論文の内容の要旨

論文題目　Cardiac Mast Cells Cause Atrial Fibrillation Through PDGF-A-mediated Fibrosis in Pressure-overloaded Hearts

和訳　　心臓肥満細胞由来の PDGF-A が圧負荷心において心房細動発症を促進する

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Introduction

Atrial fibrillation (AF) is a supraventricular arrhythmia that is characterized by rapid and fibrillatory atrial activation with an irregular ventricular response. AF remains the most common arrhythmia encountered in clinical practice, and is associated with an increased risk of stroke, heart failure, and overall mortality. Several cardiovascular disorders predispose to AF, such as coronary artery disease, valvular heart disease, congestive heart failure, and hypertension, especially when LV hypertrophy is present. The most important histopathological change in AF is atrial fibrosis. Experimental studies using animal models have indicated that interstitial deposition of dense ECM proteins causes separation between bundles of atrial myocytes, and disturbs cell-to-cell impulse propagation. In addition, atrial fibrosis potentially exaggerates myocardial ischemia by hampering oxygen diffusion, and alters the electrophysical and biomechanical properties of atrial myocytes, allowing the initiation and perpetuation of AF. The mechanisms underlying the development of atrial fibrosis in AF remain unclear, but evolving evidence has suggested that

inflammation is profoundly implicated in the process of the structural remodeling in the atrium. Inflammatory infiltrates were observed in the atrium of AF patients and animal models. However, it remains to be fully elucidated how inflammation is linked to the development of structural remodeling as a susceptible AF substrate in stressed hearts.

Mast cells function as key effector cells during allergic and immune responses through releasing preformed or newly synthesized bioactive products. Recent studies have implicated mast cells in inflammation and tissue remodeling. Indeed, mast cells reside in many tissues including the heart, and participate in the inflammatory process underlying several cardiovascular disorders, such as atherosclerosis, aortic aneurysm, heart failure, viral myocarditis, and ventricular arrhythmia during ischemia/reperfusion injury. Here we demonstrate that mast cells infiltrate the atrium of pressure-overloaded mice, and contribute to the pathogenesis of atrial fibrosis and AF susceptibility. Mechanistically, up-regulation of PDGF-A mediates the fibrogenic effect of mast cells in promoting AF. These results provide mechanistic insights into the pathogenic role of mast cells in promoting an AF substrate in stressed hearts.

Results

Atrial burst stimulation induces AF in pressure-overloaded hearts

To develop a model of AF associated with LV hypertrophy, we induced pressure overload in mice by producing transverse aorta constriction (TAC). At 10 days after TAC operation, LV became hypertrophied and heart-to-body-ratio increased. The ECGs recorded at 10 days after TAC operation revealed no episode of spontaneous AF in either TAC- or sham-operated mice. To test the inducibility of AF, we applied programmed electrical stimulation directly to right atrium under Langendorff-perfusion at 10 days after the operation. The induction of AF was attainable and reliably reproducible with programmed electrical stimulation of right atrium. AF was induced more frequently and the duration of AF episode was significantly longer in TAC-operated hearts than in sham-operated hearts.

Mast cells are accumulated and activated in the atrium of TAC-operated mice

To assess the contribution of mast cells to atrial arrhythmogenicity, we evaluated the contents of mast cells in atrium by staining histological sections with toluidine blue and avidin. The number of infiltrating mast cells increased 2.5-fold in TAC-operated mice on day 10 when comparing to sham-operated mice. We also observed a marked increase of mast cell activation with the presence of degranulation in TAC-operated mice.

Stabilization of mast cells by cromolyn attenuates AF in TAC-operated hearts

To test the importance of mast cells in the pathogenesis of AF, we systemically administered mast cell stabilizer cromolyn to TAC-operated mice and evaluated the AF inducibility. At 10 days after TAC operation, the degranulation of mast cells was almost completely inhibited under cromolyn treatment, however the mast cell contents were not decreased. Comparing to vehicle-treated mice, cromolyn-treated mice showed a remarkable reduction in the incidence and duration of AF episode after atrial burst stimulation under Langendorff-perfusion. The similar results were obtained in *in vivo* model under transesophageal atrial pacing, although the overall AF inducibility was decreased in *in vivo* model. In addition, mast cell stabilization by cromolyn remarkably attenuated fibrotic changes in the atrium of TAC-operated mice. These results suggest that stabilization of mast cells prevents atrial structural remodeling and AF inducibility in TAC-operated mice.

Reconstitution with BM cells from mast cell-deficient W/W^{v} mice attenuates AF in TAC-operated hearts

To further examine the role of mast cells in AF, we utilized mast cell-deficient WBB6F1-*Kit*$^{W/W\text{-}v}$ (W/W^{v}) mice carrying compound heterozygous mutations of *c-kit* (*Kit*^{W}, null; *Kit*$^{W\text{-}v}$, dominant negative). To circumvent the undesirable effects by altered c-kit signaling in non-hematopoietic cells, we reconstituted C57BL/6 mice with bone marrow (BM) cells from W/W^{v} mice or their control WBB6F1-*Kit*$^{+/+}$ (+/+) mice. The mast cells were absent in the atrium of mice reconstituted with BM cells from W/W^{v} mice. The incidence and duration of AF episode after atrial burst stimulation was remarkably reduced in mice reconstituted with W/W^{v} BM. Furthermore, atrial fibrosis was attenuated in mice reconstituted with W/W^{v} BM, compared with the mice reconstituted

with +/+ BM.

BMMCs co-cultured with cardiac myocytes or fibroblasts release PDGF-A to promote fibrinogenesis

To delineate mast cell-derived effectors that are involved in the promotion of atrial fibrosis, we examined the gene expressions of fibrosis-related effectors in BM-derived mast cells (BMMCs) after co-culture with cardiac myocytes or fibroblasts. We found that the mRNA level of murine *Pdgfa* in BMMCs was prominently elevated after co-culture with neonatal rat cardiac myocytes or fibroblasts. Furthermore, the mRNA level of *Pdgfa* was up-regulated in the atrium at 10 days after TAC operation, and it was significantly attenuated by reconstitution with W/W^v BM. These results suggest that mast cells infiltrating the atrium are activated to increase *Pdgfa* gene expression. We also found that the PDGF-AA concentration increased more than 3-fold in the supernatant of BMMCs and cardiac fibroblast co-culture , and this increase was remarkably blunted by stabilization of BMMCs with cromolyn during the co-culture. The conditioned medium of co-culture promoted cell proliferation of cardiac fibroblasts. The *Col3a1*mRNA expression in cardiac fibroblasts was up-regulated after co-culture with BMMCs, and it was blunted by the treatment with cromolyn or anti-PDGFR-α antibody. Thus, BMMC-derived PDGF-A can induce cell proliferation and collagen gene expression in cardiac fibroblasts.

Administration of PDGF-AA enhances AF susceptibility in normal hearts

To examine functional significance of atrial *Pdgfa* up-regulation in the development of AF substrate, we administered PDGF-AA or vehicle to non-operated mice and applied atrial burst stimulation. Administration of PDGF-AA for 10 days induced systemic tissue fibrosis, which was particularly prominent in atrium as compared with in ventricle. As a consequence, PDGF-AA-treated hearts showed a significant increase in the incidence and duration of AF episode after atrial burst stimulation under Langendorff-perfusion, compared with vehicle-treated hearts.

Neutralization of PDGFR-α attenuates AF in TAC-operated hearts

Finally, to examine the role of PDGF-A in the pathogenesis of AF substrate, we inhibited the actions of PDGF-A in TAC-operated hearts by systemic injection of a neutralizing antibody against

PDGFR-α (APA5). Neutralization of PDGFR-α induced a marked reduction in the incidence and duration of AF episode both after atrial burst stimulation under Langendorff-perfusion and after transesophageal atrial pacing *in vivo*. In addition, atrial fibrosis was attenuated in TAC-APA5 mice, compared with TAC-IgG mice. Thus, the effects of cromolyn treatment or BM reconstitution from W/W^v mice on AF substrate were reproduced by neutralization of PDGFR-α in TAC-operated hearts. These results suggest that PDGF-A mediates the deleterious effects of mast cells to promote atrial fibrosis and AF inducibility.

Conclusion

Our present study demonstrated a hitherto unknown role of mast cells in the development of a susceptible AF substrate. Mast cells were accumulated and activated in the atrium of pressure-overloaded mice, and pharmacological stabilization or genetic depletion of mast cells prevented atrial structural remodeling and reduced the incidence and duration of AF following atrial burst stimulation. Notably, infiltrating mast cells induced up-regulation of PDGF-A in the atrium and neutralization of PDGFR-α prevented atrial fibrosis and AF inducibility, indicating a pivotal role of PDGF-A in mast cell-triggered AF. Our study provides insights that mast cell-PDGF-A axis will be a promising therapeutic target as the upstream prevention of AF in stressed hearts.