Biochemical and Biophysical Research Communications 409 (2011) 7–13

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Blockade of lysophosphatidic acid receptors LPAR1/3 ameliorates lung fibrosis induced by irradiation

Lu Gan [a,1], Jian-Xin Xue [b,c,1], Xin Li [b], De-Song Liu [e], Yan Ge [a], Pei-Yan Ni [a], Lin Deng [a], You Lu [a,b,*], Wei Jiang [a,d,*]

[a] State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, PR China
[b] Department of Thoracic Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu, PR China
[c] Laboratory of Stem Cell Biology, West China Hospital, Sichuan University, Chengdu, PR China
[d] Molecular Medicine Research Center, West China Hospital, Sichuan University, Chengdu, PR China
[e] Department of Pediatrics, Sichuan Provincial Hospital of Women and Children, Chengdu, PR China

ARTICLE INFO

Article history:
Received 31 March 2011
Available online 23 April 2011

Keywords:
Radiation lung fibrosis
LPARs
VPC12249
CTGF

ABSTRACT

Lung fibrosis is a common and serious complication of radiation therapy for lung cancer, for which there are no efficient treatments. Emerging evidence indicates that lysophosphatidic acid (LPA) and its receptors (LPARs) are involved in the pathogenesis of fibrosis. Here, we reported that thoracic radiation with 16 Gy in mice induced development of radiation lung fibrosis (RLF) accompanied by obvious increases in LPA release and *LPAR1* and *LPAR3* (*LPAR1/3*) transcripts. RLF was significantly alleviated in mice treated with the dual LPAR1/3 antagonist, VPC12249. VPC12249 administration effectively prolonged animal survival, restored lung structure, inhibited fibroblast accumulation and reduced collagen deposition. Moreover, profibrotic cytokines in radiation-challenged lungs obviously decreased following administration of VPC12249, including transforming growth factor β1 (TGFβ1) and connective tissue growth factor (CTGF). *In vitro*, LPA induced both fibroblast proliferation and *CTGF* expression in a dose-dependent manner, and both were suppressed by blockade of LPAR1/3. The pro-proliferative activity of LPA on fibroblasts was inhibited by siRNA directed against CTGF. Together, our data suggest that the LPA–LPAR1/3 signaling system is involved in the development of RLF through promoting fibroblast proliferation in a CTGF-dependent manner. The LPA–LPAR1/3–CTGF pathway may be a potential target for RLF therapy.

1. Introduction

Lung cancer is the leading cause of cancer deaths worldwide [1,2]. As a conventional treatment, radiation therapy (RT) benefits approximately 30–40% of lung cancer patients, but leads to radiation-induced lung injury in 20–30% of these patients [3,4]. Radiation lung fibrosis (RLF), a late and progressive stage of radiation-induced lung injury, greatly compromises the quality of life of patients, and even causes death [5]. The elucidation of the exact molecular mechanisms of RLF and the development of new strategies for its prevention and treatment are urgently needed.

RLF generally occurs 4–6 months after RT and is characterized by fibroblast proliferation and activation, collagen deposition, and destruction of lung structure [6]. Profibrotic cytokines have been demonstrated to play important roles in the development of RLF, including transforming growth factor β1 (TGFβ1) and connective tissue growth factor (CTGF) [5,7]. TGFβ1 is a multifunctional cytokine, which is involved in proliferation of fibroblasts, production of collagen and extracellular matrix, phenotypic modulation of fibroblasts into myofibroblasts, and epithelial–mesenchymal transition during the fibrotic process [5,8]. Recently, CTGF, as a direct or indirect downstream mediator of the TGFβ1 signaling pathway, has gained increasing attention for its crucial roles in sustaining fibrosis [9]. Therefore, molecules that reduce the expression of TGFβ1 and/or CTGF have the potential to prevent and treat RLF.

Lysophosphatidic acid (LPA) is a small bioactive phospholipid, known to regulate several cellular processes via binding to and activating its five high-affinity G protein-coupled receptors (LPAR1–5) [10]. Recently, emerging evidence indicates that the LPA–LPARs signaling system is associated with fibrosis [9,11–13]. LPA levels are significantly elevated in human bronchoalveolar lavage fluid from idiopathic pulmonary fibrosis patients, and LPAR1 mediates the development of bleomycin-induced lung fibrosis

* Corresponding authors. Address: Department of Thoracic Oncology, Cancer Center, West China Hospital, Sichuan University, 37#, Guo Xue Lane, Chengdu 610041, PR China (Y. Lu), Molecular Medicine Research Center, West China Hospital, Sichuan University, 1#, Ke Yuan 4th Road, Gao Peng Street, Chengdu 610041, PR China (W. Jiang).
E-mail addresses: radyoulu@hotmail.com (Y. Lu), wcumsjw72@hotmail.com (W. Jiang).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.04.084

[12,13]. Previously, we have revealed that LPA and its receptors LPAR1 and LPAR3 (LPAR1/3) are involved in radiation pneumonitis, the early stage of radiation-induced lung injury, by regulating inflammatory cascade responses [14]. However, the exact roles of LPA and LPARs in RLF have remained unclear and need to be further delineated.

In the present study, we hypothesized that the LPA–LAPRs signaling system might participate in the development of RLF. We initially observed that RLF was accompanied by the upregulation of LPA levels and *LPAR1/3* transcripts in lung tissue. Animal survival rate, damaged lung structure, fibroblast accumulation and collagen deposition were partially restored in irradiated mice treated with the dual LPAR1/3 antagonist, VPC12249. VPC12249 administration simultaneously suppressed the expressions of profibrotic cytokines *in vivo*, including TGFβ1 and CTGF. Moreover, in *in vitro* experiments, LPA stimulated fibroblast proliferation in a dose-dependent manner. This LPA-induced proliferation of fibroblasts was significantly inhibited by blockade of LPAR1/3 and inactivation of CTGF. Therefore, the LPA–LPAR1/3–CTGF signaling pathway may contribute to RLF by promoting fibroblast proliferation.

2. Materials and methods

2.1. Animal model and administration of VPC12249

The investigation was performed in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and approved by the Animal Care and Use Committee of Sichuan University. C57BL/6 mice (8–10-weeks old and ~20 g weight) were used. After anesthetizing with an intraperitoneal (IP) injection of chloral hydrate (3.5%, 10 ml/kg), mice were subjected to 16 Gy thoracic irradiation using 200 kVp X-rays, and then housed with food and acidified water *ad libitum* for 6 months. VPC12249 (Avanti Polar Lipids, dissolved in 3% fatty acid-free BSA/PBS solution) was injected IP into mice prior to and post-irradiation (1.0 mg/kg per dose, 2 times per week). Non-irradiated mice were injected with Vehicle and served as controls.

2.2. LPA quantification

LPA was extracted from fresh whole lung tissue and quantified following the manufacturer's instructions as previously described (Chinese Twofishes, China) [14].

2.3. Histopathology

Paraffin-embedded sections were stained with hematoxylin and eosin (H&E) or Masson's trichrome for collagen. Simultaneously, they were also stained with anti-TGFβ1 (1:200, Santa Cruz), anti-CTGF (1:600, Abcam), anti-FSP1 (1:200, Abcam) or anti-α-SMA (1:200, Abcam) according to standard immunohistochemical protocols [15].

2.4. Messenger RNA (mRNA) extraction and reverse transcription-PCR

Total RNA was isolated and reverse transcribed to cDNA by M-MLV reverse transcriptase (Invitrogen). The real-time quantitative PCRs were carried out with SYBR® Premix Ex Taq™ II real-time PCR kit (Takara). The primers used are listed in Supplementary Table 1. The level of *GAPDH* mRNA in each sample was used as an internal control. Relative quantities were analyzed by the $2^{-\Delta\Delta Ct}$ method. All reactions were carried out in duplicate.

2.5. Western blot analysis

Total protein was extracted as described [14,16]. Equivalent amounts of proteins (25–40 μg) were separated by 15% SDS–PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). The membranes were sequentially incubated with antibodies overnight at 4 °C. Immunoreactivity was detected with an enhanced chemiluminescence kit (Millipore). β-Actin was used as a loading control.

2.6. Cell culture, drug treatment and transfection with synthetic small interfering RNA

Mouse embryonic fibroblasts (MEFs) were isolated from 13.5 days C57BL/6J embryos as described previously [16]. MEFs and human fetal lung fibroblasts (MRC-5s) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Three hours after serum starvation, LPA was added [11]. In the experiments involving VPC12249 treatments, the cells were pretreated with 100 μM VPC12249 for 1 h prior to the addition of 2 μM LPA [13].

A mouse-specific CTGF small interfering RNA (siRNA) was chemosynthesized as previously described [17]. Human CTGF-specific siRNA and scrambled siRNA (the non-targeting negative control) was purchased from Santa Cruz Biotechnology and Ambion, respectively. The siRNA was transfected into fibroblasts using Lipofectamine 2000 (Invitrogen). Six hours after transfection, LPA (2 μM) was added and cell viability was assessed 24 h after co-incubation.

2.7. Cell growth assay

Cell viability was assessed by Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) and Hoechst 33258 nuclei staining (Nanjing KeyGen Biotech, China) according to the manufacturer's instructions. Apoptotic cells were identified by brightly stained condensed chromatin or nuclear fragments in their nuclei.

2.8. Statistical analysis

All data were represented as mean ± SD with at least three independent experiments. The statistical significances among groups were analyzed by one-way ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered to represent a significant difference.

3. Results

3.1. Elevations of LPA levels and LPAR1/3 expressions in RLF

Radiation-induced LPA release was significantly increased in a time-dependent manner (Fig. 1A). *LPAR1–5* genes were co-expressed in radiation-challenged lungs, with *LPAR1* and *LPAR3* being the predominant transcripts (Fig. 1B). As compared with non-irradiated mice, the expressions of *LPAR1/3* mRNA were increased dramatically and reached peaks on day 7 post-radiation (3.5-fold and 2.3-fold, respectively). Subsequently, they decreased gradually and rose again from day 60 post-radiation. These data suggested that LPA and LPAR1/3 were associated with the development of RLF.

3.2. Attenuation of RLF by selectively blocking LPAR1/3

To further confirm the roles of LPAR1/3 in RLF, VPC12249 was administered IP to the irradiated mice. Survival was monitored for 24 weeks. Only 50% of mice in RT + Vehicle group survived to 24 weeks, while VPC12249 administration resulted in 70% survival (Fig. 1C).

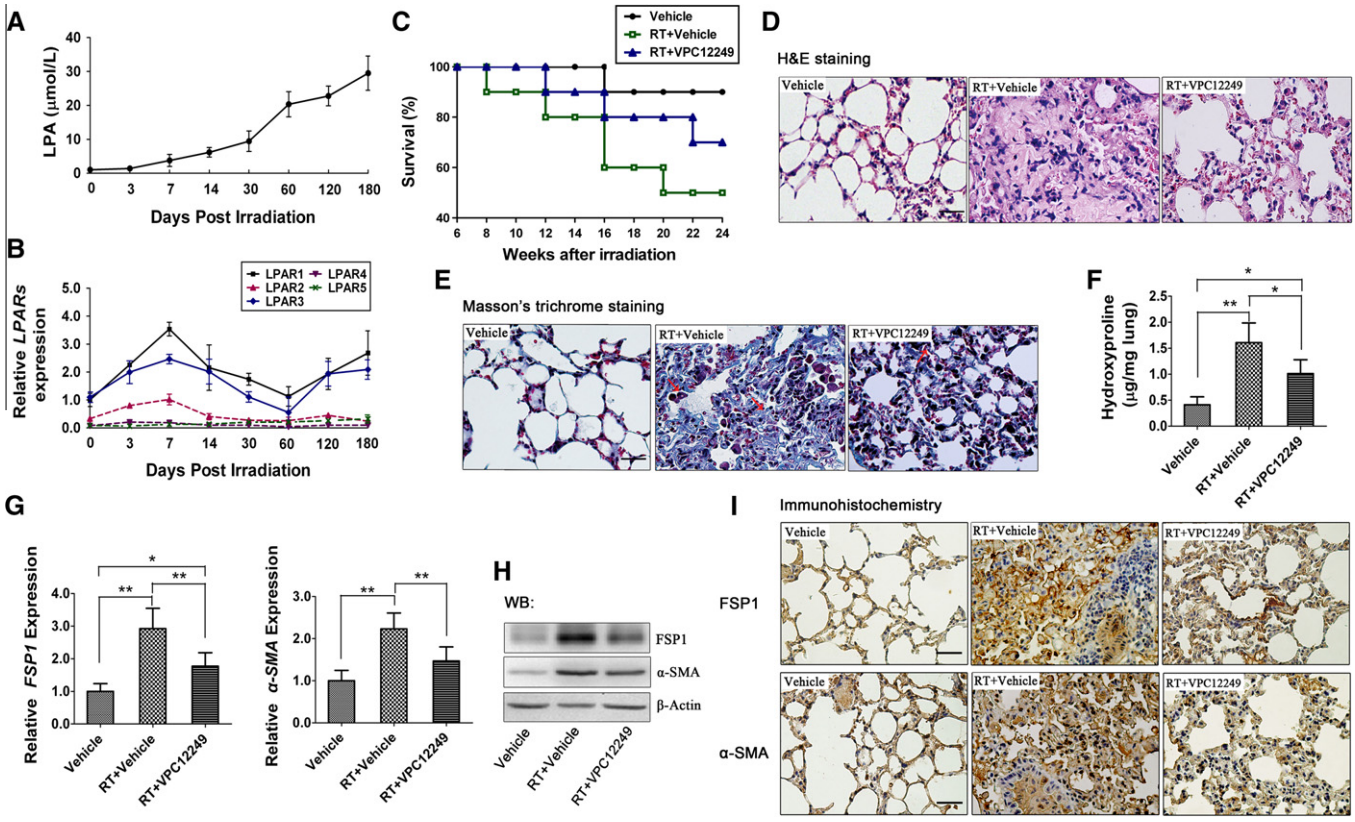

Fig. 1. The roles of the LPA–LPAR1/3 signaling system in RLF. (A) LPA levels in lung homogenate at different time points post-radiation ($n = 5$). (B) Relative expression of *LPARs* in radiation-challenged lungs, as was determined by real-time PCR using the *GAPDH* gene for normalization ($n = 5$). (C) Effect of VPC12249 on survival of irradiated mice ($n = 10$). (D, E) Histological assessment of VPC12249 treatment on RLF, representative photomicrographs of H&E staining (D) and Masson's trichrome staining (E) of lung sections. Examples of focal fibrotic lesions (arrows) are marked. Bar, 50 μm. (F) The hydroxyproline content in lung homogenate from irradiated mice with or without VPC12249 treatment. (G, H) Real-time PCR analysis of *FSP1* and *α-SMA* transcripts (G), and Western blot analysis of FSP1 and α-SMA protein expressions (H) obtained from radiation-challenged lungs (one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$). (I) Immunohistochemical staining of FSP1 and α-SMA in lung sections. Bar, 50 μm.

Six months after radiation, histopathological fibrotic changes in lung tissue were observed in the RT + Vehicle group, exhibiting significant destruction of alveolar structure, notable thickening of alveolar septa and dramatic interstitial hyperplasia (Fig. 1D). Meanwhile, Masson's trichrome staining showed that radiation resulted in marked collagen deposition in the alveolar septa and the areas around the bronchiolar tissue (Fig. 1E), which was consistent with the measurement of collagen content, as assessed by the levels of hydroxyproline (4.0-fold increase; Fig. 1F). VPC12249 administration evidently restored lung structure and reduced collagen deposition.

The fibrotic process is accompanied by fibroblast accumulation and myofibroblast activation [9]. Fibroblast-specific proteins 1 (FSP1) and α-smooth muscle actin (α-SMA) are usually used as markers of fibroblasts and activated myofibroblasts, respectively [18,19]. The results of real-time quantitative PCR (Fig. 1G) and Western blot analyses (Fig. 1H) showed that radiation induced marked increases in the expressions of *FSP1* and *α-SMA*. Immunohistochemical staining revealed that the FSP1- and α-SMA-positive loci increased within the fibrotic areas in radiation-challenged lungs (Fig. 1I), suggesting the accumulation of fibroblasts and activation of myofibroblasts. Importantly, all the alterations were visibly restored by VPC12249 administration, suggesting that profibrogenic activity of LPA was mediated by LPAR1/3 in RLF.

3.3. Downregulation of TGFβ1 and CTGF in irradiated mice by VPC12249

It has been demonstrated that TGFβ1 is involved in the fibrotic process via activating CTGF directly or indirectly [9]. In our present study, the expressions of *TGFβ1* and *CTGF* in lung were significantly elevated post-radiation, while the upregulation was obviously abolished by VPC12249 treatment (Fig. 2A and B). The results also were confirmed by immunohistochemical staining, showing that the prevention of LPA from binding to LPAR1/3 reduced the protein levels of TGFβ1 and CTGF in radiation-challenged lungs (Fig. 2C).

3.4. Effects of LPA on fibroblast proliferation in vitro

To investigate whether VPC12249 protects against fibrosis *in vivo* by inhibiting the proliferation of fibroblasts, MEFs and MRC-5s were used *in vitro* to test this hypothesis. The endogenous LPARs were detected in MEFs (Fig. 3A) and MRC-5s (Fig. 3D) by real-time PCR analysis, showing that *LPAR1–5* genes were co-expressed in them and *LPAR1* transcripts were predominant. The proliferation of fibroblasts was induced by LPA stimulation in a dose-dependent manner (Fig. 3B and E). Twenty-four hours after incubation with 2 μM LPA, the viabilities of MEFs and MRC-5s increased 2.23-fold and 1.78-fold, respectively, and these increases were suppressed in the present of VPC12249 (Fig. 3C and F). These results were paralleled by visualization of the nucleus by Hoechst 33258 staining (Fig. 3G and H). Results suggested that LPA stimulated fibroblast growth via LPAR1/3. Hoechst 33258 staining also identified apoptotic cells. There were no differences among the Vehicle, LPA, LPA + VPC12249 groups (data not shown), all of which eliminated the cytotoxicity of the pharmacological reagents.

3.5. Effects of LPA on TGFβ1 and CTGF expressions in fibroblasts

In MEFs, LPA induced a rapid and transient increase (7.1-fold after 3 h) in *CTGF* mRNA expression, and a weak increase (1.5-fold

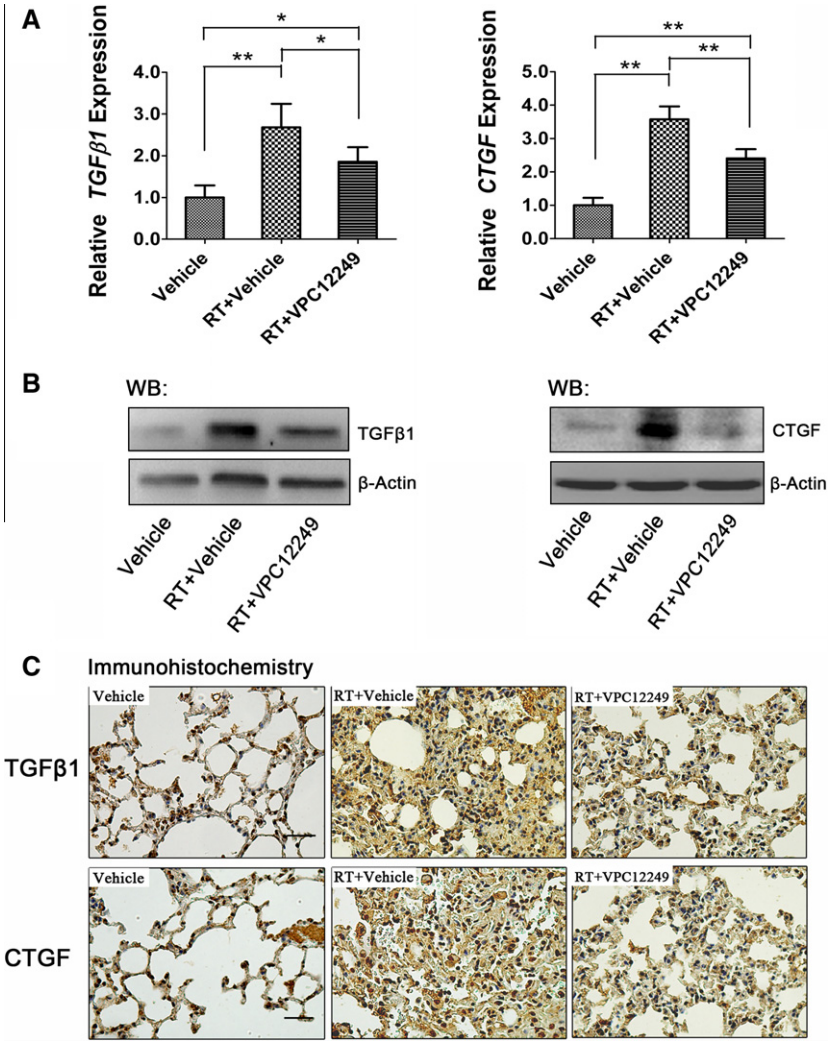

Fig. 2. Downregulation of TGFβ1 and CTGF in irradiated mice via VPC12249 administration. The mRNA and protein levels of TGFβ1 and CTGF in irradiated mice with or without VPC12249 treatment were determined by real-time PCR (A), Western blot (B) and immunohistochemical analysis (C), respectively (one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$).

after 3 h) in *TGFβ1* mRNA levels (Fig. 4A). In addition, the augment of *CTGF* transcripts triggered by LPA stimulation was dose-dependent (Fig. 4B). A similar tendency in the change of *TGFβ1* and *CTGF* transcripts was observed in MRC-5s exposed to LPA (Fig. 4D and E). LPA-induced *CTGF* expression was inhibited in the cells treated with VPC12249 (Fig. 4C and F), suggesting that the LPA–LPAR1/3 signaling system regulated the expression of *CTGF*.

3.6. LPA-induced fibroblast proliferation mediated by CTGF

To further validate whether the pro-proliferative activity of LPA on fibroblasts was mediated by CTGF, a siRNA approach was used to silence the expression of *CTGF* in cells. *CTGF* siRNA successfully decreased expression of CTGF protein in MEFs and MRC-5s (Fig. 4G and H, respectively). Importantly, the LPA-induced fibroblast proliferation was significantly inhibited by siRNA targeting the *CTGF* gene, suggesting that LPA stimulated fibroblast proliferation in a CTGF-dependent manner.

4. Discussion

LPA has been implicated in the etiology of numerous fibrosis diseases [11,13,15,17]; adverse stimulation usually causes a dramatic increase in LPA release. In the present study, the thoracic radiation-induced LPA release is elevated immediately and continuously within the 180-day post-radiation period, strongly arguing for the involvement of LPA in development of experimental pulmonary fibrosis.

LPARs have been identified to mediate the biological functions of LPA [10,20]. Owing to their significant heterogeneity, distribution in various tissue, expression patterns and downstream signaling pathways, LPA has diverse and widespread effects on various biological processes [21]. Recently, by using knockout mice and selective antagonists, emerging evidence regarding the diverse and even contradictory roles of LPARs in various diseases has been generated. LPAR1 deficiency induces psychiatric diseases in rodents [22], but ameliorates neuropathic pain [23], higher adiposity [24] and fibrosis [11,13,15,17]. Selective blockade of LPAR2 aggravates radiation-induced enteritis [25] and ischemia–reperfusion-induced renal injury [26]. However, blockade of LPAR3 protects against renal injury [26].

In our present study, *LPAR1* and *LPAR3* are highly expressed in radiation-challenged lungs. Radiation-induced changes in the expressions of the *LPAR1/3* genes develop in two stages, suggesting that LPAR1/3 might exert various effects at different stages of radiation-induced lung injury. The first stage occurs during radiation-induced acute pulmonary inflammation, and has been demon-

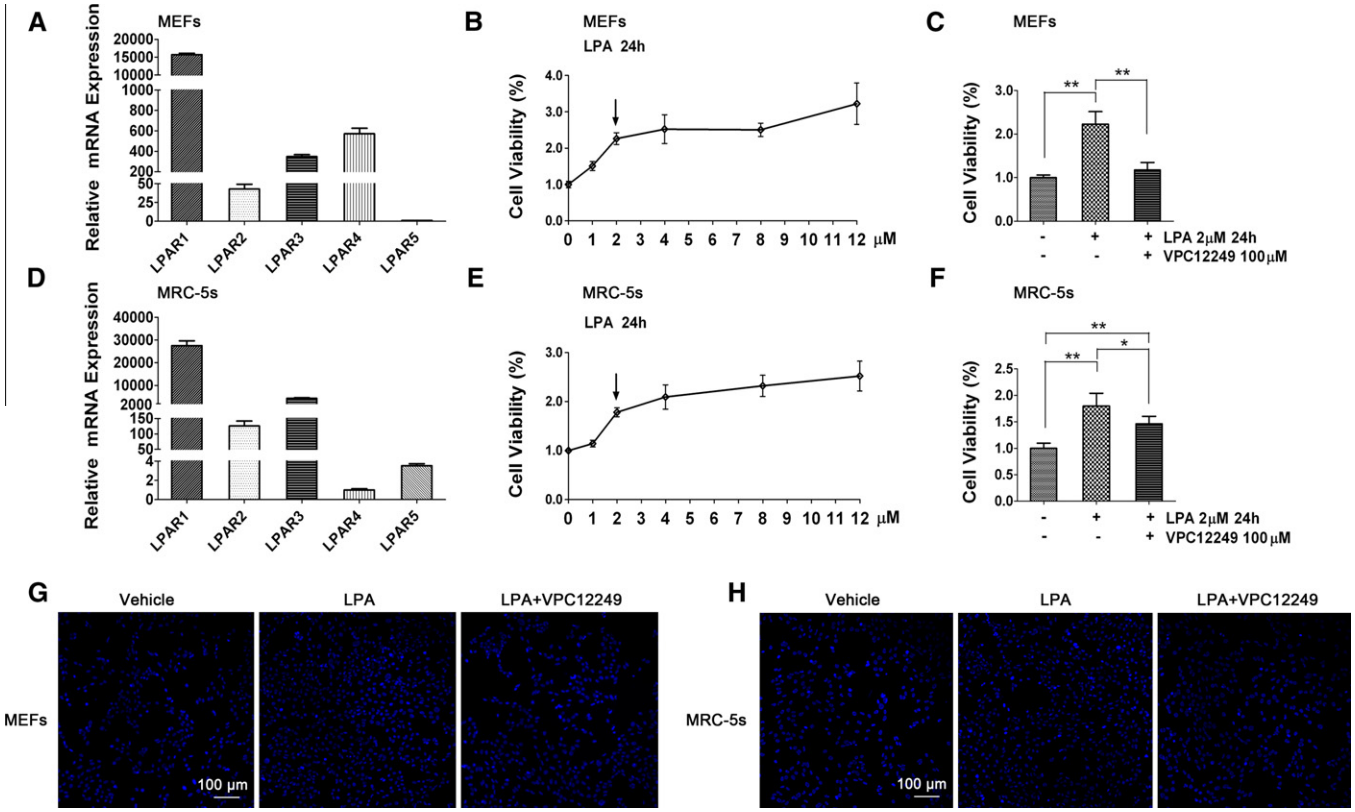

Fig. 3. The pro-proliferative activity of LPA on fibroblasts mediated by activation of LPAR1/3. Relative expressions of *LPAR1–5* were determined in MEFs (A) and MRC-5s (D) by real-time PCR analysis. The viabilities of cells were increased by various concentrations of LPA treatments for 24 h (B, E), and LPA-induced cell proliferation was inhibited by VPC12249 (C, F) determined by CCK-8 assay (one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$). (G, H) Nuclei visualized by Hoechst 33258 staining.

strated by our previous study [14]. The second stage indicates the potential roles of LPAR1/3 in fibrosis, which is subsequently confirmed by using the LPAR1/3 antagonist, VPC12249. Results show that RLF is dramatically ameliorated by blockade of LPAR1/3, which is consistent with Tager's report [13] that LPAR1 participates in the fibrosis induced by bleomycin. In the future, the exact effects of LPAR1 and LPAR3 in RLF should be explored by selectively and individually blocking these receptors.

Fibrosis is typified by excessive collagen deposition, mainly generated by fibroblasts [17] and myofibroblasts [27]. Many antifibrotic agents used in clinical trails or animal models attenuate fibrosis by inhibiting fibroblast proliferation or inducing apoptosis [28]. Abnormal fibroblast proliferation appears and persists during the onset and progression of RLF [29]. In our present study, we quantify the expressions of FSP1 and α-SMA to evaluate the accumulations of fibroblasts and myofibroblasts, respectively. Results show that RLF is accompanied by obvious fibroblast accumulation and myofibroblast activation, which are efficiently inhibited by VPC12249 administration. This suggests that the LPA–LPAR1/3 signaling system regulates fibroblast growth in fibrosis.

LPA has been known to induce mitogenic responses in many cell types, but the pro-proliferative activity of LPA is cell-type-specific [30,31]. The cell selectivity and regulation mechanism remains undefined. It may be associated with the specific expression profiling of LPARs in different cells and the dissimilar transduction pathway activated by different LPARs. In our present study, LPA promotes lung fibroblast growth *in vitro*, which is suppressed by VPC12249 treatment. Combined with *in vivo* experimental results, all the data indicate that LPA-induced fibroblast proliferation in RLF is mediated by LPAR1/3. Based on the ubiquitous chemotactic effect of LPA [32], the potential roles of LPA-induced fibroblast migration in RLF should be further determined.

Many profibrotic factors are involved in the fibrotic process. TGFβ1 has been identified to be highly associated with the pulmonary fibrosis [28], but not sufficient to perpetuate fibrosis [33,34]. CTGF, as an important downstream molecule in the TGFβ1 signaling pathway, is essential for fibroblast proliferation [35]. Recently, it has been demonstrated that CTGF promotes lung fibrosis post-radiation in a TGFβ1-independent manner [7]. LPA stimulation upregulates CTGF expression in many cell types, including human renal fibroblasts [36], mouse renal epithelial cells [11], mouse skeletal myoblasts [37] and MEFs [38]. Our present study shows that blockade of LPAR1/3 obviously suppresses LPA-induced elevation of CTGF expression in MEFs and MRC-5s, and LPA-induced fibroblast proliferation is inhibited by knockdown of CTGF expression. Consequently, the experiments *in vivo* and *in vitro* indicate that the profibrogenic signal of LPA in RLF is transduced by LPAR1/3 and mediated by activation of CTGF. Interestingly, in contrast to CTGF expression, TGFβ1 is significantly downregulated by blocking LPAR1/3 *in vivo*, but slightly upregulated in fibroblasts with LPA stimulation *in vitro*. This implies that LPA might increase TGFβ1 expression in other lung cell types after radiation and/or regulate TGFβ1 expression via specific avenues different from CTGF. Therefore, it would be interesting to further identify the possible effects of TGFβ1 on LPA-induced lung fibrosis.

In summary, to our knowledge, the present study provides evidence for the first time that (1) elevated LPA levels and LPAR1/3 expression are associated with RLF, (2) RLF is significantly alleviated in mice by blockade of LPAR1/3, which is paralleled by obvious decreases in fibroblast accumulation and CTGF expression, and (3) LPA induces fibroblast proliferation via LPAR1/3 *in vitro*, and is dependent on CTGF activation but not TGFβ1. These observations demonstrate that the LPA–LPAR1/3–CTGF pathway contributes to the development of RLF through promoting fibroblast

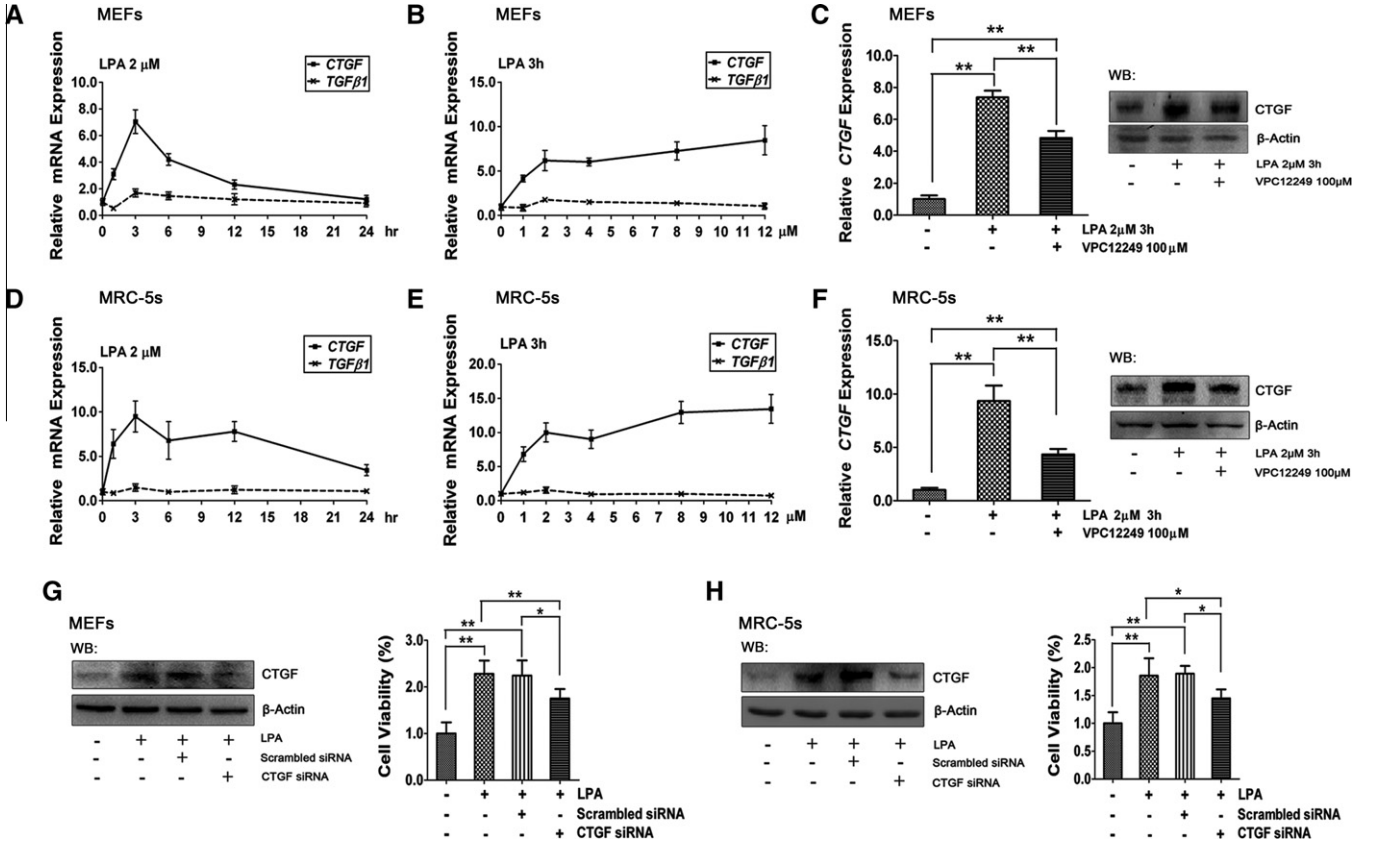

Fig. 4. LPA-induced fibroblast proliferation mediated by CTGF. *CTGF* and *TGFβ1* mRNA levels were quantified in MEFs (A, B) and MRC-5s (D, E) exposed to 2 μM LPA for increasing time periods or to various concentrations of LPA for 2 h. (C, F) LPA-induced elevation of CTGF expression was restored in fibroblasts pretreated with VPC12249, analyzed by real-time PCR and Western blot. (G, H) siRNA-directed *CTGF* gene silence inhibited LPA-induced fibroblast proliferation. The insets in (G) and (H) showed siRNA-mediated knockdown of CTGF as determined by Western blot (left) (one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$).

proliferation. This study has shed new light on the understanding of the molecular mechanisms of RLF and suggests a potential therapeutic avenue for fibrotic diseases.

Acknowledgments

This work was supported by National Natural Science Foundation of China (NSFC 30870734 to Dr. Y. Lu and 30871017, 31071001 to Dr. W. Jiang), a Key Project in the Science and Technology Pillar Program from Sichuan Science and Technology Bureau (2009FZ0067 to Dr. W. Jiang). We thank *Medjaden* Bioscience Limited for assisting in the preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.04.084.

References

[1] V. Beral, R. Peto, UK cancer survival statistics, BMJ 341 (2010) c4112.
[2] A. Jemal, R. Siegel, J. Xu, E. Ward, Cancer statistics, CA Cancer J. Clin. 60 (2010) 277–300.
[3] C. Tabata, Y. Kadokawa, R. Tabata, M. Takahashi, K. Okoshi, Y. Sakai, M. Mishima, H. Kubo, All-trans-retinoic acid prevents radiation- or bleomycin-induced pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 174 (2006) 1352–1360.
[4] T.J. Robnett, M. Machtay, E.F. Vines, M.G. McKenna, K.M. Algazy, W.G. McKenna, Factors predicting severe radiation pneumonitis in patients receiving definitive chemoradiation for lung cancer, Int. J. Radiat. Oncol. Biol. Phys. 48 (2000) 89–94.
[5] P.G. Tsoutsou, M.I. Koukourakis, Radiation pneumonitis and fibrosis: mechanisms underlying its pathogenesis and implications for future research, Int. J. Radiat. Oncol. Biol. Phys. 66 (2006) 1281–1293.
[6] A.E. Para, A. Bezjak, I.W. Yeung, J. Van Dyk, R.P. Hill, Effects of genistein following fractionated lung irradiation in mice, Radiother. Oncol. 92 (2009) 500–510.
[7] C. Li, M. Idowu, Z. Vujaskovic, Y. Oh, M.S. Anscher, Role of connective tissue growth factor (IGFBP-8) in radiation-induced lung fibrosis (RILF), Int. J. Radiat. Oncol. Biol. Phys. 72 (2008) S426–S427.
[8] G.J. Prud'homme, Pathobiology of transforming growth factor beta in cancer, fibrosis and immunologic disease, and therapeutic considerations, Lab. Invest. 87 (2007) 1077–1091.
[9] J.P. Pradere, J. Gonzalez, J. Klein, P. Valet, S. Gres, D. Salant, J.L. Bascands, J.S. Saulnier-Blache, J.P. Schanstra, Lysophosphatidic acid and renal fibrosis, Biochim. Biophys. Acta 1781 (2008) 582–587.
[10] K. Noguchi, D. Herr, T. Mutoh, J. Chun, Lysophosphatidic acid (LPA) and its receptors, Curr. Opin. Pharmacol. 9 (2009) 15–23.
[11] J.P. Pradere, J. Klein, S. Gres, C. Guigne, E. Neau, P. Valet, D. Calise, J. Chun, J.L. Bascands, J.S. Saulnier-Blache, J.P. Schanstra, LPA1 receptor activation promotes renal interstitial fibrosis, J. Am. Soc. Nephrol. 18 (2007) 3110–3118.
[12] K. Ley, A. Zarbock, From lung injury to fibrosis, Nat. Med. 14 (2008) 20–21.
[13] 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.
[14] J. Xue, L. Gan, X. Li, J. Li, G. Qi, Y. Wu, X. Fu, Q. Mao, R. Ao, J. Lang, Y. Lu, Effects of lysophosphatidic acid and its receptors LPA(1/3) on radiation pneumonitis, Oncol. Rep. 24 (2010) 1515–1520.
[15] F.V. Castelino, J. Seiders, G. Bain, S.F. Brooks, C. 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.
[16] Y. Ge, W. Jiang, L. Gan, L. Wang, C. Sun, P. Ni, Y. Liu, S. Wu, L. Gu, W. Zheng, F.E. Lund, H.B. Xin, Mouse embryonic fibroblasts from CD38 knockout mice are resistant to oxidative stresses through inhibition of reactive oxygen species production and Ca(2+) overload, Biochem. Biophys. Res. Commun. 399 (2010) 167–172.

[17] M. Ponticos, A.M. Holmes, X. Shi-wen, P. Leoni, K. Khan, V.S. Rajkumar, R.K. Hoyles, G. Bou-Gharios, C.M. Black, C.P. Denton, D.J. Abraham, A. Leask, G.E. Lindahl, Pivotal role of connective tissue growth factor in lung fibrosis: MAPK-dependent transcriptional activation of type I collagen, Arthritis Rheum. 60 (2009) 2142–2155.
[18] T.A. Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases, J. Clin. Invest. 117 (2007) 524–529.
[19] W.E. Lawson, V.V. Polosukhin, O. Zoia, G.T. Stathopoulos, W. Han, D. Plieth, J.E. Loyd, E.G. Neilson, T.S. Blackwell, Characterization of fibroblast-specific protein 1 in pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 171 (2005) 899–907.
[20] M.E. Lin, D.R. Herr, J. Chun, Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance, Prostaglandins Other Lipid Mediat. 91 (2009) 130–138.
[21] J.W. Choi, D.R. Herr, K. Noguchi, Y.C. Yung, C.W. Lee, T. Mutoh, M.E. Lin, S.T. Teo, K.E. Park, A.N. Mosley, J. Chun, LPA receptors: subtypes and biological actions, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 157–186.
[22] S.M. Harrison, C. Reavill, G. Brown, J.T. Brown, J.E. Cluderay, B. Crook, C.H. Davies, L.A. Dawson, E. Grau, C. Heidbreder, P. Hemmati, G. Hervieu, A. Howarth, Z.A. Hughes, A.J. Hunter, J. Latcham, S. Pickering, P. Pugh, D.C. Rogers, C.S. Shilliam, P.R. Maycox, LPA1 receptor-deficient mice have phenotypic changes observed in psychiatric disease, Mol. Cell. Neurosci. 24 (2003) 1170–1179.
[23] M. Inoue, M.H. Rashid, R. Fujita, J.J. Contos, J. Chun, H. Ueda, Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling, Nat. Med. 10 (2004) 712–718.
[24] M.F. Simon, D. Daviaud, J.P. Pradere, S. Gres, C. Guigne, M. Wabitsch, J. Chun, P. Valet, J.S. Saulnier-Blache, Lysophosphatidic acid inhibits adipocyte differentiation via lysophosphatidic acid 1 receptor-dependent down-regulation of peroxisome proliferator-activated receptor gamma2, J. Biol. Chem. 280 (2005) 14656–14662.
[25] W. Deng, E. Shuyu, R. Tsukahara, W.J. Valentine, G. Durgam, V. Gududuru, L. Balazs, V. Manickam, M. Arsura, L. VanMiddlesworth, L.R. Johnson, A.L. Parrill, D.D. Miller, G. Tigyi, The lysophosphatidic acid type 2 receptor is required for protection against radiation-induced intestinal injury, Gastroenterology 132 (2007) 1834–1851.
[26] M.D. Okusa, H. Ye, L. Huang, L. Sigismund, T. Macdonald, K.R. Lynch, Selective blockade of lysophosphatidic acid LPA3 receptors reduces murine renal ischemia–reperfusion injury, Am. J. Physiol. Renal Physiol. 285 (2003) F565–F574.
[27] V.V. Petrov, R.H. Fagard, P.J. Lijnen, Stimulation of collagen production by transforming growth factor-beta1 during differentiation of cardiac fibroblasts to myofibroblasts, Hypertension 39 (2002) 258–263.
[28] M. Selman, T.E. King, A. Pardo, Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy, Ann. Intern. Med. 134 (2001) 136–151.
[29] H.P. Rodemann, M. Bamberg, Cellular basis of radiation-induced fibrosis, Radiother. Oncol. 35 (1995) 83–90.
[30] W.H. Moolenaar, LPA: a novel lipid mediator with diverse biological actions, Trends Cell Biol. 4 (1994) 213–219.
[31] G. Tigyi, D.L. Dyer, R. Miledi, Lysophosphatidic acid possesses dual action in cell proliferation, Proc. Natl. Acad. Sci. USA 91 (1994) 1908–1912.
[32] E.K. Kim, S.J. Yun, K.H. Do, M.S. Kim, M. Cho, D.S. Suh, C.D. Kim, J.H. Kim, M.J. Birnbaum, S.S. Bae, Lysophosphatidic acid induces cell migration through the selective activation of Akt1, Exp. Mol. Med. 40 (2008) 445–452.
[33] R.M. Strieter, B. Mehrad, New mechanisms of pulmonary fibrosis, Chest 136 (2009) 1364–1370.
[34] W.D. Hardie, S.W. Glasser, J.S. Hagood, Emerging concepts in the pathogenesis of lung fibrosis, Am. J. Pathol. 175 (2009) 3–16.
[35] G.R. Grotendorst, M.R. Duncan, Individual domains of connective tissue growth factor regulate fibroblast proliferation and myofibroblast differentiation, FASEB J. 19 (2005) 729–738.
[36] J. Heusinger-Ribeiro, M. Eberlein, N.A. Wahab, M. Goppelt-Struebe, Expression of connective tissue growth factor in human renal fibroblasts: regulatory roles of RhoA and cAMP, J. Am. Soc. Nephrol. 12 (2001) 1853–1861.
[37] C. Cabello-Verrugio, G. Cordova, C. Vial, L.M. Zuniga, E. Brandan, Connective tissue growth factor induction by lysophosphatidic acid requires transactivation of transforming growth factor type beta receptors and the JNK pathway, Cell. Signal. 23 (2011) 449–457.
[38] C. Stortelers, R. Kerkhoven, W.H. Moolenaar, Multiple actions of lysophosphatidic acid on fibroblasts revealed by transcriptional profiling, BMC Genomics 9 (2008) 387.