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Expression of PDGFR-α in Patients with Valvular Atrial Fibrillation

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Abstract

Objective: To investigate the expression of Platelet-derived growth factor receptor alpha (PDGFR-α) in patients who have valvular atrial fibrillation. **Methods:** In this research, eighty-four patients with rheumatic heart disease who were going to undertake cardiac surgery were included. The subjects were divided into two groups: the AF group and the sinus rhythm group, the quantities are 39 and 45 respectively. Before the surgery, baseline demographics, physical examination, routine laboratory testing, echocardiography, echocardiographic data and additional clinical data were available for all patients. The right atrial tissue of the subjects was separated during surgery, with an area of approximately 0.3 - 0.5 mm^3. Immunofluorescence staining was used to analyze the distribution of PDGFR-α of atrial tissue. mRNA of PDGFR-α in atrial tissue were determined by real-time quantitative PCR (Polymerase Chain Reaction); Western-Blot technique was used to measure the protein of PDGFR-α in atrial tissue. **Results:** There were no significant differences ($P > 0.05$) in sex ratio, age, blood pressure, blood biochemistry, and other aspects of medical history between the two groups. However, the right and left atrium diameters in the AF group were markedly larger than those in the SR group ($P < 0.05$). Moreover, the mRNA and protein of PDGFR-α from right atrial tissue were obviously higher in the AF group than that in the SR group ($P < 0.05$). **Conclusion:** The expression of PDGFR-α in the right atrial tissue of patients with atrial fibrillation was found to be significantly higher.

Keywords

Rheumatic Heart Disease, Atrial Fibrillation, Atrial Remodeling, Platelet-Derived Growth Factor Receptor-α, Cardiology

1. Introduction

Nowadays, in clinic, AF (atrial fibrillation) is the most common supraventricular

arrhythmia, and also one of the key factors of life-threatening diseases such as stroke and congestive heart failure [1] [2]. PDGFR-α is a member of Platelet-derived growth factors (PDGFs), PDGFR-α is mainly distributed in cells of mesenchymal or glial origin: fibroblasts, chondrocytes, osteoblasts, glial cells and smooth muscle cells. PDGFR-α It plays an important role in the development of myocardial fibrosis by binding to its specific ligand PDGF-A. This study collected the right atrial appendage tissue, blood and basic clinical data of patients with clinical valvular atrial fibrillation, and observed PDGFR-α distribution and expression of PDGFR-α in the atrium of patients with valvular atrial fibrillation. It lays a theoretical foundation for the relationship between atrial fibrosis and atrial fibrillation.

2. Materials and Methods

2.1. Patient Enrollment and Data Collecting

Among 84 patients admitted to the hospital for cardiac valve disease and underwent cardiac valve replacement in 2012, 39 were AF patients and 45 were SR patients. Inclusion criteria: patients with rheumatic valvular disease, senile degenerative valvular disease and valvular malformation who underwent cardiac valve replacement with or without AF. Exclusion criteria: people over 65 years old, liver and kidney failure, hyperthyroidism, cardiomyopathy, chronic pulmonary heart disease, left ventricular EF value less than 40%, accompanied by obvious acute infection, tumor and other diseases. The selected patients were divided into the sinus rhythm group and the AF group. All selected subjects signed the informed consent form and passed the review by the ethics committee.

2.2. Treatment of Clinical Data and Specimens

Collect and sort out the clinical baseline data, biochemical examination, ECG and imaging examination data of patients. All the selected patients who are on an empty stomach collected 5 mL of elbow vein blood in the morning and put it in a refrigerator at four degrees Celsius for three hours. The serum was separated by a centrifuge (room temperature, twenty minutes, 1000 r/min), and stored at −80˚C. During cardiac surgery, the right atrial tissue (0.3 - 0.5 cm^3) of selected patients was taken, and after removing blood, adipose tissue and other impurities, each atrial tissue was cut into three pieces for storage.

2.3. Analysis of PDGFR-α in Atrial Tissue of Patients in AF Group and SR Group by Immunofluorescence Technique Protein Expression

The fresh right atrium tissue obtained from surgery was frozen and then embedded with OTC embedding gel. The frozen section machine 7 μm thick slices, attached to the slide, placed at room temperature for 30 minutes, fixed in four degrees Celsius acetone for 10 min, dried at room temperature and stored in the refrigerator for standby. Slices were rinsed with 0.01 mol/L PBS for 10 min × 3

times; Normal bovine serum was blocked for 30 min; 0.01 mol/L PBS rinsing for 10 min × 1 time; PDGFR-α The first antibody was diluted at 1:150, incubated at 37˚C for 1 hour, and stayed at 4˚C overnight; 0.01 mol/L PBS rinsing for 10 min × 3 times; Add fluorescent secondary antibody working solution PDGFR-α, Sheep anti-mouse/FITC, diluted 1:100, incubated at 37˚C for 1 hour; 0.01 mol/L PBS rinsing for 10 min × 3 times; Add DAPI dye working solution (1:100 dilution) and incubate at room temperature for 15 minutes; 0.01 mol/L PBS rinsing for 10 min × 3 times; Buffer glycerin seal; Observe and take photos under laser confocal microscope.

2.4. Determination of PDGFR-α in Atrial Tissue by Real-Time Fluorescent Quantitative PCR MRNA Expression of

To detect the relative amount of mRNA expression, extract the total RNA from the atrial tissue of selected patients, and then reverse transcribe it into cDNA using real-time fluorescence quantitative PCR. The target primer sequence is upstream primer 5'-GCTACATCATTCCTGCC-3'; Downstream primer 5'-AGGTGGAACTGCTGGAACC-3'. Real-time fluorescence quantitative PCR program: 95˚C for 3 min for 40 cycles, 95˚C for 10 seconds, 57.3˚C for 10 s and 72˚C for 30 s. The experiment was repeated three times. At the end of the reaction, read the CT values and dissolution and amplification curves of each reaction well for data analysis.

2.5. Determination of PDGFR-α in Atrial Tissue by Western Blot Protein Expression

Extract total protein from atrial tissue, use BAC kit to measure the total protein concentration, draw a standard curve, and calculate the actual mass concentration of the sample (μg/μL). Electrophoresis on 100 g/LSDS-PAGE gel for 2 h at 80 mV. PVDF film rotates for 70 min at 250 mA. Seal at room temperature for 2 hours in the skimmed milk powder sealing solution, and wash the film with TBST three times. Join PDGFR-α (1:500 dilution) β-Actin (1:500 dilution) first antibody, incubated at 4˚C for a night; After washing the film three times, add the second antibody (1:500 dilution) and incubate it at room temperature for two hours, TBST wash the film three times for 10 min, and put it in the strong chemiluminescence reagent ECL substrate for the chemical reaction for 5 min, and the darkroom exposure shows the specific protein signal. Use the GelDoc100 imaging system for image processing and record the results. Use the target strip and internal reference (The expression of target protein-β was semi-quantitatively analyzed by the ratio of actin) band absorbance area integral.

2.6. Statistical Analysis

In the experiment, all data in the experiment were analyzed using the SPSS19.0 statistical software package. The mean ± standard deviation is used to represent

the measured data, and it is proved that the data conforms to the normal distribution through the normality test. In the two sets of data in the experiment, the mean values were compared using a t-test, and the ratios were compared using the χ^2 test. $P < 0.05$ has statistical significance.

3. Results

3.1. Analysis of Basic Clinical Data of SR Group and AF Group

Patients participating in the experiment were divided into SR (including 19 males and 26 females) group and AF group, in AF group, 39 cases (male/female, 24/15). The characteristics of baseline data of patients are shown as follows. From the analysis of the results in the table, between the two groups there is no marked difference in the statistical treatment of various medical history data such as sex constituent ratio, age, blood pressure, blood routine, biochemical indicators, etc. ($P > 0.05$), which is comparable, see Table 1.

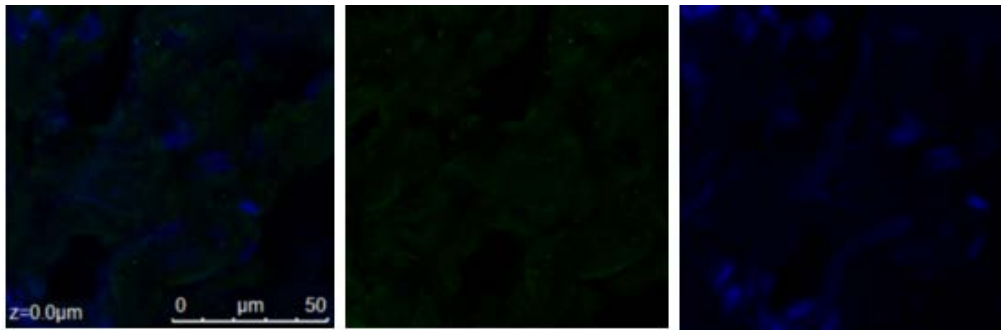
Table 1. Clinical characteristics.

	AF (n = 39)	SR (n = 45)	t/χ^2 values	P
Basic data				
Age (years)	51.1 ± 9.0	48.1 ± 10.9	−1.405	0.164
Sex	24/15	19/26	3.083	0.086
Male/Female (n)				
SBP* (mmHg)	112.4 ± 10.4	115.8 ± 14.3	1.242	0.218
DBP* (mmHg)	71.7 ± 7.4	70.5 ± 11.5	−0.567	0.572
Laboratory				
Examinations WBC* (10^9/l)	6.29 ± 1.55	6.0 ± 1.91	−0.763	0.448
RBC* (10^{12}/l)	4.55 ± 0.67	4.44 ± 0.51	−0.811	0.420
HB* (g/l)	137.1 ± 16.6	135.3 ± 16.8	−0.482	0.631
CR* (μmol/l)	92.47 ± 12.46	91.58 ± 12.43	−0.225	0.823
Echocardiograp				
Hic Parameters				
LVEF* (%)	53.10 ± 5.90	54.47 ± 5.90	1.041	0.301
LAD* (mm)	58.03 ± 13.93*	49.55 ± 17.17*	−2.420	0.018
RAD* (mm)	26.88 ± 4.81*	24.32 ± 4.26*	1.721	0.038

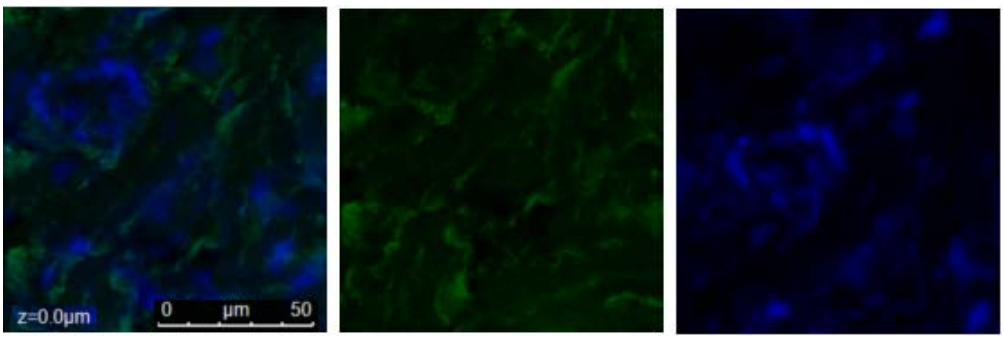
SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; RBC, red blood cell; HB, hemoglobin; CR, serum creatinine; LVEF, left ventricular ejection fraction; LAD, left atrium diameter; RAD, right atrium diameter.

3.2. Detection of PDGFR-α in Atrial Tissue of Patients in SR Group and AF Group by Laser Confocal Imaging after Double Labeling of Blue-Green Fluorescent Protein Immunofluorescence Staining Results

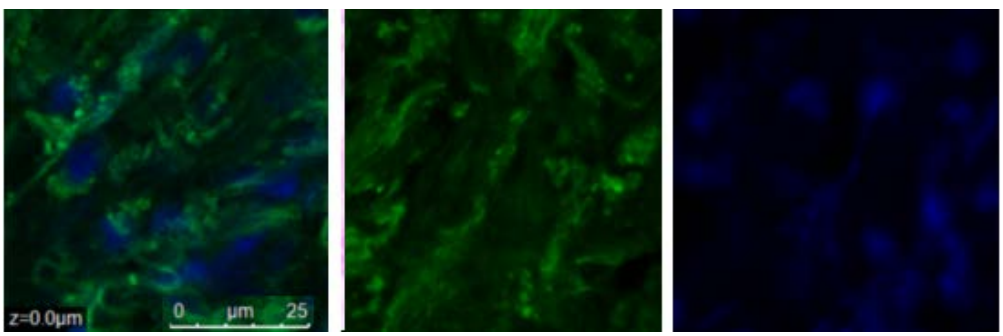
Using immunofluorescence technology to detect the protein expression of PDGFR-α. Laser confocal microscopy showed: PDGFR-α is expressed in green fluorescence, and the nucleus is blue fluorescence. PDGFR-α in the SR group and AF group. The protein was positive and localized on the cell membrane near the nucleus of the myocardium or fibroblasts. As shown in Figure 1. PDGF-A protein expression was weak in the SR group, and for PDGFR-α in the AF group the fluorescence intensity and density of protein expression were apparently greater than those of the SR group.



(a)



(b)



(c)

Figure 1. PDGFR-α of atrial tissue in SR group and AF group Confocal immunofluorescence laser × 500 times ((a) negative control; (b) SR group; (c) AF group).

3.3. Amplification Curve and Dissolution Curve

The dissolution curves of real-time fluorescence quantitative PCR were all single peaks, indicating that the amplification reaction was specific. As shown in Figure 2, PDGFR-*α*, the inflection point of the mRNA amplification curve is clear, the exponential phase and the platform phase are obvious, the slope of the exponential phase is large, and the curve parallelism is good, indicating that the amplification efficiency of each reaction is similar.

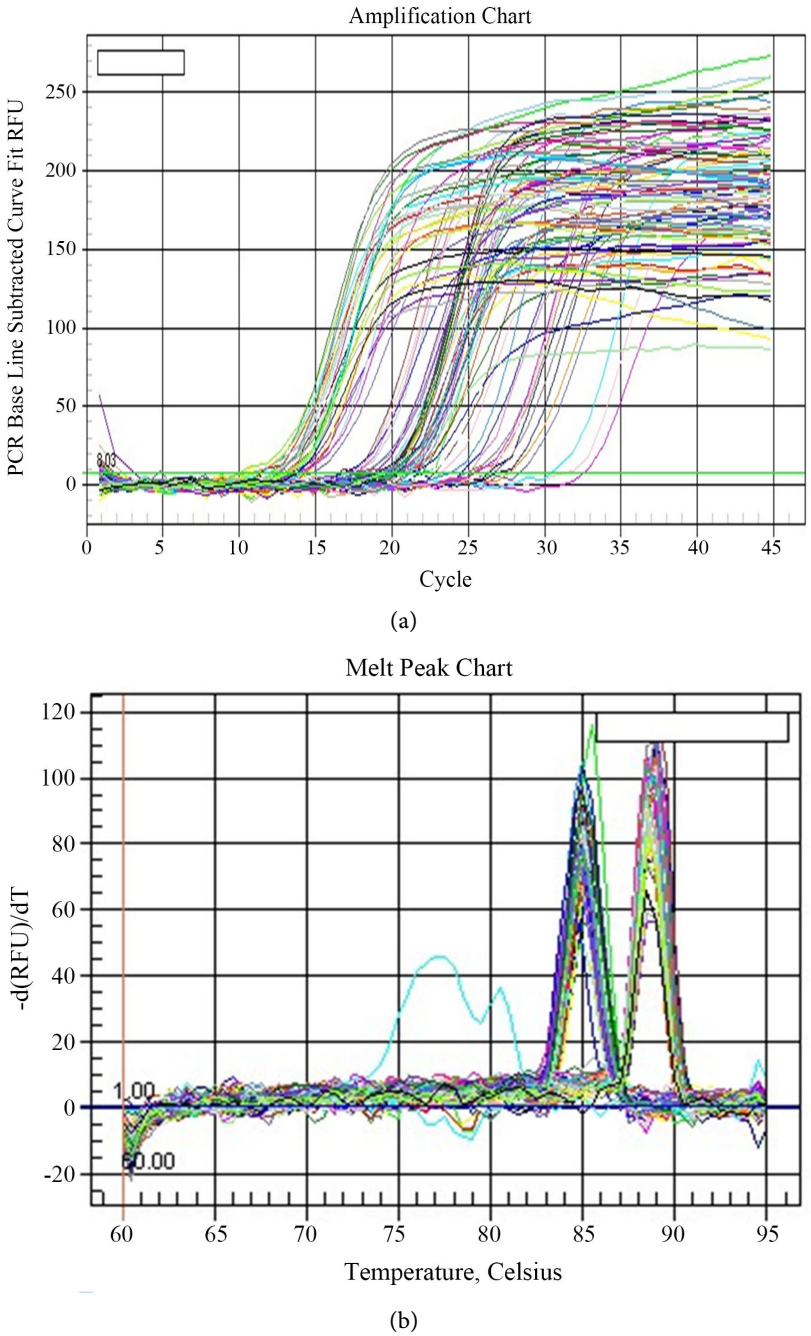


Figure 2. PDGFR-*α* mRNA amplification curve and fusion curve (a) amplification curve, (b) fusion curve.

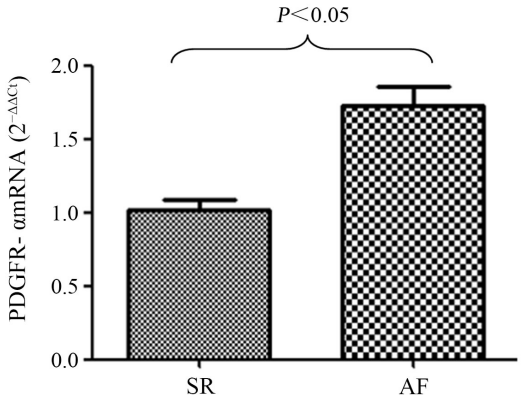


Figure 3. PDGFR-*α* of atrial tissue in the SR group and AF group. Comparison of mRNA expression.

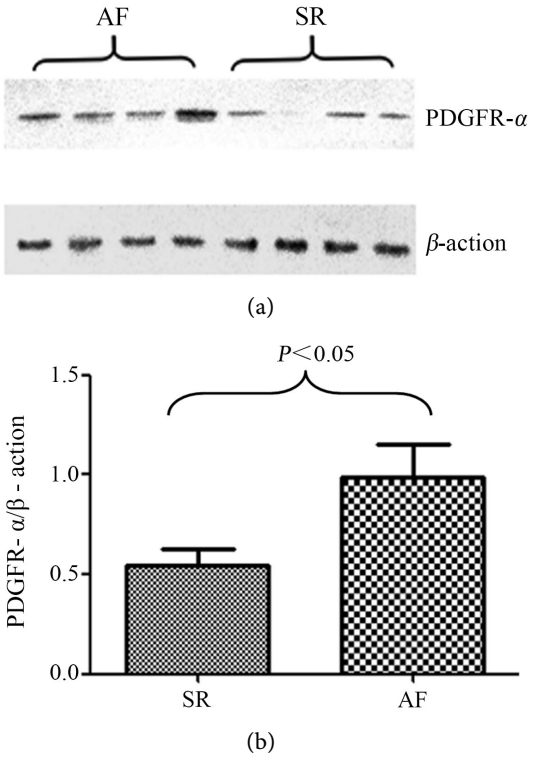


Figure 4. PDGFR-*α* of atrial tissue in the SR group and AF group. Comparison of protein expression.

3.4. Determination of PDGFR-*α* in Atrial Tissues of Patients in SR Group and AF Group by Real-Time Fluorescent Quantitative PCR MRNA Relative Expression of ($2^{-\Delta\Delta Ct}$)

PDGFR-*α* in atrial tissue was detected in the relative mRNA expression ($2^{-\Delta\Delta Ct}$) in the AF group and SR group was 1.725 ± 0.135 and 1.017 ± 0.068, respectively. After statistical processing, the result shows PDGFR-*α*, the relative expression of mRNA ($2^{-\Delta\Delta Ct}$) in the AF group was also markedly greater than SR group, with remarkable statistics significance between groups ($P < 0.05$, t = 3.922), as shown in **Figure 3**.

3.5. PDGFR-α of Atrial Tissue between SR Group and AF Group Results of Protein Level Determination

Application of Western Blot Technique to measure PDGFR-α in atrial tissue of patients' relative expression of proteins. The relative expression of the protein was scanned and imaged with the GelDoc100 imaging system, and the results were recorded. Relative expression of PDGFR-α/β-Action) in the AF group and SR group were 0.989 ± 0.162 and 0.546 ± 0.08, respectively. After statistical treatment, the results showed that the PDGFR-α of AF patients. Relative expression of PDGFR-α/β-Action) was also significantly higher than that of the SR group, with a significant statistical difference between groups ($P < 0.05$, t = 2.482), as shown in **Figure 4**.

4. Discussion

PDGFs are 24 ku cationic glycoproteins, mainly present in platelets α Granules and also existing in damaged endothelial cells, macrophages migrating under the endothelium, smooth muscle cells, fibroblasts, mesangial cells and other cells. These cells release a large amount of PDGFs in the form of autocrine and paracrine [3] [4] chain amplification reactions, and mediate the interaction between endothelium and stroma in various tissues [5] [6]. PDGFs can promote the mitosis and chemotaxis of fibroblasts and vascular smooth muscle cells, and also regulate the content of collagen, fibronectin, proteoglycan, hyaluronidase, and collagenase in the extracellular matrix, in connective tissue remodeling playing an important role. Due to its important role in tissue repair, fibrosis, tumor cell proliferation, and immune response, it has become a research hotspot for many scholars. PDGF-A is a member of the PDGF family. Its peptide chain consists of 196 and 211 amino acid residues. It is highly expressed in the heart, skeletal muscle and pancreas [7] [8] [9] and passes through the specific receptor PDGFR-α on the plasma membrane. It binds and crosses the plasma membrane to transmit information to the cell interior, and generates response through a series of actions. PDGFR-α is mainly distributed in cells of mesenchymal or glial origin: osteoblasts, chondrocytes, fibroblasts, glial cells and smooth muscle cells.

Studies confirm PDGF-A and its receptor PDGFR-α play an important role in the development of myocardial fibrosis. PDGFR-α was in acute rejection of heart transplantation. The expression of PDGF-A and PDGF cmRNAs was significantly up-regulated in chronic heart transplantation rejection [10]. Studies confirm PDGF-A and its receptor PDGFR-α play a significant role in the development of myocardial fibrosis. The expression of PDGFR-α was significantly up-regulated in acute cardiac allograft rejection, while the expression of PDGF-A and PDGF-cmRNAs was significantly up-regulated in chronic cardiac allograft rejection [10]. Relevant studies confirm that: in the process of myocardial fibrosis, PDGFR-α is more important than its ligand PDGF-A. Intravenous PDGFR-α Specific antibodies can alleviate atrial fibrosis [10] [11]. In previous studies, the characteristics of cardiac structural remodeling were compared between the AF group and SR group. The studies indicated that the atrial diameters in the atrial

fibrillation group were apparently greater than those in the SR group; Atrial tissue in the atrial fibrillation group has visible fibrosis changes; the relative expression of type I collagen mRNA ($2^{-\Delta\Delta Ct}$) in the atrial muscle samples from patients with atrial fibrillation was markedly higher than that in patients with sinus rhythm. It is suggested that the occurrence and development of atrial fibrosis in patients with rheumatic valvular disease and atrial fibrillation may be related to the up-regulation of the transcription level of type I collagen gene, the increase of synthesis and secretion of type I collagen, which leads to the abnormal accumulation of collagen in atrial tissue and the imbalance of I/III collagen deposition ratio, which provides a pathological matrix for the occurrence and maintenance of atrial fibrillation [12] [13] [14]. Whether PDGF/PDGFR signal is involved in the occurrence, development and maintenance of atrial fibrosis due to valvular atrial fibrillation by up-regulating the transcription level of type I collagen gene, it leads to the increase of fibroblast synthesis and secretion of type I collagen, and abnormal accumulation of collagen in atrial tissue remains to be further studied.

In this part, real-time fluorescence quantitative PCR was used to determine PDGFR-α in atrial muscle tissue samples of patients with sinus rhythm and atrial fibrillation mRNA relative expression. PDGFR-α was found in atrial muscle tissue samples of patients with AF, and the relative expression of mRNA in the AF group was markedly higher than that in the SR group ($P < 0.05$). Furthermore, the relative expression of PDGFR-α protein in atrial muscle tissue samples of patients with AF was also significantly greater than that in the SR group ($P < 0.05$), as determined by the Western Blot technique. This provides a theoretical basis for further studies on the relationship between the PDGF/PDGFR signaling pathway [15]-[20] and atrial fibrosis in patients with AF.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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