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Review

Evolution and clinical implications of the myofibroblast concept

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1. Development of the myofibroblast concept

The phenomena of wound contraction and scar retraction are known since old ages (for review see [1]). In the first part of our century, the work of Carrel and Lecomte du Noüy has contributed to the notion that the forces producing wound contraction are generated within the granulation tissue itself [2]. These forces were generally considered to depend on extracellular matrix rearrangements; however, Abercrombie and co-workers reported, in the fifties, that fibroblasts exert tractional forces in vitro [3]. Similarly, Hoffmann-Beerling showed that addition of ATP to permeabilized fibroblasts in culture produces a contraction of their cytoplasm [4]. In this context, and in the context of emerging work on cytoskeleton morphology and function [5], the ultrastructural observation made in our laboratory in 1971 that during granulation tissue evolution fibroblasts acquire smooth muscle (SM) cell features, such as the presence of cytoplasmic microfilament bundles [6], lead to the proposition that these cells are the source of the force producing wound contraction, and probably connective tissue retraction during fibrotic phenomena. Shortly thereafter, it was shown that strips of granulation tissue isolated and placed in a pharmacological bath would contract and relax under the influence of substances which are known to be capable of contracting and relaxing SM cells [7,8]. It is noteworthy (particularly because this observation has never been developed) that granulation tissues from different locations respond differently to the same agonist or antagonist stimulus, suggesting that the capacity to react with contraction to a given stimulus by fibroblastic cells depends on their location [8]. The name myofibroblast was suggested for this modified and possibly contractile fibroblast [7].

During the next few years, several laboratories reported by means of ultrastructural techniques the presence of myofibroblasts in several lesions characterized by fibrosis and retraction such as fibromatoses [9], liver cirrhosis [10], pulmonary [11] and renal [12] fibrosis. The presence of myofibroblasts was also shown in normal tissues in locations where a certain degree of tension was needed to function, such as the theca externa of the ovary [13], the alveolar septum [14] and the intestinal pericriptal cells [15]. Hence, the concept was developed that in both normal and pathological situations myofibroblasts develop in response to traction stimuli and can exert a force producing tissue resistance and, particularly in pathological cases, tissue deformation (for review see [16]).

Because of the emerging concept that non-muscle cells contain contractile proteins, which up to that time had been considered typical of muscle cells, and because of the description of other cytoskeletal structures, such as microtubules and intermediate filaments, a considerable effort was made by our as well as some other laboratories in the characterization of contractile and cytoskeletal elements present in myofibroblasts (for review see [17]). It was shown that myofibroblasts contain higher amounts of polymerized actin, as compared to normal fibroblasts in vivo [18]. It was also shown that normal tissues containing myofibroblasts, such as the lung alveolus, would produce contraction upon the stimulus of agents contracting SM cells [19]. A significant advance was the production of an antibody against α-SM actin [20], one of the six actin isoforms present in mammalian tissues and considered to be typical for vascular SM cells [21]. The use of this antibody showed that α-SM actin is expressed temporarily in granulation tissue of an experimental wound and disappears when the scar develops [22]. Thus, an actin isoform typical of SM cells is expressed during wound healing in granulation tissue fibroblasts; this expression coincides with the phenomenon of wound contraction. Studies by several laboratories demonstrated the presence of α-SM actin in myofibroblastic cells present in fibrotic tissues of different organs such as the liver [23,24], the lung [25], the kidney [26], the heart [27,28] and the breast [29–31]; moreover, α-SM actin was suggested as the most significant marker of myofibroblastic cells, at least in pathological settings [17]. The presence of myofibroblasts in myocardial granulation tissue following an infarct has been shown by means of ultrastructure [32] to persist for a long time. The finding was confirmed by immunohistochemistry [33]. It is noteworthy that in old myocardial scars,

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fibroblastic cells still express α-SM actin, probably because of the continuous tension present in these areas [27]. In myocardial, as well as in cutaneous granulation tissue myofibroblasts are spatially highly organized. Recently, it has been shown that the myocardial myofibroblasts express a homologue of drosophila tissue polarity gene, frizzled, when they migrate into granulation tissue; this may represent the signal for alignment [28]. Another phenomenon in which the modulation between fibroblast and myofibroblast could play an important role is the fibrosis development during heart failure. Angiotensin II has been suggested to be important for fibrosis formation in this situation [34].

Further work demonstrated that myofibroblasts, according to the pathological situation, may express other markers of SM cells, such as SM myosin heavy chains and desmin, the intermediate filament protein characteristic of muscle cells [16,17]. Other muscular proteins described in myofibroblasts were caldesmon, SM22 and tropomyosin [31]. α-SM actin is the SM cell marker generally present in myofibroblasts of normally healing granulation tissue; other markers appear in more permanent fibrotic situations such as liver cirrhosis and become notably expressed in the stroma reaction to epithelial tumors, e.g. breast cancer [30,31,35,36]. Thus, it is possible to conclude that during the evolution of fibrosis, fibroblasts acquire SM cell features; these appear temporarily in normal wound healing and more permanently in irreversible fibrotic situations. The presence of myofibroblasts was shown to coincide with collagen type III expression in Dupuytren's nodules [37]; this collagen isoform is known to be present in remodeling connective tissue. In addition to these early biochemical observations, more precise in situ hybridization studies showed that myofibroblasts, characterized by the expression of α-SM actin, were the cells responsible for collagen type I mRNA production in pulmonary fibrosis [38].

Studies aimed at the elucidation of the mechanisms regulating the transition between granulation tissue and scar formation demonstrated that apoptosis is the mechanism through which myofibroblasts disappear during this transition [39]. This observation clarified an important aspect of wound healing and suggested that hypertrophic scar formation may be the consequence of a lack of myofibroblast apoptosis at the appropriate time of healing.

The role of myofibroblasts in wound contraction was confirmed by work showing that during fetal wound healing, when regeneration phenomena rather than scarring predominate, there is no appearance of myofibroblasts [33].

2. Agents influencing myofibroblast evolution

Another important aspect of myofibroblast research has been the work explaining the factors which influence the development and the disappearance of these cells. Essentially, this work has concentrated on cytokines and growth factors. The observation that γ-interferon decreases the expression of α-SM actin in SM cells [40] suggested us to verify this possibility in granulation tissue and fibrotic tissues. Indeed, γ-interferon decreases α-SM actin expression in cultured fibroblasts and in vivo as well [41]. The most extensive studies on this subject have been done using the model of liver fibrosis [23,42]. Moreover, pilot clinical studies have suggested that γ-interferon application is efficient in reducing hypertrophic scars and fibromatosis lesions [43].

By testing several cytokines in an experimental model consisting of their topical application to the rat subcutaneous tissue through an osmotic mini pump, it was shown that TNF, IL-1 and PDGF do not stimulate myofibroblast formation, even when they elicit a granulation tissue response, whereas TGF-β1 and -β2, and more surprisingly GM-CSF, regularly stimulate the appearance of α-SM actin rich myofibroblasts [44,45]. TGF-β1 and -β2 appear to exert a very powerful α-SM actin synthesis, stimulating activity in cultured fibroblasts [46,47]. If one considers that TGF-β is known since a long time to exert a profibrotic activity and specifically to stimulate collagen type I synthesis [48], one can conclude that this cytokine is probably the most critical for the development of scarring and fibrosis. It is noteworthy that TGF-β can induce α-SM actin expression in cultured fibroblastic cells in the absence of serum, that is in the absence of cell replication which indicates a de novo synthesis of this protein [46]. The action of GM-CSF appears to be indirect in that, when GM-CSF is applied to fibroblastic cells in culture, no α-SM actin synthesis is stimulated whereas α-SM actin rich myofibroblasts are seen after local administration of GM-CSF in the subcutaneous tissue. The same is true when GM-CSF is applied either directly, or through a viral vector to the lung alveoli [49]. Here the administration of GM-CSF is followed by TGF-β1 expression and then by α-SM actin and collagen expression [49]. Some preliminary research indicates that IL-10 could also play a role in the stimulation of myofibroblast development in the kidney [50]. Taken together, these results suggest that local GM-CSF application could, either directly or indirectly through the accumulation of stimulated macrophages, elicit an important expression of TGF-β1 which in turn would induce α-SM actin and collagen expression, thus resulting in fibrotic changes or scarring.

A more recent observation in our laboratory has shown that the action of TGF-β1 is mediated through the ED-A portion of cellular fibronectin [51]. This conclusion is based on the observation that antibodies specific for the ED-A sequence inhibit the action of TGF-β in inducing both α-SM actin and collagen mRNA and protein expression. Thus, myofibroblast development probably depends on both cytokine stimulation and extracellular matrix organization. It is noteworthy that TGF-β1 elicits the synthesis of ED-A fibronectin in vitro [52].

3. Mechanisms of myofibroblast activity

Observations by several laboratories have indicated that fibroblastic cells in vitro exert mainly isometric traction forces rather than an isotonic contractile activity [53]. It is probable that a similar mechanism takes place in vivo during granulation tissue contraction. It has been postulated that cytoskeletal elements interconnecting different cells are organized as a tensegrity structure in which cytoskeletal components such as microtubules or intermediate filaments exert a resistance to the tension produced by contractile elements such as actin and myosin [54]. In this context, an interesting aspect of myofibroblast research has concerned the role of α-SM actin in the generation of myofibroblast contractile forces. In addition to being a marker of myofibroblast differentiation, α-SM actin could be functionally important for myofibroblast contraction if one takes into account the fact that α-SM actin is expressed throughout the philogenetic scale in cells whose main function is contraction, such as vascular SM cells or myoepithelial cells. Actin isoforms are very conserved and exhibit relatively small differences in amino acid sequence, which probably correspond to functional differences. We have recently characterized the sequence corresponding to the epitope of the monoclonal antibody against α-SM actin which is Ac-EEED and shown that this sequence is important for α-SM actin polymerization [55]. Indeed the incubation of α-SM actin but not of other actin isoforms with the α-SM actin specific antibody or with its Fab fragment accelerates α-SM actin polymerization. Conversely, the microinjection of the epitopic peptide into fibroblastic or SM cells inhibits selectively α-SM actin polymerization. This observation leads one to conceive the possibility of eliminating selectively α-SM actin from stress fibers of a given cell and this offers a tool for testing the role of this protein in fibroblastic cell shape maintenance and contractile activity. Recent work has shown that inhibition of α-SM actin expression in SM cells and/or fibroblasts increases their motility and likely decreases their contractile activity [56]. Hence, it is possible that α-SM actin plays an important role for the development of myofibroblastic force; if this is verified by in vivo experiments, one could envisage the possibility of influencing wound contraction by treating wounds or fibrotic tissues with the α-SM actin N-terminal peptide.

In conclusion, it appears that during the last 25 years the concept that cell activity is the main source of force generation within granulation tissue has gained general acceptance. How force is produced remains to be determined, but it is increasingly accepted that the presence of α-SM actin is instrumental for this action. Many of the factors influencing synthesis and disappearance of α-SM actin are presently known and it is now possible to envisage the study of its role in myofibroblast activity. We are confident that during the next years more data will become available explaining the biological activity of the myofibroblast and possibly allowing the modulation of its activity in order to accelerate and reduce wound contraction and fibrosis formation.

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References

[1] Majno G. The healing hand: man and wound in the ancient world. Cambridge: Harvard University Press, 1975.
[2] Carrel A, Hartmann A. Cicatrization of wounds. I. The relation between the size and the rate if its cicatrization. J Exp Med 1916;24:429–450.
[3] Abercrombie M, Flint MH, James DW. Wound contraction in relation to collagen formation is scorbutic guinea pigs. J Embryol Exp Morphol 1956;4:167–175.
[4] Hoffmann-Beerling H. Adenosintriphosphat als Betriebsstoff von Zellbewegungen. Biochim Biophys Acta 1954;14:182–194.
[5] Bray D. Cytoplasmic actin: a comparative study. In: Gordon J, editor. Cold Spring Harbor symposia on quantitative biology: the mechanisms of muscle contraction. Cold Spring Harbor: The Cold Spring Harbor Laboratory, 1973:567–571.
[6] Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 1971;27:549–550.
[7] Majno G, Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR. Contraction of granulation tissue in vitro: similarity to smooth muscle. Science 1971;173:548–550.
[8] Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G. Granulation tissue as a contractile organ. A study of structure and function. Exp Med 1972;135:719–734.
[9] Gabbiani G, Majno G. Dupuytren's contracture: fibroblast contraction. Am J Pathol 1972;66:131–146.
[10] Ramadori G, Veit T, Schwögler S, et al. Expression of the gene of α-smooth muscle-actin isoform in rat liver and in rat fat-storing (Ito) cells. Virchows Arch B Cell Pathol 1990;59:349–357.
[11] Adler KB, Low RB, Leslie KO, Mitchell J, Evans JN. Biology of disease. Contractile cells in normal and fibrotic lung. Lab Invest 1989;60:473–485.
[12] Johnson RJ, Iida H, Alpers CE, et al. Expression of smooth muscle cell phenotype by rat mesangial cell sin immune complex nephritis. J Clin Invest 1991;87:847–858.
[13] Czernobilsky B, Shezen E, Lifschitz-Mercer B, et al. Alpha smooth muscle actin (α-SM actin) in normal human ovaries, in ovarian stromal hyperplasia and in ovarian neoplasma. Virchows Arch B Cell Pathol 1989;57:55–61.
[14] Kapanci Y, Assimacopoulos A, Irlé C, Zwahlen A, Gabbiani G. Contractile interstitial cells in pulmonary septa. J Cell Biol 1974;60:375–392.
[15] Sappino AP, Dietrich PY, Widgren S, Gabbiani G. Colonic pericryptal fibroblasts. Differentiation pattern in embryogenesis and phenotypic modulation in epithelial proliferative lesions. Virchows Arch A Pathol Anat 1989;415:551–557.
[16] Desmoulière A, Gabbiani G. Modulation of fibroblastic cytoskeletal features during pathological situations: the role of extracellular matrix and cytokines. Cell Motil Cytoskeleton 1994;29:195–203.
[17] Sappino AP, Schürch W, Gabbiani G. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulation. Lab Invest 1990;63:144–161.

[18] Low RB, Chaponnier C, Gabbiani G. Organization of actin in epithelial cells during regenerative and neoplastic conditions. Correlation of morphologic, immunofluorescent, and biochemical findings. Lab Invest 1981;44:359–367.
[19] Evans JN, Adler KB. The lung strip: evaluation of a method to study contractility of pulmonary parenchyma. Exp Lung Res 1981;2:187–195.
[20] Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 1986;103:2787–2796.
[21] Gabbiani G, Schmid E, Winter S, et al. Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific α-type actin. Proc Natl Acad Sci USA 1981;78:298–302.
[22] Darby I, Skalli O, Gabbiani G. α-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 1990;63:21–29.
[23] Schmitt-Gräff A, Krüger S, Bochard F, Gabbiani G, Denk H. Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 1991;138:1233–1242.
[24] Friedman SL. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. New Engl J Med 1993;328:1828–1835.
[25] Kapanci Y, Ribaux C, Chaponnier C, Gabbiani G. Cytoskeletal features of alveolar myofibroblasts and pericytes in normal human and rat lung. J Histochem Cytochem 1992;40:1955–1963.
[26] Diamond JR, van Goor H, Ding G, Engelmyer E. Myofibroblasts in experimental hydronephrosis. Am J Pathol 1995;146:121–129.
[27] Sun Y, Weber KT. Angiotensin converting enzyme and myofibroblasts during tissue repair in the rat heart. J Mol Cell Cardiol 1996;28:851–858.
[28] Blankesteijn WM, Essers-Janssen YPG, Verluyten MJA, Daemen MJA, Smits JFM. A homologue of Drosophila tissue polarity gene frizzled is expressed in migrating myofibroblasts in the infarcted rat heart. Nat Med 1997;3:541–544.
[29] Schürch W, Seemayer TA, Lagacé R. Stromal myofibroblasts in primary invasive and metastatic carcinoma. A combined immunological, light and electron microscopic study. Virchows Arch A 1981;391:125–139.
[30] Ronnov-Jessen L, Petersen OW, Koteliansky VE, Bissell MJ. The origin of the myofibroblasts in breast cancer. J Clin Invest 1995;95:859–873.
[31] Lazard D, Sastre X, Frid MG, Glukhova MA, Thiery JP, Koteliansky VE. Expression of smooth muscle-specific proteins in myoepithelium and stromal myofibroblasts of normal and malignant human breast. Proc Natl Acad Sci USA 1993;90:999–1003.
[32] Vracko R, Thorning D. Contractile cells in rat myocardial scar tissue. Lab Invest 1991;65:214–227.
[33] Estes JM, Vande Berg JS, Adzick NS, MacGillivray TE, Desmoulière A, Gabbiani G. Phenotypic and functional features of myofibroblasts in sheep fetal wounds. Differentiation 1994;56:173–181.
[34] Weber KT. Extracellular matrix remodeling in heart failure. A role for de novo angiotensin II generation. Circulation 1997;96:4065–4082.
[35] Chiavegato A, Bochaton-Piallat ML, D'Amore E, Sartore S, Gabbiani G. Expression of myosin heavy chain isoforms in mammary epithelial cells in myofibroblasts from different fibrotic settings during neoplasia. Virchows Arch 1995;426:77–86.
[36] Schmitt-Gräff A, Desmoulière A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity. Virchows Arch 1994;425:3–24.
[37] Gabbiani G, Le Lous M, Bailey AJ, Bazin S, Delaunay A. Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural an immunologic study. Virchows Arch B Cell Pathol 1976;21:133–145.
[38] Zhang K, Rekhter MD, Gordon D, Phan SH. Myofibroblasts and their role in lung collagen gene expression during pulmonary during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. Am J Pathol 1994;145:114–125.
[39] Desmoulière A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. Am J Pathol 1995;146:56–66.
[40] Hansson GK, Hellstrand M, Rymo L, Rubbia L, Gabbiani G. Interferon γ inhibits both proliferation and expression of differentiation-specific α-smooth muscle actin in arterial smooth muscle cells. J Exp Med 1989;170:1595–1608.
[41] Desmoulière A, Rubbia-Brandt L, Abdiu A, Walz T, Macieira-Coelho A, Gabbiani G. α-smooth muscle actin is expressed in a subpopulation of cultured and cloned fibroblasts and is modulated by γ-interferon. Exp Cell Res 1992;201:64–73.
[42] Shi Z, Wakil AE, Rockey DC. Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. Proc Natl Acad Sci USA 1997;94:10663–10668.
[43] Pittet B, Rubbia-Brandt L, Desmoulière A, et al. Effect of γ-interferon on the clinical and biological evolution of hypertrophic scars and Dupuytren's disease: an open pilot study. Plast Reconstr Surg 1994;93:1224–1235.
[44] Rubbia-Brandt L, Sappino AP, Gabbiani G. Locally applied GM-CSF induces the accumulation of α-smooth muscle actin containing myofibroblasts. Virchows Arch B Cell Pathol 1991;60:73–82.
[45] Serini G, Gabbiani G. Modulation of α-smooth muscle actin expression in fibroblasts by transforming growth factor-β isoforms: an in vivo and in vitro study. Wound Rep Reg 1996;4:277–287.
[46] Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-β1 induces α-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103–111.
[47] Ronnov-Jessen L, Petersen OW. Induction of α-smooth muscle actin by transforming growth factor-β1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. Lab Invest 1993;68:696–707.
[48] Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. New Engl J Med 1994;331:1286–1292.
[49] Xing Z, Tremblay GM, Sime PJ, Gauldie J. Overexpression of granulocyte-macrophage colony-stimulating factor induces pulmonary granulation tissue formation and fibrosis by inductions of transforming growth factor-β1 and myofibroblast accumulation. Am J Pathol 1997;150:59–66.
[50] Chadban SJ, Tesch GH, Foti R, Atkins RC, Nikolic-Paterson DJ. Interkeukin-10 is a mesangial cell growth factor in vitro and in vivo. Lab Invest 1997;76:619–627.
[51] Serini G, Bochaton-Piallat ML, Zardi L, Gabbiani G. ED-A fibronectin mediates the TGF-β 1 induction of the myofibroblastic phenotype. Abstract.
[52] Balza E, Borsi L, Allemmanni G, Zardi L. Transforming growth factor β regulates the levels of different fibronectin isoforms in normal cultured fibroblasts. FEBS Lett 1988;228:42–44.
[53] Harris AK, Wild P, Stopak D. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science 1980;208:177–179.
[54] Ingber DE, Dike L, Hansen L, et al. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. Int Rev Cytol 1994;150:173–224.
[55] Chaponnier C, Goethals M, Janmey PA, Gabbiani F, Gabbiani G. The specific NH_2-terminal sequence Ac-EEED of α-smooth muscle actin plays a role in polymerization in vitro and in vivo. J Cell Biol 1995;130:887–895.
[56] Ronnov-Jessen L, Petersen OW. A function for filamentous α-smooth muscle actin: retardation of motility in fibroblasts. J Cell Biol 1996;134:67–80.