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Tissue Repair, Contraction and the Myofibroblast

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PREFACE

Thirty-four years after the first description of the myofibroblast, the number of publications concerning this cell is very impressive and continuously expanding, and the work on the myofibroblast involves many laboratories throughout the world. The myofibroblast has been implicated in developmental and physiological phenomena, as well as in a variety of pathological situations, going from wound healing and fibrotic changes to asthma and cancer invasion. Many aspects of myofibroblast biology have been clarified, such as the role of TGF-β and ED-A cellular fibronectin in its differentiation and the role of α-smooth muscle actin in tension production by this cell; however several important problems concerning myofibroblast origin, function and participation in pathological processes remain to be solved.

The purpose of this book, as well of the Meeting "Tissue Repair, Contraction and the Myofibroblast" that took place in Nyon, near Geneva, Switzerland on November 18-20, 2004, is to put together the most recent advances in the understanding of myofibroblast biology and to present the main directions of research taking place worldwide to explore new aspects of myofibroblast physiological and pathological activities, such as: mechanisms of force generation by the myofibroblast; myofibroblast origin and diversity; interaction of the myofibroblast with other cells, normal and malignant epithelial cells in particular; and participation of the myofibroblast in the development of fibrosis in various organs. If we consider the animated and constructive discussions that took place during the Nyon Meeting, we are sure that this book will inspire new research in these fields.

This book would not have existed without the help of the European Tissue Repair Society and the Swiss National Science Foundation as well as the several Sponsors who are listed in the acknowledgments.

We hope that it will be the first of a long and fruitful series.

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INTRODUCTION

The Evolution of the Concept of Myofibroblast:
Implications for Normal and Pathological Tissue Remodeling

Alexis Desmoulière, Christine Chaponnier and Giulio Gabbiani*

Abstract

The recognition of the role of the myofibroblast in granulation tissue contraction and connective tissue remodeling during fibrocontractive diseases has allowed a theoretical and practical progress in the understanding of these pathologies. The observation that TGF-β is the key cytokine in myofibroblast differentiation, correlated with its role in collagen synthesis promotion, shows a coordinated mechanism in connective tissue remodeling. Recent work has furnished new knowledge concerning the molecular mechanisms of tension production by the myofibroblast and indicated that the N-terminal peptide of α-smooth muscle actin exerts an inhibitory action on myofibroblast contraction. Moreover the multiple derivation, both local and from circulating cells, of the myofibroblast begins to be understood. These data point to the myofibroblast as a major regulator of connective tissue remodeling and in turn of epithelial organization.

Introduction

After the first description of the myofibroblast in granulation tissue of an open wound by means of electron microscopy, as an intermediate cell between the fibroblast and the smooth muscle cell (SMC),[1] the myofibroblast has been identified both in normal tissues, particularly in locations where there is a necessity of mechanical force development (for a review, see ref. 2), and in pathological tissues, in relation with hypertrophic scarring, fibromatoses and fibrocontractive diseases (for a review, see ref. 3) as well as in the stroma reaction to epithelial tumors (for a review, see ref. 4). More recently myofibroblasts have been described in the deep dermis of patients with systemic sclerosis (for a review, see ref. 5) and in the bronchial submucosa of asthmatic patients (for a review, see ref. 6).

In an attempt to verify whether the myofibroblast expresses markers of the SMC phenotype, our laboratory has shown that α-SM actin, the actin isoform typical of vascular SMCs, is synthesized during fibroblast/myofibroblast modulation.[7] Indeed the presence of this protein represents at present the best marker of the myofibroblastic phenotype (for a review, see ref. 8).

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When present in the myofibroblast, α-SM actin localizes in stress fibers, an organelle that has been at first considered characteristic of cultured cells, but is also present in vivo, particularly during myofibroblast differentiation. Myofibroblasts can also express other proteins characteristic of SMCs, such as SM-myosin heavy chains, according to the pathological situation;[9] however they have not been shown up to now to express late markers of SMC differentiation, such as smoothelin.[10] This allows distinguishing between the two phenotypes.

Fibroblast/Myofibroblast Transition

The mechanisms of fibroblast/myofibroblast transition have been the object of intensive investigation. Transforming growth factor (TGF)-β is now accepted as the most important factor in this transition since it stimulates both the synthesis of collagen type I[11] and of α-SM actin by fibroblastic cells.[12,13] Connective tissue growth factor has also been proposed to play a role in myofibroblast differentiation.[14] It is now accepted that during the healing of an open wound, fibroblast/myofibroblast transition begins with the appearance of the protomyofibroblast, which contains stress fibers expressing only cytoplasmic β- and γ-actin isoforms.[10] This first transition is not yet well explored, but it probably depends on mechanical tension development. Then follows the appearance of the differentiated myofibroblast under the influence of mechanical tension as well as of chemical mediators, such as TGF-β (Fig. 1). It should be noted that the action of TGF-β in stimulating both collagen type I and α-SM actin synthesis strictly depends on the presence of cellular fibronectin and in particular of the ED-A splice variant of this glycoprotein.[15] Thus myofibroblast differentiation is a complex process, regulated by at least a cytokine, an extracellular matrix component as well as the presence of mechanical tension (Fig. 1).

During the healing of an open wound, when epithelial reconstruction is achieved, an important wave of apoptosis is observed in the underlying granulation tissue affecting small vessel cells (endothelial cells, pericytes) and myofibroblasts, thus leading to the formation of scar tissue.[16] The lack of apoptosis has been suggested as one of the mechanisms involved in the development of hypertrophic scars and possibly of other fibrotic changes. However this possibility has not yet been thoroughly explored.

The local derivation of fibroblastic cells from preexisting fibroblasts during wound healing has remained a dogma since the early work of Ross et al.[17] Subsequent work by several laboratories has shown that indeed local fibroblasts are a major source of myofibroblasts; however myofibroblasts can derive also from local mesenchymal cells such as SMCs, pericytes, hepatic stellate cells or mesangial cells.[18] The derivation of myofibroblasts from SMCs is particularly interesting in view of the recently described different mechanisms of contraction of these two cells (see below). In the last years the possibility that myofibroblasts derive from local epithelial cells or from blood bone marrow derived cells, which was suggested very early in the literature (for review see refs. 17,18), has been again convincingly proposed. Thus, it appears that tubular epithelial cells of the kidney are at least in part the source of myofibroblasts during interstitial fibrosis[19] and that mesothelial cells can originate myofibroblasts during peritoneal fibrosis.[20] Moreover it is more and more accepted that a variable proportion of myofibroblasts present in different pathological situations, e.g., liver[21] and pulmonary[22] fibrosis, are bone marrow derived. In this respect it is noteworthy that the description of circulating cells, called fibrocytes, which localize in areas of repair[23-25] and are probably an important source of myofibroblasts.[26] Clearly the identification and characterization of such cells may have important implications for the understanding of reparative and fibrotic changes of many organs and for the planning of therapeutic strategies.

Role of α-SM Actin in Tension Generation

As discussed above, α-SM actin is the most used marker of the myofibroblastic phenotype (for a review, see ref. 27). However the question as to whether this protein is instrumental in force production by the myofibroblast has been debated for a long time. Recently our

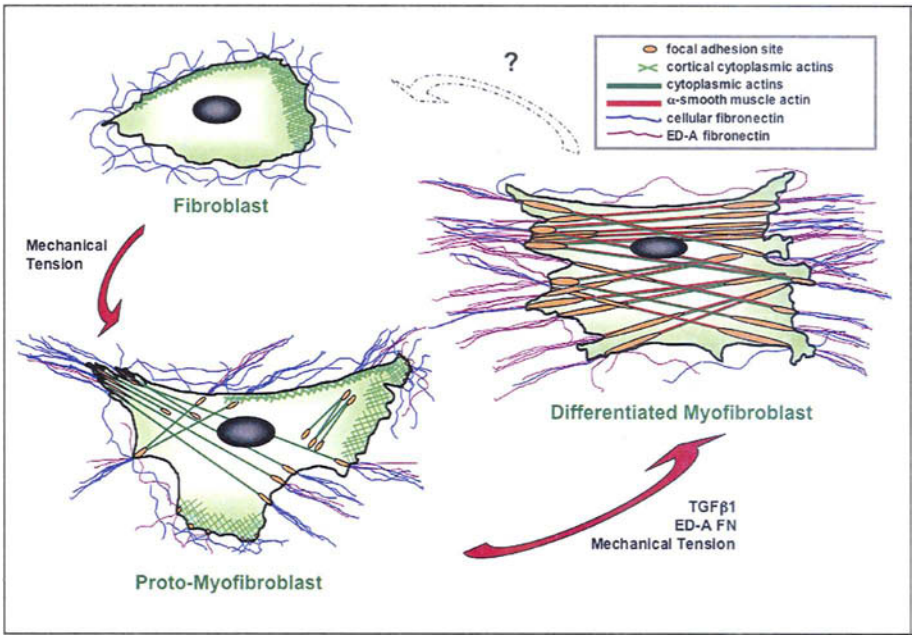

Figure 1. Two-stage model of myofibroblast differentiation. In vivo, fibroblasts might contain actin in their cortex but they neither show stress fibres nor do they form adhesion complexes with the extracellular matrix. Under mechanical stress, fibroblasts will differentiate into proto-myofibroblasts, which form cytoplasmic actin-containing stress fibers that terminate in fibronexus adhesion complexes. Proto-myofibroblasts also express and organize cellular fibronectin—including the ED-A splice variant—at the cell surface. Functionally, these cells can generate contractile force. TGF-β1 increases the expression of ED-A fibronectin. Both factors, in the presence of mechanical stress, promote the modulation of proto-myofibroblasts into differentiated myofibroblasts that are characterized by the de novo expression of α-smooth muscle actin in more extensively developed stress fibres and by large fibronexus adhesion complexes (in vivo) or supermature focal adhesions (in vitro). Functionally, differentiated myofibroblasts generate greater contractile force than proto-myofibroblasts, which is reflected by a higher organization of extracellular fibronectin into fibrils. (From ref. 10, ©2002 Nature Publishing Group, with permission.)

laboratory has shown that there is a good correlation between α-SM actin expression and the capacity of producing deformations in the silicone substrate on which fibroblastic cells are cultured; moreover transfection of swiss 3T3 fibroblasts with α-SM actin cDNA results in an increased contractility, which is significantly higher compared to that of fibroblasts transfected with the cDNA of α-cardiac actin or γ-cytoplasmic actin.[28] The increase in contractility takes place in the absence of any other change in protein expression, in particular of myosin heavy chain expression. These results strongly suggest that α-SM actin plays a direct role in tension production by myofibroblasts.

Two other observations have helped in pinpointing the mechanism of α-SM actin participation in tension production by myofibroblasts: (1) the decrease of the critical concentration for α-SM actin polymerization by the Fab fragment of the specific antibody for this protein, suggesting that binding of α-SM actin epitope facilitates incorporation of the protein into filaments of stress fibers;[29] and (2) the identification of the epitopic sequence for this antibody, i.e., the N-terminal sequence AcEEED.[29] The identification of a putative compound that in the cell would bind α-SM actin in a way similar to that of the antibody and thus increase its incorporation into stress fibers has not yet been possible, but we have shown that

microinjection in cultured myofibroblasts of the epitopic sequence decreases significantly and selectively α-SM actin incorporation into stress fibers. The sequence Ac-EEED is very acidic and does not penetrate spontaneously in cells. In order to perform more systematic studies, we have coupled it with an Antennapedia sequence that facilitates cell penetration.[30] We have seen that such fusion peptide inhibits myofibroblast contractility both in vitro and in vivo, using an experimental model of splinted wound in the rat that facilitates the study of wound contraction by eliminating the role of epithelialisation.[31] These observations open the possibility to influence wound contraction and/or myofibroblast dependent connective tissue remodeling in vivo and thus may be the basis for a new therapeutic strategy concerning several connective tissue diseases in which the myofibroblast appears a key player.

The fusion peptide should also represent a useful tool for the understanding of the molecular mechanisms regulating myofibroblast driven connective tissue remodeling. It is now accepted that wound contraction, as well as probably contracture formation, depends on the continuous long lasting production of isometric tension by single myofibroblasts (for a review, see ref. 32). The resulting connective tissue retraction can be stabilized by extracellular matrix deposition. This complex dynamic process could explain connective tissue remodeling in normal wound healing and in pathological situations such as liver cirrhosis or pulmonary fibrosis. Recent work aimed to understand the mechanism of force generation by stress fibers has indicated that tension production by the myofibroblast is regulated differently with respect to the classical Ca^{++} depending reversible SM contraction and is rather under the control of a Rho/Rho kinase and myosin phosphatase related pathway.[33,34] These findings establish for the first time a clear difference between myofibroblast and SMC in terms of contraction mechanisms and suggest that the myofibroblast utilizes a more primitive mechanism of force production that bears some analogies with the extracellular matrix remodeling taking place during embryonic development.[35] In this respect it is noteworthy that myofiboblasts have been described in embryonic tissues of various species, including man,[36] but their possible participation to developmental phenomena has not been explored. If these assumptions will be verified, one can propose for the myofibroblast a physiological role during development and in normal tissues where the production of mechanical tension is required, and a role in the evolution of normal and pathological wound healing as well as of fibrocontractive diseases. It has been suggested that during development connective tissue remodeling plays an important role in epithelial morphogenesis, implying a cross talk between epithelial and mesenchymal cells.[35] A pathological counterpart of this phenomenon could be the cross talk that starts to be understood between epithelial cancer cells and myofibroblasts of the stroma reaction.[37]

Conclusions and Perspective

The concept of myofibroblast has generated a significant amount of research during the last thirty years. It appears that rather than being a typical contractile cell, the myofibroblast plays a remodeling function that is necessary during development and repair phenomena. Many aspects of myofibroblast biology are not yet clear. We indicate arbitrarily here some of them that stimulate particularly our curiosity:

1. Very little has been done in the field of myofibroblast and of fibroblast heterogeneity, although early observations have shown that the agonists stimulating myofibroblast contraction are different for myofibroblasts derived from different organs.[38] Recent work has described markers distinguishing among different fibroblastic phenotypes.[39] Work along these lines would bring an important contribution to the understanding of fibroblast biology and function.
2. Myofibroblast apoptosis is a well-established phenomenon,[16] but its mechanisms are at present mysterious. Their understanding will help explaining the onset of pathological scarring and of fibrocontractive diseases.
3. A clear knowledge of the cellular origin of myofibroblasts in different pathological phenomena will be instrumental for the planification of therapeutic strategies.