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Eplerenone inhibits atrial fibrosis in mutant TGF-β1 transgenic mice

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The purpose of the present study was to study the impacts of eplerenone (EPL), an antagonist of mineralocorticoid receptors (MR), on atrial fibrosis in a mouse model with selective fibrosis in the atrium, and to explore the possible mechanisms. Using mutant TGF-β1 transgenic (Tx) mice, we first demonstrated that EPL inhibited atrial fibrosis specifically and decreased macrophage accumulation in the atria of these mice. Results from immunohistochemistry and western blotting showed that EPL attenuated protein expression of fibrosis-related molecules such as connective tissue growth factor (CTGF) and fibronectin in the atria of Tx mice. In culture, EPL inhibited gene expression of fibrosis-related molecules such as fibronectin, α-SMA, and CTGF in TGF-β1-stimulated atrial fibroblasts. Finally, using a co-culture system, we showed that TGF-β1-stimulated atrial fibroblasts induced migration of macrophages and this was blocked by EPL. EPL also blocked TGF-β1-induced gene expression of intedeukin-6 (IL-6) in atrial fibroblasts. Therefore, we conclude that EPL attenuated atrial fibrosis and macrophage infiltration in Tx mice. TGF-β1 and IL-6 were involved in the impacts of EPL on activation of atrial fibroblasts and interactions between fibroblasts and macrophages.

eplerenone, atrial fibrosis, atrial fibroblasts, macrophages, TGF-β1

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INTRODUCTION

Atrial fibrillation (AF) is the most common type of arrhythmia in clinical practice and posts great challenges to medical treatment (Allessie et al., 2002; Nattel, 2002). Atrial fibrosis is a hallmark of atrial structural remodeling that critically contributes to the occurrence and maintenance of AF (Burstein and Nattel, 2008). Therefore, targeting atrial fibrosis is an important strategy to treat AF.

Renin-angiotensin-aldosterone system plays important roles in atrial fibrosis (Ehrlich et al., 2006; Mayyas et al., 2013). Various drugs have been used in clinics to block this system. However, long-term therapeutic effects are not satisfactory, likely because of the failure to reduce production of aldosterone, the endogenous agonist of mineralocorticoid receptors (MR) (Struthers, 2004; Ehrlich et al., 2006; Duan, 2014). MR antagonists have been shown to inhibit atrial fibrosis in animal models such as myocardial infarction that is accompanied by ventricular fibrosis (Milliez et al., 2005). It has remained unclear whether the impacts of MR antagonists on the atrium are dependent on the ventricular changes.

Fibroblasts and transforming growth factor-β1 (TGF-β1) are essential in atrial fibrosis (Burstein and Nattel, 2008). Activated TGF-β1 and angiotensin II promote the produc-

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tion of each other and both induce fibrosis-related molecules in fibroblasts, contributing to atrial fibrosis (Burstein and Nattel, 2008; Zhang et al., 2015b). Our previous study has shown that in the presence of angiotensin II, activated fibroblasts induces the infiltration of macrophages that in turn promote the proliferation of fibroblasts (Chen et al., 2015b). However, the impacts of MR antagonists on fibroblasts and their interactions with macrophages are not completely understood.

In the current study, we aim to investigate the effects of MR antagonist on atrial fibrosis and macrophages infiltration using MHC-transforming growth factor (TGF)-β1cys^{33}ser transgenic (Tx) mice that have fibrosis in atria selectively and to explore the underlying mechanisms.

RESULTS

EPL inhibits atrial fibrosis and macrophages infiltration

To investigate whether EPL specifically suppresses atrial fibrosis, we treated Tx mice with EPL diet. Masson's trichrome staining demonstrated that untreated Tx mice had significant atrial fibrosis and this was markedly suppressed by EPL treatment (Figure 1A and B). We have previously shown that macrophages are involved in the process of atrial fibrosis (Chen et al., 2015b). Thus, we used MAC2 to detect macrophages in atria. The results showed that Tx mice had more infiltrated macrophages in atria than wild type mice and that EPL treatment significantly inhibited macrophage infiltration in the atria of Tx mice (Figure 1C and D).

EPL suppresses fibrosis-related molecules in atria

To further analyze the pathological consequences, we next examined protein levels of fibrosis-related molecules in atria. Results from both immunohistochemistry and western blotting showed that EPL attenuated expression of CTGF and fibronectin in the atria of Tx mice (Figure 2), further demonstrating the inhibitory effects of EPL on atrial fibrosis.

EPL suppresses expression of fibrosis-related genes in fibroblasts

We next examined expression of fibrosis-related genes in primary atrial fibroblasts. TGF-β1 markedly increased the expression of fibronectin, α-smooth muscle actin (α-SMA) and CTGF, all of which have been shown to play important roles in atrial fibrosis (Figure 3). EPL significantly attenuated TGF-β1-induced expression of these genes and it did not affect the expression in un-stimulated fibroblasts at baseline (Figure 3).

EPL inhibits fibroblasts-induced macrophage migration

Finally, we investigated the impacts of EPL on interactions between atrial fibroblasts and macrophages using a co-culture system. Transwell assay showed that when co-cultured with TGF-β1-stimulated atrial fibroblasts, more migration of macrophages was observed, whereas EPL significantly suppressed macrophage migration (Figure 4A and B). Furthermore, TGF-β1 increased IL-6 gene expression of atrial fibroblasts in the co-culture system and such elevation was blocked by EPL (Figure 4C). These results implied that IL-6 might be involved in the suppressive effects of EPL on fibroblast-macrophage interactions and then macrophage migration. In co-culture system using un-stimulated fibroblasts, EPL alone did not affect migration of macrophages or IL-6 expression of fibroblasts (Figure 4B and C).

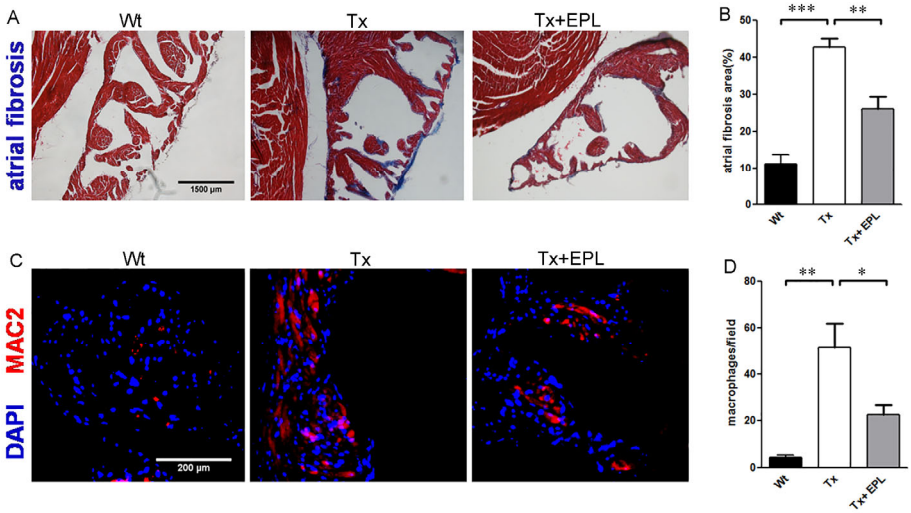

Figure 1 Eplerenone (EPL) inhibits atrial fibrosis and macrophage infiltration in TGF-β1 transgenic mice (Tx). A, Representative Masson's trichrome staining of atrial samples. Fibrotic areas stained blue. Scale bar, 1,500 μm. B, Quantification of atrial fibrosis. C, Representative immunofluorescence staining of macrophages using MAC2 in atrial samples. Scale bar, 200 μm. D, Quantification of MAC2 positive macrophages. Data are presented as mean±SEM. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

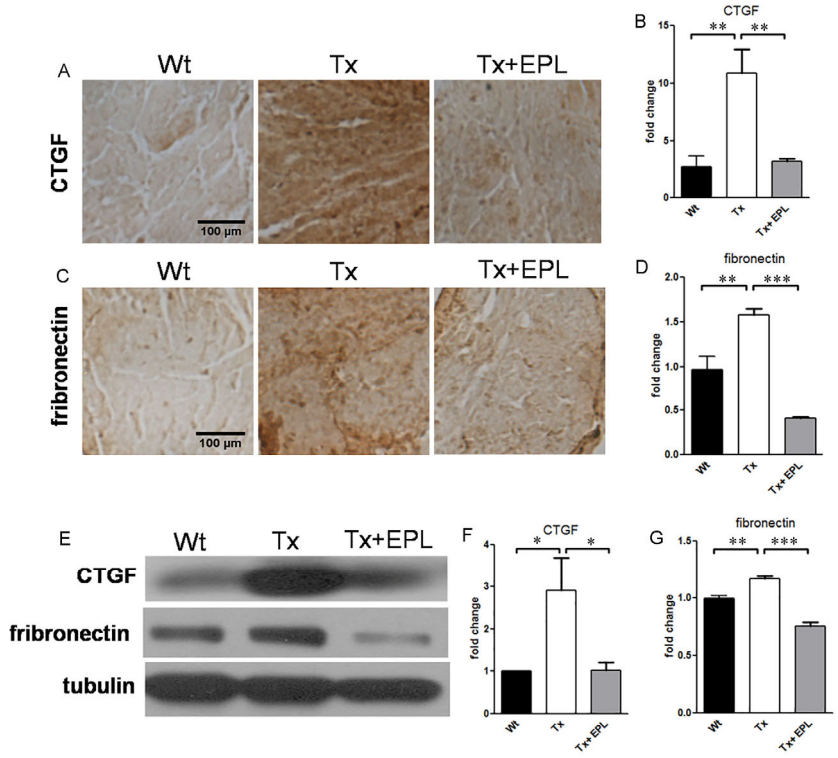

Figure 2 Eplerenone (EPL) inhibits expression of fibrosis-related molecules in atria of TGF-β1 transgenic mice (Tx). A, Representative immunohistochemical staining of CTGF in atrial samples. Positive areas stained brown. B, Quantification of CTGF positive areas. C, Representative immunohistochemical staining of fibronectin in atrial samples. Positive areas stained brown. D, Quantification of fibronectin positive areas. E, Western blotting analysis of CTGF and fibronectin in atrial samples. F, Quantification of CTGF detected by western blotting. G, Quantification of fibronectin detected by western blotting. Scale bars, 100 μm. Data are presented as mean±SEM. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

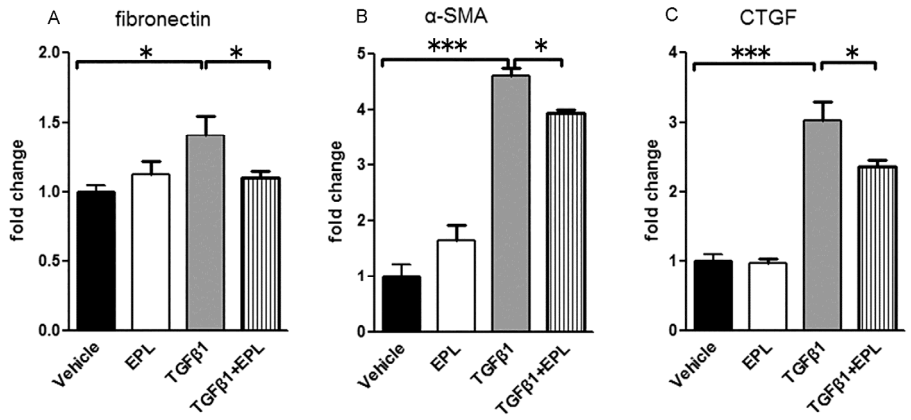

Figure 3 Eplerenone (EPL) inhibits fibrosis-related gene expression induced by TGF-β1 in fibroblasts. Primary atrial fibroblasts were treated with vehicle (DMSO), EPL, TGF-β1, or TGF-β1+EPL. Gene expression of fibronectin (A), α-SMA (B), and CTGF (C) was measured using QRT-PCR. Data are presented as mean±SEM. *, $P<0.05$; ***, $P<0.001$.

DISCUSSIONS

Our results demonstrated that EPL inhibited fibrosis, macrophage infiltration, and expression of fibrosis-related molecules in atria of mice that expressed constitutively active TGF-β1. EPL suppressed TGF-β1-induced expression of fibrosis-related molecules in atrial fibroblasts *in vitro*. TGF-β1-sitmulated fibroblasts induced macrophage migration and this was blocked by EPL. Finally, TGF-β1 induced IL-6 expression was also inhibited by EPL in atrial fibroblasts.

Evidence supports that MR antagonists are beneficial for AF. Spironolactone, an MR antagonist, has been demonstrated to prevent re-occurrence of AF in patients with nor-

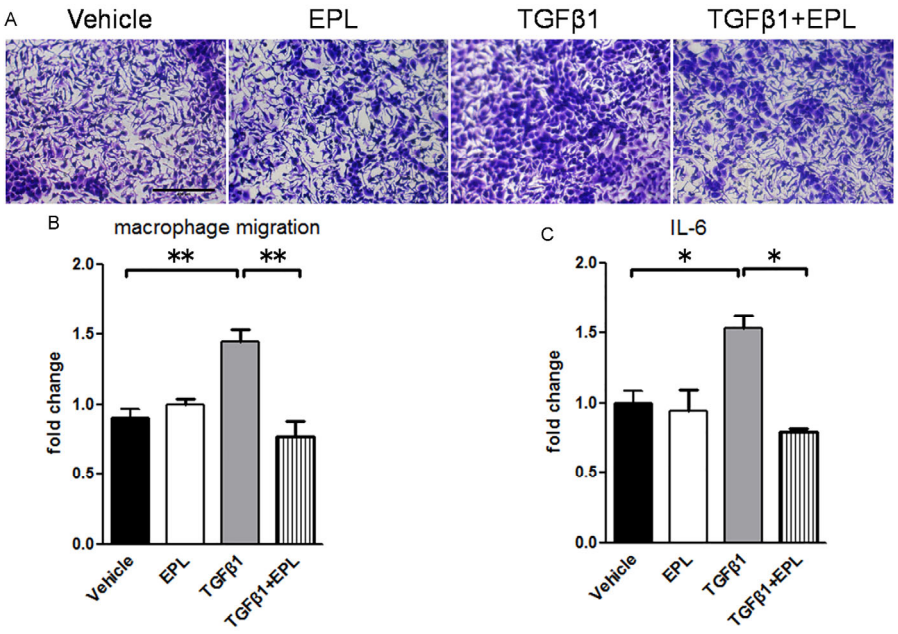

Figure 4 Eplerenone (EPL) inhibits fibroblasts-induced macrophage migration. A, Representative staining of macrophages in Transwell assay. Primary atrial fibroblasts were first stimulated with vehicle (DMSO), EPL, TGF-β1, or TGF-β1+EPL, and then cultured in regular media in bottom chambers of Transwell plates. Macrophages were subsequently added to inserts of the Transwell plates. Scale bar, 400 μm. B, Quantification of macrophage migration. C, IL-6 gene expression detected by QRT-PCR in fibroblasts. Data are presented as mean±SEM. *, $P<0.05$; **, $P<0.01$.

mal left ventricular systolic function (Dabrowski et al., 2010). EPL has been shown to reduce the incidence of new onset of AF in patients with systolic heart failure (Swedberg et al., 2012). In addition, spironolactone attenuates structural remodeling of the atria and improves atrial electrical remodeling in long-term rapid pacing-induced AF dog model (Zhao, 2010). Our results showed that EPL reduced atrial fibrosis in TGF-β1 transgenic mice, further supporting the beneficial effects of MR antagonists in AF.

Our results suggest that EPL inhibits atrial fibrosis through interfering with TGF-β1signaling. First, the *in vivo* data showed that EPL inhibited atrial fibrosis mice that expressed constitutively active form of TGF-β1. Second, the *in vitro* data demonstrated that EPL inhibited TGF-β1-induced expression of fibrosis-related molecules in fibroblasts. These results imply that MR antagonist may inhibit atrial fibrosis via its suppression on TGF-β1 signaling and expression of fibrosis-related molecules. Consistently, spironolactone has been shown to suppress aldosterone-induced expression of fibrosis-related molecules in fibroblasts (Lavall et al., 2014). A recent report has demonstrated that MR deficiency in endothelial cells prevented Western diet-induced profibrotic signaling (TGF-β1/Smad pathway) in cardiac ventricles (Jia et al., 2015), suggesting that endothelial MR directly impacts TGF-β1/Smad pathway. It is plausible that MR affects this pathway and mediates the effects of EPL in atrial fibroblasts and ultimately affects atrial fibrosis. However, this remains to be further delineated in future investigations.

Our study showed that EPL blocked interactions between fibroblasts and macrophages under stimulation of TGF-β1 and IL-6 may be involved. Infiltrated macrophages in the heart release various pro-inflammatory cytokines to interact with other cells, leading to cardiac remodeling (van Amerongen et al., 2007; Ma et al., 2012). Macrophages are important in triggering the differentiation of fibroblasts into myofibroblasts mainly through TGF-β1-dependent pathways (Wynn, 2007). IL-6 has been independently related to cardiovascular events and death in AF patients (Roldan et al., 2012; Aulin et al., 2015). In addition, IL-6 plays an important role in the recruitment and survival of macrophages (Kothari et al., 2014). These findings and our results together suggest that interactions between macrophages and fibroblasts are critical in the process of atrial fibrosis and that a TGF-β1/IL-6 axis may mediate the impacts of EPL on such interactions.

The current study has several limitations. First, the exact function of infiltrated macrophages was not delineated *in vivo*. Second, it remains to be further determined whether IL-6 mediated the interactions between fibroblasts and macrophages as well as macrophage migration both *in vivo* and *in vitro*. These aspects are worth more investigations in the future.

In short, we provided evidence that EPL inhibited fibrosis, macrophage infiltration, and fibrosis-related molecules in atria of mice with selective atrial fibrosis. Further, TGF-β1 and IL-6 might be part of the mechanisms through which EPL suppressed fibroblast activation and fibroblast/macrophage interactions as well as ultimately atrial fibrosis.

MATERIALS AND METHODS

Animal studies

We used 3.5 months old male mice in our study (n=5 per group). Tx mice with selective atrial fibrosis were generated as previously described (Nakajima et al., 2000). Littermate wild type (Wt) mice were used as controls. Eplerenone (EPL, 200 mg kg^{-1} day^{-1}) was mixed in mouse chow (Purepharm, Zhejiang). At day 75, all animals were euthanized and hearts were collected and fixed in 4% paraformaldehyde solution. All animal studies were approved by the Institutional Animal Care and Use Committee of Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Histological analysis

Fixed cardiac samples were cut into 5 μm sections. Masson trichrome (Sigma, St. Louis, USA) were used to stain fibrotic areas in atria. The ratio of atrial fibrosis area to total atrial area was calculated. For immunohistochemistry, sections were stained with antibody against CTGF (1∶200, Santa Cruz, USA) or fibronectin (1∶200, Abcam, UK). Images were captured using Olympus microscope attached to a computerized imaging system and analyzed by Image-Pro Plus 5.0. For immunofluorescence, sections were stained with antibody against MAC2 (1∶250, eBioscience, USA) and 4′,6-diamidino-2-phenylindole (DAPI) (1∶1, Life technologies, USA) was used to detect nuclei. Fluorescent signals were captured by a fluorescence microscope and five images were randomly chosen for quantification of macrophages.

Cell culture

Atrial fibroblasts were isolated from C57BL/6J mice (Chen et al., 2015a) and then cultured in Dulbecco's Modified Eagle's Medium (DMEM) -F12 supplemented with 20% fetal bovine serum in a 37°C humidified incubator with 5% CO_2. The media were replaced every 2 days. Cells were cultured in DMEM-F12 with 1% serum for 24 h and then stimulated by TGF-β1 (5 ng mL^{-1}, Pepro tech, USA) for 24 h. In EPL group, fibroblasts were pre-treated with EPL (20 μmol L^{-1}, Abcam, USA) for 2 h then treated with continued EPL or TGF-β1+EPL.

To isolate primary macrophages, C57BL/6J mice were injected intraperitoneally with 1 mL sterile 10% thioglycollate medium (Scharlaus, Spain). Three days later, macrophages were collected by intraperitoneal lavage and cultured as previously described (Usher et al., 2010).

Quantitative RT-PCR (qRT-PCR)

qRT-PCR was performed as we previously described (Zhang et al., 2015a). Primers sequences are: fibronectin, 5′-GAAGGTTTGCAACCCACTGT-3′ (forward), 5′-TCTGCAGTGTCGTCTTCACC-3′ (reverse), α-smooth muscle actin (α-SMA), 5′-ACAGAGGCACCACTGAACCCTAAG-3′ (forward), 5′-ACAATCTCACGCTCGGCAGTAGTC-3′ (reverse); Connective tissue growth factor (CTGF), 5′-GGGCCTCTTCTGCGATTTC-3′ (forward), 5′-ATCCAGGCAAGTGCATTGGTA-3′ (reverse); IL-6, 5′-GAGGATACCACTCCCAACAGACC-3′ (forward); 5′-AAGTGCATCATCGTTGTTCATACA-3′ (reverse); 18S, 5′-TTGATTAAGTCCCTGCCCTTTGT-3′ (forward), 5′-CGATCCGAGGGCCTCACTA-3′ (reverse).

Western blotting analysis

Proteins were extracted from mouse atria and underwent western blotting analysis as previously described (Chen et al., 2015a). Primary antibodies are: CTGF (1∶500, Santa Cruz), fibronectin (1∶1000, Abcam), and tubulin (1∶5000, Sigma, St. Louis).

Transwell assay

Fibroblasts were plated in the bottom chamber of 24-well plates and incubated at 37°C with 5% CO_2 overnight. After being cultured in 1% serum medium for 24 h, fibroblasts were stimulated by EPL, TGF-β1 or TGF-β1+EPL. After another 24 h, the medium was changed to 1% serum medium without TGF-β1 or EPL. At the same time, mouse peritoneal macrophages were harvested and added (1×10^5 cells mL^{-1}) into transwell inserts with polycarbonate membranes (8.0 μm pores, Millipore, USA). Macrophages were allowed to migrate for 24 h in medium containing 1% serum at 37°C. After staining, 5 high power fields (×200) were counted to determine the average numbers of migrated macrophages per high power field.

Statistical analysis

Statistical analysis was performed by Graphpad Prism 5 software. The data were expressed as means±SD. The differences between groups were assessed by one-way ANOVA. A value of P<0.05 was considered statistically significant.

Compliance and ethics *The author(s) declare that they have no conflict of interest. All applicable institutional and/or national guidelines for the care and use of animals were followed.*

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Aulin, J., Siegbahn, A., Hijazi, Z., Ezekowitz, M.D., Andersson, U., Connolly, S.J., Huber, K., Reilly, P.A., Wallentin, L., and Oldgren, J. (2015). Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. Am Heart J 6,

1151–1160.
Burstein, B., and Nattel, S. (2008). Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. J Am Coll Cardiol 8, 802–809.
Chen, X., Liu, X., Wang, Q., Zhang, M., Guo, M., Liu, F., Jiang, W., and Zhou, L. (2015a). Pioglitazone inhibits angiotensin II-induced atrial fibroblasts proliferation via NF-kappaB/TGF-beta1/ TRIF/TRAF6 pathway. Exp Cell Res 1, 43–55.
Chen, X., Zhang, D., Zhang, M., Guo, M., Zhan, Y., Liu, F., Jiang, W., Zhou, L., Zhao, L., Wang, Q., and Liu, X. (2015b). TRIF promotes angiotensin II-induced cross-talk between fibroblasts and macrophages in atrial fibrosis. Biochem Biophys Res Commun 1, 100–105.
Dabrowski, R., Borowiec, A., Smolis-Bak, E., Kowalik, I., Sosnowski, C., Kraska, A., Kazimierska, B., Wozniak, J., Zareba, W., and Szwed, H. (2010). Effect of combined spironolactone-beta-blocker +/− enalapril treatment on occurrence of symptomatic atrial fibrillation episodes in patients with a history of paroxysmal atrial fibrillation (SPIR-AF study). Am J Cardiol 11, 1609–1614.
Duan, S. (2014). Mineralocorticoid receptor: a critical player in vascular remodeling. Sci China Life Sci 57, 809–817.
Ehrlich, J.R., Hohnloser, S.H., and Nattel, S. (2006). Role of angiotensin system and effects of its inhibition in atrial fibrillation: clinical and experimental evidence. Eur Heart J 5, 512–518.
Nakajima, H., Nakajima, H.O., Salcher, O., Dittiè, A.S., Dembowsky, K., Jing, S., and Field, L.J. (2000). Atrial but notventricular fibrosis in mice expressing a mutant transforming growthfactor-beta(1) transgene in the heart. Circ Res 86, 571–579.
Zhao, J., Li, J., Li, W., Li, Y., Shan, H., Gong, Y., and Yang, B. (2010). Effects of spironolactone on atrial structuralremodelling in a canine model of atrial fibrillation produced byprolonged atrial pacing. Br J Pharmacol 159, 1584–1594.
Jia, G., Habibi, J., DeMarco, V.G., Martinez-Lemus, L.A., Ma, L., Whaley-Connell, A.T., Aroor, A.R., Domeier, T.L., Zhu, Y., Meininger, G.A., Barrett Mueller, K., Jaffe, I.Z., and Sowers, J.R. (2015). Endothelial mineralocorticoid receptor deletion prevents diet-Induced cardiac diastolic dysfunction in females. Hypertension 6, 1159–1167.
Kothari, P., Pestana, R., Mesraoua, R., Elchaki, R., Khan, K.M., Dannenberg, A.J., and Falcone, D.J. (2014). IL-6-mediated induction of matrix metalloproteinase-9 is modulated by JAK-dependent IL-10 expression in macrophages. J Immunol 1, 349–357.
Lavall, D., Selzer, C., Schuster, P., Lenski, M., Adam, O., Schafers, H.J., Bohm, M., and Laufs, U. (2014). The mineralocorticoid receptor promotes fibrotic remodeling in atrial fibrillation. J Biological Chem 10, 6656–6668.
Ma, F., Li, Y., Jia, L., Han, Y., Cheng, J., Li, H., Qi, Y., and Du, J. (2012). Macrophage-stimulated cardiac fibroblast production of IL-6 is essential for TGF beta/Smad activation and cardiac fibrosis induced by angiotensin II. PLoS One 5, e35144.
Allessie, M., Ausma, J., and Schotten, U. (2002). Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovasc Res 54, 230–246.
Mayyas, F., Alzoubi, K.H., and Van Wagoner, D.R. (2013). Impact of aldosterone antagonists on the substrate for atrial fibrillation: aldosterone promotes oxidative stress and atrial structural/electrical remodeling. Int J Cardiol 6, 5135–5142.
Nattel, S. (2002). New ideas about atrial fibrillation 50 years on. Nature, 219–226.
Milliez, P., Deangelis, N., Rucker-Martin, C., Leenhardt, A., Vicaut, E., Robidel, E., Beaufils, P., Delcayre, C., and Hatem, S.N. (2005). Spironolactone reduces fibrosis of dilated atria during heart failure in rats with myocardial infarction. Eur Heart J, 2193–2199.
Roldan, V., Marin, F., Diaz, J., Gallego, P., Jover, E., Romera, M., Manzano-Fernandez, S., Casas, T., Valdes, M., Vicente, V., and Lip, G.Y. (2012). High sensitivity cardiac troponin T and interleukin-6 predict adverse cardiovascular events and mortality in anticoagulated patients with atrial fibrillation. J Thromb Haemost 8, 1500–1507.
Struthers, A.D. (2004). The clinical implications of aldosterone escape in congestive heart failure. Eur J Heart Fail 5, 539–545.
Swedberg, K., Zannad, F., McMurray, J.J., Krum, H., van Veldhuisen, D.J., Shi, H., Vincent, J., Pitt, B., and EMPHASIS-HF Study Investigators. (2012). Eplerenone and atrial fibrillation in mild systolic heart failure: results from the EMPHASIS-HF (Eplerenone in Mild Patients Hospitalization And SurvIval Study in Heart Failure) study. J Am Coll Cardiol 18, 1598–1603.
Usher, M.G., Duan, S.Z., Ivaschenko, C.Y., Frieler, R.A., Berger, S., Schutz, G., Lumeng, C.N., and Mortensen, R.M. (2010). Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice. J Clin Invest 9, 3350–3364.
van Amerongen, M.J., Harmsen, M.C., van Rooijen, N., Petersen, A.H., and van Luyn, M.J. (2007). Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. Am J Pathol 3, 818–829.
Wynn, T.A. (2007). Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 3, 524–529.
Zhang, W., Zheng, X., Du, L., Sun, J., Shen, Z., Shi, C., Sun, S., Zhang, Z., Chen, X., Qin, M., Liu, X., Tao, J., Jia, L., Fan, H., Zhou, B., Yu, Y., Ying, H., Hui, L., Liu, X., Yi, X., Liu, X., Zhang, L., and Duan, S. (2015a). High salt primes a specific activation state of macrophages, M(Na). Cell Res 8, 893–910.
Zhang, Y., Wang, J., Li, H., Yuan, L., Wang, L., Wu, B., and Ge, J. (2015b). Hydrogen sulfide suppresses transforming growth factor-β1-induced differentiation of human cardiac fibroblasts into myofibroblasts. Sci China Life Sci 58, 1126–1134.