Int J Clin Exp Med 2018;11(6):5699-5706
www.ijcem.com /ISSN:1940-5901/IJCEM0068034

Original Article

Increased expression of the TSP-1/TGF-β/MMP-9 axis in atrial fibrillation related to rheumatic heart disease

Zhonglu Yang[1,2], Huishan Wang[2]

[1]*Fourth Military Medical University, Xi'an 710032, China;* [2]*Department of Cardiovascular Surgery, General Hospital of Shenyang Military Command, Shenyang 110016, China*

Received October 27, 2017; Accepted March 19, 2018; Epub June 15, 2018; Published June 30, 2018

Abstract: Atrial fibrosis is a hallmark of atrial fibrillation (AF). Thrombospondin-1 (TSP-1), a matricellular protein, has been shown to play an important role in fibrogenesis. However, its role in atrial fibrosis of AF has not been defined. A total of 60 patients with sinus rhythm (SR) or AF were enrolled in this study. ELISA, Western blot, and Masson's trichrome staining were used to detect expression of TSP-1, transforming growth factor-β (TGF-β), matrix metalloproteinase-9 (MMP-9), and atrial fibrosis. The degree of atrial fibrosis and left atrial diameter (LAD) was significant higher in patients with AF than in the patients with SR. Expression of TSP-1, TGF-β, and MMP-9 in serum and right appendage (RAA) were upregulated in patients with AF as compared with patients with SR. In addition, serum TSP-1, TGF-β, and MMP-9 levels were positively correlated with atrial fibrosis in AF.

Keywords: TSP-1, TGF-β, MMP-9, atrial fibrosis, atrial fibrillation

Introduction

Atrial fibrillation (AF) has been widely recognized as the most common arrhythmia in clinical practice. It is a major cause of morbidity and mortality as the patients with AF are highly susceptible to heart failure and stroke [1]. Although the mechanisms underpinning development of AF are still under investigation, one of the major pathological changes in AF is atrial fibrosis [2].

Thrombospondin-1 (TSP-1), a matricellular protein, exhibits a wide range of biological properties, and its profibrotic property has gained increasing attention in various diseases including hepatic fibrosis [3, 4], cardiac fibrosis [5] and renal fibrosis [6, 7]. Transforming growth factor-β (TGF-β), a multifunctional cytokine, has been considered as an important mediator in atrial fibrillation [8, 9]. An accumulating body of evidence has illustrated that TSP-1 is an activator of TGF-β in hepatic fibrosis [4] and renal fibrosis [7]. However, relatively little information is available about the role of TSP-1/TGF-β in atrial fibrosis. Matrix metalloproteinase-9 (MMP-9) is a subtype of proteolytic enzymes and has been shown to regulate extracullular matrix degradation, contributing to pathogenesis of cardiovascular diseases including atrial fibrillation [10]. Several studies have demonstrated that treatment with TSP-1 upregulated expression of MMP-9 in gastric adenocarcinoma cells [11] and oral squamous cell carcinoma cells [12]. In addition, increasing evidence has suggested an association between TGF-β and MMP-9 in myocardium fibrosis [13, 14]. *In vitro* experiments demonstrated that TGF-β induced MMP-9 expression in human kidney glomerular endothelial cells [15] and retinal pigment epithelial cells [16]. Thus we hypothesized that the TSP-1/TGF-β/MMP-9 aixs contributes to atrial fibrosis in atrial fibrillation.

Subjects and methods

Study subjects and specimen collection

All procedures were approved by the Ethics Committee of the General Hospital of Shenyang Military. A total of 60 patients with rheumatic heart disease undergoing valve replacement surgery in the General Hospital of Shenyang Military were enrolled in this study. The patients were divided into two groups: sinus rhythm (SR) group consisted of 30 patients with SR and atrial fibrillation (AF) group consisted of 30 patients with AF. All the patients had routine

Table 1. Clinical data of patients

Parameters	SR	AF	*P* value
Basic data			
Sex, M/F (n)	16/14	18/12	0.7948
Age (years)	52.5±9.8	54.0±10.9	0.5690
BMI (kg/m^2)	22.2±2.7	23.2±3.2	0.1839
NYHA class I/II/III/IV (n)	2/11/17/0	1/14/15/0	0.6642
Echocardiographic parameters			
LVDd (cm)	5.8±0.8	5.9±1.1	0.8937
LVDs (cm)	4.1±0.7	4.5±0.9	0.0735
EF (%)	54.3±4.1	52.0±5.1	0.0562
LAD (cm)	4.2±0.6	5.3±0.8	0.0010
Pre-operative drugs (n)			
Calcium antagonists (n)	12	16	0.4379
Beta blocker (n)	10	12	0.7892
ACEI or ARB (n)	14	17	0.6058
Digitalis (n)	11	14	0.6010
Diuretics (n)	9	14	0.2882

SR, sinus rhythm; AF, atrial fibrillation; BMI, body mass index; NYHA, New York Association; LVDd, left ventricular diastolic diameter; LVDs, left ventricular end-systolic dimension; LAD, left atrial diameter; EF, ejection fraction; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

pre-operative 2-dimensinal color echocardiography, 12-lead ECG and 24 hour ambulatory electrocardiogram. Patients with sick sinus syndrome, renal disease, cardiomyopathy, or hyperthyreosis were not included in this study.

Blood samples were collected from the subjects' peripheral vein and then centrifuged to obtain serum samples. The right appendage (RAA) was dissected under extracorporeal circulation. The tissue was trimmed to remove fat, washed with cold PBS, and divided into two parts: one part was frozen in liquid nitrogen and stored at -80°C for Western blot analysis; the other part was fixed with 4% paraformaldehyde and then embedded in paraffin for Masson's trichrome staining.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of TSP-1 (Abcam, Carbridge, MA, USA), TGF-β (R&D Systems, Minneapolis, MN, USA) and MMP-9 (R&D Systems, Minneapolis, MN, USA) were quantitated with commercially available ELISA kits according to the manufacturer's instructions.

Western blot

RAA tissues were homogenized using a pellet pestle motor. Protein samples were lysed in RIPA buffer and centrifuged at full speed for 10 min. The protein concentration in the supernatant was determined using the bicinchoninic acid protein assay kit. Equal amounts of protein were analyzed by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel and electrophoretically transferred onto polyvinylidene difluoride membranes. After blocking with blocking buffer containing 5% low-fat milk diluted with TBST, the membranes were incubated overnight at 4°C with TSP-1 antibody (1:200, Abcam, Carbridge, MA, USA), TGF-β (1:500, Santa Cruz, CA, USA), MMP-9 (1:1000, Santa Cruz, CA, USA), Col I (1:500, Abcam, Carbridge, MA, USA), Col III (1:500, Abcam, Carbridge, MA, USA). The membranes were then washed and incubated with secondary antibody at 1:10000 dilution for 1 hour before detection. The blots were detected using an enhanced chemiluminescence reagent and exposed to X-ray films. All samples from each group were also probed with an anti-β-actin antibody (1:2000, Santa Cruz, CA, USA) to correct for sample loading. The band intensities were quantified using Quantity One analysis software.

Masson's trichrome staining

RAA tissues were fixed in 4% paraformaldehyde for 48 hours, embedded in paraffin, and serial sections were cut. The sections were subjected to Masson's trichrome staining. Myocardial cells were stained red and collagens were stained blue. Collagen volume fraction (CVF) was detected to measure the percentage of fibrous tissue using Image Pro Plus software.

Statistical analysis

All data are represented as means ± SD. Statistical analysis was conducted using Sigma-Stat software (SPSS, Chicago, IL). The significance of difference between two groups

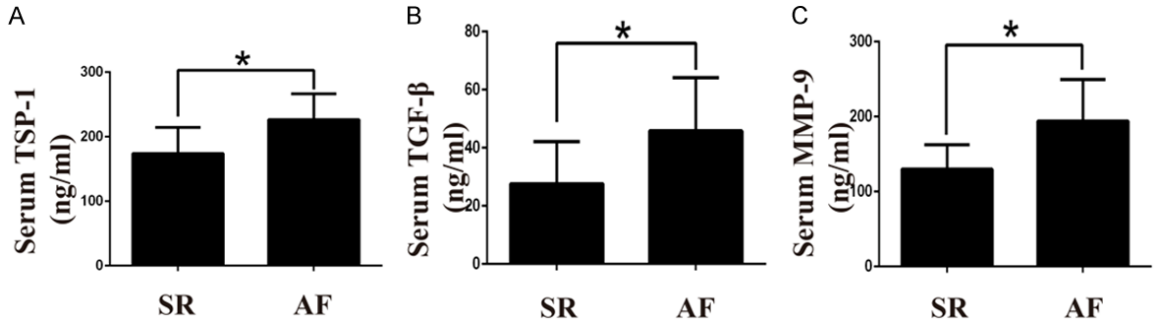

Figure 1. Serum levels of TSP-1, TGF-β, and MMP-9 in SR and AF groups. A. Increased serum TSP-1 levels in AF group. B. Increased serum TGF-β levels in AF group. C. Increased serum MMP-9 levels in AF group. *p<0.05 between groups; **p<0.01 between groups; n=30 in each group.

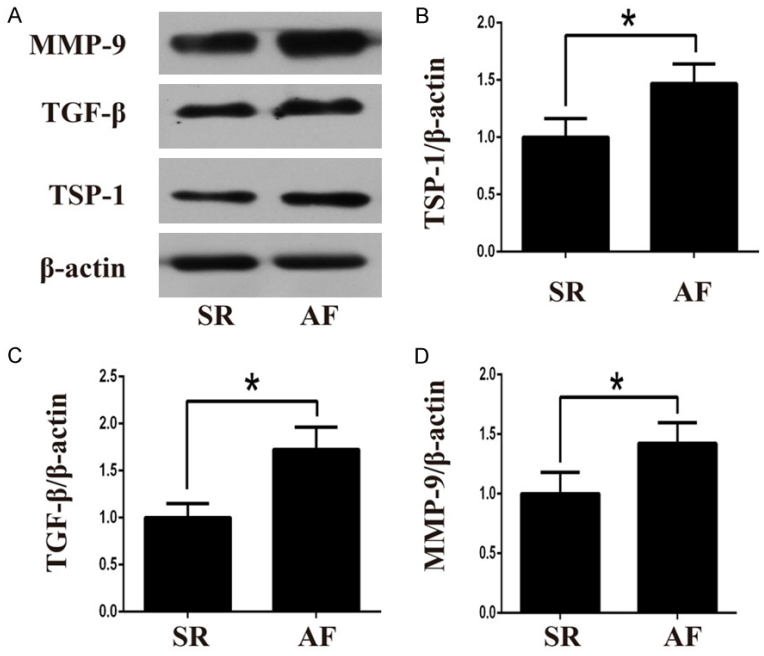

Figure 2. Protein expression of TSP-1, TGF-β, and MMP-9 in RAA tissues of the two groups. A. Representative Western blot of TSP-1, TGF-β, and MMP-9. B-D. Quantitative analysis of expressions of TSP-1, TGF-β, and MMP-9 in SR and AF groups. *p<0.05 between groups; n=30 in each group.

was evaluated by unpaired student's t-test. Categorical variables were compared using Chi-square analysis. Correlation analyses were carried out using pearson/spearman correlations. A *P* value of 0.05 was considered as the significance threshold.

Results

Clinical findings

In this study, we enrolled 60 patients undergoing valve replacement surgery, 30 patients had sinus rhythm and other 30 patients had AF. Patient characteristics were summarized in **Table 1**. There were no significant differences between age, sex, BMI, NYHA class, and preoperative drugs. LVDd and LVDs were larger in patients with AF than those of patients with SR, but it did not reach significance. However, patients with AF exhibited significantly larger LAD than patients with SR.

Increased TSP-1, TGF-β and MMP-9 levels in patients with AF

As shown in **Figure 1A**, serum TSP-1 was higher in AF group than in SR group (p<0.05). The serum levels of TGF-β and MMP-9 had a similar trend in patients with AF (both p<0.05, **Figure 1B**, **1C**). Furthermore, Western blot was performed to detect the protein expression of TSP-1, TGF-β, and MMP-9 in RAA of patients. Similar to serum TSP-1, the protein levels of TSP-1 in RAA tissues of AF were upregulated 1.5 fold compared to that in the SR group (p<0.05, **Figure 2A**). In addition, Western blot revealed a significant upregulation of TGF-β in AF group compared to SR group (p<0.05, **Figure 2B**), which was accompanied by increased MMP-9 expression in the AF group (p<0.05, **Figure 2C**).

Increased atrial fibrosis in RAA of patients with AF

The next aim of the investigation was to examine atrial fibrosis. Masson's trichrome staining

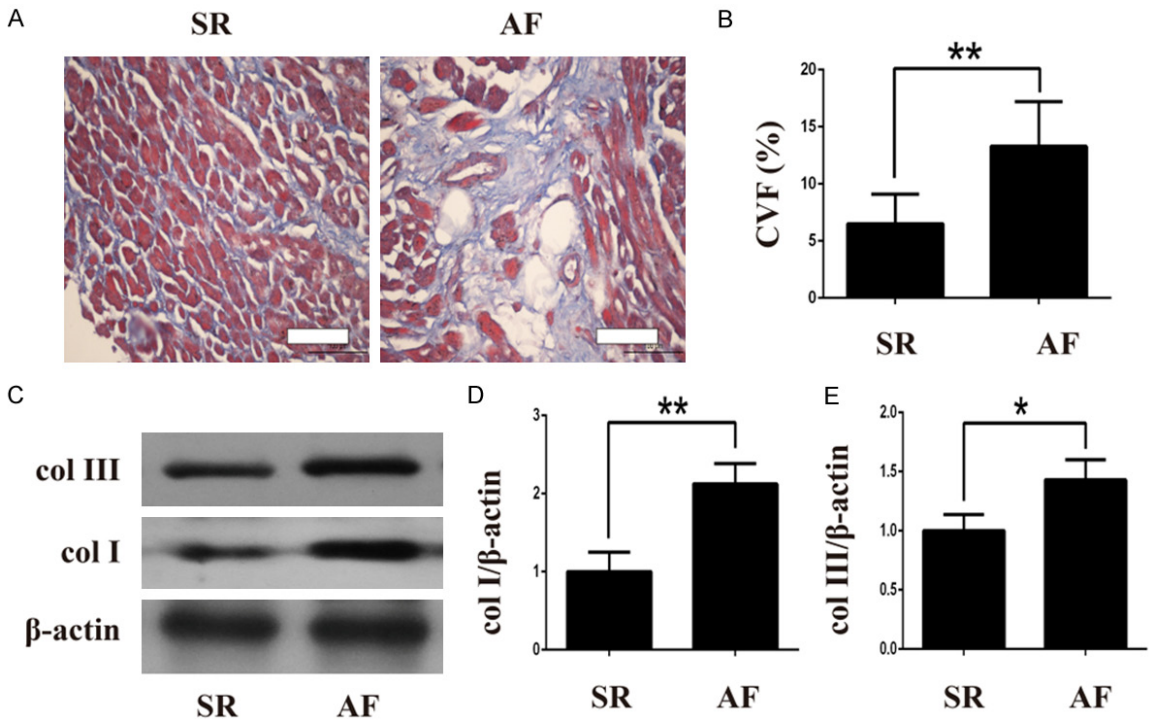

Figure 3. Atrial fibrosis in SR and AF groups. A. Representative Masson's trichrome staining in RAA of SR and AF groups. B. Quantitative analysis of fibrosis in RAA by CVF. C. Representative Western blot of col I and col III in RAA of SR and AF groups. D. Quantitative analysis of col I expression in RAA. E. Quantitative analysis of col III expression in RAA. *p<0.05 between groups; **p<0.01 between groups; Bar=100 μm; n=30 in each group. RAA, right appendage; Col I, collagen I; Col III, collagen III; CVF, Collagen volume fraction.

demonstrated an increased collage deposition in RAA tissues of patients with AF compared to patients with SR (p<0.05, **Figure 3A**, **3B**). In addition, collagen protein expression confirmed the fibrosis levels in atrial tissues. Representative images from Western blot assay and summarized data were presented in **Figure 3C-E**. A significant increase of col I and col III were observed in patients with AF (p<0.01 and p<0.05, respectively).

Correlation between serum TSP-1/TGF-β/MMP-9 and clinical parameters

More and more studies indicated the relationship between atrial fibrosis and LAD, thus we determined the correlation between TSP-1/TGF-β/MMP-9 and LAD in patients with AF. As illustrated in **Figure 4A** and **4C**, serum TSP-1 and MMP-9 were all positively correlated with LAD (both p<0.05). Markedly, serum TGF-β correlated strongly with LAD (p< 0.01, **Figure 4B**). However, no association between serum TSP-1, TGF-β, MMP-9, and EF values was found in patients with AF (**Figure 4D-F**).

Correlation between CVF levels and serum TSP-1/TGF-β/MMP-9 levels

Increasing evidence has indicated the role of TSP-1, TGF-β, and MMP-9 in fibrosis. The results shown in **Figure 4** indicate that serum TSP-1 and TGF-β levels are positively correlated with CVF, a marker of fibrosis (both p<0.05, **Figure 5A** and **5B**). Furthermore, the correlation between serum MMP-9 and CVF was also significant (p<0.05, **Figure 5C**).

Discussion

Overwhelming evidence from clinical and laboratory investigations has demonstrated that LAD and atrial fibrosis are markers of AF [17, 18]. In accordance to previous studies, we found larger LAD in patients with AF compared to patients with SR. Interstitial fibrosis of atrial myocardium induced electrical uncoupling of adjacent muscle bundles, led to disturbed electrical continuity, and contributed to AF development. However, AF in turn promoted atrial fibrosis over time [19]. Thus, it was likely that AF induced AF via a positive feedback

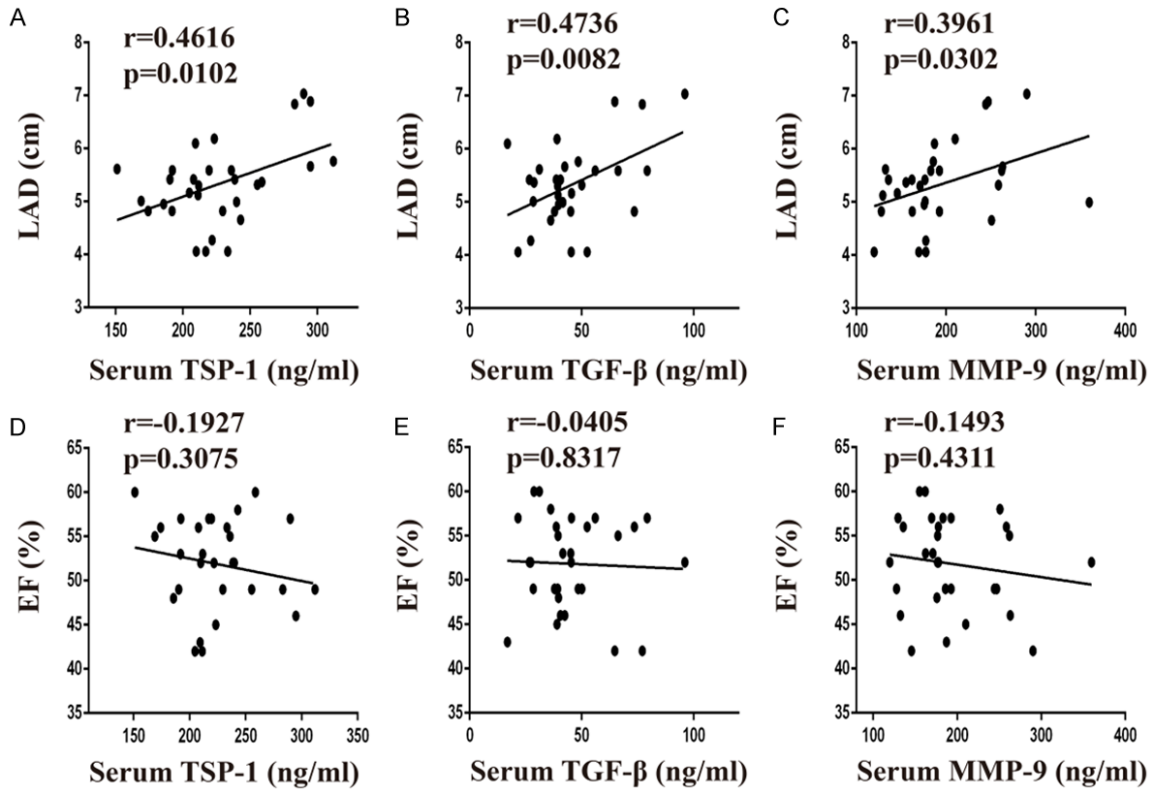

Figure 4. TSP-1/TGF-β/MMP-9 correlated to LAD and EF in patients with AF. A. Correlation between serum TSP-1 levels and LAD in AF group. B. Correlation between serum TGF-β levels and LAD in AF group. C. Correlation between serum MMP-9 levels and LAD in AF group. D. Correlation between serum TSP-1 levels and EF in AF group. E. Correlation between serum TGF-β levels and EF in AF group. F. Correlation between serum MMP-9 levels and EF in AF group.

involving atrial fibrosis. Consistent with the findings of others, the present data demonstrate increased atrial fibrosis in patients with AF, as evidenced by increased Masson's trichrome staining and upregulated expression of collagens. However, the underlying mechanisms that lead to atrial fibrosis remained unknown.

TSP-1 was originally identified as a matricellular glycoprotein from active platelets [20], which was subsequently followed by various other studies. Procter and colleagues reported higher plasma TSP-1 levels in chronic AF compared to onset AF [21]. Furthermore, plasma TSP-1 concentrations were correlated with platelet hyperaggregability in chronic AF patients [21, 22]. In the present study, both serum TSP-1 and TSP-1 protein expression in atrial tissues were significantly increased in patients with AF compared to patients with SR. In the past decade, much attention has been devoted assessing the role of TSP-1 in fibrosis. Genetic inhibition of TSP-1 reversed the upregulated expression of collagen in diabetic myocardium [23]. In addition, TSP-1 was found to mediate high glucose-induced collagen type III synthesis and TGF-β expressions in cardiac fibroblasts [23]. Cao and colleagues indicated that aging mice exhibited enhanced expression of TSP-1 and TGF-β in hearts, and paralleled by increased myocardium fibrosis [24], which may be regulated to micro RNA-18/19 [25]. However, there is a paucity of data about the role of TSP-1 in atrial fibrosis. In the current study, we demonstrated that serum TSP-1 levels were positively correlated with atrial fibrosis and LAD, suggesting the possible role of TSP-1 in atrial fibrosis in AF.

TGF-β, a pro-fibrotic factor, has been considered as a critical player in AF. A considerable number of studies have shown upregulation of TGF-β in patients with AF [9, 17, 26]. A significant increase in susceptibility to atrial fibrillation was observed in transgenic animals with overexpression of TGF-β [27, 28]. In addition, TGF-β level was an independent predictor of AF recurrence in patients with catheter ablation and surgery ablation [8, 17, 29]. Consistent

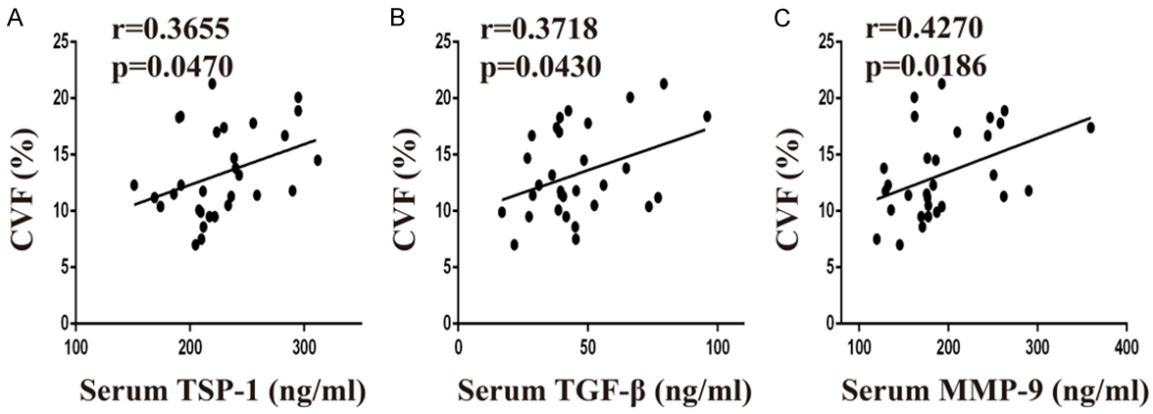

Figure 5. Correlation between TSP-1/TGF-β/MMP-9 and CVF in patients with AF. A. Serum TSP-1 correlated to CVF in AF group. B. Serum TGF-β correlated to CVF in AF group. C. Serum MMP-9 correlated to CVF in AF group.

with the findings of others, TGF-β levels in serum and atrial tissues were higher in patients with AF than in patients with SR. In addition, a positive correlation between TGF-β and atrial fibrosis was observed in this present study. Supporting these statements, Wu et al. demonstrated that TGF-β was positively correlated with markers of fibrosis such as collagen I, collagen III and CVF [17]. To further define the mechanistic link between TGF-β and atrial fibrosis, several downstream factors of TGF-β such as Smad3, E-cadherin, STAT3, and collagen [9, 30] were reported previously. More and more reports have demonstrated that TSP-1-mediated activation of TGF-β contributes to fibrosis [23, 31, 32]. Therefore, together with the previous findings and the present study, it is not inconceivable that TSP-1 could promote atrial fibrosis via activation of TGF-β in AF, although further studies are clearly warranted to elucidate the molecular mechanisms.

MMP-9, a novel fibrotic and inflammatory marker, was upregulated in AF and correlated with recurrence after ablation [33-35]. Our findings are consistent with the studies showing that patients with AF exhibited increased MMP-9 in serum and atrial tissues. In addition, serum MMP-9 levels are correlated with LAD and atrial fibrosis. A previous study reported that TGF-β-induced downstream expression was blunted by MMP-9 deletion in ageing mice, suggesting that MMP-9-mediated activation of TGF-β contributed to expression of fibrotic markers [13]. These results further support the hypothesis that MMP-9 promotes atrial fibrosis via activation of TGF-β. However, TGF-β could upregulate MMP-9 expression in kidney glomerular endothelial cells [15], suggesting that MMP-9 exhibits a positive feedback to induce TGF-β activation and expression. Interestingly, TSP-1 has been shown to promote progression of cancer by upregulation of MMP-9 [11, 36]. Considering the findings together, both TSP-1 and MMP-9 were inducers of TGF-β, and TGF-β which in turn induced MMP-9 expression.

In conclusion, we present evidence showing increased expression of TSP-1, TGF-β, and MMP-9 and increased atrial fibrosis in patients with AF. Another interesting finding in the present study was that serum TSP-1, TGF-β, and MMP-9 levels were all positively correlated with atrial fibrosis in patients with AF. The increased expression of the TSP-1/TGF-β/MMP-9 axis contributed to atrial fibrosis in patients with AF.

Acknowledgements

This work was supported by the Scientific and Technological Project of Liaoning Province, China (2012225005).

Disclosure of conflict of interest

None.

Address correspondence to: Huishan Wang, Department of Cardiovascular Surgery, General Hospital of Shenyang Military Command, 83 Cultural Road, Shenhe District, Shenyang 110016, China. Tel: +86 13309885095; E-mail: moxsihrt329@163.com

References

[1] Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH Jr, Zheng ZJ, Forouzanfar MH,

Naghavi M, Mensah GA, Ezzati M and Murray CJ. Worldwide epidemiology of atrial fibrillation: a global burden of disease 2010 study. Circulation 2014; 129: 837-847.

[2] Nattel S and Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. J Am Coll Cardiol 2014; 63: 2335-2345.

[3] Narmada BC, Chia SM, Tucker-Kellogg L and Yu H. HGF regulates the activation of TGF-beta1 in rat hepatocytes and hepatic stellate cells. J Cell Physiol 2013; 228: 393-401.

[4] Kondou H, Mushiake S, Etani Y, Miyoshi Y, Michigami T and Ozono K. A blocking peptide for transforming growth factor-beta1 activation prevents hepatic fibrosis in vivo. J Hepatol 2003; 39: 742-748.

[5] Gonzalez-Quesada C, Cavalera M, Biernacka A, Kong P, Lee DW, Saxena A, Frunza O, Dobaczewski M, Shinde A and Frangogiannis NG. Thrombospondin-1 induction in the diabetic myocardium stabilizes the cardiac matrix in addition to promoting vascular rarefaction through angiopoietin-2 upregulation. Circ Res 2013; 113: 1331-1344.

[6] Bige N, Shweke N, Benhassine S, Jouanneau C, Vandermeersch S, Dussaule JC, Chatziantoniou C, Ronco P and Boffa JJ. Thrombospondin-1 plays a profibrotic and pro-inflammatory role during ureteric obstruction. Kidney Int 2012; 81: 1226-1238.

[7] Nakagawa T, Lan HY, Glushakova O, Zhu HJ, Kang DH, Schreiner GF, Bottinger EP, Johnson RJ and Sautin YY. Role of ERK1/2 and p38 mitogen-activated protein kinases in the regulation of thrombospondin-1 by TGF-beta1 in rat proximal tubular cells and mouse fibroblasts. J Am Soc Nephrol 2005; 16: 899-904.

[8] Kishima H, Mine T, Takahashi S, Ashida K, Ishihara M and Masuyama T. The impact of transforming growth factor-beta1 level on outcome after catheter ablation in patients with atrial fibrillation. J Cardiovasc Electrophysiol 2017; 28: 402-409.

[9] Chang SH, Yeh YH, Lee JL, Hsu YJ, Kuo CT and Chen WJ. Transforming growth factor-beta-mediated CD44/STAT3 signaling contributes to the development of atrial fibrosis and fibrillation. Basic Res Cardiol 2017; 112: 58.

[10] Lewkowicz J, Knapp M, Tankiewicz-Kwedlo A, Sawicki R, Kaminska M, Waszkiewicz E and Musial WJ. MMP-9 in atrial remodeling in patients with atrial fibrillation. Ann Cardiol Angeiol (Paris) 2015; 64: 285-291.

[11] Albo D, Shinohara T and Tuszynski GP. Up-regulation of matrix metalloproteinase 9 by thrombospondin 1 in gastric cancer. J Surg Res 2002; 108: 51-60.

[12] Hayashido Y, Nakashima M, Urabe K, Yoshioka H, Yoshioka Y, Hamana T, Kitano H, Koizumi K and Okamoto T. Role of stromal thrombospondin-1 in motility and proteolytic activity of oral squamous cell carcinoma cells. Int J Mol Med 2003; 12: 447-452.

[13] Chiao YA, Ramirez TA, Zamilpa R, Okoronkwo SM, Dai Q, Zhang J, Jin YF and Lindsey ML. Matrix metalloproteinase-9 deletion attenuates myocardial fibrosis and diastolic dysfunction in ageing mice. Cardiovasc Res 2012; 96: 444-455.

[14] Zhao T, Zhao W, Chen Y, Li VS, Meng W and Sun Y. Platelet-derived growth factor-D promotes fibrogenesis of cardiac fibroblasts. Am J Physiol Heart Circ Physiol 2013; 304: H1719-1726.

[15] Zhao Y, Qiao X, Wang L, Tan TK, Zhao H, Zhang Y, Zhang J, Rao P, Cao Q, Wang Y, Wang Y, Wang YM, Lee VW, Alexander SI, Harris DC and Zheng G. Matrix metalloproteinase 9 induces endothelial-mesenchymal transition via notch activation in human kidney glomerular endothelial cells. BMC Cell Biol 2016; 17: 21.

[16] Kimura K, Orita T, Liu Y, Yang Y, Tokuda K, Kurakazu T, Noda T, Yanai R, Morishige N, Takeda A, Ishibashi T and Sonoda KH. Attenuation of EMT in RPE cells and subretinal fibrosis by an RAR-gamma agonist. J Mol Med (Berl) 2015; 93: 749-758.

[17] Wu H, Xie J, Li GN, Chen QH, Li R, Zhang XL, Kang LN and Xu B. Possible involvement of TGF-beta/periostin in fibrosis of right atrial appendages in patients with atrial fibrillation. Int J Clin Exp Pathol 2015; 8: 6859-6869.

[18] Xue XD, Huang JH and Wang HS. Angiotensin II activates signal transducers and activators of transcription 3 via Rac1 in the atrial tissue in permanent atrial fibrillation patients with rheumatic heart disease. Cell Biochem Biophys 2015; 71: 205-213.

[19] Yue L, Xie J and Nattel S. Molecular determinants of cardiac fibroblast electrical function and therapeutic implications for atrial fibrillation. Cardiovasc Res 2011; 89: 744-753.

[20] Lawler JW, Slayter HS and Coligan JE. Isolation and characterization of a high molecular weight glycoprotein from human blood platelets. J Biol Chem 1978; 253: 8609-8616.

[21] Procter NE, Ball J, Liu S, Hurst N, Nooney VB, Goh V, Stafford I, Heresztyn T, Carrington M, Ngo DT, Hylek EM, Isenberg JS, Chirkov YY, Stewart S, Horowitz JD and Investigators S. Impaired platelet nitric oxide response in patients with new onset atrial fibrillation. Int J Cardiol 2015; 179: 160-165.

[22] Procter NE, Ball J, Ngo DT, Chirkov YY, Isenberg JS, Hylek EM, Stewart S and Horowitz JD. Platelet hyperaggregability in patients with atrial fibrillation. Evidence of a background proinflammatory milieu. Herz 2016; 41: 57-62.

[23] Tang M, Zhou F, Zhang W, Guo Z, Shang Y, Lu H, Lu R, Zhang Y, Chen Y and Zhong M. The role of thrombospondin-1-mediated TGF-beta1 on collagen type III synthesis induced by high glucose. Mol Cell Biochem 2011; 346: 49-56.
[24] Cai H, Yuan Z, Fei Q and Zhao J. Investigation of thrombospondin-1 and transforming growth factor-beta expression in the heart of aging mice. Exp Ther Med 2012; 3: 433-436.
[25] van Almen GC, Verhesen W, van Leeuwen RE, van de Vrie M, Eurlings C, Schellings MW, Swinnen M, Cleutjens JP, van Zandvoort MA, Heymans S and Schroen B. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. Aging Cell 2011; 10: 769-779.
[26] Zhang D, Liu X, Chen X, Gu J, Li F, Zhang W and Zheng Y. Role of the MAPKs/TGF-beta1/TRAF6 signaling pathway in atrial fibrosis of patients with chronic atrial fibrillation and rheumatic mitral valve disease. Cardiology 2014; 129: 216-223.
[27] Choi EK, Chang PC, Lee YS, Lin SF, Zhu W, Maruyama M, Fishbein MC, Chen Z, Rubart-von der Lohe M, Field LJ and Chen PS. Triggered firing and atrial fibrillation in transgenic mice with selective atrial fibrosis induced by overexpression of TGF-beta1. Circ J 2012; 76: 1354-1362.
[28] Polejaeva IA, Ranjan R, Davies CJ, Regouski M, Hall J, Olsen AL, Meng Q, Rutigliano HM, Dosdall DJ, Angel NA, Sachse FB, Seidel T, Thomas AJ, Stott R, Panter KE, Lee PM, Van Wettere AJ, Stevens JR, Wang Z, MacLeod RS, Marrouche NF and White KL. Increased susceptibility to atrial fibrillation secondary to atrial fibrosis in transgenic goats expressing transforming growth factor-beta1. J Cardiovasc Electrophysiol 2016; 27: 1220-1229.
[29] Wu CH, Hu YF, Chou CY, Lin YJ, Chang SL, Lo LW, Tuan TC, Li CH, Chao TF, Chung FP, Liao JN and Chen SA. Transforming growth factor-beta1 level and outcome after catheter ablation for nonparoxysmal atrial fibrillation. Heart Rhythm 2013; 10: 10-15.
[30] Chen X, Xu J, Jiang B and Liu D. Bone morphogenetic protein-7 antagonizes myocardial fibrosis induced by atrial fibrillation by restraining transforming growth factor-beta (TGF-beta)/smads signaling. Med Sci Monit 2016; 22: 3457-3468.
[31] Xie XS, Li FY, Liu HC, Deng Y, Li Z and Fan JM. LSKL, a peptide antagonist of thrombospondin-1, attenuates renal interstitial fibrosis in rats with unilateral ureteral obstruction. Arch Pharm Res 2010; 33: 275-284.
[32] Poczatek MH, Hugo C, Darley-Usmar V and Murphy-Ullrich JE. Glucose stimulation of transforming growth factor-beta bioactivity in mesangial cells is mediated by thrombospondin-1. Am J Pathol 2000; 157: 1353-1363.
[33] Wu G, Wang S, Cheng M, Peng B, Liang J, Huang H, Jiang X, Zhang L, Yang B, Cha Y, Jiang H and Huang C. The serum matrix metalloproteinase-9 level is an independent predictor of recurrence after ablation of persistent atrial fibrillation. Clinics (Sao Paulo) 2016; 71: 251-256.
[34] Manfrini O, Cenko E, Ricci B and Bugiardini R. Post cardiovascular surgery atrial fibrillation. Biomarkers determining prognosis. Curr Med Chem 2017; [Epub ahead of print].
[35] Huxley RR, Lopez FL, MacLehose RF, Eckfeldt JH, Couper D, Leiendecker-Foster C, Hoogeveen RC, Chen LY, Soliman EZ, Agarwal SK and Alonso A. Novel association between plasma matrix metalloproteinase-9 and risk of incident atrial fibrillation in a case-cohort study: the Atherosclerosis Risk in Communities study. PLoS One 2013; 8: e59052.
[36] Qian X, Rothman VL, Nicosia RF and Tuszynski GP. Expression of thrombospondin-1 in human pancreatic adenocarcinomas: role in matrix metalloproteinase-9 production. Pathol Oncol Res 2001; 7: 251-259.