mimicking cells.

We have not observed loss of reactivity to jacalin in tissue included in paraffin for more than 10 years. Good results are obtained in formalin- and alcohol-fixed specimens. In some cases the positivity was weaker. This weakness could be explained, as happens with other lectins, because of varying degree of formalin fixation artefacts (Ree, 1983).

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References

Alpers C.E.. Beckstead J.H. and Newman A.B. (1984). Malignant fibrous histiocytoma, myelomonocytic leukemia, and Hodgkin's disease arising in an elderly man. Cancer 53, 1943-1947.

Alroy J., Ucci A.A. and Periera M.E.A. (1988). Lectin histochemistry: an update. In: Advances in immunohistochemistry. DeLellis R.A. (ed). New York. Raven Press. pp 93-131.

Aucouturier P., Mihaesco E., Mihaeso C. and Pred'homme J.L. (1987). Characterization of jacalin, the human IgA and IgD binding lectin from Jackfruit. Mol. Immunol. 24, 503-511.

Brooks J.S.J., Livolsi V. and Pietra G.G. (1990). Mesothelial cell inclusions in mediastinal lymph nodes mimicking metastatic carcinoma. Am. J. Clin. Pathol. 93, 741-748.

Caballero T., Urdiales M., Aneiros J. and Nogales F. (1984). Carcinoma mamario con células gigantes multinucleadas parecidas a osteoclastos: Estudio immunohistoquímico y ultraestructural. Patología 17, 339-348.

Elias J.M. (1990). The lymph node biopsy. In: Immunohistopathology: A practical approach to diagnosis. American Society of Clinical Pathology. Chicago. pp 274-275.

Franklin W.A. (1983). Tissue binding of lectins in disorders of the breast. Cancer 51, 295-300.

Gaffey M.J., Lack E.E., Christ M.L. and Weis L.M. (1991). Anaplastic thyroid carcinoma with osteoclast-like giant cells. A clinicopathologic, immunohistochemical and ultrastructural study. Am. J. Surg. Pathol. 15, 160-168.

Gould E., Perez J., Albores-Saavedra J. and Legaspi A. (1989). Signet ring cell sinus histiocytosis. A previously unrecognized histologic condition mimicking metaplastic adenocarcinoma in lymph nodes. Am. J. Clin. Pathol. 92, 509-512.

Grogan T.M., Payne C.M., Richter L.C. and Rangel C.S. (1985). Signet ring cell lymphoma of T-cell origin. An immunocytochemical and ultrastructural study relating giant vacuole formation to cytoplasmic sequestration of surface membrane. Am. J. Surg. Pathol. 9, 684-692.

Hagiwara K., Collet-Cassart D., Kobayashi K. and Vaerman J.P. (1988). Jacalin: isolation, characterization, and influence of various factors on its interaction with human IgA1, as assessed by precipitation and latex agglutination. Mol. Immunol. 25, 69-83.

Howard D.R. and Barsakis J.G. (1981). Peanut agglutinin: A new marker for tissue histiocytes. Lab. Invest. 44, 29A.

Isaacson P., Jones D.B., Milward-Sadler G.H., Judd M.A. and Payne S. (1981). Alpha-1 antitrypsin in human macrophages. J. Clin. Pathol. 34, 982-990.

Kadin M.E. (1982). Possible origin of the reed-Sternberg cell from an interdigitating reticulum cell. Cancer Treat. Rep. 66, 601-608.

Kirkeby S., Bog-Hansen T.C. and Moe D. (1992). Mosaic lectin and enzyme staining patterns in rat skeletal muscle. J. Histochem. Cytochem. 40, 1511-1516.

Krogerus L. and Andersson L.C. (1990). Different lectin binding patterns in primary breast cancers and their metastases. Cancer 66, 1802-1809.

Laitinen L., Lehtonen E. and Virtanen I. (1989). Differential expression of galactose and N-acetylgalactosamine residues during fetal development acetylgalactosamine residues during fetal development and postnatal maturation of rat glomeruli as revealed with lectin conjugates. Anat. Rec. 223, 311-321.

Meister P. and Nathrath W. (1980). Immunohistochemical markers of histiocytic tumors. Hum. Pathol. 11, 300-301.

Norton A.J. and Isaacson P.G. (1989). Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. I. Range of antibodies and staining patterns. Histopathology 14, 437-466.

Prause J.U., Jensen O.A., Paschides K., Stouhase A. and Vangsted P. (1989). Conjunctival cell glycoprotein pattern of healthy persons and of patients with primary Sjogren's syndrome. Light microscopical investigation using lectin probes. J. Autoimmun. 2, 495-500.

Ree H.J. (1983). Lectin histochemistry of malignant tumors: Concanavalin A: a new histochemical marker for macrophage-histiocytes in follicular lymphoma. Cancer 51, 1639-1646.

Ree H.J. (1986). Concanavalin A-binding histiocytes in Hodgkin's disease. Cancer 58, 87-95.

Ree H.J. and Hsu S.M. (1983). Lectin histochemistry of malignant tumors: Peanut agglutinin (PNA) receptors in follicular lymphoma and follicular hyperplasia: An immunohistochemical study. Cancer 51, 1631-1638.

Roholl P.J.M., Kleijne J., Pijper H.W. and van Unnik J.A.M. (1985a). Comparative immunohistochemical investigations of marker for malignant histiocytes. Hum. Pathol. 16, 763-771.

Roholl P.J.M., Keijne J., vas Basten C.D.H., van der Putte S.C. and van Unnik J.A.M. (1985b). A study to analyse the origin of tumor cells in malignant fibrous histiocytomas. A multiparametric characterization. Cancer 56, 2809-2815.

Roholl P.J.M., Kleijne J., Prins M.E.F., Hooijkaa H., Vroom M. and van Unnik J.A.M. (1988). Immunologic marker analysis of normal and malignant histiocytes. Hum. Pathol. 89, 187-194.

Rosen P.P. and Oberman H.A. (1992). Invasive lobular carcinoma. In: Tumors of the mammary gland. Atlas of tumor pathology. Third series. Armed Forces Institute of Pathology. Washington. D.C. pp 168-175.

Sheibani K. and Battifora H. (1988). Signet- ring cell melanoma. A rare morphologic variant of malignant melanoma. Am. J. Surg. Pathol. 12, 28-34.

Subramony C. and Lewin J.R. (1985). Nevus cells within lymph nodes. Possible metastases from a benign intradermal nevus. Am. J. Clin. Pathol. 84, 220-223.

Taylor C.R. (1978). Immunoperoxidase techniques: Practical and

theoretical aspects. Arch. Pathol. Lab. Med. 102, 113-121.

Ueda T., Aozasa K., Yamamura T., Tsujimoto M., Ono K. and Matsumoto K. (1987). Lectin histochemistry of malignant fibrohistiocytic tumors. Am. J. Surg. Pathol. 11, 257-262.

Wickramasinghe S.N. (1992). bone marrow. In: Histology for pathologists. Sternberg S.S. (ed). Raven Press. New York. p 17.

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