The myofibroblast:
phenotypic characterization as a prerequisite to understanding its functions in translational medicine

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- Introduction
- The evolving definition of the myofibroblast: promoting an understanding of biology and pathology
  - From early ultrastructural criteria to a comprehensive definition
  - Immunohistochemistry brings a less precise definition
  - Consequences of an imprecise definition in normal tissue biology – so-called myofibroblasts in bladder and gut
- The myofibroblast in fibrotic conditions, tumour stroma and tissue engineering – a therapeutic target in translational medicine
  - *De novo* myofibroblastic differentiation and functions of myofibroblasts in granulation tissue, fibroproliferative conditions and tumour stroma
  - Bone-marrow-derived circulating fibrocytes, epithelial-mesenchymal transformation and the origin of myofibroblasts
- Conclusions

Abstract

The phrase 'translational research' conveys the idea of the pursuit of applications for the treatment of human disease. The myofibroblast, long known for having a role in wound-healing, and for its presence in fibrotic conditions and tumour stroma, is becoming a focus for translational research, not least through its increasingly documented role as a tumour-promoting cell. In fibroproliferative conditions, cancer and tissue engineering, the myofibroblast, derived partly and possibly from circulating bone-marrow-derived cells and epithelial-to-mesenchymal transformation, is attracting great attention. In cancer, this cell was initially regarded as a barrier to tumour dissemination, but there is now a growing body of evidence to indicate that it is an active participant in tumour progression. While the involvement of the myofibroblast in these pathological processes is pushing the myofibroblast into the limelight of translational medicine as a target for potential anti-fibrotic and anti-cancer therapy, there are still numerous indications from the literature that the myofibroblast is a poorly understood cell in terms of its differentiation. Partly, this is due to a failure to appreciate the contribution of electron microscopy to understanding the nature of this cell. This paper, therefore, is devoted to detailing the principal phenotypic characteristics of the myofibroblast and promotes the argument that understanding how the myofibroblast carries out its roles in normal biological and in pathological processes will be enhanced by a sound understanding of its cellular differentiation, which in turn arguably demands a significant ultrastructural input.

Keywords: myofibroblast ● fibrosis ● cancer ● tumour stroma ● ultrastructure ● therapy

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Introduction

The myofibroblast is known primarily for its role in wound healing [1, 2], and in this capacity it promotes the health of the mammalian organism. The myofibroblast persists in abnormal wound healing and is also present in other clinically and histopathologically distinct fibroproliferative conditions [3–7]. In addition, it can be present in large numbers in tumour stroma, particularly of epithelial cancers [3–7], and in this respect, it has a negative impact on health by promoting tumour progression. We now have a great deal of detailed information from experimental and diagnostic pathologists on the processes in which myofibroblasts participate in fibroses and in the promotion of cancer – indeed, the cell is now being mentioned as a target for therapeutic intervention in translational medicine [8, 9]. And yet, there is a great deal of evidence from the literature indicating a failure to understand the cellular differentiation of the myofibroblast. This is partly because the initial definition was largely based on electron microscopy, and in recent years this technique has been somewhat neglected by experimentalists and cancer cell biologists: to a significant degree, it has been displaced by other techniques, such as immunohistochemistry, confocal microscopy and molecular biological procedures.

This paper promotes a precise definition of the myofibroblast in order to promote a better understanding of the nature of this cell in its role as a major player in translational medicine in fibrotic conditions, cancer and tissue engineering. The review also illustrates how a precise definition, emphasising cellular structure, can inform normal cell biology and pathology thinking.

The evolving definition of the myofibroblast: promoting an understanding of biology and pathology

From early ultrastructural criteria to a comprehensive definition

A comprehensive definition of the myofibroblast has been promoted in recent years [10–15], which

Table 1 Comprehensive definition of the myofibroblast

- Spindle-cell or stellate-cell morphology (Fig. 1)
- Palely eosinophilic cytoplasm
- An abundant pericellular matrix
- Immunophenotype:
  ➢ Vimentin positive
  ➢ α-smooth-muscle actin positive (Fig. 1)
  ➢ Smooth-muscle myosin negative
  ➢ Non-muscle myosin positive
  ➢ Virtually no desmin (Fig. 1)
  ➢ Fibronectin positive (Fig. 1) especially the extra domain A variant
- Ultrastructure (Fig. 2)
  ➢ Prominent rough endoplasmic reticulum
  ➢ A Golgi apparatus producing collagen secretion granules
  ➢ Peripheral myofilaments
  ➢ Fibronexus junctions (no lamina)
  ➢ Gap junctions.

includes light microscopy features as seen in haematoxylin and eosin sections, immunophenotype and ultrastructure. The definition of the fully differentiated myofibroblast is given in Table 1.

It has taken more than three decades for this definition to evolve since the first use of the term *myofibroblast* in the early 1970s. The term was introduced by Majno *et al.* [2] for the cells found in experimental wound-healing which had also been described by the same group as 'modified fibroblasts' [1]. These cells had the prominent rough endoplasmic reticulum (rER) of fibroblasts and the contractile myofilaments of smooth-muscle cells (although these were few and located under the membrane) (Figs. 2A and 3). In addition, a cell-surface component described as basement membrane-like material was noted. At that time, basal lamina ('basement membrane') of epithelial cells and the similar so-called 'external' lamina of, for example, nerve-sheath and muscle cells, was the only well-known structure to be found on cell surfaces. In granulation tissue myofibroblasts, this material was felt to be not quite like a true lamina: it was irregularly deposited and possessed a filamentous substructure, and so the term, basement membrane-like material, was used. Subsequently, this was construed as the 'microtendon', a structure transferring contractile force to, and attaching the cell to extracellular matrix [16], while further studies [12, 17, 18] revealed that this material was fibronectin, and that it was organised into fibrils, ribbons or sheets. This
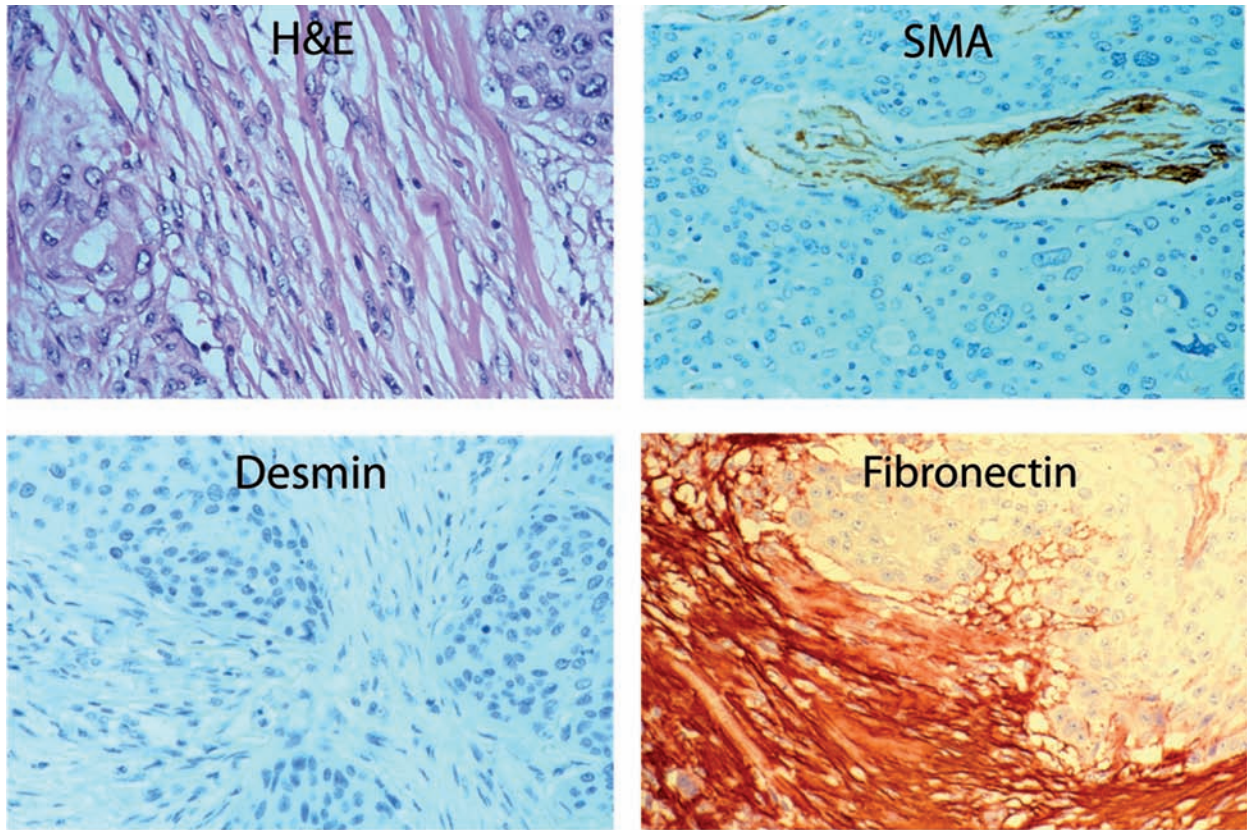
Fig. 1 Light microscopy features of reactive myofibroblasts from the stroma of squamous cell carcinoma.

cell-to-matrix junction, consisting of an aligned myofilament bundle and fibronectin fibril contacting one another through a point at the cell surface (Fig. 4), was given the name *fibronexus* [19], and has been argued as being a highly characteristic marker of the myofibroblast [10–15]. However, the fibronectin fibril and fibronexus have not always been fully understood: in particular the fibronectin fibril has sometimes been confused with true lamina, and this has led to interpretational uncertainty (see below).

Immunohistochemistry brings a less precise definition

During the 1980s, imunohistochemistry underwent significant development and among investigators of cell biology and diagnostic pathologists it became a preferred technique: pathologists, for instance, liked the more direct correlation possible between immunostained and haematoxylin and eosin sections. Myofibroblasts were found to contain α-smooth-muscle actin (SMA), while lesional myofibroblasts (but not granulation tissue or tumour stroma myofibroblasts) also sometimes expressed desmin [20] (Fig. 1). An immunohistochemically based definition became desirable and a *spindled cell expressing SMA* has become and continues to be one of the most popular definitions of the myofibroblast [21–25]. However, a spindled cell positive for SMA (or containing the equivalent to SMA in the form of modestly developed actin filament bundles), is an imprecise definition for the myofibroblast because a number of normal cells conform to this definition [15, 26] (Table 2).

In normal tissues, it is true that there is little difficulty in identifying these cells by cell morphology and histological context alone, but in pathological processes and in particular cancer, these various forms of differentiation may be harder to identify as

these tissues become genetically and phenotypically more complex. In pathology, the list of SMA-positive spindled cells (or spindled cells with a few peripheral myofilaments) is even larger, and includes transforming epithelia (for example, cells in anterior capsular cataract and tubulointerstitial fibrosis) and a wide variety of tumours as histogenetically distinct as desmoplastic malignant melanoma and spindle-cell carcinoma [27]. Suffice it to say, that electron microscopy can distinguish between these cell types, and so can offer a more confident interpretation of myofibroblastic differentiation in a pathological context.

In addition to SMA, some lesional myofibroblasts contain the intermediate filament protein, desmin [20] and this observation seems to have been translated into the erroneous but widespread idea that desmin is a general marker of myofibroblasts. In fact, granulation tissue and tumour stroma myofibroblasts, which have been argued as constituting the nearest approach to the normal myofibroblast with which to assess myofibroblastic differentiation in pathological cells, almost entirely lack desmin [28] (Fig. 1): further, desmin negativity rather than positive desmin staining should be seen as a more appropriate indicator of myofibroblastic differentiation. In a lesion, positive desmin staining (in the right context) can indicate either true smooth-muscle or myofibroblastic differentiation, and strong and diffuse staining should always prompt an investigator to prefer true smooth muscle differentiation. Desmin, in the context of a lamina demonstrated by electron microscopy, would strongly suggest true smooth-muscle differentiation, an indication of the value of ultrastructure in the clear assignment of a differentiation to a cell [13, 15, 29].

Consequences of an imprecise definition in normal tissue biology – so-called myofibroblasts in bladder and gut

As noted above, immunohistochemistry, highly important as this technique is, has introduced an element of imprecision into the definition of the myofibroblast, and inevitably some interpretational confusion. Indirectly, the technique added further interpretational uncertainty in that the popularity of immunohistochemistry has partly contributed to a loss of ultrastructural interpretational expertise among pathologists and investigators of cell biology, one specific area of interpretational difficulty being the confusion between true lamina and fibronectin.

Table 2 Normal cells of spindled morphology expressing smooth-muscle actin (SMA)

- True smooth-muscle cells
- Pericytes
- Myoepithelium
- Interstitial Cells of Cajal
- Intestinal subepithelial (pericryptal) fibroblasts
- Myofibroblasts
- Endothelium
- Fibroblastic reticulum cells of lymphoid tissue
- Interstitial cells of the alveolar septa
- Testicular myoid and stromal cells
- Cells of thecae and capsules.

Partly because the fibronectin fibril and fibronexus were first described in the cell biological literature [17–19], it took some time, at least in pathology, for this structure to be fully appreciated. Initially, a lack of awareness of the fibronexus led many investigators to use the combination of rER and myofilaments as the ultrastructural criterion for the myofibroblast—again, an imprecise definition. Also, the early term for the fibronectin fibril, basement membrane-like material, led many investigators to equate it with true lamina: as a result, real basal or external lamina, on occasion and erroneously, has been interpreted as a marker of the myofibroblast [30, 31].

Yet other authorities have maintained that myofibroblasts have both fibronectin fibrils and lamina on their surface [5, 6, 32]. Against this view is the argument that the structures interpreted as lamina are really focal profiles of fibronectin close to the cell surface membrane but not exhibiting the co-linearity with myofilaments or the projection out into the matrix on account of sectioning geometry (see [10–12, 14, 15] for discussions on the distinction of lamina from fibronectin). It is accepted that a single profile of a fibronectin fibril out of context can resemble lamina (Fig. 2A), but there are several significant differences (Fig. 2A and B). In the same tissue (fixed, therefore, under the same conditions for more valid comparison) lamina is paler-staining than fibronectin [13]; fibronectin has a finely filamentous, longitudinal substructure, a co-linearity with myofilaments, and projects out into the matrix away from the cell surface. Lamina, by contrast, does not have an orientated filamentous substructure and faithfully follows the contour of the cell surface (Fig. 2B).
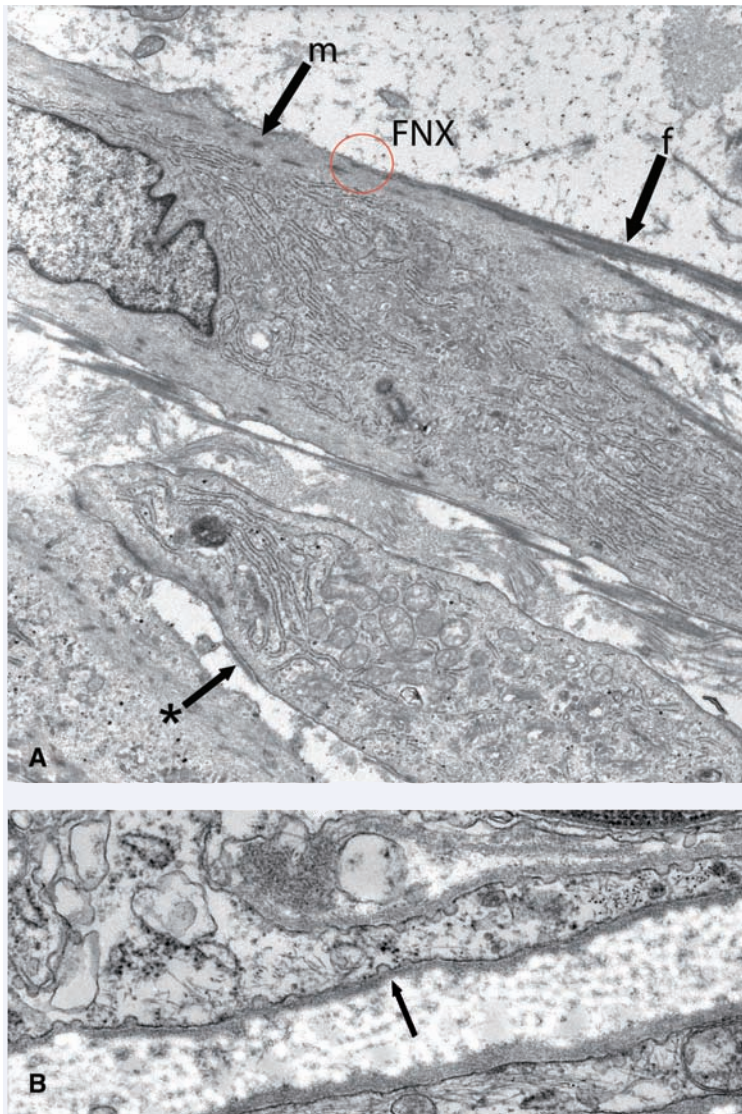
Fig. 2 (**A**): Tumour stroma myofibroblast from squamous cell carcinoma. It shows abundant rER cisternae in an ordered array, peripheral myofilaments (m) and a colinear extracellular fibronectin fibril at the fibronexus (FNX). Note how the fibronectin fibril is dense and straight, and projects away from the cell surface into matrix. The asterisked arrow indicates a focal area of fibronectin adjacent to the cell surface and mimicking lamina. Reproduced with permission from BC Decker Inc (Hamilton, Canada) from Fig. 2 in *Journal of Otolaryngology*. 1996; 25: 361–2. (**B**): 'External' lamina (arrow) around a perineurial cell from a normal cutaneous nerve, showing a paler staining quality and faithful adherence to the cell surface contour.

Eyden's studies have consistently argued that there is no evidence for lamina on the myofibroblast surface, in spite of the fact that some studies have shown the presence of lamina proteins in the extracellular matrix of myofibroblasts [33–35]. Here, presumably, these proteins are not organised into an ultrastructurally identifiable supramolecular structure.

Certainly, there has been erroneous interpretation and misleading hypothesising as a result of these points of ultrastructural confusion, even in the context of normal cell and tissue biology. In the lamina propria of the urinary bladder, for example, cells possessing plasmalemmal caveolae, plaques, myofilaments and lamina have been described as myofibroblasts [30]: however, the unambiguous lamina illustrated, in the context of other features, indicates that these cells are smooth-muscle in nature, not myofibroblastic. Another study equated an interstitial type of cell with 'myofibroblast', and quoted Wiseman *et al.* as having found 'human suburothelial cells with the typical ultrastructure of myofibroblasts' [36], a re-iteration of an interpretational error. These suburothelial interstitial cells, in having a lamina, are arguably smooth-muscle cells, somewhat modified, it is true, by having fewer myofilaments than fully differentiated smooth-muscle cells, but smooth-muscle cells none-the-less.

A further example of a normal tissue being interpreted as containing myofibroblasts is the gut, the cells concerned being the *subepithelial* or *pericryptal fibroblasts*, to use a terminology from the days of electron microscopy before the advent of immunohistochemistry [37, 38]. Immunohistochemistry, however, revealed SMA in these cells, a well-known marker of myofibroblasts [20]. The relatively restricted use of electron microscopy and therefore the limited diffusion of the ultrastructurally based definition for the myofibroblast has popularised in this area of research the definition noted above, spindled morphology and SMA [39, 40], the imprecision of which is emphasised by use at one and the same time of both 'myofibroblast' and 'myofibroblast-like' [39]. To use the term, myofibroblast, for a spindled or stellate cell positive for SMA fails to take account of other similar cells, among them true smooth-muscle cells, even poorly differentiated ones (Table 2), while to include, for example, the pericyte as a type of myofibroblast [40] ignores its distinctive ultrastructure [14, 15] as a primitive kind of smooth-muscle cell.

Pericryptal cells are also desmin and caldesmon positive, which are markers more suggestive of true smooth-muscle than myofibroblastic differentiation [41–43]. Although incidental ultrastructural observations on the human pericryptal cells also suggest a primitive form of smooth-muscle differentiation to judge from the plaques, lamina and myofilaments [44], these cells none-the-less remain incompletely documented in terms of ultrastructure and ultraimmunophenotype: they may yet prove to be cells with intermediate characteristics, not entirely conforming to any one of the clearly defined cell types illustrated in Figure 3.

Much the same can be said for the interstitial cell of Cajal (ICC). This cell, a component of normal, non-traumatized gut musculature, has been interpreted as showing smooth-muscle features – namely, myofilaments (although these are few), lamina, attachment plaques and caveolae [45–47] (although Min and Seo [48] argue that ICCs are more fibroblastic). These features alone would exclude a myofibroblastic nature, but in addition, the cytoplasm contains abundant vimentin filaments, prominent smooth endoplasmic reticulum and usually a paucity of rER – further features which would not be typical of a myofibroblast. Finally, fibronexuses, the archetypal structural marker of the myofibroblast, have not been found on ICCs.

The importance of an appropriate nomenclature lies in the fact that calling the interstitial cells of the bladder, the pericryptal cells and ICCs of the normal gut by the common name of myofibroblast (a) hides a wealth of structural and biological diversity within this broad group of cells; (b) could lead to inappropriate hypothesising by making undue comparisons between these normal cells and 'true' myofibroblasts (those from granulation tissue and tumour stroma); and (c) disseminates the view that myofibroblasts are components of normal tissues. With regard to this last point, the overwhelming evidence is that true myofibroblasts are reactive cells (except for their neoplastic counterparts) and are not a component of normal tissues [14, 15, 49–51], and they arise in conditions of trauma (wounds) or other forms of pathology (fibroses, tumour stroma). The view that they are normal cells (*i.e.* are found in non-traumatised tissues) is misleading. It is perhaps more appropriate to think in terms of all normal tissues' having or being able to recruit stem cells capable of transforming into myofibroblasts, but to say that normal tissues contain myofibroblasts, or that myofibroblasts are ubiquitous [4, 25, 52–56] paints an unrepresentative picture of normal tissues.

The cells documented as possible myofibroblasts from other, diverse sites, such as external thecae, capsules, alveolar septa, bone marrow and lymph nodes [5, 25, 40] either conform to the imprecise immunohistochemical definition (SMA positivity in a spindled cell) [57], or lack the full complement of ultrastructural features as outlined above for the myofibroblast (Table 1): in particular, they invariably lack fibronexus junctions and some possess unambiguous lamina (*e.g.*, [58–60]), which in the context of rare myofilaments, amounts to a low level of smooth-muscle differentiation. In some cases, myofibroblastic character has been interpreted from observations on cultured cells [59], and *in vitro* a variety of initially non-myofibroblastic cells can transdifferentiate into myofibroblasts [15]. Finally, however, while many of these cells might be expressing an exceedingly low level of myofibroblastic differentiation (as suggested by the SMA staining), they are so far from well-differentiated myofibroblasts that it would be misrepresentational to think of them as true myofibroblasts and to think of normal tissues as harbouring unambiguous myofibroblasts.

However, periodontal ligament and the lamina propria of the testis are notable exceptions. The development of myofibroblastic differentiation (detailed below) requires a number of factors, which include transforming growth factor-β (TGF-β) and physical stress [61–65]. Periodontal ligaments are indeed subjected
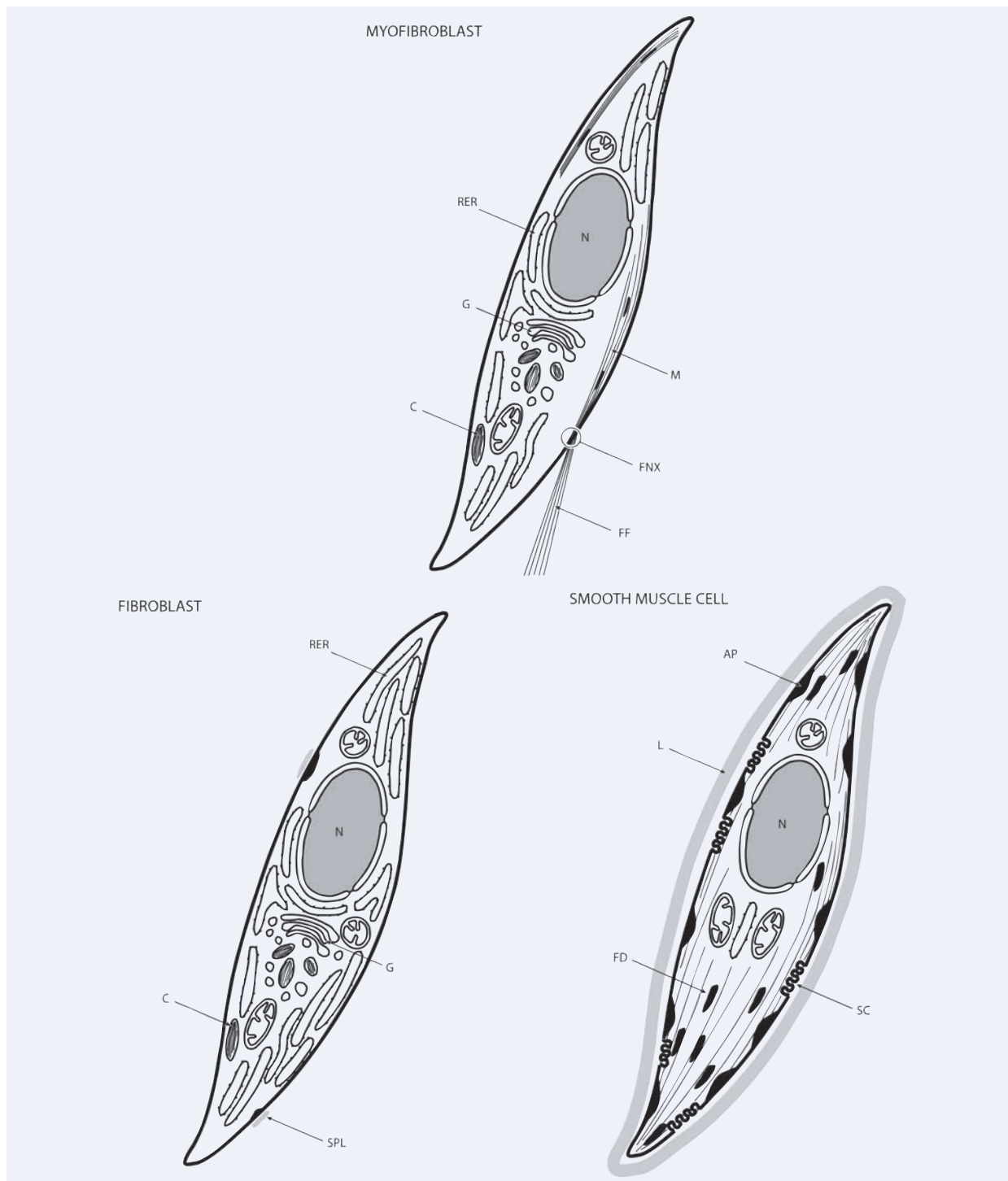
Fig. 3 Diagrammatic representation of the myofibroblast, in comparison with the fibroblast and the smooth-muscle cell. Abbreviations: AP, attachment plaque; c, collagen secretion granule; FD, focal density; FF, fibronectin fibril; FNX, fibronexus; G, Golgi apparatus; L, lamina; M, myofilament bundle; N, nucleus; RER, rough endoplasmic reticulum; SC, surface (plasmalemmal) caveolae; SPL, subplasmalemmal linear density (focal adhesion). Drawing by Paul Chantry.

to the continual stress of tooth movement during mastication [66–67], while the testicular lamina propria is also regarded as a dynamic tissue, where myofibroblasts with cell-to-matrix devices very similar to modestly developed fibronexuses have been described [68].

No one would deny that the stark classification of stromal cells into fibroblasts and smooth-muscle cells as in Fig. 3 is a simplification and should be used as a guide (see [69]), and that probably there are many anatomical sites where stromal cells have slight variations in phenotype reflecting distinctive local functions [40]. But, it seems reasonable to use the term, myofibroblast, where sufficient imunophenotypic and ultrastructural data permit, and where these data provide a clear distinction from archetypal fibroblasts and smooth-muscle cells.

The myofibroblast in fibrotic conditions, tumour stroma and tissue engineering – a therapeutic target in translational medicine

De novo myofibroblastic differentiation and functions of myofibroblasts in granulation tissue, fibroproliferative conditions and tumour stroma

The presence of myofibroblasts in granulation tissue and in fibrotic conditions, and the awareness of their status in tumour stroma as cells promoting the progression of cancer [70–72], emphasise the myofibroblast as a target for anti-fibrotic and anti-cancer therapy [8, 9, 73–77] and as a focus for study in stem cell biology and tissue engineering [78], itself a field with enormous translational research potential. Therapeutic intervention will be most successful by exploiting one or more of the myofibroblast's crucial or specific biochemical pathways.

Several of the steps in the development of the myofibroblastic phenotype are known for granulation tissue and tumour stroma myofibroblasts. In a formidably complex molecular scenario, which is beyond the scope of this review, the following are some of the principal elements:

- Platelet-derived growth factor (PDGF) released from the blood of a wound acts as a mitogen or chemo-attractant for surrounding fibroblasts

- Stress fibres develop containing cytoplasmic actins by a stress-related process (in protomy ofibroblasts)
- TGF-β induces synthesis of extra domain A (EDA) fibronectin
- EDA fibronectin facilitates the synthesis of α-SMA
- α-SMA becomes incorporated into stress fibres, which therefore become bundles of myofilaments with focal densities
- A variety of proteins, some initially associated with the simple focal adhesions of fibroblasts, show increased expression, while new proteins are expressed, which create molecular adhesions between α-SMA-bearing myofilament bundles, the cell membrane and fibronectin fibrils (fibronexuses) in fully differentiated myofibroblasts. These proteins include:
  ➢ α-SMA
  ➢ EDA cellular fibronectin,
  ➢ α5 β1 and αvβ3 integrins
  ➢ Vinculin, paxillin, talin
  ➢ α-actinin
  ➢ cytoplasmic β- and γ-actins
  ➢ Tensin
  ➢ Focal adhesion kinase (FAK) [7, 63, 79–81].

Tumour myofibroblasts arise and participate in an even more complex molecular picture (see, *e.g.*, [55, 71, 82] for further details), of which the following are some of the main points and activities:

- Some of the main indications that myofibroblasts promote tumours included the observation that cancer cells injected into wounds grew far more than when injected into normal skin [83]; myofibroblasts facilitate neoplastic growth of prostatic epithelium in immunodeficient mice [70]; and they lead to greater and more vascularised tumour growth in co-mingling experiments [23]. More recently, a numerical association between tumour stromal myofibroblasts and poor prognosis has been demonstrated [84, 85].
- As in granulation tissue, PDGF and TGF-β switch on myofibroblast differentiation.
- PDGF attracts and stimulates mitosis of mesenchymal cells. Some TGF-β is cancer-cell derived, and this also acts as a chemo-attractant for surrounding fibroblasts and then promotes myofibroblastic differentiation: thus there is intimate cross-talk between cancer and host cells.
- TGF-β promotes tumour progression in a number of situations: to mention two out of many (see [55], in a prostate carcinoma cell line, it causes the for-

mation of larger tumours and more extensive metastatic disease *in vitro* and *in vivo* [86], while anti-TGF-β antibodies inhibit lung metastases of mammary cancer cells in athymic mice [87].

- TGF-β indirectly recruits monocytes and macrophages which produce matrix degrading molecules such as metalloproteinases, which loosen the physical structure of matrix and facilitate tumour cell migration [88], while some of the degradation products may also act to favour progression of tumour cells [89].
- TGF-β induces vascular endothelial growth factor (VEGF) molecules, which are neo-angiogenic, and further furnish tumour cells with the physical opportunity of metastasis.
- Urokinase-type plasminogen activator and its receptor are involved in extracellular matrix turnover, leading to cell migration and invasion [90].
- Myofibroblasts are often found in the tumour-stroma interface, and may form a barrier between tumour cells and host immune cells, thereby interfering with the host's ability to mount an immune defence against cancer [23, 55].

De Wewer and Mareel [55] have emphasized the need for a detailed understanding of myofibroblastic activity and in particular the biochemical communication between myofibroblasts and tumour cells as a pre-requisite for the development of therapeutic applications. Many of the studies that have led to our current understanding of these complex processes have lacked an ultrastructural input: so, is there a role for the ultrastructurally-enhanced definition of the myofibroblast – a key player – in such studies?

The ultrastructural definition of the myofibroblast tells us that in particular this cell has what we might think of as a very physical involvement with its extracellular space, through the fibronexus and its fibrillar fibronectin. This is a feature which does not attract much attention in experimental cancer cell biology studies. And yet the surface of any cell is of paramount importance in its dealings with the outside world. What are the possible roles of the cell-surface fibronectin of these myofibroblasts? It has been known for a long time that fibronectin promotes a variety of cellular functions (cell attachment, differentiation, proliferation and migration) and acts as a 'migratory substrate' for embryonic cells [91–93]. It is not surprising, therefore, that fibronectin has been found to influence the migration of tumour cells [94, 95]. Moreover, EDA fibronectin is known to activate

matrix metalloproteinase production [96], which, as noted above, function in matrix degradation as part of the tumour progression process. The fibronexus itself, as a physical junction between cell and matrix, could have several functions. An early idea was of a structure permitting the transfer of intracellular contractility to the extracellular matrix to achieve overall tissue contraction and wound-closure in granulation tissue [32]. In tumour stroma, the cell-to-matrix adhesive role of the fibronexus may be exploited for the purpose of creating extracellular matrix domains to provide stable areas for biochemical communication. Finally, the fibronectin fibril itself might have mechanosensing properties, being able to detect physical stress or tension in the extracellular matrix and use the energy of this stress to activate signalling molecules [63, 65]: these would precipitate a molecular cascade leading to number of cellular activities, such as differentiation, migration, mitosis – all of importance to the tumour progression process.

Bone-marrow-derived circulating fibrocytes, epithelial-mesenchymal transformation and the origin of myofibroblasts

Quite apart from the activity of myofibroblasts once located within granulation tissue, a focus of fibrosis or a tumour site, their origin is also inextricably bound up with the development of therapeutic intervention for these diseases. The traditional view has been that myofibroblasts arise from quiescent resident mesenchymal cells in the surrounding tissues – fibroblasts being the mainly implicated progenitor, but also pericytes, smooth-muscle cells and even endothelium [97]. Increasingly, however, attention is being focussed on to two other mechanisms – derivation from bone-marrow-derived circulating fibrocytes and origin from epithelial-mesenchymal transformation.

Bone-marrow-derived circulating cells (sometimes referred to as 'fibrocytes') have been demonstrated recently with the ability to localize to and populate tissue sites. These include normal tissues [98–99], granulation tissue [21, 100, 101], fibrosing conditions [102, 103] and tumour stroma [56, 104, 105] (see Quan *et al.* [106] for an excellent recent review and further references). Some of this research uses elegant experimentation: for example, Direkze *et al.* [56]

employed green fluorescent protein (GFP) staining for transplanted male cells into female GFP-negative recipients, to show how bone-marrow-derived cells populate tumour stroma. However, ultrastructural studies in these areas of research are few, and the maximum confidence of interpretation stemming from electron microscopy in the characterisation of cell types is not being fully exploited. Further, not all lesions involve repair by circulating cells: in athero-sclerosis, while there is evidence that circulating pro-genitor cells regenerate endothelium [107], other data suggest that smooth-muscle cells healing ather-osclerotic plaques are from local tissue origin [108].

Epithelial-mesenchymal transition or transforma-tion (EMT) is the second major mechanism postulat-ed to provide an origin for myofibroblasts in fibrosing conditions [109] and tumour stroma [82,110] and is currently receiving considerable attention. The concept is based on findings of the kind typified in tubulointer-stitial fibrosis. Here, stromal cells in the interstitium are SMA-positive and interpreted as myofibroblasts; the tubular epithelium concomitantly loses some of its epithelial phenotype, assumes some mesenchymal features (vimentin, SMA, desmin, fibroblast-specific-protein-1) and is hypothesized as developing into myofibroblasts [73]. Again, ultrastructural studies in these areas of research are few, and some insights from electron microscopy risk being lost, in the sense that an ultrastructural input may modify some of the interpretations being formulated in EMT. Overwhelmingly, these studies use SMA as a marker for the myofibroblast, with the attendant imprecision that that definition brings. We ourselves have studied tubulointerstitial fibrosis and found that SMA-positive stromal cells in the interstitium mostly correspond with cells which by electron microscopy have a non-descript cytoplasm and sparse bundles of actin fila-ments at the cell periphery [111]. Indeed, myofibrob-lasts as fibronexus-bearing cells were found to be uncommon [111]. Probably, many of the cells in tubu-lointerstitial fibrosis which are spindled and SMA-pos-itive are not fully differentiated myofibroblasts.

This point needs exploration because immunohisto-chemistry itself is not very good at demonstrating lev-els of differentiation, and one can argue that there is an increase in myofibroblastic differentiation in a spec-trum from primitive SMA-negative stromal cells (non-myofibroblastic cells) to SMA-positive stromal cells lacking fibronexuses (cells which some might argue are showing a low level of myofibroblastic differentiation,
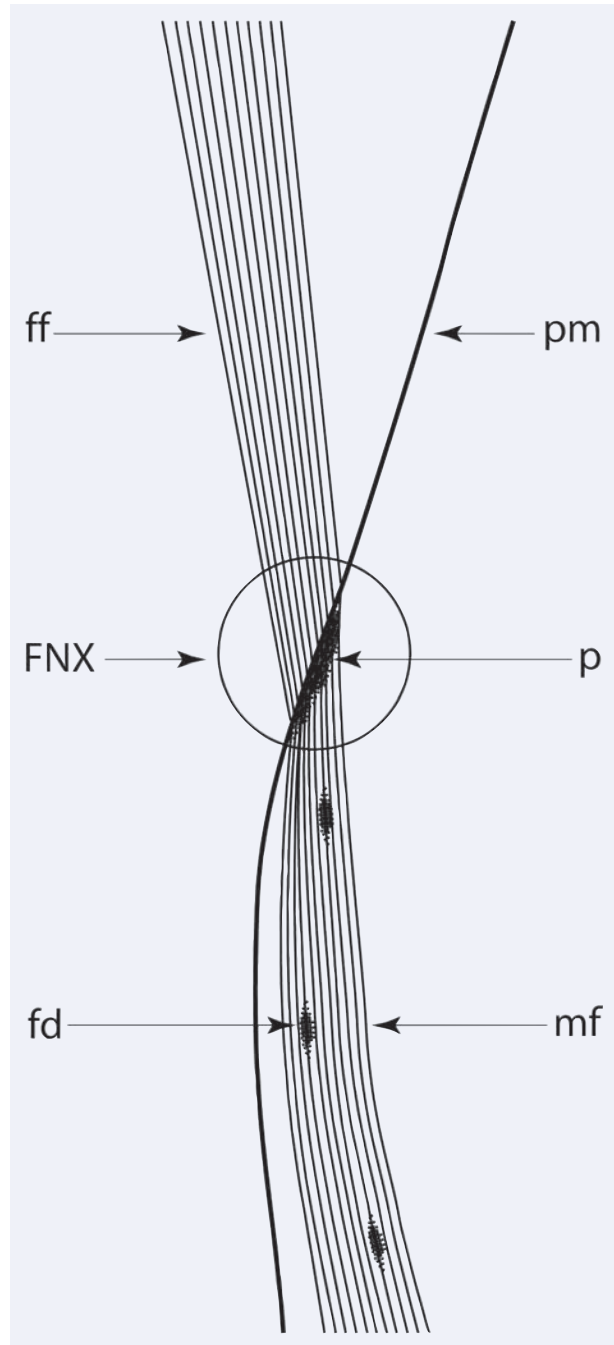

Fig. 4 Diagrammatic representation of the fibronexus (FNX), showing the connection through the membrane at a plaque (p) of myofilaments (mf) and fibronectin fila-ments (ff). fd, focal density; pm, plasmalemma. Drawing by Paul Chantry. Reproduced with permission from *Histology Histopathology*. 2001; 16: 57–70.
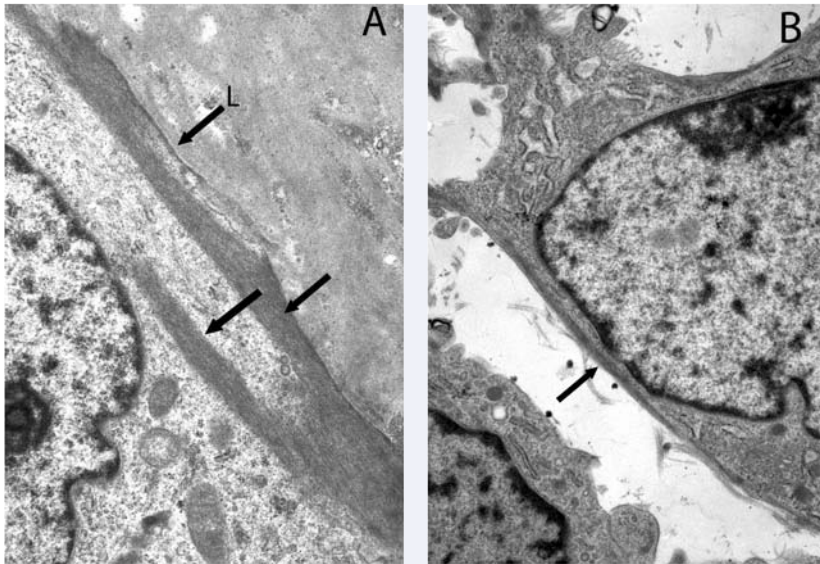
Fig. 5 (**A**): Peripherally located bundles of actin filaments in epithelium of renal tubulointerstitial fibrosis. (**B**): Stromal cell showing some rough endoplasmic reticulum and a slender bundle of actin filaments, but no fibronexuses. Reproduced with permission from *Journal of Submicroscopic Cytology and Pathology.* 2003; 35: 221–33.

that is, 'ambiguous myofibroblasts') to fully differentiated myofibroblasts which would be SMA-positive fibronexus-bearing cells. One of the undoubted qualities of electron microscopy is that it can indeed provide information on a level of differentiation. In this respect, the reference to 'complete transition to … mesenchymal phenotype …' [112] is open to dispute without the techniques to demonstrate it, and electron microscopy is one such technique.

Even when electron microscopy is performed, care is needed for appropriate interpretation. In a paper espousing EMT in the kidney [113], figures have purported to show myofibroblasts as a final product of EMT, whereas the images arguably show either epithelium itself or endothelium [14, 15] but, given the absence of any rER or fibronexuses, not myofibroblasts. In diabetic nephropathy, interstitial cells have been described conforming to a definition of the myofibroblast as a cell which, *inter alia*, possesses basement membrane [114]: in the context of a hypothesis attempting to show myofibroblast derivation from epithelium, the finding of mesenchymal cells in the interstitium bearing lamina could be compatible with the presence of detached, but epithelial cells and not myofibrobasts. This morphological 'evidence' for derivation of myofibroblasts from epithelium has subsequently been quoted in support of EMT [73, 115]. In addition to the lack of ultrastructural evidence of complete epithelial-to-myofibroblast transformation, there is inconsistent demonstration of a molecular marker for the myofibroblast, the αSMA

gene [116, 117]. This argument does not deny the existence of EMT as a possible source of fibrogenic cells, but argues that it is uncertain how many of these SMA-positive and fibroblast-specific-protein-1-positive cells are fully differentiated myofibroblasts and raises the question of whether EMT is complete or partial. This is important because of the distinctive fibronectin-rich molecular architecture of the surface of the fully differentiated myofibroblast, which must have implications for how this cell behaves.

Conclusions

The myofibroblast is becoming recognised as a target for translational medicine, since it is a significant cellular participant in granulation tissue and a variety of human pathology. Electron microscopy is the technique, which, *par excellence*, provides an unambiguous means of identifying the myofibroblast by means of several cell structural features – prominent rER, peripheral smooth-muscle myofilaments and fibronexus junctions – in the context of spindle cell morphology and an immunophenotype of positivity for SMA and EDA fibronectin. Electron microscopy is also valuable for providing an assesment of the level of myofibroblastic differentiation in populations of spindled cells positive for SMA. Many of the experimentalists creating new insights into the genesis and evolution of major diseases involving myofibroblasts eschew

electron microscopy in favour of light microscopy techniques, including immunohistochemistry and confocal microscopy, and other procedures. Light microscopy definitions of the myofibroblast are imprecise, especially in pathology, and there is room in experimental studies in wound healing, fibroproliferative conditons, tissue engineering and cancer cell biology for an extra dimension of interpretational confidence in identifying the differentiation of participating cells, which comes from electron microscopy. An increasingly sophisticated understanding of myofibroblast activities at the molecular level will enhance the possibilities of targeting this cell for therapeutic purposes.

Acknowledgements

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