

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF ELASTOFIBROMAS

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Elastofibromas are slow-growing and rare soft-tissue tumors. The etiology and pathogenetic mechanisms are still controversial and there are only a few studies in the literature investigating the histochemical, immunohistochemical, and genetic features to determine the pathogenesis.

We investigated the cellular composition of lesions with a diagnosis of elastofibroma in 17 patients by using histochemical and immunohistochemical methods.

There were 17 cases with a mean age of 53.5 years. Mean lesion diameter was 6.6 cm. The immunohistochemical method showed vimentin and factor XIIIa positivity in all cases. Four cases had focal myoglobin positivity in the spindle-shaped cells between the collagen fibers. Spindle cells were positive for CD34 in 8 cases. Smooth muscle actin, desmin, type 4 collagen and laminin were negative in all cases. The elastic nature of the abnormal fibers was shown histoch with Verhoeff elastin staining and aldehyde fuchsin staining in all cases.

Our results have shown that the concurrent positivity of factor XIIIa and CD34 in the cells forming the lesion might show that the lesion originates from primitive dermal mesenchymal cells. In addition, the myoglobin positivity found in some cases indicates the possibility of a myofibroblastic origin of elastofibromas.

Key words: elastofibroma, immunohistochemistry, CD34, factor XIIIa.

Introduction

Elastofibromas are rare, benign, slow-growing soft tissue tumors and were first described in 1961 by Jarven and Saxi [1, 2]. They are characteristically located in the distal scapular region [3-5]. They are usually seen in middle-aged and elderly individuals [6]. Morphologically, there is dense collagen mixed with globular or droplet type abnormal elastic fibers as well as a normal fatty tissue component [6, 7]. The real etiology and pathogenetic mechanisms of elastofibromas are still controversial. There are only a few studies in the literature investigating the histochem-

ical, immunohistochemical, and genetic features to determine the pathogenesis [8].

We investigated the cellular composition of 17 elastofibroma lesions using histochemical and immunohistochemical methods in this study and discuss the clinicopathological data of the cases in comparison with the literature.

Material and methods

A total of 17 cases diagnosed with elastofibroma at the Ministry of Health, Izmir Bozyaka Training and Research Hospital's Department of Pathology between the years of 2000 and 2012 were retro-

spectively analyzed in this study. Clinical data such as age, gender, and location of the lesion were taken from the medical records of the cases. Four-micron thick sections were taken from the tissues that were fixed with 10% formalin and embedded in paraffin. The sections were then deparaffinized and stained with hematoxylin and eosin. Histochemical and immunohistochemical methods were also applied to the sections to determine the cellular components. Histochemically, Masson trichrome, Verhoeff elastin stain, Giemsa, toluidine blue and aldehyde fuchsin-alcian blue stains; immunohistochemically, smooth muscle actin (1A4, 1/200, Spring Bioscience), desmin (D33, 1/50, Thermo Scientific), myoglobin (MGN01, 1/200, Spring Bioscience), CD34 (QBEND/10, 1/200, Thermo Scientific), vimentin (SP20, 1/200, Spring Bioscience), type 4 collagen (CIV22, 1/50, Dako Cytomation, Denmark), laminin (polyclonal rabbit, Biogenex), and factor XIIIa (AC-1A1, 1/100, Thermo Scientific) antibodies were applied to the lesions.

Results

Clinical data

The mean age of the 17 patients was 53.5 years (oldest 73, youngest 28 years). There were 13 females and 4 males. All lesions were located in the dorsal scapular region. All patients underwent surgical resection. Complete resection was performed in 16 patients while one case with incomplete resection underwent a re-excision 2 months later. None of the cases suffered a recurrence.

Macroscopic findings

All lesions had grayish-white surface areas with irregular contours mixed with fatty tissue areas. The mean diameter of the tumors was 6.6 cm.

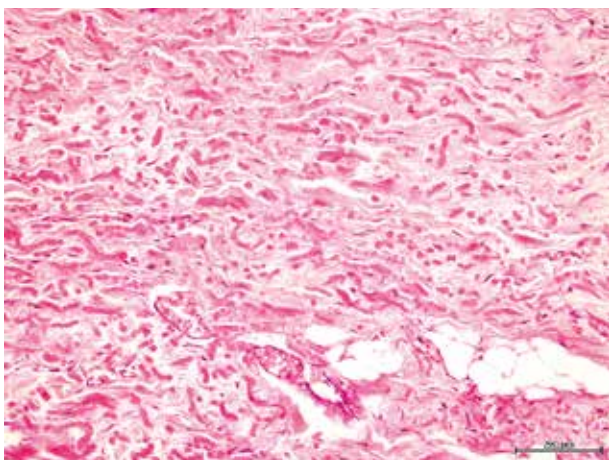


Fig. 1. Abnormal elastic fibers in fibrocollagenous stroma with spindle or stellate cells (HE, magnification 200×)

Microscopic findings

The lesion was composed of a variable amount of mature adipose tissue with spindle-like and satellite collagen bands together with abnormal elastic fibers embedded in this fibrocollagenous matrix under light microscopic examination (Fig. 1). Elongated or drop-like abnormal elastic fibers were observed.

Histochemical findings

The elastic nature of the abnormal fiber was shown histochemically with Verhoeff elastin staining and aldehyde fuchsin staining in all cases (Fig. 2). Collagen bands in the lesion were determined with Masson trichrome staining. In addition, the presence of mast cells was shown with toluidine blue and Giemsa staining.

Immunohistochemical findings

Diffuse positivity was found with vimentin and factor XIIIa in all cases (Figs. 3, 4). Four cases had focal myoglobin positivity in the spindle-shaped cells between the collagen fibers (Fig. 5). Spindle cells were found to be CD34-positive in 8 cases (Fig. 6). Smooth muscle actin, desmin, type 4 collagen and laminin were negative in all cases.

Discussion

Although elastofibromas are well defined in terms of clinical data, treatment approach and histopathological diagnostic criteria, the pathogenetic mechanisms and their real nature are still controversial.

Electron microscopic findings of elastofibromas in previous studies report the spindle cells in the lesion to be fibroblastic or myofibroblastic in nature [9-11]. Elastofibromas are believed to be a reactive process caused by microtraumas [11]. Taking this into account, Ramos *et al.* [9] postulate elastofibromas to

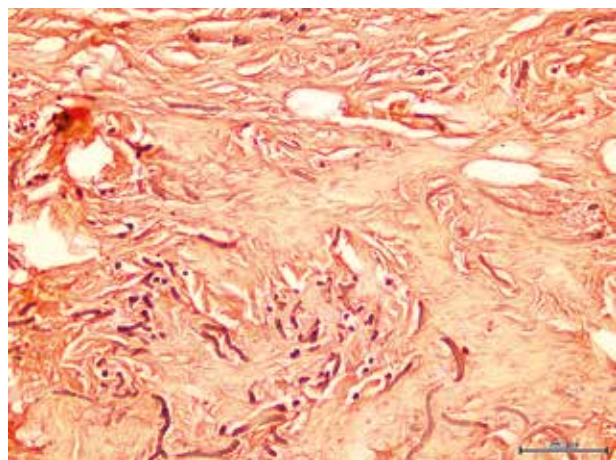


Fig. 2. Elongated and globular elastic fibers are seen. (Verhoeff Elastin Stain, magnification 200×)

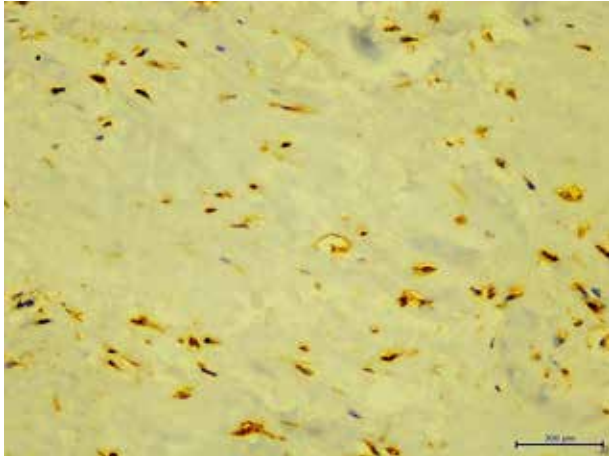


Fig. 3. Vimentin was diffusely positive in spindle-shaped cells (vimentin, magnification 400×)

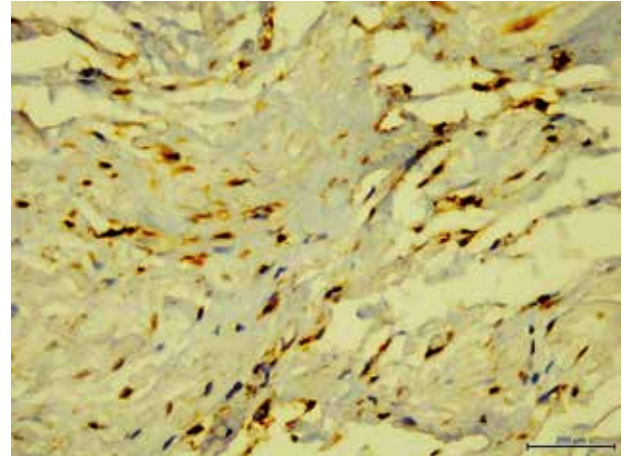


Fig. 4. Diffuse positive factor XIIIa immunostaining in elastofibroma (factor XIIIa, magnification 400×)

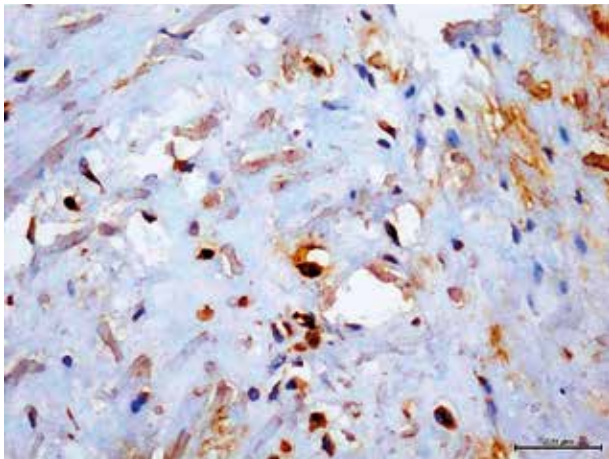


Fig. 5. Focal positive immunostaining by myoglobin in elastofibroma (myoglobin, magnification 400×)

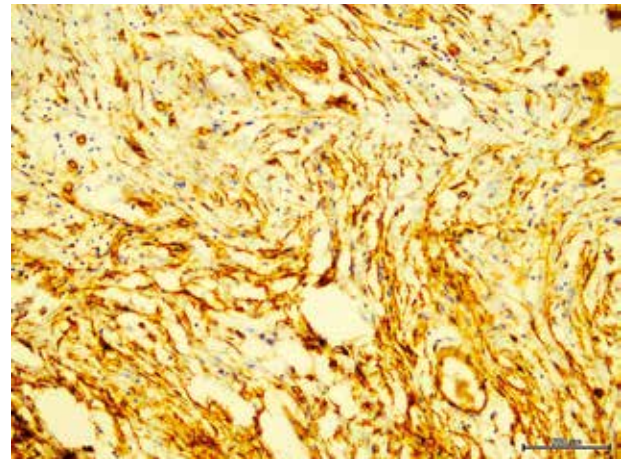


Fig. 6. CD34 was expressed in spindle-shaped cells (CD34, magnification 400×)

be possibly of myofibroblastic origin as the main cell components in reactive processes are myofibroblasts. Ruelas *et al.* found actin and desmin positivity in all 4 of their cases as the evidence of a myofibroblastic nature [12]. Spindle cells were actin and desmin negative in our series but the myoglobin positivity in 4 cases may indicate possible myofibroblastic differentiation, similar to the findings of Ruelas *et al.*

Later studies have made a possible myofibroblastic nature controversial. Hisaoka *et al.* analyzed 14 elastofibroma cases and reported that they could not find, in any case, stress fibers or a cell-stroma connecting region (fibronexus), which are ultrastructural evidence of myofibroblastic differentiation [6]. The same investigators also could not find positivity with actin and desmin and reported that the previously described myofibroblastic differentiation was not seen in their lesions [6]. Similar to Hisaoka *et al.*, there are other publications defining actin and desmin negativity in elastofibromas and reporting them to be of fibroblastic nature and not myofibroblastic [4, 5, 8, 13].

Elastofibromas are very commonly located in the interscapular region and some authors therefore believe these lesions to be a reactive process stemming from degeneration in connective tissues caused by the constant friction of the shoulder on the ribcage with repetitive movements [5, 7, 10, 14]. However, there are studies that report the lesion to be more neoplastic than reactive by demonstrating chromosomal instability and clonal chromosomal aberrations as evidence [6, 15, 16]. Chromosomal studies were not performed in our cases but none of the patients reported recurrent severe physical activity supporting a reactive process in the anamnesis.

The possibility of genetic inheritance in the pathogenesis of elastofibromas has also been discussed. The ABCC6 gene, previously described in pseudoxanthoma elasticum cases [17], was investigated in four elastofibroma cases in another study but could not be detected [7]. However, Nagamine *et al.* reported a positive family history in 1/3 of the cases in a series

of 170 patients in another study investigating possible genetic inheritance [18]. None of our cases had a positive family history.

A large number of studies in the literature have defined diffuse CD34 positivity in spindle-shaped stromal cells [4, 6, 8]. CD34 positivity was observed in 8 of our cases, in accordance with the literature.

An interesting immunohistochemical finding in the present study is the factor XIIIa positivity in all cases. Only a few studies have investigated factor XIIIa positivity in elastofibromas. Yamazaki *et al.* reported factor XIIIa positivity in all 5 of their cases, similar to our study [19]. Narvaez *et al.* defined 2 dendritic cell-type in the dermis: a CD34 (–) and factor XIIIa-positive [20]. Silverman and Tamsen reported the CD34 and factor XIIIa (+) cells to be primitive mesenchymal cells and to be present together in some dermal tumors, but they did not define this association in elastofibromas [21]. This association was found in approximately half of the cases in our series, and is considered to be a finding supporting a primitive mesenchymal origin of the cells making up the elastofibroma.

Gray *et al.* investigated the relationship between Kaposi sarcoma spindle cells and dermal dendrocytes and reported the number of factor XIIIa (+) cells to be high in plaque and patchy stage tumors and decreasing in nodular stage tumors, whereas the CD34-positive spindle cells differentiating towards endothelium increased in the nodular stage [22]. According to Silverman, these findings of Gray *et al.* indicate that factor XIIIa-positive cells cause clonal CD34-positive cell proliferation [21]. This speculation may also be valid for elastofibroma pathogenesis and the CD34 (+) cells detected in elastofibromas may be spindle cells that are differentiated in the fibroblastic or myofibroblastic direction following activation by factor 13a (+) dermal dendrocytes. The presence of CD34 (+) in all 4 of our patients with focal myoglobin positivity may indicate the possibility of myofibroblastic differentiation in primitive mesenchymal cells.

Mast cells are normally located in the dermis and are active in fibroblast stimulation with some cytokines present in their granules, and also serve in the reconstruction of extracellular matrix in wound healing with the proteases they contain [23]. There are also publications that report mast cells to secrete fibroblast growth factor [24]. A study examining 7 elastofibroma cases showed the presence of mast cells in 5 of them [4]. We found the presence of varying amounts of mast cells with toluidine blue and Giemsa histochemical staining in 10 of our 17 cases, in accordance with the literature. The presence of mast cells in elastofibroma cases may be responsible for the fibroblastic proliferation of the lesion.

In conclusion, our findings of CD34 and vimentin positivity with actin and desmin negativity support the fibroblastic nature of the lesion, and the positivity of factor XIIIa associated with CD34 is interpreted as evidence of the lesion being derived from dermal mesenchymal cells. However, determination of myoglobin positivity in some cases with CD34 (+) positivity made us consider that CD34 (+) mesenchymal cells could be differentiated in the myofibroblastic direction. Therefore, the possibility of a myofibroblastic origin of the lesion could not be completely excluded. Immunohistochemical studies in larger series may provide more accurate information on the cellular composition of elastofibromas.

The authors declare no conflict of interest.

References

1. Nishio J, Isayama T, Iwasaki H, Naito M. Elastofibroma dorsii: diagnostic and therapeutic algorithm. *J Shoulder Elbow Surg* 2012; 21: 77-81.
2. Yáñez S, Val-Bernal JF, Echevarría MA, Landeras R, Izquierdo J, Gallardo E. Retrospective analysis of 6 cases of elastofibroma dorsii. *Actas Dermosifiliogr* 2008; 99: 644-647.
3. Muratori F, Esposito M, Rosa F, Liuzza F, Magarelli N, Rossi B, Folath HM, Pacelli F, Maccauro G. Elastofibroma dorsii: 8 case reports and a literature review. *J Orthop Traumatol* 2008; 9: 33-37.
4. Banu Dogan Gun, Burak Bahadır, Kemal Behzatoğlu, Mustafa Özkan Gün, Şükrü Oğuz Özdamar. Elastofibroma: a clinicopathologic and immunohistochemical study of seven cases and literature review. *APMIS* 2007; 115: 115-119.
5. Neri A, Caruso S, Bettarini F, Falzarano SM, Di Martino M, Vuolo G, Pedrazzani C, Marrelli D, Roviello F. Elastofibroma dorsii: three cases of personal experience. *G Chir* 2009; 30: 96-99.
6. Hisaoka M, Hashimoto H. Elastofibroma: clonal fibrous proliferation with predominant CD34-positive cells. *Virchows Arch* 2006; 448: 195-199.
7. Naouri M, Michenet P, Chassaing N, Martin L. Immunohistochemical characterization of elastofibroma and exclusion of ABCC6 as a predisposing gene. *Br J Dermatol* 2007; 156: 755-758.
8. Kuroda N, Hamaguchi N, Ohara M, Hirouchi T, Mizuno K, Hayashi Y, Lee GH. Elastofibroma: a histochemical, immunohistochemical, and ultrastructural study of two patients. *Med Mol Morphol* 2008; 41: 179-182.
9. Ramos CV, Gillespie W, Narconis RJ. Elastofibroma. A pseudotumor of myofibroblasts. *Arch Pathol Lab Med* 1978; 102: 538-540.
10. Christopher DM Fletcher, K. Krishnan Unni, Fredrik Mertens. *WHO Pathology & Genetics Tumors of Soft Tissue and Bone*. IARC Press, Lyon 2002.
11. Franz M, Enzinger, Sharon W. *Wiess. Soft Tissue Tumors*. Third Edition. Mosby-Year Book, Inc. 1995.
12. Ruelas Villavicencio AL, Aponte Paredes PS, Ortiz Hidalgo C. CD-34 expression in elastofibroma. *Clinicopathological, histochemical, and immunohistochemical study of four cases*. *Rev Invest Clin* 1999; 51: 11-16.
13. Kayaselçuk F, Demirhan B, Kayaselçuk U, Ozerdem OR, Tuncer I. Vimentin, smooth muscle actin, desmin, S-100 protein, p53, and estrogen receptor expression in elastofibroma and nodular fasciitis. *Ann Diagn Pathol* 2002; 6: 94-99.

14. Marcos J, Rodríguez Russo P, Lima S, Rodríguez EL. Elastofibroma dorsi: an unusual case of a subscapular nodular mass. *J Clin Rheumatol* 2004; 10: 21-24.
15. Vanni R, Marras S, Faa G, Uccheddu A, Dal Cin P, Sciot R, Samson I, Van den Berghe H. Chromosome instability in elastofibroma. *Cancer Genet Cytogenet* 1999; 111: 182-183.
16. Batstone P, Forsyth L, Goodlad J. Clonal chromosome aberrations secondary to chromosome instability in an elastofibroma. *Cancer Genetics and Cytogenetics* 2001; 128: 46-47.
17. Chassaing N, Martin L, Mazereeuw J, Barrié L, Nizard S, Bonafé JL, Calvas P, Hovnanian A. Novel ABCC6 mutations in pseudoxanthoma elasticum. *J Invest Dermatol* 2004; 122: 608-613.
18. Nagamine N, Nohara Y, Ito E. Elastofibroma in Okinawa. A clinicopathologic study of 170 cases. *Cancer* 1982; 50: 1794-1805.
19. Yamazaki K. An ultrastructural and immunohistochemical study of elastofibroma: CD 34, MEF-2, prominin 2 (CD133), and factor XIIIa-positive proliferating fibroblastic stromal cells connected by Cx43-type gap junctions. *Ultrastruct Pathol* 2007; 31: 209-219.
20. Narvaez D, Kanitakis J, Faure M, Claudy A. Immunohistochemical study of CD34-positive dendritic cells of human dermis. *Am J Dermatopathol* 1996; 18: 283-288.
21. Silverman, Jeffrey S, Tamsen, Ali. CD34 and Factor XII-Ia-Positive Microvascular Dendritic Cells and the Family of Fibrohistiocytic Mesenchymal Tumors. *Am J Dermatopathol* 1998; 20: 533-536.
22. Gray MH, Trimble CL, Zirn J, McNutt NS, Smoller BR, Varghese M. Relationship of factor XIIIa-positive dermal dendrocytes to Kaposi's sarcoma. *Arch Pathol Lab Med* 1991; 115: 791-796.
23. Hebda PA, Collins MA, Tharp MD. Mast cell and myofibroblast in wound healing. *Dermatol Clin* 1993; 11: 685-696.
24. Inoue Y, King TE Jr, Barker E, Daniloff E, Newman LS. Basic fibroblast growth factor and its receptors in idiopathic pulmonary fibrosis and lymphangiomyomatosis. *Am J Respir Crit Care Med* 2002; 166: 765-773.

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