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Fibrosis: The sixth element

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Abstract

Disease-related injury in any organ triggers a complex cascade of cellular and molecular responses that culminates in tissue fibrosis. This monograph describes the molecular, cellular and immunological characteristics that occur in an inflammatory process, until it ends in fibrosis; and particularly when the phenomenon persists, leading to an activation that does not remit from the effector cells, chronifying the process, healing the tissue or the organ, generating specific pathologies and dysfunction. The article is illustrated with some examples of fibrotic diseases, discussing their pathogenesis, clinical picture and treatment, particularly in light of new immunopathogenic findings. The preponderant role of early therapeutic interventions to block the evolution towards tissue scar is highlighted. In the end, recommendations are made about the avenues of investigation that must be followed in this final stage of the inflammatory process.

Introduction

Inflammation is a term credited to Celsus. It was used as a metaphor because the dermal response to injury was reminiscent of a fire, characterized by redness (rubor), heat (calor), swelling (tumor), and pain (dolor), and some of these Latin terms are used today in medical schools. Virchow described a fifth change, loss of function (*functio laesa*). A sixth change, repair, could also be added, because a new growth occurs after tissue injury, just like after the fire occurs in the forests, prairies and even cities in an attempt to preserve the function and life [1]. When injury and inflammatory responses are abrogated, resorption of extracellular matrix proteins occurs, promoting organ repair. When chronic injury persists, the unremitting activation of effector cells results in the continuous deposition of extracellular matrix, progressive scarring and organ damage [2].

Rockey and others have proposed 4 phases of the fibrogenic response. The first phase is the beginning of the response to the injury to the organ. The second phase is the activation of effector cells and the third is the elaboration of extracellular matrix proteins (ECM). The fourth phase is the deposit of these proteins in a dynamic model, in which, not only the deposit is increased, but the reabsorption of ECM is also reduced, promoting the progression to fibrosis and finally the failure of the organ [2]. The "wounding response" is activated by complex activities within different cells that generate specific molecules with programmed action pathways. The cellular component includes inflammatory cells (eg, polymorphonuclear cells, macrophages, T lymphocytes, etc.), epithelial and endothelial cells and fibrogenic effector cells that are the ones that will produce and release ECM proteins. These effector cells include fibroblasts, myofibroblasts, cells derived from bone marrow, fibrocytes, epithelial cells in transition to mesothelial cells (EMT), endothelial cells in transition to mesothelial cells (EndMT), and mesothelial cell in transition to mesenchymal cells (MMT). A number of molecules are critical to transmit and control information between the cells that suffer the initial injury and the effector cells that produce ECM. The deformity of the organ produces dysfunction, but it must be emphasized that this is a dynamic, active and ductile phenomenon, although the fibrogenic plasticity varies from organ to organ.

Pathogenesis

Both acute and chronic inflammation can develop fibrosis. Inflammation damages the epithelial and endothelial cells leading to the production and release of inflammatory mediators (cytokines, chemokines and others) that recruit a wide range of inflammatory cells (PMNs, eosinophils, basophils, mastocells, T lymphocytes and macrophages). These cells, in turn, release profibrotic mediators which activate effector cells that manage the fibrogenic process [3]. The molecular factors that direct and control the fibrotic process are broad and complex. One of these factors, which is practically present in almost all fibrotic processes, is the transforming growth factor beta (TGF-β). This factor involves the binding of a ligand to a serine-threonine kinase type II receptor that recruits and phosphorylates to type I receptor. This type I receptor then phosphorylates SMADs that modulate the gene expression of the genes that code for the activity of fibrogenic factors. SMAD is an acronym from the fusion of Caenorhabditis elegans Sma genes and the Drosophila Mad Mothers against decapentaplejic proteins to transducer signals. Actually TGF-β is a superfamily that involves multiple cascades of signals [4]. TGF-β is a potent stimulator of fibrogenic cells for the production of ECM proteins [5,6].

Other profibrotic factors that stimulate ECM production are: platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), IL-1, IL-4, IL -6, IL-10, TNF-α. Also vasoactive peptides play an important role in the deposition process of ECM, such as angiotensin II and endothelin I [7]. This latter peptide has a role in fibrosis in practically all organs by acting on the receptor coupled to G protein of endothelin-A or on the cellular receptor of endothelin-B or both [8]. Integrins, which

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bind extracellular matrix to cells, are also critical in the pathogenesis of fibrosis [9].

These factors will stimulate the effector cells to release ECM. The matrix proteins that form the fibrotic scar are mainly collagen I and III (synthesized from pro-collagen), cellular fibronectin, basement membrane proteins such as laminin, proteoglycans and aggrecan. Myofibroblasts express smooth muscle proteins, including actin (ACTA2), which is contractile. The contraction of these cells contributes to the distortion of the parenchymal architecture, but this remodeling confers a dynamism character to the tissue and may have important therapeutic implications [2]. The fibrogenic process therefore involves an interrelation between biosynthesis, deposition and ECM degradation. The synthesis is counterbalanced by degradation by matrix metalloproteinases (MMPs) (collagenases and gelatinases), whose activity in turn is controlled by tissue inhibitors of metalloproteinases (TIMPs). Collectively MMPs and TIMPs determine the general rate of ECM degradation [10,11]. In addition, the process is governed by the elimination of effector cells by senescence, apoptosis and autophagy.

The role of genetics in the pathogenesis of fibrosis goes "in crescendo". For example, in the kidney, fibrosis is a prominent change of karyomegalic interstitial nephritis, which is caused by mutation in the gene encoding Fanconi anemia-associated nuclease 1 (FANI) [12]. In the liver, PNLAP3 is important in ethanol-mediated fibrosis and associated with fatty liver disease and there are a number of candidate genes that may be important in hepatic fibrosis induced by infection with the hepatitis C virus [13-15]. Primary myelofibrosis (PMF) can be associated with mutations of the JAK2, CALR, MPL genes [16]. HCM (hypertrophic cardiomyopathy) is a relatively common genetic disorder of cardiac sarcomeres, characterized by left ventricular hypertrophy and represents the most common cause of sudden cardiac death among young athletes [17]. Mutations in TERT, TERC, PARN, RTL1, genes involved in the maintenance of telomere length, are associated with an increased risk of idiopathic pulmonary fibrosis (IPF) [18,19]. Variations in genes (DSP, AKAP13, CTNNA, and DPP9) that are responsible for cell adhesion, integrity, and mechanotransduction (the generation of electrical signals from mechanical stimuli) also confer a predisposition for IPF [20,21].

A single-nucleotide polymorphism (rs35705950) in the promoter region of MUC5B substantially increases the risk of IPF [22]. MUC5B codes for mucin 5B, a glycoprotein required for airway clearance and innate immune responses to bacteria [23]. The rs35705950 minor allele leads to overexpression of mucin 5B in small-airway epithelial cells, to universal finding in patients with IPF (regardless of the MUC5B genotype). Although the mechanism linking mucin 5B overexpression and IPF risk remains unknown, some researchers have hypothesized that aberrant mucociliary clearance may lead to alterations in the lung microbiome and innate immune responses that promote IPF [24-26].

The epigenetic regulation of gene expression, which includes but is not limited to DNA methylation, post-translational modifications of chromatin histones, and regulatory microRNAs (miRs), is important in fibrosis. The miRs play a role in expanding genetic regulation. In diabetic nephropathy, TGF-β promotes the expression of mi-R192, which results in collagen deposition [27], and miR-19b regulates TGF-β signals in hepatic stellate cells [28]. In the heart, miR-21, miR-29, miR-30 and miR-133 participate in the remodeling of the myocardial matrix [29]. Fibroblasts from patients with IPF have global changes in DNA methylation, which are not found in normal lung fibroblasts [30].

Clinical examples

Fibrosis is a pathological change of disease in virtually all organs (Table 1). Here are some examples of fibrosis,

Cardiac fibrosis

Heart failure (HF) is a malignant and fatal disease. The incidence of heart failure is 1-2% in developed countries and increases to 10% in those over 70 years, with only 35% of survival to 5 years after diagnosis [31]. A key mechanism of HF is cardiac remodeling, which includes 2 aspects: injury to cardiomyocytes and myocardial fibrosis. There are two types of myocardial fibrosis: fibrosis by replacement and interstitial fibrosis (reactive). Reactive fibrosis occurs in perivascular spaces and corresponds to a fibrogenic response similar to that of other tissues; fibrosis due to replacement occurs at the site of myocyte loss, as occurs in necrosis due to myocardial infarction. Both lead to ECM deposit.

The damaged myocardium releases DAMPs (danger-associated molecular patterns) that are molecules that activate macrophages, mastocells and lymphocytes at the site of injury. For example, one of these molecules (CCL-2 = C-C motif chemokine 2) recruits monocytes inducing their proliferation and mobilization of bone marrow into the inflammatory compartment as precursors of macrophages [32]. Macrophages (M1 and M2) release inflammatory mediators (which attract more cells amplifying inflammation) and profibrotic factors (TGF-β, PDGF, IL-10, angiotensin II, and endothelin I) that activate fibrogenic effector cells [33]. Inhibiting macrophage infiltration could prevent the development of fibrosis [34]. Mastocells release, in addition to the fibrogenic factors, tryptase and chymase. The latter, via angiotensin II, can activate the TGF-β / SMAD axis and promote fibrosis. Blocking TGF-β with antibodies could suppress the production of collagen induced by chymase [31]. Infiltration with T lymphocytes is associated with progression of HF. There are 4 subsets of T lymphocytes: T helper (Th1 and Th2), T regulatory (Treg) and

Table 1. Fibrogenesis and organ system. Selected organs and associated diseases are highlighted

Eye	Strabismus
Skin	Scleroderma
	Keloid
	Nephrogenic systemic fibrosis
Pulmonary fibrosis	Restrictive lung disease
	Pulmonary hypertension
	Right-side heart failure
Cardiac fibrosis	Diastolic dysfunction
	Heart failure
	Arrhythmia
Renal fibrosis	Chronic kidney disease
	Hypertension, anemia
	Electrolytes disturbances
Cirrhosis	Portal hypertension
	Ascites
	Gastroesophageal varices
	Hepatorenal syndrome
	Hepatopulmonary syndrome
	Portopulmonary syndrome
	Hepatic encephalopathy
	Hepatocellular cancer
Pancreatic fibrosis	Chronic pain
	Diabetes mellitus
	Malabsorption
	Cancer

Th17. Th1 media collagen-cross-linking in left ventricle leading to diastolic dysfunction; Th2 releases IL-13 and IL-14 and Th17 produces IL-17, all of which promote the production of collagen. Treg attenuates myocardial fibrosis and is the main mechanism of protection from injury to cardiomyocytes.

Although proinflammatory cytokines, ROS, TGF-β, renin-aldosterone-angiotensin regulate the process of myocardial fibrosis, recent studies describe some other emerging molecules involved in the fibrogenic process. Such is the case of cardiotrophine-1, nicotinamide adenine dinucleotide phosphate oxidase and several matricellular proteins that are involved in the activation of myofibroblasts and collagen cross-linking. These molecules are under intense investigation as diagnostic and therapeutic objectives [35,36].

TGF-β1 (one of the 3 isoforms of TGF-β) activates the conversion of fibroblasts (the most abundant cell in the myocardium) to myofibroblasts and activates the production of collagen, mainly I and III [37]. Cardiomyopathy associated with TGF-β1 is associated with valvular thickening, valvular dysfunction, systolic and diastolic dysfunction, and electrophysiological abnormalities and repolarization disorders. The collagen in the septa generates areas of arrhythmogenic fibrosis by inducing a slow discontinuation of the conduction and also an alteration of the reentry circuits due to spatial heterogeneity [38-41]. Fibrotic scar in heart correlates strongly with arrhythmias and sudden cardiac death.

Markers of myocardial fibrosis such as Galactin-3 and ST-2 (a member of the IL-1 receptor family) have produced dissimilar results [42]. miRNAs and pro-Collagen Type I (PICP) and Amino-Terminal Pro-Peptide of Pro-Collagen Type III (P III NP) are not very specific because they are not only associated with myocardial but also hepatic and pulmonary fibrosis.

Nuclear Magnetic Resonance (MR) with Gadolinium is a recent method of research and diagnosis. Last Gadolinium Enhancement (LGE) is defined excessively in the myocardium due to the lengthening of the extracellular space by the deposit of ECM. The patterns of LGE-MR could help identify the causes of heart failure, especially cardiomyopathies [43]. At the prognostic level, the amount of high-scale threshold on CMR correlates positively with the possibility of adverse cardiovascular outcomes in patients with end C & D heart failure (adjusted hazard ratio 1.46 / 10% increase in LGE: $p = 0.003$) [44]. There is no "best threshold" that perfectly matches the extent of the fibrosis and the intensity of the LGE signal is not precise enough to differentiate the types of fibrosis (for example, the interstitial vs. the replacement). T1 "mapping" has a prominent advantage to detect diffuse fibrosis because it evaluates the relaxation time of myocardial tissue.

The ECV (extracellular volume fraction) evaluates myocardial fibrosis by measuring T1 mapping analysis pre- and post-contrast. Myocardial ECV is higher in patients with non-ischemic dilated cardiomyopathy than in normal individuals, and may identify early interstitial fibrosis. ECV can be an important method to assess mechanical and physiological abnormalities in patients with HCM, a relatively common genetic disorder of cardiac sarcomeres that presents with hypertrophy of the left ventricle and is the most frequent cause of sudden death among young athletes [45]. Although endomyocardial biopsy could be considered as the "gold standard" for detecting myocardial fibrosis, the ethical and moral regulations and the invasive nature do not allow apply it in a generalized way.

Hepatic fibrosis

Liver fibrosis is a frequent and potentially fatal complication of many chronic diseases that affect hepatocytes or biliary cells, and represents a high medical and economic burden. Although it is true that molecular knowledge of pathogenesis has advanced considerably at the experimental level, the transfer to the clinical part is limited and pharmacological treatment is generally effective only in experimental models [46]. The different etiological agents damage the hepatocytes, inducing an inflammatory response that involves the local vascular system and the immune system, causing the systemic mobilization of endocrine and neurological mediators. This response involves endothelial cells, stellate cells and resident immune cells (macrophages = Kupffer cells, dendritic cells and mastocells), which contain surface receptors that sense bacterial toxins (PAMPs) and molecules released from the injured tissue (DAMPs) by releasing a variety of different inflammatory and profibrotic mediators within the liver tissue. Numerous molecular pathways participate, as do those found in other organs, but a pathway that seems to be exclusive until this time of liver is the one that uses the toll-like receptor 4 (TLR4) [47]. TLR4 is activated on the surface of the stellate cells by bacterial intestinal lipopolysaccharides derived from the intestine (translocated bacteria), activating fibrogenic effector cells, linking fibrosis and microbiome [48]. Although a variety of effector cells synthesize ECM, hepatic stellate cells appear to be the major source of ECM in the liver. There is sufficient evidence that these cells, similar to pericytes, undergo myofibroblastic transformation in response to injury.

Hepatic fibrosis can be caused by different etiologies including genetic disorders, chronic viral infection, excessive alcohol consumption, autoimmune attack, metabolic disorders, decreased bile flow, venous obstruction and parasitic infection [49]. But also excess lipids and other lipotoxic agents produce endoplasmic reticulum stress, impaired mitochondrial function and oxidative stress in parenchymal and non-parenchymal hepatic cells, and modifications in the microbiotic composition of the gastrointestinal tract or its integrity may be associated with nonalcoholic fatty liver disease and hepatic fibrosis [50]. The progression of liver disease can be triggered by the acidic bile composition. Bile acids are amphipathic molecules with manifold physiological functions. On the one hand, they facilitate the emulsification of dietary fats and assist the intestinal absorption of lipids and fat-soluble vitamins [51]. On the other hand, they act like hormones and are embedded in a complex signal cascades, The most important targets of bile acids are the farnesoid X receptor (FXR) and the G protein-coupled membrane receptor 5 (TGR5), which activate the expression of genes involved in the metabolism of bile acids, lipids and carbohydrates. Bile acids also have antimicrobial action [52].

The end result of hepatic fibrogenesis is cirrhosis, an ominous parenchymal lesion that underlies a wide range of devastating complications that have adverse effects on survival. Portal hypertension, a devastating result of injury, develops during the fibrogenic response after disruption of the normal interaction between sinusoidal endothelial cells and hepatic stellate cells; the resulting activation and contraction of pericytes-like stellate cells leads to sinusoidal constriction and increased intrahepatic resistance. This increase in resistance in turn activates abnormal signaling by smooth-muscle cells in mesenteric vessels. An increase in angiogenesis and collateral blood flow follows, resulting in an increase in mesenteric blood flow and a worsening of portal hypertension [50]. The major clinical sequelae of portal hypertension, variceal hemorrhage and ascites, emerge relatively late, after the portal pressure rises to a hepatic venous pressure gradient of more than 12 mm Hg [53].

Renal fibrosis

Renal fibrosis is characterized by an excessive deposit of ECM in the interstitial compartment, leading to scar formation [54]. The kidneys are susceptible to hypertension and diabetes, the two leading causes of renal fibrosis. As in other organs, renal fibrosis is mediated by cellular elements and molecular elements, but among the factors that stimulate ECM in renal fibrosis, TGF-β1 is the main responsible. It is the most potent and ubiquitous profibrotic factor, which acts through intracellular signals such as protein-kinases and transcription factors, and is involved not only in ECM deposition, but also in hypertrophic renal proliferation and renal cell apoptosis. The renin-angiotensin-aldosterone system is particularly important in renal fibrosis induced by hypertension [55]. Myofibroblasts are the major source of ECM: α-smooth muscle actin-positive myofibroblasts. A pathogenic pathway of renal fibrosis is the defects of the metabolism of fatty acids in tubular epithelial cells with accumulation of intracellular lipids [56]. Basically what exists is an enzymatic defect in the oxidation of fatty acids (FAO). TGF-β1, the most potent profibrotic cytokine, inhibits the expression of carnitine palmitoyltransferase 1 (CPT1), the rate-limiting enzyme in FAO, and that decreases fatty acid metabolism. Accumulated fatty acids damage cells that generate signals for the proliferation of myofibroblasts and renal fibrogenesis [57].

Another pathogenic pathway is the program of transformation of epithelial cells to mesenchymal cells, which generates fibrogenesis (EMT). Therefore, inhibiting the EMT program and blocking or inducing (genetic or pharmacological ablation) a reduction in the population of myofibroblasts could in the future reduce the progression to fibrosis after injury [58,59]. Injury to any of the elements of renal cellular architecture (glomeruli, tubules, interstitium and capillaries) triggers the EMC deposit. The location determines the clinical consequence. The immune deposits are located in the glomeruli producing glomerulonephritis, while the injury of the tubulo-interstitial environment (NSAIDs, infection, polycystic kidney disease, urinary obstruction) produces tubulo-interstitial fibrosis [60].

Glomerular fibrosis, regardless of the cause, diminishes renal blood flow, which leads to hypoxia and the activation of hypoxia-inducible factor 1, which in turn triggers nephron collapse and fibrotic replacement by means of rarefaction [61]. Regardless of the initiating insult, renal fibrosis leads to loss of function and organ failure. Homeostasis can be maintained with a glomerular filtration rate as low as approximately 10% of the normal rate. As the mechanisms maintaining homeostasis are progressively disrupted, anemia develops and the regulation of electrolyte balance and pH is disrupted.

Pulmonary fibrosis

The family of interstitial lung diseases is characterized by cellular proliferation, interstitial inflammation, fibrosis, or a combination of such findings within the alveolar wall that is not due to infection or cancer [62]. Interstitial fibrosis is the predominant phenotype in most cases. The majority of patients with interstitial fibrosis ultimately receive a diagnosis of chronic hypersensitivity pneumonitis (due to mold or bird exposure), pulmonary sarcoidosis, an underlying autoimmune disease, drugs, environmental exposures (e.g., silica dust or asbestos), or if no cause is identified, an idiopathic interstitial pneumonia. The most common idiopathic interstitial pneumonia is idiopathic pulmonary fibrosis (IPF), a chronic, progressive, fibrotic interstitial lung disease of unknown cause, often with characteristic imaging and histologic appearances that occurs primarily in older adults [63]. The increased rate of hospitalizations and deaths suggests an increased burden of a disease that occurs worldwide. The incidence of IPF is higher in the USA and Europe (3-9 cases per 100,000 persons-year) and the prevalence is 494 cases per 100,000 in adults over 65 years [64,65].

The chronic history of exercise dyspnea is practically universal in this entity, accompanied by chronic cough without purulence and fatigue. With the progression, bilateral Velcro-like crackles, clubbing and acrocyanosis appear. The three typical functional findings are reduced DLCO, hypoxemia at rest or desaturation with exertion, and a normal or low FVC. Conventional radiography shows nonspecific changes or bilateral basal reticular abnormalities. High resolution CT (supine, inspiratory and expiratory decubitus and with thin reconstruction [<1.25 mm]), in the absence of a specific cause of fibrosis, will identify the UIP pattern (usual interstitial fibrosis), diagnosis of IPF. Lung biopsy will be done only if the combination of clinical, functional and imaging data is not diagnostic, and if the histological result is expected to influence the therapeutic decision. The procedure of choice is thoracoscopy of multiple lobes and avoiding the most fibrotic areas. Progressive pulmonary fibrosis also leads to pulmonary hypertension, right-sided heart failure, and ultimately respiratory failure. It is frequently confused with heart failure or COPD [66].

A conceptual model of pathogenesis is that the recurrent subclinical epithelial injury is superimposed on an accelerated epithelial aging, which leads to an aberrant repair of the injured alveoli and deposition of ECM by myofibroblasts. Apparently senescence of epithelial cells is a central phenotype that favors pulmonary fibrosis [67]. Shortened telomeres, oxidative stress, proteostatic dysregulation, endoplasmic reticulum stress, and mitochondrial dysfunction decrease epithelial alveolar cell proliferation and increase the secretion of profibrotic mediators [68,69]. A study has identified abundance of *prevotella*, *veillonella* and *escherichia* in the bronchioloalveolar lavage of patients with IPF and abundance of *streptococcus* and *staphylococcus* have been associated with an increased risk of disease progression [70,71]. A number of non-genetic risk factors for IPF have been identified. Older age, male sex, and cigarette smoking are considered risk factors for IPF [72]. Observational data have implicated gastroesophageal reflux, [73], obstructive sleep apnea [74], air pollution [75], herpesvirus infection [76], and certain occupational exposures in interstitial lung disease.

Other form of fibrosis

Fibrosis also occurs in the joints, bone marrow, brain, eyes, intestines, peritoneum and retroperitoneum, pancreas, mediastinum and skin, and in these cases is driven by typical cellular and molecular processes. Mediastinal fibrosis is a rare disease mainly associated with histoplasmosis (USA), blastomycosis, aspergillosis, mucormycosis and cryptococcosis. It has also been associated with other granulomatous diseases (tuberculosis and sarcoidosis), autoimmune processes (Behçet's disease, rheumatoid arthritis, systemic lupus erythematosus), neoplasms (lung adenocarcinoma, Hodgkin's lymphoma), and radiotherapy [77]. Clinical features include the involvement of mediastinal structures such as superior vena cava, inferior vena cava, pulmonary arteries and veins, esophagus, and heart. Postulated pathogenic mechanisms include an abnormal autoimmune response to chronic infection and a fibrotic response that invades the mediastinum [78]. Retroperitoneal fibrosis is a rare condition characterized by inflammation and fibrosis in the retroperitoneal space; Most cases are idiopathic, but secondary causes include drugs, infections, autoimmune and inflammatory stimuli, and radiation. The major clinical sequelae of this condition are related to its involvement with structures in the

retroperitoneum, including arteries (leading to chronic renal failure) and ureters (leading to hydronephrosis). Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by a clonal proliferation derived from stem cells that usually, but not always, is accompanied by mutations (already described in the pathogenesis section), abnormal expression of cytokines, bone marrow fibrosis, splenomegaly, extramedullary hematopoiesis, constitutional symptoms, cachexia, leukemic progression and reduced survival [79].

In certain cancers, fibrosis is linked to TGF-β-integrin signaling [80]. In scleroderma, the prototypical fibrosing skin disease, skin fibroblasts and myofibroblasts are activated through the TGF-β–SMAD signaling pathway [81]. Nephrogenic systemic fibrosis, a debilitating condition that is marked by widespread organ fibrosis, occurs in patients with renal insufficiency who have been exposed to gadolinium-based contrast material. Initial systemic inflammatory-response reactions and the reaction of gadolinium (Gd3+) ions with circulating proteins and heavy metals lead to the deposition of insoluble elements in tissue. Since no effective therapies have been identified, prevention is key. A recently recognized IgG4-related disease appears to involve autoimmune-driven inflammation that provokes fibrosis in multiple organs, including the pancreas, retroperitoneum, lung, kidney, liver, and aorta [82].

Therapy

Fibrosis and resultant organ failure account for at least one third of deaths worldwide [83]. Since fibrosis is common and has adverse effects in all organs, it is an attractive therapeutic target. Elimination of the inciting stimulus is the first and most efficacious approach. Fibrosis of parenchymal tissue usually progresses slowly, which suggests that therapy may be required for extended periods; slowing the progression of fibrosis may be a more realistic therapeutic goal than eliminating it. Fibrosis occurs by means of a dynamic process that involves the synthesis and deposition of extracellular matrix, and its reversion occurs by means of the elimination of effector cells and shifts in the balance of matrix synthesis and degradation. Although it is not clear what pathogenic or clinical factors promote reversibility, the regression of fibrosis has been shown to lead to improved clinical outcomes. The best indication that fibrosis is reversible and that this reversibility has positive effects on clinical outcomes is based on the treatment of liver and pulmonary fibrosis [84,85].

Heart

During the past several decades, the medical treatment of HF has dramatically progressed. Pharmacologic therapies in clinical use for heart failure that target the primary underlying disease appear to have a secondary effect on fibrosis. Traditional medical interventions to improve the prognosis of HF are beta-blockers, ACE inhibitors, and aldosterone antagonists. These three first-line interventions are called the "Golden Triangle" of HF treatment.

New approaches include decreasing production or increasing collagen degradation. Seralaxin is a recombinant form of human relaxin-2, inhibits cross-linking and collagen synthesis, and has entered into phase III clinical studies for heart failure [86]. Some studies indicate that the diuretic torasemide can inhibit lysyl-oxidase to limit the speed of collagen crosslinks and thus reduce myocardial fibrosis. The anti-TGF-β1 antibody can suppress collagen synthesis, but it is not specific for myocardium, since the inhibition of TGF-β1 signals is systemic and may generate unexpected results in the attempt to suppress myocardial fibrosis. Studies in animals blocking miRNAs (anti-miRs) with oligonucleotides appear to reduce interstitial fibrosis, but there are still no clinical studies.

There are drugs that target the degradation of collagen. There are selective and non-selective inhibitors of MMPs. Batimastat, marimastat, GM-6001 (ilomastat or gelardin), PD-166793 and ONO-4817 are non-selective inhibitors. However, semi-selective inhibitors (PY-2 and 1, and 2-HOPO-2) produce better results than non-selective inhibitors, since they are unstable due to proteolysis. PD-166793 and CGS270223A can improve re-perfusion ischemia [31]. CMT3 (chemically modified tetracycline-3) can inhibit MMP-2 and MMP-9 and therefore cardiac remodeling. Obviously more studies are required to identify more specific objective sites [87]. A promising idea for the treatment of cardiac fibrosis is based on the premise that cardiac fibroblasts can be reprogrammed into cardiomyocyte-like cells [88,89]. It is not yet known if this type of therapy can be used in human.

Liver

The process of hepatic fibrosis is dynamic. Since hepatocytes are capable of regeneration, liver fibrosis may be especially amenable to therapeutic intervention, and even cirrhosis can be reversed [90,91]. Eradication of HCV infection, antiviral therapy for HBV infection, glucocorticoid therapy for autoimmune hepatitis, phlebotomy for hemochromatosis, relief of biliary obstruction, and cessation of alcohol consumption in alcoholic hepatitis each clearly reverses fibrotic change, and many of these treatments improve clinical outcomes [92,93].

Despite recent enthusiasm with the use of antioxidants in fibrosis and experimental studies, solid clinical efficacy data in humans are very limited [46]. Inhibition of liver damage can be implemented in several ways; namely: fibropreventive, fibrostatic or fibrolytic drugs. Obeticolic acid (OCA, and INT-747), has anticolestastial and hepatoprotective properties, increases insulin sensitivity, modulates the metabolism of fats and has anti-inflammatory and antifibrotic properties. Both alternatives are under clinical evaluation and OCA significantly reduced fibrosis in clinical work in patients with NASH [94]. OCAs and similar drugs target FXR receptors. Best known are the endogenous agonists of these FXR receptors such as chenodeoxycholic acid, deoxycholoic acid, cholic acid and lithocholic acid. In general, these medications improve glucose metabolism, increase insulin sensitivity, reduce hepatic lipogenesis and increase β-oxidation.

Drugs that reduce the intestinal absorption of cholesterol, increase bile flow and change the hydrophobicity index of the pool of bile acids, cause anti-inflammatory effects. UDCA (steroid bile acid ursodeoxycholic acid) and nor UDCA (short chain homolog) increase the cholehepatic shunt, are also therapeutic options [95,96]. An alternative that requires future studies in humans is to neutralize osteopontin (increases TGF-β and induces fibrogenesis) and the use of pentraxins which are proteins that bind directly to monocytes, neutrophils and macrophages, modifying their activation, dissemination and polarization and inhibiting their differentiation to fibrocytes [97,98].

Recent strategies are in development. For example, induce apoptosis, senescence or deactivation of the cells that produce ECM [99]. Sequestering profibrotic cytokines could be effective. For example, sequestering TGF-β and its receptors, or PDGF and its signals with multikinases and aptamers, was investigated experimentally and in preclinical studies [100]. An antibody against lysyl-oxidase-like 2 (LOXL2), involved in collagen crosslinking; simtuzumab, has not shown activity in patients with hepatic fibrosis [101]. Microbiotic is

the sum and composition of the microorganisms of the gastrointestinal tract. It is conceivable that manipulating it with antifibrotic microbes could, in the future, be established as a treatment in human liver disease [102,103].

Viral eradication (antiviral drugs), removal of parasites (praziquantel for *Schistosma*), and suspension of toxins or drugs can restore liver architecture. It should be remembered that this fibrotic process is dynamic and even advanced fibrosis can be recovered [104]. Portal thrombosis and venous obstruction are frequent in liver cirrhosis and advanced fibrosis. Use of low molecular weight heparin (enoxaparin) can prevent portal vein thrombosis, hepatic decompensation, and reduce mortality in patients with advanced liver cirrhosis [105].

NAFLD (non-alcoholic fatty liver disease) (afflict 30% of the general population in the Western world), NASH (non-alcoholic steatohepatitis), *diabetes mellitus*, and metabolic syndrome could be prevented with a healthy diet, weight loss and regular physical activity, but the hectic and stressful nature of modern life, and the industry's propaganda of foods rich in sugars and fats impede such adjustments. Several pharmacological attempts are under scrutiny. In 516 patients with biopsy-proven NASH, the use of thiazolidinedione (rosiglitazone or pioglitazone) was associated with improvement in NASH resolution and fibrosis, even in patients without diabetes [106]. Other candidate drugs are: the glucagon-like peptide-1 receptor (GCP-1R), and the FXR agonist as OCA (INT-747) that is currently in phase 2 and 3 studies in various types of fibrosing liver disease. Vitamin E has beneficial effects on liver enzymes, steatosis, inflammation, hepatocyte ballooning and hepatic fibrosis [107]. Selonsertib (CS-4997), an inhibitor of apoptosis signal-regulating kinase (ASK1) has been shown to reduce fibrosis in patients with NASH and fibrosis in stages 2-3 [108]. Other drugs such as cenicriviroc and elafibronor are still under investigation [109-111].

Kidney

Like the therapies used to treat cardiac fibrosis, those typically used to prevent renal fibrosis target the underlying disease processes and as such involve the treatment of hypertension and diabetes. One target is the renin–angiotensin system. This approach involves the use of ACE inhibitors and angiotensin-receptor blockers that ameliorate renal damage and fibrosis through multiple pathways, including the suppression of the actions of TGF-β [112]. Therapies based on the antagonism of aldosterone that make use of mineralocorticoid receptor antagonists have been shown to inhibit or slow the progression of fibrosis in humans [113]. Novel approaches to the treatment of fibrosis of the kidneys include those that target bone morphogenetic protein-7, NADPH oxidase (NOX) (NOX1 and NOX4), and the SMAD3 and SMAD4 pathways [114].

Lung

The lung presents special challenges with regard to therapy targeting fibrosis. On the one hand, the lung has easily measured clinical features that allow for assessment of lung function, a surrogate for fibrosis. On the other hand, pulmonary fibrosis appears to be less dynamic than fibrosis occurring in other organ systems. Non-pharmacologic management strategies help patients with IPF live healthier, more normal lives, and the importance of these approaches cannot be overemphasized. Smoking cessation should be a priority for patients who are actively using tobacco products. Influenza, pneumococcal, and other age-appropriate vaccines should be administered [85]. Clinical practice guidelines strongly recommend supplemental oxygen for patients with IPF [63]. Oxygen administration reduces exertional dyspnea and improves exercise tolerance [115]. An oxyhemoglobin saturation of 88% or less at rest, during exertion, or during sleep should prompt initiation of home oxygen therapy [116]. Pulmonary rehabilitation, a structured exercise program designed for adults with advanced lung disease, has been shown to improve exercise capacity and health-related quality of life for patients with IPF [117].

Only a minority of patients with IPF receive a transplant [118]. Lung transplantation can prolong survival and improve quality of life for highly selected candidates [119,120]; however, only 66% of transplant recipients with IPF survive for more than 3 years after transplantation and only 53% survive for more than 5 years [118]. Common complications include primary graft dysfunction, acute and chronic forms of allograft rejection, cytomegaloviral and other infections, and cancer [118]. IPF has not been shown to recur in the allograft. Referral to a transplantation center should be made at the time of diagnosis, since the evaluation process and waiting time can last for months to years [121]. Common contraindications include recent cancer, advanced non-pulmonary organ failure, and lack of a reliable social support system [122]. Poverty, meager health budgets and little experience of health personnel with this tool threaten the possibility of implementing them successfully in third world countries,

Treatment guidelines for IPF include a strong recommendation against the use of prednisone in combination with azathioprine and oral N-acetylcysteine, a regimen associated with an increase in mortality by a factor of 9, as compared with placebo. Interferon-γ, [123], endothelin antagonists, [124] and warfarin [125] are ineffective or harmful in patients with IPF. The Food and Drug Administration has appropriately warned consumers against various unapproved stem-cell "therapies" advertised for the treatment of IPF [126]. Although current guidelines recommend the use of antacid therapy to treat IPF, there are no data from clinical trials to support this recommendation [63]. More recent data suggest that antacid therapy may increase the risk of respiratory infections in patients with IPF [127].

Two medications, nintedanib and pirfenidone, have been shown to be safe and effective in the treatment of IPF; both are recommended for use in patients with IPF [63]. In placebo-controlled, randomized trials, each drug has been shown to slow the rate of FVC decline by approximately 50% over the course of 1 year [128,129]. Both have shown some efficacy in reducing severe respiratory events, such as acute exacerbations, and hospitalization for respiratory events [130,131]. Pooled data and meta-analyses suggest that these agents may reduce mortality [132,133]. The cost of each medication is estimated to exceed $100,000 annually. Again, in low-income countries it is very difficult to implement these therapies on a regular basis for patients with IPF due to its high cost, especially that they are of indefinite use.

Nintedanib is a tyrosine kinase inhibitor that targets growth factor pathways, including those downstream from VEGF receptors 1, 2, and 3, CGF receptors 1, 2, and 3, and PDGF receptor. Patients should initially be prescribed 150 mg of nintedanib, to be taken by mouth twice daily. The dose can be decreased to 100 mg twice daily if unmanageable side effects occur. The medication should be taken with food and can be continued indefinitely. Patients taking nintedanib commonly have diarrhea, which can often be managed with antidiarrheal agents [128]. Cases of drug-induced liver injury have been reported. Liver function should be tested at baseline, monitored monthly for the first 3 months, and then monitored as clinically indicated. Since nintedanib is associated with a small increase in the risk of bleeding, this agent

should be used with great caution, if at all, in patients receiving full-dose anticoagulant therapy. Atheroembolic events, including myocardial infarction, have also been reported with nintedanib [128]. Caution should be used when treating patients with cardiovascular risk factors, including those who have coronary artery disease.

Pirfenidone has a number of anti-inflammatory and anti-fibrotic effects, including inhibition of collagen synthesis, down-regulation of TGF-β and TNF-α, and a reduction in fibroblast proliferation [134]. Pirfenidone is prescribed in an escalating-dose fashion over a 14-day period: 267 mg (one capsule) by mouth three times daily for 1 week, 534 mg (two capsules) three times daily for 1 week, and 801 mg (three capsules) three times daily thereafter. Patients can subsequently be transitioned to an 801-mg tablet three times daily. Pirfenidone must be taken with food and can be continued indefinitely. Common side effects, such as anorexia, nausea, and vomiting, [129] can often be ameliorated by judicious use of antacids and antiemetic agents. In some cases, side effects are severe enough to require a lower total daily dose (six to eight capsules daily). A photosensitive rash can also occur. Liver function should be monitored periodically.

Pirfenidone and nintedanib provide similar benefits [135,136]. Recent data on treatment that combines these agents suggest clinically significant gastrointestinal side effects [137]. It is difficult to recommend one agent over the other, since there have been no head-to-head comparisons.

There are several possible approaches for the management of cough in IPF, though none are universally effective. Thalidomide be used to ameliorate cough in patients with IPF [138]. An observational study suggests that pirfenidone may attenuate cough [139]. The P2X3 antagonist AF-219/MK-7264 (gefapixant) suppresses idiopathic cough [140]; a trial of this agent in patients with IPF has been completed (NCT02502097). Finally, an inhaled cromolyn preparation was shown to ameliorate cough in patients with IPF [141].

Future directions

Fibrosis is a hallmark of pathologic remodeling in numerous tissues and a contributor to clinical disease. Thus, it is important to understand the central mechanisms underlying the fibrogenic process. A major conserved cellular element is the activated fibroblast, also known as a myofibroblast, which produces abundant amounts of ECM. Some of the major conserved molecular processes involve TGF-β, PDGF, CTGF, vasoactive compounds (endothelin-1 and angiotensin II), and integrin–extracellular matrix signaling pathways. The fact that tissue fibrosis is remarkably plastic suggests that many of these major elements of disease pathogenesis may emerge as targets of novel therapeutic interventions. Novel preventive interventions, evolving use of screening biomarkers, and the eventual ability to target newly discovered risk factors for fibrosis could lead to a decline in the incidence of associated diseases in coming years. Advances in therapeutics, including individualized approaches and interventions to halt collagen deposition, may turn the associated pathologies into lifelong chronic diseases.

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Author contribution

This work was only carried out by the author. Author AA contributed in the planning, data collection, data analysis, writing and critical review. AA read and approved the final manuscript.

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