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The biology of the myofibroblast

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The mechanisms leading to the retraction of the granulation tissue during wound healing have not been fully elucidated to date [reviewed in 1]. Our laboratory several years ago described that fibroblasts present in granulation tissue exhibit several ultrastructural features of smooth muscle cells, including the presence of microfilament bundles with dense bodies scattered within [2]. These cells, called myofibroblasts, have been proposed to play a retractile role in several conditions such as granulation tissue contraction, parenchymal organ retraction, fibromatosis and stromal reaction to epithelial tumors [reviewed in 3]. The coincidence of the presence of myofibroblasts with retractile phenomena has supported this hypothesis. However, direct proof of the presence and activity of contractile elements in myofibroblasts has been possible only after suitable techniques have been developed to localize and quantify cytoskeletal and contractile proteins within the affected organs. For this purpose, the advances in the understanding of cytoskeletal and contractile element morphology and biochemistry in different cells have been of great value [reviewed in 4]. Presently, we know that the cytoskeleton of mesenchymal cells is composed of intermediate filaments which consist of a single protein named vimentin. In muscle cells, however, most intermediate filaments have been shown to contain another related, but not identical, protein which is called desmin. However, vascular smooth muscle cells always express vimentin, and only a proportion of them contains in addition desmin. Moreover, desmin has been increasingly found in a number of nonmuscle mesenchymal cells such as endothelial cells [5, 6], podocytes [7] and stromal cells from various locations [7–10]. Another marker of tissue origin is the presence of a specific actin isoform, since the six actin isoform expressing mammals show a tissue specific distribution [4]. In particular, α-smooth muscle actin is present in all smooth muscle cells. Finally, isoforms of myosin heavy and light chains can also be typical of smooth muscle (particularly under normal conditions) and hence assist in identifying cells which are involved in different pathological changes [11].

Using different markers, we have defined four cytoskeletal phenotypes among myofibroblasts: 1) phenotype V represented by myofibroblasts positive for vimentin only; 2) phenotype VA represented by myofibroblasts positive for vimentin and α-smooth muscle actin; 3) phenotype VAD represented by myofibroblasts positive for vimentin, α-smooth muscle actin and desmin; and 4) phenotype VD represented by myofibroblasts positive for vimentin and desmin. When normally healing granulation tissue is studied with these criteria, during granulation tissue contraction a high proportion of myofibroblasts have been found to develop the expression of α-smooth muscle actin, but not of desmin and smooth muscle myosin [12], and hence acquire, at least in part, smooth muscle features. When contraction stops and the wound is fully epithelialized, myofibroblasts containing α-smooth muscle actin disappear, probably by apoptosis, and the scar classically becomes less cellular and composed of typical fibroblasts with well developed rough endoplasmic reticulum and no more microfilaments, nor α-smooth muscle actin. In more permanent retractile conditions and in particular in kidney, lung or liver fibrosis, myofibroblasts expressing α-smooth muscle actin are constantly present, and in addition, a proportion of them also express desmin [13]. However, at present, no myofibroblast expressing smooth muscle myosin has been described [11, 14]. On the basis of these results, we propose that during the development of fibrocontractive diseases, fibroblasts acquire contractile features and produce the centripetal force leading to retraction. For this purpose, myofibroblasts have the capacity of developing connections to the surrounding extracellular matrix and hence to act on the whole tissue [3]. Traction rather than contraction forces have been shown to be responsible for the retractile activity of cultured fibroblasts on their substratum [15]. In analogy with these observations, we suggest that the retractile activity of myofibroblasts during fibrotic changes is more dependent in isometric contraction than on isotonic contraction.

What remains to be explored are the mechanisms leading to the development of cytoskeletal features similar to those of smooth muscle cells in fibroblasts, including the factors which can regulate, in vivo and in vitro, the appearance of α-smooth muscle actin and desmin. The more likely candidates for these actions are cytokines, which can be locally liberated by vascular cells, inflammatory cells and fibroblastic cells themselves, as well as extracellular matrix components which have been shown to influence the shape, the replication and the development of cytoskeletal features in fibroblastic and smooth muscle cells [reviewed in 3]. Working along these lines, we have observed that γ-interferon, a cytokine mainly produced by T-helper lymphocytes is capable of inhibiting the expression of α-smooth muscle actin in both smooth muscle [16] and fibroblasts [17]. When γ-interferon is applied to Dupuytren's nodules, it produces an improvement of the retractile conditions and in hypertrophic scars, in addition to the reduction of the size of the lesion, it elicits the disappearance of α-smooth muscle actin in myofibroblasts (unpublished observation).

Although further studies are needed to confirm these preliminary results, we feel that work in these directions can help not only the understanding of the pathogenesis of fibrocontractive diseases, but also may reveal future directions for treatment. In this respect, we have observed in an experimental rat model that the application of GM-CSF to the subcutaneous tissue induces not only the proliferation of fibroblasts and the formation of ultrastructurally typical myofibroblasts, but also the expression of α-smooth muscle actin in a significant proportion of these cells [18]. GM-CSF is mainly known for its hematopoietic effect [19], but some extra-hematopoietic activity has been attributed to this factor. Thus, GM-CSF stimulates migration of human endothelial cells [19] and proliferation of different non-hematopoietic cells of mesenchymal origin in vitro, such as endothelial cells [20], bone marrow fibroblast precursors, and several transformed cell lines [21]. Moreover, in transgenic mice expressing GM-CSF, fibrotic nodules developed in areas where macrophages accumulate [22]. These lesions have been interpreted as following chronic macrophage activation induced by GM-CSF. However, another study did not detect any side effects in response to long-term GM-CSF treatment in mice [23]. Clearly, these experiments need further confirmation, but they indicate that progress in the understanding of cytokine effects on fibroblastic cells may furnish explanation for the mechanisms leading to the development of a contractile phenotype in fibroblasts. It is well known that heparin and heparan sulfates inhibit smooth muscle cell replication and increase the expression of α-smooth muscle actin in these cells [24]. We have also observed that heparin and heparan sulfates exert similar action on fibroblastic cells and hence they could also participate in the regulation of fibroblastic phenotype during wound healing and different retractile diseases (unpublished observation).

In conclusion, the early observation that fibroblasts modify their phenotype during wound healing and fibrocontractive diseases has been implemented by several biochemical and functional findings which support the idea that myofibroblasts are a key cell for the understanding of retractile phenomena. Further studies on the factors regulating the phenotype of myofibroblasts will be useful for understanding their behavior in vivo, and possibly modifying this behavior during the different clinical settings.

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