Functions of galectin-3 and its role in fibrotic diseases

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Abbreviations: ABC, ATP binding cassette; Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; AECs, alveolar epithelial cells; AGEs, advanced glycosylation end product-binding proteins; α-SMA, α-smooth muscle actin; BALF, bronchoalveolar lavage fluid; BMC, bone marrow cell; CTGF, connective tissue growth factor; dcSSc, diffuse cutaneous SSc; E-cad, epithelial cadherin; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EGR-1, early growth response-1; EMT, epithelial-mesenchymal transition; ERK1/2, extracellular signal-regulated kinase 1/2; FB, fibro-
blasts; Gal-3, galectin-3; GM-CT-01, galactomannan; GR-MD-02, galactoarabinorhamnogalaturonan; HF, heart failure; HSCs, hepatic stellate cells; IFN-γ, interferon-γ; IGFBP-rP1, insulin-like growth factor binding protein-related protein 1; IL, interleukin; LAP, latency-associated peptide; MFb, myofibroblasts; MMP, matrix metalloproteinases; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; PF, pulmonary fibrosis; PKC, protein kinase C; SSc, systemic sclerosis; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VE-cad, vascular endothelial-cadherin; VM, vasculogenic mimicry; VSMCs, vascular smooth muscle cells.
Abstract
Fibrotic diseases occur in a variety of organs that lead to continuous organ injury, function decline, even failure. Nevertheless, there are currently limited effective treatment options. Galectin-3 (Gal-3) is a pleiotropic lectin which plays an important role in cell proliferation, adhesion, differentiation, angiogenesis and apoptosis. Accumulating evidence indicates that Gal-3 activates a variety of pro-fibrotic factors, promotes fibroblasts proliferation and transformation, and mediates collagens 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 pro-fibrotic role of Gal-3 and 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 fibrosis shares the core features including epithelial and endothelial injury and dysfunction, abnormal proliferation of myofibroblasts (MFb), 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 have been confirmed to regulate the activation of ECM-producing cells in the pro-fibrotic processes (Speca et al., 2012; Friedman et al., 2013). Despite considerable researches of the molecular mechanisms and treatment trials for tissue fibrosis, current solid and unequivocal therapeutic options remain limited. As the severe tissue scarring and accompanied end stage fibrosis are irreversible in most situations. Thus, greater efforts are still needed to identify the common and unique mechanisms of fibrosis, all of which are aimed to look for effective anti-fibrotic targets and drugs.

It appears to be widely accepted that investigating the targets which are aberrantly expressed in the animal models and fibrotic patients are meaningful and promising to unearth the therapeutic strategies for fibrotic diseases. Till now, numerous researches have put into the field of organ fibrosis and several polypeptide mediators have been found important to the fibrotic process such as platelet-derived growth factor (PDGF), insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1), connective tissue growth factor (CTGF), and transforming growth factor (TGF)-β1 (Bonner, 2004;
Sureshbabu et al., 2011; Yu et al., 2013; Guo et al., 2014; Hao et al., 2014; Ma et al., 2014). However, effective alternative targets and therapies are urgently required.

Galectin-3 (Gal-3), a multifunctional protein of a growing family of β-galactoside-binding animal lectins, is mainly produced by macrophage and implicated in a variety of biological events such as inflammation and angiogenesis (Liu et al., 1995; Nangia-Makker et al., 2000; Sano et al., 2000; Zuberi et al., 2004; Henderson et al., 2008). Recently, there is mounting evidence demonstrating that Gal-3 is activated in fibrotic models and abnormally increased in fibrotic patients, while Gal-3 inhibitions protect against fibrotic disorders (Nishi et al., 2007; Yang et al., 2008; Lok et al., 2010; Lopez-Andrés et al., 2012; Mackinnon et al., 2012; Taniguchi et al., 2012; Yu et al., 2013; Bayes-Genis et al., 2014). It suggests that Gal-3 may be an important mediator and effective therapeutic target for tissue fibrosis. Thus, fully understand the functions of Gal-3 and its role in fibrotic diseases may yield pivotal insights to the pathogenesis of fibrosis and delineate novel strategies for the therapeutic applications.

**Superstructure and location**

Gal-3, formerly known as the Mac-2 antigen, is a chimera-type ~30kDa 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 (MMP); 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, nucle-
us and cell surface, and eventually secreted into the extracellular and circulation by various cell types including macrophages and monocytes (Inohara and Raz, 1994; Shibata et al., 2005; Kapucuoğlu et al., 2009). A large number of reports show that Gal-3 is highly expressed and secreted by macrophages which mediate chronic and acute inflammation, innate and adaptive immunity as well as surfactant homeostasis (Liu et al., 1995; Reynolds et al., 2005; MacKinnon et al., 2008).

Cell receptors and ligands

The activation of Gal-3 is involved in 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 (EGFR) 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 has a high-affinity binding for the advanced glycosylation end product-binding proteins (AGEs) in macrophages, astrocytes, and endothelial cells, independently of which increased Gal-3 expression may also play a special role in tissue remodeling due to its adhesive and growth regulatory effects (Pricci et al., 2000). CD98, known to be important for cell fusion, adhesion, and amino acid transport, has also been demonstrated to be a 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, chronic inflammatory and fibrotic diseases (Dong and Hughes, 1997). Furthermore, Gal-3 can induce apoptosis of Jurkat cells through binding receptors such as CD45 (Xue et al.,
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 exerts Gal-3 activity on 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 increase of 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 physiological and pathological processes including cell apoptosis, adhesion, migration, angiogenesis and inflammatory responses (Fig. 1).

Cell apoptosis. Apoptosis is an important physiological process ensuring 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 its anti-apoptotic activity may be through 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 (beta(1) integrin) and resulted in activation of mitochondrial 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 via inhibiting cysteine 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). It also showed that primary CD95 (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 anti-apoptotic molecule Gal-3 was expressed only in type I cells, revealing that endogenous Gal-3 determines the routing of CD95 apoptotic signaling pathways (Fukumori et al., 2004).

However, Nucling, a novel apoptosis-associated molecule, mediated apoptosis by inhibiting Gal-3 expression via interfering with the nuclear translocation process of nuclear factor-κB (NF-κB) signaling (Liu et al., 2004). It also showed that Gal-3 silencing sensitized multidrug resistance cells to epirubicin by inhibiting ATP binding cassette (ABC) transporters and activating the mitochondrial apoptosis pathway through modulation of the β-catenin/GSK-3β pathway (suppressed β-catenin and increased GSK-3β expression) in human colon cancer cells (Lee et al., 2013). Taken together, 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 the adhesion between neutrophils and laminin but also mediated the combination with IgE by which Gal-3 impelled the loss of L-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 activated monocytes, resulting in the positive regulation of other adhesion molecules and cell adhesion to fibronectin (Danella 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 calcium-sensitive and PKC-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 (PKC) α (Moncada et al., 2013; Gao et al., 2014). Gal-3 could up-regulate the expression of vascular endothelial-cadherin (VE-cad), enhance invasion of IL-8 and promote vasculogenic mimicry (VM) due to restraining early growth response-1 (EGR-1) (Mourad-Zeidan et al., 2008). Moreover, Gal-3 could impel the production of IL-8,
which functioned as a chemoattractant 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 on account of inducing 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 (VCAM-1), while alpha(4) 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 diseases, 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). Another research demonstrated that Gal-3 knockdown 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 pathological conditions (Henderson et al., 2008). Recent evidence suggests that Gal-3 plays a key role in the development of fibrosis in liver, vascular, renal, cardiac 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 the anti-inflammatory actions (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 the inflammatory mediators (Mallat and Lotersztajn, 2013).

It was confirmed that Gal-3 stimulated HSCs proliferation via initiating 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 up-regulated in hepatocytes, while in Gal-3/- mice and Gal-3/- 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 based on serum proteomics (Cheung et al., 2010). A recent work implicated bone mar-
row cell (BMC) therapy as a method of treating cirrhotic mice, with which the Gal-3 positive cells were markedly lower, the degree of liver fibrosis and the expression of collagen I were reduced (de Oliveira et al., 2012). Treating liver fibrosis SD rats with GR-MD-02 (galactoarabino-rhamnogalaturonan) or GM-CT-01 (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 pro-fibrotic function of Gal-3 in promoting HSCs proliferation, which may be partly due to the role of Gal-3 in enhancing HSCs migration and adhesion, thus increasing ECM accumulation. Moreover, blockade of Gal-3 may provide an alternative therapeutic approach to attenuate 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 over-expression enhanced collagen I synthesis in rat vascular smooth muscle cells (VSMCs), while Gal-3 inhibition with modified citrus pectin or siRNA blocked 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−/− 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 biological 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 (dcSSc), but not in the mid-stage 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 to 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 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 in different renal diseases with variable etiology progresses to end-stage renal failure (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 anti-fibrotic 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 degrees of renal damage and fibrosis were 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 up-regulation of Gal-3 expression, while transplanting kidneys into Gal-3-/- mice reduced interstitial fibrosis with reduced activation of MFb and expression of collagen I, YM1 (a marker of alternative macrophage activation) and IL-4 (Dang et al., 2012). Moreover, Gal-3 expression was up-regulated in mouse renal fibrosis and Gal-3 deficiency inhibited renal MFb accumulation/activation and fibrosis, while specific depletion of macrophages using CD11b-DTR mice reduced fibrosis severity after the model and Gal-3 deficiency did not af-
fect macrophage recruitment or macrophage pro-inflammatory cytokine profiles in response to interferon-γ (IFN-γ)/lipopolysaccharide (Henderson et al., 2008). Amazingly, further study affirmed that Gal-3 secretion by macrophages was critical in the activation of renal FB to a pro-fibrotic 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 due 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 as irreversible damage in various cardiovascular diseases and eventually 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 pro-inflammatory
effects by activating macrophages as well as mediate the proliferation of ECM-producing cells. In a Gal-3 induced SD rat model, macrophages and mast cells infiltration and cardiac fibrosis were enhanced, while TGF-β/Smad3 signaling pathway was activated (Liu et al., 2009). However, N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), 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 SD 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). It 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 (collagen 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 im-
Portance 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 cells increase, FB proliferation, FB to MFb transformation, EMT, pro-coagulant 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 through inducing EMT, ECM production, and apoptosis of alveolar epithelial cells (AECs) in PF, and 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 galectin 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 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 (BALF) in PF patients but lower after receiving corticosteroid therapy; TNF-α and IFN-γ could induce high expression of Gal-3 in U937 monocytes; Gal-3 also induced production of TNF-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 WT and Gal-3-/- primary lung FB; moreover, Gal-3-/- AECs showed reduced EMT in response to TGF-β1 without stimulating α-SMA expression or reducing E-cadherin (E-cad) 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-β1-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 BALF and serum compared with patients with control subjects, and this rose sharply during an acute exacerbation, suggesting that Gal-3 may be a marker of active PF in patients (Mackinnon et al., 2012). In patients with Hermansky-Pudlak syndrome that are associated with PF, high levels of intracellular Gal-3 are found only in dermal FB which may be due to abnormal intracellular trafficking (Cullinane et al., 2014). These surveys suggest that Gal-3 inhibition may be an exciting novel therapeutic option to treat patients with PF. However, it is essential to elucidate the availability of Gal-3 inhibitors for treating PF in preclinical studies first.

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, limited strategies have been effective in preventing these diseases in the clinic until recently. Thus, given the difficulty in investigating therapeutic targets and drugs for tissue fibrosis, there is still a very active area of research. Gal-3 is a kind of multifunctional proteins 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 level has been proven higher in both patients with organ fibrosis and fibrotic models, while Gal-3 inhibitors (such as GR-MD-02, GM-CT-01, Ac-SDKP, N-acetyllactosamine 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 factors release, inflammatory cells (macrophages, monocytes, etc.) activation, 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 due 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 anti-fibrotic 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 patients with cardiac fibrosis, HF, active SSc, and active PF (Lok et al.,
However, further studies are necessary to ensure the accurate role of Gal-3 and its inhibitors in renal fibrosis, and expound the importance of Gal-3 as a marker of cardiac fibrosis to predict 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 plays a direct role in fibrogenesis such as SSc and vascular fibrosis. Till now, few in vivo or in vitro studies have been conducted to clarify the role of Gal-3 in SSc and other organ fibrosis such as cystic fibrosis.

Besides, additional investigations are necessary to clarify the dual intra- and extra-cellular functions of Gal-3 in tissue fibrosis. Firstly, the in-depth researches of the functional network of Gal-3 associated with MAPK/ERK1/2 pathway, caspase activation, β-catenin/GSK-3β signaling, EMT process, and TGF-β/Smads signaling will be helpful to enrich the pathogenesis of organ fibrosis. Secondly, the compounds which have hydrophilic substituent at the taloside O2 position may enhance specificity for binding human Gal-3, and further researches involved in these selective and high affinity Gal-3 inhibitors may be effective to attenuate fibrosis (Téllez-Sanz et al., 2013). Thirdly, it is pivotal to determine whether the inhibitors used should target Gal-3 in extra- or intra-cellular 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 writing of the manuscript: L.C. Li, J. Li, J. Gao
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of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis.


Footnotes

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Figure legends

Fig. 1. The role of Gal-3 in cell apoptosis, adhesion, migration, angiogenesis and inflammation. ABC, ATP binding cassette; EGR-1, early growth response-1; ERK1/2, extracellular signal-regulated kinase 1/2; Gal-3, galectin-3; IL, interleukin; VCAM-1, vascular cell adhesion molecule-1; VE-cad, vascular endothelial-cadherin; VM, vasculogenic mimicry.

Fig. 2. The pro-fibrotic network of Gal-3 secreted by macrophages and fibroblasts in tissue fibrosis. α-SMA, α-smooth muscle actin; ECM, extracellular matrix; Gal-3, galectin-3; IL, interleukin; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.
Table 1. The levels of Gal-3 in fibrotic patients and its role in vivo and in vitro fibrotic models

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<td>Gal-3↑, collagen I↑</td>
<td>BMC</td>
<td>de Oliveira et al., 2012</td>
</tr>
<tr>
<td>cirrhotic mice</td>
<td>Gal-3-positive</td>
<td>GR-MD-02, GM-CT-01</td>
<td>Traber et al., 2013</td>
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<tr>
<td>SD rats</td>
<td>Gal-3-positive</td>
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<tr>
<td>Patients</td>
<td>Gal-3-binding protein↑</td>
<td></td>
<td>Henderson et al., 2006;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cheung et al., 2010</td>
</tr>
<tr>
<td>Vascular fibrosis</td>
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<tr>
<td>VSMCs</td>
<td>Collagen I↑</td>
<td>Modified citrus pectin, Gal-3 siRNA</td>
<td>Calvier et al., 2013</td>
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<tr>
<td>Gal-3-/- mice</td>
<td>Inflammation↓, collagen I↓</td>
<td>Gal-3-/-</td>
<td>Calvier et al., 2013</td>
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<td>Systemic sclerosis</td>
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<tr>
<td>Patients</td>
<td>Gal-3↑</td>
<td>-</td>
<td>Taniguchi et al., 2012;</td>
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<td>Koca et al., 2014</td>
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<tr>
<td>Renal fibrosis</td>
<td>Total collagen↑, MFb↓, ECM↓, Endo180↓</td>
<td>Gal-3-/-</td>
<td>Okamura et al., 2011</td>
</tr>
<tr>
<td>Gal-3-/- mice</td>
<td>MFb↓, collagen I↓, YM1↓, IL-4↓</td>
<td>Gal-3-/-</td>
<td>Dang et al., 2012</td>
</tr>
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<td>Gal-3-/-/CD11b-DTR mice</td>
<td>Gal-3 ↓, FB activation ↓</td>
<td>Gal-3-/-, depleted macrophages</td>
<td>Henderson et al., 2008</td>
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<td>Cardiac fibrosis</td>
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<tr>
<td>FB</td>
<td>Gal-3 induced collagen↑, cyclin D1↑</td>
<td>-</td>
<td>Sharma et al., 2004;</td>
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<td>TGRmRen2-27 rats</td>
<td>Gal-3↑, activated macrophages</td>
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<td>SD rat</td>
<td>Gal-3 induced TGF-β/Smad3↑, macrophages↑, mast cells↑</td>
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<td>Sharma et al., 2004;</td>
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<tr>
<td>Gal-3-/- mice</td>
<td>Collagen↓</td>
<td>Gal-3-/-, N-acetyllactosamine</td>
<td>Yu et al., 2013</td>
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<td>Pulmonary fibrosis</td>
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<td>U937 monocytes</td>
<td>TNF-α and IFN-γ induced Gal-3↑</td>
<td>-</td>
<td>Nishi et al., 2007</td>
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<td>THP-1 macrophages</td>
<td>Gal-3 induced TNF-α↑, IFN-γ↑</td>
<td>-</td>
<td>Nishi et al., 2007</td>
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<td>NIH-3T3 FB</td>
<td>Collagen↑</td>
<td>-</td>
<td>Nishi et al., 2007</td>
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<td>Gal-3-/- mice</td>
<td>EMT↓, MFb↓, collagen↓, β-catenin↓</td>
<td>Gal-3-/-, TD139</td>
<td>Mackinson et al., 2012</td>
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<td>Patients</td>
<td>Gal-3↑</td>
<td>Corticosteroid</td>
<td>Nishi et al., 2007; Mackinson et al., 2012</td>
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</table>

Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; α-SMA, α-smooth muscle actin; BALF, bronchoalveolar lavage fluid; BMC, bone marrow cell; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ERK1/2, extracellular signal-regulated. 
kinase 1/2; FB, fibroblasts; Gal-3, galectin-3; GM-CT-01, galactomannan; GR-MD-02, galactoarabinorhamnogalacturonan; HF, heart failure; HSCs, hepatic stellate cells; IFN-γ, interferon-γ; IL, interleukin; MFb, myofibroblasts; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VSMCs, vascular smooth muscle cells.
Figure 1

Anti-apoptosis

Bcl-2 sequence similarity
Cysteine protease ↓

Pro-apoptosis

Cytochrome c ↑
Caspase-3 ↑
Mitochondrial apoptosis ↑
ABC transporters ↓

Apoptosis

Inflammation

VCAM-1 ↑
Eosinophil trafficking
Th1/Th2 balance

Adhesion/migration

L-selectin ↓
ERK1/2 ↑
IL-8 ↑
Monocytes activation
PKC-dependent pathway

Angiogenesis

Gal-3

VE-cad ↑
IL-8 ↑
VM ↑
EGR-1 ↓
Figure 2

- CXC chemokine
- TNF-α
- IL-4, IL-8

Macrophages

- Gal-3

Stress, injury...

Fibroblasts

Myofibroblasts (α-SMA*)

TGF-β1

Myofibroblasts

Collagenses

Inflammation

Tissue injury

Tissue fibrosis

ECM adhesion