Biochemical and Biophysical Research Communications 464 (2015) 100–105

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

TRIF promotes angiotensin II-induced cross-talk between fibroblasts and macrophages in atrial fibrosis

Xiao-Qing Chen [a], Dao-Liang Zhang [a], Ming-Jian Zhang [b], Meng Guo [b], Yang-Yang Zhan [b], Fang Liu [b], Wei-Feng Jiang [a], Li Zhou [a], Liang Zhao [a, *], Quan-Xing Wang [b, **], Xu Liu [a, *]

[a] Department of Cardiology, Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai, PR China
[b] National Key Laboratory of Medical Immunology, Second Military Medical University, Shanghai, PR China

ARTICLE INFO

Article history:
Received 16 April 2015
Accepted 3 May 2015
Available online 5 June 2015

Keywords:
Atrial fibrosis
Atrial fibroblasts
Macrophages
TRIF

ABSTRACT

Aims: Atrial fibroblasts and macrophages have long been thought to participate in atrial fibrillation (AF). However, which specific mediator may regulate the interaction between them remains unclear.
Methods and results: We provided the evidence for the involvement of Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF), an important inflammation-related molecule, in the pathophysiology of AF. Patients with AF showed higher levels of angiotensin II (AngII) and TRIF expression and larger number of macrophages infiltration in left atria appendage than individuals with sinus rhythm (SR). In the cell study, AngII induced chemokines expressions in mouse atrial fibroblasts and AngII-stimulated atrial fibroblasts induced the chemotaxis of macrophages, which were reduced by losartan and TRIF siRNA. Meanwhile, AngII-stimulated atrial fibroblasts proliferation was enhanced by macrophages.
Conclusions: Our data demonstrated that TRIF may be a crucial factor promoting the interaction between atrial fibroblasts and macrophages, leading to atrial fibrosis.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia [1], however, its underlying pathophysiology remains incompletely understood. An increasing number of findings show that atrial fibrosis and inflammation are important components in AF pathophysiology [1–3]. Meanwhile, atrial fibroblasts play a vital role in atrial fibrosis and AF, both by inducing arrhythmogenesis caused by fibroblast-cardiomyocyte electric interactions and by promoting collagen production [1]. Besides, study also provides the evidence that macrophages infiltrate in endocardium in the process of AF [4]. Thus, atrial fibroblasts and macrophages contribute greatly to AF. However, the mechanisms underlying the interaction between fibroblasts and macrophages are largely unknown.

Our previous study shows that Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) is an important molecule in Angiotensin II(AngII)-induced atrial fibrosis and atrial fibroblasts proliferation [5]. Tumor necrosis factor receptor associated factor 6 (TRAF6) is an important inflammation-related molecule [6]. And TRIF, an upstream cascade of TRAF6, associates with TRAF6 directly with or without AngII stimulation [5,7]. Thus, we believe that TRIF may be the key mechanistic link in the cross-talk between atrial fibroblasts and macrophages.

To test this hypothesis, we evaluated the TRIF expression, fibrosis and macrophages infiltration in atria of patients with first-time mitral/aortic valve replacement surgery. Besides, we also assessed the role of TRIF in the cross-talk between macrophages and fibroblasts in the presence of AngII.

2. Materials and methods

2.1. Study population

The study protocol was approved by the local ethics committee and informed consent was obtained from all patients. The

Abbreviations: AF, atrial fibrillation; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing IFN-β; AngII, angiotensin II; TRAF6, tumor necrosis factor receptor associated factor 6; SR, sinus rhythm; LA, left atrial appendage.

* Corresponding authors. 241 West Huaihai Road, Shanghai 200030, PR China.
** Corresponding author. 800 Xiangyin Road, Shanghai 200433, PR China.
E-mail addresses: zhaol_zg@163.com (L. Zhao), wqxejd@126.com (Q.-X. Wang), liuxu_xk@163.com (X. Liu).

http://dx.doi.org/10.1016/j.bbrc.2015.05.131
0006-291X/

study was performed conforming the declaration of Helsinki. The study population consisted of consecutive patients (n = 82) with rheumatic heart disease who underwent mitral/aortic valve replacement in the Shanghai Chest Hospital during the period from February 1, 2013 to October 30, 2013, excluding those with hyperthyroidism, coronary heart disease, dilated cardiomyopathy, diabetes or chronic pulmonary heart disease. 35 patients were diagnosed with permanent AF (AF group) and 47 patients without AF (sinus rhythm [SR] group). Tissue samples of left atrial appendage (LA) were obtained from all patients during surgery. Patients did not receive drugs at least 12 h before surgery.

2.2. AngII concentrations

The experimental procedure to determine AngII concentrations in LA by ELISA (Assay Max, Assay Pro, USA) was previously described [8].

2.3. Histological analysis

Fixed atrial samples from patients were cut into 4–5 mm sections. To measure fibrotic areas, sectioned samples were stained with masson trichrome. The fibrotic areas were calculated as the ratio of total fibrosis area to total section area. Sections underwent immunohistochemical staining with the antibodies against CD68 (1:200) and TRIF (1: 200) (both Abcam, Cambridge, MA). Images were captured by an Olympus microscope attached to a computerized imaging system and analyzed by Image-Pro Plus 5.0.

2.4. Isolation and culture of mouse atrial fibroblasts and peritoneal macrophages

The experimental procedure to isolate mouse atrial fibroblasts was performed as we previously described [5]. Fibroblasts in passages 2–3 were placed in serum-free medium for 24 h before AngII (1 μM) stimulation.

C57BL/6J mice were injected intraperitoneally with 1 mL sterile 10% thioglycollate medium (Scharlaus, Barcelona, Spain). Three days later, macrophages were collected by PBS intraperitoneal lavage [9]. After collection, macrophages were being passaged for experiment the next day. The animal study was approved by the local ethics committee and relevant procedures were followed the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA).

2.5. Quantitative real time PCR and small-interfering RNA (siRNA) transfection

The experimental procedures of quantitative real time PCR and siRNA transfection were performed as we previously described [10]. Primers used for the amplification of mice CCL2, CCL7, CCL12, CXCL10 and GAPDH genes were as follows: CCL2, '5-GCCAACTCTCACTGAAGCC-3' (forward), '5-GCTGGTGAATGAGTAGCAGC-3' (reverse); CCL7, '5-GCTGCTTTCAGCATCCAAGTG-3' (forward), '5-CCAGGGACACCGACTACTG-3' (reverse); CCL12,'5-TCCTCAGGTATTGGCTGGAC-3' (forward), 5-TGGCTGCTTGTGATTCTCCT-3 (reverse); CXCL10, '5-CGTCATTTTCTGCCTCATCCT-3' (forward), '5-TGGTCTTAGATTCCGGATTCAG-3' (reverse); GAPDH, 5′-TGACCACAGTCCATGCCATC-3′ (forward), 5′-GACGGACACATTGGGGGTAG-3′ (reverse). GAPDH was used as internal control. The siRNA targeting mice TRIF was as follows: TRIF (sense:5′-GCUAUGUAACACACCGCUGTT-3′, antisense:5′-CAGCGGUGUGUUACAUAGCTT-3′).

2.6. Western blot analysis

Proteins were extracted from mouse atrial fibroblasts or human atria and underwent western blot analysis as we previously described [11]. Primary antibodies were as follows: TRIF (1:1000, Abcam, Cambridge, MA) and GAPDH (1:5000, Cell Signaling Technology). And GAPDH was used as internal control.

2.7. Transwell

Model transwell assays were performed as previously described [12]. In brief, mouse atrial fibroblasts were plated in the bottom chamber of 24-well plate and incubated at 37 °C 5% CO_2 overnight. Then fibroblasts were stimulated by AngII (1 μM) after cultured in serum-free medium for 24 h. At the same time, mouse peritoneal macrophages were harvested and added (1×10^5 cells/ml) into transwell inserted with a polycarbonate membrane containing 8.0 μm pores (Millipore, USA); Next, macrophages were allowed to migrate for 24 h in the presence of AngII (1 μM) in the 37 °C, 5% CO_2 incubator. After that, transwell insert was removed from the chamber and submerged in PBS to remove unattached cells. Then macrophages were fixed by 10% formalin for 15 min and stained by hematoxylin for 30min. At last, 5 high power fields (× 200) were counted to determine the average macrophage number migrated per high power field.

2.8. Preparation of AngII-treated macrophage conditioned medium and proliferation assay of fibroblasts

Mouse peritoneal macrophages were cultured in serum-containing complete medium for 24 h after isolation from mouse. Then macrophages were stimulated by AngII (1 μM) for 24 h. After that, medium was collected.

Fibroblast proliferation was determined by cell counting kit-8 (CCK-8, Dojindo, Japan) according to the manufacturer's protocol. Briefly, cells were plated in a 96-well plate. Atrial fibroblasts were incubated with 1 μM AngII or macrophage conditioned medium for 24 h. After treatment, cell viability was determined by reading the optical density at 450 nm.

2.9. Statistical analysis

Statistical analysis was performed by SPSS 19.0 software. The data were expressed as means ± SD or percentage. The differences between groups were assessed by one-way ANOVA followed by Dunnett post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. TRIF and atrial fibrosis

To test TRIF expression in atria, we performed immunohistochemical studies in specimens derived from LA of patients undergoing mitral/aortic valve replacement (n = 82). The characteristics of the two groups were statistically similar in all baseline variables (data were not shown). However, individuals with concomitant AF showed substantially higher TRIF expression (Fig. 1A and B) and AngII concentration (Fig. 1C). To determine whether inflammation responses contribute to AF, we detected infiltration of $CD68^+$ macrophages in atria. As immunohistochemistry described, CD68 (Fig. 1A) expression was higher in AF group compared with SR group, underscoring the concept that increased inflammation was a reflection of AF. Meanwhile, as was shown in Fig. 1A and D, atria fibrosis also increased significantly in AF group.

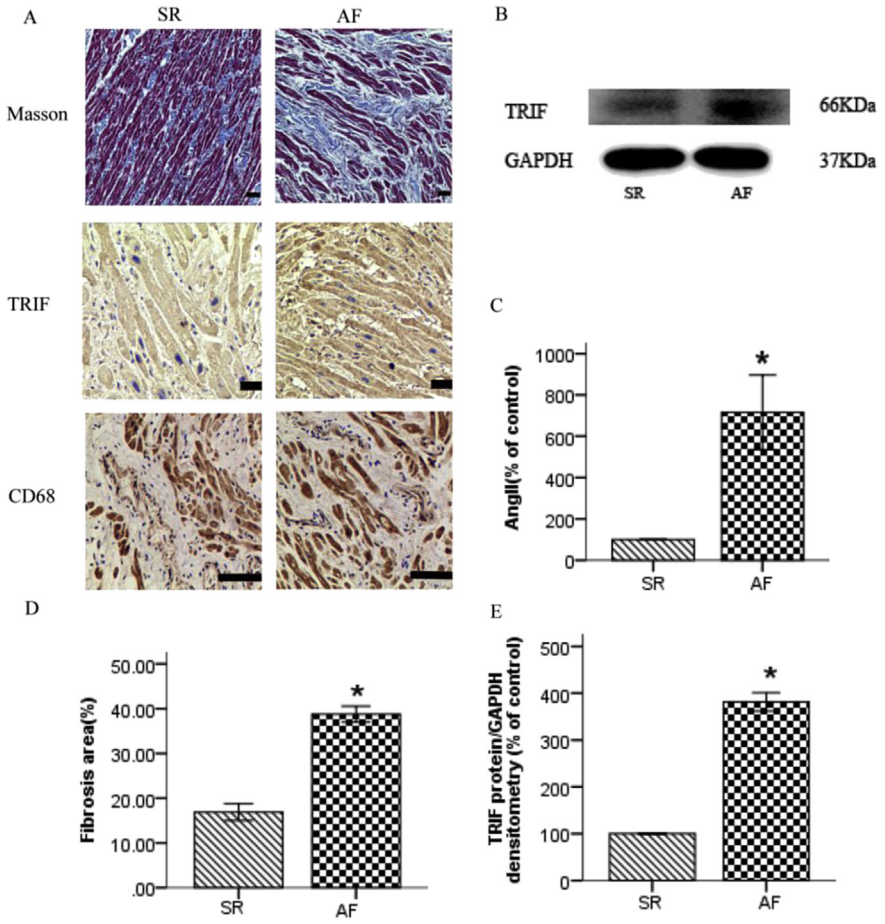

Fig. 1. Fibrosis, AngII concentration, TRIF and CD68 expressions in LA of human. Representative samples of each group were shown in A&B. AF group (n = 35) showed increased AngII concentration (C), fibrosis (D) and TRIF expression (E) compared with SR group (n = 47). Original magnification, × 100. Data represent the mean ± SD. *P < 0.05 vs. SR.

3.2. TRIF and macrophages chemotaxis, fibroblasts proliferation

To further characterize the molecular mechanism of above observation, we assessed TRIF expression in AngII-treated mouse atrial fibroblasts in vitro by western blot. And the finding was that AngII significantly increased the expression of TRIF, which was partly reversed by AT1R antagonist (losartan) [5]. This observation indicated that TRIF expression in atrial fibroblasts was partly AngII-dependent. Besides, In consideration of infiltration of macrophages in atria of AF patients, we performed transwell assays to explore the underlying mechanisms. As was shown in Fig. 2A and B, AngII-treated fibroblasts induced chemotaxis of macrophages, which was suppressed by losartan or TRIF siRNA. Taken together, these observations reinforced the idea that chemotaxis of macrophages induced by AngII-treated fibroblasts was TRIF-dependent.

In order to determine the role of infiltrated macrophages, we tested the proliferation of atrial fibroblasts by CCK-8. As shown in Fig. 2C, the proliferation of atrial fibroblasts after being treated with AngII-treated macrophage conditioned medium became faster compared to that of control cells with the stimulation of AngII (1 μM).

3.3. TRIF and chemokine expression

Given that chemotaxis of macrophages was regulated by a variety of chemokines, we determined the expressions of chemokines in atrial fibroblasts. In support of above concept that AngII-enhanced macrophages infiltration partly depended on TRIF, we confirmed that AngII-induced CCL2 (Fig. 3A), CXCL10 (Fig. 3B), CCL7 (Fig. 3C) and CCL12 (Fig. 3D) expressions were partly reversed by TRIF siRNA.

4. Discussion

Our main findings of the present study were as follows: (1) Compared with SR group, patients with AF showed higher degree of fibrosis, higher TRIF expression, as well as larger number of macrophages infiltration in LA. (2) AngII-treated atrial fibroblasts induced the chemotaxis of macrophages, which could be reversed by losartan or TRIF siRNA. (3) Chemokines expressions were increased by AngII in atrial fibroblasts, which was reversed by losartan or TRIF siRNA. (4) AngII-stimulated atrial fibroblasts proliferation was enhanced by macrophages.

Clinical investigations reported multiple associations between vulnerability to AF and circulating levels of cytokines, C-reactive protein, complement [13–15]. Whether this inflammatory phenotype represented an epiphenomenon in AF or was causally linked to the initiation and progression of AF remained unclear. Notably, TRAF6, an important inflammation-related molecule [6], participated in AngII-stimulated activation of Ikappa B kinase, cardiomyocyte hypertrophy and atrial fibroblasts proliferation [10,16,17]. Since TRIF was an important upstream cascade of TRAF6, we focused on its expression in AF specimens and our exploration showed that the high expression of TRIF was

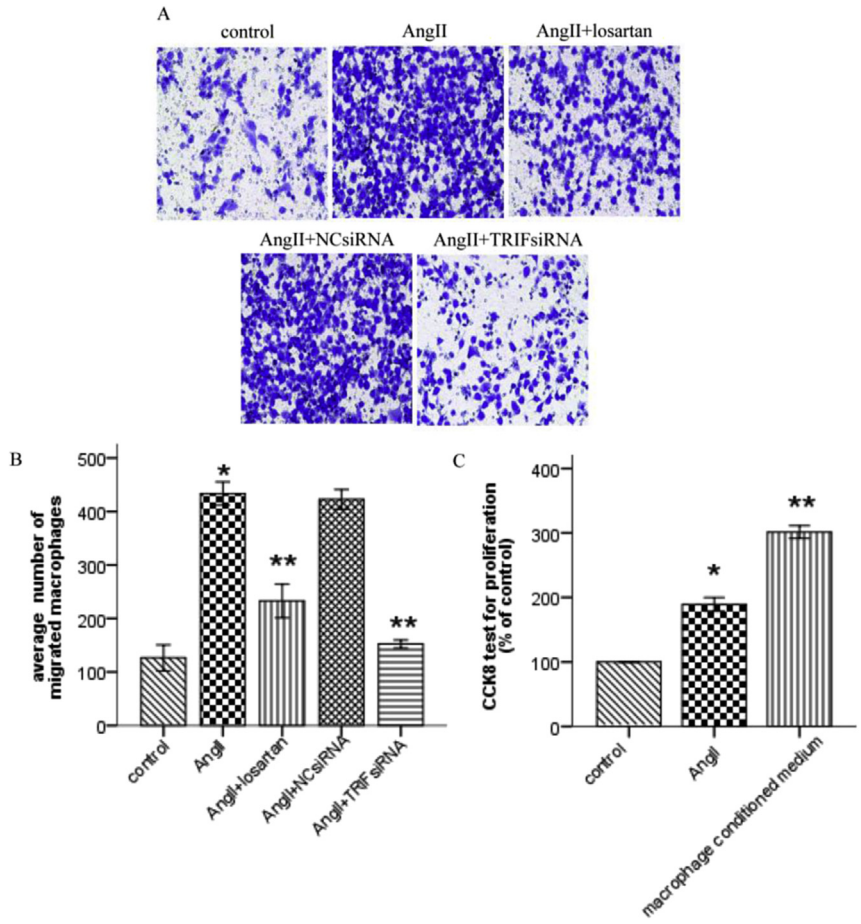

Fig. 2. TRIF involved in AngII-induced cross-talk between atrial fibroblasts and macrophages. Atrial fibroblasts were pretreated with losartan (10 mmol/L) (AT1R antagonist) for 30 min and then stimulated with AngII for 24 h. Atrial fibroblasts were transfected with TRIF siRNA or NC siRNA for 48 h or pretreated with losartan, and then incubated with AngII for 24 h. Transwell assays was performed to determine the number of migrated macrophages in the presence of atrial fibroblasts (A). AngII-induced migration of macrophages was supressed by losartan or TRIFsiRNA (B). AngII-stimulated atrial fibroblast proliferation was enhanced by AngII-treated macrophage conditioned medium (C). Data represent means ± SD of three independent experiments. Original magnification, × 200.*P < 0.05 vs.control, **P < 0.05 vs. AngII or NCsiRNA.

accompanied with serious atrial fibrosis and large amount of macrophages infiltration. In consideration of AngII's central role in subcellular mediators linking atrial inflammation and fibrosis, we tested its concentrations in atria. And we found that patients with AF showed substantially elevated AngII concentration compared with SR group, which was consistent with previous study [8].

Despite above mechanism unrevealed, it was reported that, as a downstream regulators of toll-like receptor 4, TRIF played a crucial role in inflammation and cardiac fibrosis [18]. And in a study that examined the TRIF mutated gene in LDLR$^{-/-}$mice, it was found that LDLR$^{-/-}$mice with lack-of-function mutations in TRIF were significantly protected from atherosclerosis, and the mice displayed fewer observed lesional macrophages [19]. In addition, it was also found that deleting TRIF in myeloid cells was sufficient to attenuate vessel inflammation and protect against atherosclerosis, as shown by reduced aortic inflammation [20]. And our previous study showed the important role of TRIF in atrial fibrosis [5]. Taken together, these findings underscored the role of TRIF as a mediator of the observed atrial inflammation and fibrosis.

Besides, we also showed the strong interaction between macrophages and atrial fibroblasts. Lots of researches reported that the mutual interaction between macrophages and fibroblasts contributed to fibrosis. According to previous findings, macrophages were found to accumulate in the perivascular space and co-localize with fibroblasts, producing collagen in hypertrophied hearts of spontaneously hypertensive rats and renovascular hypertensive rats [21,22]. And it was demonstrated later that macrophages accumulation played a crucial role as the upper stream event of myocardial fibrosis in the later phase through transforming growth factor β induction and fibroblast activation [23]. Furthermore, the production of profibrotic cytokines by accumulated macrophages was found as important contribution to fibrotic processes by activating fibroblast proliferation [24]. Then activated fibroblasts expressed profibrotic substances, which self-amplified ongoing fibrotic tissue formation [25]. Notably, in this study, we noticed that atrial fibroblasts participated in the process of chemotaxis of macrophages, then macrophages further promoted the proliferation of atrial fibroblasts. Taking above evidences together into consideration, we believed that fibrotic process was related to the activation of the positive feedback loop created by macrophages and activated fibroblasts.

Also, we demonstrated the contribution of TRIF to macrophage infiltration induced by atrial fibroblasts. As our previous study showed, AngII stimulated atrial fibroblasts proliferation by inducing TRIF expression [5]. It was reported that chemokine neutralization significantly protected fibrosis formation by inhibiting fibrocyte recruitment [26]. Consistent with it, our data showed that AngII-induced chemokines expressions of atrial fibrobalsts were reversed by TRIF siRNA. As a result, macrophage infiltration significantly reduced.

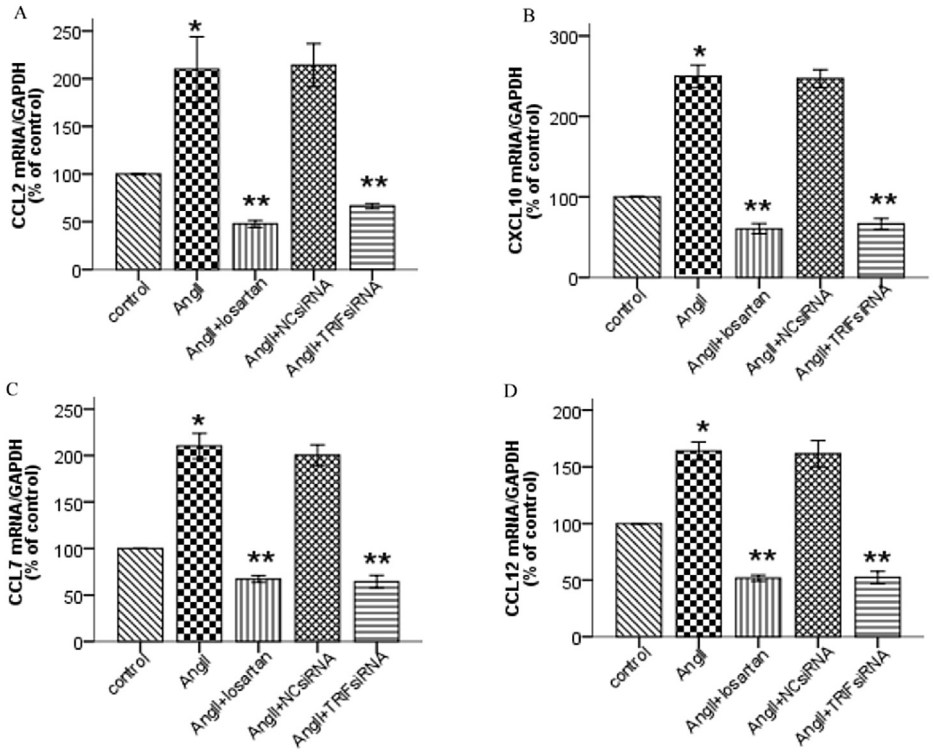

Fig. 3. Chemokines expressions in AngII-treated mouse atrial fibroblasts. Atrial fibroblasts were pretreated with losartan or transfected with TRIFsiRNA or NCsiRNA and then incubated with AngII for 24 h. Quantitative real time PCR was performed to detect mRNA level of chemokines. AngII-induced CCL2 (A), CXCL10 (B), CCL7 (C) and CCL12 (D) expressions were reduced by losartan or TRIFsiRNA. Data represent means ± SD of three independent experiments. *P < 0.05 vs.control, **P < 0.05 vs. AngII or NCsiRNA.

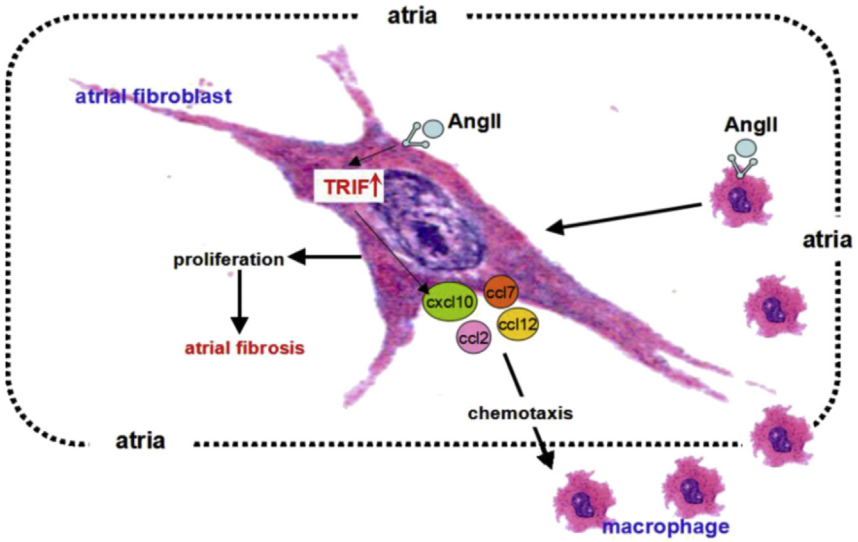

Fig. 4. Cross-talk between atrial fibroblasts and macrophages in atria. AngII interacted with its receptor and then stimulated the activation of atrial fibroblasts, inducing the high expression of TRIF. Then, TRIF enhanced the expression of chemokines, inducing the chemotaxis of macrophages in atria. Meanwhile, AngII activated macrophages. As a result, macrophages further induced the proliferation of atrial fibroblasts, leading to atrial fibrosis.

Chemokines involved in above process had been studied a lot with a lot of attention focused on CCl2. Kolattukudy et al. demonstrated that targeted overexpression of CCL2 gene in adult mouse heart muscle induced chronic diffuse infiltration of macrophages [27]. This finding was consistent with the one showing that CCL2 induction was associated with adventitial macrophage accumulation in the aortic wall of hypertensive rats treated with a continuous infusion of AngII [28]. Besides CCL2, some additional factors were required to recruit macrophages and activate inflammatory process [29]. In this study, we found that chemotaxis of macrophages was accompanied by the inductions of CCL2, CCL7, CCL12 and CXCL10 expressions.

Taken together, we believed that TRIF promoted AngII-induced cross-talk between atrial fibroblasts and macrophages, creating a long-term positive feedback loop that contributed to atrial fibrosis (Fig. 4).

In short, the evidences provided here—TRIF played an important role in AngII-induced cross-talk between atrial fibroblasts and macrophages—suggest that TRIF was involved in atrial fibrosis and inflammation, which were the pathophysiology of AF. Given that current pharmacological strategies to treat AF are, for the most part, of limited clinical efficacy and directed mainly at ion channels, TRIF may serve as a potential new target of treatment in this disease.

5. Study limitations

Our current study had some limitations that need to be mentioned. First, we did not verify the above phenomenon in vivo by TRIF knock-out mice model. Second, we did not evaluate the effect of TRIF on AngII-induced atrial inflammation in vitro by TRIF overexpression.

Acknowledgements

This work was supported by National Nature Science Foundation of China [grant number: 30871083] and Doctoral Innovation Fund Projects from Shanghai Jiao Tong University School of Medicine [grant number: BXJ201442].

Conflict of interest

None declared.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.131.

References

[1] Y.K. Iwasaki, K. Nishida, T. Kato, S. Nattel, Atrial fibrillation pathophysiology: implications for management, Circulation 124 (2011) 2264–2274.
[2] S. Nattel, M. Harada, Atrial remodeling and atrial fibrillation: recent advances and translational perspectives, J. Am. Coll. Cardiol. 63 (22) (2014) 2335–2345.
[3] T.T. Issac, H. Dokainish, N.M. Lakkis, Role of inflammation in initiation and perpetuation of atrial fibrillation: a systematic review of the published data, J. Am. Coll. Cardiol. 50 (2007) 2021–2028.
[4] T. Yamashita, A. Sekiguchi, Y.K. Iwasaki, T. Date, K. Sagara, H. Tanabe, H. Suma, H. Sawada, T. Aizawa, Recruitment of immune cells across atrial endocardium in human atrial fibrillation, Circ. J. 74 (2) (2010) 262–270.
[5] X.Q. Chen, X. Liu, Q.X. Wang, M.J. Zhang, M. Guo, F. Liu, W.F. Jiang, L. Zhou, Pioglitazone inhibits angiotensin II-induced atrial fibroblasts proliferation via NF-κB/TGF-β1/TRIF/TRAF6 pathway, Exp. Cell. Res. 330 (1) (2015) 43–55.
[6] M. Landström, The TAK1-TRAF6 signalling pathway, Int. J. Biochem. Cell. Biol. 42 (2010) 585–589.
[7] S. Sato, M. Sugiyama, M. Yamamoto, Y. Watanabe, T. Kawai, K. Takeda, S. Akira, Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling, J. Immunol. 171 (2003) 4304–4310.
[8] O. Adam, K. Theobald, D. Lavall, M. Grube, H.K. Kroemer, S. Ameling, H.J. Schäfers, M. Böhm, U. Laufs, Increased lysyl oxidase expression and collagen cross-linking during atrial fibrillation, J. Mol. Cell. Cardiol. 50 (2011) 678–685.
[9] D.G. Alleva, E.B. Johnson, F.M. Lio, S.A. Boehme, P.J. Conlon, P.D. Crowe, Regulation of murine macrophage proinflammatory and anti-inflammatory cytokines by ligands for peroxisome proliferator-activated receptor-gamma: counter-regulatory activity by IFN-gamma, J. Leukoc. Biol. 71 (2002) 677–685.
[10] J. Gu, X. Liu, Q.X. Wang, H.W. Tan, M. Guo, W.F. Jiang, L. Zhou, Angiotensin II increases CTGF expression via MAPKs/TGF-β1/TRAF6 pathway in atrial fibroblasts, Exp. Cell. Res. 318 (2012) 2105–2115.
[11] J. Gu, X. Liu, Q.X. Wang, M. Guo, F. Liu, Z.P. Song, D.D. Zhang, Beneficial effects of pioglitazone on atrial structural and electrical remodeling in vitro cellular models, J. Mol. Cell. Cardiol. 65 (2013) 1–8.
[12] R. Shen, E.R. Drelichman, D. Bimczok, C. Ochsenbauer, J.C. Kappes, J.A. Cannon, D. Tudor, M. Bomsel, L.E. Smythies, P.D. Smith, GP41-specific antibody blocks cell-free HIV type 1 transcytosis through human rectal mucosa and model colonicepithelium, J. Immunol. 184 (2010) 3648–3655.
[13] R.J. Aviles, D.O. Martin, C. Apperson-Hansen, P.L. Houghtaling, P. Rautaharju, R.A. Kronmal, R.P. Tracy, D.R. Van Wagoner, B.M. Psaty, M.S. Lauer, M.K. Chung, Inflammation as a risk factor for atrial fibrillation, Circulation 108 (2003) 3006–3010.
[14] N. Sata, N. Hamada, T. Horinouchi, S. Amitani, T. Yamashita, Y. Moriyama, K. Miyahara, C-reactive protein and atrial fibrillation. Is inflammation a consequence or a cause of atrial fibrillation? Jpn. Heart. J. 45 (2004) 441–445.
[15] D.S. Conway, P. Buggins, E. Hughes, G.Y. Lip, Prognostic significance of raised plasma levels of interleukin-6 and C-reactive protein in atrial fibrillation, Am. Heart. J. 148 (2004) 462–466.
[16] P. Doyon, M.J. Servant, Tumor necrosis factor receptor-associated factor-6 and ribosomal S6 kinase intracellular pathways link the angiotensin II AT1 receptor to the phosphorylation and activation of the IkappaB kinase complex in vascular smooth muscle cells, J. Biol. Chem. 285 (2010) 30708–30718.
[17] S.J. Watkins, G.M. Borthwick, R. Oakenfull, A. Robson, H.M. Arthur, Angiotensin II-induced cardiomyocyte hypertrophy in vitro is TAK1-dependent and Smad2/3-independent, Hypertens. Res. 35 (2012) 393–398.
[18] Y. Ma, X. Zhang, H. Bao, S. Mi, W. Cai, H. Yan, Q. Wang, Z. Wang, J. Yan, G.C. Fan, M.L. Lindsey, Z. Hu, Toll-like receptor (TLR) 2 and TLR4 differentially regulate doxorubicin induced cardiomyopathy in mice, PLoS One 7 (2012) e40763.
[19] L.K. Curtiss, A.S. Black, D.J. Bonnet, P.S. Tobias, Atherosclerosis induced by endogenous and exogenous toll-like receptor (TLR)1 or TLR6 agonists, J. Lipid. Res. 53 (2012) 2126–2132.
[20] A.M. Lundberg, D.F. Ketelhuth, M.E. Johansson, N. Gerdes, S. Liu, M. Yamamoto, S. Akira, G.K. Hansson, Toll-like receptor 3 and 4 signalling through the TRIF and TRAM adaptors in haematopoietic cells promotes atherosclerosis, Cardiovasc. Res. 99 (2013) 364–373.
[21] N. Hinglais, D. Heudes, A. Nicoletti, C. Mandet, M. Laurent, J.B. Bariéty J,Michel, Colocalization of myocardial fibrosis and inflammatory cells in rats, Lab. Invest. 70 (1994) 286–294.
[22] A. Nicoletti, D. Heudes, C. Mandet, N. Hinglais, J. Bariety, J.B. Michel, Inflammatory cells and myocardial fibrosis: spatial and temporal distribution in renovascular hypertensive rats, Cardiovasc. Res. 32 (1996) 1096–1107.
[23] F. Kuwahara, H. Kai, K. Tokuda, M. Takeya, A. Takeshita, K. Egashira, T. Imaizumi, Hypertensive myocardial fibrosis and diastolic dysfunction: another model of inflammation? Hypertension 43 (2004) 739–745.
[24] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease, Nat. Med. 18 (2012) 1028–1040.
[25] A. Desmoulière, A. Geinoz, F. Gabbiani, G. Gabbiani, Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts, J. Cell. Biol. 122 (1993) 103–111.
[26] B.B. Moore, L. Murray, A. Das, C.A. Wilke, A.B. Herrygers, G.B. Toews, The role of CCL12 in the recruitment of fibrocytes and lung fibrosis, Am. J. Respir. Cell. Mol. Biol. 35 (2006) 175–181.
[27] P.E. Kolattukudy, T. Quach, S. Bergese, S. Breckenridge, J. Hensley, R. Altschuld, G. Gordillo, S. Klenotic, C. Orosz, J. Parker-Thornburg, Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle, Am. J. Pathol. 152 (1998) 101–111.
[28] Q. Capers 4th, R.W. Alexander, P. Lou, H. De Leon, J.N. Wilcox, N. Ishizaka, A.B. Howard, W.R. Taylor, Monocyte chemoattractant protein-1 expression in aortic tissues of hypertensive rats, Hypertension 30 (1997) 1397–1402.
[29] L. Gu, S.C. Tseng, B.J. Rollins, Monocyte chemoattractant protein-1, Chem. Immunol. 72 (1999) 7–29.