Functions of Galectin-3 and Its Role in Fibrotic Diseases

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ABSTRACT

Fibrotic diseases occur in a variety of organs and lead to continuous organ injury, function decline, and even failure. Currently effective treatment options are limited. Galectin-3 (Gal-3) is a pleiotropic lectin that plays an important role in cell proliferation, adhesion, differentiation, angiogenesis, and apoptosis. Accumulating evidence indicates that Gal-3 activates a variety of profibrotic factors, promotes fibroblast proliferation and transformation, and mediates collagen production. Recent studies have defined key roles for Gal-3 in fibrogenesis in diverse organ systems, including liver, kidney, lung, and myocardial. To help set the stage for future research, we review recent advances about the role played by Gal-3 in fibrotic diseases. Herein we discuss the potential profibrotic role of Gal-3, inhibition of which may represent a promising therapeutic strategy against tissue fibrosis.

Introduction

Tissue fibrosis is a progressive, severely debilitating disease characterized by superabundant accumulation of extracellular matrix (ECM) leading to excessive tissue scarring, organ injury, function decline, and even failure (Insel et al., 2012; Speca et al., 2012; Friedman et al., 2013). Recent advances indicate that organ fibroses share core features that include epithelial and endothelial injury and dysfunction; abnormal proliferation of myofibroblasts (MPb), smooth muscle cells and stellate cells, and ECM deposition (Bonner, 2004; Speca et al., 2012; Friedman et al., 2013). In addition, a variety of cytokines, chemokines, growth factors, and angiogenic factors regulate the activation of ECM-producing cells in profibrotic processes (Speca et al., 2012; Friedman et al., 2013). Despite considerable research into the molecular mechanisms of and treatment trials for tissue fibrosis, current solid and unequivocal therapeutic options remain limited. As the severe tissue scarring that accompanies end-stage fibrosis is irreversible in most situations, greater efforts are still needed to identify the common and unique mechanisms of fibrosis, all of which need to be aimed at finding effective antifibrotic targets and drugs.

It appears to be widely accepted that investigating the targets that are aberrantly expressed in animal models and fibrotic patients promises to unearth new therapeutic strategies for fibrotic diseases. Up to the present time numerous research efforts in the field of organ fibrosis have identified several polypeptide mediators important to the fibrotic process, such as platelet-derived growth factor, insulin-like growth factor binding protein–related protein 1, connective tissue growth factor, and transforming growth factor (TGF-β). Nevertheless, effective alternative targets and therapies are still urgently required.

Galectin-3 (Gal-3), a multifunctional protein of an expanding family of β-galactoside–binding animal lectins, is mainly produced by macrophages, and is implicated in a variety of biologic events, such as inflammation and angiogenesis (Nangia-Makker et al., 2000; Sano et al., 2000; Zuberi et al., 2004; Henderson et al., 2004; Sureshbabu et al., 2011; Yu et al., 2013; Guo et al., 2014; Hao et al., 2014; Ma et al., 2014). Galectin-3 may be an important mediator of and effective therapeutic target for tissue fibrosis. Thus, a more complete understanding of the functions of Gal-3 and its role in fibrotic diseases may yield pivotal insights into the pathogenesis of fibrosis and delineate novel strategies that lead to therapeutic applications.

Abbreviations: AECs, alveolar epithelial cells; Aldo, aldosterone; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ERK1/2, extracellular signal–regulated kinase 1/2; FB, fibroblasts; Gal-3, galectin-3; GSK-3β, glycogen synthase kinase 3β; HF, heart failure; HSCs, hepatic stellate cells; IL, interleukin; MFb, myofibroblasts; PF, pulmonary fibrosis; α-SMA, α-smooth muscle actin; SSc, systemic sclerosis; TGF-β, transforming growth factor-β.
Superstructure and Location

Gal-3, formerly known as the Mac-2 antigen, is a chimeratype ∼30-kDa carbohydrate-binding protein (Hughes 1994; Henderson et al., 2008). It is composed of a short NH2 terminal domain, which decides specific cellular targets; a repetitive collagen-like sequence, which serves as a substrate for matrix metalloproteinases; and a carboxyl terminal domain that contains the carbohydrate-binding region (Henderson et al., 2006; Mourad-Zeidan et al., 2008). Gal-3 can be expressed in cytoplasm, nucleus, and cell surface, and eventually is secreted into the extracellular matrix and circulation by various cell types, including macrophages and monocytes (Inohara and Raz, 1994; Shibata et al., 2005; Kapucuoglu et al., 2009). A large number of reports show that Gal-3 is mainly secreted by macrophages that mediate chronic and acute inflammation, innate and adaptive immunity, as well as surfactant homeostasis (Reynolds, 2005; MacKinnon et al., 2008).

Cell Receptors and Ligands

The activation of Gal-3 involves several receptors and ligands. Notably, a recent study confirms that the superstructure of galectins at the cell surface can bind cell-surface receptors such as epidermal growth factor receptor, which is a potent mitogen for collagen-producing mesenchymal cells (Partridge et al., 2004; Martinelli et al., 2011; Fuchs et al., 2014). Besides, Gal-3 exhibits high-affinity binding for the advanced glycosylation end product–binding proteins in macrophages, astrocytes, and endothelial cells. Independently, increased Gal-3 expression may also play a special role in tissue remodeling because of its adhesive and growth-regulative effects (Pricci et al., 2000). CD98, known to be important for cell fusion, adhesion, and amino acid transport, is also a demonstrated receptor for Gal-3 (Dong and Hughes, 1997; Dalton et al., 2007). The mechanistic link among interleukin (IL)-4, Gal-3, and CD98 can drive alternative macrophage activation and chronic inflammatory and fibrotic diseases (Dong and Hughes, 1997). Furthermore, Gal-3 can induce apoptosis of Jurkat cells by binding receptors such as CD45 (Xue et al., 2013).

In addition, some ligands for Gal-3 have been identified, including various glycoforms of ECM glycoproteins, such as laminin and integrins (Yang et al., 2008; Hynes, 2009; Margadant et al., 2012). Blocking cell adhesion on laminin substrata elicits Gal-3 activity in cell-matrix interactions and cell motility (Sato and Hughes, 1992), while Gal-3 expression enhances β1 integrin–mediated cell adhesion to fibronectin and laminin (Margadant et al., 2012). It also demonstrates that Gal-3 is involved in the integrin β1–induced epithelial-mesenchymal transition (EMT)–like phenomenon that is characterized by loss of cell-cell contacts and cell scattering and an increase in cell migration and fibronectin fibrillogenesis (Margadant et al., 2012).

Functions of Gal-3

Gal-3 has the ability to bind to cell surface and ECM glycans and affect a variety of physiologic and pathologic processes, including cell apoptosis, adhesion, migration, angiogenesis, and inflammatory responses (Fig. 1).

Cell Apoptosis. Apoptosis is an important physiologic process that ensures a balance between cellular proliferation and turnover in nearly all tissues. It was shown that Gal-3 had significant sequence similarity with Bcl-2, a well characterized suppressor of apoptosis, and human leukemia T cells transfected with Gal-3 cDNA displayed higher growth rates, indicating that Gal-3’s antiapoptotic activity may occur via the cell death–inhibition pathway that involves Bcl-2 (Yang et al., 1996). Another study revealed that extracellular Gal-3 induced apoptosis in activated T cells by binding to CD7 and CD29 (β1 integrin) and this resulted in activation of mitochondri al apoptosis events, including cytochrome c release and caspase-3 activation (Fukumori et al., 2003). Furthermore, exorbitant Gal-3 rendered breast carcinoma cells resistant to apoptosis by inhibiting cytokine protease, while extracellular Gal-3 directly induced death of human thymocytes and T cells by binding to CD45 and CD71 (Akahani et al., 1997; Stillman et al., 2006). Fukumori et al. (2004) also showed that primary CD85 (APO-1/Fas) was involved in apoptotic signaling pathways, one regulated by the large amount of active caspase-8 (type I) formed at the death-inducing signaling complex and the other by the apoptogenic activity of mitochondria (type II), but the antiapoptotic molecule Gal-3 was expressed only in type I cells, revealing that endogenous Gal-3 determines the routing of CD95 apoptotic signaling pathways.

However, Nucling, a novel apoptosis-associated molecule, mediated apoptosis by inhibiting Gal-3 expression by interfering with the nuclear translocation process of nuclear factor-κB signaling (Liu et al., 2004). The same study also showed that Gal-3 silencing sensitized multidrug resistance cells to epirubicin by inhibiting ATP-binding cassette transporters and activating the mitochondrial apoptosis pathway through modulation of the β-catenin/glycogen synthase kinase 3β (GSK3β) pathway (suppressed β-catenin and increased GSK-3β expression) in human colon cancer cells (Lee et al., 2013). Together, these studies show that Gal-3 has a dual role in regulating apoptosis.

Cell Adhesion and Migration. Cell adhesion is essential for the genesis and maintenance of both three-dimensional structure and normal function in tissues. The biochemical entities mediating cell adhesion are multiprotein complexes comprising the adhesion receptors, the extracellular matrix molecules, and the adhesion plaque proteins (Gumbiner, 1996; Aplin et al., 1998). Extracellular Gal-3 is becoming established as a mediator of cell-to-ECM adhesive interactions as well as a variety of extracellular processes, such as kidney development, angiogenesis, autoimmune disorders, endocytosis, and possibly exocytosis (Ochieng et al., 2004). It was shown that Gal-3 not only promoted adhesion between neutrophils and laminin but also mediated the combination with IgE by which Gal-3 impelled the loss of ζ-selectin and production of IL-8 to induce natural immunity response (Sato and Hughes, 1992; Nieminen et al., 2005). A recent report indicated that Gal-3 might bridge monocytes to laminin and also activate monocytes, resulting in the positive regulation of other adhesion molecules and cell adhesion to fibronectin (Danella Polli et al., 2013). Further study reported that Gal-3 promoted adhesion of corneal epithelial cells onto collagen IV, while exogenous Gal-3 enhanced epithelial cell–wound healing (Yabuta et al., 2014). It also reported that circulating Gal-3 could promote cell migration and activate extracellular signal–regulated kinase 1/2 (ERK1/2) through a calcium-sensitive and protein kinase C–dependent pathway (Gao et al., 2014).
Angiogenesis. Angiogenesis, a hallmark of wound healing and inflammatory diseases, is regulated by several “classic” factors, such as protein kinase C-α (Moncada de la Rosa et al., 2013; Gao et al., 2014). Gal-3 could upregulate the expression of vascular endothelial-cadherin, enhance invasion of IL-8, and promote vasculogenic mimicry by restraining early growth response-1 (Mourad-Zeidan et al., 2008). Moreover, Gal-3 could impel the production of IL-8, which functioned as a chemo-attractant for neutrophilic granulocytes, macrophages, endothelial cells, and mast cells (Nieminen et al., 2005). Likewise, Gal-3 could directly stimulate capillary tube formation of endothelial cells in vitro and induce angiogenesis (Nangia-Makker et al., 2000).

Inflammation. Acute inflammation is the normal response of vascularized tissues to injury, irritation, and infection, while chronic inflammation is a harmful process that occurs through failure to resolve acute inflammation or persistence of an inflammatory stimulus (Nathan and Ding, 2010; Pera et al., 2014). Gal-3 is a novel and potent inflammatory protein, largely owing to macrophage activation and migration (Liu and Hsu, 2007; MacKinnon et al., 2008; Hsu et al., 2009). It was reported that eosinophil-expressed Gal-3–mediated rolling and adhesion on vascular cell adhesion molecule-1, while α4 integrin–mediated eosinophil rolling on immobilized Gal-3; meanwhile, eosinophil-expressed Gal-3 interacted with immobilized Gal-3 through the carbohydrate recognition domain of Gal-3 during eosinophil trafficking (Rao et al., 2007). In patients with severe chronic obstructive pulmonary disease, there were increased Gal-3 expression and neutrophil accumulation in the small airway epithelium, along with epithelial proliferation and airway obstruction (Pilette et al., 2007). Other research demonstrated that Gal-3 knockout mice developed a lower Th2 response but a higher Th1 response, which played a significant role in the processes of inflammation and fibrosis (Zuberi et al., 2004; Kikuchi et al., 2010).

Gal-3 in Fibrotic Diseases

Gal-3 secretion in fibroblasts (FB) and macrophages can be stimulated by stress, such as heat shock and irradiation (Sato and Hughes, 1994; Kasper and Hughes, 1996). It was reported that Gal-3 had a hand in EMT, scar formation, and tissue architecture disruption under pathologic conditions (Henderson et al., 2008). Recent evidence suggests that Gal-3 plays a key role in the development of fibrosis in liver, blood vessels, kidney, heart, and lung (Nishi et al., 2007; Henderson et al., 2008; Ho et al., 2012; Jiang et al., 2012; Calvier et al., 2013) (Fig. 2).

Liver Fibrosis. Liver fibrosis is reversed, stabilized, or prevented in 57–79% of patients by conventional treatment regimens, mainly by anti-inflammatory treatments (Czaja, 2014). This process is driven by a heterogeneous population of hepatic MFb, which mainly derive from hepatic stellate cells (HSCs) and portal FB that facilitate hepatocyte interactions via inflammatory mediators (Mallat and Lotersztajn, 2013).

It was confirmed that Gal-3 stimulated HSC proliferation by initiating the ERK1/2 signaling pathway, while thiodigalactoside (a potent inhibitor of β-galactoside binding) attenuated the effects (Maeda et al., 2003). Moreover, Gal-3−/− mice exerted an attenuated fibrogenic response with reduced expression of α-smooth muscle actin (α-SMA) and procollagen α1 (I) (Jiang
et al., 2012). In established human fibrotic liver disease, Gal-3 expression was upregulated in hepatocytes, while in Gal-3<sup>−/−</sup> mice and Gal-3<sup>−/−</sup> HSCs, liver fibrosis and TGF-β activation were attenuated with reduced α-SMA and procollagen (I) expression (Henderson et al., 2006). In a clinical investigation, Gal-3 binding protein was introduced as a candidate-marker of hepatitis C–related fibrosis on the basis of serum proteomics (Cheung et al., 2010). A recent work implicated bone marrow cell therapy as a method of treating cirrhotic mice in which Gal-3–positive cells were markedly fewer and the degree of liver fibrosis and expression of collagen I were reduced (de Oliveira et al., 2012). Treating liver fibrosis Sprague-Dawley rats with galactoarabino-rhamnogalaturonan or galactomannan, two galectin protein inhibitors, markedly reduced fibrosis with reduction in portal- and septal-Gal-3–positive macrophages (Traber et al., 2013). These data indicate a significant profibrotic function of Gal-3 in promoting HSC proliferation, which may be partly attributable to the role of Gal-3 in increasing ECM secretion via enhancing HSC migration and adhesion. Moreover, blockade of Gal-3 may provide an alternative therapeutic approach to attenuating liver fibrosis.

**Vascular Fibrosis.** Vascular fibrosis is associated with the renin-angiotensin-aldosterone system, oxidative stress, inflammatory cytokines, and the imbalance of endothelium-derived cytokine secretion (Lan et al., 2013). Gal-3 is a mediator of angiogenesis and plays a crucial role in vascular fibrosis (Nangia-Makker et al., 2000; Mourad-Zeidan et al., 2008; Calvier et al., 2013). As observed, Gal-3 overexpression enhanced collagen I synthesis in rat vascular smooth muscle cells, while Gal-3 inhibition with modified citrus pectin or siRNA blocked aldosterone (Aldo)-induced collagen I synthesis (Calvier et al., 2013). In hypertensive Aldo-treated rats, Gal-3 expression was increased with vascular hypertrophy, inflammation, and fibrosis, while spironolactone or modified citrus pectin treatment reversed the above effects (Calvier et al., 2013). In addition, Aldo increased aortic Gal-3 expression, inflammation, and collagen I production in wild-type mice, whereas no changes occurred in Gal-3<sup>−/−</sup> mice (Calvier et al., 2013). These findings suggest a key role for Gal-3 in Aldo-induced vascular fibrosis, and interfering with Gal-3 function may be effective for its treatment. Nevertheless, further studies are still needed to clarify whether Gal-3–mediated angiogenesis contributes to vascular fibrosis.

**Systemic Sclerosis.** Systemic sclerosis (SSc), or scleroderma, is an autoimmune disease of unknown etiology characterized by progressive fibrosis with FB activation (Haak et al., 2014; Koca et al., 2014). Gal-3 is implicated in a variety of biologic processes, including fibrosis, angiogenesis, and immune activation, all of which are involved in the development of SSc (Taniguchi et al., 2012). It was reported that serum Gal-3 levels were significantly decreased in early diffuse cutaneous SSc, but not in the midstage or late-stage, compared with the control subjects (Taniguchi et al., 2012). However, serum Gal-3 levels were higher in SSc patients with both digital ulcers and elevated right ventricular systolic pressure than in those without each symptom (Taniguchi et al., 2012). A recent clinical study showed that the serum Gal-3 level was higher in the SSc patient group compared with the control group, but it was not correlated with the disease activity and severity indexes, and it was higher in the active SSc group than in the inactive SSc group (Koca et al., 2014). These data suggest that Gal-3 may be related to the developmental process of skin sclerosis in diffuse cutaneous SSc and pulmonary vascular involvements in total SSc, as well as being a prominent biomarker of disease activity in SSc. However, it is not clear how Gal-3 regulates its related cellular processes, such as inflammation and angiogenesis, which are involved in SSc based on clinical observations as mentioned above.
Renal Fibrosis. Renal fibrosis is a common pathway of progression to end-stage renal failure in different renal diseases with variable etiologies (Conway and Hughes, 2012). The activation of FB and MFB, endoplasmic reticulum stress, microvascular rarefaction, and tissue hypoxia promote scar formation and renal fibrogenesis (Chiang et al., 2011; Liu, 2011). As the prevalence of end-stage renal disease is constantly on the rise, the lack of established antifibrotic therapies is a considerable unmet need in clinical practice (Tampe and Zeisberg, 2014). Therefore, it is essential to identify key factors that initiate tubulointerstitial inflammation and subsequent renal fibrosis.

It has been confirmed that the degree of renal damage and fibrosis was more extensive in Gal-3−/− mice with increased total collagen, but there was a corresponding decrease of MFB, ECM synthesis, and Endo180 (a receptor for intracellular collagen degradation) expression (Okamura et al., 2011). This suggests that Gal-3 may protect renal tubules from chronic injury by limiting apoptosis and enhancing matrix remodeling and fibrosis attenuation. Specifically, transplanting kidneys into C57BL6 mice was associated with interstitial fibrosis and upregulation of Gal-3 expression, while transplanting kidneys into Gal-3−/− mice reduced interstitial fibrosis with reduced activation of MFB and expression of collagen I, YML (a marker of alternative macrophage activation), and IL-4 (Dang et al., 2012). Moreover, Gal-3 expression was upregulated in mouse renal fibrosis, and Gal-3 deficiency inhibited renal MFB accumulation/activation and fibrosis, while specific deletion of macrophages using CD11b-DTR mice reduced fibrosis severity after the model and Gal-3 deficiency did not affect macrophage recruitment or macrophage proinflammatory cytokine profiles in response to interferon-γ/lipopolysaccharide (Henderson et al., 2008). Amazingly, further study confirmed that Gal-3 secretion by macrophages was critical in the activation of renal FB to a profibrotic phenotype (Henderson et al., 2008). In these studies, the controversial role of Gal-3 in renal fibrosis was investigated, but Gal-3 deficiency led to consistent reduction of MFB activation. In addition, these results provide evidence that the effects of Gal-3 in renal fibrosis may be attributable to its role in modulating inflammation and cell apoptosis. Further studies, such as the application of Gal-3 inhibitors in renal fibrosis models, are necessary to more accurately define the role of Gal-3 in renal fibrosis.

Cardiac Fibrosis. Cardiac fibrosis is considered irreversible damage in various cardiovascular diseases, among them overt heart failure (HF) (Roubille et al., 2014). However, the diagnosis and potential treatments of cardiac fibrosis are limited. Recent evidence shows that Gal-3 is a novel prognostic marker of cardiac fibrosis, and it is associated with increased risk of HF and mortality, and thus Gal-3 may exert a critical role in cardiac fibrosis (Lok et al., 2010; Ho et al., 2012).

A comprehensive microarray study indicates that Gal-3 expression was increased specifically in homozygous transgenic TGRmRen2-27 rats that later rapidly developed HF, and Gal-3 colocalized with activated myocardial macrophages with Gal-3 binding sites in rat cardiac FB and ECM (Sharma et al., 2004). Furthermore, recombinant Gal-3 induced cardiac FB proliferation, collagen production, and cyclin D1 expression (Sharma et al., 2004). This suggests that Gal-3 may exert proinflammatory effects by activating macrophages as well as mediating the proliferation of ECM-producing cells. In a Gal-3−/− induced Sprague-Dawley rat model, macrophage and mast cell infiltration and cardiac fibrosis were enhanced, while TGF-β/Smad3 signaling pathway was activated (Liu et al., 2009). However, N-acetyl-seraly-aspartyl-lysyl-proline, a naturally occurring tetrapeptide, prevented the above effects (Liu et al., 2009). In a recent therapeutic study, Gal-3−/− mice, C56Bl6/J mice, TGRmRen2-27 rats, and Sprague-Dawley rats were used to test the therapeutic role of Gal-3 inhibition in cardiac fibrosis induced by angiotensin II infusion or transverse aortic constriction (Yu et al., 2013). The study also showed that Gal-3−/− mice and administration of Gal-3 inhibitor N-acetyllactosamine to REN2 rats after transverse aortic constriction attenuated myocardial fibrosis; meanwhile, N-acetyllactosamine decreased collagen production (collagens I and III), collagen processing, and deposition in Gal-3−/− induced FB (Yu et al., 2013).

As a biomarker in serum levels of extracellular cardiac matrix, Gal-3 was associated with death or HF hospitalization in age- and gender-adjusted analyses; moreover, Gal-3 > 30 ng/ml was associated with death or HF hospitalization, revealing that increased Gal-3 is associated with adverse long-term cardiovascular outcomes (Lopez-Andrés et al., 2012). However, recent head-to-head comparison of fibrosis biomarkers in chronic HF revealed the superiority of ST2 over Gal-3 in risk stratification, and the incremental predictive contribution of Gal-3 to the clinical risk factors was trivial (Bayes-Genis et al., 2014). Thus, drugs antagonizing Gal-3 may be potential therapeutic candidates for the prevention of HF with extensive fibrosis. However, its importance as a marker of cardiac fibrosis to predict clinical outcomes of HF is controversial, and thus further studies are still needed.

Pulmonary Fibrosis. Pulmonary fibrosis (PF), characterized by excessive ECM deposition, is involved in abnormal inflammatory cell increase, FB proliferation, FB-to-MFB transformation, EMT, procoagulant signaling, and oxidative stress (He et al., 2012; Todd et al., 2012; Wollin et al., 2014). TGF-β has been assigned a clear pathogenic role by its induction of EMT, ECM production, and apoptosis of alveolar epithelial cells (AECs) in PF, whereas inhibiting TGF-β activity reduces PF (Kim et al., 2006; Sureshbabu et al., 2011; Kurotsu et al., 2014; Wollin et al., 2014). Gal-3, involved in TGF-β signaling, has also been investigated in PF (Mackinnon et al., 2012).

An early study showed that the total galec4tin concentration in lung was dramatically increased in irradiation-induced lung inflammation and repair in rats with an increase of Gal-3-positive interstitial and alveolar macrophages. Interestingly, Gal-3 was prominently expressed at the surface of newly formed type I AECs and to a lesser extent at the apical surface of type II AECs (Kasper and Hughes, 1996). Nishi and colleagues first provided evidence that Gal-3 was specifically increased in bronchoalveolar lavage fluid in PF patients but lower after they received corticosteroid therapy; tumor necrosis factor-α and interferon-γ could induce high expression of Gal-3 in U937 monocytes; Gal-3 also induced production of tumor necrosis factor-α and IL-8 in THP-1 macrophages; and stimulated NIH-3T3 FB to induce migration and collagen synthesis (Nishi et al., 2007). These data suggest that the role of Gal-3 in inflammation and cell migration may be crucial for the development of PF. However, Gal-3−/− mice showed significant attenuation of adenoviral TGF-β1-induced fibrosis, including EMT, MFB activation, and collagen production, but with no difference in proliferation between wild-type and Gal-3−/− primary lung FB; moreover, Gal-3−/− AECs showed reduced expression in response to TGF-β1 without stimulating α-SMA expression or reducing E-cadherin in response to TGF-β1 stimulation (Mackinnon et al., 2012). Further examination showed that Gal-3 deletion...
reduced phosphorylation and nuclear translocation of β-catenin but had no effect on p-Smad2/3; however, TD139, an inhibitor of Gal-3, blocked TGF-β-induced β-catenin activation and attenuated the late-stage progression of PF after bleomycin (Mackinnon et al., 2012).

Interestingly, stable PF patients had elevated levels of Gal-3 in bronchoalveolar lavage fluid and serum compared with patients with control subjects, and this rose sharply during treatment with PF. However, it is essential first to inhibit may be an exciting novel therapeutic option for treating patients with PF. These surveys suggest that Gal-3 inhibition may be an exciting novel therapeutic option for treating patients with PF. However, it is essential first to elucidate the availability of Gal-3 inhibitors for treating PF in preclinical studies.

### Conclusion and Future Directions

Fibrosis is typically the result of maladjusted tissue-repair response of organs to injury, inflammation, or stress. Although a large body of experimental evidence has partly illuminated the evoked mechanisms of fibrogenesis, effective strategies to prevent these diseases in the clinic have been limited until recently. Thus, given the difficulty in investigating therapeutic targets and drugs, tissue fibrosis is still a very active area of research. Gal-3 is a type of multifunctional protein that exhibits a variety of functions in cellular processes, including inflammation, apoptosis, angiogenesis, adhesion, and migration, and thus takes part in the development of several diseases. Recent evidence shows that Gal-3 contributes to several fibrotic diseases.

Gal-3 levels have been proven higher in both patients with organ fibrosis and fibrotic models, while Gal-3 inhibitors (such as galactoarabino-ham nogalacturonan, galactomannan, N-acetyl-seryl-asparyl-lysyl-proline, N-acetyltallosamine, and TD139) attenuate fibrosis both in vivo and in vitro studies (Liu et al., 2009; Mackinnon et al., 2012; Taniguchi et al., 2012; Traber et al., 2013; Yu et al., 2013) (Table 1). In the process of tissue fibrosis, Gal-3 promotes inflammatory factor release, inflammatory cell activation (macrophages, monocytes, etc.), and tissue injury in different organs (Nishi et al., 2007; Liu et al., 2009). In addition, Gal-3 enhances the proliferation of ECM-producing cells, including FB and MFb, which may be partly attributable to the functions of Gal-3 in facilitating the migration and adhesion of such cells, as well as its role in regulating cell apoptosis (Nishi et al., 2007; Henderson et al., 2008; Okamura et al., 2011; Dang et al., 2012). The enigmatic role of Gal-3 in fibrotic diseases and the antifibrotic effect of Gal-3 inhibition in fibrogenesis raise the possibility that Gal-3 inhibition may be a novel potent therapeutic strategy for treating tissue fibrosis. Moreover, Gal-3 level may be a prominent and reliable biomarker in

### TABLE 1

The levels of Gal-3 in fibrotic patients and its role in in vivo and in vitro fibrotic models

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<th>Mechanisms</th>
<th>Gal-3 Inhibition</th>
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<td>Liver fibrosis</td>
<td>HSCs</td>
<td>Gal-3 initiates ERK1/2</td>
<td>Thiodigalactoside</td>
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<td></td>
<td>Gal-3/− mice</td>
<td>TGF-β p, α-SMA ↓, procollagen I ↓</td>
<td>Gal-3/−</td>
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<td></td>
<td>Cirrhotic mice</td>
<td>Gal-3 ↑, collagen I ↑</td>
<td>BMC</td>
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<td>SD rats</td>
<td>Gal-3-positive macrophages ↑</td>
<td>GR-MD-02, GM-CT-01</td>
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<td>Patients</td>
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<td>Vascular fibrosis</td>
<td>VSMCs</td>
<td>Collagen I ↑</td>
<td>Modified citrus pectin, Gal-3 siRNA</td>
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<td>Systemic sclerosis</td>
<td>Gal-3/− mice</td>
<td>Inflammation ↓, collagen I ↓</td>
<td>Gal-3/−</td>
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<td>Patients</td>
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<td>Renal fibrosis</td>
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<td></td>
<td>Gal-3/− mice</td>
<td>Total collagen ↓, MFb ↓, ECM ↓, Endo180 ↓, MFb ↓, collagen I ↓, YM1 ↓, IL-4 ↓</td>
<td>Gal-3/−</td>
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<td>Gallieater, depleted macrophages ↑</td>
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<td>Cardiac fibrosis</td>
<td>FB</td>
<td>Gal-3/−-induced collagen ↓, cyclin D1 ↑</td>
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<td>TGRmRen2-27 rats</td>
<td>Gal-3/−-induced macrophages ↑</td>
<td>Ac-SDKP</td>
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<td>SD rat</td>
<td>Gal-3/−-induced macrophages ↑, mast cells ↑</td>
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<td>Gal-3/− mice</td>
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<td>THP-1 macrophages</td>
<td>Gal-3 ↑</td>
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<td>NIH-3T3 FB</td>
<td>Gal-3/−-induced TNF-α ↑, IFN-γ ↑</td>
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<td></td>
<td>Gal-3/− mice</td>
<td>Collagen ↑</td>
<td>Gal-3/−/TD139</td>
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<td>Patients</td>
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Ac-SDKP: N-acetyl-seryl-asparyl-lysyl-proline; BMC: bone marrow cell; GM-CT-01, galactomannan; GR-MD-02, galactoarabino-ham nogalacturonan; IFN-γ, interferon-γ; siRNA, small interfering RNA; TNF-α, tumor necrosis factor-α; VSMCs, vascular smooth muscle cells.
patients with cardiac fibrosis, HF, active SSC, and active PF (Lok et al., 2010; Lopez-Andrés et al., 2012; MacKinnon et al., 2012; Koca et al., 2014).

However, further studies are necessary to ensure an accurate depiction of the role of Gal-3 and its inhibitors in renal fibrosis, and to expand the importance of Gal-3 as a marker in cardiac fibrosis and for predicting clinical outcomes of HF (Henderson et al., 2008; Okamura et al., 2011; Ho et al., 2012; Bayes-Genis et al., 2014). Moreover, it is not clear whether the functions of cell apoptosis and angiogenesis mediated by Gal-3 play a direct role in fibrogenesis, such as in SSC and vascular fibrosis. Until now, few in vivo or vitro studies have been conducted to clarify the role of Gal-3 in SSC and other organ fibroses, such as cystic fibrosis.

Besides, additional investigations are necessary to clarify the dual intra- and extracellular functions of Gal-3 associated with the mitogen-activated protein kinase/ERK1/2 pathway, caspase activation, β-catenin/GSK-3β signaling, EMT process, and TGF-β/Smads signaling will help clarify the pathogenesis of organ fibrosis. Second, the compounds that have hydrophilic substituent at the taloside O2 position may enhance specificity for binding human Gal-3, and further research involving these selective and high-affinity Gal-3 inhibitors may be an effective route to attenuating fibrosis (Téllez-Sanz et al., 2013). Thirdly, it is pivotal to determine whether the inhibitors used should target Gal-3 in extra- or intracellular space in different types of fibrosis. Hopefully, all of these may provide enough evidence for the crucial role of Gal-3 in fibrotic diseases and shed light on the rational design of drugs against Gal-3 in fibrosis.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: L. Li, J. Li, Gao.

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