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メタデータ	言語: English 出版者: 公開日: 2017-10-03 キーワード (Ja): キーワード (En): 作成者: Takuwa, Yoh, Ikeda, Hitoshi, Okamoto, Yasuo, Takuwa, Noriko, Yoshioka, Kazuaki メールアドレス: 所属:
URL	http://hdl.handle.net/2297/32827

Sphingosine-1-phosphate as a mediator involved in development of fibrotic diseases

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Abstract

Fibrosis is a pathological process characterized by massive deposition of extracellular matrix (ECM) such as type I/III collagens and fibronectin that are secreted by an expanded pool of myofibroblasts, which are phenotypically altered fibroblasts with more contractile, proliferative, migratory and secretory activities. Fibrosis occurs in various organs including the lung, heart, liver and kidney, resulting in loss of normal tissue architecture and functions. Myofibroblasts could originate from multiple sources including tissue-resident fibroblasts, epithelial and endothelial cells through mechanisms of epithelial/endothelial-mesenchymal transition (EMT/EndMT), and bone marrow-derived circulating progenitors called fibrocytes. Emerging evidence in recent years shows that sphingosine-1-phosphate (S1P) acts on several types of target cells and is engaged in pro-fibrotic inflammatory process and fibrogenic process through multiple mechanisms, which include vascular permeability change, leukocyte infiltration, and migration, proliferation and myofibroblast differentiation of fibroblasts. Many of these S1P actions are receptor subtype-specific. In these actions, S1P has multiple cross-talks with other cytokines, particularly transforming growth factor-β (TGFβ), which plays a major role in fibrosis. The cross-talks include the regulation of S1P production through altered expression and activity of sphingosine kinases in fibrotic lesions, altered

expression of S1P receptors, and S1P receptor-mediated transactivation of TGFβ signaling pathway. These cross-talks may give rise to a feed-forward, amplifying loop between S1P and TGFβ, and possibly with other cytokines in stimulating fibrogenesis. Another lysophospholipid mediator lysophosphatidic acid has also been recently implicated in fibrosis. The lysophospholipid signaling pathways represent novel, promising therapeutic targets for treating refractory fibrotic diseases.

Keywords: sphingosine-1-phosphate; lysophosphatidic acid; S1P receptor; fibrosis; myofibroblast; fibrocyte; epithelial-mesenchymal transition

Introduction

Fibrosis is usually associated with chronic inflammation induced by various stimuli including infections, immune reactions, chemical irritation, mechanical injuries and irradiation, and involves massive deposition of extracellular matrix (ECM) including type I and III collagens, fibronectin, elastin and proteoglycan by an expanded fibroblastic pool [1, 2]. Fibrosis occurs in various organs and tissues including the heart, lung, liver, kidney and skin, resulting in distorted organ architecture and serious dysfunction. For example, the development of fibrosis in the heart causes increased stiffness of the myocardium and aberrant signaling within myocardium, resulting in progressive cardiac failure [3]. Pulmonary fibrosis disrupts normal alveolar architecture and impairs gas exchange with reduced vital capacity and oxygen diffusion [4]. Liver fibrosis (cirrhosis) leads to hepatic failure and portal hypertension.

Fibroblasts are the key cells that are involved in fibrogenesis [1-3]. In healthy tissues, fibroblasts are quiescent; they primarily maintain ECM component homeostasis by regulating synthesis and degradation of ECM. In the process of fibrosis, injured epithelial and non-epithelial cells release cytokines that attract inflammatory cells to primary sites of insult. Infiltrating inflammatory cells then release additional cytokines that provoke further damages in the tissues and also stimulate fibroblasts to migrate,

proliferate and secrete de novo ECM components (Figure 1) [5]. Fibroblasts thus activated at sites of fibrogenesis acquire smooth muscle features including elevated expression of α-smooth muscle actin (αSMA) and increased contractility [1-5]. These contractile fibroblasts are termed myofibroblasts. Myofibroblasts can originate from various cell types including tissue-resident fibroblasts, epithelial and endothelial cells through processes of epithelial/endothelial-mesenchymal transition (EMT/EndMT), vascular pericytes, and bone marrow-derived circulating fibroblast-like cells termed fibrocytes, but most are likely generated from tissue-resident stromal fibroblasts [1,4-6].

Among various cytokines and growth factors implicated in fibrogenesis, transforming growth factor-β (TGFβ), which is released from damaged parenchymal cells, inflammatory cells and myofibroblasts themselves, potently induces myofibroblastic differentiation and promotes the synthesis of ECM, playing a pivotal role in fibrogenesis [5]. TGFβ is also one of the most powerful inducer of EMT. Connective tissue growth factor (CTGF) is another fibrogenic cytokine that acts downstream of TGFβ and enhances TGFβ-driven responses. Platelet-derived growth factor (PDGF) is a powerful mitogen and chemoattractant for fibroblasts. Other cytokines including tumor necrosis factor-α, interleukin-1 and interleukin-6 and vasoactive peptides including endothelin and angiotensin II (Ang II) are also implicated

in myofibroblastic differentiation and their activation, thus fibrogenesis.

Sphingosine-1-phosphate (S1P) is a blood-borne lysophospholipid mediator that exerts a variety of activities including the regulation of cell migration, proliferation and differentiation. S1P research during the past two decades has elucidated well appreciated, diverse actions of S1P, which, however, may not yet reflect the whole picture of pleiotropic actions of this enigmatic mediator. S1P was initially shown to be potently mitogenic for fibroblasts in the early 90's [7]. Subsequently, S1P was also found to regulate migration of fibroblasts [8, 9]. More recently, S1P was shown to induce a myofibroblastic phenotype through trans-activating TGFβ signaling pathway [10]. Furthermore, S1P maintains vascular barrier integrity and regulates leukocyte trafficking [11], thus playing an important role in regulating inflammation, which underlies fibrosis when it is prolonged. In fact, recent studies (see below) reported the findings to suggest that S1P signaling pathway is involved in fibrotic diseases. This article reviews current knowledge of the role of S1P signaling pathway as a novel key factor in understanding cellular and molecular mechanisms of fibrosis in various organs.

Cardiac fibrosis

In the myocardium, the S1P-synthesizing enzymes sphingosine kinases (SphKs) and

S1P receptors $S1P_1$, $S1P_2$ and $S1P_3$ are expressed, with the order of reported abundance in receptor subtypes being $S1P_1$>> $S1P_3$> $S1P_2$ for cardiomyocytes and $S1P_3$>> $S1P_1$> $S1P_2$ for cardiac fibroblasts (CFs) [12]. In vasculature, $S1P_1$ and $S1P_3$ are predominantly expressed in endothelial cells to mediate eNOS stimulation and vasorelaxation [13, 14], whereas $S1P_2$ and $S1P_3$ are the major subtypes in smooth muscle to mediate vasocontraction via Ca^{2+} mobilization and Rho activation [13, 15, 16]. Whether S1P induces vasorelaxation or contraction in a vascular bed could be determined largely by the S1P receptor subtype expression and the balance between vasodilator and constrictor signals. It was demonstrated that S1P induced relaxation of rat coronary artery as an overall effect, which was mediated mainly via endothelial $S1P_3$ [17], whereas S1P reduced coronary perfusion in mice independently of eNOS [18]. In the heart, acute S1P (and its carrier HDL) action is cardioprotective against ischemia/reperfusion injury [12, 14, 19, 20 for review], which has attracted much attention from researchers of both basic and clinical medicine. S1P protects cultured rat neonatal cardiomyocytes from ischemia-induced cell death [21] and suppresses ischemia/reperfusion injury ex vivo [22]. In vivo studies using S1P receptor-null mice showed that $S1P_3$, either alone [14] or in combination with $S1P_2$ [23], mediates cardioprotective effect of S1P and HDL against ischemia/reperfusion injury (Figure 2). The cardioprotective action of $S1P_3$ seems to

involve $S1P_3$-mediated activation of endothelial NO synthase (eNOS) [14]. Studies using mice with genetic deletion of SphK1 [24] and SphK2 [25] demonstrated that endogenous S1P, either in perfusates or in cardiac tissues, is cardioprotective ex vivo. S1P generation in the heart is upregulated in response to a transient ischemia, suggesting the contribution of the SphK-S1P axis to ischemic preconditioning and ischemic post-conditioning [26]. Mice heterozygous for deletion of the S1P-degradating enzyme S1P lyase gene and wild-type mice receiving an SPL inhibitor, which showed elevated S1P levels in both plasma and cardiac tissues, had reduced sensitivity to ischemia/reperfusion injury, raising a possibility of cardioprotection with an S1P lyase inhibitor in clinical settings [27]. However, a recent study provided evidence that excess S1P signaling for prolonged period of time could rather exert a deleterious effect, i.e. promote cardiac fibrosis.

In the normal human adult heart cardiac fibroblasts (CFs) are the most abundant cell type and constitute approximately 60% of total cells in cardiac tissues while cardiomyocytes occupy 30% and the remaining 10% are vascular endothelial and smooth muscle cells [28]. This fact suggests crucial roles of CFs in normal cardiac physiology. CFs originally stem from the proepicardium during embryonic stage [29]. Just after birth under a drastic hemodynamic change, CFs rapidly proliferate and

actively produce ECM in neonatal heart, thus beginning to provide structural support against mechanical stretch and ensure myocardial integrity, which persists lifelong with constant and steady-state turnover of ECM. In addition, although CFs are not an excitable cell type, they communicate with cardiomyocytes via gap junctions, and electrically respond to mechanical stretch with cyclical changes in the membrane potential [30, 31], thus contributing to synchronized excitation of atria and ventricles. Upon ischemia and I/R injury acute derangements in cellular functions of cardiomyocytes and CFs could lead to generation of fatal arrhythmia. After survival through acute myocardial infarction CFs proliferate and produce ECM to form a scar tissue that substitutes for necrotic and apoptotic loss of cardiomyocytes to achieve cardiac repair. In a variety of chronically ill conditions of ischemic and non-ischemic etiology, CFs respond to external stimuli with phenotypic changes, which include increased cell proliferation, migration, and changes in production and degradation of ECM, depending upon external stimuli that are as diverse as mechanical stretch, altered oxygen tension, proinflammatory and profibrotic cytokines and growth factors [32]. CFs thus activated or myofibroblasts produce a variety of bioactive substances, which in turn act in an autocrine/paracrine fashion on cardiomyocytes and CFs themselves, provoking cardiac fibrosis with or without cardiomyocyte hypertrophy and functional

decompensation. Myofibroblasts in cardiac remodeling may be derived from circulating bone marrow-derived fibrocytes and epithelial cells through EMT, in addition to resident CFs which originally stem from the proepicardium during embryonic stage [33]. Deposition of myofibroblast-derived ECM including abundant type I/III fibrillar collagen confers stiffness to myocardium, which leads to impaired cardiac relaxation and contraction, i.e. heart failure. CFs/myofibroblasts thus play the major role in pathological cardiac remodeling and represent a target of therapeutic intervention.

Previous studies have shown that local renin-angiotensin-aldosterone system (RAS) and TGFβ play crucial roles in inducing pathological cardiac remodeling [32, 34]. Cardiomyocytes and CFs express all the components of RAS. Myofibroblasts are the major source of Ang II, which induces synthesis and secretion of TGFβ and other proinflammatory and profibrotic cytokines including TNFα, IL-6, and endothelin-1 (ET-1) through AT_1 receptor (AT_1R). TGFβ induces Smad-dependent transcriptional upregulation of connective tissue growth factor and collagen synthesis [35]. TGFβ signaling in the heart may also be transactivated by Ang II [36]. AT_1R activation in cardiomyocytes induces activation of the Rho family small GTPases, RhoA and Rac1, leading to cardiac remodeling including hypertrophy and fibrosis [37]. Rac1 activation is located upstream of NADPH oxidase-mediated production of ROS [38], a mediator of

cardiac remodeling. Moreover, AT_1R activation in vascular endothelial cells inhibits eNOS through a Rho-dependent mechanism [39], which could participate in cardiac fibrosis. These mechanisms provide the molecular basis for well established treatment for pathological cardiac remodeling and heart failure with AT_1R blockers (ARB) or angiotensin converting enzyme (ACE) inhibitors and statins, the latter being an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that produces mevalonate essential for membrane targeting of Ras and Rho family small GTPases.

Transgenic mice that overexpressed SphK1 under a universal promoter developed spontaneous cardiomyocyte degeneration and fibrosis without hypertrophy, which started by 12 weeks and progressed with age [40]. SphK activity and S1P content in the transgenic heart were 20- and 1.7-fold elevated over those of the wild type littermates, respectively, without any change in sphingosine or ceramide content. Plasma and serum S1P levels were comparable to those in wild type mice. Transgenic mice grew up normally with normal blood pressure, blood cell counts and blood biochemistry. Transgenic heart tissues displayed increases in GTP-bound active forms of RhoA and Rac1, and an oxidative stress marker malondialdehyde. Administration of either an HMG CoA reductase inhibitor or an antioxidant N-2-mercaptopropyonylglycine nearly completely protected transgenic hearts from fibrosis, whereas an AT_1R antagonist was

without any effect. Interestingly, cardiac fibrosis in SphK1-transgenic mice was substantially alleviated in $S1P_3^{-/-}$ genetic background. SphK1-transgenic/$S1P_3^{+/+}$ heart tissues showed higher levels of Smad3 phosphorylation over SphK1-transgenic/$S1P_3^{-/-}$ hearts, suggesting that activation of TGFβ signaling is involved in $S1P_3$-dependent development of cardiac fibrosis. It is likely that basal TGFβ expression level in the myocardium was sufficient for $S1P_3$-mediated amplification of TGFβ signaling, since increased expression of TGFβ in the myocardium of SphK1-transgenic mice was not observed. In ischemia/reperfusion injury experiments, SphK1-transgenic mice exhibited a reduction in infarcted area compared with wild-type mice. Thus, transgenic overexpression is also cardioprotective against acute ischemia/reperfusion injury. These observations altogether suggest that, in contrast to the beneficial effects of HDL-associated S1P which induces activation of endothelial $S1P_3$-eNOS axis and myocardial $S1P_3$-phosphatidylinositol 3-kinase (PI3K)-Akt axis, persistent and exaggerated stimulation of $S1P_3$ in the myocardium leads to cardiac fibrosis in vivo through multiple mechanisms, which include transactivation of TGFβ signaling pathway and activation of RhoA and Rac1 small GTPases [41, 42] with downstream signaling involving reactive oxygen species (ROS) generation [43].

According to the observations in cultured fibroblasts derived from other tissues [44,

45], TGFβ signaling could be located either upstream or downstream of S1P receptors in cardiac fibroblasts. S1P induced myofibroblast differentiation of murine CFs in a manner dependent on $S1P_2$ and RhoA [46], while TGFβ stimulation upregulated both the expression and activity of SphK1 in CFs. Furthermore, the stimulatory effect of TGFβ on collagen expression was dependent upon SphK1 and $S1P_2$, and inhibited by anti-S1P monoclonal antibody. Although crossing of the SphK1-transgenic mice with $S1P_2$-null mice failed to produce double mutant mice in our laboratory, possible in vivo contribution of $S1P_2$ in TGFβ-mediated cardiac fibrosis deserves further investigation.

Pulmonary fibrosis

Pulmonary fibrosis is an intractable lung disease with a high mortality rate [47]. Chronic inflammation due to viral infection, radiotherapy and inhalation of environmental toxic substances or epithelial injury of unknown cause underlie pulmonary fibrosis. Epithelial injury results in apoptotic cell death and/or abnormal activation of alveolar epithelial cells with the production of cytokines and other mediators, leading to infiltration of inflammatory cells and expansion of fibroblasts and myofibroblasts through proliferation and activation of resident fibroblasts, stimulation of EMT, and recruitment of circulating fibrocytes (Figure 3) [47-49]. Activated

myofibroblasts secrete excessive amounts of ECM, resulting in destruction of lung architecture.

In recent years, there is growing evidence that S1P and another lysophospholipid mediator lysophosphatidic acid (LPA) are involved in the development of lung fibrosis. Lung fibroblasts express multiple lysophospholipid receptor subtypes including LPA_{1-3} and $S1P_{1-3}$, and respond to LPA and S1P with differentiation into myofibroblasts and stimulation of migration [10]. In fibroblasts LPA and S1P activate Cl^- channel activity, which plays an important role in myofibroblast differentiation by the lysophospholipid mediators [50]. Tager et al. showed that LPA is a crucial factor in the progression of pulmonary fibrosis [51]. LPA was detected in bronchoalveolar lavage fluid (BALF) in patients with pulmonary fibrosis of unknown cause (idiopathic pulmonary fibrosis (IPF)). In bleomycin-induced lung fibrosis model of mice, LPA concentration in BALF was increased after bleomycin challenge. Mice lacking LPA_1, which was the most abundant LPA receptor subtype in lung fibroblasts, was protected from bleomycin-induced pulmonary fibrosis and mortality, indicating a profibrotic role of LPA_1. LPA_1 deletion suppressed fibroblast recruitment and vascular leakage, which are pathogenically linked to pulmonary fibrosis, but did not alter leukocyte infiltration in bleomycin-treated mice. They also observed that fibroblasts obtained from patients with

IPF most abundantly express LPA_1 among LPA-specific receptor subtypes and that pharmacological blockade of LPA_1 abolished chemotaxis of fibroblasts toward LPA or BALF from IPF patients. LPA-LPA_1 signaling also promoted apoptosis of epithelial cells in bleomycin-induced lung injury [52]: apoptosis of alveolar and bronchial epithelial cells after bleomycin challenge was attenuated in LPA_1-deficinet mice compared with wild-type mice. In contrast, stimulation of LPA_1 rendered lung fibroblasts resistant to apoptotic stimuli. Thus, this action of LPA_1 allows fibroblasts to persist in areas of fibrosis. These results indicate that LPA signaling through LPA_1 contributes to development of pulmonary fibrosis through its actions on fibroblasts, vasculature and epithelial cells. In a mouse model of radiation-induced lung fibrosis, the content of LPA and the expression of LPA_1 and LPA_3 in the lung were increased [53]. The administration of $LPA_{1/3}$ antagonist VPC12249 reduced fibroblast accumulation and collagen deposition in the lung, and increased mice survival. LPA_2 is also implicated in lung fibrosis. LPA-LPA_2 is engaged in the activation of TGF-β, which is a major fibrogenic cytokine in the pathogenesis of lung fibrosis [48, 49]. LPA_2 is involved in $\alpha_v\beta_6$ integrin-mediated activation of TGF-β through G_q protein, RhoA, and Rho kinase in epithelial cells [54]. Expression levels of LPA_2 receptor and $\alpha_v\beta_6$ integrin are upregulated in bleomycin-injured lung of mice and in lung fibrotic areas in patients with

interstitial pneumonia.

These results suggest that LPA and its receptors are potential targets for treatment of pulmonary fibrosis. Recently, an orally available LPA_1 antagonist AM966 has been demonstrated to protect mice against bleomycin-induced lung fibrosis [55]. This LPA_1-antagonist alleviated bleomycin-induced lung injury including vascular leakage, inflammation and fibrosis, and reduced TGF-β1 level in BALF. Another orally available LPA_1-selective antagonist AM095 also effectively attenuated bleomycin-induced increases in collagen accumulation and infiltrating inflammatory cells in BALF in mice [56]. More recently, the LPA_1-selective antagonist AM152 was granted Orphan Drug Status by the FDA for the treatment of IPF [57].

S1P has also been implicated in lung fibrosis although precise mechanisms of the S1P involvement are still ill-defined. A previous study [58] showed that bleomycin treatment of NIH3T3 cells led to an increase in cellular S1P level. The expression of SphK1 was increased in lung fibrotic regions in bleomycin-administered mice [59]. In patients with IPF, S1P levels in serum and BALF were increased compared with normal subjects and correlated with lung functions in IPF patients [60]. In lung tissues from IPF patients, increased expression of SphK1 protein was observed. These observations suggested that S1P may be involved in IPF. Fibroblasts mainly express $S1P_1$, $S1P_2$ and

$S1P_3$ [60, 61]. $S1P_1$ mediates migration and proliferation of mouse embryonic fibroblasts (MEFs) in response to S1P [62, 63]. In contrast, $S1P_2$ mediate inhibition of chemoattractant-directed cell migration and proliferation in MEFs and human lung fibroblast HLF-1 cells [60-62]. Thus, the balance between $S1P_1$ and $S1P_2$ signaling seems to critically regulate recruitment and proliferation of fibroblasts in fibrotic lungs.

Several recent studies suggest that S1P regulates myofibroblast differentiation: S1P induced αSMA expression and morphologic change in human lung fibroblasts WI38 in a manner dependent on Rho kinase but independent of pertussis toxin-sensitive G_i [64]. FTY720, whose phosphorylation product is a potent agonist for all S1P receptors except $S1P_2$, induced myofibroblast differentiation of fibroblasts via $S1P_3$ [10]. This FTY720 effect was mediated by Smad3. On the other hand, TGFβ induced myofibroblast differentiation via S1P and its receptors. TGFβ1 treatment of human WI38 lung fibroblasts increased expression of αSMA and SphK1 [59]. SphK inhibition by chemical inhibitors or siRNA-mediated knockdown attenuated TGFβ1-induced upregulation of αSMA and fibronectin. Moreover, knockdown of either $S1P_2$ or $S1P_3$ but not $S1P_1$ inhibited TGFβ1-induced αSMA expression. The addition of the Rho kinase inhibitor Y27632 also suppressed TGFβ1-induced αSMA expression. Thus, TGFβ1 induced myofibroblast differentiation through SphK1-$S1P_2$/$S1P_3$-Rho kinase pathway.

Myofibroblasts in fibrotic lesions may also be derived from epithelial cells through EMT. S1P induced EMT in alveolar type II epithelial A549 cells via $S1P_2$ and $S1P_3$ [60]. S1P-induced EMT was dependent on TGFβ-Smad3 pathway, Rho-Rho kinase and ROS. TGFβ1-induced EMT was accompanied by the upregulation of SphK1 expression and partially mediated by SphK1, $S1P_2$/$S1P_3$, Rho kinase and ROS. Furthermore, a recent study [65] showed that genetic deletion of SphK1 protected mice from bleomycin-induced fibrosis and mortality with attenuated expression of αSMA, fibronectin and collagen, and phosphorylation of Smad2/3. These observations together suggest that the feed-forward cross-talk between S1P and TGFβ, which amplifies profibrotic signals, is existent.

Previous studies demonstrated that S1P-$S1P_1$ pathway enhances endothelial barrier function in vivo and in vitro [66]. Shea et al. showed that repeated administration of FTY720 or the $S1P_1$-selective agonist AUY954 into mice increased vascular leak and mortality after bleomycin challenge [67]. Continued exposure to the $S1P_1$ agonists also resulted in exacerbation of lung fibrosis. These observations, together with other studies [68, 69], indicate that $S1P_1$-agonists can act as functional antagonists of $S1P_1$ in vivo through the mechanism of $S1P_1$-agonist-induced internalization and degradation of $S1P_1$ and consequently impair the barrier-protective function of $S1P_1$ on endothelial cells.

These observations also suggest that vascular hyperpermeability is an important component of the fibrogenic responses to lung injury and point to the possibility that targeting endothelial $S1P_1$ may be a useful therapeutic tactic.

As described above, several reports suggested that S1P might be involved in lung fibrosis. However, compared with LPA, it remains ill-defined whether S1P is profibrotic or anti-fibrotic as an overall effect and by what exact mechanisms S1P could modify lung fibrosis. It is necessary to further explore the roles of S1P signaling pathway in lung fibrosis, particularly by utilizing specific inactivation of S1P receptor subtypes and/or modulation of SphK isoforms. The studies of patients with IPF showed that the signaling pathways of LPA and S1P may be stimulated in lung fibrotic lesions as suggested by increased levels of LPA and S1P in BALF, elevated expression of SphK1 in lung fibrotic lesions and augmented chemotactic responses of fibroblasts from IPF patients [51, 60]. However, the available data are still limited, and whether LPA and S1P have a causal role in IPF awaits further investigation.

Systemic sclerosis

Systemic sclerosis (scleroderma) is a connective tissue disease of unknown cause and is characterized by progressive extensive multi-organ fibrosis [70]. Fibrosis is most

prominent in the skin, lung, heart, kidney and gastrointestinal tract. The pathogenesis of systemic sclerosis involves interactions between vasculopathy, inflammation and autoimmunity, and fibrosis. As the earliest events, tissue injury by various insults including viral infection and oxidative stress may result in vascular injury. Vascular injury causes endothelial activation and dysfunction, leading to vascular permeability changes, increased endothelial expression of leukocyte adhesion molecules, altered secretion of vasoactive mediators, and platelet activation. These events result in leukocyte adhesion to the endothelium, platelet aggregation and thrombosis, vascular remodeling with intimal and medial hypertrophy, and eventually vascular obliteration with tissue hypoxia. The lesions at the early stage are also characterized by infiltration of T lymphocytes and monocytes. Release of cytokines and growth factors from the infiltrating leukocytes, activated endothelium and other cell types in the lesions induces further tissue damage and wound healing responses comprising recruitment of fibroblasts, myofibroblast differentiation, and ECM accumulation by the expanded pool of fibroblasts/myofibroblasts. Progressive fibrosis is the primary cause of death in systemic sclerosis and refractory to current therapy. Therefore, discovery of novel mediators to drive fibrogenesis is expected to provide effective therapeutic targets for progressive fibrosis in systemic sclerosis.

Several lines of recent studies provided a rationale for the hypothesis that LPA and S1P might be new mediators for systemic sclerosis. Patients with systemic sclerosis exhibited elevated (2.2-fold) serum LPA levels compared with healthy subjects [71, 72]. However, serum activity of the key LPA-producing enzyme autotaxin and serum level of the LPA precursor lysophosphatidylcholine were not different between patients and control subjects, suggesting that elevated serum LPA in patients may be due to reduced degradation of LPA. Analysis of LPA species revealed that serum level of arachidonyl-LPA but not other LPA species was higher in systemic sclerosis patients. Arachidonyl-LPA is shown to have a relatively high platelet-activating potency and chemotactic activity for immature dendritic cells. The same study also demonstrated that serum level of S1P was approximately 1.4-fold increased in systemic sclerosis patients than in control subjects. Two other studies [73, 74] using dermal fibroblasts derived from systemic sclerosis patients demonstrated altered responses of fibroblasts to LPA and S1P. Fibroblasts from systemic sclerosis patients showed increased activity of LPA-activated Cl^- channel, which is implicated in myofibroblast differentiation [50], and elevated αSMA expression compared with normal fibroblasts [73]. Fibroblasts from systemic sclerosis patients also showed decreased expression of $S1P_1$/$S1P_2$ and increased expression of $S1P_3$, compared with normal fibroblasts [74]. S1P induced

phosphorylation of Smad3 via $S1P_1/S1P_2$ in systemic sclerosis fibroblasts whereas closely related S1P receptor agonist dihydro-S1P rather inhibited Smad3 phosphorylation, although the molecular mechanism underlying the differential effects of S1P and dihydro-S1P was unknown.

Subcutaneous injection of bleomycin in mice, which is a commonly used animal model for dermal fibrosis in systemic sclerosis, induces collagen accumulation in the skin [70]. Bleomycin-induced dermal thickening and collagen accumulation was nearly abrogated in LPA_1-KO mice but not LPA_2-KO mice [75]. Subcutaneous bleomycin injection stimulated Smad 2 phosphorylation and αSMA expression in the dermis. Stimulation of dermal Smad2 phosphorylation and αSMA expression was markedly inhibited in LPA_1-KO mice, indicating the contribution of LPA_1 to activation of TGFβ/Smad pathway and fibrosis in the skin. Administration of the LPA_1-selective antagonist AM095 markedly inhibited bleomycin-induced dermal fibrosis. In addition, S1P and LPA may be involved in vasculopathy, autoimmunity and inflammation in systemic sclerosis through their actions on vascular endothelium, inflammatory cells including macrophages and mast cells, and immune cells [11, 16, 76].

In summary, these observations suggest that LPA/S1P signaling have the potential as attractive new therapeutic targets for fibrosis in systemic sclerosis.

Liver fibrosis

Irrespective of the insults, such as virus, alcohol abuse or drugs, the wound healing response generally occurs in the liver upon injury, and the persistence of this response may result in liver fibrosis. It is now well known that among the cells in the liver, hepatic stellate cells play a major role in liver fibrosis (Figure 4). During the process of liver fibrosis, hepatic stellate cells undergo a phenotypic change to myofibroblasts. This change has been termed as activation, in which enhanced proliferation, extracellular matrices production, contractility and motility are among its main features [77-79]. Many fibrogenic factors in the liver such as TGFβ or PDGF have been evaluated with a focus on the effects on this hepatic stellate cell activation [79].

Regarding hepatic stellate cells and S1P, S1P was first shown to stimulate rat hepatic stellate cell proliferation via an extracellular mechanism, suggesting that S1P could be a pro-fibrogenic factor in the liver [80]. However, the contrary finding of antiproliferative properties of S1P as an intracellular mediator was reported in human hepatic myofibroblats [81]. Although the discrepancy between these two results *in vitro* has not been solved yet, whether S1P might be pro-fibrogenic or anti-fibrogenic in the liver was later determined with S1P receptor-deficient mice *in vivo*. In mice, deletion of $S1P_1$ is

known to result in embryonic lethality, while deletion of $S1P_2$- or $S1P_3$-deleted mice are viable and cause no obvious abnormality in the liver [82]. However, once acute liver injury was induced in $S1P_2$-deficient mice, a response of hepatic stellate cells (or hepatic myofibroblasts) to the insult was dramatically affected, in which the wound healing responses determined by accumulation of hepatic myofibroblasts was profoundly reduced. Furthermore, S1P enhanced proliferation of cultured hepatic myofibroblasts from wild-type mice but not those from $S1P_2$-deficient mice [83]. These results suggest a pro-fibrogenic property of S1P via $S1P_2$ in the liver. Indeed, liver fibrosis caused by repeated administration of carbon tetrachloride was shown to be reduced in $S1P_2$-deficient mice, suggesting that S1P plays a stimulatory role in liver fibrosis via $S1P_2$ [84]. It has been shown that $S1P_2$ is preferentially coupled to $G_{12/13}$ and hence Rho [85]. In line with this, the enhancement of Rho and Rho kinase activity in the liver during the process of fibrosis after bile duct ligation was reduced in $S1P_2$-deficient mice [86]. Collectively, the contribution of S1P to the progress of liver fibrosis may be mediated by Rho and Rho kinase activation via $S1P_2$.

Recently, a potential role of bone marrow-derived cells in liver fibrosis is gaining attention. There are a number of studies reporting that bone marrow-derived cells migrate into the liver, transdifferentiate to hepatic myofibroblasts and may participate in

the progress of liver fibrosis. In contrast, it has also been shown that bone marrow-derived cells in the liver contribute to the regression of liver fibrosis. Thus, controversy exists regarding the role of bone marrow-derived cells in liver fibrosis [87]. From the standpoint of bone marrow-derived cells as pro-fibrogenic cells, S1P was shown to play an important role in the progress of liver fibrosis caused by bile duct ligation [88] or repeated administration of carbon tetrachloride [89] to mediate bone marrow-derived cell migration into the liver. These effects were shown to be exerted via $S1P_3$, because suramin as $S1P_3$ antagonist attenuated the pro-fibrogenic effect of S1P [88, 89]. Furthermore, the contribution of $S1P_1$ and $S1P_3$ to S1P-induced migration was reported in human hepatic stellate cell line [90] or human hepatic myofibroblasts [91]. Thus, so far there are two lines of evidence showing that S1P is pro-fibrogenic, and the exact contribution of S1P receptors, $S1P_1$, $S1P_2$ and $S1P_3$, to liver fibrosis should be further elucidated.

Because hepatic stellate cells reside around hepatic sinusoid, which is unique vasculature in the liver, contraction of those cells results in increased pressure of hepatic sinusoid and hence of portal vein, which is linked to hepatic sinusoid. Thus, it has been speculated that hepatic stellate cells may play a direct role in the pathogenesis of portal hypertension, one of major complications of liver fibrosis. S1P was shown to enhance

contractility of hepatic stellate cells *in vitro* [80], and further to increase portal vein pressure *ex vivo* in rats with Rho and Rho kinase activation via $S1P_2$ [92]. Furthermore, it has been recently shown that the intravenous administration of $S1P_2$ antagonist, JTE-013, promptly reduces portal vein pressure with Rho kinase inactivation in rats with portal hypertension caused by bile duct ligation [86]. It should be noted that this effect of $S1P_2$ antagonist is selective for portal vein pressure in rats with portal hypertension, which may be explained by selective enhancement of $S1P_2$ expression in stellate cells of the liver. These results suggest that S1P importantly contributes to the pathophysiology of portal hypertension via $S1P_2$, and that $S1P_2$ antagonist merits consideration as a therapeutic agent for portal hypertension.

Regarding S1P level in the blood stream in liver fibrosis, plasma S1P level was shown to be reduced, and the inverse correlation was revealed between serum hyaluronic acid level, a marker of liver fibrosis, and plasma S1P level in patients with chronic hepatitis C, suggesting that plasma S1P level may be reduced during the process of liver fibrosis. In line with this speculation, plasma S1P level was shown to be reduced in association with the progress of liver fibrosis in rats with carbon tetrachloride administration [93]. It is established that red blood cells and platelets contribute importantly to the regulation of plasma S1P level [94, 95]. In addition, liver may be also

substantially involved in the regulation of plasma S1P level.

The dynamic change of plasma S1P level and S1P receptors expressions in the liver in the process of liver fibrosis and the substantial effect of S1P receptor knockout on liver fibrosis have been described. Although all these findings strongly suggest an important role of S1P in the pathophysiology of liver fibrosis, little is known regarding the signaling pathways operating in the liver so far. Thus, this point should be elucidated in the future, and also the therapeutic strategy for liver fibrosis focusing on the regulation of S1P effects should be further established.

Conclusion and perspectives

Emerging evidence highlights the new role of S1P signaling pathway in fibrosis, which is often associated with chronic inflammation. The previous studies using mouse models of fibrosis suggest that S1P may affect fibrogenesis through affecting vascular barrier function, inflammatory cell recruitment, and migration, proliferation and myofibroblast differentiation of fibroblasts. S1P could exert receptor subtype-specific, distinct effects on each of these processes. In fibrotic lesions, a variety of cytokines and mediators may regulate expression and activity of SphKs, thereby altering S1P production. However, it remains undefined what roles locally produced and blood S1P

plays in fibrogenesis, respectively. Moreover, S1P signaling pathway seems to cross-talk with TGFβ signaling pathway at multiple steps. This cross-talk amplifies cellular mechanisms for stimulating fibrogenesis. Details in cellular and molecular mechanisms underlying S1P regulation of fibrogenesis still remain to be clarified. Information concerning the role of the lysophospholipid mediators in human fibrotic diseases is scarce. In addition, it is necessary to reveal similarities and dissimilarities in human fibrotic diseases and animal models of fibrosis, because most of animal data on the pathogenesis of fibrotic diseases were obtained using particular animal models of each fibrotic disease. Nevertheless, available evidence demonstrates that lysophospholipid signaling pathways of S1P and also LPA are novel, promising therapeutic targets for treating fibrotic diseases.

Acknowledgments

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology in Japan, funds from the Kanazawa University Strategic Research Development Program, and the IPNU Research Promotion Program.

References

[1] T.A. Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases, J. Clin. Invest. 117(2007) 524-529.

[2] P. Camelliti, T.K. Borg, P. Kohl, Structural and functional characterization of cardiac fibroblasts, Cardiovasc. Res. 65(2005)40-51.

[3] R.D. Brown, S.K. Ambler, M.D. Mitchell, C.S. Long, The cardiac fibroblast: Therapeutic target in myocardial remodeling and failure, Annu. Rev. Pharmacol. Toxicol. 45(2005)657-687.

[4] T.A. Wynn, Integrating mechanisms of pulmonary fibrosis, J. Exp. Med. 208 (2011) 1339-1350.

[5] K.E. Porter, N.A. Turner, Cardiac fibroblasts: At the heart of myocardial remodeling, Pharmacol. Thera. 123(209)255-278.

[6] H. Guarino, A.Tosoni, M. Nebuloni, Direct contribution to organ fibrosis: epithelial-mesenchymal transition, Human Pathol. 40(2009) 1365-1376.

[7] H. Zhang, N.N. Desai, A. Olivera, T. Seki, G. Brooker, S. Spiegel, Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation, J. Cell Biol. 114(1991) 155-167.

[8] Y. Sadahira, F. Ruan, S. Hakomori, Y. Igarashi, Sphingosine 1-phosphate, a specific endogenous signaling molecule controlling cell motility and tumor cell invasiveness, Proc. Natl. Acad. Sci. U. S. A. 89(1992) 9686-9690.

[9] J.P. Hobson, H.M. Rosenfeldt, L.S. Barak, A. Olivera, S. Poulton, M.G. Caron, S. Milstien, S. Spiegel, Role of the sphingosine-1-phosphate receptor EDG-1 in PDGF-induced cell motility, Science. 291(2001) 1800-1803.

[10] C.D. Keller, P.R. Gil, M. Tölle, M. van der Giet, J. Chun, H.H. Radeke, M. Schäfer-Korting, B. Kleuser, Immunomodulator FTY720 induced myofibroblast differentiation via the lysophospholipid receptor $S1P_3$ and Smad3 signaling, Am. J. Pathol. 170 (2007) 281-292.

[11] S. Spiegel, S. Milstien, The outs and the ins of sphingosine-1-phosphate in immunity. Nat. Rev. Immunol. 11(2011)403-415.

[12] C.K. Means, J.H. Brown, Sphingosine-1-phosphate receptor signalling in the heart, Cardiovasc. Res. 82 (2009) 193-200.

[13] M. Forrest, S.-Y. Sun, R. Hajdu, J. Bergstrom, D. Card, G. Doherty, J. Hale, C. Keohane, C. Meyers, J. Milligan, S. Mills, N. Nomura, H. Rosen, M. Rosenbach, G.-J. Shei, I. I. Singer, M. Tian, S. West, V. White, J. Xie, R. L. Proia, and S. Mandala, Immune cell regulation and cardiovascular effects of sphingosine

1-phosphate receptor agonists in rodents are mediated via distinct receptor subtypes, J. Pharmacol. Exp. Ther. 309 (2004) 758-68.

[14] G.Theilmeier, C. Schmidt, J. Herrmann, P., M. Schäfers, I. Herrgott, J. Mersmann, J. Larmann, S. Hermann, J. Stypmann, O. Schober, R. Hildebrand, R. Schulz, G. Heusch, M. Haude, K. von Wnuck Lipinski, C. Herzog, M. Schmitz, R. Erbel, J. Chun, B. Levkau, High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the $S1P_3$ lysophospholipid receptor, Circulation 114 (2006) 1403–1409.

[15] Y. Ryu, N. Takuwa, N. Sugimoto , S. Sakurada, S. Usui, H. Okamoto, O. Matsui, Y. Takuwa, Sphingosine-1-phosphate, a platelet-derived lysophospholipid mediator, negatively Regulates cellular Rac activity and cell migration in vascular smooth muscle cells, Circ. Res. 90 (2002) 325-332.

[16] Y. Takuwa, Y. Okamoto, K. Yoshioka, N. Takuwa, Sphingosine-1-phosphate signaling in physiology and diseases, BioFactors 38 (2012) in press.

[17] K.M. Mair, E. Robinson, K.A. Kane, S. Pyne, R.R. Brett, N.J. Pyne, S. Kennedy, Interaction between anandamide and sphingosine-1-phosphate in mediating vasorelaxation in rat coronary artery, Br. J. Pharmacol. 161 (2010) 176-192.

[18] B. Levkau, S. Hermann, G. Theilmeier, M. van der Giet, J. Chun, O. Schober, M. Schäfers M. High-density lipoprotein stimulates myocardial perfusion in vivo. Circulation. 110(2004)3355-3359.

[19] K. Sattler, B. Levkau, Sphingosine-1-phosphate as a mediator of high-density lipoprotein effects in cardiovascular protection, Cardiovasc. Res. 82 (2009) 201-11.

[20] J.S. Karliner, Sphingosine kinase and sphingosine 1-phosphate in cardioprotection, J. Cardiovasc. Pharmacol. 53(2009) 189-97

[21] J.S. Karliner, N. Honbo, K. Summers, M.O. Gray, E.J. Goetzl, The lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid enhance survival during hypoxia in neonatal rat cardiac myocytes, J. Mol. Cell. Cardiol. 33 (2001) 1713–1717.

[22] Z.Q. Jin, H.Z. Zhou, P. Zhu, N. Honbo, D. Mochly-Rosen, R.O. Messing, E.J. Goetzl, J.S. Karliner, M.O. Gray, Cardioprotection mediated by sphingosine-1-phosphate and ganglioside GM-1 in wild-type and PKC epsilon knockout mouse hearts, Am. J. Physiol. Heart Circ Physiol. 282 (2002) H1970-7.

[23] C.K. Means, C.Y. Xiao, Z. Li, T. Zhang, J.H. Omens, I. Ishii, J. Chun, H.J. Brown, Sphingosine 1-phosphate $S1P_2$ and $S1P_3$ receptor-mediated Akt activation protects against in vivo myocardial ischemia-reperfusion injury, Am. J. Physiol. Heart Circ

Physiol 292 (2007) H2944–H2951.
[24] Z.Q. Jin, J. Zhang, Y. Huang, H.E. Hoover, D.A. Vessey, J.S. Karliner, A sphingosine kinase 1 mutation sensitizes the myocardium to ischemia/reperfusion injury, Cardiovasc. Res. 76 (2007) 41-50.
[25] D.A. Vessey, L. Li, Z-Q. Jin, M. Kelley, N. Honbo, J. Zhang, J.S. Karliner, A Sphingosine kinase form 2 knockout sensitizes mouse myocardium to ischemia/reoxygenation injury and diminishes responsiveness to ischemic preconditioning, Oxid. Med. Cell. Longev. 2011(2011) 961059.
[26] D.A. Vessey, L. Li, N. Honbo, J.S. Karliner, Sphingosine 1-phosphate is an important endogenous cardioprotectant released by ischemic pre- and postconditioning, Am. J. Physiol. Heart Circ Physiol. 297 (2009) H1429-35.
[27] Z.Q. Jin, H. Fyrst, M. Zhang, A.D. Borowsky, L. Dillard, J.S. Karliner, J.D. Saba, S1P lyase: a novel therapeutic target for ischemia-reperfusion injury of the heart. Am. J. Physiol. Heart Circ Physiol. 300 (2011) H1753-61.
[28] B.I. Jugdutt, Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation 108 (2003) 1395–1403.
[29] T.K. Borg, K. Rubin, E. Lundgren,K. Borg, B. Obrink, Recognition of extracellular matrix components by neonatal and adult cardiac myocytes. Dev. Biol. 104 (1984) 86–96.
[30] K. Goshima, Y. Tonomura, Synchronized beating of embryonicmousemyocardial cells mediated by FL cells in monolayer culture. Exp. Cell Res. 56 (1969) 387–392.
[31] L. Chilton, W.R. Giles, G.L. Smith, Evidence of intercellular coupling between co-cultured adult rabbit ventricular myocytes and myofibroblasts, J. Physiol. 583 (2007) 225-36.
[32] K.E. Porter, N.A. Turner, Cardiac fibroblasts: at the heart of myocardial remodeling, Pharmacology & Therapeutics 123 (2009) 255–278
[33] A.F. Moorman, V.M. Christoffels, Cardiac chamber formation: development, genes, and evolution. Physiol. Rev. 83 (2003) 1223–1267.
[34] E.E. Creemers, Y.M. Pinto, Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovasc. Res. 89 (2011):265-72.
[35] S. Rosenkranz, TGF-β1 and angiotensin networking in cardiac remodeling. Cardiovasc. Res. 63 (2004) 423–432.
[36] J.J. Schultz, S.A. Witt, B.J. Glascock,M.L. Nieman, P.J. Reiser, S.L. Nix,T.R. Kimball, T. Doetschman, TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. J. Clin. Invest. 109 (2002) 787–796.
[37] U. Laufs, H. Kilter, C. Konko, S. Wassmann, M. Boehm, G. Nickenig, Impact of

HMG CoA reductase inhibition on small GTPases in the heart, Cardiovasc. Res. 53 (2002) 911–920

[38] P.L. Hordijk, Regulation of NADPH oxidases: the role of Rac proteins, Circ. Res. 98 (2006) 453-62.

[39] A. Shatanawi, M.J. Romero, J.A. Iddings, S. Chandra, N.S. Umapathy, A.D. Verin, R.B. Caldwell, R.W. Caldwell, Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/p38 mitogen-activated protein kinase/arginase pathway, Am. J. Physiol. Cell Physiol. 300(2011) C1181-92.

[40] N. Takuwa, S. Ohkura, S. Takashima, K. Ohtani, Y. Okamoto, T. Tanaka, K. Hirano, S. Usui, F. Wang, W. Du, K. Yoshioka, Y. Banno, M. Sasaki, I. Ichi, M. Okamura, N. Sugimoto, K. Mizugishi, Y. Nakanuma, I. Ishii, M. Takamura, S. Kaneko, S. Kojo, K. Satouchi, K. Mitumori, J. Chun, Y. Takuwa, $S1P_3$-mediated cardiac fibrosis in sphingosine kinase 1 transgenic mice involves reactive oxygen species, Cardiovasc. Res. 85(2010):484-93.

[41] Y. Takuwa, Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors, Biochim. Biophys. Acta 1582 (2002) 112-20.

[42] Y. Takuwa, Y. Okamoto, K. Yoshioka, N. Takuwa, Sphingosine-1-phosphate signaling and biological activities in the cardiovascular system, Biochim. Biophys. Acta. 1781 (2008) 483-8.

[43] J.H. Brown, D.P. Del Re, M.A. Sussman, The Rac and Rho hall of fame: a decade of hypertrophic signaling hits, Circ. Res. 98 (2006) 730-42.

[44] C.D. Keller, P. Rivera Gil, M. Tölle, M. van der Giet, J. Chun, H.H. Radeke, M. Schäfer-Korting, B. Kleuser, Immunomodulator FTY720 induces myofibroblast differentiation via the lysophospholipid receptor S1P3 and Smad3 signaling, Am. J. Pathol. 170 (2007) 281-92.

[45] F.Cencetti, C. Bernacchioni, P.Nincheri, C.Donati, P. Bruni, Transforming growth factor-beta1 induces transdifferentiation of myoblasts into myofibroblasts via up-regulation of sphingosine kinase-1/S1P3 axis, Mol. Biol. Cell. 21 (2010) 1111-24.

[46] N. Gellings Lowe, J.S. Swaney, K.M. Moreno, R.A. Sabbadini, Sphingosine-1-phosphate and sphingosine kinase are critical for transforming growth factor-beta-stimulated collagen production by cardiac fibroblasts, Cardiovasc. Res. 82 (2009) 303-12.

[47] T.E. King Jr., A. Pardo, M. Seiman, Idiopathic pulmonary fibrosis, Lancet 6736 (2011) 60052-60054.

[48] R.M. Strieter, B. Mehrad, New mechanisms of pulmonary fibrosis, Chest 136

(2009) 1364-1379.
[49] T. Kisseleva, D.A. Brenner, Fibrogenesis of parencymal organs, Proc. Am. Thorac. Soc. 5 (2008) 338-342.
[50] Z. Yin, M.A. Watsky, Choride channel activity in human lung fibroblasts and myofibroblasts. Am. J. Physiol. 288 (2005) L1110-L1116.
[51] A.M. Tager, P. LaCamera, B.S. Shea, G.S. Campanella, M. Selman, Z. Zhao, V. Polosukhin, J. Wain, B.A. Karimi-Shah, N.D. Kim, W.K. Hart, A. Pardo, T.S. Blackwell, Y. Xu, J. Chun, A.D. Luster, The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak, Nat. Med. 14 (2008) 45-54.
[52] M. Funke, Z. Zhao, Y. Xu, J. Chun, A.M. Tager, The lysophosphatidic acid receptor LPA1 promotes epithelial cell apoptosis after lung injury, Am. J. Respir. Cell Mol. Biol. 46 (2012) 355-364.
[53] L. Gan, J.-X. Xue, X. Li, D.-S. Liu, Y. Ge, P.-Y. Ni, L. Deng, Y. Lu, W. Jiang, Blockade of lysophosphatidic acid receptors LPAR1/3 ameliorates lung fibrosis induced by irradiation, Biochem. Biophys. Res. Commun. 409 (2011) 7-13.
[54] M.Y. Xu, J. Porte, A.J. Knox, P.H. Weinreb, T.M. Maher, S.M. Violette, R.J. McAnulty, D. Sheppard, G. Jenkins, Lysophosphatidic acid induces αvβ6 integrin-mediated TGF-βactivation via the LPA2 receptor and the small G protein $G\alpha_q$, Am. J. Pathol. 174 (2009) 1264-1279.
[55] J.S. Swaney, C. Chapman, L.D. Correa, K.J. Stebbins, R.A. Bundey, P.C. Prodanovich, P. Fagan, C.S. Baccei, A.M. Santini, J.H. Hutchinson, T.J. Seiders, T.A. Parr, P. Prasit, J.F. Evans, D.S. Lorrain, A novel, orally active LPA_1 receptor antagonist inhibits lung fibrosis in the mouse bleomycin model, Brit. J. Pharmacol. 160 (2010) 1699-1713.
[56] J.S. Swaney, C. Chapman, L.D. Correa, K.J. Broadhead, G. Bain, A.M. Santini, J. Darlington, C.D. King, C.S. Baccei, C. Lee, T.A. Parr, R. Roppe, T.J. Seiders, J. Ziff, P. Prasit, J.H. Hutchinson, J.F. Evans, D.S. Lorrain, Pharmacokinetic and pharmacodynamic characterization of an oral lysophosphatidic acid type LPA_1 receptor-selective antagonist, J. Pharmacol. Exp. Ther. 336 (2011) 693-700.
[57] “Amira Pharmaceuticals receives Orphan Drug Status for a novel LPA1 antagonist, AM152, for the treatment of idiopathic pulmonary fibrosis” 2011. http://www.amirapharm.com/articles/AM152 Orphan Drug.html.
[58] R. Dhami, X. He, E.H. Schuchman, Acid sphingomyelinase deficiency attenuates bleomycin-induced lung inflammation and fibrosis in mice, Cell. Physiol. Biochem. 26 (2010) 749-760.

[59] Y. Kono, T. Nishiuma, Y. Nishimura, Y. Kotani, T. Okada, S. Nakamura, M. Yokoyama, Sphingosine kinase 1 regulates differentiation of human and mouse lung fibroblasts mediated by TGF-β1, Am. J. Respir. Cell Mol. Biol. 37 (2007) 395-404.

[60] J. Milara, R. Navarro, G. Juan, T. Peiró, A. Serrano, M. Ramón, E. Morcillo, J. Cortijo, Sphingosine-1-phosphate is increased in patients with idiopathic pulmonary fibrosis and mediates epithelial to mesenchymal transition, Thorax 67 (2012) 147-156.

[61] M. Hashimoto, X. Wang, L. Mao, T. Kobayashi, S. Kawasaki, N. Mori, M.L. Toews, H.J. Kim, D.R. Cerutis, X. Liu, S.I. Rennard, Sphingsine 1-phosphate potentiates human lung fibroblast chemotaxis through the $S1P_2$ receptor, Am. J. Respir. Cell Mol. Biol. 39 (2008) 356-363.

[62] S.K. Goparaju, P.S. Jolly, K.R. Watterson, M. Bektas, S. Alvarez, S. Sarkar, L. Mel, I. Ishii, J. Chun, S. Milstien, S. Spiegel, The $S1P_2$ receptor negatively regulates platelet-derived growth factor-induced motility and proliferation, Mol. Cell. Biol. 25 (2005) 4237-4249.

[63] J.S. Long, V. Natarajan, G. Tigyi, S. Pyne, N.J. Pyne, The functional PDGFβ receptor-S1P1 receptor signaling complex is involved in regulating migration of mouse embryonic fibroblasts in response to platelet derived growth factor, Prostaglandins Other Lipid Mediat. 80 (2006) 74-80.

[64] Y. Urata, Y. Nishimura, T. Hirase, M. Yokoyama, Sphingosine 1-phosphate induces alpha-smooth muscle actin expression in lung fibroblasts via Rho-kinase, Kobe J. Med. Sci. 51 (2005) 17-27.

[65] L. Hunag, P. Fu, W. Ma, D. He, B. Mathew, J.G. Garcia, V. Natarajan, Sphingosine kinase 1 deficiency protects bleomycin induced mouse lung fibrosis, Am. J. Respir. Crit. Care Med. 183 (2011) A2152.

[66] J. G. Garcia, F. Liu, A. D. Verin, A. Birukova, M. A. Dechert, W. T. Gerthoffer, J. R. Bamberg, D. English, Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. J. Clin. Invest. 108 (2001) 689-701.

[67] B.S. Shea, S.F. Brooks, B.A. Fontaine, J. Chun, A.D. Luster, A.M. Tager, Prolonged exposure to sphingosine 1-phosphate receptor-1 agonists exacerbates vascular leak, fibrosis, and mortality after lung injury, Am. J. Respir. Cell Mol. Biol. 43 (2010) 662-673.

[68] D. Marsolais, H. Rosen, Chemical modulators of sphingosine-1-phosphate receptors as barrier-oriented therapeutic molecules, Nat. Rev. Drug Discov. 8 (2009) 297-307.

[69] M.L. Oo, S. H. Chang, S. Thangada, M.T. Wu, K. Rezaul, V. Blaho, S. I. Hwang, D. K. Han, T. Hla, Engagement of $S1P_1$-degradative mechanisms leads to vascular leak in mice, J. Clin. Invest. 121(2011) 2290-300.

[70] J. Varga, D. Abraham, Systemic sclerosis: a prototypic multisystem fibrotic disorder, J. Clin. Invest. 117(2007) 557-567.

[71] A. Tokumura, L. D. Carbone, Y. Yoshioka, J. Morishige, M. Kikuchi, A. Postlethwaite, M. A. Watsky, Elevated serum levels of arachidonoyl-lysophosphatidic acid and sphingosine 1-phosphate in systemic sclerosis, Int. J. Med. Sci. 6(2009) 168-176.

[72] D. Pattanaik, A. E. Postlethwaite, A role for lysophosphatidic acid and sphingosine 1-phosphate in the pathogenesis of systemic sclerosis, Discov. Med. 10(2010):161-167.

[73] Z. Yin, L. D. Carbone, M. Gotoh, A. Postlethwaite, A. L. Bolen, G. J. Tigyi, K. Murakami-Murofushi, M. A. Watsky, Lysophosphatidic acid-activated Cl- current activity in human systemic sclerosis skin fibroblasts, Rheumatology (Oxford). 49(2010):2290-2297.

[74] S. Bu, Y. Asano, A. Bujor, K. Highland, F. Hant, M. Trojanowska, Opposite effects of dihydrosphingosine 1-phosphate and sphingosine 1-phosphate on transforming growth factor-beta/Smad signaling are mediated through the PTEN/PPM1A-dependent pathway, Arthritis Rheum. 62(2010):2117-2126.

[75] F. V. Castelino, J. Seiders, G. Bain, S. F. Brooks, C. D. King, J. S. Swaney, D. S. Lorrain, J. Chun, A. D. Luster, A. M. Tager, Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma, Arthritis Rheum. 63(2011):1405-1415.

[76] T. Mutoh, R. Rivera, J. Chun, Insights into the pharmacological relevance of lysophospholipid receptors, Br. J. Pharmacol. 165(2012):829-844.

[77] D.C. Rockey, C.N. Housset, S.L. Friedman, Activation-dependent contractility of rat hepatic lipocytes in culture and in vivo, J. Clin. Invest. 92(1993) 1795-1804.

[78] K. Ikeda, T. Wakahara, Y.Q. Wang, H. Kadoya, N. Kawada, K. Kaneda, In vitro migratory potential of rat quiescent hepatic stellate cells and its augmentation by cell activation, Hepatology 29 (1999) 1760-1767.

[79] S.L. Friedman, Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury, J. Biol. Chem. 275(2000) 2247-2250.

[80] H. Ikeda, Y. Yatomi, M. Yanase, H. Satoh, H. Maekawa, I. Ogata, Y. Ozaki, Y. Takuwa, S. Mochida, K. Fujiwara, Biological activities of novel lipid mediator sphingosine 1-phosphate in rat hepatic stellate cells, Am. J. Physiol. Gastrointest

Liver Physiol.279(2000) G304-310.
[81] J. Davaille, C. Gallois, A. Habib, L. Li, A. Mallat, J. Tao, T.L evade, S. Lotersztajn, Antiproliferative properties of sphingosine 1-phosphate in human hepatic myofibroblasts. A cyclooxygenase-2 mediated pathway, J. Biol. Chem. 275(2000) 34628-34633.
[82] J.W. Choi, C.W. Lee, J. Chun, Biological roles of lysophospholipid receptors revealed by genetic null mice: an update,BiochimBiophys Acta.1781(2008)531-539.
[83] V. Serriere-Lanneau, F. Teixeira-Clerc, L. Li, M. Schippers, W. de Wries, B. Julien, J. Tran-Van-Nhieu, S. Manin, K. Poelstra, J. Chun, S. Carpentier, T. Levade, A. Mallat, S. Lotersztajn, The sphingosine 1-phosphate receptor S1P2 triggers hepatic wound healing, Faseb J. 21 (2007) 2005-2013.
[84] H. Ikeda, N. Watanabe, I. Ishii, T. Shimosawa, Y. Kume, T. Tomiya, Y. Inoue, T. Nishikawa, N. Ohtomo, Y. Tanoue, S. Iitsuka, R. Fujita, M. Omata, J. Chun, Y. Yatomi, Sphingosine 1-phosphate regulates regeneration and fibrosis after liver injury via sphingosine 1-phosphate receptor 2, J. Lipid. Res. 50(2009) 556-564.
[85] H. Okamoto, N. Takuwa, T. Yokomizo, N. Sugimoto, S. Sakurada, H. Shigematsu, Y. Takuwa, Inhibitory regulation of Rac activation, membrane ruffling, and cell migration by the G protein-coupled sphingosine-1-phosphate receptor EDG5 but not EDG1 or EDG3, Mol. Cell. Biol. 20 (2000) 9247-9261.
[86] Y. Kegeyama, H. Ikeda, N. Watanabe, M. Nagamine, Y. Kusumoto, M. Yashiro, Y. Satoh, T. Shimosawa, K. Shinozaki, T. Tomiya, Y. Inoue, T. Nishikawa, N. Ohtomo, Y. Tanoue, H. Yokota, T. Koyama, K. Ishimaru, Y. Okamoto, Y. Takuwa, K. Koike, Y. Yatomi, Antagonism of sphingosine 1-phosphate receptor 2 causes a selective reduction of portal vein pressure in bile duct-ligated rats, Hepatology in press
[87] Y. Inagaki, R. Higashiyama, Interplay between bone marrow and liver in the pathogenesis of hepatic fibrosis, Hepatol. Res.(2012)in press.
[88] C. Li, X. Jiang, L. Yang, X. Liu, S. Yue, L. Li, Involvement of sphingosine 1-phosphate (SIP)/S1P3 signaling in cholestasis-induced liver fibrosis, Am. J. Pathol. 175(2009) 1464-1472.
[89] C. Li, Y. Kong, H. Wang, S. Wang, H. Yu, X. Liu, L. Yang, X. Jiang, L. Li, L. Li. Homing of bone marrow mesenchymal stem cells mediated by sphingosine 1-phosphate contributes to liver fibrosis, J. Hepatol. 50(2009) 1174-1183.
[90] C. Li, S. Zheng, H. You, X. Liu, M. Lin, L. Yang, L. Li, Sphingosine 1-phosphate (S1P)/S1P receptors are involved in human liver fibrosis by action on hepatic myofibroblasts motility, J. Hepatol. 54(2011) 1205-1213.
[91] X. Liu, S. Yue, C. Li, L. Yang, H. You, L. Li, Essential roles of sphingosine

1-phosphate receptor types 1 and 3 in human hepatic stellate cells motility and activation, J. Cell. Physiol. 226(2011)2370-2377.

[92] H. Ikeda, K. Nagashima, M. Yanase, T. Tomiya, M. Arai, Y. Inoue, K. Tejima, T. Nishikawa, N. Watanabe, M. Omata, K. Fujiwara, Sphingosine 1-phosphate enhances portal pressure in isolated perfused liver via S1P2 with Rho activation, Biochem.Biophys. Res. Commun. 320 (2004) 754-759.

[93] H. Ikeda, R. Ohkawa, N. Watanabe, K. Nakamura, Y. Kume, H. Nakagawa, H. Yoshida, S. Okubo, H. Yokota, T. Tomiya, Y. Inoue, T. Nishikawa, N. Ohtomo, Y. Tanoue, K. Koike, Y. Yatomi, Plasma concentration of bioactive lipid mediator sphingosine 1-phosphate is reduced in patients with chronic hepatitis C, Clin. Chim. Acta. 411(2010)765-770.

[94] Y. Yatomi, F.Ruan, S.Hakomori, Y.Igarashi, Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets, Blood 86(1995)193-202.

[95] R. Pappu, S.R. Schwab, I. Cornelissen, J.P. Pereira, J.B. Regard, Y. Xu, E. Camerer, Y.W. Zheng, Y. Huang, J.G. Cyster, S.R. Coughlin, Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate, Science. 316(2007) 295-298.

Figure legends

Figure 1. Myofibroblastic differentiation and production of extracellular matrix and cytokines by myofibroblasts. Activated fibroblasts or myofibroblasts are derived from several different sources including resident stromal fibroblasts, epithelial cells (epithelial-mesenchymal transition (EMT)), endothelial cells (endothelial-mesenchymal transition (EndMT)), and bone marrow-derived fibrocytes. Myofibroblasts synthesize and deposit ECM components, which mainly include collagen type I and smaller amounts of collagen type III, fibronectin, elastin, laminin, proteoglycan and glycosaminoglycan, and release various cytokines and mediators, which stimulate myofibroblasts in a paracrine manner. Infiltrating inflammatory cells, parenchymal cells and other cells also release cytokines and mediators. TGFβ, transforming growth factor-β; IL-6, interleukin-6; Ang II, angiotensin II.

Figure 2. Receptor subtype-specific cardioprotective and cardio-damaging effects of S1P. S1P exerts a cardioprotective effect through $S1P_3$-mediated eNOS stimulation in endothelial cells (EC) and generation of Akt-mediated survival signal in cardiomyocytes (CM). This effect is a relatively quick response. In contrast, S1P induces cardiac remodeling (myocardial cell death and cardiac fibrosis) through $S1P_3$/$S1P_2$-mediated

Rho GTPase activation and transactivation of TGFβ signaling pathway. TGFβ stimulates SphK1 to increase S1P production, which further exacerbates cardiac damage. The transactivation of TGFβ signaling pathway by $S1P_3$ and stimulation of SphK1 by TGFβ constitute a feed-forward amplifying signaling loop, which leads to cardiac damage. PLC, phospholipase C; PI3K, phosphatidylinositol 3-kianse; MFb, myofibroblast.

Figure 3. Pathologic fibrotic response to lung injury and its modulation by S1P. Damage to lung epithelial cells is associated with apoptosis of epithelial cells, the loss of epithelial/endothelial barrier, resulting in re-epithelialization and increased vascular permeability. Apoptotic cells, recruited inflammatory cells, or abnormally activated alveolar epithelial cells produce mediators that induce the formation of fibroblasts and myofibroblasts through the proliferation of resident mesencymal cells, recruitment of circulating fibrocytes, and stimulation of EMT. Activated myofibroblasts secrete excessive amounts of ECM, mainly collagen, resulting destruction of lung architecture. Growing evidence suggest the role of S1P in vascular barrier protection, regulation of migration and proliferation of fibroblasts and myofibroblast differentiation (boxed). LPA is also implicated in lung fibrosis through mechanisms involving epithelial

damaging and vascular barrier disruption.

Figure 4. Involvement of hepatic stellate cells in liver fibrosis and its regulation by S1P receptors. Various liver injury results in liver fibrosis. Hepatic stellate cells, pericytes of sinusoid, play a major role in liver fibrosis. During the process of liver fibrosis, hepatic stellate cells proliferate and undergo differentiation into myofibroblasts, which produce extracellular matrix proteins. S1P is involved in these processes primarily via $S1P_2$. $S1P_2$ also mediates contraction of stellate cells, resulting in portal hypertension. $S1P_1$ and $S1P_3$ may also be involved in myofibroblast differentiation and activation through stimulating proliferation and migration. Another source of myofibroblasts in the fibrotic liver is circulationg bone marrow-derived fibrocytes, which may be recruited by the action of $S1P_3$.

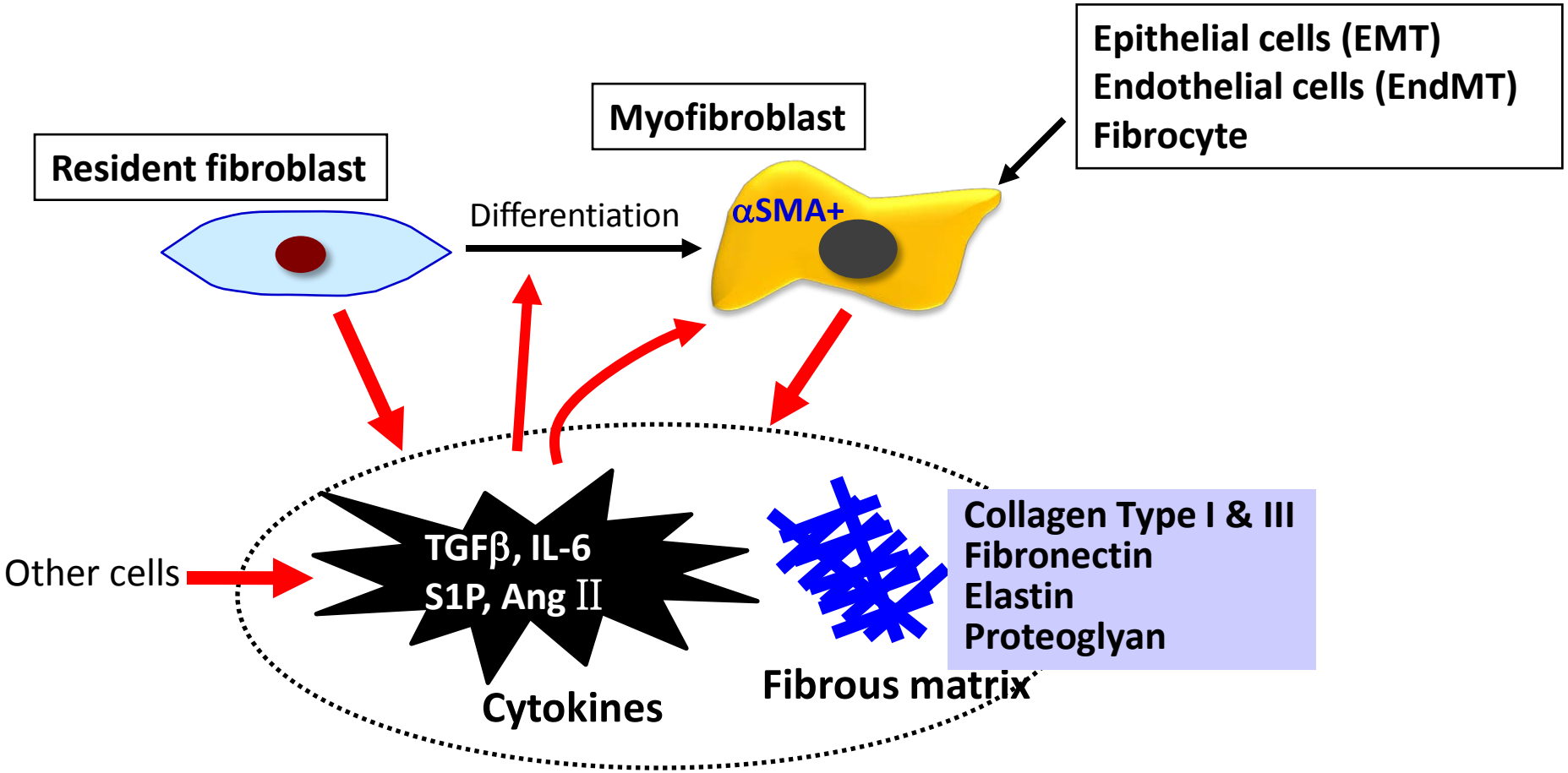
Epithelial cells (EMT)
Endothelial cells (EndMT)
Fibrocyte
Myofibroblast
Resident fibroblast
Differentiation
αSMA+
Other cells
TGFβ, IL-6
S1P, Ang II
Cytokines
Fibrous matrix
Collagen Type I & III
Fibronectin
Elastin
Proteoglyan

Figure 1

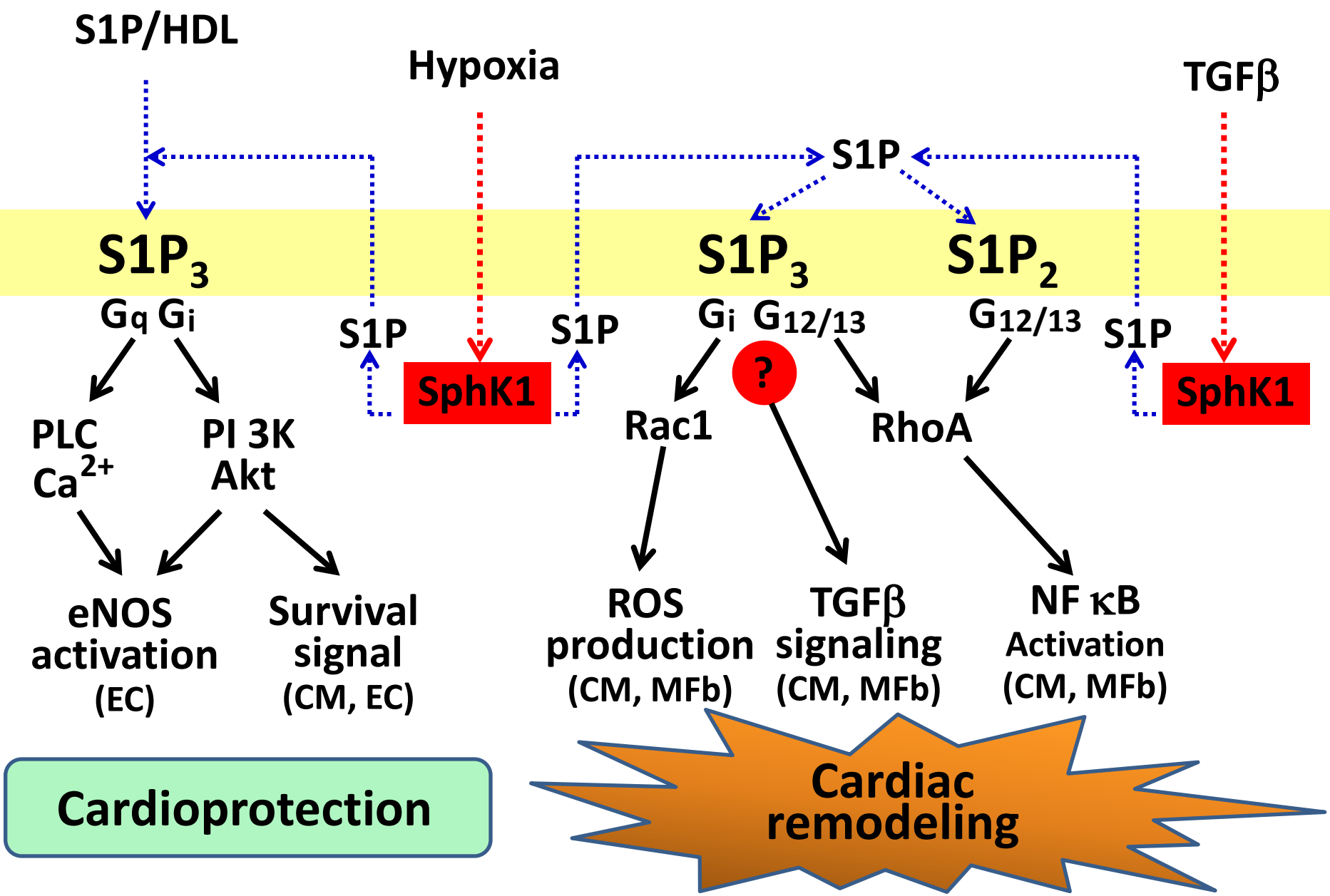
S1P/HDL
Hypoxia
TGFβ
S1P
S1P3
S1P3
S1P2
Gq Gi
S1P
SphK1
S1P
Gi G12/13
?
G12/13
S1P
SphK1
PLC
Ca2+
PI 3K
Akt
Rac1
RhoA
eNOS
activation
(EC)
Survival
signal
(CM, EC)
ROS
production
(CM, MFb)
TGFβ
signaling
(CM, MFb)
NF κB
Activation
(CM, MFb)
Cardioprotection
Cardiac
remodeling

Figure 2

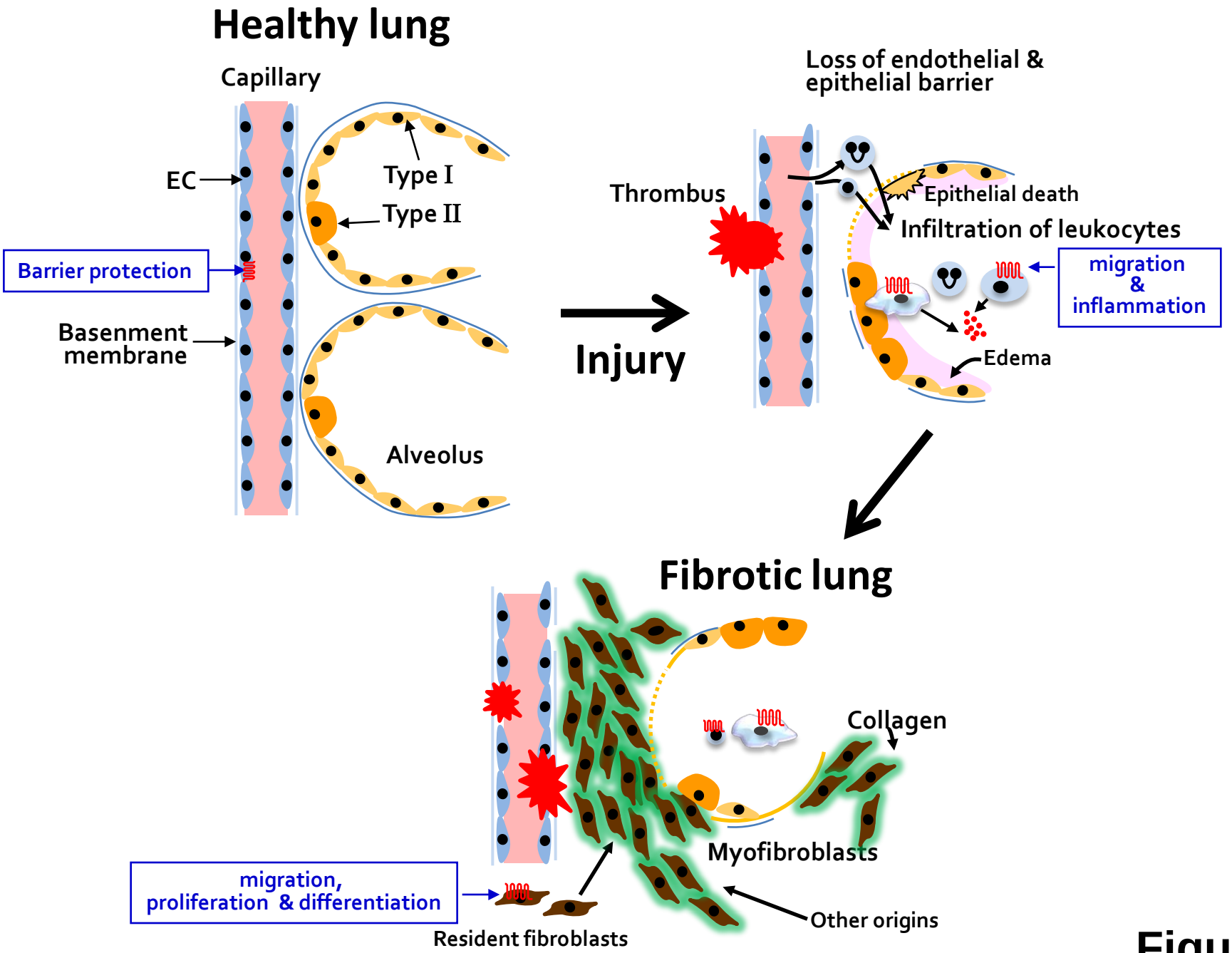
Healthy lung
Capillary
EC
Type I
Type II
Barrier protection
Basenment membrane
Alveolus
Injury
Loss of endothelial & epithelial barrier
Thrombus
Epithelial death
Infiltration of leukocytes
migration & inflammation
Edema
Fibrotic lung
Collagen
Myofibroblasts
Other origins
migration, proliferation & differentiation
Resident fibroblasts

Figure 3

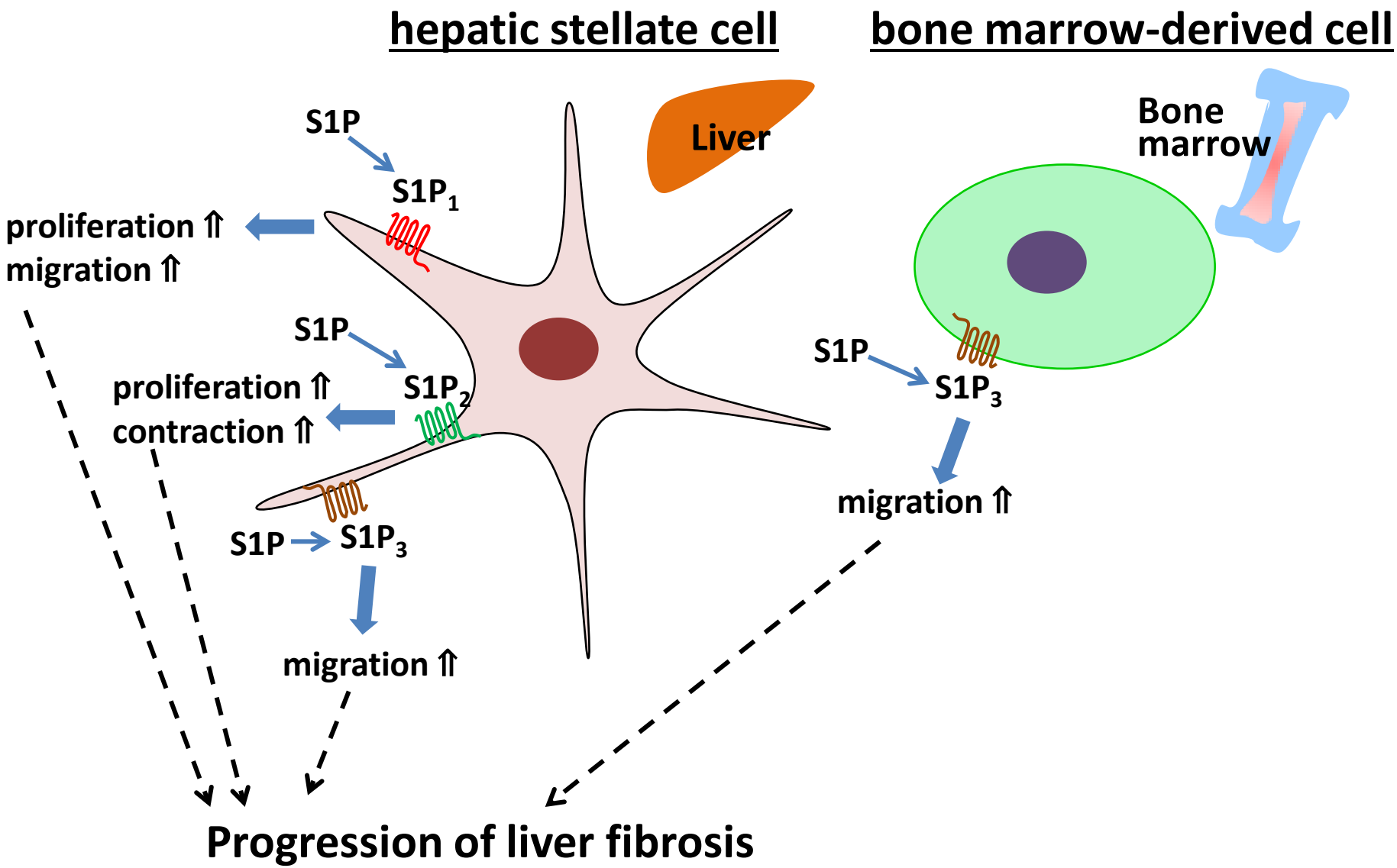
hepatic stellate cell
bone marrow-derived cell
Liver
Bone marrow
S1P
S1P1
proliferation ⇑
migration ⇑
S1P
S1P2
proliferation ⇑
contraction ⇑
S1P
S1P3
migration ⇑
S1P
S1P3
migration ⇑
Progression of liver fibrosis

Figure 4