Kaohsiung Journal of Medical Sciences (2012) **28**, 545–549

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ORIGINAL ARTICLE

Expression of MMP-2, MMP-9 and MMP-11 in dermatofibroma and dermatofibrosarcoma protuberans

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Received 23 June 2011; accepted 7 November 2011
Available online 27 August 2012

KEYWORDS
Dermatofibroma;
Dermatofibrosarcoma protuberans;
MMP-2;
MMP-9;
MMP-11

Abstract Dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) are the spindle cell mesenchymal neoplasms of the dermis and subcutis. Their histogenesis still remains uncertain and controversial. Traditionally, CD34 and factor XIIIa or other markers have been widely used to distinguish these two diseases. However, the results of these markers reveal overlapping and they lack specificity. Formalin-fixed, paraffin-embedded blocks were collected from the biopsied cases in Kaohsiung Medical University Hospital in Taiwan between 2004 and 2006. This study included 19 cases of DF and 17 cases of DFSP. Immunohistochemical analysis using antibodies CD34, matrix metalloproteinases (MMP)-2, MMP-9, and MMP-11 was performed. We found that the expression of CD34, MMP-2 and MMP-11 shows significant statistical differences in Immunohistochemistry (IHC) study positive or negative reactivity (positive of CD34 in DFSP and positive of MMP-2 and MMP-11 in DF; $p = 0.03$, $p < 0.001$, and $p < 0.001$, respectively) between DF and DFSP. The result for expression of MMP-9 reveals no differences. The results indicate that the pathogenesis of DF and DFSP are affected by different expressions of extracellular matrix proteins. Metalloproteinases may play a direct role in these two diseases. Since no single marker can completely distinguish DF from DFSP, a combination of more than two or three stains may elevate the accuracy of diagnosis.

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doi:10.1016/j.kjms.2012.04.017

Introduction

Dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) are both mesenchymal neoplasms of spindle-shaped cell tumors in the dermis and subcutis. Histologically, DFSP tends to diffusely infiltrate the dermis and invade into subcutaneous tissue, along the fibrous septa of fat, resulting in a honeycomb appearance. DF is predominantly a dermal lesion but may involve the upper part of the dermis sometimes. DF variant as cellular fibrous histiocytoma sometimes involves subcutaneous fat. Therefore, in clinical practice, it challenges pathologists to make a diagnosis according to routine hematoxylin and eosin (H&E) staining in some gray-zone cases. In addition, histogenesis remains uncertain and controversial until now. For prognosis, DFSP has a significantly higher risk of local recurrence [1] than DF and can metastasize especially in the case of the fibrosarcomatous variant [2]. Therefore, it is important to make a correct diagnosis for prognosis prediction and treatment strategy.

Traditionally, CD34 and factor XIIIa have been widely used to distinguish these two diseases [3–5]. Other immunohistochemical (IHC) markers such as COX-2 [6], CD117 [7], Nestin [8], Apo D [9], and High mobility group A (HMG-A) [10] have also been studied. However, the results of these markers leave an overlap and a lack of the specificity. In a previous study, the matrix metalloproteinases (MMPs) family is known to be capable of regulation of connective tissue turnover by cell-matrix interactions [11]. It also has been suggested that increased proteolysis is a potential mechanism for cancer cells to penetrate the basement membrane, enter the interstitial stroma and metastasize to distant sites [12]. In our study, we evaluate the expression of MMP-2, MMP-9, and MMP-11, which are synthesized by dermal fibroblasts in DF and DFSP, and in an attempt to differentiate and understand the pathogenesis of these two neoplasms.

Materials and methods

Formalin-fixed, paraffin-embedded blocks of dermatofibroma and dermatofibrosarcoma protuberans were collected from biopsies in Kaohsiung Medical University Hospital between 2004 and 2006. The H&E slides were reviewed to confirm the diagnosis. This study included 19 cases of DF and 17 cases of DFSP. We chose only extremely confirmed cases of DF and DFSP, diagnoses of which were made according to the criteria published in *Health Organization Classification of Skin Tumors* [13]. The study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-980340).

IHC staining was performed using the MMP-2, MMP-9, MMP-11 (1:75, NeoMarkers, Union City, CA, USA) and CD34 (1:50, DAKO, Glostrup, Denmark) antibody. Then, a Ventana (Tucson, AZ, USA) automated instrument was used for IHC staining according to the manufacturer's guidelines. Finally, the tissue sections were counterstained by Mayer hematoxylin and dehydrated in increasing grades of alcohols cleared in xylene and mounted. Negative controls were obtained by replacing the primary antibody with nonimmune serum.

IHC studies were evaluated semiquantitatively according to the method of a previously published paper [14]. Scoring was semiquantitative based on the number of spindled tumor cells stained in the whole tumor. Positive reactivity was defined as cytoplasmic and was graded in the following manner: 0, no reactivity; +1, 1–10% of tumor cells reactive; +2, 11–25% of tumor cells reactive; +3, 26–50% of cells reactive; +4, 51–75% % of cells reactive; and +5, with > 75% of cells reactive. For further analysis, using a cut-off point to define two groups of negative and positive staining, −, 1+ staining patterns were regarded as negative, and 2+ ~ 5+ staining patterns were regarded as positive. Two pathologists blinded to the clinical outcome independently evaluated the immunostaining patterns. If a discrepancy was present, the pathologists re-analyzed the slides together and reached a consensus regarding the final score. For statistical analysis, the correlations of CD34, MMP-2, MMP-9, and MMP-11 expression were assessed using the Chi-square test. All statistical analyses were performed with the SPSS 15.0 statistical software program (Chicago, IL, USA). The p values less than 0.05 were considered to be statistically significant.

Results

The results of immunoreactivities for CD34, MMP-2, MMP-9 and MMP-11 in DF and DFSP are summarized in Table 1.

Immunoreactivity for CD34

Several endothelial cells and spindle cells in the reticular dermis in both DF and DFSP were demonstrated by CD34 staining as positive internal control. In most cases of DF, CD34 was completely negative (15 of 19, 80%). This is shown in Fig. 1. Of the other four DF cases, two cases showed a weakly positive (Score 2) staining and two cases disclosed a strong positive (Score 5) staining. On the other hand, CD34 expression was noted as strong positive level (Score 5) in 12 of the 17 DFSPs and five cases were negative. The CD34 IHC reactivity was significantly more positive in DFSP compared with that in DF ($p = 0.03$).

Table 1 MMP-2, MMP-9, MMP-11 and CD 34 Immunoreactivity in DF and DFSP.

	DF ($n = 19$)	DFSP ($n = 17$)	p value*
CD34			
Positive	4	12	0.03
Negative	15	5	
MMP-2			
Positive	19	3	< 0.001
Negative	0	14	
MMP-9			
Positive	19	17	1.0
Negative	0	0	
MMP-11			
Positive	17	5	< 0.001
Negative	2	12	

*p value was determined by Chi-square test.
DF = dermatofibroma; DFSP = dermatofibrosarcoma protuberans; MM = matrix metalloproteinases.

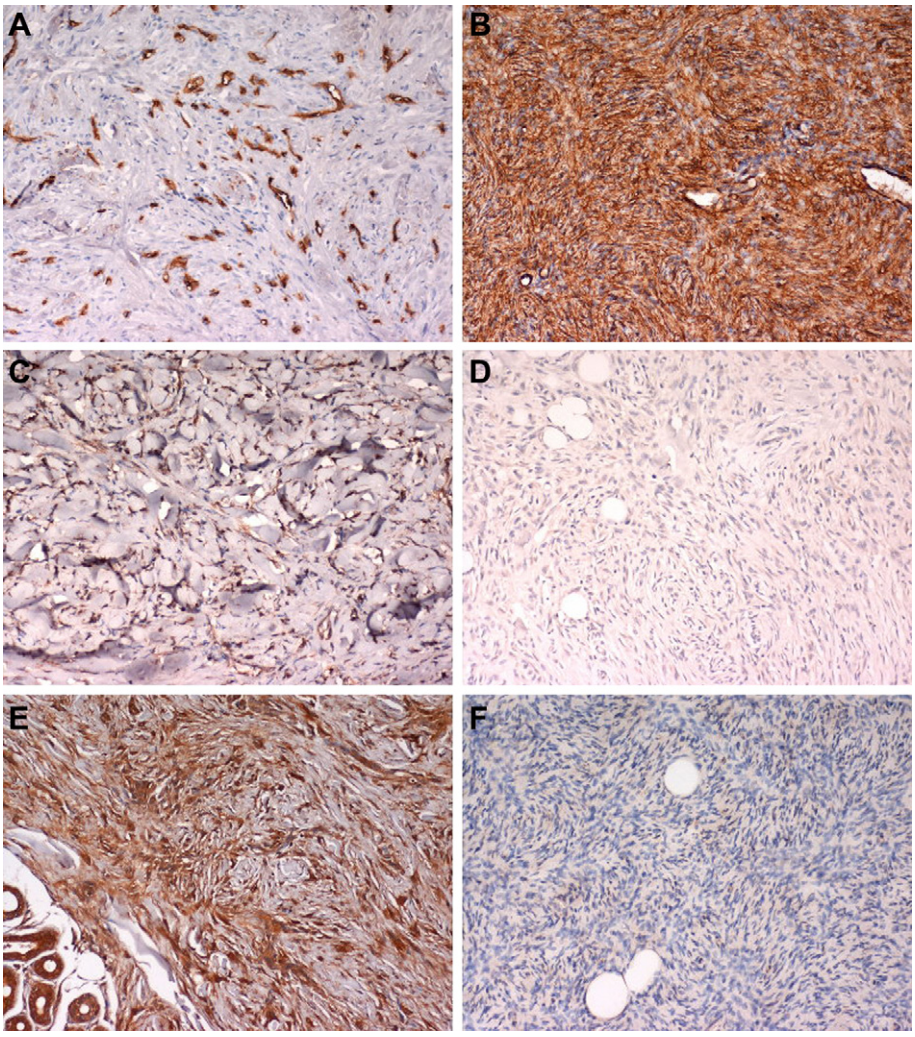

Figure 1. Immunohistochemical expression of DF; and DFSP stained with antibody to CD34, MMP-2, and MMP-11. Original magnification ×200. (A) Only endothelial cells among the tumor cells show immunoreactivity to CD34 in DF; (B) the tumor cells show strong immunoreactivity to CD34 in DFSP; (C) the tumor cells show intense immunoreactivity to MMP-2 in DF; (D) the tumor cells show no immunoreactivity to MMP-2 in DFSP; (E) the tumor cells show intense immunoreactivity to MMP-11 in DF; (F) the tumor cells show no immunoreactivity to MMP-11 in DFSP. DF = dermatofibroma; DFSP = dermatofibrosarcoma protuberans; MMP = matrix metalloproteinases.

Immunoreactivity for MMP-2

In IHC expression of MMP-2 stain, 19 cases (100%) of DF showed positive cytoplasmic staining (> 10% tumor cells). The results are shown in Fig. 1. Seven cases showed moderate staining (Score 2—3) and 12 cases were strong positive (Score 4—5). By contrast, only three cases (17.6%) of DFSP showed weak expression (Score 2—3) of MMP-2. The other 14 cases were negative for MMP-2 stain (9 Score 0 and 5 Score 1). The MMP-2 IHC reactivity was significantly more positive in DF compared with that in DFSP ($p < 0.001$).

Immunoreactivity for MMP-9

IHC expression of MMP-9 stain, 19 cases (100%) of DF showed strong positive (19 cases score 5) and 17 cases of DFSP showed moderate-to-strong positive (3 cases score 4 and 14 cases score 5). There was no statistical significance between these two neoplasms in MMP-9 staining.

Immunoreactivity for MMP-11

Immunoreactivity for MMP-11 was expressed in the cytoplasm of the spindle cells in DFs. At low-power view, tumor cells were positively stained and contrasted strongly with the surrounding adjacent dermis or subcutaneous fat. Eccrine ducts were also stained as a positive internal control [14]. In DF, ST3 was positive (Score 2—5) in most cases (17/19, 89%). Only two cases shows negative for MMP-11 stain (Score 1). By contrast, in 12 of 17 DFSPs, the tumor cells were negative for MMP-11 (71%). Five cases of DFSP showed moderate to strong positive (Score 3—5) MMP-11 staining. The MMP-11 IHC reactivity was significantly more positive in DF than in DFSP ($p < 0.001$).

Table 2 Previous Studies of MMP-2, MMP-9, and MMP-11 in DF and DFSP.

Reference	DF: positive/total case (%)			DFSP: positive/total case (%)		
	MMP-2	MMP-9	MMP-11	MMP-2	MMP-9	MMP-11
Weinrach DM et al. [1]	21/45 (47%)	9/45 (20%)		0/41(0%)	0/36 (%)	—
Unden et al. [26]	—	—	19/19 (100%)	—	—	0/7 (0%)
Thewes et al. [27]	—	—	23/40 (57.5%)	—	—	1/8 (12.5%)
Cribier et al. [14]	—	—	40/40 (100%)	—	—	0/40 (0%)
H. J. Kim [13]	—	—	23/23 (100%)	—	—	1/17 (5.9%)

DF = dermatofibroma; DFSP = dermatofibrosarcoma protuberans; MM = matrix metalloproteinases.

Discussion

DF and DFSP are the both spindle-shaped cell mesenchymal neoplasms of the dermis and subcutis. Statistically, DFSP has higher risk of recurrence than DF [1] and it can metastasize especially in the case of the fibrosarcomatous variant [2]. However, their pathogenesis remains still unclear. Histologically, DFSP is difficult to differentiate from DF variants due to sometime identical characteristics. Therefore, making the correct diagnosis according to H&E staining is still a challenge for dermatopathologists in some cases.

In previous studies, numerous IHC markers were been used to distinguish DF from DFSP. Traditionally, CD34 and factor XIIIa were most widely used as the tools to differentiate these two diseases [3—5]. Other IHC markers, such as COX-2 [6], CD117 [7], Nestin [8], Apo D [9], CD44, hyaluronate [15] , D2-40 [16], CD163 [17], and HMG-A [10], have also been studied. Ultrastructural study [18] or gene analysis [19—21] have also been performed. However, these markers or techniques leave an overlap and they lack specificity.

The MMPs family is known to be capable of regulation of connective tissue turnover by cell-matrix interactions [11]. MMPs play a key role in cancer progression. For example, MMP-1, MMP-2, and MMP-9 are involved in the initial breakdown of collagen and basement membrane components during tumor growth and invasion [22]. The expression of MMP-2 and MMP-9 was found to correlate with ras-mediated cellular transformation and to be a function of malignant potential [23]. Nevertheless, to date, few studies have investigated the expression of MMP-2, MMP-9, and MMP-11 in DF and DFSP [1,24—27]. The possible pathogenesis of these two diseases is still controversial.

Therefore, we chose gelatinase A/type IV collagenase (MMP-2, 72kb), gelatinase B/type IV collagenase (MMP-9, 92kb) and stromelysin-3 (MMP-11) as the IHC markers based on the following evidence. First, DF and DFSP are composed of predominant spindle-shaped cell resembling fibroblasts. We wished to demonstrate the interactions of MMPs and fibroblasts. Second, MMP-11 was found to be a useful marker for the differential diagnosis of DF and DFSP, whereas DFSPs were rarely positive for MMP-11 stain [24,27,28]. We wished to compare different IHC markers to distinguish DF from DFSP (Table 2) [1,13,14,26,27].

In our study, we found significant IHC expression ($>$ 10% tumor cells) of CD34 in DFSP, MMP-2 (100%) and MMP-11 (89%) in DF. The mean $\pm$ standard deviation (SD) IHC score of CD34, MMP-2, and MMP-11 shows significant statistical differences (0.03, $<$ 0.001, and $<$ 0.001, respectively) between DF and DFSP. The expression of CD34, MMP-2, and MMP-11 is similar to the previous studies in DF and DFSP [1,3—5,14,24,25,27—29]. Although a higher expression of MMP-2 was found in a lot of cancers, it seemed that MMP-2 was not regulated only just at the transcriptional level, but also that it was governed by other enzyme activators and inhibitors [1]. Several factors may mediate and influence MMP-2 expression. Since zymography is a certain way to evaluate the functions of MMPs, further study of MMP-2 function in cells is needed.

Alternatively, the result of expression of MMP-9 in our study is quite different from the Weinrach study [1]. Nine of 45 cases in DF and none in DFSP in their study are positive for MMP-9. All of our cases, both DF and DFSP, are strongly positive for MMP-9 staining. These discrepancies may be attributed to several factors, such as case numbers, immunohistochemical techniques, scoring methods, and even antigen retrieval.

Our study suggests that the pathogenesis of DF and DFSP are affected by different interactions of extracellular matrix proteins. MMPs may play a direct role in the progression of these two diseases. Further research is therefore necessary to determine the mechanisms in more detail. For some cases in which a definite diagnosis by H&E staining is difficult, IHC study can provide an assistant role. As no single marker can distinguish DF from DFSP completely, a combination of more than two or three stainings (data not shown) may elevate the accuracy of diagnosis.

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