Dysregulated Atrial Gene Expression of Osteoprotegerin/ Receptor Activator of Nuclear Factor- κ B (RANK)/ RANK Ligand Axis in the Development and Progression of Atrial Fibrillation

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Background: Atrial structural remodeling is increasingly emphasized in initiation and perpetuation of atrial fibrillation (AF). Osteoprotegerin (OPG)/receptor activator of nuclear factor- κ B (RANK)/RANK ligand (RANKL) axis, a key regulatory system in bone homeostasis, was recently linked to some cardiovascular disorders for its regulatory functions to myocardial remodeling. It was hypothesized that OPG/RANK/RANKL axis is involved in the development and progression of AF by regulating atrial structural remodeling.

Methods and Results: Clinical data, and biopsies of right atrial appendage were collected from sex- and age-matched subjects: 24 persistent AF patients, 24 paroxysmal AF patients, 24 sinus rhythm patients undergoing isolated mitral valve surgery and 24 healthy heart donors. AF groups had higher atrial gene expression of OPG/RANK/RANKL axis and RANKL/OPG ratio, particularly in paroxysmal AF. This upregulated expression and activity were positively correlated with higher regulatory indicators of atrial structural remodeling as reflected by higher transcripts of tumor necrosis factor (TNF)-related apoptosis-inducing ligand, matrix metalloproteinase (MMP)-2 and MMP-9, pro-inflammatory factors TNF- α and interleukin-6, and higher ratios of MMP-9/tissue inhibitor of metalloproteinase (TIMP)-1 and MMP-2/TIMP-2 in AF.

Conclusions: The present findings suggest a potential role for known mediators of bone metabolism in the development and progression of AF and possibly represent new targets for therapeutic intervention in this disorder. (*Circ J* 2011; **75**: 2781–2788)

Key Words: Atrial fibrillation; Atrial structural remodeling; Osteoprotegerin; Receptor activator of nuclear factor- κ B; Receptor activator of nuclear factor- κ B ligand

Atrial fibrillation (AF) is the most common cardiac arrhythmia and its prevalence is increasing all over the world. It can induce and aggravate heart failure (HF) and increase the risk of thromboembolism.¹ The fundamental mechanism underlying AF had long been considered to be electrical remodeling,² but limited success rates and uncertain long-term outcomes of current AF therapies based on this hypothesis of electrical remodeling, indicate that the pathophysiology of AF is much more complex than previously thought.³ In the recent decade, increasing experimental and clinical stud-

ies have demonstrated that atrial structural remodeling, especially interstitial fibrosis,⁴ myocardial apoptosis⁵ and inflammation,⁶ occurs in the initiation and perpetuation of AF, creating more favorable substrates for AF. Therefore, targeting the regulation of atrial structural remodeling may be a promising therapeutic direction.

Osteoprotegerin (OPG)/receptor activator of nuclear factor- κ B (NF- κ B; RANK)/RANK ligand (RANKL) axis is an essential pathway for paracrine signaling in bone metabolism.⁷ Recent experimental, clinical, and epidemiological data, how-

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Table 1. Real-Time PCR Primers

Target	Sequence (5'→3')	GenBank accession no.	Melting temperature (°C)
OPG	(+)-AAGGGCGCTACCTTGAGATAG (-)-GCAAAGTGTATTTGCTCTGGG	NM_002546	61
RANKL	(+)-GCCAGCAGAGACTACCAAG (-)-GAGGGCCACGAACATGGAG	NM_003701	62
RANK	(+)-GCTCCTCCATGTACCAAGTGG (-)-ACTGTCAGAGGTAGTAGTCATT	NM_003839	61
TRAIL	(+)-AGTGAGAGAAAGAGGTCTCAG (-)-CCAGAGCCTTTTCATTCTTGA	NM_003810	60
MMP-2	(+)-GCCCCAGACAGGTGATCTTG (-)-GCTTGCGAGGGAAGAAGTTGT	NM_004530	63
MMP-9	(+)-GGGACGCAGACATCGTCATC (-)-TCGTCATCGTCGAAATGGGC	NM_004994	63
TIMP-1	(+)-CTTCTGCAATCCGACCTCGT (-)-CCCTAAGGCTTGGAAACCTTT	NM_003254	62
TIMP-2	(+)-AAGCGGTCAAGTGAAGAAG (-)-GGGGCCGTGTAGATAAACTCTAT	NM_003255	61
TNF- α	(+)-ACCCTCAGACGCCACATCCC (-)-GTCCCGGATCATGCTTTCAGT	NM_004428	58
IL-6	(+)-CGGGAACGAAAGAGAAGCTC (-)-AGGGAAGGCAGCAGGCAACAC	NM_000600	58
GAPDH	(+)-ATGGGGAAGGTGAAGGTCG (-)-GGGGTCATTGATGGCAACAATA	NM_002046	61

(+), forward primers; (-), reverse primers.

PCR, polymerase chain reaction; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor- κ B ligand; RANK, receptor activator of nuclear factor- κ B; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; IL, interleukin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Mitral Valve Surgery Patient Characteristics

Parameters	SR	PaAF	PeAF	P value
M/F (n)	10/14	10/14	10/14	1.000
Age (years)	46.0 $\pm$ 10.1	45.9 $\pm$ 10.0	45.9 $\pm$ 9.4	0.999
Heart rate (beats/min)	79 $\pm$ 6	84 $\pm$ 12	83 $\pm$ 17	0.276
Mean artery pressure (mmHg)	89.8 $\pm$ 10.7	90.4 $\pm$ 9.6	87.0 $\pm$ 9.9	0.381
C-reactive protein (mg/L)	2.97 $\pm$ 0.94	4.29 $\pm$ 2.09*	4.80 $\pm$ 1.52**	<0.001
Duration of valvular disease (years)	5.3 $\pm$ 4.6	7.1 $\pm$ 4.1	10.1 $\pm$ 5.7**†	0.003
NYHA class I/II/III/IV (n)	4/8/10/2	3/8/11/2	1/10/12/1	0.853
Echocardiography parameters				
LVDd (mm)	49.8 $\pm$ 8.4	49.8 $\pm$ 7.2	51.7 $\pm$ 9.6	0.648
EF (%)	64.4 $\pm$ 3.1	62.8 $\pm$ 4.6	63.5 $\pm$ 4.6	0.442
LAD (mm)	43.0 $\pm$ 7.0	51.9 $\pm$ 8.4**	57.9 $\pm$ 9.1**†	<0.001
RAD (mm)	39.9 $\pm$ 4.2	42.7 $\pm$ 4.8*	45.8 $\pm$ 6.4**†	0.003
PASP (mmHg)	32.8 $\pm$ 8.5	42.2 $\pm$ 11.5**	49.9 $\pm$ 15.7**	<0.001
Left atrial thrombus (n)	0	3	6**	0.032
Preoperative length of stay (days)	10.4 $\pm$ 4.7	15.4 $\pm$ 5.3**	14.5 $\pm$ 6.3*	0.004
Cause of mitral valve disease (n)				
Rheumatic/Degenerative	16/8	17/7	23/1**†	0.032
Preoperative anti-arrhythmics (n)				
Digitalis	22	22	22	1.000
Calcium-channel blocker	2	1	3	0.580
β -blocker	4	7	8	0.395
ACE-I	3	7	8	0.211

Data given as mean $\pm$ SD or number of patients.

*P<0.05, **P<0.01 (SR is reference group); †P<0.05 (PaAF is reference group).

SR, sinus rhythm; PaAF, paroxysmal atrial fibrillation; PeAF, persistent atrial fibrillation; NYHA, New York Heart Association; LVDd, left ventricular end-diastolic diameter; EF, ejection fraction; LAD, left atrial diameter; RAD, right atrial diameter; PASP, pulmonary artery systolic pressure; ACE-I, angiotensin-converting enzyme inhibitor.

Figure 1. Gene expression of (A) osteoprotegerin (OPG), (B) receptor activator of nuclear factor- κ B ligand (RANKL), (C) receptor activator of nuclear factor- κ B (RANK), (E) tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and (D) RANKL/OPG and (F) TRAIL/OPG ratios in right atrial appendages of mitral valve surgery patients with persistent atrial fibrillation (PeAF), paroxysmal atrial fibrillation (PaAF) and sinus rhythm (SR) matched with normal controls (NC). GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Data given as mean $\pm$ SEM. *P<0.05 between groups; **P<0.01 between groups.

ever, have also linked this axis to a range of cardiovascular disorders, including diabetic macroangiopathy,⁸ aortic aneurysm,⁹ cardiac valvular disease,¹⁰ and different manifestations of atherosclerosis.¹¹ As members of the tumor necrosis factor (TNF) superfamily, the roles of OPG and RANKL had been established in the regulation of inflammatory responses¹² and extracellular matrix remodeling,¹³ and the exertion of anti-apoptotic effects.¹⁴ As an illustration of these widespread effects, an interesting study using human myocardial tissue and a rat HF model demonstrated that interactions among OPG, RANKL and RANK might be implicated in the pathogenesis of HF through different mechanisms such as promo-

tion of matrix degradation and inflammation.¹⁵ Moreover, a significant association was recently identified between circulating OPG concentration and AF incidence in a community-based study.¹⁶

According to the aforementioned findings, we hypothesized that the OPG/RANK/RANKL axis is involved in the development and progression of AF by regulating atrial structural remodeling. To test this hypothesis, the aim of the present study was to measure gene expression of OPG, RANK and RANKL in right atrial appendage (RAA) tissues of AF and sinus rhythm (SR) patients matched with normal controls (NC). Furthermore, to explore the possible mechanism, we investi-

Figure 2. Gene expression of (A) matrix metalloproteinase (MMP)-9, (B) MMP-2, (C) tissue inhibitor of metalloproteinase (TIMP)-1, (D) TIMP-2, and (E) MMP-9/TIMP-1 and (F) MMP-2/TIMP-2 ratios in the right atrial appendages of mitral valve surgery patients with persistent atrial fibrillation (PeAF), paroxysmal atrial fibrillation (PaAF) and sinus rhythm (SR) matched with normal controls (NC). GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Data given as mean±SEM. **P<0.01 between groups.

gated gene expression of regulatory indicators reflecting apoptosis, matrix degradation and inflammation in RAA tissues.

Methods

Patients

We have been building a biobank for AF research in the First Affiliated Hospital of Nanjing Medical University and the Affiliated Drum Tower Hospital of Nanjing University Medical School since November 2007. It was constructed according to the Helsinki Declaration and approved by the ethics committees of Nanjing Medical University and Nanjing University. On receiving written informed consent, we collected atrial

tissue during surgery, preoperative venous blood and detail clinical data.

In the present study, we included the patients undergoing isolated mitral valve surgery and excluded some of them due to the following criteria: (1) age>65 years or history of cancer; (2) abnormal thyroid function; (3) occurrence of bone fracture within the previous 6 months; (4) concomitant coronary artery disease; (5) aortic or valvular calcification; (6) bacterial endocarditis or rheumatic activity; and (7) complicated diabetes or renal dysfunction (serum creatinine >136 μmol/L). Finally, we recruited 24 patients with preoperative paroxysmal AF (PaAF), 24 sex- and age-matched patients with persistent AF (PeAF) and 24 sex- and age-matched patients with SR. NC were 24

sex- and age-matched healthy heart donors. They were trauma victims and were free of cardiovascular pathology and documented AF.

Tissue Collection and Storage

The RAA tissue for each patient was obtained from the cannulation site before starting extracorporeal circulation. The surgical maneuver was the same in each patient to ensure consistency of sampling site. RAA specimens were obtained from donor hearts before perfusion. After excision, RAAs were immediately snap-frozen in liquid nitrogen for real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) assay.

Real-Time Quantitative RT-PCR

The primers of OPG/RANK/RANKL axis, TNF-related apoptosis-inducing ligand (TRAIL), matrix metalloproteinase (MMP)-2 and MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2, and pro-inflammatory factors TNF- α and interleukin (IL)-6 were synthesized by Invitrogen, Hong Kong (Table 1). Total RNA was isolated from frozen RAAs by acid-phenol extraction in the presence of chaotropic salts (TRIzol, Invitrogen) and subsequent isopropanol ethanol precipitation. The synthesis of cDNA was carried out with the reverse transcriptase kit (Promega, US) according to the manufacturer's instructions. Real-time PCR was performed using the LightCycler 480 system (Roche Diagnostics, Switzerland) with a total volume of 20 μ l containing 10 μ l 2 $\times$ Master Mix SYBR Green I (Takara, Japan), 0.25 μ mol/L forward primers, 0.25 μ mol/L reverse primers, 2 μ l cDNA template, and H₂O to a final volume of 20 μ l. The protocol of real-time PCR consisted of 40 cycles, and cycling parameters were as follows: denaturation at 94°C for 10 s, annealing at 58–63°C (Table 1) for 15 s and extension at 72°C for 20 s. The results were analyzed using Roche LightCycler 480 software. Data of target mRNA copies were calculated relative to human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta C_t}$ method. The measurements of each sample were performed in triplicate.

Statistical Analysis

For the comparison among groups, 1-way ANOVA (normally distributed) or Mann–Whitney test (2 groups, non-normally distributed) and Kruskal–Wallis test (n groups, non-normally distributed) were used for continuous variables, and the chi-squared test was utilized for categorical variables. Correlation analysis (Pearson or Spearman) was used to assess the association between atrial gene expression of OPG/RANK/RANKL axis and mRNA levels of TRAIL, MMPs, TIMPs and pro-inflammatory factors in RAAs. The criterion of statistical significance used was a 2-sided $P < 0.05$. Statistical analysis was performed with GBSTAT (version 9.0, Dynamic Microsystems).

Results

Clinical Characteristics

The SR, PaAF, PeAF and NC groups all had 24 sex- and age-matched subjects, therefore age and gender distribution were equal among them. C-reactive protein and pulmonary artery systolic pressure, as well as preoperative length of stay were higher or longer in the 2 AF groups than in the SR group. Duration of valvular disease was longer in PeAF than SR and PaAF. Left and right atrial diameters were largest in PeAF, followed by PaAF and SR. The proportion of rheumatic etiol-

ogy and left atrial thrombus was higher in PeAF than in SR, and the former was also higher in PeAF than PaAF. Other parameters, summarized in Table 2, were not significantly different among groups.

Gene Expression of OPG/RANK/RANKL Axis in RAAs

On real-time quantitative RT-PCR an increasing gradient of gene expression of OPG, RANKL and RANK was seen in the NC, SR and AF groups, although there was no difference between PaAF and PeAF (Figures 1A–C). RANKL/OPG ratio (Figure 1D) was highest in PaAF, followed by PeAF, SR and NC.

Gene Expression of TRAIL, MMPs, TIMPs and Pro-Inflammatory Factors in RAAs

Gene expression of TRAIL was highest in PeAF and PaAF, between which there was no significant difference ($P = 0.07$), followed by SR and NC (Figure 1E). Moreover, the TRAIL/OPG ratio was lowest in NC and SR, between which there was no significant difference ($P = 0.585$), followed by PaAF and

Table 3. Correlations Between Atrial Gene Expression of OPG/RANK/RANKL Axis and Transcripts of TRAIL, MMPs, TIMPs and Pro-Inflammatory Factors in RAAs

	Correlation coefficient			
	OPG	RANK	RANKL	RANKL/OPG
TRAIL	0.542**	-0.026	0.105	0.246*
MMP-2	0.198	0.307**	0.424**	0.613**
MMP-9	0.178	0.272**	0.312**	0.568**
TIMP-1	0.032	-0.143	-0.152	-0.260*
TIMP-2	-0.065	-0.175	-0.189	-0.332**
MMP-9/TIMP-1	0.391**	0.286**	0.423**	0.669**
MMP-2/TIMP-2	0.379**	0.311**	0.528**	0.715**
TNF- α	0.225*	0.193	0.373**	0.625**
IL-6	0.202	0.142	0.258*	0.476**

*P<0.05, **P<0.01.

RAA, right atrial appendage. Other abbreviations see in Table 1.

PeAF (Figure 1F).

Gene expression of MMP-2 gradually increased in NC, SR, PaAF and PeAF (Figure 2B). TIMP-1 mRNA level was similar in all the groups (Figure 2C), whereas TIMP-2 was upregulated in both SR and PaAF compared with NC and PeAF (Figure 2D). MMP-9 level and MMP-9/TIMP-1 ratio were highest in PeAF and PaAF, between which there was no significant difference ($P=0.781$ and $P=0.212$, respectively), followed by SR and NC (Figures 2A,E). MMP-2/TIMP-2 ratio was found to be higher only in PeAF, compared with PaAF, SR and NC (Figure 2F).

The TNF- α mRNA level was highest in PeAF and PaAF, between which there was no significant difference ($P=0.830$), followed by SR and NC (Figure 3A). Additionally, IL-6 level was highest in PeAF and lowest in NC, whereas the middle 2 groups were not significantly different ($P=0.540$; Figure 3B).

Correlations Between Gene Expression of OPG/RANK/RANKL Axis and Transcripts of TRAIL, MMPs, TIMPs and Pro-Inflammatory Factors in RAAs

As shown in Table 3, TRAIL correlated positively with OPG and RANKL/OPG ratio. MMP-2 and MMP-9 correlated positively with RANK, RANKL and RANKL/OPG ratio. A negative correlation was found between TIMP-1, TIMP-2 and RANKL/OPG ratio. Moreover, MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios were correlated positively with OPG, RANK, RANKL and RANKL/OPG ratio. TNF- α and IL-6 were correlated positively with RANKL and RANKL/OPG ratio. A weak but significant positive correlation was also found between TNF- α and OPG ($P=0.042$).

Discussion

The present study has explored for the first time the alterations of atrial expression of the OPG/RANK/RANKL axis in the process of development and progression of AF. An increasing gradient of gene expression of OPG, RANKL, RANK and RANKL/OPG ratio was identified in NC, SR and AF groups. RANKL/OPG ratio, a more reliable marker of OPG/RANK/RANKL axis activity,¹⁵ was simultaneously found to be higher in PaAF than PeAF. Furthermore, the axis was statistically correlated with gene expression of TRAIL, the MMP/TIMP system and pro-inflammatory factors in RAAs. Therefore, the present data support the findings of Schnabel et al¹⁶ and reinforce the growing evidence that the OPG/RANK/RANKL axis might be one of the regulatory systems in the atrial structural

remodeling of AF.

Apoptosis has been noted in the atrial tissue of both pacing-induced AF animals¹⁷ and chronic AF patients.¹⁸ In the pacing-induced AF model, apoptosis was also demonstrated to occur prior to other arrhythmogenic structural remodeling.¹⁷ Moreover, we previously showed that a higher degree of apoptosis was associated with early recurrence of AF after surgery.¹⁹ TRAIL, another member of the TNF superfamily, is able to mediate apoptosis via the caspase pathway by binding to its receptors.²⁰ Interestingly, a pilot clinical study recently found elevated levels of soluble TRAIL in AF patients and observed that the concentration of TRAIL decreased after SR restoration, although they did not confirm its atrial origin.²¹ As a decoy receptor, OPG can combine TRAIL and inhibit TRAIL-induced apoptosis. It was first demonstrated in endothelial cells,²² then in rheumatoid fibroblast-like synovial cells²³ and a cancer cell.²⁰ In the present study we have provided the first evidence of enhanced atrial gene expression of TRAIL and higher TRAIL/OPG ratio in AF, particularly in PeAF. It is intriguing to speculate that activation of apoptosis by TRAIL, which can be neutralized by formation of TRAIL/OPG complex, may play an important role in the development and progression of AF.

Interstitial fibrosis, the most predominant structural remodeling in AF, results from an excessive accumulation of collagen fibers within atria, particularly due to disruption of the equilibrium between synthesis and degradation of collagen.²⁴ The degradation of collagen is mainly regulated by MMPs and TIMPs.²⁵ The expression of gelatinases (MMP-2 and -9) and their inhibitors (TIMP-1 and -2) is dysregulated when atrial fibrosis occurs,²⁵ and the increase of the corresponding ratios can promote fibrosis.²⁶ In the present study we found increased transcript of MMP-2 and MMP-9 and decreased gene expression of TIMP-2 in RAAs of AF (particularly PeAF) patients, which is in accordance with the previous findings.^{25,27,28} A concomitant increase of atrial MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios, however, although demonstrated in failing heart previously,²⁶ is noted for the first time in AF in the present study. More importantly, we have also identified a convincingly positive correlation between MMP/TIMP ratios and the OPG/RANK/RANKL axis.

In clinical and experimental HF, 2 previous studies demonstrated that upregulation of RANKL contributed to enhanced MMP activity (MMP-2 and -9 in particular), as well as a modest decrease in TIMP expression in human fibroblasts, which suggested a potential mechanism by which activation of the OPG/RANK/RANKL axis might result in matrix degradation, adverse ventricular remodeling, and worsening myocardial function.^{15,29} In support of this deduction, Halapas et al recently found that increased serum levels of RANKL and MMP-1/TIMP-1 were positively correlated with each other, 6 months after myocardial infarction, suggesting a potential regulatory effect of RANKL on myocardial healing after myocardial infarction.¹¹ In addition, anti-RANKL or OPG treatment were further demonstrated to completely inhibit the induction of IL-17 on the upregulation of MMP-1 in cardiac fibroblasts, as well as to inhibit myocardial fibrosis.³⁰ As a compensatory mechanism to neutralize the effect of RANKL, OPG may theoretically represent a protective counter-regulatory response aimed at limiting the induction of MMP activity by inhibiting RANKL binding to RANK. Moran et al, however, recently reported that OPG could increase MMP-9 activity (2-fold) and MMP-2 activity (1.5-fold) in THP-1 cells,⁹ suggesting that it acts as a double-edged sword in matrix degradation. Thus, together with the present findings, it is not inconceivable that enhanced atrial

expression and activity of the OPG/RANK/RANKL axis could promote matrix degradation within atria by stimulating the activity of the MMP/TIMP system, ultimately contributing to the development and progression of AF.

Several pathways are activated by this ligand–receptor interaction, but of particular importance is the NF- κ B pathway, which is activated in human AF atria, and is the bridge between inflammation and AF.³¹ In fact, there is increasing evidence that AF is associated with inflammatory conditions, such as cardiac surgery, myocarditis and pericarditis.⁶ Patients with lone AF were also found to have existing myocarditis within atrial tissues.³² Moreover, we previously demonstrated that atrial TNF- α and IL-6 levels were associated with early recurrence of AF after surgery.¹⁹ Inflammation can perpetuate adverse electrical and structural remodeling and promote development and chronicity of AF.³²

Ueland et al recently demonstrated enhanced RANKL expression in T cells from HF patients,¹⁵ and it is tempting to hypothesize that the interaction between T cells expressing RANKL and dendritic cells,³³ for example, in the failing myocardium, may represent a link between adaptive and innate immune responses, leading to both persistent and increased inflammation in HF patients.¹⁵ In an in vitro study, microvascular endothelial cells, in the presence of RANKL, were seen to promote intimal migration of monocytes.³⁴ Additionally, higher RANKL/OPG ratios were found in some inflamed tissues.³⁵ To our knowledge, OPG acts as a decoy receptor, binding to RANKL, preventing the interaction between RANK and RANKL.⁷ Therefore OPG appears to have 2 different roles in this scenario. On the one hand, its neutralizing function may be considered beneficial, with OPG rises representing a partly protective counter-regulatory response aimed at limiting inflammation. On the other hand, binding of OPG with RANKL possibly inhibits the rapid clearance of RANKL in atria, stabilizing its levels, thus seemingly augmenting its pro-inflammatory actions.

Study Limitations

First, by protocol, left atrial tissue was not obtained. Thus, possible differences between right and left atrial expression of these indicators could not be investigated. Second, atrial mRNA expression was investigated without measurement of protein expression and location, pathological and binding experiments, or plasma level determinations. Such additional data might have provided more insight into the pathophysiology. Finally, due to the nature of the experiment, the present data could not confirm a causal relationship between the OPG/RANK/RANKL axis and atrial structural remodeling. Future animal research should address this issue.

Conclusions

The present study has shown that enhanced atrial gene expression and activity of the OPG/RANK/RANKL axis may contribute to the development and progression of AF by regulating atrial structural remodeling. This suggests a potential role for known mediators of bone homeostasis in the pathogenesis of AF and possibly represents new targets for therapeutic intervention in this disorder.

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Supplemental Files

Supplemental File 1

Table S1. Correlations Between Atrial Gene Expression of OPG/RANK/RANKL Axis, Regulatory Indicators of Atrial Structural Remodeling and PASP, LAD, RAD

Please find supplemental file(s);
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