Immunohistochemical Study of Fibrohistiocytic Tumors of the Skin

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Background: Histologic distinction between various fibrohistiocytic tumors of the skin may sometimes be difficult. Recently, several immunohistochemical markers of "histiocytes" and "facultative fibroblasts" have been introduced and used for the study of some fibrohistiocytic tumors of the skin.

Objective: The purpose of this study is to determine whether immunostaining with MAC 387, antibodies to S-100 protein, factor Xllla(FXllla) and CD 34 allows distinction between various fibrohistiocytic tumors of the skin in formalin-fixed, paraffin-embedded specimens.

Methods: Paraffin-embedded specimens of dermatofibroma, keloid, hypertrophic scar, dermatofibrosarcoma protuberans(DFSP), neurofibroma, and juvenile xanthogranuloma were investigated with MAC 387, antibodies to S-100 protein, CD 34 and FXllla using avidin-biotin-peroxidase complex method.

Results: In all fibrous dermatofibromas (n=26), 20-90% of constituent cells were positive for FXllla. Focal or diffuse CD 34 reactivity was present in DFSP (n=2). Weak reactivity for CD 34 and consistent labeling for S-100 protein were found in neurofibromas (n=5). Tumor cells showed negative for FXllla, CD 34 and S-100 protein in keloids (n=2), hypertrophic scars (n=6), and juvenile xanthogranulomas (n=5). MAC 387 did not label tumor cells of the fibrohistiocytic tumors we have studied.

Conclusion: Immunostaining of paraffin-embedded specimens with antibodies to S-100 protein, FXllla and CD 34 may be useful in the differential diagnoses of fibrohistiocytic tumors of the skin. (Ann Dermatol 7:(2)121~126, 1995)

Key Words: CD34 Factor Xllla, Fibrohistiocytic tumors of the skin, MAC 387, S-100 protein.

Fibrohistiocytic tumors of the skin may sometimes be difficult to diagnose clinically as well as histologically. The distinction between dermatofibrosarcoma protuberans (DFSP) and dermatofibroma is an especially well-known challenge for the dermatopathologist. Recent papers have continued to introduce putative paraffin section markers or "histiocytes" and "facultative fibroblasts", and they have been used for the study of fibrohistiocytic tumors of the skin. S-100 protein, originally regarded as a nervous system specific protein, is now known to be present in several nonneural cells such as Langerhans' cells, melanocytes, and chondrocytes, and in tumors derived from these cell types' and immunohistochemical staining using anti-S-100 protein antibody became a standard for the diagnosis of Langerhans cell histiocytosis. MAC-387 is a murine monoclonal antibody raised against monocytes that labels cells of the monocyte-macrophage lineage in granulomatous inflammation of the skin². Factor Xllla (FXllla) labels the normal dermal population of fixed connective tissue cells, or fibrocytes, which have

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Table 1. Details of antibody employed

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antibody Source</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-S-100 protein</td>
<td>Dako Japan</td>
<td>1:600</td>
</tr>
<tr>
<td>MAC 387</td>
<td>Dako Japan</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-factor Xllla</td>
<td>Calbiochem</td>
<td>1:400</td>
</tr>
<tr>
<td>Anti-HPCA-1 (CD 34)</td>
<td>Becton Dickinson</td>
<td>1:10</td>
</tr>
</tbody>
</table>

Table 2. Immunohistochemical staining of fibrohistiocytic tumors

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total no. of cases</th>
<th>S-100</th>
<th>MAC 387</th>
<th>FXllla</th>
<th>CD 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatofibroma</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>+(26/28)*</td>
<td>-</td>
</tr>
<tr>
<td>Keloid</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertrophic scar</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DFSP</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Neurofibroma</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+(focal)</td>
</tr>
<tr>
<td>Juvenile xanthogranuloma</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Tumor cells were negative for FXllla in cellular types of dermatofibroma

been renamed dermal dendrocytes because of their characteristic dendritic processes. A similar population of dendritic cells, which express the antigen CD 34, has also recently been identified in human reticular dermis.

In this study, we examined S-100 protein, MAC 387, FXllla, and CD 34 expressions on several fibrohistiocytic tumors, and neurofibroma. Neurofibroma were included in this study because it is relatively common and a histologic differentiation of neurofibroma from fibrohistiocytic tumors sometimes is needed. We found that these were very useful markers to establish a definite diagnosis of fibrohistiocytic tumors of the skin.

**MATERIALS AND METHODS**

**Tissue**

Twenty-eight cases of dermatofibroma, 2 cases of keloid, 6 cases of hypertrophic scar, 2 cases of DFSP, 5 cases of neurofibroma, and 5 cases of juvenile xanthogranuloma were collected from the pathologic files of Department of Pathology, Seoul National University Hospital. Hematoxylin and eosin stained sections of all lesions were reviewed and the diagnoses were confirmed. All tissue had been fixed in formalin and then paraffin-embedded according to conventional procedure.

**Immunoperoxidase staining**

Immunohistochemical studies on the formalin-fixed paraffin-embedded tissue specimens were performed using an avidin-biotin-peroxidase complex method. Briefly, four to six μm thick sections were cut, deparaffinized in xylene, and rehydrated in ethanol. Endogenous peroxidase activity was blocked by 30 minutes incubation with 3% hydrogen peroxide in absolute methanol. Background staining was minimized by pre-incubation with normal horse serum or normal rabbit serum (1:20 dilution). Sections were then incubated for 1 hour at room temperature with antibody to S-100 protein (Dako Japan, Kyoto, Japan) and MAC 387 antibody (Dako Japan, Kyoto, Japan), antibody to factor Xllla (Calbiochem, La Jolla, CA, U.S.A.), antibody to CD 34 (anti-HPCA-1, Becton Dickinson, Mountain View, CA, U.S.A.). (Table 1). Staining with the immunoperoxidase avidin-biotin complex kit (Vector Laboratories, Burlingame, CA, U.S.A.) followed, using 3, 3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, U.S.A.) as the chromogen. Sections were counterstained with Mayer's hematoxylin.

**Evaluation**

The intensity of staining was graded as negative, weakly staining, or strongly staining. The distribution of positive staining was described as focal (less than 50%), or diffuse (more than 50%).

**RESULTS**

The results are summarized in table 2.
Dermatofibroma

In the 28 cases of dermatofibroma we studied, the vast majority of the constituent cells were not labeled by anti-S-100 protein and MAC 387. Only occasional individual cells within the tumors showed labeling for S-100 protein or with MAC 387. MAC 387 positive cells did not correspond with the presence of foamy or hemosiderin-containing macrophages. FXIIIa labeling was found in 26 cases of fibrous variants of dermatofibroma.

In contrast, tumor cells showed negative for FXIIIa in 2 cases of cellular variants of dermatofibroma. Although FXIIIa labeling was a consistent finding in fibrous dermatofibromas, there were some variation in the intensity of immunoperoxidase reaction product from lesion to lesion and in different parts of the individual dermatofibromas. FXIIIa was expressed mainly on the spindle-shaped and stellate tumor cells. Stellate cells, which were situated at the periphery of the tumor showed a strong positive reaction(Fig. 1). In the center of the dermatofibromas, intensity of labeling with FXIIIa was generally weak and only a small percentage of the cells labeled. The tumor cells did not express the CD 34 antigen in dermatofibroma, and CD 34 positive cells were limited to the vascular endothelium and margin of dendritic cells.
in the reticular dermis. CD34 helped to identify uncannalized vessels not readily seen with routine H&E stains (Fig. 2).

**Keloid and hypertrophic scar**

In the 2 keloids and 6 hypertrophic scars, the spindle shaped fibroblasts were consistently negative for S-100 protein, FXIIla and with MAC 387. Stellate cells, which were situated above the tumor mass showed positive for FXIIla (Fig. 3). Two of the 6 hypertrophic scars contained scattered FXIIla positive dendritic cells in the tumor mass. However, these cells were morphologically distinct from the spindle shaped fibroblasts in the hypertrophic scar and appeared as if trapped within the tumor mass. The spindle shaped fibroblasts were also negatively stained with anti-CD34 antibody, while the endothelial cells in tumor tissue were CD34 positive.

**Dermatofibrosarcoma protuberans (DFSP)**

None of the tumor cells of the 2 DFSP were positive for S-100 protein and with MAC 387. FXIIla was not expressed on tumor cells in any of the cases of DFSP although its staining pattern was variable; in one case, FXIIla-positive dendritic cells were scattered among the tumor cells, whereas in the other case, no FXIIla positive cells were present in the tumor tissue. Both the 2 cases of DFSP showed positive CD34 staining. However, the staining pattern was not uniform. One case showed a diffuse positive staining of the tumor mass (Fig. 4) while in the other case, some tumor strands showed linear staining, with other tumor cells unstained.

**Neurofibroma**

Diffuse positive staining of the tumor cells for S-100 protein was found in all 6 cases of neurofibroma (Fig. 5). Tumor cells were negative with MAC 387. Except for rare isolated cells, interpreted as trapped residual connective tissue cells, no positive FXIIla tumor cells were found in the neurofibroma. Focal reactivity for CD34 was found in all the neurofibromas we have studied, mainly on wavy fibers (Fig. 6). Endothelial cells in tumor tissue also showed positive for CD34.

**Juvenile xanthogranuloma**

In the 5 juvenile xanthogranulomas, none or few of tumor cells were positive for S-100 protein and with MAC 387. Touton giant cells showed no labeling with MAC 387. The majority of tumor cells were FXIIla negative. Only a few "trapped" FXIIla positive cells were seen within the tumors. The tumor cells of juvenile xanthogranuloma were negatively stained with anti-CD34 antibody, while endothelial cells in tumor tissue were CD34 positive.

**DISCUSSION**

Dermatofibromas, known also as fibrous
histiocytomas, histicytomas, and sclerosing hemangiomases, are relatively common fibrohistiocytic tumors of the skin. Although the clinical and histologic features of dermatofibroma are well known, no unequivocal agreement exists about whether the primary cell is a fibroblast or a histiocyte. Cerio et al. have postulated that dermatofibromas represent a proliferative lesion of dermal dendrocytes different in category and histogenesis from other fibroelastic lesions of the skin with the aid of two recently introduced antibodies: anti-FXIIa and MAC 387. According to Cerio et al., most cells in dermatofibromas react intensely with FXIIa, whereas MAC 387-positive cells, that is, macrophages are few. In our study, FXIIa labeling was a consistent finding in all the fibrous dermatofibromas whereas none of tumor cells were positive for FXIIa in cellular dermatofibromas. dermatofibromas have been divided into "fibrous" lesions composed entirely or almost entirely of fibroblasts and collagen, and "cellular" lesion composed to a significant degree of phagocytic cells with the appearance of histiocytes. In the fibrous types of dermatofibromas, 20-90% of the constituent cells showed labeling for FXIIa, with the most prominent labeling at the peripheral or active margin of the lesion as Cerio et al. reported. Headington suggests that 'dermal dendrocyte' is a stem cell which has potential, under an appropriate stimulus, to develop fibroblastic or phagocytic activities. It appears that FXIIa labeling tends to be reduced or lost in dermatofibroma as the cells acquire fibroblastic or macrophage activity. Negative reactivity for FXIIa in cellular dermatofibroma that we observed may be the result of the maturation of the tumor cells. Other fibrohistiocytic lesions that we have studied, such as keloids, hypertrophic scars, DFSP, neurofibromas, and juvenile xanthogranulomas were negative for FXIIa. In both the fibrous type and cellular type of dermatofibroma, scattered small capillaries with prominent endothelial cells could be found with anti-CD 34. This was a very characteristic finding in all of the dermatofibroma. Since CD 34 expression helped to identify unciliated vessels in dermatofibromas not readily seen with routine H&E stains, immunohistochemical staining with anti-CD 34 antibody can also be a help to establish a diagnosis of dermatofibroma. DFSP resembles deep growing dermatofibroma, nodular fascitis, neurofibroma and neural sheath tumors. Recently, several reports on CD 34 staining of DFSP have been published. Although Aiba et al. and Kutzner reported strong and consistent expression of CD 34 in all the cases of DFSP they have studied, Cohen et al. and Altman et al. reported few cases of DFSP which did not react with anti-CD 34 antibody. While examining CD 34 expression on various fibrohistiocytic tumors immunohisto logically, we found that both the 2 DFSP are CD 34 positive, whereas other fibrohistiocytic tumors, such as dermatofibroma, keloid, hypertrophic scar, and juvenile xanthogranuloma, are CD 34 negative. The staining pattern of CD 34 is not uniform in DFSP. One case demonstrated a generalized positive staining of the tumor cells whereas the other case showed a focal positive staining of the tumor cells. CD 34 was focally reactive in portions of all the neurofibromas we have studied, mainly on the wavy fibers. These findings are interesting in view of the neural origin theory of DFSP. Cohen et al. also reported focal or diffuse positive staining of neurofibromas with anti-CD 34. However, CD 34 reactivity could not be detected in neurofibromas by Kutzner. In our study, S-100 protein consistently expressed in all the tumor cells of neurofibromas. This result proved to be helpful in the distinction of neurofibroma from dermatofibroma since we misdiagnosed 1 case of neurofibroma as dermatofibroma clinically. Although S-100 protein is sporadically present within a variety of mesenchymal tumors, diffuse staining of most tumor cells was characteristic in tumors of neural origin. All the fibrohistiocytic tumors we studied were negative with MAC 387. Thus a combination of staining patterns for FXIIa, CD 34, S-100, and MAC 387 can make the diagnosis of the fibrohistiocytic tumors of the skin more reliable, i.e. many tumor cells in fibrous dermatofibroma are FXIIa(+), CD 34(−), S-100(−), MAC 387(−), many tumor cells in DFSP are FXIIa(−), CD 34(+),S-100(−), MAC 387(−). Many tumor cells in keloid and hypertrophic scar, and juvenile xanthogranuloma are FXIIa(−), CD 34(−), S-100(−), MAC 387(−). Fortunately, it is relatively easy to differentiate juvenile xanthogranuloma from keloid and hypertrophic scar with routine H&E stains. Our study demonstrated that differential expression of "fibrohistiocytic" markers in fibrohistiocytic tumors of the skin may become a useful immunohistoloechemical adjunct in the differential diagnosis of these tumors.
REFERENCES


