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Co-ordinate expression of alpha5 beta1 integrin and fibronectin in Dupuytren's disease

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Summary

The expression of alpha5 beta1 ($\alpha5\beta1$) integrin and its extracellular ligand fibronectin was studied immunohistochemically in 23 cases of Dupuytren's disease using an immunoperoxidase method for light microscopic visualization. All cases consisted of multiple nodules showing a variable degree of cellularity and fibrosis. Depending on the histological appearance of these nodules, each case was assigned to the three following phases: proliferative, involutional and residual. Alpha5 beta 1 integrin was detected in the highly cellular areas of both proliferative and involutional phases where fibronectin was simultaneously expressed in the extracellular matrix (ECM). Diversely $\alpha5\beta1$ and fibronectin disappeared from the hypocellular areas of involutional phase, undergoing fibrotic transformation, and from the fibrotic connective tissue of residual phases. These findings indicate that the expression pattern of $\alpha5\beta1$ integrin correlates with the presence in the ECM of the corresponding ligand fibronectin during the different phases of Dupuytren's disease. We suggest that $\alpha5\beta1$ integrin, linking fibronectin to stromal cells of both proliferative and involutional phases, may be involved in the contractile processes occuring in Dupuytren's disease.

Key words: Dupuytren's disease − alpha5 beta1 integrin − fibronectin

Introduction

Alpha5 beta1 ($\alpha5\beta1$) integrin is a specific cellular receptor for fibronectin (Hynes, 1987; Ruoslahti, 1991), widely expressed on epithelial, endothelial cells, and fibroblasts (Ruck et al., 1994). This cellular receptor and its extracellular ligand fibronectin are involved in transmembrane signalling events that control morphogenetic processes (Adams and Watt, 1990; Rahilly and Fleming, 1992; Roman and McDonald, 1992), tissue regeneration (Pujades et al., 1992), and migration of normal and tumoral cells (Giancotti and Rouslahti, 1990). Several in vitro and in vivo studies have shown that alterations in expression or distribution of $\alpha5\beta1$ integrin on the cell surface may imply changes in the cells adhesive properties, leading to defects of morphogenesis (Yang et al., 1993) and tumoral transformation (Plantefaber and Hynes, 1989).

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Dupuytren's disease is a superficial fibromatosis consisting of a single nodule or an ill defined conglomerate of several nodular masses intimately associated with portions of the palmar aponeurosis and sometimes subcutaneous fat (Enzinger and Weiss, 1988). Depending on the degree of cellularity and fibrosis of nodules, three different morphologic phases are observed during the course of Dupuytren's disease: proliferative, involutional and residual (Luck, 1959). During these phases extracellular matrix (ECM) undergoes changes in the expression of several glycoproteins. The proliferative and involutional phases show a predominance of type III collagen and fibronectin whereas large amounts of type I collagen are present in the residual phase (Meister et al., 1979; Tomasek et al., 1986; Tomasek and Haaksma, 1991; Schurch et al., 1992).

Although the expression of fibronectin has been studied in Dupuytren's disease (Tomasek et al., 1986; Tomasek and Haaksma, 1991), to our knowledge no detailed information is available on the expression of its cellular receptor $\alpha5\beta1$ integrin. The purpose of the present study was to analyze immunohistochemically the expression of $\alpha5\beta1$ integrin and fibronectin during the different phases of Dupuytren's disease.

Materials and Methods

Light microscopy. Macroscopic nodular masses within resected palmar aponeurotic specimens, derived from 23 patients affected by palmar fibromatosis (Dupuytren's disease), were fixed in 10% buffered formaldehyde for 12 h and paraffin-embedded. Sections of 4 μm were cut and stained with hematoxylin-eosin, van Gieson stain for collagen, and silver impregnation for the evaluation of the fiber content as previously reported (Meister et al., 1979). Depending on the degree of cellularity and fibrosis, the nodules were assigned to the three following phases: proliferative, involutional and residual (Luck, 1959). Since all cases studied by us consisted of multiple nodules showing considerable variation in their histological pattern, classification of the disease phase was based on the predominant histological appearance as summarized in Table 1.

Table 1. Correlation between disease phase and expression of fibronectin and alpha5 beta1 integrin in 23 cases of Dupuytren's disease

Disease phase	Number of cases	Fibronectin	alpha5 beta1 integrin
Proliferative	10	+ + +	+ + +
Involutional	6	+ + −	+ + −
Residual phase	7	− − −	− − −

+ + + = diffuse strong immunoreactivity; + + − = strong immunoreactivity restricted to cellular areas; − − − = no immunoreactivity

Immunohistochemistry. From ech case, samples of tissue were snap-frozen in liquid nitrogen. Consecutive 4 μm frozen sections were cut and air-dried for 20 min. They were then fixed in cold acetone for 10 min at room temperature and air-dried for 20 min. The frozen sections were immunostained by the avidin-biotin peroxidase (ABC) complex (Vector Laboratories, Burlingame, CA, USA). They were incubated for 1 h at room temperature with the primary antibodies. Biotinylated (ABC method) secondary antibodies (DAKO, Denmark) were each applied for 30 min at room temperature. Bound peroxidase was visualized using 0.05% 3.3'-diaminobenzidine tetrahydrochloride (Sigma) as chromogenic substrate for 10 min at room temperature. Control sections were incubated with 0.01 M phosphate-buffered saline (PBS) instead of primary antibodies. *Antibodies.* The following antibodies were used: polyclonal antibody anti-fibronectin (DAKO, dilution 1 : 400) and monoclonal antibody against integrin $\alpha5\beta1$ (DAKO, dilution 1 : 200).

Results

Light microscopy. Proliferative phase nodules of Dupuytren's disease were characterized by high stromal cellular density and marked vascularization while ECM contained few mature collagen fibers. The involutional phase nodules also showed high cellularity but stromal cells tended to be aligned in the same direction. In some areas a fibrotic transformation of ECM started to develop, enveloping individual stromal cells. The residual phase nodules were hypocellular and their ECM was represented by thick band of collagen giving them a tendon-like appeerence.

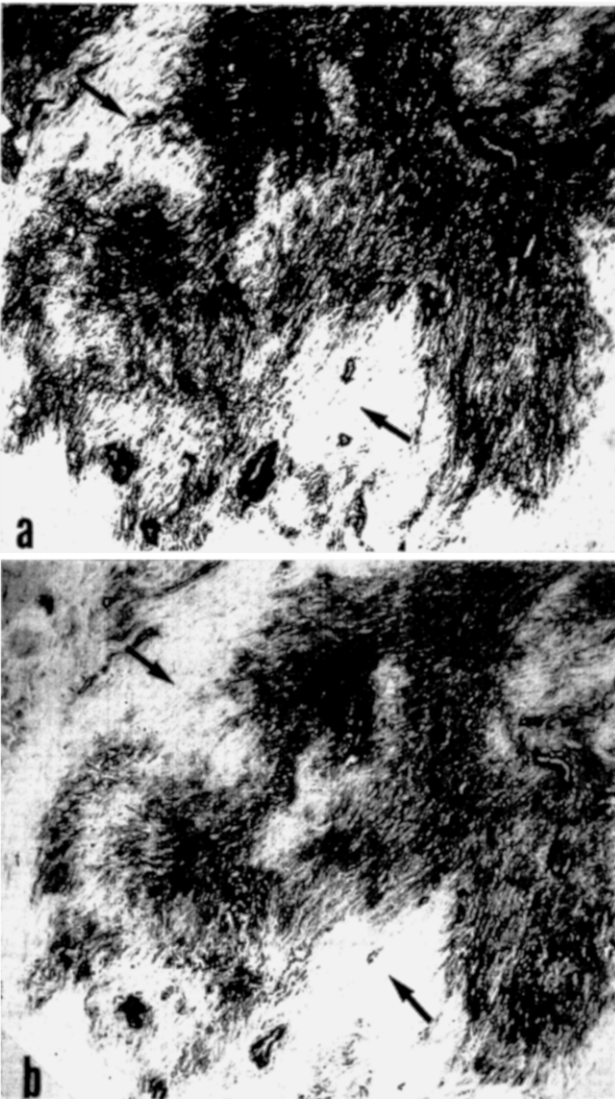*Immunohistochemistry.* Immunohistochemical results are summarized in Table 1.

Fig. 1. ABC peroxidase, haematoxylin. Involutional phase of Dupuytren's disease. Serial sections stained by fibronectin (a) and $\alpha5\beta1$ integrin (b) show immunoreactivity in the cellular areas and no reaction in those areas undergoing fibrotic transformation (arrows). Endothelial cells of capillaries are also stained. ×80

Immunostaining for fibronectin showed a strong reaction in the ECM surrounding the highly cellular areas in the proliferative and involutional phases (Fig. 1 a). The expression of this glycoprotein disappeared from those areas of the involutional phase where a fibrosis started to develop (Fig. 1 a) and from the fibrotic connective tissue of the residual phase. In each phase fibronectin was detected around capillary walls and in the subendothelial basement membrane and muscolar layer of small blood vessels.

By examining serial sections, immunostaining for $\alpha 5\beta 1$ integrin showed a distribution pattern similar to that observed for fibronectin (Fig. 1 b). $\alpha 5\beta 1$ integrin was expressed in the same cellular areas where fibronectin was detected. It was present on stromal cells of the highly cellular areas of proliferative and involutional phases whereas it disappeared from the hypocellular fibrotic areas of both involutional and residual phases. $\alpha 5\beta 1$ integrin was always expressed by endothelial cells of capillary walls and small blood vessels.

Discussion

In the present study we showed a close relationship between $\alpha 5\beta 1$ integrin expression and the presence of its corresponding extracellular ligand fibronectin, during the different phases of Dupuytren's disease. Fibronectin and $\alpha 5\beta 1$ integrin were markedly expressed in the highly cellular areas of both proliferative and involutional phases whereas they disappeared from the hypocellular fibrotic connective tissue of involutional and residual phases.

Since previous immunohistochemical and electron microscopic studies on Dupuytren's disease have demonstrated that stromal cells of both proliferative and involutional phases are mainly myofibroblasts (Gabbiani and Majno, 1972; Iwasaki et al., 1984; Schurch et al., 1992), we suggest that stromal cells expressing $\alpha 5\beta 1$ integrin in our study are myofibroblasts. It has been proposed that the contraction process in Dupuytren's disease is an active cellular process due to myofibroblasts which are believed to be the cell type responsible for the contractile events (Gabbiani and Majno, 1972; Chiu and McFarlane, 1978; Tomasek et al., 1987; Tomasek and Haaksma, 1991). These cells are connected to the extracellular matrix by a transmembrane complex, called, fibronexus consisting of intracellular microfilaments of actin in continuity with extracellular fibronectin fibers (Tomasek and Haaksma, 1991). Contractile forces generated by intracellular actin microfilaments in these cells are probably transmitted through this complex to the surrounding ECM. Although the detection of integrins by immunohistochemical techniques do not always imply their functional activity (Pignatelli and Vessey, 1994), the simultaneous and co-ordinated expression of $\alpha 5\beta 1$ integrin on stromal cells and fibronectin in the surrounding ECM suggests that this cellular receptor may be involved in contraction processes occuring in Dupuytren's disease. The restricted expression of both $\alpha 5\beta 1$ integrin and fibronectin to proliferative and involutional phases of Dupuytren's disease, in which ECM remodelling and contraction processes occur (Gabbiani and Majno, 1972; Meister et al., 1979; Schurch et al., 1992), lead us to hypothesize that $\alpha 5\beta 1$ integrin, linking fibronectin to stromal cells, may serve to transmit contractile forces generated by these cells to the surrounding ECM. It still remains to be determinated whether $\alpha 5\beta 1$ integrin is a component of fibronexus.

Acknowledgments

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