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Review Article

Experimental systems to study the origin of the myofibroblast in peritoneal fibrosis

Manreet Padwal, Peter J. Margetts*

Division of Nephrology, Department of Medicine, McMaster University, St. Joseph's Hospital, Hamilton, Ontario, Canada

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A B S T R A C T

Peritoneal fibrosis is one of the major complications occurring in long-term peritoneal dialysis patients as a result of injury. Peritoneal fibrosis is characterized by submesothelial thickening and fibrosis which is associated with a decline in peritoneal membrane function. The myofibroblast has been identified as the key player involved in the development and progression of peritoneal fibrosis. Activation of the myofibroblast is correlated with expansion of the extracellular matrix and changes in peritoneal membrane integrity. Over the years, epithelial to mesenchymal transition (EMT) has been accepted as the predominant source of the myofibroblast. Peritoneal mesothelial cells have been described to undergo EMT in response to injury. Several animal and *in vitro* studies support the role of EMT in peritoneal fibrosis; however, emerging evidence from genetic fate-mapping studies has demonstrated that myofibroblasts may be arising from resident fibroblasts and pericytes/perivascular fibroblasts. In this review, we will discuss hypotheses currently surrounding the origin of the myofibroblast and highlight the experimental systems predominantly being used to investigate this.

Introduction

Peritoneal dialysis (PD) is a widely used renal replacement therapy for patients with end-stage renal disease [1,2]. The healthy peritoneum is a semipermeable membrane consisting of a superficial mesothelial layer, a basement membrane, and a thin submesothelial zone (Fig. 1A) [3]. Long-term exposure to bioincompatible dialysis solutions, uremia [4], and intermittent peritonitis [5] leads to a low-grade chronic inflammation, triggering a reparative response. This response normally provides restoration of tissue structure with minimal loss of function. However, this reparative mechanism may become dysregulated. This results in tissue fibrosis with deposition of extracellular matrix (ECM), angiogenesis, and eventual membrane failure (Fig. 1B). The appearance of myofibroblasts in the submesothelial tissue is a critical component of peritoneal membrane fibrosis and angiogenesis [5].

The myofibroblast in fibrosis

The myofibroblast is a specialized contractile cell that expresses both fibroblast and smooth muscle cell–like characteristics [6]. Myofibroblasts are motile cells and therefore possess stress fibers and ruffled cell membranes due to changes in the actin cytoskeleton [7]. Stress fibers are composed of bundles of actin microfilaments and contractile proteins. Actin microfilaments terminate at the cell surface in a specialized adhesion complex termed the fibronexus that becomes linked to extracellular fibronectin fibrils [7]. This creates a

* Corresponding author. Division of Nephrology, Department of Medicine, McMaster University, St. Joseph's Hospital, 50 Charlton Avenue East, Hamilton, Ontario L8N 4A6, Canada.
E-mail address: margetts@mcmaster.ca (PJ Margetts).

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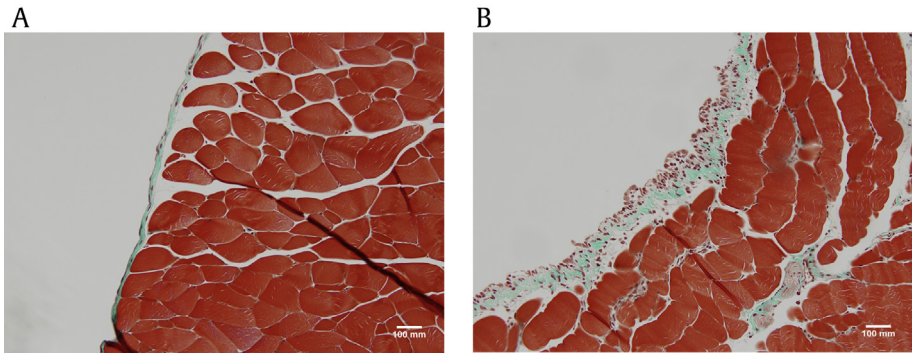

Figure 1. Histology of the peritoneal membrane. (A) Healthy mouse peritoneum taken from the anterior adbominal wall consists of superficial mesothelial layer and a thin submesothelial zone. (B) Exposure to an adenovirus expressing transforming growth factor beta results in submesothelial thickening, angiogenesis, and increased cell number in the peritoneum.

mechanotransduction system that allows for transmission of force from stress fibers to the ECM. Similarly, mechanical signals from the extracellular environment can activate intracellular signaling pathways [8]. Expression of alpha smooth muscle actin (α-SMA) is a key marker that distinguishes the myofibroblast from the fibroblast [9]. Although the function of the myofibroblast has not fully been elucidated, the myofibroblast has mostly been studied for its role in wound healing and its presence during pathological conditions such as fibrosis. Originally, myofibroblasts were observed in granulation tissue of healing wounds. They were observed to produce the contractile force required during wound contraction. More recently, several studies have identified the myofibroblast as an important element in the production of ECM and progression of fibrosis [7,10].

In a healthy environment, stromal cells including fibroblasts and perivascular cells remain quiescent, producing little ECM and few actin interactions between cells and cell to matrix [8]. In response to injury, myofibroblasts accumulate at the site of injury where they produce ECM in response to cytokines from local cells [6,8]. Transforming growth factor beta (TGF-β) is a key cytokine that regulates expression of α-SMA and type 1 collagen in the myofibroblast [9,11]. Moreover, the differentiation process of the myofibroblast is largely regulated by TGF-β. In addition to TGF-β, myofibroblasts are also stimulated by changes in the mechanical microenvironment and high extracellular stress [7,8]. During normal conditions, the ECM maintains its cross-linked structure; however, tissue injury results in remodeling of the ECM and changes in the mechanical environment. In response to this change in ECM structure and TGF-β signaling, precursor cells begin to acquire contractile stress fibers [7]. During this time, myofibroblasts express some features that are similar to fibroblasts such as actin stress fibers. This transitional cell has been characterized as the protomyofibroblast [8,12]. The protomyofibroblast begins to acquire more contractile activity with the expression of α-SMA in the stress fibers and can now be characterized as a myofibroblast. ECM stiffness is one promoter of myofibroblast maturation [12,13]. During the final stages of wound healing, the ECM regains its normal structure and the myofibroblasts undergo apoptosis [8,12].

The last component of the wound-healing response involves replacement of injured cells with new cells and resolution of scar tissue to preserve functional and structural integrity of the organ [14]. However, this is not always the case depending on the type and duration of injury as well as the severity of damage. Often, chronic injury may result in the inability to replace scar tissue with functional tissue resulting in irreversible fibrosis [14]. Therefore, fibrosis is largely characterized as accumulation of ECM including collagen, proteoglycans, and fibronectin [10]. The myofibroblast is the primary effector cell that is activated during this process [10]. Myofibroblast populations have been noted in abnormal wound healing in several vital organs such as the kidney, lung, liver, and heart [11,15,16].

Myofibroblasts have also emerged as the main effector cell contributing to peritoneal fibrosis. The presence of the myofibroblast contributes to the loss of integrity of the peritoneal membrane in long-term PD patients by inducing collagen deposition resulting in structural and functional changes in the peritoneal membrane [17]. In PD patients, the myofibroblast is dominant in the peritoneal membrane even before fibrosis is present in contrast to the normal healthy peritoneum [18].

It is challenging to study stromal cells; fibroblasts do not express any specific markers resulting in difficulties distinguishing them from other cells [10]. Several studies have used fibroblast specific protein (FSP) 1 (also known as S100A4) as a fibroblast-specific marker [19]. Recent evidence demonstrates FSP1 is not specific to fibroblasts and is also expressed in other cells such as monocytes [40]. Myofibroblast expression of α-SMA is increasingly being used as the method of identifying the myofibroblast. However, this marker still presents some challenges as α-SMA is also expressed by other cells of the mesenchymal lineage such as vascular smooth muscle cells [10].

Origin of the myofibroblast: current hypotheses

As the myofibroblast is the key effector cell in progression of fibrosis, it is of scientific and practical interest to elucidate the origin of this cell. This is a controversial area of research. In various research studies, myofibroblasts have been shown to derive from different cellular sources including resident fibroblasts [8,16], epithelial cells via epithelial to mesenchymal transition (EMT) [20], endothelial cells (endothelial to mesenchymal transition) [21], perivascular cells [22], and bone marrow–derived cells [19,23]. Interestingly, the original hypothesis was that myofibroblasts arose from locally residing mesenchymal cells such as resident fibroblasts and pericytes/