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Differential expression of factor XIIIa and CD34 in cutaneous mesenchymal tumors

The histogenetic relationship amongst various dendritic cells of the dermis which may express markers including factor XIIIa (FXIIIa) or CD34 remains unclear. In this study we utilized a sensitive indirect immunoperoxidase staining technique to identify CD34 and FXIIIa, as well the monocyte/macrophage markers KP-1 and MAC 387 expression in a variety of cutaneous dermal tumors of mesenchymal origin to see if differential expression of CD34 vs FXIIIa exists. Tumors studied included dermatofibroma (DF) (N = 10), keloid (N = 9), atypical fibroxanthoma (AFX) (N = 3), and dermatofibrosarcoma protuberans (DFSP) (N = 7). DF were all composed of FXIIIa+ spindle-shaped and stellate tumor cells (mean score = 4.9 or ≥ 75% FXIIIa+) as previously reported, but these cells rarely (< 10%) expressed CD34. Six of 7 DFSP were found to be > 75% CD34+ and FXIIIa negative, while one DFSP was negative for both CD34 and FXIIIa. In all DFSP, there were trapped FXIIIa+ cells which were distinct from the spindle-shaped tumor cells. AFX showed sparse populations of FXIIIa+ cells in the stroma (mean score = 1.33 or 10–25% positive), which were distinct from the atypical giant cells characteristic of these tumors. Keloid similarly contained trapped FXIIIa+ cells (mean score = 0.44 or < 5% positive) that were distinct from the spindle-shaped fibroblasts of the tumor mass. Dendritic and spindle-shaped cells within these tumors were consistently both KP-1 and Mac-387 negative, while all lesions studied were characterized by scattered round, histiocytic cells which were KP-1+ and/or Mac-387+ irrespective of tumor cell type. We suggest that these tumors can be delineated by their relative degrees of FXIIIa and CD34 expression and that these neoplasms may be a useful link with which to study the relationship between CD34+ cells and dermal dendrocytes.

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Recent work has demonstrated the presence of populations of dendritic cells in the dermis. One subset of these cells are thought to be of bone marrow origin and are known as dermal dendrocytes (DD). They express factor XIIIa (FXIIIa) and have been shown to compose certain cutaneous tumors such as dermatofibromas (1). FXIIIa+ DD may be phagocytic and have been proposed to be antigen presenting cells (APC) distinct from Langerhans cells and other APC of the skin (2, 3). A similar population of dendritic cells, which express the antigen CD34, has also recently been identified in human reticular dermis, often in coexistence with FXIIIa+ DD (4). CD34 has been described as a

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Table 1. Mesenchymal tumor expression of factor XIIIa and CD34

Tumor type	Factor XIIIa score*	CD34 score*
Dermatofibroma	4.90 ± 0.10** (n = 10)	0.10 ± 0.10 (n = 10)
Atypical fibroxanthoma	1.33 ± 0.34 (n = 3)	0.00 ± 0.00 (n = 3)
Keloid	0.44 ± 0.18 (n = 9)	0.00 ± 0.00 (n = 9)
Dermatofibrosarcoma protuberans	1.0 ± 0.00 (n = 7)	4.43 ± 0.57*** (n = 7)

* The values expressed are the mean ± SEM (based on the scale below) for cells of dendritic/spindle-shaped morphology within the tumor mass. In the keloids, atypical fibroxanthomas and dermatofibrosarcoma protuberans lesions FXIIIa+ cells were trapped within the tumor mass yet distinct from tumors cells. The semiquantitative average of 10–40X high power fields within the dermal tumor mass was scored as:

0 = none of tumor cells.
1 = < 10% of tumor cells.
2 = 10–25% of tumor cells.
3 = 25–50% of tumor cells.
4 = 50–75% of tumor cells.
5 = > 75% of tumor cells.

** = Significant pairwise comparison vs keloid and dermatofibrosarcoma protuberans ($p < 0.05$).

*** = Significant pairwise comparison vs keloid and dermatofibroma ($p < 0.05$).

hematopoietic progenitor antigen is expressed by < 1% of bone marrow cells. It is unclear if CD34+ dendritic cells may denote a similar stem cell population in the skin. If so, these cells may serve as a reservoir of mesenchymal precursor cells which could differentiate upon appropriate stimulation into FXIIIa+ DD (3, 4).

The current study employs a sensitive immunoperoxidase staining technique to identify populations of both CD34+ and FXIIIa+ dendritic cells within tumors of mesenchymal origin including, dermatofibromas (DF), atypical fibroxanthoma (AFX), dermatofibrosarcoma protuberans (DFSP), and keloid. We report significant differential expression of FXIIIa and CD34 in these cutaneous tumors.

Material and methods

Patients

Subjects were identified by diagnosis from biopsy records on file in the Departments of Pathology at Henry Ford Hospital, and the University of Michigan Medical Center. Included in this study were serial paraffin sections from 10 DF, 9 keloids, 3 AFX, and 7 DFSP. Original hematoxylin and eosin slides were reviewed to confirm the recorded diagnoses.

Indirect immunoperoxidase

Indirect immunoperoxidase (IPX) staining was performed on 4 μm paraffin-imbedded sections which were deparaffinized in xylene and dehydrated in ethyl alcohol. Sections were incubated for 40 min with trypsin 0.1% (Sigma, St. Louis, MO) in phosphate buffered saline 0.05M (PBS), with $CaCl_2$ 0.1%. Endogenous peroxidase activity was inhibited with equal proportions of 100% methanol and 3% hydrogen peroxide, prior to blockage with horse serum and exposure to primary antibodies for 1 h at room temperature. Antibodies used were diluted in PBS with 0.5% bovine serum albumin and included: anti-human FXIIIa (1:400) and FXIIIs (1:400) (Calbiochem, LaJolla, CA), Mac-387 (1:50), KP-1 (1:50) (Dako, Carperteria, CA), and CD34 (1:10) (HPCA-1, Becton Dickinson, Mountainview, CA). Antibodies were visualized using the avidin/biotin peroxidase technique (Vectastain ABC Kit, Vector Labs Inc, Burlingame, CA). The chromagen was 3-amino, 9-ethyl carbazole (AEC-Sigma, St. Louis, MO). All post-fixation procedures were performed at room temperature. An irrelevant, isotype-matched antibody was included in each experiment as a control.

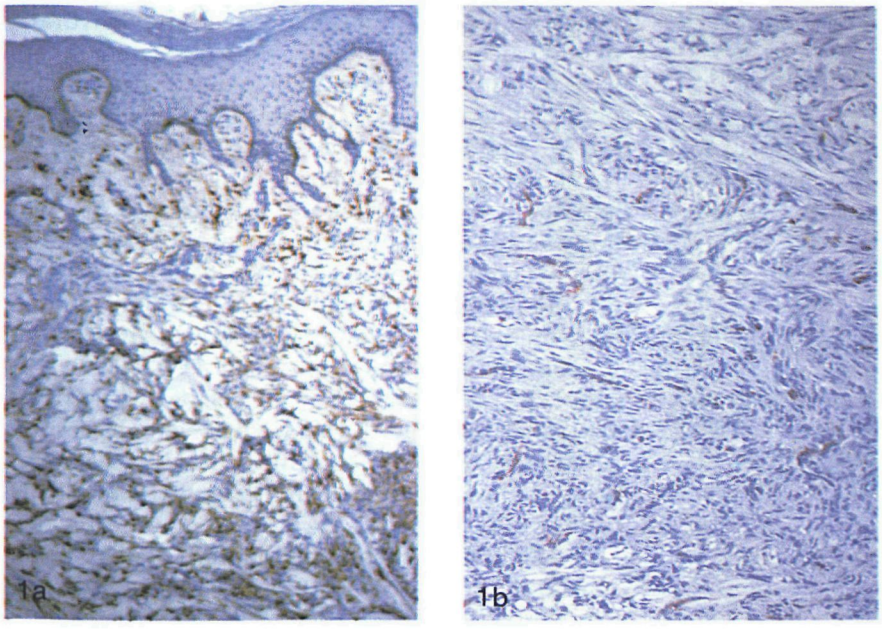
Dendritic and spindle-shaped dermal cells from within these tumors with positive staining by the IPX technique were evaluated semiquantitatively by scoring the average number positive cells within the dermal tumor mass in 10 high-power fields (40X), employing the following scoring system: 0 = no dendritic/spindle-shaped cell staining, 1 = < 10% of dendritic/spindle-shaped cell staining, 2 = 10–25% of dendritic/spindle-shaped cell staining, 3 = 25–50% of dendritic/spindle-shaped cell staining, 4 = 50–75% of dendritic/spindle-shaped cell staining, 5 = > 75% of dendritic/spindle-shaped cell staining. Only those cells with visible nuclei and discernable processes were included in this analysis.

Statistical analysis

A non-parametric analysis of variance (Kruskal-Wallis test) was first performed for the mean FXIIIa and CD34 expression within each disease group. To study FXIIIa and CD34 staining among the DF, keloid, AFX, and DFSP groups, pairwise comparisons were used with Bonferoni's correction for multiple comparisons.

Results

The results are summarized in Table 1. All DF (N = 10) studied expressed FXIIIa on the majority of spindle-shaped and stellate tumor cells (Fig. 1a). The average score (± SEM) of DF tumor cell expression of FXIIIa was 4.90 ± 0.10. CD34 staining of these tumor cells was rare, and CD34+ cells were limited to the vascular endothelium and a margin of



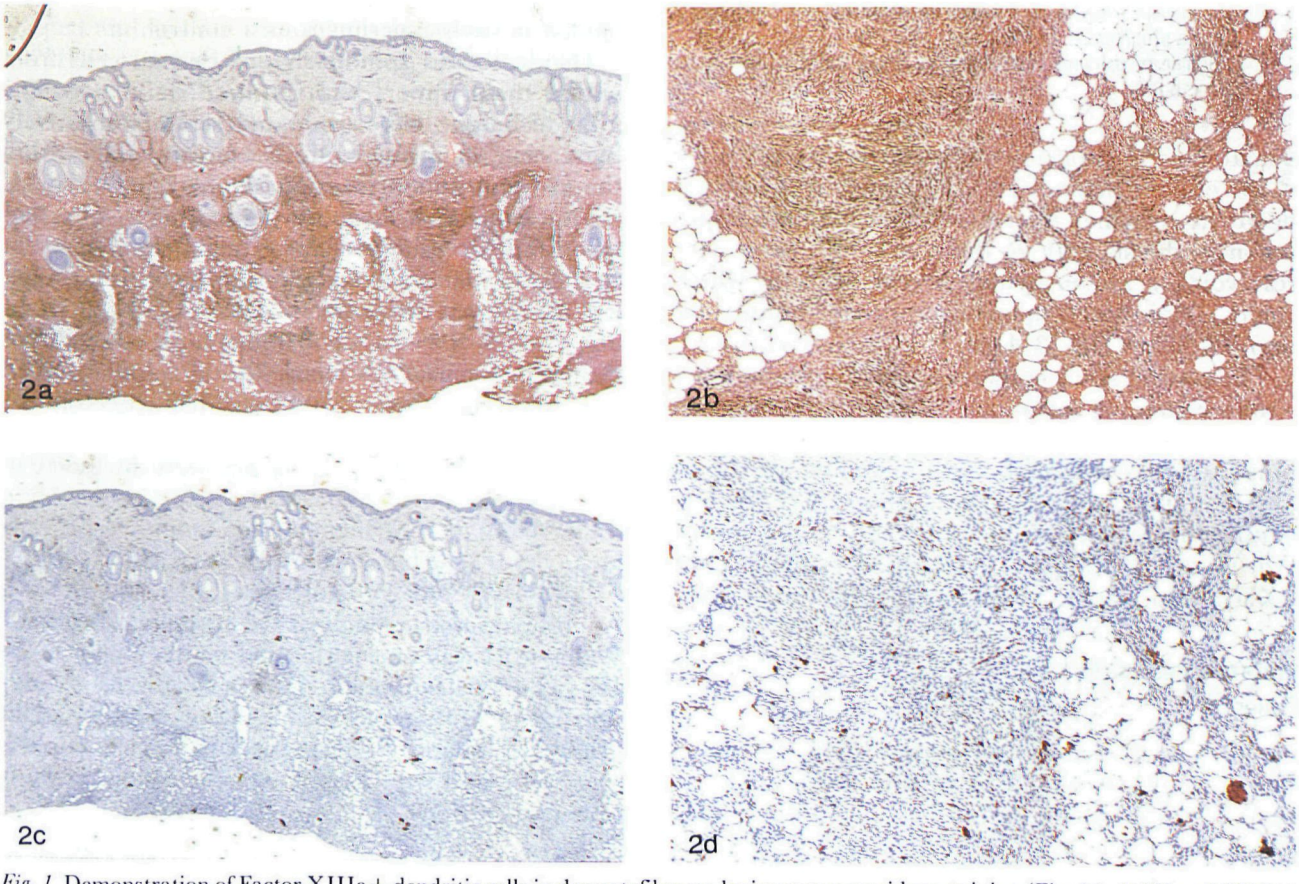


Fig. 1. Demonstration of Factor XIIIa+ dendritic cells in dermatofibroma by immunoperoxidase staining (Fig. 1A, × 10) and CD34+ cells limited the vessels in the same lesion (Fig. 1B, × 50).

Fig. 2. Demonstration of the universal expression of CD34 by spindle-shaped tumors cells in dermatofibrosarcoma protuberans by immunoperoxidase staining (Fig. 2A, × 5, 2B, × 25) and the sparce number of FXIIIa+ cells scattered throughout the same lesion (Fig. 2C, × 5, 2D, × 25). Note how CD34 allows demonstration of the deeply invasive aspects of the tumor in the subcutaneous fat.

dendritic cells in the reticular dermis with similar morphology as previously described (Fig. 1b) (4).

Many of the stromal cells in AFX (N = 3) were dendritic and some expressed FXIIIa (mean score = 1.3 ± 0.35). CD34 staining was rarely seen on any of these dendritic cells. In none of the AFX were >25% of lesional cells FXIIIa+ and these cells composed a separate population of supporting cells, readily distinguished from the atypical giant cells and foam cells characteristic of AFX.

Four of 9 keloids also contained scattered FXIIIa+ dendritic cells in the tumor mass. These cells were morphologically distinct from the spindle-shaped fibroblasts in the keloid and appeared as if trapped within the tumor mass. CD34 expression in a keloid was limited to the vascular endothelium.

CD34 consistently stained all the large, atypical, spindle-shaped tumor cells within 6/7 DFSP, whereas one case was repeatedly CD34 negative. The mean CD34 score for the 7 cases was 4.43 ± 0.57. CD34 delineated the deeper, more invasive aspects of the tumors (Figs 2a,b). All DFSP studied showed scattered FXIIIa+ small dendritic cells of typical DD morphology (Figs 2c,d). The mean score for FXIIIa expression by the 7 DFSP studied was 1.0, consistent with this scattering of normal appearing DD trapped within the lesions.

Similar to prior reports, we found Mac-387 and KP-1 did not identify DD in paraffin sections (2, 5). In the tumors studied, Mac-387 and KP-1 stained scattered dermal cells characterized by large histiocytic cells, pale nuclei, abundant cytoplasm and a lack of distinct dendritic processes.

Statistically significant increases in FXIIIa+ cells were shown for the DF group as compared to the keloids and DFSP ($p < 0.05$) CD34+ cells were significantly increased in the DFSP group as compared to the DF and keloid groups ($p < 0.05$). Comparisons of AFX vs DFSP and AFX vs DF were not statistically relevant due to the small numbers of cases.

Discussion

Dendritic cells of the dermis have recently been the focus of intensive work. Cerio et al. defined dermal dendrocytes as a population of dendritic cells in the upper dermis of normal skin which express FXIIIa as well as HLA-DR (5). These cells also express CD14, CD45, and CD54, suggesting a mononuclear cell origin for DD (2). In normal human skin, FXIIIa+ DD are located perivascularly and limited to the papillary and upper reticular dermis. A morphologically similar, CD34+/FXIIIa negative population of dendritic cells has been recently identified and is concentrated immediately below the FXIIIa+ DD in the deep reticular dermis (3, 4). CD34 is a monomeric 115-kd glycoprotein expressed on hematopoietic progenitor cells in bone marrow as well as on endothelial cells in the skin and a wide variety of vascular and spindle-shaped cell tumors (4, 6–8).

FXIIIa and CD34 are both reported to be expressed by populations of spindle-shaped tumor cells within Kaposi's sarcoma (KS) (4, 9, 10). CD34 and FXIIIa co-expression by various proportions of spindle-shaped cells within KS lesions has led to speculation of a histogenetic connection between these two cell populations. KS cells were previously felt to be a neoplastic proliferation of endothelial cells alone, but have been recently suggested to be related to DD as well (9).

Although the sample sizes studied were not large, the current study demonstrates that differential expression of CD34 and FXIIIa by dendritic cells within benign and malignant mesenchymal dermal tumors is more regularly seen than co-expression, and may prove to be a useful method to delineate these lesions histologically. Similar to recent reports, we found the spindle-shaped cells in 6/7 DFSP to be CD34+ and FXIIIa negative (8, 9). Our finding in DF and keloid demonstrated that there are distinct FXIIIa/CD34 profiles for each of these benign tumors as well. If supported by larger studies, differential FXIIIa vs CD34 expression in these tumors may become a useful immunohistochemical adjunct in histologic delineation as in general: DF are FXIIIa+ and CD34 negative, DFSP are FXIIIa negative and CD34+, and keloids are both FXIIIa and CD34 negative.

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