The myofibroblast in health and disease

El miofibroblasto en tejidos normales y en situaciones patológicas

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SUMMARY

The myofibroblast is essential for the integrity of the mammalian body by virtue of its role in wound-healing, but it can also threaten it by its ability to promote tumour development. It is an almost universal cellular component in mammalian lesions, but not a typical component of normal untraumatised tissues. Partly because of its absence from normal tissue, it has not been part of conventional histology teaching. This has contributed to difficulties in appreciating the nature of the myofibroblast and defining it. This paper documents the features of the myofibroblast which provide a definition for the myofibroblast needed by scientists interested in the mechanism of disease and pathologists wanting to diagnose myofibroblastic lesions. Light microscopy features emphasised for defining the myofibroblast include: spindled cell morphology, an abundant matrix, immunostaining for α-smooth-muscle actin (in the absence of desmin and h-caldesmon) and the ED-A splice variant of cellular fibronectin. By electron microscopy, rough endoplasmic reticulum, peripherally located smooth-muscle type myofilaments, a Golgi apparatus producing collagen-secretion granules and fibronexus junctions are important. The fibronexus is emphasised as a distinctive organelle for identifying the myofibroblast and lamina is emphasised as absent. The mechanism by which myofibroblasts arise in granulation tissue and promote tumour development, and the how the above definition can be used in diagnosing myofibroblastic lesions, is discussed.

Keywords: Myofibroblast, ultrastructure, fibronexus, myofibroblastic sarcoma.

RESUMEN

El miofibroblasto es fundamental para la integridad del organismo de los mamíferos dado su papel en la curación de las heridas, pero puede resultar deletéreo por su capacidad para producir tumores. Se trata de un componente celular prácticamente universal en lesiones de mamíferos, pero no es un componente típico del tejido normal no traumatizado. Debido, en parte, a su ausencia en tejidos normales, no suele formar parte de la enseñanza convencional de la histología, lo que ha dificultado su estudio y definición. El presente trabajo documenta las características del miofibroblasto con el fin de proporcionar una definición para científicos interesados en los mecanismos de enfermedad y para histopatólogos involucrados en el diagnóstico de lesiones miofibroblásticas. Las características histológicas que permiten la identificación del miofibroblasto son: morfología fusocelular, abundante matriz y positividad inmunohistoquímica para α-actina específica de músculo liso (en ausencia de desmina y h-caldesmón) y EDA-fibronectina. En microscopía electrónica los hallazgos más importantes son: evidencia de retículo endoplásmico rugoso bien desarrollado, miofilamentos subplasmalemales de tipo muscular liso, aparato de Golgi con gránulos de secreción de colágeno y uniones tipo fibronexo que se considera como una organela característica; no debe encontrarse lámina externa. En el presente trabajo se comentan los mecanismo por los que el miofibroblasto aparece en el tejido de granulación y da lugar a tumores y como la anterior definición puede aplicarse al diagnóstico de las lesiones miofibroblásticas.

Palabras clave: Miofibroblasto, ultraestructura, fibronexo, sarcoma miofibroblástico.

Rev Esp Patol 2008; 41 (1): 3-10

INTRODUCTION

The myofibroblast is an unusual and an interesting cell for a number of reasons. It is a cellular version of the *Jeckyll and Hyde* character in Robert Louis Stevenson's novel, in that it can have a benign or a malign influence depending on circumstances. By virtue of its role in wound-healing, it can promote health, but it can also endanger it by promoting the development of tumours. Understanding the biological complexity of this cell has

Recibido el 7/2/07. Aceptado el 12/7/07.
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been hindered by the fact that it has not been easy to define. First: it is not, essentially, a «normal» cell: *i.e.*, in contrast to such well known cells as smooth-muscle cells, endothelium and pericytes, it is not found in normal untraumatised tissues. Consequently, the myofibroblast has not traditionally been featured in histology textbooks and courses for medical and science students. Second: the myofibroblast is of interest because it harbours within itself two phenotypes normally found in other cells – the fibroblast and smooth-muscle cell.

This review offers a definition of the myofibroblast, which is essential for scientists investigating the mechanism of disease in which these cells participate, and for pathologists who need to diagnose myofibroblastic lesions: it also discusses a number of aspects of myofibroblast biology which are still the subject of controversy and debate.

DEFINITION OF THE MYOFIBROBLAST: CRITERIA FOR IDENTIFICATION

It is clear from the literature that there are differing definitions of the myofibroblast, a situation partly reflecting the different scientific backgrounds of investigators (for example, whether they work in and have trained in pathology, anatomy or cell-biology) and their selective interest in the published literature.

The myofibroblast was originally defined by purely ultrastructural criteria in 1971 (1). Since then, immunohistochemistry has added its own component to the definition, while the myofibroblast also, of course, has distinctive morphological features in histological sections which cannot be divorced from the definition. So, the current definition is a complex one, reflecting the input from different techniques:

- Spindle-cell or stellate-cell morphology
- A pericellular matrix containing *inter alia* collagen and glycosaminoglycans
- Palely eosinophilic and prominent cytoplasm
- Immunophenotype:
  - Vimentin positive
  - α-smooth-muscle actin positive (?-SMA)
  - non-muscle myosin positive
  - minimal levels of desmin and smooth-muscle myosin
  - EDA cellular fibronectin positive
- Ultrastructure
  - prominent rough endoplasmic reticulum (rER)
  - a Golgi apparatus producing collagen secretion granules
  - modestly developed and frequently peripherally located
  - myofilaments with focal densities
  - gap junctions
  - fibronexuses consisting of converging myofilaments and
  - external fibronectin fibril
  - absence of lamina.

This definition applies to the fully differentiated myofibroblast as found in granulation tissue or tumour stroma. But in these tissues, some cells, we presume, are evolving into a high level of myofibroblastic differentiation from more primitive precursors, and so may lack some of these features. This lesser degree of differentiation may also be seen in neoplastic myofibroblasts, where, in addition and for example, nucleoli can be expected to be enlarged, and immunophenotype expanded, reduced or aberrant (e.g., cytokeratin may be present).

DESMIN STAINING IN MYOFIBROBLASTS

In normal cells, desmin is the archetypal intermediate filament protein of muscle cells, including, of course, smooth-muscle cells: desmin is not significantly expressed in either granulation tissue or tumour stromal myofibroblasts (2,3). As such, it should not be regarded as a primary marker of the myofibroblast. However, since the study of Skalli et al (4) many authors have used desmin as a confirmatory marker for the myofibroblast. The study of Skalli et al investigated desmin in a wide range of tissues – including normal tissues such as dermis, normally healing granulation tissue, as well as lesional cells such as those in fibromatosis, and it is important to note that desmin was present only in lesional myofibroblasts.

In order to identify myofibroblastic differentiation in lesional cells, it is necessary to have a definition for what one might call the *normal cellular counterpart*: as mentioned above and further detailed below, myofibroblasts very largely do not exist in normal tissues, so it is necessary to identify the nearest tissue to normal, and this has been argued as the granulation tissue and tumour stromal myofibroblast. One therefore needs to be careful when assigning a cell differentiation on the basis of desmin staining in lesional cells. Strong staining will indeed suggest true smooth-muscle differentiation: lesser levels of staining will be ambiguous, and an electron microscopy input is suggested to make the distinction between smooth-muscle and myofibroblastic differentiation. In addition, if desmin staining is co-expressed with lamina, this will give stronger support to an interpretation of true smooth-muscle differentiation.

THE SPECIAL IMPORTANCE OF THE FIBRONEXUS AS A MYOFIBROBLAST MARKER

At least in the field of pathology, the *fibronexus* (or *fibronexus junction*) is less well known as a myofibrob-

last marker than rER and myofilaments. Partly, this is because it was first documented in the non-pathology literature (by Singer in 1979 in the journal, *Cell*) (5) and some time has been needed for this structure to become embedded in the pathology literature.

The fibronexus is a discrete area on the myofibroblast cell surface where the intracellular myofilaments and the extracellular fibronectin filaments (forming the fibronectin fibril) converge. The myofilaments attach to subplasmalemmal actin-binding proteins, which in turn attach to transmembrane integrins, which, on the cell exterior, attach to fibronectin. The myofilament bundle and fibronectin fibril are therefore in indirect contact and are seen, in appropriate sections, to be co-linear (5-12). The fibronexus is, therefore, a cell-to-matrix junction, ensuring a degree of adhesion to or contact with the extracellular matrix.

Much uncertainty in the field of myofibroblast biology has been generated by confusing the fibronectin fibril with lamina («external lamina»), one of the few other structures associated with the external surface of cells. The fibronectin fibril and lamina are quite different structures, with different functions and different significances for cell differentiation.

The fibronectin fibril has the following features, which distinguish it from lamina (figs. 1-3):

- it is denser and straighter than lamina
- it has a longitudinal and finely filamentous substructure, which is lacking in lamina
- it projects (usually at a small angle) from the cell surface into the extracellular space, whereas lamina usually follows the contours of the cell with which it is associated
- it is co-linear with intracellular myofilaments
- it is often seen «attaching» to the cell surface at a localised cell surface inclination.

Immuno-electronmicroscopy has confirmed the fibronectin content of the fibronectin fibril (8,10,13). The

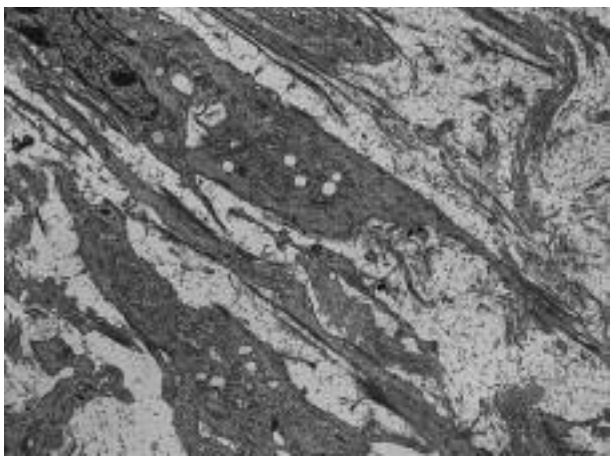

Fig. 1: A myofibroblast and myofibroblast processes in squamous cell carcinoma stroma. rER and peripheral myofilaments are present, and dense fibrillar fibronectin is evident at cell surfaces.

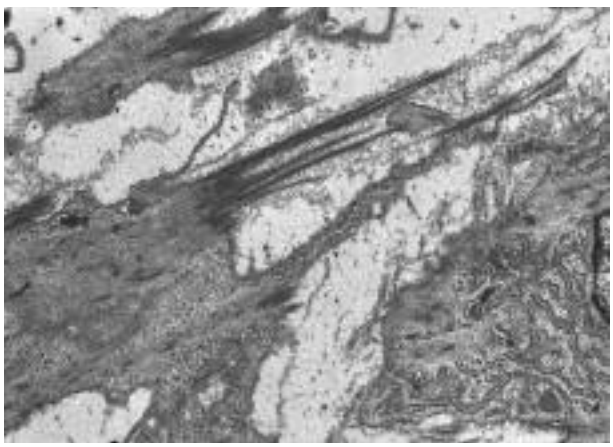

Fig. 2: Detail of myofilaments and fibronectin fibrils forming a fibronexus. The fibronectin projects out into the matrix.

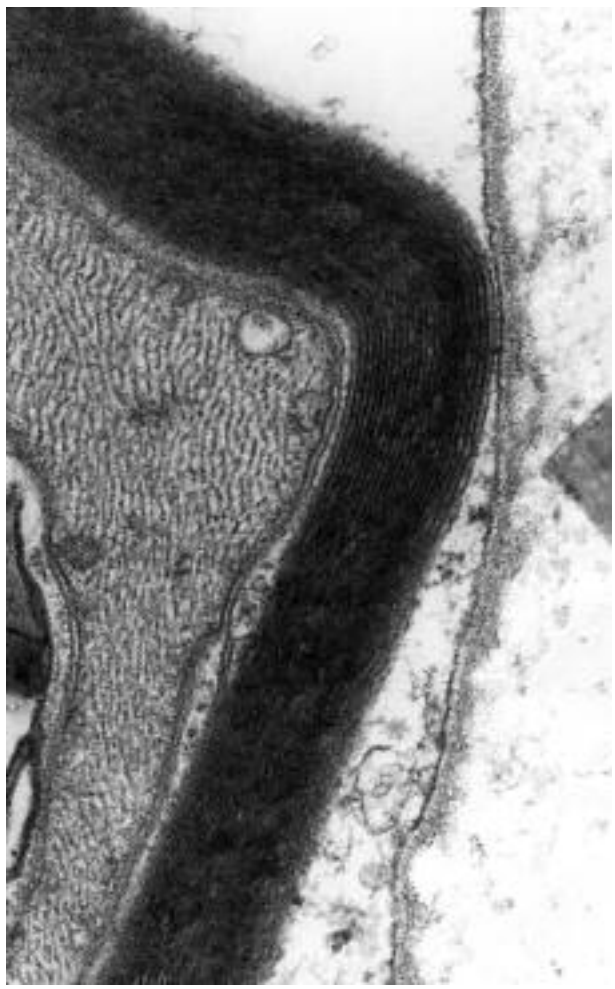

Fig. 3: Lamina over the surface of a Schwann cell: note how it follows the contour of the cell surface, in contrast to the fibronectin in figures 1 and 2.

structures immuno-ultrastructurally labelled by anti-fibronectin antibodies in the study by Tamm *et al* (14) are also probably related to fibronectin fibrils. It is likely that the fibronectin at the fibronexus contains the myofibroblast-specific ED-A isoform (15,16). While lamina also contains some fibronectin, it is rich in proteins such as laminin, type IV collagen and proteoglycans (17).

On the specificity of the fibronexus, the published literature points to it as a highly characteristic marker organelle of the myofibroblast (18-21). However, like nearly all markers, whether immunohistochemical or ultrastructural, it is not completely specific. Rare examples of endothelium exhibit fibronexuses (22,23), mostly in the aorta, possibly as an adaptation to haemodynamic stress. In attenuated form, they have been noted in certain vascular smooth-muscle cells (9,24), whereas in bovine arteriosclerosis, structures resembling fibronectin fibrils appear to be prominent (25). In addition, normal cells, which in the *in vivo* state are non-myofibroblastic, can assume myofibroblastic features including formation of the fibronexus when cultured *in vitro*: examples include fibroblasts, smooth muscle cells and epithelium (5,26). This represents transdifferentiation towards the myofibroblast phenotype of an initially non-myofibroblastic cell.

ABSENCE OF THE MYOFIBROBLAST FROM NORMAL TISSUES

The myofibroblast is archetypally found in granulation tissue, non-neoplastic fibrosing or fibro-contractive conditions, and tumour stroma (18,19,27-32). Like nearly all cells, in addition, it can be neoplastic (see below). Apart from the tumoral counterpart, the myofibroblast is essentially a *reactive* cell, and by that is meant a cell appearing in conditions generated by externally applied trauma or inherent abnormality, as in tumour stroma.

This essentially reactive nature of the non-neoplastic myofibroblast conflicts with a number of references in the literature describing the myofibroblast as being found in normal organs and tissues (27,28,33,34). It is probably true that all tissues harbour mesenchymal or fibroblastic cells which have the *potential* to become activated to a myofibroblast as a result of externally applied trauma, but to say that all tissues have myofibroblasts implies that all tissues are traumatised, which is clearly not the case. It is more reasonable, perhaps, to take the view that *some* stromal cells showing very minor degrees of myofibroblastic differentiation resulting from comparably minor and possibly transient states of trauma or stress. Consequently, most stromal cells in normal tissues do not show significant myofibroblastic differentiation, and those that do, show it only to a very minor degree. The periodontal myofibroblast and the interstitial cells of the mammalian testis are two exceptions.

BIOLOGICAL FUNCTIONS OF THE MYOFIBROBLAST

Wound-healing

This process, by which physical integrity of mammalian tissues is ensured after injury, consists of several steps. One of the earliest is the transdifferentiation of surrounding resident stromal cells, fibroblasts as often as not, into myofibroblasts. This process involves the switching on of non-muscle actin in cells having a spindled or flattened morphology, rER, and stress fibres lacking α-SMA: they have been referred to as *protomyofibroblasts* (35,36). The protomyofibroblast is, therefore, one of the earliest phases in the transition of a fibroblast to a myofibroblast.

Several studies have indicated that the entire process leading to the complete myofibroblast phenotype requires the concerted action of growth factors (such as transforming growth factor β, [TGFβ]), matrix molecules, and a mechanically stressed environment (15,16,35-38). Two factors appear to be important in the earliest stages of the development of the myofibroblast: platelet-derived growth factor (PDGF) released from the blood in a wound acting as a mitogen or chemo-attractant for resident fibroblasts (39); and PDGF interacting with these PDGF-receptor bearing cells and, with the involvement of other cytokines, precipitating them into a differentiation process which ultimately leads to the myofibroblast.

As already mentioned, another early signal for protomyofibroblast formation is mechanical tension. The effect of mechanical stress in the formation of stress fibres (bundles of actin filaments) attached to membranes was demonstrated by Brandes *et al* (40) (see also: 15,16,41). Endothelial cells in stressed tissues showed enhanced actin filament bundles compared with non-stressed specimens. It is arguable that the appearance of α-SMA in cardiac fibroblasts results from ventricular pressure overload (42) and is an example of stress-related myofibroblastic development. The stressing would appear to be a reactive mechanism by which cell and matrix cohesion is ensured, in much the same way, perhaps, that aortic endothelial cells elaborate fibronexus junctions with the extracellular matrix under conditions of high haemodynamic stress (22).

Before inducing α-SMA, however, TGFβ induces the synthesis of fibronectin, and collectively TGFβ and fibronectin direct α-SMA synthesis. TGFβ regulates the levels and isoform patterns of fibronectin (43), especially the ED-A and ED-B splice-variants (44), which then exert a «permissive» action for the expression of α-SMA (35,39,45). The ED-A variant of fibronectin, in particular, can be regarded as important a marker for the myofibroblast as α-SMA.

Myofibroblasts and tumour promotion

There is growing evidence that myofibroblasts promote tumour development, and act in concert with neoplastic cells (46-53). This conflicts with the early idea that abundant matrix synthesized by the myofibroblast formed a physical barrier inhibiting tumour cell movement and amounted to a protective measure for the host (54-56).

The tumour-promoting effect is probably based on the direct cytokine-stimulation of cancer cells, the maintenance of vascularity, but also partly on the capacity of myofibroblasts to produce enzymes which degrade either matrix (50) or molecules which enhance the structural integrity of matrix, such as lysyl oxidase (this promotes collagen and elastin crosslinking, and is decreased in invasive compared with *in situ* carcinomas – 57). Matrix-degrading enzymes include metalloproteinases (58,59): in principle, such activity would create easier physical access for neoplastic cells to the vasculature –an early step in the metastatic process– but also it seems likely that it would produce new molecules with enhanced activity with regard, for example, to the migratory activity needed to access vessels (59).

Another currently prevalent idea is that myofibroblasts create a physical barrier between carcinoma cells, on the one hand, and the macrophages and T cells, on the other, which are part of the system attempting to mount an immune defence on the part of the body again the cancer (50,52). The many images in the published literature of myofibroblasts in close association with carcinoma cells would be consistent with this idea (28,60).

Mechano-reception and the detection of stress

The fibronexus is regarded as a transmembrane cell-to-matrix adhesive or junctional device. While its functions are not unambiguously clear as yet, it may have a role in transferring the intracellular contractility through the cell surface to the matrix, in such processes as wound-contraction (35,61). At the same time, it has been recognised that fibroblasts, not having differentiated into myofibroblasts, can contract tissue matrices, presumably by tractional forces (62). More recently, the idea has been proposed that fibronexuses may detect tension in the extracellular matrix (15,16,35,63) and thereby act as *mechano-transducers*, converting the energy of external mechanical stress into biological activity (cell-signalling, *de novo* protein synthesis and new phenotypes). This is an exciting area of investigation where physics and biology interface.

ORIGIN AND FATE

Myofibroblasts have traditionally been argued as deriving mainly from locally resident fibroblasts (62,64,65) but also smooth-muscle cells, pericytes (3,52,66), macrophages (67,68), as well as other «more specialised» cells such as hepatic stellate cells (66) and epithelium (69). Ultimately, they may derive from bone marrow via circulating blood-borne fibrocytes (70-74).

Towards the conclusion of wound-healing, myofibroblasts, along with cells of the neovasculature, disappear by apoptosis (66,75). When the apoptotic mechanism fails, prolonged scarring results, leading to such conditions as hypertrophic scar and keloid.

MYOFIBROBLASTIC DIFFERENTIATION IN TUMOURS AND TUMOUR-LIKE LESIONS

Using the definition detailed above, it is clear that a spectrum of myofibroblastic differentiation exists in the tumours and tumour-like lesions that are often referred to as *fibroblastic/myofibroblastic*. The lesions containing the most highly differentiated myofibroblasts include nodular and proliferative fasciitis, Dupuytren's disease and the other (myo)fibromatoses, inflammatory myofibroblastic tumour, and some myofibroblastic sarcomas (76).

There are good grounds based on ultrastructure and desmin immunostaining for regarding some of the lesions widely regarded and referred to as myofibroblastic as showing, rather, a low level of true smooth-muscle differentiation. While many of the ultrastructurally examined fibromatoses exhibit fibronexus junctions and so are fully myofibroblastic, a few others have the lamina indicative of smooth-muscle cells (77) or are strongly desmin-positive (78). By contrast, nodular fasciitis is uniformly negative for desmin (79,80), further emphasising its «true» or «complete» myofibroblastic phenotype.

The features of attachment plaques, caveolae and lamina have been seen in a number of myofibroblastomas (81-85) and angiomyofibroblastoma (86), which, in the absence of fibronexus junctions, suggest true smooth-muscle differentiation.

In poorly differentiatiated spindle cell tumours, the distinction between myofibroblastic differentiation and, for example, leiomyosarcomatous differentiation becomes difficult, particularly, when a cell has rER and peripheral myofilaments but no identifiable fibronexuses. This raises the question: does one need the fibronexus for the identification of myofibroblastic differentiation in a spindle-cell sarcoma? Although in earlier papers the fibronexus was indeed emphasised as an essential part of the definition of the myofibroblast (9), it might now be more reasonable to think in terms of levels of differentiation and levels of diagnostic confidence. If, in a spindle-cell sarcoma, there are features of myofibroblastic differentiation (delicate α-smooth-muscle actin, rER, myofilaments, no lamina, no desmin, no h-caldesmon) but no fibronexuses, it may still be reasonable to call this a

myofibroblastic sarcoma (a poorly differentiated one) *in the absence of any other compelling diagnosis*. However, *maximum diagnostic confidence* of a myofibroblastic tumour would be achieved by identifying fibronexuses, which in turn requires electron microscopy.

ACKNOWLEDGEMENT

Several parts of this review derive from my two papers published in the Journal of submicroscopic Cytology and Pathology in 2005 (76,87). I am most grateful to the Managing Editor, Laura Neri, for kind permission to make use of the text and figures in these papers for this review.

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