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Abstract

Myofibroblasts (MFs) play a critical role in the progression of chronic inflammatory and fibroproliferative diseases in different tissues/organs, whatever the etiology. Fibrosis is preceded and sustained by persistent injury and inflammatory response in a profibrogenic scenario involving mutual interactions, operated by several mediators and pathways, of MFs and related precursor cells with innate immunity cells and virtually any cell type in a defined tissue. These interactions, mediators and related signaling pathways are critical in initiating and perpetuating the differentiation of precursors cells into MFs that in different tissues share peculiar traits and phenotypic responses, including the ability to proliferate, produce ECM components, migrate and contribute to the modulation of inflammatory response and tissue angiogenesis. Literature studies related to liver, lung and kidney fibrosis have outlined a number of MF-related core regulatory fibrogenic signaling pathways conserved across these different organs and potentially targetable in order to develop effective antifibrotic therapeutic strategies.

Keywords.

Myofibroblasts; chronic inflammatory diseases; liver fibrosis; lung fibrosis; kidney fibrosis; profibrogenic pathways; antifibrotic therapeutic strategies.

Abbreviations

Angiotensin-converting enzyme, ACE; airway-centered fibrosis, ACF; advanced alveolar epithelial cells, AEC2; glycation end products, AGEs; angiotensin I, Ang I; angiotensin II, AT-II; angiotensin Type 1 receptor, AT1R; α-smooth muscle actin, α-SMA; autotoxin, ATX; bile duct ligation, BDL; bone marrow-derived cells, BMdC; bone morphogenetic protein, BMP; carbon tetrachloridechemokine, CCl_4; (C-C motif) ligand 2, CCL2; C-C chemokine receptor type 2, CCR2; chenodeoxycholic acid, CDC; chronic inflammatory diseases, CID; CLD, chronic liver disease; CX3CL1, chemokine (C-X3-C motif) ligand 1; chronic hypersensitivity pneumonitis, CrHP; v-crk sarcoma virus CT10 oncogene homolog, CRK; CSF1, colony stimulating factor 1; connective tissue growth factor, CTGF/CCN2; cysteine-rich angiogenic inducer 61, CYR61; DAMPs damage-associated molecular patterns; dendritic cells, DC; Desert hedgehog, Dhh; Dickkopf-1, Dkk-1; Dishevelled, Dvl; ECM, extracellular matrix; epidermal growth factor, EGF; epithelial mesenchymal transition, EMT; endothelial-to-mesenchymal transition, EndoMT; extracellular signal-regulated protein kinase, ERK; endothelin-1, ET-1; ET receptor antagonists, ERAs; erythropoietin, EPO; fatty acids, FA; focal adhesion kinase, FAK; fibroblast growth factor b, FGFb; FGF receptor, FGFR; fibrotic non-specific interstitial pneumonia, fNSIP; farnesoid X receptor, FXR; Frizzled receptor, Fzd; growth factor, GF; green fluorescent protein, GFP; G protein-coupled receptors, GPCRs; growth factor receptor bound protein 2, GRB2; heparin-binding EGF-like growth factor, HB-EGF; epidermal growth factor receptor Erb-B, HER/Erb; high fat diet, HFD;

Hedgehog, Hh; hydrogen peroxide, H_2O_2; hepatic stellate cell, HSC; hypoxia-inducible factor 1α, HIF1α; interferon-γ, IFN-γ; insulin-like growth factor 1, IGF-1; insulin-like growth factor binding proteins, IGFBP; Indian hedgehog, Ihh; interleukin, IL; idiopathic pulmonary fibrosis, IPF; inducible nitric oxide synthetase, iNOS; ischemic–reperfusion, IR; interferon regulatory factor IRF; Janus-associated kinase, Jak1; Janus kinase-2, JAK-2; c-Jun-NH_2-terminal kinases, JNK; keratinocyte growth factor, KGF; latency-associated peptide, LAP; LIM kinase 1, LIMK1; lipopolysaccharide, LPS; liver X receptors, LXRs; lysophosphatidic acid, LPA; mitogen-activated protein kinase, low-density lipoprotein receptor related protein-5/6, LRP5/LRP6; liver sinusoidal endothelial cells, LSECs; mitogen-activated protein kinase, MAPK; macrophage inflammatory protein, MIP; matrix metalloproteases, MMPs; mannose receptor C-type 1, Mrc1; myocardin-related transcription factor, MRTF; NADPH oxidase, NOX; non-alcoholic fatty liver disease, NAFLD; non alcoholic steatohepatitis, NASH; Notch intracellular domain, NICD; natural killer, NK; natural killer T, NK-T; nuclear factor kappa B, NF-kB; nucleoside triphosphate diphosphohydrolase-2, NTPD2; nuclear receptors, NRs; superoxide anion, $O_2^{\bullet-}$; obeticholic acid, OCA; pathogen-associated molecular patterns, PAMPs; partitioning defective 6, PAR6; primary biliary cirrhosis, PBC; platelet-derived growth factor, PDGF; programmed cell death ligands 1, PD-L1 and 2 PD-L2; prostaglandins, PE; protein kinase B, PKB; phosphoinositide-3-kinase, PI3K; phospholipase C, PLC; peroxisome proliferator-activated receptors, PPARs; pattern recognition receptors, PRRs; primary sclerosizing cholangitis, PSC; protein tyrosine kinase 7, PTK7; Patched, Ptch; Pentraxin-2, PTX2; receptor for advanced glycation end-products, RAGE; renin-angiotensin system, RAS; receptor tyrosine kinase-like orphan receptor 2, ROR2; reactive oxygen species, ROS; retinoid X receptor, RXR; receptor-like tyrosine kinase, RYK; sphingosine-1-phosphate, S1P; small interfering RNA, siRNA; stromal cell-derived factor-1, SDF-1; Src homology-2-containing, SHC; Sonic hedgehog, Shh; small heterodimer partner, SHP; Smoothened, Smo; sphingosine kinase 1, SphK1; v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog, SRC; signal transducer and activator of transcription, STAT; PDZ-binding motif, TAZ; transforming growth factor α, TGF-α; transforming growth factor β1, TGF-β1; transforming growth factor receptor, TGFR; tissue inhibitors of metalloproteinase, TIMP; Toll-like receptors, TLRs; tumor necrosis factor α, TNF-α; T regulatory, Treg; thrombospondin type I repeat, TSR; usual interstitial pneumonia, UIP; ursodeoxycholic acid, UDCA; unilateral ureteral obstruction, UUO; vascular endothelial growth factor A, VEGF-A; vascular endothelial growth factor receptor, VEGFR; von Willebrand factor type C, VWC; Wingless-related Integrase,Wnt; yes-associated protein, YAP.

1. Introduction

Fibrosis is usually defined as an excessive deposition of extracellular matrix (ECM) components (i.e. fibrous connective tissue) within and around chronically damaged tissue and, whatever the specific etiology and the tissue or organ involved, it represents the final common pathological outcome of several chronic inflammatory diseases (CID) [1-3]. In conditions of severe tissue injury or, more frequently, persistent chronic tissue injury and/or dysregulated wound healing response, fibrosis is the net result of persisting fibrogenesis. Accordingly, persisting fibrogenesis can be envisaged as a dynamic and highly integrated molecular, tissue and cellular process, potentially reversible, that can drive the progression of a CID towards permanent scarring, tissue/organ failure and eventually death as observed in end-stage kidney, liver and lung diseases but also in heart failure [1-9]. In addition, fibrosis also represents a major pathological feature in several autoimmune diseases and can influence tumor invasion and metastasis as well as other clinical settings like chronic graft rejection or the development of some progressive myopathies [1-3]. Along these lines, fibrotic progression in different tissues and organs can be triggered by several etiological agents or conditions, including persistent infections by defined micro-organisms, chronic exposure to toxins or other agents/drugs inducing tissue injury, inherited genetic disorders, chronic autoimmune-mediated inflammation as well as conditions of altered metabolism (i.e., those included in the definition of metabolic syndrome) and hypertension [1-10].

Whatever the etiology or initiating agent and the specific target tissue or organ involved, current literature unequivocally indicate that a feature common to practically all fibrotic diseases is represented by the activation of ECM-producing myofibroblasts (MFs), key profibrogenic cells responsible for disease progression that can originate from different cell sources or processes. The crucial role of MFs in fibrotic diseases is believed to rely on the peculiar responses exhibited by these cells, including the proliferative attitude, the ability to produce ECM components, to migrate in response to chemotactic stimuli and to actively contribute to the modulation of inflammatory/immune response and tissue angiogenesis.

According to the crucial key pathogenic role of MFs in fibrotic diseases progression and the substantial lack of treatment strategies to specifically target the pathogenesis of fibrosis this review is dedicated to offer a number of pertinent information and messages with a major focus on liver, lung and kidney fibrosis. Pertinent to the scope of the present review we will first offer an overview of mediators, molecular mechanisms and cellular interactions (i.e., the pro-fibrogenic

scenario) that are critical in initiating and perpetuating the differentiation of quiescent precursors cells into activated MFs, then favoring disease progression. Since in almost any CID fibrosis is preceded and sustained by inflammatory response a first focus will be on the relationships between activation of innate and adaptive immunity cells and fibrosis regulation [1-12]. The major properties and functional responses currently attributed to the MF phenotype will be then rapidly recapitulated, with a section also dedicated to recall actual knowledge and intense literature debate concerning the heterogeneous cellular origin of MFs. The remaining part of this review will be then dedicated to the analysis of major common (i.e., conserved across different tissues and organs) MFs-related core pro-fibrogenic signaling pathways that can be potentially targeted in order to develop effective antifibrotic therapeutic strategies. We will not here analyze the role of integrins and related signaling pathways, the emerging role of miRNAs or the issues of ECM remodeling and reversion of fibrosis (including killing of MFs and/or their senescence) since these aspects will be specifically addressed by other reviews in this issue.

2. Innate and adaptive immunity - related mediators, signaling and cells in regulating fibrosis

In almost all conditions of tissue injury any significant loss of epithelial and/or endothelial cells is followed by the activation of complex and interrelated wound healing programs that are triggered in order to rapidly restore the original tissue homeostasis, and this always means activation of acute inflammatory response and of innate immunity cells (mainly resident macrophages, neutrophils and dendritic cells). This complex inflammatory response can be triggered by either pathogen-associated molecular patterns (PAMPs) from invading microorganisms and/or damage-associated molecular patterns (DAMPs) released by dead or dying cells, and involves activation, proliferation and recruitment of a variety of hematopoietic cells engrafting the injured tissue (including neutrophils, monocyte/macrophages, B and T lymphocytes, NK cells, mesenchymal stem cells or other myeloid cells) as well as of tissue resident cells (including resident macrophages, fibroblasts or other precursors of MFs, epithelial and endothelial cells, adult stem cells) [1-3,12-14].

The release of cytokines, chemokines, growth factors and other mediators, including reactive oxygen species (ROS) and other redox-related mediators, by either activated/damaged epithelial cells or activated innate immunity cells may also activate the adaptive immune response. The wound healing response is designed to eliminate or counteract the injurious agent or condition as well as to transiently activate at the same time quiescent precursor cells to MFs, that is to drive

angiogenesis, to produce ECM components and release mitogenic growth factors to sustain cellular repopulation of the injured tissue resulting resolution of inflammation and restoration of the normal tissue architecture. However, (see the general scheme in Figure 1) if the agent or condition leading to tissue injury is not removed or contained, chronic tissue injury will ensue leading to a chronic and/or dysregulated wound healing response, characterized by the overlapping of perpetuation of tissue injury, chronic inflammation and then persistent activation of repair and regeneration, resulting in persisting fibrogenesis, fibrosis, functional impairment, tissue/organ failure and death [1-3]. Major events, mediators and cells involved of this pro-fibrogenic scenario are summarized in Figure 1 and briefly addressed in the following subsections, taking in mind a few critical concepts for the progression of fibrotic diseases that can apply to any CID: i) perpetuation of tissue injury results from either chronic exposure to the specific etiology/agent or chronic inflammatory response as operated through several mediators and ROS; ii) chronic recruitment/activation of either innate or adaptive immunity cells can result in a "pro-fibrogenic environment" characterized by multiple interactions between involved cells as well as by the up- and/or dysregulated synthesis and release of cytokines, chemokines, growth factors, ROS and other mediators; this "environment" can favor chronic activation of wound healing and fibrogenesis and significantly impair regeneration/repopulation of the injured tissue; (iii) the effectiveness of "pro-fibrogenic environment, usually paralleled by altered/inefficient remodeling, can be additionally modulated by tissue hypoxia and angiogenesis that, as proposed in chronic liver disease (CLD) progression, have been suggested to sustain and even drive fibrogenesis and vascular changes [15-17]; iv) in defined conditions, as shown by experimental and clinical studies (for example in a pre-cirrhotic state for CLD) [4,5], fibrosis is potentially reversible, with reversion depending on either removal of exposure to the specific etiology or to effective therapy.

2.1 Cells of innate immunity in regulating fibrosis

2.1.1 Monocytes and macrophages in optimal wound healing

Macrophages exhibit a major "regulatory" activity practically in any stage of repair and fibrosis [13,14] and this is due to their ability to display a remarkable plasticity and to adapt their behavior in response to environmental signals and cues, giving rise to different populations of macrophages with distinct functions [18]. Indeed in conditions of tissue injury, particularly if chronic, high numbers of inflammatory monocytes are recruited from bone marrow (typically $Ly6c^{hi}$ and CX_3CR1^{lo} monocytes, expressing receptors for CCL2 and stromal cell-derived factor-1

or SDF1 that operate as major chemoattractants) to the site of injury where often exceed the population of resident macrophages [12,13]. Both recruited and resident macrophages can proliferate and undergo functional changes in response to the overall signals/mediators available in the local tissue environment [19,20] and several laboratories have provided evidence that distinct monocyte and macrophage activation states, with specialized and critically timed roles, operate in potentially interconnected conditions like tissue repair, regeneration, and fibrosis [21-23]. As recently reviewed [13], either tissue resident macrophages or those recruited from bone marrow in a tissue injury environment can undergo critical functional changes (i.e., in response to PAMPs, DAMPs, ROS, GFs, cytokines and chemokines and other mediators) leading to three main dominant phenotypes: i) the inflammatory macrophage, ii) the wound healing or tissue repair macrophage and iii) the resolving macrophage. These distinct phenotypes in an ideal response to injury and under optimal control of tissue repair are timely correlated and involved in a logical sequence, with each phenotype playing specific and critical roles at different stage of the process, although is still unclear whether the single macrophage may undergo conversion of phenotype as a consequence of signals present in the local microenvironment or this may involve distinct functional subsets of monocytes and macrophages. The classical inflammatory macrophage (M1) phenotype, sometime indicated as M(interferon-γ [IFN-γ]) phenotype, is usually the dominant phenotype in the early response following injury. This phenotype expresses receptors for CX3CL1 and CSF1 and originate from the activation of resident macrophages or recruited bone marrow-derived monocytes following interactions with DAMPs, PAMPs as well as other danger signals (ATP, cholesterol or sodium monourate crystals) from the injured microenvironment. In particular, the M1 polarization is mainly oriented through IFN-γ and TLR-activated interferon regulatory factor (IRF) / signal transducer and activator of transcription -1 (STAT1) signaling, and M1 phenotype exhibits high levels of expression and autocrine/paracrine release of major inflammatory cytokines (IL-1β, TNFα and IL-6), high production of reactive nitrogen species and ROS, promotion of Th1 response as well as by a strong microbicidal and tumoricidal activity.

In a later phase the tissue repair macrophage phenotype become predominant in the presence of mainly IL-4, also referred to as alternatively activated M(IL-4)-like phenotype, and/or IL-13 , with both cytokines operating through STAT6 signaling. The activation of the tissue repair phenotype leads to multiple actions/responses operating through the increased expression of several GFs, including platelet-derived growth factor (PDGF), transforming growth factor β1 (TGF-β1), transforming growth factor α (TGF-α), insulin-like growth factor 1 (IGF-1), Wingless-related

integrase 3A (WNT3A), and vascular endothelial growth factor a (VEGF-A). These and other additional mediators can promote i) proliferation of parenchymal and or stromal cells survived to injury, ii) elicit angiogenesis, iii) stimulate fibroblasts and other precursors to migrate and differentiate into MFs as well as, in the presence of severe injury, iv) activation of local adult stem cells and/or progenitor cells. In a final phase the $Ly6c^{lo}$ resolving or anti-inflammatory macrophages, in response mainly to IL-10 (via a STAT3-mediated signaling) and other signals (including possibly phagocytosis), become dominant and offer a critical contribute to switch off the inflammatory response by secreting a variety of anti-inflammatory mediators, mainly IL-10, TGF-β1 and mannose receptor, C type 1 (Mrc1) and by expressing cell-surface receptors like programmed cell death ligands 1 (PD-L1) and 2 (PD-L2) [24-26].

This ideal scenario can be altered by either chronic injury or by dysregulated tissue repair that may result in uncontrolled expression of inflammatory mediators, ROS and growth factors or deficiencies in the generation of inhibitory/anti-inflammatory macrophages, overall leading to chronic inflammatory diseases and fibrosis [13,14].

2.1.2 Pro-inflammatory and pro-fibrotic macrophages in fibrosis

Under conditions of CID two main messages related to the role of macrophage phenotypes are quite clear from the literature: i) pro-inflammatory macrophage can significantly exacerbate tissue injury and then influence the fibrotic progression of the CID; ii) macrophages exhibiting "pro-fibrotic or pro-fibrogenic" properties have been described by several laboratories [12-14, 27]. The first mentioned issue has been unequivocally established by studies showing that progression of the CID and fibrosis can be prevented by impairing the recruitment of destructive monocytes in the injured tissue. As a typical example, studies performed using CCR2 knockout mice and the chronic CCl_4 murine model of liver fibrosis have unequivocally shown that CCL2-mediated bone marrow egression of monocytes and recruitment into chronically injured liver is critical for experimental fibrosis progression. The significant reduction of fibrosis observed in CCR2 knockout mice was depending on the reduced accumulation in injured liver parenchyma of $Ly6c^{hi}/CD11b^{+}/F480^{+}$ macrophages likely derived from CCL2/CCR2 signaling - mediated recruitment of $Ly6c^{hi}$ inflammatory monocytes [28,29]. The population of $Ly6c^{hi}/CD11b^{+}/F480^{+}$ and iNOS producing macrophages was proposed as the main "profibrogenic" macrophage population in the specific CCl_4 murine model of liver fibrosis [29]. Studies performed in relation to fibrosis in other tissues or organs have confirmed macrophages (with $Ly6c^{hi}$ monocyte-derived macrophages still playing a

major pro-fibrogenic role) as potential pro-fibrotic effectors in other conditions of progressive CID involving lung, kidney, heart and skin [30-33].

An exacerbation of the tissue damaging action of pro-inflammatory macrophages may also occur in the presence of factors able to prevent accumulating tissue macrophages from converting from a pro-inflammatory to a reparative phenotype, as shown for different pathological conditions of CID in which a sustained production of pro-inflammatory cytokine by macrophages (like sustained production of TNFα or of IL-1β through NLRP3 inflammasome activation) was the major driver of persistent inflammation and fibrosis [34-36].

According to what proposed some years ago [1], a contribution to the progression of CID towards fibrosis can also rely on the persistent activation or sustained recruitment of M(IL-4)-like cells. A first study supporting this concept was provided for the bleomycin model of lung fibrosis in which the experimental strategy to deplete monocytes and macrophages resulted in the reduction of fibrosis associated with a decrease in the expression of markers of alternative macrophage activation [32]. Very similar results were obtained in experimental models of liver fibrosis following depletion of monocytes and macrophages [12,37]. In several models of fibrosis increased production and activation of TGF-β1 by macrophages [1-9], that can sustain activation of precursor cells into MFs, has been reported to be relevant for fibrotic disease progression, particularly in the early phases of the disease, as specifically shown in models of lung fibrosis mimicking idiopathic pulmonary fibrosis (IPF) [38]. Along these lines, it should be noted that pro-fibrogenic Ly6c^{hi} hepatic macrophages also express high levels of the TGFβ-activating protein thrombospondin -1 [39]. In addition, TGF-β1-driven fibrogenesis in the lung has been reported to be dependent on hypoxia and hypoxia-inducible factor 1α (HIF1α), and indeed silencing of HIF-1α expression reduced the development of bleomycin-induced fibrosis by markedly down-regulating TGF-β1 production in alveolar macrophages [40]. It has also been suggested that macrophages may sustain fibrosis not only by releasing TGF-β1 and other direct profibrogenic factors but also by orchestrating local inflammatory reactions that maintain fibrotic responses or by blocking the emergence of pro-resolution pathways [13,28,41,42].

Macrophage have been reported to affect activation of MFs precursor or to directly modulate MFs responses also by other mechanisms: i) by directly enhancing the survival and activation of MFs, as shown in a murine study for liver macrophages being able to enhance MFs survival through stimulation of nuclear factor kappa B (NF-kB) activity in activated hepatic stellate cells

[43]; ii) by releasing galectin-3, markedly up-regulated in renal fibrosis, which has been shown in a model of renal fibrosis to be critical for the activation of renal fibroblasts to MFs [44]; iii) by producing and releasing, in addition to TGFβ1, a number of critical growth factors, interleukins and chemokines, including the powerful mitogen PDGF (also chemotactic for MFs) and of course the classical Th2 cytokines IL-4 and IL-13 which can also directly stimulate the synthesis of collagen type I by MFs, as shown for example for hepatic MFs [12,13]; to this list one should add critical chemokines like CCL2, CCL8 and CCL7 (formerly known as MCP1, MCP2 and MCP3, respectively) that are all able to recruit MFs [1-6,12,13].

2.1.3 Role of other innate immunity cells in CID and fibrosis

Although macrophages display a major role, some studies have implicated in progressive fibrosis other cells of the innate immunity. Evidence that neutrophils may play some role in fibrogenesis is quite limited and sometimes controversial, at least in some tissue or organ. For example, in two liver-centered experimental studies using two different efficient strategies to deplete or limit the number neutrophils (an antiserum to neutrophils or the use of CXCR2 – deficient mice) no significant prevention in fibrosis was reported [45,46]. Indeed, for the liver at least this seems consistent with the view that neutrophils may rather contribute to collagen degradation during resolution by releasing matrix metalloproteases (MMPs) [47]. More convincing data supporting a pro-fibrotic role of neutrophils have been provided by a study performed in the murine models of bleomycin- and hypersensitivity pneumonitis–induced pulmonary fibrosis [48].

A putative pro-fibrogenic role has been suggested for liver dendritic cells (DC) [49] which are known to significantly modulate hepatic immunity [12,50]. By using two murine models of liver fibrosis these authors showed that fibrosis development is associated with a significant expansion of DC and, in turn, that an efficient depletion of DC was preventing fibrosis. Interestingly, the action of DC in fibrotic liver was attributed to the ability to stimulate surrounding non parenchymal cells to produce inflammatory mediators (mainly TNFα and IL-6) as well as to induce hepatic stellate cells, NK cells, and T cells to express and release several mediators that modulate positively inflammation and proliferation with the overall result to trigger a potent immune response.

2.2 Cells of the adaptive immunity in regulating fibrosis

Several studies performed on different models of tissue fibrosis or, where available, in the correspondent human conditions, have shown that almost all functional lineages of cells derived by

naïve $CD4^+$ lymphocytes under conditions of tissue injury and involved in adaptive immunity, can have a role in regulating fibrosis [1].

A first general message is that a T_H2 cytokine response, mediated by IL-4, IL-5 and IL-13, can usually act as a potent driver of progressive fibrosis [1,12,51]. In particular, IL-13 has emerged as a major T_H2 cytokine in mediating fibrosis as shown in different models, tissue and organs [1] including liver fibrosis of different etiology [52-54], IPF and experimental lung fibrosis [55,56]. The action of IL-13 has been attributed either to its ability to stimulate the production and activation of TGF-β [57] as well as to promote fibrosis by directly activating the synthetic and proliferative properties of MFs or related precursors cells [58-61]. In particular the pro-fibrogenic action of IL-13 is controlled by the abundance of the IL-13Rα1 signaling receptor and IL-13Rα2 decoy receptor expressed on MFs, with fibrosis being exacerbated when the expression of the decoy receptor is low or absent [62-64]. However, it should be noted that a murine study performed in mice deficient in IL-13Rα2 showed that these mice were more resistant to IL-1β- and IL-17-driven inflammation, a data that indicate that IL-13Rα2 is likely to operate as a critical regulator of both T_H17-mediated inflammation and T_H2-driven fibrosis [65].

The overall scenario is even more complex since T_H17 - related response has been described as both pro-inflammatory and pro-fibrotic [1]. $CD4^+$ T_H17 lymphocytes represent a subset of T helper lymphocytes expressing the pro-inflammatory cytokine IL-17A, the latter emerging as an additional pro-fibrogenic mediator whose expression has been described to be involved in lung, liver and myocardial fibrosis [66-68]. This is associated with neutrophilia and increased recruitment of neutrophils that contribute to tissue injury and fibrosis as well as mortality, as shown in lung fibrosis and in patients affected by IPF [69-71]. Of interest, upregulation of IL-17A and then the T_H17 - related response has been shown to be upstream mechanistically related to the pro-inflammatory cytokines IL-1β and IL-23 [66,72] and is known to linked also to TGF-β1.

In a sharp contrast with T_H2 and T_H17 immunity, T_H1 response has been reported to mainly exhibit anti-fibrotic activity in liver, lung and renal fibrosis as a consequence of the release of IFN-γ by T_H1 lymphocytes [73-75] as well as by natural killer (NK) and natural killer T (NK-T) cells [76,77]. The anti-fibrotic action of IFN-γ is a complex one and operates by exerting an inhibitory action on the TGF-β1 pro-fibrotic activity, by inhibiting phosphorylation of Smad3 and related activation of TGF-β1-dependent genes (for example procollagen type I and III), or by a pathway involving Janus-associated kinase (Jak1)/Stat1 signaling resulting in up-regulation of Smad7

expression that prevents the interaction of Smad3 with the TGF-β receptor [78,79]. Finally IFN-γ has been also reported to prevent the differentiation induced by T_H2 cytokine–induced of $CD14^+$ peripheral blood monocytes into fibrocytes, a population of fibroblast-like cells believed to contribute to fibrosis progression in many tissues and organs [80].

A peculiar role is perhaps exhibited by $CD4^+/CD25^+/Foxp3^+$ T regulatory (Treg) cells that, significantly induced in fibrotic diseases, have been reported to either suppress or promote fibrosis. According to their ability to release immunosuppressive cytokines like IL-10 and TGF-β1, Treg cells should be able to inhibit progressive fibrosis by mainly affecting the inflammatory response as proposed for different different fibrotic diseases, including HCV-related liver fibrosis and fibrosis in IPF patients [81,82]. However, the role of Treg cells is still unclear since some studies reported a rather opposite scenario, suggesting that Treg cells, possibly through their ability to synthetize and release TGF-β1, may operate as pro-fibrogenic cells [83,84].

3. Myofibroblasts: heterogeneous cellular origin and major phenotypic responses

MFs represent critical cells in wound healing response since they actively regulate connective tissue remodeling by uniquely combining the ECM-synthesizing features of fibroblasts with the peculiar cytoskeletal characteristics of contractile smooth muscle cells. They are easily recognized by the immune-positivity for α-smooth-muscle actin (α-SMA), the most reliable in vivo marker for these cells and a relevant player in contractile force production [85,86]. As a matter of fact, α-SMA expression is controlled (either activated or repressed at the level of its promoter) by a number of transcription factors and is also epigenetically regulated [reviewed in 86]. MFs represent, in any tissue or organ analyzed, a heterogenous population of α-SMA-positive cells and they can originate from fibroblasts but also from several other cell sources [1-10,15,85-89]. Fibroblasts and other precursor cells are known to undergo controlled and transient activation into the MF phenotype during normal tissue repair, particularly following an acute tissue injury, which is a fundamental step for restoring tissue integrity [85,86]. Once restored the original condition, this transient activation is followed by the disappearance of MFs either by apoptosis or by a reversion of the phenotype. However, as it can happen under conditions of severe or chronic injury as well as of dysregulated wound healing response and in the presence of the "profibrogenic environment", the activity of MFs becomes excessive and persistent through time, significantly contributing to tissue/organ fibrosis and dysfunction [1-10,85,86].

3.1 Cellular origin of MFs in progressive, fibrotic chronic inflammatory diseases

3.1.1 The cellular sources of hepatic MFs

For a pathologist the term "hepatic myofibroblast" is a very familiar one which applies to an heterogeneous population of α-SMA-positive cells sharing a mesenchymal-like ultrastructural phenotype and easily identified by immunohistochemistry in fibrotic and cirrhotic human liver specimens [4-6,12,15,87, 90-93]. Hepatic MFs are highly proliferative and contractile cells able to contribute to liver fibrogenesis and CLD progression whatever the etiology, then including conditions of chronic by hepatitis B and C viruses, non-alcoholic fatty liver disease or NAFLD, chronic exposure to drugs or toxins (mainly excess ethanol consumption in western countries), autoimmune-mediated injury (primary biliary cirrhosis or PBC, primary sclerosizing cholangitis or PSC) or inherited diseases (hereditary hemochromatosis, Wilson's disease). According to a common scheme, that applies also for other tissues and organs, hepatic MFs, as effectors of liver fibrogenesis, can act as a cellular crossroad that integrate incoming paracrine or autocrine signals (including growth factors, pro-inflammatory cytokines, chemokines, proangiogenic mediators, adipokines, ROS and others) released from any hepatic (and extrahepatic) cell populations involved and/or available in the chronically injured microenvironment [4-6,12,15]. In this brief section we will just recapitulate present knowledge concerning the cellular origin of hepatic MFs.

Hepatic stellate cells (HSCs) are actually considered as the major source of liver MFs and in normal liver they reside in the subendothelial space of Disse, are characterized by the presence of cytoplasmic processes defined as intersinusoidal (or interparenchymal) and subendothelial that establish contact with hepatocytes, liver sinusoidal endothelial cells (LSECs), other adjacent HSC and nerve endings [94-96]. HSC in normal liver act as liver specific pericytes, store and metabolize vitamin A and retinoids and synthetize and remodel ECM components in the space of Disse. Since the previous review article in this issue is specifically dedicated to this peculiar liver cell type [97] here it is sufficient to say that extensive literature data indicate that hepatic MFs originate in CLD of any etiology mainly from HSCs through a process defined as activation/trans-differentiation. This interpretation has been emphasized by an elegant fate tracing experimental study that, by employing a novel Cre-transgenic mouse able to label 99% of HSCs, showed that as much as 82-96% of liver MFs originated directly from HSC in different experimental murine models of chronic liver injury usually employed to reproduce human CLD of toxic, cholestatic and metabolic (NAFLD/non alcoholic steatohpeatitis or NASH) origin [98].

Hepatic MFs have been also reported to originate from portal fibroblasts, which are liver resident fibroblasts located in the portal tract mesenchyme that surrounds bile ducts and express a rather specific marker profile including fibulin 2, elastin, IL-6, cofilin 1 and the ecto-ATPase nucleoside triphosphate diphosphohydrolase-2 (NTPD2) [99,100]. These cells have been originally suggested as a significant source of MFs during the course of biliary fibrosis but fate tracing studies, reviewed in ref. [100], including the one mentioned before [98], have somewhat reduced the emphasis on this point. However, is still interesting and re-evaluated the hypothesis [101] that portal fibroblasts and MFs may represent the earliest cell populations activated after injury to the biliary epithelium; indeed it has been suggested that HSCs and portal fibroblasts may occupy different niches, with the HSCs niche being induced by hypoxia during liver parenchymal injury and the portal fibroblast niche by the ductular reaction following biliary injury [102]. According to the latter interpretation, MFs from HSC may mediate liver wound healing whereas portal MFs may regulate scar formation.

Hepatic MFs have been also described to originate from bone marrow-derived cells (BMdC) with the original study describing that a significant percentage of MF-like cells in human livers were positive for markers that could only derive from BMdC recruited in the chronically injured parenchyma (i.e, carrying Y chromosome, as found in the liver of females that received a bone marrow transplant from male donors before developing HCV-related CLD) [103]. Other reports confirmed this hypothesis suggesting that hepatic MFs may originate from either mesenchymal stem cells [104,105] or from α-SMA negative BMdC defined as fibrocytes [106] recruited in the chronically damaged liver parenchyma. However, there is agreement that the overall quantitative contribution by BMdC to MFs population is quite limited [87,107].

Finally, as for other types of organ fibrosis, MFs have been suggested to originate from either hepatocytes or from biliary epithelial cells following a process of epithelial-to-mesenchymal transition (EMT). This issue is highly controversial for CLD, as for other tissues and organs, and has generated an intense debate in the field [4-6,10,15,87,92,93,108]. The prevailing view in the field is that the involvement of EMT as profibrogenic mechanism in progressive CLD is likely, if any, of minor relevance, as suggested by several elegant but mostly negative fate tracing studies [98,109-112]. However, as a note of caution, although at present no relevant liver-related studies have been produced, it should be noted that very recently major concepts related to EMT process have been re-evaluated suggesting that EMT should be envisaged as "a set of multiple and dynamic transitional states between the epithelial and mesenchymal phenotypes", as opposed to the original idea of "a process involving a single binary decision" [113]. In particular, some studies performed

on experimental models of chronic renal injury have proposed that, at least for kidney fibrosis, EMT and its intermediate states may be involved through a partial activation of the EMT program (reviewed in ref. [113,114]).

3.1.2 The origin of MFs in renal fibrosis

Renal fibrosis is a common end-point of several chronic kidney disease (CKD) in which aberrant and excessive deposition of ECM components is usually appreciated in both glomeruli and interstitial regions, with major causes of CKD being diabetes, hypertension, HIV infection and obesity, or consequent to primary kidney like focal segmental glomerulosclerosis and acute kidney injury [115-120]. As in CID of other tissues and organs, α-SMA positive MFs have been recognized as major ECM producing cells and then actively contributing to fibrosis. In CKD settings MFs can be found in the renal interstitium and, although to a less extent, in glomeruli in different animal models of renal fibrosis. Similar to what previously described for hepatic MFs, also in renal fibrosis the contribution of various precursor cells to the development of kidney MFs and renal fibrosis is still a matter of intense debate and controversy [9,10,121-126]. This may depend on different aspects, including the particular protocol of kidney chronic injury employed as well as the choice of the genetic background of mice (including transgenic mice) used in the experiments, with C57Bl6 background, used for example by the group of Duffield [121], considered as more correct to develop features of CKD than the BalbC background used by others [125]. Critical studies performed on properly designed transgenic reporter mice expressing green fluorescent protein (GFP) under the control of collagen 1a1 promoter and enhancer (col1a1-GFP mouse) showed that GFP-positive collagen 1a1-producing cells overlapped remarkably with α-SMA positive cells, with just 1% of GFP positive cells being α-SMA negative. However, approx. 25% of α-SMA positive cells resulted negative for GFP, demonstrating that kidney MFs may represent a heterogeous cell population [121,122]. At present five major cellular sources of MFs have been proposed in these years [9,10,121-126].

Kidney resident fibroblasts, routinely identified in vivo as cells positive for PDGFR-β and CD73 and the absence of markers for other cell lineages [10,122], were the first cells proposed as kidney MFs precursors. Kidney fibroblasts residing in the interstitium synthetize and remodel interstitial ECM, communicate with either epithelial cells and endothelial cells and then contribute to tissue homeostasis. In addition, certain subpopulations of renal fibroblasts in the deep cortex and outer medulla of the kidney produce erythropoietin (EPO) in response to hypoxia; moreover, these EPO-producing fibroblasts under hypoxia proliferate and spread from the deep cortex to the capsule. A study employing transgenic mice allowing to trace EPO-producing resident fibroblasts

has shown that these cells should represent the main source of MFs in three distinct experimental models of kidney fibrosis, including unilateral ureteral obstruction (UUO), folic acid nephropathy, and severe ischemic–reperfusion (IR) injury [127]. However, other Authors, by using multiple genetically engineered mice, have proposed that MFs derived from resident fibroblasts may account up to 50 % of all α-SMA positive cells detected in the UUO experimental murine model of kidney fibrosis [125], with the remaining MFs deriving from bone marrow precursors (35%) or following either endothelial-to-mesenchymal transition (EndoMT, 10%) or EMT program (less than 5%). Intriguingly, it has been reported that medullary MFs (not cortical ones) derived from resident fibroblasts uniquely express Wnt4 [128] and that a subset of medullary fibroblasts seems to share characteristics in common with hepatic stellate cells, including ability to store vitamin A and express cytoglobin, the latter being still expressed by MFs derived from these splanchnic fibroblasts in the UUO model [129].

Another potential source of MFs in CKDs has been identified in pericytes, although this hypothesis is mainly based on a single relevant lineage-tracing study by Humphreys et al. [130] that, originally designed to investigate whether tubular epithelial cells may become fibroblasts through an EMT process, in the end concluded that a very significant number of αSMA-positive MFs apparently originated from pericytes in the UUO model and under conditions of ischemia reperfusion (IR) injury. Indeed, as discussed in ref. [10], the possibility exists that, as proposed for other tissues and organs, pericytes and resident (i.e., perivascular) fibroblasts in the kidney may represent overlapping rather than distinct cell populations, both contributing primarily to kidney MFs population, as reported in a study of some years ago [121].

Different laboratories, by using several transgenic mice for lineage tracing and various protocols to induce experimental CKDs, have provided consistent evidence indicating that a percentage of MFs, variable from 8-10% up to 20-50% depending on the specific mice model and protocol adopted (most studies employed the UUO protocol of kidney injury), may originate from bone marrow - derived cells usually referred to as fibrocytes due to their nature of collagen-producing cells of hematopoietic origin (reviewed in ref. [10] and references therein). This general feature has been confirmed in a paper investigating a quite large number of specimens from patients with different types of CKD [131], and just a single negative experimental study has been reported in the literature [121] in which less of 0.1% of MFs was suggested to derive from fibrocytes. However, since fibrocytes and monocytes are cells that share common markers (CD45, CD11b, CD16/32, CD68, major histocompatibility complex II, and fibroblast-specific protein 1), the unresolved question here as well as in other tissues/organs is to establish whether (and how many)

fibrocytes are a subpopulation of collagen-producing monocytes or macrophages or may develop, under the pressure of a pro-inflammatory and pro-fibrotic microenvironment, from monocytes recruited to the site of injury [132-134].

Whether kidney MFs may derive from epithelial kidney cells through an EMT process is, similarly to what described for CLDs, highly controversial and a matter of intense debate [9,10,119-124]. Although pioneer studies in the field proposed EMT as a major process sustaining fibrosis progression in chronically injured kidney [135-137] more recent rigorous lineage fate tracing studies offered a rather different scenario suggesting either a minor (i.e., less than 5% MFs derived via EMT) [125] or absent contribution of EMT to kidney MFs population [130,138,139]. However, as previously mentioned and reviewed [113,114], two recent studies have proposed that in conditions of CKD just a partial EMT program may be activated. These studies suggested that although renal epithelial cells may not directly generate MFs, deletion of Twist or Snail (i.e., major EMT-related transcription factors) in renal epithelial cells can significantly reduce interstitial fibrosis in different murine models like UUO, folic acid administration or nephrotoxic-serum-induced nephritis [140,141]. These studies suggested, in particular, that renal epithelial cells may undergo a partial EMT, with one study pointing to perturbations in transporter proteins and cell-cycle regulation [141] and the other study showing that epithelial cells, although losing epithelial markers, remained integrated in the tubules [140]. These studies suggested that injured epithelial cells may still release mediators and/or exosomes to the interstitium to promote the differentiation of fibroblasts into MFs, as well as recruitment of BMd cells and macrophages, then overall sustaining fibrogenesis.

A somewhat similar debate is active on the hypothesis that kidney MFs may also originate from endothelial cells through the involvement of a process similar to EMT and defined as endothelial-to-mesenchymal transition (EndoMT). Here however, although some criticisms to protocols and murine models has been raised, data supporting the role of EndoMT seem more consistent than those supporting EMT [142-144] with the proposed percentage of MFs derived from endothelial cells ranging from less than 10% [125] up to 25 % [144].

3.1.3 The origin of MFs in lung fibrosis

Pulmonary fibrosis occurs in humans in a variety of clinical settings, constituting a relevant cause of morbidity and mortality, with characteristic features including focal accumulation of cells with fibroblast/MFs-like morphology and excessive production and deposition of ECM components [7,8]. Pulmonary fibrosis is indeed heterogeneous and, as recently reviewed, at least 3 distinctive

pathologic patterns of pulmonary fibrosis can be recognized, including usual interstitial pneumonia (UIP), fibrotic non-specific interstitial pneumonia (fNSIP) and airway-centered fibrosis (ACF) [8]. In addition, distinctive histopathological characters are also based primarily on the distribution of lung fibrosis, either diffuse or patchy, as well as on the anatomic fibrosis location that could be either interstitial, air centered or subpleural/paraseptal. Although several form of lung injury and etiologies (autoimmune, environmental, infective, etc.) have been reported to result in pulmonary fibrosis and a certain degree of histopathologic overlap between the major types of pulmonary fibrosis may occur, the UIP pattern is typically observed in the lung of patients with idiopathic pulmonary fibrosis (IPF) [7,8,145]. IPF, in particular, is a fatal lung disease of still uncertain etiology which, in addition to the presence of fibroblast foci, is recognized by the peculiar histopathological character of microscopic honeycomb remodeling (or bronchiolization) of alveoli [146,147]. Concerning the other two major patterns, the fNSIP pattern is primarily seen in association with connective tissue disease and, to a less extent, to other etiologies, whereas the prototype of ACF pattern is usually observed in patients with chronic hypersensitivity pneumonitis (CrHP) [8].

Not surprisingly, the origin of lung MFs is currently believed to be as heterogeneous as reported for CLD and CKD. Once again resident fibroblasts [148] and/or adventitial fibroblasts of blood vessels [149] (pericytes?) have been the first cells proposed as the most obvious source of α-SMA positive MFs, particularly in the bleomycin experimental model of lung fibrosis. It should be underlined that the bleomycin-induced pulmonary fibrosis in mice, which is widely employed as an in vivo experimental model for IPF, whether administered intra-tracheally or systemically, indeed results in the development of patchy fibrotic lesions followed by slow repair, i.e., a pattern quite far from the progressive pathological remodeling typically seen in human pulmonary fibrosis [150].

As a second putative source of lung MFs some Authors proposed the already mentioned population of bone marrow - derived fibrocytes, known to be recruited in injured lung. This hypothesis was first proposed by an in vitro study employing fibrocytes (i.e., BM-derived cells characterized as positive for collagen I, CD11b, CD13, CD34, CD86 and MCH class II antigens) [151]. Later, an identical conclusion was drawn from a study in which adult mice were durably engrafted with BM-derived cells obtained from GFP-positive transgenic mice: in these engrafted/chymeric mice, exposure to bleomycin resulted in an increase of GFP(+) cells also expressing type I collagen; however these cells, when isolated and cultured, did not express α-SMA [152]. Other studies have confirmed this hypothesis either experimentally [153,154] or by identification of fibrocytes in the blood of patients with IPF [155,156].

As for CKDs and CLDs, it was then later proposed by several laboratories that the cellular origin of at least part of pulmonary MFs was represented by lung epithelial cells, including type 2 alveolar epithelial cells (AEC2) [157-162] through an EMT process. These studies were either performed in vitro or by using more specific in vivo lineage-tracing experiments using mainly the bleomycin protocol to induce lung fibrosis. In some of these studies [160,161] Authors estimated that EMT of alveolar epithelial cells may have contributed up to 50% of the population of pro-fibrogenic cells in experimental conditions of bleomycin-induced lung fibrosis. These data were supported by human data that were obtained by immunohistochemical analysis of archive specimens, with the expression of EMT-associated transcription factors, such as Snail, Slug and Twist or images showing "transitional cells" being presented as evidence for the involvement of EMT. More specific studies have reported less enthusiastic data regarding the involvement of EMT process in lung fibrosis and the excellent report by Rock et al. [163] may serve as a relevant and reliable example. In this study authors employed different genetic tools and transgenic mice in order to follow the fates of specific cell types in the bleomycin-induced model of pulmonary fibrosis to finally reasonably exclude either a quantitatively major origin of α-SMA positive MFs from either pericyte-like or, by using two different lineage tracing approaches, AEC2 cells. Rather, these Authors described that under experimental bleomycin-induced injury, AEC2 cells were significantly converted into AEC1 cells.

3.2 Major phenotypic responses operated by MFs

As mentioned in the previous sections, the cellular source of MFs during the development of organs fibrosis is currently believed to be heterogeneous and represents a matter of intense debate and controversy. What is clear is that in different tissues/organs a complex network of intracellular events can induce and sustain the so called process of activation of putative precursor cells into MFs which includes regulatory controls affecting transcription, translation, post-translation and epigenetics, among others, representing the response of precursor and/or transiently activated cells to the profibrogenic environment. However, although unavoidable differences exists between liver, kidney and lung MFs [1-14], at least some of the major phenotypic responses operated by these profibrogenic cells, during and/or following the process of activation, may be considered as remarkably similar and sometimes homologous, whatever the cellular source and organ involved. In this section we will briefly recapitulate the most relevant and common phenotypic responses (Figure 1) operated by a prototype MF during tissue/organ fibrosis under the pressure of several mediators (including cytokines, growth factors, chemokines, vasoactive peptides, angiogenic factors, ROS and others) as a necessary introductory piece of knowledge for the following

paragraphs on core targetable signaling pathways. Of course this will unavoidably exclude some responses that may be more relevant for some specific MF-like cell (for example, the contractility of hepatic MFs).

3.2.1 Synthesis and remodeling of ECM

Increased synthesis of ECM components is one of the most obvious profibrogenic actions of any activated MF in a scenario of persistent chronic injury, with TGF-β1 being by far the most potent cytokine able to enhance production of fibrillary collagens (mainly Type I and III), α-SMA, laminin and fibronectin (to name few relevant examples), and overall to drive differentiation of putative precursor cells into MFs and perpetuate their activation [1-10, 15,17,164-166]. This can be associated, as shown in hepatic MFs, to a dysregulation of the expression of genes coding for enzyme involved in ECM remodeling, leading to an up-regulation of the expression of tissue inhibitors of metalloproteases (TIMPs) and a down-regulation or inefficient removal of excess fibrillary collagen by metalloproteases (MMPs) [3-6,15,17]. TGF-β1 can be released by either resident macrophages or activated macrophages following monocyte recruitment from peripheral blood as well as by activated MFs in an autocrine/paracrine loop. Several other mediators have been proposed to affect the expression of either some ECM components and/or MMPs or TIMPs involved in ECM remodeling but of particular relevance is the case of ROS, either released by injured epithelial/parenchymal cells or overproduced in relation to the activation of critical NADPH-oxidase isoforms, associated to the interaction of several ligands (growth factors, cytokines and other active peptides) with their cognate receptors. Indeed, several cells in the different tissues/organs, including epithelial cells, macrophages, endothelial cells as well as MFs and infiltrating leukocytes, can express multiple NOX isoforms, either phagocytic or non-phagocytic, being then able to contribute to ROS generation [1-10, 15,17,164-170]. As an example, ROS as well as other oxidative stress - related mediators like the aldehydic end-product of lipid peroxidation 4-hydroxy-2,3-nonenal (HNE) have been reported to up-regulate expression of pro-collagen type I and TIMP-1 and MCP-1 (CCL2) by activated-, MF-like, HSCs (HSC/MFs), possibly through activation of specific signal transduction pathways and transcription factors, including activation of JNKs, AP-1 and, only for ROS, NF-kB [3-6,15,17,167].

3.2.2 Proliferation and survival

The MF mesenchymal-like phenotype during the progression of CID is usually characterized by a high proliferative attitude which is the net result of increased availability in the profibrogenic

environment of growth factors released by surrounding cells and able to stimulate mitogenesis, as well as an increased expression of related receptors expressed by MFs [1-10, 15,17,164-166]. The most potent mitogen for transiently activated precursor cells and MFs is represented by platelet-derived growth factor (PDGF), with increased expression of the corresponding α- or β-receptor subunit (PDGF-Rα or PDGF-Rβ) representing a common marker of activated precursors or MFs. This is relevant since PDGF, in addition to stimulate proliferation, is also a potent chemoattractant for these cells as well, more generally, for cells of mesenchymal origin. Moreover, at least for hepatic HSC/MFs, autocrine/paracrine expression of PDGF and up-regulation of related receptors is sustained by TGF-β1. Other growth factors and mediators have been reported to stimulate proliferation of MFs although the mitogenic mediators may vary according to the specific tissue involved. As an example of this complexity, liver activated and MF-like HSC (HSC/MFs) [4-6,11,12,15, 171]) have been reported to proliferate in response to a long list of mediators including at least transforming growth factor (TGF)-α, epidermal growth factor (EGF), thrombin, keratinocyte growth factor, connective tissue growth factor (CTGF), now renamed as CCN-2 (one of the six homologous matricellular and cysteine rich proteins belonging to the novel CCN family) and hereafter indicated as CTGF/CCN-2, bFGF and the adipokine leptin. As a further example, in IPF the list of other peptide mediators proposed to stimulate lung MFs to proliferate (i.e., in addition to PDGF), can include members of the IL-6 family (IL-6, IL-11 and oncostatin M or OSM) and CCL21 through the CCR7 receptor (reviewed in ref.[172]). According to this extremely complex scenario, several of the signals than can induce MFs to proliferate (plus likely TGFβ1) are also believed to concur to the increased survival attitude of MFs that in their state of persistent activation are characterized by resistance to the induction of apoptosis, allowing these cells to survive in a potentially hostile environment like the one usually found in CID [1-10, 15,17,164-166]. Human HSC/MFs, for example, have been reported to be resistant to most pro-apoptotic stimuli due to Bcl-2 overexpression and up-regulation of PI3K/C-Akt signaling and to survive to significantly high levels of ROS [173,174], with several mediators (in addition to mitogens and TGFβ1) suggested to operate as survival signals for hepatic MFs (see ref. [164].

3.2.3 Pro-inflammatory role

MFs, by expressing relevant receptors when activated, represent a target for several cytokines and other inflammatory mediators during the development of any CID. In turn, persistently activated MFs have been shown to also actively contribute to sustain inflammatory response by releasing critical pro-inflammatory mediators with two major examples being represented by the ability to release the two chemokines CCL2 and CCL21 [1-10, 15,17,164-166,

171,172]. In addition, several laboratories (reviewed in ref. [175,176]) have reported that inflammasome activation in either macrophages and MFs, particularly NLRP3 inflammasome, by either DAMPs released by damaged epithelial cells or PAMPs, may contribute to fibrosis in different tissues. Since inflammasome inhibition has been described to reduce for example lung fibrosis MFs may then also contribute to fibrosis progression at least by releasing IL-1β, possibly in relation to ROS levels and NADPH oxidase involvement (reviewed in ref. [175]). As a matter of fact, the ability of MFs to produce and release cytokines, interleukins and chemokines is also relevant in regulating and/or modulating interactions with cells of innate and adaptive immunity [1-14].

3.2.4 Migration in response to chemoattractants or ROS

An additional typical feature of activated precursor cells and/or of MFs is represented by the acquisition of the ability to migrate in the scenario of chronic tissue injury. This occurs in response to a number of chemoattractant peptide mediators released in the profibrogenic environment by other cell populations (epithelial cells, macrophages, endothelial cells, platelets and immune cells) or by activated MFs themselves in a autocrine/paracrine loop, with part of these mediators being entrapped in the ECM [1-10,17,164]. The list of chemoattractants effective on MFs is quite impressive but if one has to refer to the most ascertained ones in different tissues PDGF can be considered as the most potent chemotactic signal. A minimal list of other chemoattractants able to stimulate MFs oriented recruitment/migration should at least include CTGF/CCN-2 as well as several chemokines of the CC- and CXC-chemokine receptor families, and of course related chemokine receptors [1-10,17,164,177]. Concerning CC-chemokines, a major role has been reported for CCL2 (monocyte-chemoattractant peptide -1 or MCP-1), CCL3 (macrophage inflammatory protein 1α or MIP-1α) bur with studies in different condition of organ fibrosis also involving CCL4 (MIP-1β), CCL20 (MIP-3α), CCL21, CCL11, CCL22 (macrophage-derived chemokine) and CCL6 (C10), among others [177]. Involvement of CC-chemokines in fibrosis has been reported to be regulated by IL-4 and IL-13 levels. For hepatic MFs, proangiogenic peptides like VEGF-A and Angiopoietin I as well as Angiotensin II can exert chemotaxis, with all these chemoattractants, including also PDGF and CCL2, involving NADPH oxidase-dependent intracellular generation of ROS through activation of ERK1/2 and JNK1/2 signaling pathways [177]. It should be noted that any significant increase in the intracellular levels of ROS can elicit migration of hepatic MFs [178] as also confirmed following exposure to hypoxic conditions, where MFs migrated by involving an early, mitochondrial-dependent ROS-mediated activation of ERK

and JNK, followed by a delayed- and HIF-1α-dependent up-regulation and release of VEGF [179,180].

3.2.5 Proangiogenic role

MFs can contribute to CID progression by modulating angiogenesis and vascular remodeling as in IPF, liver fibrosis and many other fibrotic diseases [1-10, 16,17, 164,177], with hypoxia-dependent angiogenesis and vascular remodeling often occurring prior to the development of fibrosis or, according to some views related in particular to liver angiogenesis in CLDs, driving tissue fibrosis [179,180-182]. The attention to angiogenesis in CLDs is justified by the fact that HSC in physiological conditions have been described to behave also as liver specific pericytes being in strict contact with sinusoidal endothelial cells [4-6,15-17]; indeed HSC and HSC/MFs, as well as other MFs in different tissues [1-10,16,17,164,177], synthetize and release several proangiogenic peptides like VEGFA, Angiopoietin 1 or 2, PDGF-BB, hedgehog ligands and also express related receptors representing then also a cellular target for these mediators [179-185]. These proangiogenic factors are believed to enhance the fibrogenic properties of MFs and to contribute to angiogenesis by eliciting paracrine signals affecting neighboring endothelial cells.

4. Major targetable pro-fibrogenic signaling pathways

Available evidence suggests that tissue/organ fibrosis is often a dynamic and, at least for some conditions, potentially reversible process. Almost all researchers working in the field are making serious efforts in order to identify targetable mechanisms, signaling pathways and mediators involved in fibrosis progression and/or resolution. A major obvious translational objective, in addition to improve the ability to non-invasively assess fibrosis, is to use this knowledge to develop selective and effective antifibrotic drugs to be then tested in clinical trials. Indeed, although several pre-clinical studies offered promising results, for most of the relevant conditions of CID we have to face a lack of pharmacological agents officially approved as "antifibrotic", with the exception of few drugs recently approved for the therapy of pulmonary fibrosis [1-10,15,17,164,177,186]. Taking into account that the best therapeutic option, whenever possible, is of course represented by the control and/or cure of the primary disease (by either eradicating the microbial/viral etiological agent, removing the exposure to exogenous or endogenous toxic compounds, suppressing immune response, affecting metabolic dysregulation, etc.) here we will analyze the most relevant and common core profibrogenic signaling pathways (summarized in Figure 2) involved in the progression of fibrosis in major organs like at least liver, lung and kidney. In particular, the focus will be on the pathways that in some way affect the

behavior of MFs and of their precursor cells within a list that is now becoming impressive (see a full list in ref. [186]). Whenever possible, we will also offer examples of findings from experimental studies that have been translated into human clinical trials for some specific CID.

4.1 Classic, growth factor-stimulated, profibrogenic signaling pathways

4.1.1 TGFβ profibrogenic signaling pathways

TGFβ is a member of the TGFβ superfamily, a highly conserved group of cytokines with three major isoforms described in mammalians (TGFβ1, -β2 and -β3). Of these isoforms TGFβ1, a ubiquitously expressed pleiotropic cytokine, is the most potent and best characterized profibrogenic factor playing a role of "master cytokine" in any human or experimental condition of organ/tissue fibrosis including then not only liver, pulmonary (both parenchymal and airway fibrosis) and renal fibrosis [1-15, 17,97,164,177] but also fibrosis of skin, heart, pancreas and gut (reviewed in ref.[186]). In addition and integration of what already previously described in this review concerning the role of this factor, TGFβ isoforms are produced by a wide variety of cell types, including in the major organs at least macrophages, MFs, endothelial cells and activated/damaged epithelial cells. When activated, particularly TGFβ1, can affect mainly macrophage and MFs by resulting in their recruitment, by leading MFs to proliferate through PDGF expression as well as by stimulating both cell type to express and release several other proinflammatory and fibrogenic cytokines [1-15,17,97,164,177,186]. TGFβ1 overall works either as a major initiating signal, driving precursor cells towards the activated MF phenotype, as well as a major mediator able to enhance and perpetuate the fibrotic response.

TGFβ1 is secreted in a latent form as a complex with the latency-associated peptide (LAP) and the active TGFβ1 itself, with LAP forming disulfide bonds to members of the latent TGFβ-binding proteins that are cross-linked to ECM proteins. This results in TGFβ1 being stored in the latent form in the ECM matrix from where it could be retrieved/activated through different mechanisms such as proteolysis by several proteases (MMP2 and MMP9, plasmin, tryptase, thrombin and elastase), acidification or oxidation, as well as interaction with integrins and thrombospondin [187-189]. Concerning integrins, for example, in some organs like kidney, liver and the biliary tract, damaged epithelial cells have been reported to directly activate TGFβ through $\alpha_v\beta_6$ integrin which is not expressed by epithelial cells in other organs. Interestingly, MFs can activate TGFβ using their own, even distinct, integrins with lung MFs being able to activate TGFβ using multiple integrins that all share the α_v subunit [190]. Once activated the TGFβ isoforms can then act on surrounding cells expressing the specific receptors activating canonical or non-canonical signaling pathways [187-189].

In the so-called canonical pathway TGFβ isoforms binds to two receptors containing serine/threonine kinase domains in their intracellular portions, known as TGFβ receptors type I (TGFβR-I) and type II (TGFβR-II), the latter being the specific receptor for TGFβ ligands. TGFβR-I is phosphorylated by TGFβR-II on ligand binding and mediates specific intracellular signaling through phosphorylation of Smad2 and Smad3. Phosphorylated Smad2/3 then interacts with Smad4 to form a complex that, following nuclear translocation, can interact with other DNA-binding transcription factors, co-activators and co-repressors, to finally binds to the promoters of TGFβ target genes, including collagen and CTGF/CCN-2, then regulating their transcription [187,188]. In turn, in the scenario of canonical signaling, Smad7 is an established negative regulator of the TGFβ pathway that operates through the binding to TGFβR-I, thereby blocking Smad2/3 activation and facilitating receptor degradation. It should be noted however, that the real response of different target cells to TGFβ isoforms, as nicely pointed out [188,189], is an extremely complex event being tightly modulated by numerous cellular context-dependent factors or determinants, including more components involved in signal transduction (ligand traps, co-receptors, inhibitory SMAD proteins and crosstalk inputs from other signaling), regulation of transcription of target genes or epigenetic status.

TGFβ has been reported to operate also through non-canonical pathways (SMAD independent) mediated by small GTPases (mainly RhoA and Cdc42) and leading to the activation of MAP kinases such as c-Jun-NH_2-terminal kinases (JNK), ERK1/2, and p38 (29, 105, 164), as well as of PI3K. Together with the canonical Smad pathway, the TGFβ-induced activation of ERK1/2 has been reported to activate CTGF/CCN-2 and type I collagen synthesis whereas the TGFβ-induced activation of JNK and p38 or of PI3K/Akt cascades have reported to contribute to differentiation of precursor cells into MFs (reviewed in [191]). In addition, two other non-canonical signaling have been described involving i) a TGFβ and bone morphogenetic protein (BMP), through type II receptors, signaling acting by direct activation of partitioning defective 6 (PAR6) and LIM kinase 1 (LIMK1) or ii) R-SMAD proteins that can participate in microRNA (miRNA) processing by Drosha complexes for the biogenesis of a subset of SMAD-binding miRNA precursors [188].

According to the prominent profibrogenic role of TGFβ signaling in recent years several studies have tested a number of different specific potential therapeutic strategies including the attempt to block circulating TGFβ1, to antagonize its receptors or to block its activation at the surface of target cells [1-10,15,164,186]. As a note of caution, it should be emphasized that since TGFβ isoforms also display relevant activities related to normal homeostasis, including inhibition

of inflammatory response, immune regulation and tumor suppression [186,192,193], potential adverse effects may follow systemic inhibition of TGFβ activity.

Nevertheless in the last years a number of preclinical studies as well as sometimes clinical trials are worth mentioning that have targeted TGFβ pathways. Whether preclinical studies are concerned, the block (at least partial) of TGFβ pathway and then fibrosis reduction has been obtained by adopting different strategies, including the following: i) the use of soluble TGFβRII, as shown in the bleomycin model of lung fibrosis [194]; ii) the use of selective and orally active TGFβRI kinase inhibitor [195]; iii) strategies designed to exacerbate the induction of the inhibitory SMAD7, as shown by either leading to increased action of YB-1, a IFN-γ related intracellular mediator up-regulating SMAD7 [196-198] or by directly overexpressing SMAD7 [199,200] in different experimental model of lung and liver fibrosis as well as of systemic sclerosis; iv) strategies affecting the Hippo pathway - related functional role of transcriptional coactivator with PDZ-binding motif (TAZ) and yes-associated protein (YAP) [201], according to the proposed relationships between Hippo pathway and TGFβ in lung fibrosis [202]; v) the attempt to block $\alpha_v\beta_6$-mediated TGF-β activation, as shown in two different mice models of pulmonary fibrosis [203,204] and also in a murine model of biliary fibrosis [190,205] by using a humanized anti-$\alpha_v\beta_6$ antibody; vi) the use of pirfenidone, a drug which is believed to act by inhibiting TGF-β production or activity, which has been shown to be effective in experimental lung fibrosis [206], cardiac and renal fibrosis in streptozotocin-diabetic rats [207], and liver fibrosis [208].

Results from clinical trials actually published by employing strategies designed to block TGF-β pathway are indeed few. An approach to be cited is the use of G1008 humanized antibody that binds and blocks the function of all TGFβ isoforms, now designed as fresolimumab, that was evaluated in early clinical trials in both fibrotic disease and cancer. This antibody was reported to be well tolerated in a Phase I clinical trial in patients with primary focal segmental glomerulosclerosis [209] and to offer some improvement in skin fibrosis parameters in multiple sclerosis patients [210]. Along these lines, although its precise mechanism of action is still unclear, pirfenidone has been reported to exert anti-inflammatory and antifibrotic action by likely attenuating TGF-β production and action; this drug has been approved for the treatment of IPF in Japan and Europe (reviewed in ref. [211]). Accordingly, pirfenidone has been reported to affect TGF-β-induced MF differentiation and fibrogenic activity of primary human lung fibroblasts [212], although its antifibrotic effects may also depend on the action on the renin-angiotensin system [213].

4.1.2 PDGF profibrogenic signaling pathway

PDGF is widely considered as a major growth factor involved in fibrosis of different tissues, including lung, kidney, liver, skin and pancreas [1-10,186] where the expression of related genes leads to the assembly of disulfide bonded homo- or heterodimers, with PDGF ligands being released by macrophages (particularly M2), platelets, MFs and sometimes by some epithelial cell types (for example, biliary epithelial cells or cholangiocytes). PDGF-A and PDGF-B subunit can form either homodimers and heterodimers (PDGF-AA, -AB, -BB) whereas PDGF-C and -D are detectable only as homodimers (PDGF-CC and -DD) [214,215]. The PDGF ligands exert their profibrogenic biological effects through their binding to the two structurally related tyrosine kinase receptors defined as PDGFR-α and PDGFR-β, with PDGF-AA and PDGF-CC inducing PDGFR-α dimerization and PDGF-BB and PDGF-DD inducing PDGFR-β dimerization in vivo [214,215]. Other combinations of ligand/receptor interactions have been described in cultured cells and the overall message is that PDGF-BB is the only dimer able to bind all the three receptor combinations (PDGFR-αα, -αβ and -ββ) whereas the other dimers have a more restricted ability. PDGF-AB can bind to PDGFR-αα and PDGFR-αβ combinations, PDGF-DD can bind to PDGFR-αβ and PDGFR-ββ whereas PDGF-AA and PDGF-CC interact primarily with PDGFR-αα [216,217]. PDGF ligands are potent peptide factors able promote tissue remodeling and wound healing during either acute or chronic injury [1-10,15,17,164,177] and elicit a signaling pathway that has been shown to regulate, in any kind of MFs and related precursor cells, cell proliferation and survival, migration, angiogenesis, cytoskeletal rearrangements as well as the synthesis of major components of ECM including collagen, glycosaminoglycans and proteoglycans [218]. PDGF ligands are also believed to contribute, during chronic tissue injury, to sustain phenotypic changes leading to the MF phenotype [1-10,15,164,177,186,219].

Following ligand-receptor interaction/dimerization the PDGF signaling pathway is known to proceed through the downstream phosphorylation of extracellular signal-regulated protein kinase / mitogen-activated protein kinase (Erk/MAPK) and protein kinase B (Akt/PKB) of the phosphoinositide-3-kinase (PI3K) pathways, a signal that is usually inducing proliferation of mesenchymal cells (precursor cells) and MFs. It should be noted, however, that some kind of cells like activated HSC and portal MFs, although expressing both receptor types, proliferate mainly in response to PDGF-BB and PDGF-DD accordingly to the fact that and in vitro and in vivo only PDGFR-β is up-regulated in these cells (reviewed in ref. [219]). Indeed, although the two different types of receptors can activate common pathways, there are two interesting differences with RasGAP binding being only activated by PDGFR-β whereas the intracellular adapter CrkII is only associated with PDGFR-α [220]. Other signaling components and/or cascades have been reported to be up-regulated by PDGF, including JAK/STAT pathway, phospholipase C(PLC)γ, JNK

isoforms, protein kinase C and p706SK [218-220], with JNK isoforms found to be particularly relevant, in addition to ERK1/2 and PI3K, in mediating PDGF-induced and redox-modulated oriented migration of activated HSC [178].

According to the major role of PDGF ligands and of their receptors in fibrosis, several studies have investigated different strategies to affect PDGF signaling pathway in experimental models of fibrosis, offering variable degrees of fibrosis inhibition, with the most relevant being the following: i) the attempt to block the expression of PDGF subunits; for example, the use of an antisense PDGF-B inhibited liver fibrogenesis in a short-term rat model of biliary fibrosis due to bile duct ligation (BDL) [221] and the genetic deletion of PDGF-C reduced fibrosis in the murine UUO model of interstitial kidney fibrosis [222]; ii) the use of strategies involving inhibition of PDGF subunits and/or their binding to the receptors; a first example comes from the field of experimental liver fibrosis with studies showing the variable efficacy of either a dominant-negative soluble PDGFR-β [223,224] or of antibodies against PDGF-B chain [225,226] or recognizing PDGF-BB [226]; a second example is represented by the use of aptamers, which are stabilized oligonucleotides, or of slow off-rate modified aptamers (SOMAmers) that bind proteins with very high specificity; PDGF-B aptamers have been reported to prevent development of progressive renal scarring in experimental glomerulonephritis [227]; iii) the use of molecules designed to block the receptor-mediated PDGF signaling, like imatinib mesylate (an inhibitor of tyrosine kinases of both receptors, also recognizing the bcr-abl fusion protein c-kit, and Flt3) [228], with early prevention of fibrosis, or sorafenib which is a potent inhibitor of pro-angiogenic vascular endothelial growth factor receptor 2 (VEGFR-2), PDGFR-β, and Raf kinases [229,230]; more recently, nilotinib, which is a second generation tyrosine kinase inhibitor 20-times more potent than imatinib, has been used to prevent experimental liver fibrosis [231,232]; iv) the use of PDGFR-β specific siRNA delivered into activated HSC by the hydrodynamics-based transfection method, a procedure that remarkably improved liver function and reduced fibrosis in two models of liver fibrosis [233,234]; v) the use of strategies designed to take advantage of known endogenous inhibitors of PDGF signaling, as reviewed in [219].

Whether the use of strategies to counteract PDGF signaling in human patients, few examples are available at present. Possibly the most remarkable example is represented by clinical trials (Phase II TOMORROW trial and two replicate Phase III INPULSIS(®) trials) that investigated efficacy and safety of nintedanib versus placebo in patients with idiopathic pulmonary fibrosis (IPF) [235-237]. Nintedanib (formerly known as BIBF 1120) is an intracellular inhibitor that targets multiple tyrosine kinases, including those associated to PDGF, FGF (see later) and VEGF receptors. Administration to IPF patients in these trials has been reported to be well tolerated and to

significantly reduce the decline in FVC, typical in IPF patients, which is consistent with a slowing of disease progression. These data have been confirmed and recently nintedanib has been approved both in the USA and in the EU for the treatment of IPF [238,239]. However, it seems correct to say that the reported efficacy of nintedanib has to be related to the fact that this molecules can target multiple tyrosine kinases and not only those associated to PDGF receptors. Indeed, in a randomized controlled trial over 96 weeks in which treatment with imatinib alone (vs placebo) was employed, no significant effect on IPF progression was observed [240].

4.1.3 CTGF/CCN-2 profibrogenic signaling pathway

CTGF, now renamed as CCN-2, is one of the six homologous matricellular and cysteine rich proteins belonging to the novel CCN family, whose members interact with and orchestrate a number of extracellular signaling and matrix molecules and have been reported to play important roles in embryo development, as well as in inflammation, tissue repair as well as in a broad range of pathological processes, including tissue fibrosis (liver, lung kidney and pancreas) and cancer [241,242]. CCN proteins share a peculiar modular structure having a N-terminal secretory peptide which is followed by four conserved domains that display sequence homologies to those found in a number of peptide, such as insulin-like growth factor binding proteins (IGFBP), von Willebrand factor type C repeat (VWC), thrombospondin type I repeat (TSR), with and a carboxyl-terminal domain containing a cysteine-knot motif. In addition, these proteins also have a non-conserved, protease-sensitive central hinge region. This region can be cleaved by several proteases (MMP1, MMP3, MMP7, MMP13 and ADAM28 in the case of CTGF/CCN2) that can dissect the proteins into two halves that then may bind distinct receptors on the surface of target cells [241,242].

At transcriptional level, the expression of CCN proteins, including CTGF/CCN-2, is up-regulated by a long list of extracellular and environmental stimuli potentially involved in CID that includes TGF-β, PDGF, TNFα, IL1β, angiotensin II (AT-II)2, FGF2 and myocardin-related transcription factor (MRTF). In addition, expression of CCN proteins is also elicited by thrombin, prostaglandins (PE), endothelin-1 (ET-1) and sphingosine-1-phosphate (S1P) (agonists of G protein-coupled receptors or GPCRs) as well as hypoxia, UV and mechanical stretch. On the other hand CTGF/CCN-2 as matricellular protein can bind integrins and HSPGs. CCN2 can bind to ECM components like fibronectin and aggrecan as well as several cell-surface receptors, such as receptor activator of NF-κB (RANK), LRP6, TrkA, fibroblast growth factor (FGF) receptor (FGFR) 2, and FGFR3. Of interest, CCN2 can direct interact in a bi-directional way with TGF-β, bone morphogenetic protein (BMP) 2, BMP4, FGF2, VEGF by modulating the signal emitted from these

molecules [241,242]. These multiple interactions are likely to represent the basis for the different biological effects exerted by CTGF/CCN-2 in different conditions of fibrosis [1-10,164,177,186].

Accordingly, CTGF/CCN-2 is overexpressed in virtually every human fibrotic disease during the course of healing, whatever the etiology, organ or tissue, being abundantly released by plateletes [243,244], with the other mentioned growth factors also inducing CTGF/CCN-2 expression at the site of injury. CTGF/CCN-2 is believed, together with PDGF, TGFβ and other growth factors and cytokines/chemokines, to contribute to transdifferentiation of precursor mesenchymal cells into fibrogenic MFs and to maintain the activated state. Along these lines, CTGF/CCN-2 is known to act synergistically with TGF-β1 to promote ECM deposition and fibrogenesis since it is not fibrogenic if administered individually [245,246]. Moreover, serum and biofluid levels of CTGF/CCN2 correlate with the severity of fibrosis in major organs (heart, kidney, liver and lung) leading to the suggestion that may represent a non-invasive biomarker for fibrosis in these diseases [247]. A number of studies suggest that precursor cells and MF, in addition to be a target for CTGF/CCN-2, may also release in an autocrine way this profibrogenic factor. Indeed, the specific overexpression of CTGF/CCN2 in profibrogenic cells is sufficient to drive fibrosis in lung, skin and kidney [248]; accordingly, its overexpression in parenchymal cells of most organs does not per se induce fibrosis in mice, but exacerbates the fibrotic response when in the presence of tissue injury [248].

Other experimental studies have provided additional evidence for the profibrogenic role of CTGF/CCN-2 indicating that inhibition or downregulation of this CCN matricellular protein can ameliorate fibrosis in many organs. In particular, the use of antisense nucleotides of specific siRNAs has been reported to reduce experimental liver fibrosis [249,250] and renal fibrosis [251,252]. Other studies related to experimental lung fibrosis have reported reduction of fibrosis in the bleomycin model following administration of PEGylated CTGF siRNA [253] or of CTGF/CCN-2 neutralizing antibodies [254]. Similarly, the administration of FG-3019, a humanized anti-CTGF monoclonal antibody, was effective in animal models of kidney and lung fibrosis [255]. Interestingly, the same antibody has been recently employed in an open-label phase 2 trial administered by intravenous infusion every 3 weeks for 45 weeks in patients with IPF, with significant positive changes reported for some patients, but results need to be confirmed in a trial including placebo [256].

It should be mentioned that in recent years another matricellular protein of the CCN family defined as cysteine-rich angiogenic inducer 61 (CYR61/CCN1) is emerging as being involved in fibrosis [186,257,258] with some studies suggesting a pro-inflammatory role of this protein and

others suggesting that CCN1 might be involved mainly in fibrosis resolution, possibly by triggering senescence of MFs and leading them to apoptosis (reviewed in ref. 257,258]. Interestingly, the administration of an adenoviral vector expressing CYR61/CCN1 prevented significantly collagen expression, attenuated TGFβ signalling and induced increased ROS generation-related cellular senescence and apoptosis in rodent fibrotic models [259].

4.1.4 HGF signaling pathway

Hepatocyte growth factor (HGF) is a pleiotropic cytokine composed of an α-chain and a β-chain both containing four kringle domains and a serine protease-like structure. HGF binds to a specific tyrosine kinase receptor identified as the c-met proto-oncogene, with the receptor undergoing autophosphorylation in tyrosine kinase domain, then resulting in the recruitment of several intracellular signaling proteins, including growth factor receptor bound protein 2 (GRB2), Src homology-2-containing (SHC), v-crk sarcoma virus CT10 oncogene homolog (CRK), phosphatidylinositol 3-kinase (PI3K), v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC) and the transcription factor signal transducer and activator of transcription (STAT-3) [260]. HGF-MET signaling, which is believed to be prominent in acquisition of the malignant characteristics in tumor cells, modulates immune cell functions and also plays an inhibitory role in the progression of chronic inflammation and fibrosis, with best characterized signaling pathways resulting in cell survival (via PI3K-AKT), migration (via focal adhesion kinase or FAK), cell cycle progression, proliferation and mobility (via Ras/ERK) [260]. In relation to fibrosis, administration of HGF has been reported to prevent the progression of experimental kidney fibrosis in the UUO model [261,262], likely by means of its prevalent anti-inflammatory action leading to disruption of NF-kB signaling and reduction of pro-inflammatory cytokines [260]. Similarly, administration of HGF or ectopic HGF expression ameliorated experimental bleomycin-induced lung fibrosis, where HGF is produced by mesenchymal and bronchial epithelial cells and alveolar macrophages [263,264]. The antifibrotic effect of HGF was reported to operate through induction of MFs apoptosis, reduction of collagen synthesis and extracellular matrix degradation due to increased activity of MMP2 and MMP9, as well as through reduction of apoptosis in alveolar epithelial cells and decrease in TNF and IL-6 levels [263,264] and by antagonizing the profibrotic actions of TGF-β1 through up-regulation of SMAD7 [265]. However, although this pathway is believed to be an attractive therapeutic target, particularly in relation to pulmonary fibrosis, a number of limitations and problems related to its use in human patients has been outlined, including a pro-carcinogenic risk [260,266].

4.1.5 EGF/EGFR signaling pathway

EGF is the best known member of a group of at least eleven ligands known to bind and activate the members of the correspondent family (EGFR) of tyrosine kinase transmembrane receptors, usually expressed, but not only, on epithelial cells. EGF, as other ligands (heparin-binding EGF-like growth factor or HB-EGF, transforming growth factor-α or TGF-α, amphiregulin, betacellulin, epigen and epiregulin) binds specifically to the EGFR/Human Epidermal Growth Factor Receptor-1 (HER1)/ErbB1 and/or HER4/ErbB4 [267-269]. EGF binding to EGFR induces its phosphorylation on specific tyrosine residues although the EGFRs can be also transactivated by stimuli not directly interacting with the EGFR ectodomain, including G-protein coupled receptor ligands, other receptor tyrosine kinase agonists, cytokines, and chemokines (most relevant examples being ET-1, Angiotensin II and TGFβ). Once activated the EGFRs, following binding of adaptor proteins (Grb2, Shc, Gab1), initiate multiple intracellular signaling pathways, including the MAPK/ERK pathway, the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways and the PI3K/Akt/NFkB pathways, involved in cell survival, proliferation, dedifferentiation, and migration.

EGF/EGFR system has been involved in lung, kidney and liver fibrosis with the overall effects ranging from favoring induction of proliferation and regeneration to antifibrogenic ones [1-10,186,270]. If liver fibrosis is concerned, EGF increases in CLD progression towards cirrhosis and hepatocellular carcinoma and a polymorphism in the human EGF gene has been identified leading to increased EGF expression and associated with increased fibrosis and cirrhosis progression [271,272]. A recent study has successfully employed the EGFR inhibitor erlotinib in various animal models of CLD reporting that the drug acted on reducing EGFR phosphorylation in HSC and the number of activated HSC, hepatocyte proliferation and liver injury, then preventing fibrotic progression [273]. Similar data have been reported for different animal models of kidney fibrosis by using either erlotinib or another EGFR inhibitor like gefitinib [274-6].

4.1.6 FGF/FGFR signaling pathways

The FGF family of ligands includes more than 20 different members classified as canonical FGFs (FGF1-10, 16-18, -20), endocrine FGFs (FGF15/19,-21,-23) and intracellular FGFs (FGF11-14), that signal through four distinct tyrosine kinase receptors (FGFR1–4) [277,278]. Although these FGF ligands were initially characterized (and named) for their ability to promote fibroblast proliferation, it is now clear that they can affect the behavior of multiple cell types, including epithelial cell proliferation. The FGF/FGFR pathway is complex and additionaly regulated by

cofactors and binding proteins, including heparin sulfate proteoglycans, the Klotho family proteins and the FGF-binding proteins [278]. Members of the FGF family function in the earliest stages of embryonic development and during organogenesis as well as in adult tissues where can mediate metabolic functions, tissue repair, and regeneration, sometimes reactivating developmental signaling pathways. The canonical members are those usually reported to be involved in chronic injury and fibrosis affecting liver, kidney, lung and pancreas [1-10,186] and the canonical intracellular signaling, following FGFR activation, leads to phosphorylation of adaptor proteins for the activation of major intracellular signaling pathways RAS/MAPK/ERK, PI3K-Akt, PLCγ and STAT.

In addition to the previously mentioned antifibrotic effects of nintedanib (a multiple inhibitor acting on FGFR1–3, VEGFR1–3 and PDGFRα and β) in condition of lung fibrosis like IPF [235-237], other studies have focused the attention of FGFs in lung fibrosis, particularly on the role of FGF-1. A very recent study has nicely showed that FGF1, administered by adenoviral vector, can prevent and even therapeutically affect TGFβ1-driven pulmonary fibrosis in the model that uses transient overexpression of TGF-β1 in the lung by adenoviral vector gene transfer [279]. In this study FGF-1 inhibited MF differentiation and induced proliferation of alveolar epithelial cells, overall regulating TGFβ1 signalling by controlling TGFβR1 expression and degradation as well as regulating FGFR1 expression. As recently discussed, FGF-1 antifibrotic action in conditions of chronic lung injury may overlap with analogue properties displayed by FGF-9 and -19 [280]. Concerning liver fibrosis, several FGFs are normally produced by hepatocytes and HSCs, but in CLD expression of FGFs in HSCs is greatly upregulated, stimulating hepatocyte regeneration and growth. Several FGF isoforms, particularly FGF-1 and -2, have been shown to directly induce HSC proliferation and activation and recently endocrine FGFs like FGF15/19 and FGF21 have been reported to regulate HSCs (reviewed in [281]). Experimental studies showed some inhibition of liver fibrosis by using inhibitors of FGFR1 like NP603 or brivanib [282,283], although the latter drug was also inhibiting VGFRs and PDGFRs. More recently, FGF-21 has been also reported to inhibit liver fibrogenesis affecting TGF-β/smad2/3 and NF-κB signaling pathways [284] .

4.1.7 VEGF/VEGFR signaling pathways

VEGF, particularly VEGF-A, is the prototype of pro-angiogenic factors and the critical involvement of VEGF and angiogenesis as well as of hypoxia (i.e., common in CID and the obvious stimulus for angiogenesis in normal and pathophysiological conditions) and hypoxia-inducible factors (HIFs) in the fibrotic progression of human and experimental CID in liver, lung, kidney and pancreas, has been proposed in the last two decades [1-10,16,17,181,182,285,286]. VEGF-A can

indeed be released by any kind of cell type in the profibrogenic scenario, including mainly epithelial cells, endothelial cells, macrophages as well as MFs and possibly also their precursor cells. According to literature data, the role of angiogenesis and experimental anti-angiogenic therapy has been extensively investigated in the field of liver fibrosis and several reviews have been published on this matter (reviewed in ref.[16,17,181,182]). The impressive amount of data available for the liver can be summarized as follows: i) hypoxia, HIFs, and then VEGF-dependent angiogenesis have been proposed to sustain and potentially driving liver fibrogenesis; this is consistent with studies showing that MFs are both target for VEGF (which stimulates oriented migration, proliferation and possibly ECM synthesis) as well as a source of either VEGF and Angiopoietin I in response to hypoxia but also of other hypoxia-independent stimuli [178-182]; ii) experimental studies have revealed that blocking hepatic angiogenesis was not only limiting fibrosis but also the genesis of portal hypertension and related complications in advanced stages of CLDs (reviewed in ref. [17,181,182,287]. In these pre-clinical studies the most common anti-angiogenic therapeutic strategy employed was designed to affect the function of VEGFR2; this was achieved by employing selective neutralizing antibodies or inhibitors of VEGFR2, drugs targeting multiple receptor tyrosine kinases like sunitinib or sorafenib or was obtained by administering CXCL9 chemokine (reviewed in ref. [182]). However, these and other strategies were not translated into clinical trials of CLDs and at present the only approved antifibrotic drug for clinical use that also act on VEGFR (but not only) is the already mentioned nintedanib, employed in the treatment of IPF. It should be also noted that, differently from liver fibrosis, in the field of CKD the feeling is that failed angiogenesis (i.e., not enough) may be central to progressive renal fibrosis, with hypoxia favoring fibrogenesis rather than angiogenesis [286].

4.1.8 Wnt signaling pathways

Wingless/Integrase-1 (Wnt) signaling is a very complex signaling pathway involving a family of secreted cysteine-rich and lipid-modified glycoproteins known as Wnt ligands [288]. These ligands signal through several membrane receptors including the classic Frizzled (Fzd) receptors, the low-density lipoprotein receptor related protein-5/6 (LRP5 and LRP6) as well as some atypical tyrosine kinases such as RYK, PTK7 and ROR2. The Wnt signalling pathway, that recognizes both a canonical β-catenin-dependent arm and a β-catenin-independent non-canonical arm, is believed to play a critical physiological role during embryonic development and in maintaining post-natal tissue homoeostasis [288,289]. Aberrant Wnt signaling is also known to be significantly associated with several pathophysiological conditions including primarily inflammatory and fibroproliferative disorders as well as malignancy [1-10,186,288-290]. Indeed,

Wnt target genes are linked to proliferation, survival, matrix protein expression, inflammatory responses and differentiation. Interestingly, several potential components/mechanisms of this signaling are potentially targetable using different strategies, as recently reviewed [291] .

The involvement of Wnt/β-catenin signaling is well documented in human conditions of lung injury, including IPF where the signaling is increased in the bronchial and alveolar epithelium in areas associated with proliferative bronchiolar lesions and fibroblast foci, with also fibroblasts and MFs being positive for nuclear β-catenin (reviewed in [290]). Accordingly, nuclear β-catenin localization is usually associated with increased expression of matrilysin/MMP7 (a prototype target of Wnt signaling) and an experimental study showed that genetic ablation of matrilysin/MMP7 resulted in a significant protection in the bleomycin model of lung fibrosis [292]. Another experimental study showed that the global knockout of the Wnt canonical co-receptor LRP5 prevented bleomycin-induced pulmonary fibrosis by decreasing β-catenin signaling and, in turn, also the expression of TGFβ [293]. This is relevant since TGFβ and Wnt signalling interact at multiple levels playing a crucial role in fibrotic diseases, with TGFβ exerting a modulatory effect on either Wnt ligands and receptor expression in lung fibrosis [294,295]. Accordingly, some experimental study has provided evidence for prevention of bleomycin-induced lung fibrosis when using strategies to block Wnt signaling, including administration into the trachea of small interfering RNA (siRNA) for β-catenin [296] or using small molecules like ICG-001, that selectively blocks the beta-catenin/CBP interaction [297], XAV939 that specifically inhibits Tankyrase 1/2, eventually leading to the degradation of β-catenin [298] and resulting in down-regulation of TGFβ1 and FGF2 expression [299]. Alternatively, in the same model the peptide mimetic NSC668036 was reported to achieve positive results by blocking interaction between Fzd receptors and Dishevelled (Dvl) [300].

In conditions of liver fibrosis Wnt/β-catenin signaling has been reported to either activate or inhibit HSC, as reviewed elsewhere [97,301], and similar therapeutic strategies have reported for pre-clinical models of liver fibrosis; reduction of fibrosis was obtained by transducing Dickkopf-1 (Dkk-1), a Wnt coreceptor antagonist, by an adenoviral vector [302] in BDL model, or by administering PRI-724, a selective inhibitor of the cAMP-response element-binding protein-binding protein (CBP)/β-catenin interaction, in the CCl4 model [303]. Whether kidney fibrosis is concerned. two recent experimental studies showed improvement of fibrosis associated to either Dkk-3 targeting in two preclinical models [304] or by using the small molecule Wnt-C59 that blocks the catalytic activity of the Wnt-acyl transferase porcupine, thereby preventing secretion of all Wnt ligands [305].

4.1.9 Hedgehog signaling pathway

Hedgehog (Hh) signaling pathway, that plays a prominent role in embryogenesis and differentiation of developing tissues and various roles in adult tissues, has been proposed to also contribute to liver, kidney, pulmonary, pancreas as well as cardiac fibrosis [97,186,306-308]. Three Hh ligands for this pathways have been described (Sonic hedgehog or Shh, Indian hedgehog or Ihh and Desert hedgehog or Dhh) that operate through binding to the two transmembrane proteins Patched (Ptch) and Smoothened (Smo). This very complex signaling pathway in its canonical cascade is usually inactive in the absence of ligands with Ptch (particularly Ptch1) inhibiting Smo membrane translocation, then resulting in the binding of transcription factor Gli to the intracellular Cos2-Fu-SuFu complex and subsequent Gli phosphorylation by several kinases (glycogen synthase kinase 3β, protein kinase A and casein kinase1) and cleavage. In the presence of Shh or other ligands Ptch activity is suppressed, Smo can translocate to the plasma membrane and interact with Cos2 avoiding Gli binding to Cos2-Fu-SuFu complex; Gli can then enter the nucleus to induce the transcription of target genes (reviewed in ref. [308]). Non-canonical Hh pathways exist that do not signal through Gli or Smo and are involved in the control of various physiologic functions in different types of tissues.

In conditions of liver fibrosis Hh ligands, which are not expressed in healthy liver, are released by either cholangiocytes or hepatocytes and can act on HSC contributing to their differentiation to activated MFs, that in turn can also secrete Hh ligands together with hepatic progenitor cells [309]. Accordingly, some preclinical studies have reported reduced liver fibrosis and decrease of MFs by treating animals with small molecules like the Smo antagonists cyclopamine [310] or GDC-0449 (vismodegib) [311,312]. Similar results have been reported for experimental kidney fibrosis employing the Smo antagonists IPI-926 (saridegib) [307] or again cyclopamine [313]. In conditions of lung fibrosis (bleomycin model or human IPF), Shh-mediated signaling has been proposed to play a role in the tissue remodeling and fibrosis [306,308] with adenovirus-mediated overexpression of Shh enhancing ECM production [314]. A further area of interest is represented by the emerging evidence that shows the association of miRNAs with Hh signaling and preclinical studies suggesting that Hh-regulating miRNAs can induce inactivation of HSCs resulting in reduced liver fibrosis [315].

4.1.10 Notch signaling pathways

Notch signaling, in addition to the established role of this signaling in organ development, regeneration and repair, is slowly emerging as an additional putative target for antifibrotic therapy,

as recently reviewed in the three major field of liver, lung and kidney fibrosis [316-318]. The peculiarity of this signaling relies in the fact that needs physical contact between the cell sending the signal, and then expressing Notch ligands belonging to the Delta or Jagged family on plasma membrane, and the cell receiving the signal through Notch receptors. In the canonical pathway the binding of Notch ligands to related receptors is followed in the "receiving" cell by proteolytic cleavage of the Notch extracellular domain by ADAM10/TACE metalloprotease, whereas the remnant intracellular receptor is further cleaved by the γ-secretase within its transmembrane domain to allow release and nuclear translocation of the Notch intracellular domain or NICD that, as a complex with other adaptor proteins, serves as transcriptional factor for target genes (Hes, Hey, p21, Myc and Sox9). The best characterized non-canonical pathway involves interaction with Wnt/β-catenin signaling although other signaling systems (NFkB, PI3K/Akt, HIF1α and TGFβ) may be also involved.

According to current knowledge Notch signaling components, up-regulated during CLD, are expressed both in epithelial and mesenchymal liver cells, with Jag1 being expressed by hepatocytes, cholangiocytes in ductular reaction and activated HSCs; in the latter cells Jag1 may stimulate α-SMA and collagen production [318]. Interestingly, inhibition of Notch signaling by using a γ-secretase inhibitor (N-[N-(3,5-difluorophenacetyl-l-alanyl)]-S-phenylglycine t-butyl ester or DAPT), reduced experimental CCl4-induced fibrosis [319]. Moreover, Notch signaling is likely to also modulate inflammatory response and M1 activation (TLR4 related) of macrophages, as suggested by experiments performed in Notch +/- mice [320].

The scenario in conditions of chronic kidney or pulmonary diseases seems remarkably similar, with Notch signaling being activated in these diseases and genetic manipulation of signaling components resulting in modulation of experimental fibrosis (reviewed in ref. [316,317]). Along these lines, the inhibition of γ-secretase improved either experimental kidney fibrosis through inhibition of TGFβ/Smad2/3 signaling pathway [321] or lung fibrosis [322].

4.1.11 Endothelins signaling pathways

The family of endothelins (ET) includes three 21-amino-acid peptides (ET-1, ET-2, and ET-3), of which ET-1 is the most biologically relevant, particularly for renal function, in either physiological and pathophysiological conditions affecting major organs (kidney, liver, lung and pancreas) [186,323,324]. ET-1 is produced by the endothelium as well as by virtually every cell type in the organism and is known to bind to two receptor isoforms defined as ETA and ETB. Under physiological conditions binding of ET-1 to ETA leads to vasoconstriction, cell proliferation and ECM deposition, whereas the binding to ETB results in vasodilation as well as inhibition of

proliferation and fibrosis [323]. However, as we will see, in some pathological conditions the binding to ETB can promote tissue injury and fibrosis. The effects of ETs are primarily exerted through local binding and then these ligands essentially acts in an autocrine and/or paracrine manner.

ET-1 is particularly relevant in regulating kidney functions such as sodium and water excretion, total and regional blood flow, mesangial contraction, podocyte function, and acid/base handling [323,324]. Pertinent to this review, a long list of conditions or mediators can up-regulate ET-1 synthesis in CKD, including several that are typically involved in the fibrotic progression of any CID like several growth factors, inflammatory cytokines, angiotensin II, oxidative stress and hypoxia, and others more properly related to kidney chronic injury (like acidemia, aldosterone, insulin and hyperglycemia in diabetic disease, proteinuria) [324]. The first preclinical observation unequivocally linking ET-1 to kidney fibrosis was obtained in ET-1 transgenic mice that spontaneously developed glomerulosclerosis and interstitial fibrosis [325] and several reports have outlined some protective effect of ET receptor antagonists (ERAs) able to block ET-1 binding to ETA (BQ-123, BQ-788, darusentan, avosentan, sitaxentan, atrasentan) or to both ETA and ETB like bosentan in animal model of diabetic nephropathy (reviewed in ref.[324,326]). However, bosentan was reported to be effective in preventing diabetes-induced experimental fibrosis (also in terms of TGFβ, collagen I, collagen IV and fibronectin expression) by some studies but not in others, with these incongruences being the consequence of the blockade of both ETA and ETB receptors (discussed in ref. [326]). Similar data for ERAs were reported also in non-diabetic experimental models of chronic nephropathy and these drugs have been also used in clinical studies on patients with CKD of different etiology. Overall ERAs, that have known adverse effects, gave some positive results, with mainly ETA antagonists, within the setting of a multiple drug therapy also involving modulation/blockade of renin-angiotensin system (RAS), proposed to be beneficial in treating CKD progression (reviewed in ref. [324]).

ERAs have been also employed in preclinical models of CLDs showing the ability to reduce significantly liver fibrosis, as first shown in an early study in which bosentan was administered in the CCl4 and BDL models [327] to more recent homologous studies, within several published, in which ETA antagonists LU 135252 [328] or ambrisentan [329] were effective in reducing fibrosis and expression of collagen I and TIMP-1 in either BDL model or in the model of NAFLD prone ob/ob mice, respectively. This is of interest recalling the fact that ET-1, up-regulated by TGFβ1, has been long known to directly stimulate proliferation as well as the synthesis of ECM components in activated HSC [4-6,17,164]. In turn, few data have been produced for lung fibrosis

although the ET system has been implicated in the pathophysiology of IPF as ET-1 and related cytokines may contribute to fibrosis development by inducing proliferation of fibroblasts, by decreasing ECM remodeling but stimulating synthesis of ECM components [1,2,88,149]. In addition, although ERAs are approved for use in pulmonary hypertension, which is a common comorbidity in patients with IPF, clinical trials with ERAs were found to be essentially ineffective in IPF patients [330].

4.1.12 Renin / Angiotensin system

The renin/angiotensin system (RAS) is known to play a key role in maintaining blood pressure homeostasis and fluid/salt balance through coordinated effects exerted on heart, blood vessels and kidneys [331]. In the classic pathway of RAS, renin operates by cleaving liver-derived precursor peptide angiotensinogen into the decapeptide Angiotensin I (Ang I), which is then further degraded into the octapeptide Angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE), the most bioactive enzyme of RAS. Ang II can exert its biological effects mainly through the binding to angiotensin Type 1 receptor (AT1R) [331]. Apart from the roles in regulating blood pressure and fluid/salt balance, RAS has entered the field of organ fibrosis since its main components are usually up-regulated [1-10,186].

Data in the field of CLDs have shown that RAS is a significant mediator of hepatic fibrosis, remarkably because AT1R is highly expressed in activated HSCs and Ang II/AT1R signaling pathway mediates in these cells increased proliferation, migration, cell contraction as well as TGFβ and type I collagen expression through increased intracellular levels Calcium and, importantly, of ROS generated by NADPH oxidase (NOX) isoforms and related activation of PKC, PI3K/Akt and MAPKs pathways (reviewed in ref. [332,333]. Experimental studies showed that both the blockade of AT1R with antagonists (losartan, irbesartan) [334,335] or its genetic manipulation [336] significantly reduced liver inflammation and fibrosis in CCl4 or BDL models. Similar results were observed in NAFLD/NASH model with fibrosis prevention by telmisartan [337,338]. More recent studies have outlined that AT1R-related fibrosis and cirrhotic complications may rely on signaling through Janus kinase-2 (JAK-2) pathway in both animal models [339] and in human HCV patients [340], potentially suggesting that JAK-2 inhibitors already approved for the use in humans, but not for CLDs and cirrhosis, may represent a future therapeutic option.

In the field of CKD, RAS inhibition is overall considered a relevant principle of nephroprotection, particularly in the context of diabetic nephropathy. Within the several reported nephroprotective effects obtained in an impressive number of preclinical studies performed by using

several drugs targeting AT1R, pertinent to the present review, are to be cited those showing prevention of either glomerulosclerosis and/or interstitial kidney fibrosis in animal models by administering candesartan, eprosartan, telmisartan and valsartan (reviewed in ref. [341]). In these studies the protection afforded towards fibrosis was found to be comparable with that obtained by using an ACE inhibitor, usually enalapril [341]. Targeting RAS system has been shown to affect also experimental lung fibrosis with Ang II exerting profibrotic action on lung fibroblasts, leading to growth factor expression, ECM synthesis and migration mediated through both AT1 and AT2 receptors, with several studies suggesting a protective role for strategies leading to ACE inhibition (reviewed in ref. [342]). However, retrospective analysis of clinical use of ACE inhibitors and/or statins indicated no significant change in survival in IPF patients [343].

4.2 The role of oxidative stress, ROS and NADPH-oxidase isoforms

Oxidative stress can be defined as an imbalance between an excessive generation of ROS and the capacity of the cells and/or tissues to eliminate, inactivate or scavenge them [167-170]. The involvement of oxidative stress, ROS and other redox-related reactive intermediates has been extensively documented in most experimental models of liver, kidney and lung fibrosis and in almost all related clinical conditions [1-10,15,17,164,166-170,290]. Oxidative stress in any CID can be the result of the direct impact of the specific etiology, resulting in increased generation of intracellular ROS by either injured epithelial cells or, following significant induction of cell death, by activated inflammatory cells. In most conditions of CID oxidative stress is also accompanied by a progressive decrease in the efficiency of antioxidant defenses. Since several excellent reviews are available on this specific matter, here it is sufficient to recall just some general major concept: i) excessive generation of ROS can favor CID progression by perpetuating cell death (necrotic, apoptotic, necroptotic or other) and then chronic inflammation; ii) ROS and other related mediators can *per se* affect/modulate the behavior of MFs and of their precursor cells by up-regulating critical genes (for example pro-collagen type I, TIMP-1, CCL2 and others) and modulating additional phenotypic responses through activation of specific signal transduction pathways and transcription factors; iii) intracellular ROS generation in MFs also represents the consequence of activation of NADPH-oxidase isoforms in response to classic pro-fibrogenic mediators acting on MFs (including TGFβ, PDGF, Ang II and most if not all those previously described in section 4.1); this significantly contributes to a persistent shift towards higher intracellular ROS levels which is believed, according to current knowledge, to perpetuate and further amplify activation of major profibrogenic signaling pathways in MFs and/or precursor cells; iv) antioxidant supplementation,

found to offer encouraging results in preclinical studies, was generally ineffective on CID progression in clinical trials [1-10,15,17,164,166-170,290].

4.2.1 NADPH-oxidase isoforms in organ fibrosis

NADPH-oxidase (NOX) is a multicomponent transmembrane enzyme complex that generates ROS like superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) from molecular oxygen using NADPH as an electron donor in response to a wide range of stimuli [344]. The prototype NOX or phagocytic NOX, present in neutrophils and macrophages, is formed by an heterodimeric membrane-bound flavocytochrome b558 complex (containing the catalytic subunit $gp91^{phox}$ or NOX2 and the regulatory subunit $p22^{phox}$) and by cytosolic regulatory components like $p47^{phox}$, $p40^{phox}$, $p67^{phox}$ and Rac. On stimulation with agonists (LPS, IFN-γ, Ang-II) these cytosolic components translocate to the membrane-bound flavocytochrome complex, leading to enzymatic activity. The non-phagocytic NOXs are expressed at low levels in various tissues/organs composed of multiple cell types and present some variations, with NOX2 being replaced by a different member of the mammalian NOX family (that comprises other six members identified as NOX1, NOX3, NOX4, NOX5, DUOX1 and DUOX2) and the complex being assembled in response to a longer list of ligands including Ang II, TNF-α, IL-1β, LPS, PDGF, PG-F2α, EGF, bFGF, IFN-γ and ET-1 [168-170].

If the liver is concerned, Kupffer cells, macrophages and other immune cells express NOX2 whereas hepatocytes and endothelial cells express NOX1, NOX2 and NOX4. Interestingly, it has been shown that MFs derived from HSCs express NOX2 and nonphagocytic NOX isoforms NOX1 and NOX4, which mediate distinct ligand-stimulated (Ang II, PDGF, TGFβ, ET1, etc) and ROS-mediated profibrogenic actions in these cells (reviewed in [168]) affecting MAPK cascades, PI3K/Akt signaling, NF-kB system and L-Type Calcium channels and then major phenotypic responses (proliferation, migration, ECM synthesis and remodeling, inflammation as well as contractility). This has provided the rationale to try to assess the efficacy of pharmacological NOX inhibitors as putative therapeutic agents to treat hepatic fibrosis, taking in mind that administration of generic antioxidants (for example Vitamin E, vitamin C, polyenyl phosphatidyl choline, or ursodeoxycholic acid, UDCA) although efficient in preclinical studies, failed to demonstrate antifibrotic efficacy in CLD patients [167,168]. Preclinical studies showed that administration of the compound GKT137831, found effective in suppressing in vitro critical pathways in HSCs, resulted in a reduction of ROS generation and liver fibrosis in mice undergoing CCl4- or BDL-induced liver fibrosis in mice by inhibiting both NOX1 and NOX4 [345,346]. However, no liver-related trial has been performed in our knowledge and indeed in most trials with CLDs patients NOX inhibition was

obtained indirectly, for example through the use of drugs blocking AT1R and then the renin angiotensin system signaling pathway (see before in section 4.1.12).

Interestingly, NOX4 also mediates lung MFs activation and fibrogenic responses in lung, particularly in IPF, whereas NOX1, NOX2 and NOX5 were unmodified, with NOX4 being up-regulated by TGFβ through Smad phosphorylation and critical for MF differentiation as shown by NOX4 silencing experiments [347,348]. The use of the NOX1/4 inhibitor GKT137831 resulted in the inhibition of major genes related to ECM deposition and principal profibrogenic pathways (including that of TGFβ) and in the prevention of lung fibrosis induced by bleomycin [349]. A very close scenario emerges from studies on kidney fibrosis, where NOX4 seems to be the NOX isoform that plays the most important role particularly in fibrosis associated to diabetic kidney disease and focal segmental glomerulosclerosis [350]. GKT137831 was reported to reduce established renal fibrosis as well as glomerular hypertrophy, mesangial matrix expansion, urinary albumin excretion and podocyte loss in two murine models of type 1 diabetes mellitus with progressive renal disease [351,352]. The renoprotective effect of GKT137831 was also replicated in a murine model of type II diabetes [353]. The GKT137831 in these years was employed in phase I studies, being well tolerated, and also in a 12 weeks treatment in a Phase II trial in patients with diabetic nephropathy. In the latter study, however, no apparent significant reduction of albuminuria (the primary efficacy end point) (reviewed in [350]).

4.3 Immune related targetable signaling pathways or molecules

As previously discussed, the involvement of innate immunity cells is critical in any form of progressive/fibroproliferative CID and several related signaling pathways, potentially targetable, have been outlined as significant [1-10,15,17]. The list may include pathways elicited by CD40L and several cytokines and chemokines as well as those related to the activation of several receptors such as Toll-like receptors (TLRs) by DAMPs or PAMPs, receptor for advanced glycation end-products (RAGE) by AGE and several others [97,186,290,354]. We will here just briefly comment some examples that has been targeted in either preclinical or clinical studies.

4.3.1 Cytokines and chemokines

Several cytokines and chemokines are known to be involved in progressive and fibroproliferative CID, but relatively few studies dealing with specific related targeting of these peptides (or of their receptors and signaling) have been published.

A typical example is represent by CCL2, a potent chemoattractant acting through the related receptor 2 (CCR2), expressed by monocytes, macrophages, T-cells, MFs and precursor cells as well as epithelial cells, and overall overexpressed in lung, liver and kidney fibrosis. CCL2 is likely to actively contribute to fibrogenesis by recruiting either monocyte/macrophages from peripheral blood (particularly $LY6c^{hi}$) and by recruiting MFS and precursor cells during chronic injury. Interestingly, bleomycin-induced lung injury and fibrosis was attenuated in animals deficient in the receptor for CCL2, CCR2, or by treatment with anti-CCL2 gene therapy [355,356]. In the field of kidney fibrosis CCR2 antagonists (propagermanium or RS-504393, CCX140-B) have been found effective in experimental models of progressive chronic injury including type 2 diabetic nephropathy and UUO [357-360].

Another interesting example is represented by IL-4 and IL-13, which are relevant mediators of innate immune activation and TH_2 responses, with several reports indicating their involvement in preclinical animal models and their overexpression (together with cognate receptors) in human fibrotic diseases (reviewed in [97,186,290,354]). IL-13 is overexpressed in the lungs of IPF patients with lung fibroblasts exhibiting increased expression of both IL-13Ra1 and IL-13Ra2 receptors; accordingly, treatment with an IL-13 targeted antibody (tralokinumab) in a murine model of IPF attenuated lung fibrosis and also restored epithelial integrity [361] and the same antibody, well tolerated, has been recently employed in a phase II clinical trials in asthma patients [362], with positive effects in a subpopulation of patients with severe asthma. Other antibodies against IL4 or IL13 or to the common receptor have been formulated and are being tested for fibrotic diseases in clinical trials [186,290,354].

4.3.2 Pentraxin 2

Pentraxin-2 (serum amyloid P or PTX2) is a human protein working as a pattern recognition receptor known to contribute to the regulation of the innate immune response and to inhibit the differentiation of monocytes into profibrotic, alternatively activated (M2) macrophages [363]. PTX2 has been detected in the sites of injury and reported to inhibit fibrosis and promotes repair and a human recombinant form of PTX2 (PRM-151) has been reported to exert protective effects in a variety of preclinical models of fibrosis in multiple tissue types (reviewed in [363]). Very recently, PRM-151 has been employed in a randomised, double-blind, placebo-controlled, multiple ascending dose trial in IPF patients with some encouraging results [364].

4.3.3 TNFα

TNFα as well as related receptors are other putative targets for therapeutical intervention in organ fibrosis. Indeed, specific anti-TNF therapy has been initially proposed for treatment of rheumatoid arthritis (the monoclonal antibody infliximab against human TNFα) and then the approach has been approved for many other chronic inflammatory diseases including Crohn's disease, ulcerative colitis, psoriatic arthritis, psoriasis and ankylosing spondylitis [365]. At present, few studies related to organ fibrosis have been performed and worth to mention is a study employing a monoclonal antibody raised against rat TNF that suppressed inflammation and renal fibrosis in a model of experimental glomerulonephritis [366], In addition, the monoclonal antibody adalimumab has been recently used in a Phase II clinical trial for patients affected by resistant (i.e., to conventional corticosteroid therapy) focal segmental glomerulosclerosis with some positive results on these particular patients [367].

4.3.4 Toll-like receptors

TLRs are pattern recognition receptors (PRRs) able to detect microbial infection and exposure as PAMPs, including bacterial protein products (particularly lipopolysaccharide or LPS), transcripts, and genomic DNA, as well as DAMPs (such as hyaluronan fragments and HMGB1). The mechanistic involvement of TLRs, best characterized for CLDs and then liver fibrosis, as reviewed in the first review of this issue [97], is also recognized for lung and kidney fibrosis with these receptors detected in cells of innate immunity, MFs and precursor cells as well as in epithelial and even endothelial cells [368]. In CLDs a relevant finding is that activated HSCs express both TLR4, a known modulator of liver fibrosis, and TLR9 and then can respond to LPS (mainly of gut origin) and to other bacterial products. Data available from literature are at present mostly from studies in which the pro-fibrotic involvement of TLRs has been tested in genetically manipulated mice to down-regulate expression of specific TLRs and overall these studies indicate that by deleting them liver and kidney fibrosis are usually prevented [97,368]. This scenario intriguingly does not apply to models of pulmonary fibrosis, with most of studies showing either no reduction or even increased lung fibrosis when inhibiting TLR2 and TLR4 signaling in contrast to the fibrosis-reducing effects of TLR inhibition observed in the liver or kidney (reviewed in [368]).

4.4 Other targetable pathways involved in fibrosis

4.4.1 Nuclear receptors

Nuclear receptors (NRs) are a superfamily of ligand-activated transcriptional factors recognized as master regulators of metabolism being also involved in the metabolic fate of nutrients. In particular, transcription factors like peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs) and farnesoid X receptor (FXR) by triggering specific molecular cascades are known to finely regulate energetic fluxes and metabolic pathways [369,370]. Dysregulation of NRs-related pathways has been outlined in various conditions of CID [1-10], although a major role is recognized in metabolic derangements related to insulin resistance IR and metabolic syndrome and then in the pathogenesis of progressive NAFLD in obese and/or Type2 diabetes patients [369,370].

4.4.1.1 Peroxisome proliferator–activated receptors (PPARs)

The PPARs members of the NR superfamily include PPAR-α, PPAR-β/δ, and PPAR-γ, known to play a general critical role in regulating cell growth, differentiation, metabolism and inflammation as well as, in the liver, also cholesterol and bile acid homeostasis, glucose and lipid metabolism [20–22]. PPAR-α is highly expressed in liver, kidney and muscle, whereas PPAR-γ is predominantly expressed in adipose tissue whereas expression of PPAR-β/δ is ubiquitous in the organism. All these receptor are typically activated by fatty acids (FA) to then form a heterodimer with retinoid X receptor (RXR) which, in turn, can interact with PPAR response elements in the target genes to regulate their expression [371,372].

Whether progressive NAFLD is concerned, activation of PPAR-α is believed to be essentially protective (i.e. by controlling the rate of FA catabolism and lipogenesis in hepatocytes). This has been shown by experimental studies reporting that genetic manipulation to down-regulate PPAR-α (PPAR-α deficient or null mice) leads to a more severe and progressive form of dietary-induced NASH and that the use of a potent PPAR-α agonist like Wy-14,643 can usually reverse NASH (reviewed in [373]). However, although in humans liver PPAR-α gene expression was negatively correlated with insulin resistance, severity of steatosis, presence of NASH and fibrosis, the use of PPAR-α agonists failed to offer positive results in human trials [371,373].

PPAR-γ expression/activity is usually increased in either murine models and in humans and studies performed in mice fed an high fat diet (HFD) showed that PPAR-γ knockdown by either genetic manipulation or by RNA interfering-adenoviral vector injection protected from hepatic steatosis [374,375]. However, PPAR-γ agonists like thiazolidinedione in preclinical studies improved steatosis and protected from NASH and fibrosis by increasing insulin sensitivity in adipose tissue and skeletal muscle, overcoming the direct steatogenic effects in hepatocytes, as well

as by up-regulating the secretion of adiponectin and the expression of its receptors in the liver and adipose tissue and possibly by also inhibiting activated HSCs [376,377]. However, in a phase III clinical trial thiazolidinediones like pioglitazone and rosiglitazone, that improved steatosis and lobular inflammation, were found ineffective with respect to fibrosis in NASH patients [378]. Interestingly, some studies in experimental models and clinical studies have started to suggest that glitazones may also inhibit the progression of renal diseases, particularly of course diabetic nephropathy [379]. However, there are a number of emerging safety concerns (for example for glytazone-induced cardiotoxicity) for these drugs that may limit the use in either NAFLD and kidney diseases. More interesting results were obtained in a phase II trial in which elafibranor (GFT-505), a dual PPARα/δ agonist, was employed, resulting in improvement of NASH parameter and cardiometabolic risk without fibrosis worsening [380].

4.4.1.2 Farnesoid X receptor (FXR)

FXR, mainly expressed in the liver and the gut, acts as a bile acid (BA) sensor regulating, together with a G protein-coupled BA receptor (GPBAR1), BA levels in hepatocytes and by mediating the signaling effects exerted by BA on glucose and lipid metabolism [381]. BA, particularly chenodeoxycholic acid (CDC), are the natural FXR ligands and activated FXR forms a heterodimer with RXR that binds to the promoter region of the two main target genes, small heterodimer partner (SHP) and FGF-19; this pathway is finalized to reduce the expression of genes involved in BA synthesis (mainly CYP7A1) [381]. Overall, the BA-activated FXR signaling can enhance insulin sensitivity and FA β-oxidation, then limiting steatogenesis, and can also reduce hepatocellular gluconeogenesis and lipogenesis. Since FXR signaling impacts both bile acid synthesis and lipid metabolism, its pharmacological activation is a putative therapeutic approach for liver diseases associated with BA mediated cell injury as well as alcoholic and non-alcoholic fatty liver disease. Indeed, FXR plays a major role in hepatic fibrosis, being reduced in human and mice fibrotic livers, but also in fibrosing kidney disorders observed in diabetics [382,383]. FXR ligands may promote a FXR-SHP regulatory cascade able to reverse or inhibit liver fibrosis and, consistent with this hypothesis, administration of the semi-synthetic CDC derivative obeticholic acid (OCA) is protective against inflammation and fibrosis in preclinical models of NAFLD [384] and in NAFLD patients [385,386]. It should be noted that two recent experimental studies have reported that activation of FXR by FXR agonists GW4064 and CDC may protect from renal fibrosis in the UUO murine model [387] and that OCA administration may reduce pulmonary fibrosis in the bleomycin model [388].

4.4.1.3 Liver X receptors (LXRs)

There are two receptor belonging to this group of NRs, LXR-α and LXR-β, that in the liver serve as lipid sensors and contribute to the regulation of major genes modulating the metabolism of cholesterol and FA [389]. Interconnections between LXRs and SREBP-1 pathways is recognized as a critical step in the cascade of events leading to steatosis, which is commonly detected in both NAFLD and chronic HCV patients, and favoring inflammatory and fibrotic changes. Along these lines, the use of the synthetic compound SR9238, able to target LXR-α and LXR-β, has been reported to suppress hepatic lipogenesis, inflammation, and steatosis in an experimental NAFLD model in mice [390]; moreover, UDCA inhibited LXR-α-mediated hepatic lipogenesis [391]. Of interest, the administration of the LXR agonist T0901317 was recently reported to attenuate lung inflammation and fibrosis induced by bleomycin in mice, with the drug suggested to abolish overexpression of TGF-β1 and to inhibit NF-κB DNA-binding activity [392].

4.4.2 Lisophosphatidic acid (LPA) and other lipids

Lysophosphatidic acid (LPA) is a water-soluble, growth factor-like, phospholipid reported to act as a signaling molecule displaying a wide range of effects in many different tissues, including the control of lipid homeostasis in the liver. LPA signaling is mediated through G-protein-coupled receptors (GPCR) and its dysregulation has been detected in a growing number of disorders including fibrosis, particularly lung fibrosis [393]. The related receptor, LPA1R, is usually expressed on fibroblasts, particularly those obtained from IPF [394] patients, and experimental studies performed using the bleomycin murine model indicate that a reduction of lung fibrosis was obtained in either LPA1 receptor knockout mice as well as in animals treated with selective LPA1R antagonists, AM966 or AM095 [395]. The profibrogenic effects of activation of LPA1R signaling may rely on LPA1R-mediated vascular leakage and increased fibroblast recruitment [394] .

In conditions of CLDs, it has been shown that the hepatocyte derived autotaxin (ATX) can concur in up-regulation of LPA levels which in turn have been reported to activate the LPA1R on HSCs. [396,397]. Pharmacological and/or genetic inhibition of ATX and/or LPA1R has been found to down-modulate HSC activation and to reduce experimental liver fibrosis and cancer [396,397]. In addition to LPA, studies on samples from IPF patients have outlined a significant increase of the levels of sphingosine-1-phosphate (S1P) and sphingosine kinase 1 (SphK1) with these levels being correlated inversely with lung function [398]. Interestingly, the administration in vivo of the S1P1 agonist FTY720 was found to worsen bleomycin-induced lung injury [399].

4.4.3 Advanced glycation end-products (AGE) and related receptor (RAGE)

Advanced glycation end products (AGEs) are the irreversible products of non-enzymatic glycation of proteins, nucleic acids, and lipids which can be over-produced in hyperglycemic or oxidative stress environments. AGEs have various structures such as N-ε-carboxymethylated lysine (CML), pentosidine or pyrroline according to the precursor molecule. AGEs involve oxidative and non-oxidative molecular rearrangements and may be involved in several disorders, particularly diabetes-related, with a toxicity that may be due to interaction with the receptor for AGEs (RAGE), to the tissue deposition of AGEs or to in situ glycation. AGEs have been reported to trigger proinflammatory and profibrogenic cellular responses that are capable of damaging tissues, often targeting various organs including heart, liver, kidney and lung [186,400-402].

AGEs have been involved in several forms of kidney pathology associated with diabetic and non-diabetic nephropathies and detected in all renal compartments in diabetic patients, including the vessels, glomeruli, tubules and interstitium [400]. RAGE is expressed at low levels in podocytes and endothelial cells in the human and murine glomerulus and has been reported to increase during disease. Accordingly, the AGE/RAGE system may lead to activation of several renal cell types,, including endothelial, tubular and mesangial cells as well as podocytes, and AGEs are believed to play a role in ECM accumulation in diabetic glomerulosclerosis, in diabetic (renovascular, microangiopathic and glomerular) and non-diabetic renal injury associated with progressive glomerulosclerosis [400]. In experimental studies diabetic nephropathy has been significantly prevented by essentially four strategies: i) by genetic deletion of RAGE as in RAGE knock out mice [403]; ii) by prevention of AGEs formation, in either unspecific way, as shown mainly in studies employing antagonists of AT1R (see in a previous section, discussed in [400]), or, more recently, specifically by employing nicousamide; this drug has been shown to also act by reducing the AGE-stimulated overexpression of TGF-β1 and CTGF [404]; iii) by administration of drug resulting in the breakdown of already formed AGEs as in studies employing alagebrium [403]; iv) block of RAGE activation using a soluble form of RAGE (sRAGE) or using antibodies against RAGE [400].

In the field of lung fibrosis the attention has been mainly focused on IPF in which AGEs and RAGE are clearly increased [400,405], with some controversies to the real profibrogenic contribution of RAGE. Indeed, a study performed using RAGE null mice outlined that the lack of RAGE resulted in a worsen evolution of bleomycin-induced pulmonary fibrosis compared with control mice; moreover, RAGE null mice spontaneously developed lung fibrosis, suggesting that RAGE may per se have a preventive role in fibrosis [406]. Very recently it has been proposed that the real determinant or driving force may be represented in IPF by the increased AGEs/RAGE ratio [407].

Finally, whether liver fibrosis is concerned, original data indicated that in experimental hepatic fibrosis RAGE expression was enhanced in activated HSC as well as in endothelial cells, inflammatory cells and activated cholangiocytes. Although HSC expressed RAGE, exposure of these cells to AGE-BSA did not alter HSC proliferation, apoptosis, fibrogenic signal transduction and fibrosis- or fibrolysis-related gene expression [402]. Other studies suggested that the AGE/RAGE system may operate in a profibrogenic way, as for example suggested by a study showing protection from fibrosis by using specific-siRNA targeting of RAGE [408]. Accordingly, in another study and by using the MCD dietary model of NAFLD the presence of a high content of AGE in the diet accelerated the progression of experimental NAFLD by exacerbating liver injury, inflammation and liver fibrosis, apparently through oxidative stress- and RAGE-dependent profibrotic effects of AGEs on activated HSCs [409].

5. Summary and future directions

As summarized in this review, MFs play a critical role in the progression of chronic inflammatory and fibroproliferative diseases in different tissues or organs, whatever the etiology. Persistent activation of these cells is sustained by chronic injury and inflammatory response in a profibrogenic scenario involving mutual interactions, operated by several mediators and pathways, of MFs and related precursor cells with innate immunity cells and virtually any cell type in a defined tissue. Our knowledge of these interactions, mediators and related signaling pathways, critical in initiating and perpetuating the differentiation of precursors cells into persistently activated MFs, is enormously increased in the last two decades also taking advantage of genetically manipulated murine models. Accordingly, because of preclinical studies, we are now fully aware of an impressive number of candidate putative targets and/or targetable pathways (the most relevant and common summarized in this review) to possibly counteract the progression of chronic inflammatory and fibroproliferative diseases. This emerging and accumulating knowledge has started to be translated to relevant clinical conditions in order to develop effective antifibrotic therapeutic strategies. Although still with some flaws as just a few therapeutic options to counteract fibrosis have been specifically approved for the use in humans, nevertheless the way is open and the correct application of emerging biomolecular technologies as well as the continuous efforts to translate data and concepts from pre-clinical studies into to human conditions represent an obligatory way to be followed.

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Figure legends

Figure 1. The pro-fibrogenic scenario in tissue/organ fibrosis leading to MFs activation

In any condition of persistent chronic tissue injury and/or dysregulated wound healing response, a complex scenario of interrelated events, cell populations and mediators, including signaling pathways, is believed to occur whatever the specific etiology or tissue involved. The prototype pro-fibrogenic scenario always involves: i) chronic injury to epithelial and /or endothelial cells that, in turn, ii) switch on activation of coagulation and platelets as well as, of critical relevance, of innate immunity cells; iii) mediators released by injured epithelial and/or endothelial cells as well as from activated inflammatory cells (either resident or recruited from peripheral blood) and likely also by cells of innate immunity create the complex environment that eventually results in the persistent activation of MFs from their precursor cells; iv) activated MFs further sustain fibrogenesis through their phenotypic responses.

Figure 2. Major targetable profibrogenic signaling pathways affecting MFs behaviour

Major profibrogenic pathways that have been described to be involved in almost all conditions of tissue/organ fibrosis. The focus is, in particular, on those core pathways reported to play a primary and common role in liver, lung and kidney fibrosis and that have been targeted by different strategies of pharmacological intervention mostly in pre-clinical studies.

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Graphical Abstract

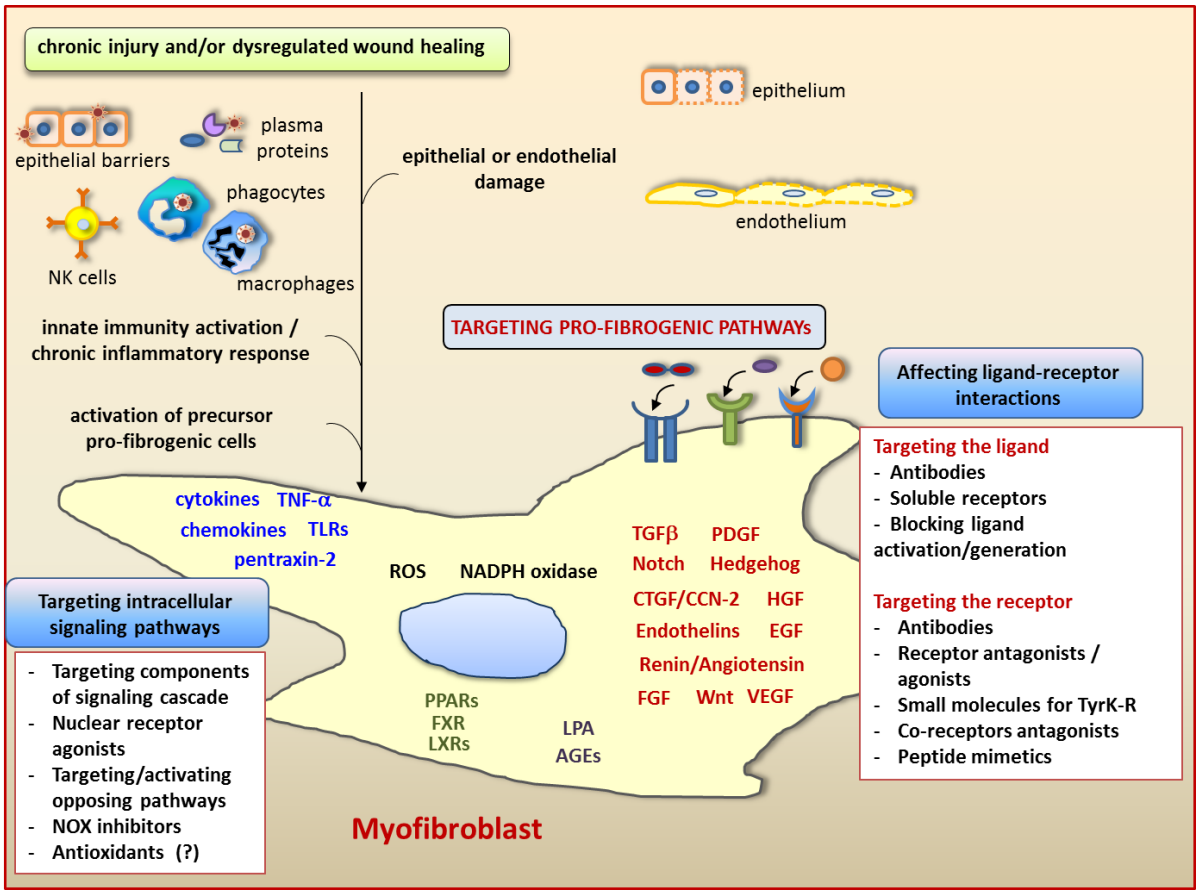

Figure 1.

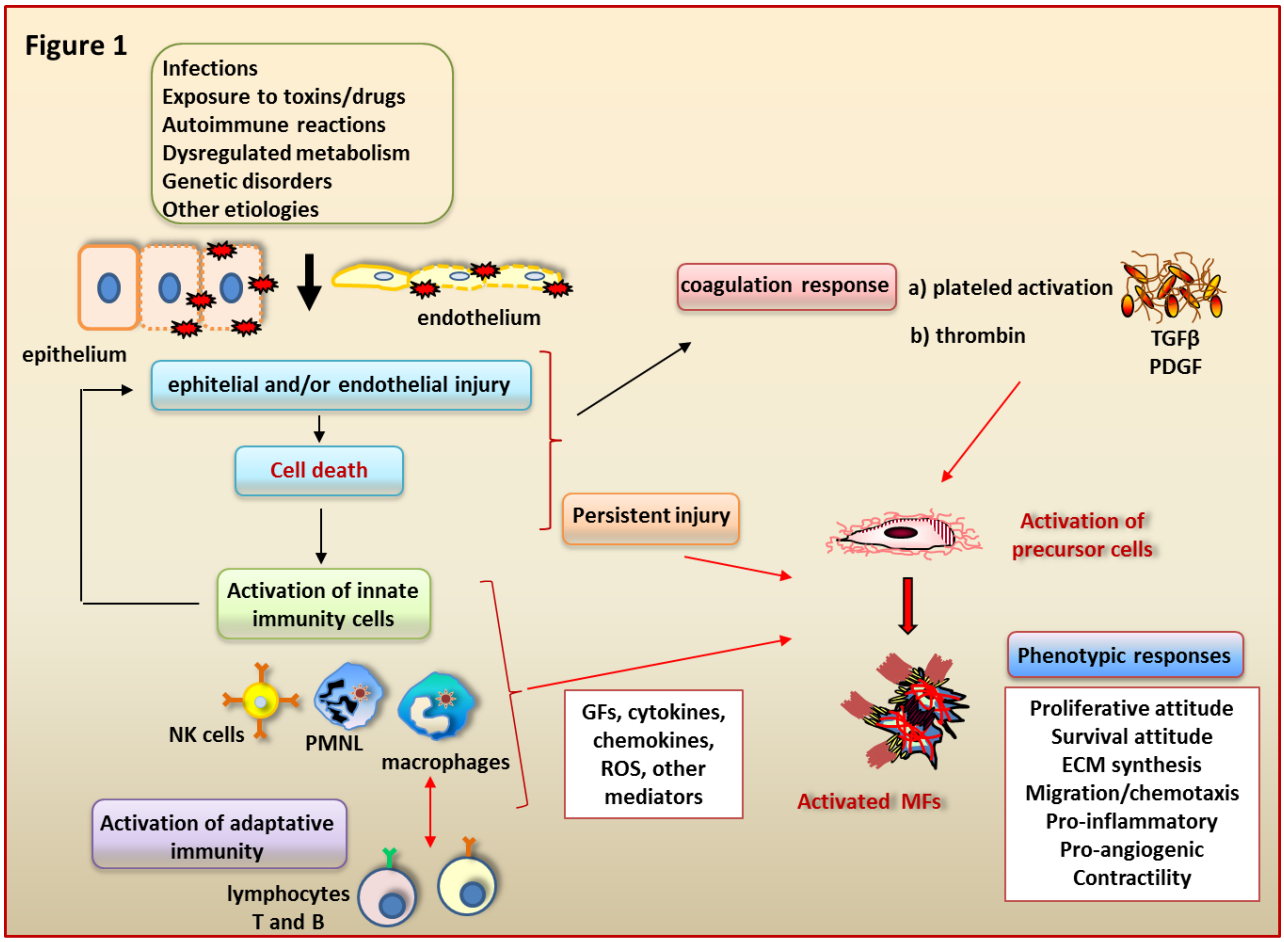
Figure 1
Infections
Exposure to toxins/drugs
Autoimmune reactions
Dysregulated metabolism
Genetic disorders
Other etiologies
epithelium
endothelium
ephitelial and/or endothelial injury
Cell death
Activation of innate immunity cells
NK cells
PMNL
macrophages
Activation of adaptative immunity
lymphocytes T and B
coagulation response
a) plateled activation
b) thrombin
TGFβ
PDGF
Persistent injury
Activation of precursor cells
GFs, cytokines, chemokines, ROS, other mediators
Activated MFs
Phenotypic responses
Proliferative attitude
Survival attitude
ECM synthesis
Migration/chemotaxis
Pro-inflammatory
Pro-angiogenic
Contractility

Figure 2.

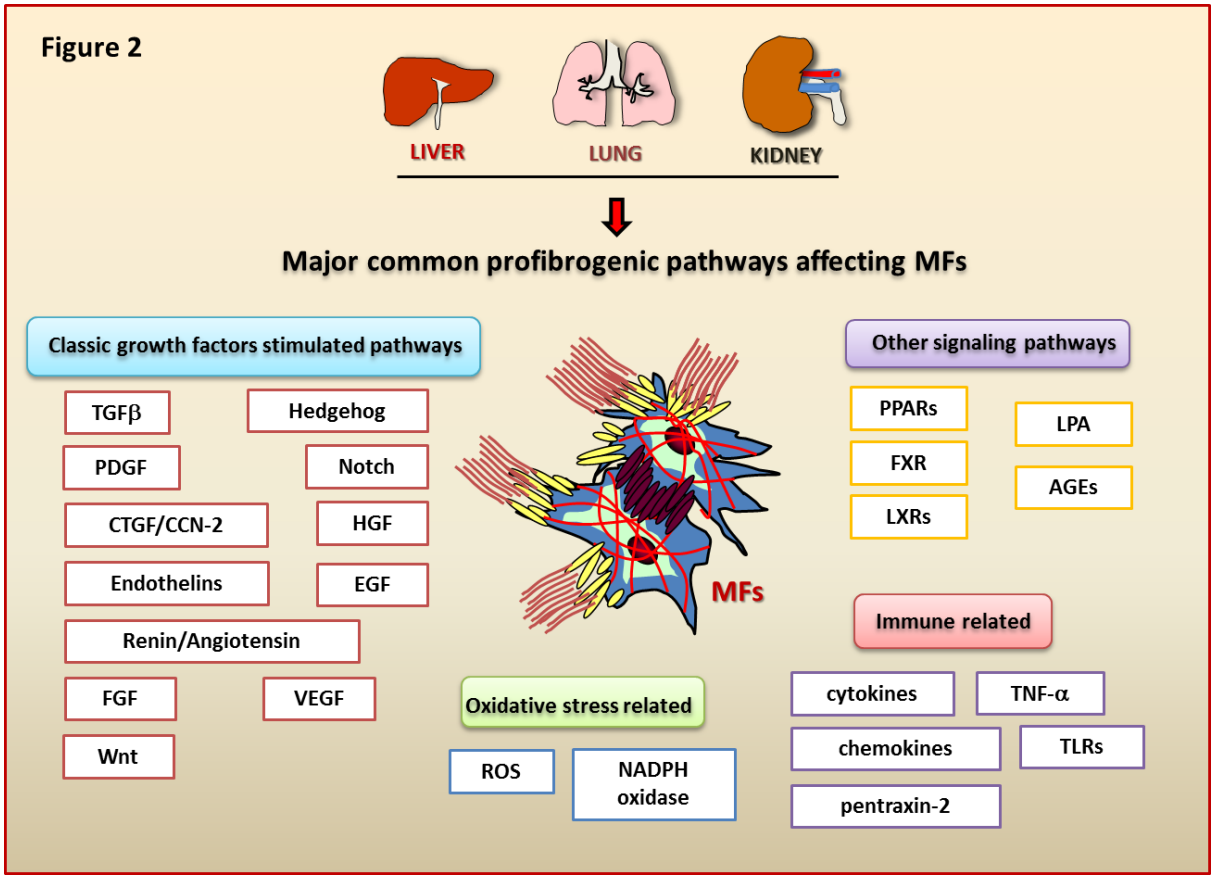
Figure 2
LIVER
LUNG
KIDNEY
Major common profibrogenic pathways affecting MFs
Classic growth factors stimulated pathways
TGFβ
Hedgehog
PDGF
Notch
CTGF/CCN-2
HGF
Endothelins
EGF
Renin/Angiotensin
FGF
VEGF
Wnt
MFs
Other signaling pathways
PPARs
LPA
FXR
AGEs
LXRs
Immune related
cytokines
TNF-α
chemokines
TLRs
pentraxin-2
Oxidative stress related
ROS
NADPH oxidase