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１．研究テーマ

細胞接着分子による心筋構造の制御に関する研究

２．本年度の研究業績

(1) 学会・研究会等における発表　有　・　無　（学会名・演題）

1．International Symposium: Cardiomyopathy and Heart Failure・Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy
2．第７回日本心不全学会学術総会・ Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy
3．第７回日本心血管内分泌代謝学会学術総会・ Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy

(2) 学会誌等に発表した論文　有　・　無　（雑誌名・論文名）

3．今後の研究計画

細胞は、細胞外マトリックスと接着し、マトリックス空間を移動し、自らの増殖や分化を能動的に制御すると考えられている。細胞の増殖分化や恒常性維持に不可欠な細胞接着は、いくつかのレセプター・ファミリーによって、巧妙に調節されている。中でも、細胞と細胞、細胞と細胞外マトリックス双方の接着にインテグリンとオステオポンチンが重要であることが示されている。一方、不全心筋では、心筋梗塞、心肥大、拡張型心筋症など原因となる疾患に関わらず、心筋リモデリングと呼ばれる機能的、構造的変化が起きる。再構築した心筋では、心筋細胞数が減少し、残存した心筋細胞への負荷が増えるため、心筋細胞は肥大し、細胞間結合が緩む。また、線維芽細胞増殖及び、細胞外マトリックス増加により、線維化が進展する。この過程に、アンジオテンシンⅡ、エンドセリンや各種サイトカインが関わることが知られている。心肥大・線維化において、レニンーアンジオテンシン系がオステオポンチンとインテグリンの発現修飾の関与をあきらかにする必要がある。これから、オステオポンチン遺伝子欠損マウスを用い、液性因子による心筋細胞肥大・線維化モデルを作成し、野生型マウスと比較し、インテグリンとオステオポンチンの関係を明らかにする。

4．指導責任者の意見

賈楠君は、平成１２年４月から当循環病態内科学分野の大学院生として在籍し、細胞接着分子による心筋構造の制御に関する研究に励んでいます。線維化の形成におけるオステオポンチンの役割を検討し、日本心不全学会学術総会などの三つの会議において研究の結果を発表しました。これから、論文を作成し、投稿する予定です。将来に彼が日本に留学の経験を生かして、日中医学の交流を深めていくことを期待できると思っています。今後とも、ご支援の程よろしくお願いいたします。

指導責任者氏名　北畠　顕　㊞

5．研究報告書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

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－日中医学協会助成事業－

アンジオテンシンIIによる心肥大において オステオポンチンは心臓の線維化と再構築に重要である

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ABSTRACT

Objective: Osteopontin (OPN) is reported to be up-regulated in several experimental models of cardiac fibrosis and remodeling however, its direct effects on those still remained unclear. In the present study, we examined the hypothesis that OPN is important for the development of cardiac fibrosis and remodeling in AII infusion model, using OPN deficient mice ($OP^{-/-}$). Moreover, we examined whether the effect of Eplerenone (Ep), a novel aldosterone receptor blocker, on the prevention of cardiac fibrosis and/or remodeling was mediated through the inhibition of OPN.

Methods and Results: WT or $OP^{-/-}$ mice ($OP^{-/-}$/AII) were treated with AII, infused at a rate 2 μg/kg/min for 4 weeks. WT mice received AII were also divided into two groups, control group (WT/AII) and Ep treatment group (WT/AII/Ep). While AII significantly elevated blood pressure (BP) in WT mice, BPs in $OP^{-/-}$/AII and WT/AII/Ep mice were significantly lower than that in WT/AII mice. AII caused both cardiac hypertrophy that was reflected by the increase of the left ventricular/body weight ratios, the myocyte areas, and the cardiac ANF mRNA expression, and cardiac fibrosis that was reflected by the increase of the perivascular fibrotic areas and the cardiac collagen I mRNA in WT mice. Ep and chronic depletion of OPN equally ameliorated the development of cardiac fibrosis, while only Ep abolished the development of cardiac hypertrophy. Interestingly, despite the reduced fibrosis, cardiac systolic function was significantly deteriorated in $OP^{-/-}$/AII mice by AII infusion.

Conclusions: These results suggest that OPN has a pivotal role in the development of AII induced cardiac fibrosis and remodeling. Moreover, the effect of Ep on the prevention of not cardiac hypertrophy but cardiac fibrosis might be partially mediated through the inhibition of OPN.

Key Words OPN, cardiac fibrosis, hypertrophy, remodeling, AII, aldosterone receptor blocker

INTRODUCTION

Osteopontin (OPN) is a recently discovered mediator of the profibrotic effects of AII.[5] Although first isolated from mineralized bone matrix, OPN has since been shown to be synthesized by several cell types, including cardiac myocytes, microvascular endothelial cells, and fibroblasts.[6] OPN appears capable of mediating diverse biological functions, including cell adhesion, chemotaxis, and signaling.[7] OPN has also been shown to interact with fibronectin and collagen, suggesting its possible role in matrix organization and/or stability.[8] AII strongly upregulates the expression of OPN in rat and human cardiac fibroblasts.[5, 9] Monoclonal antibody directed against OPN completely blocked the mitogenic effect of AII on cultured rat cardiac fibroblasts.[5] Recently, the expression of OPN in heart was reported to increase coincidently with the development of heart failure.[10] Moreover, recent report demonstrated that myocardial infarction caused exaggerated left ventricular dilation and reduced collagen deposition in OPN deficient mice. These results suggest that OPN has a pivotal role in the cardiac fibrosis and cardiac remodeling.

MATERIALS AND METHODS

Mice The creation of the OPN deficient mouse ($OP^{-/-}$) used in this study has been described previously. Eight-week-old male OPN KO (n=20) after 8 backcrosses to C57BL/6 and age-matched C57BL/6 male WT (n=50) mice were used. Mice were housed under climate-controlled conditions with a 12-hour light/dark cycle and were provided with standard food and water ad libitum. All protocols were approved by local institutional guidelines. Chronic Administration of Pressor Dose of AII An osmotic minipump (model 2004, Alza Corp) was implanted subcutaneously into each mice. Pressor doses of AII (2 μg/kg/min) and saline were administered for 4 weeks. WT and $OP^{-/-}$ mice (group $OP^{-/-}$/AII) were treated with AII. WT mice received AII were also divided into two groups, control group (group WT/AII) and Ep treatment group (group WT/AII/Ep). Ep treatment was done as previously described. Briefly, mice received rodent chow containing 0.32% Na^+ and 0.83% K^+ (No. 7012, Harland Teklad), and Ep was administered by chow supplemented with Ep at 2 mg/g (Research Diets). Echocardiographic Analysis Transthoracic echocardiography was performed at the end of the study using an EUB 8000 echocardiographic instrument (Hitachi-Medico, Tokyo, Japan) with our originally developed 10-MHz imaging transducer as described previously. Mice were anesthetized with pentobarbiturate (70mg/kg IP). After a good-quality two-dimensional image was obtained, M-mode images of the left ventricle were recorded. Intraventricular septum thickness, end-diastolic left ventricular internal diameter (EDD), end-systolic left ventricular internal diameter (ESD), and left ventricular posterior wall thickness were measured. All measurements were performed by use of the leading edge–to–leading edge convention adopted by the American Society of Echocardiography. Percent fractional shortening (%FS) was calculated as %FS=[(EDD-ESD)/EDD]x100 to estimate the cardiac systolic function. Isovolumic relaxation time (IRT) was measured to estimate the cardiac diastolic function. IRT was corrected by each RR interval time to compensate for the HR variance. RT-PCR Analysis Total RNA isolated from left ventricular myocardium was used for first-strand cDNA synthesis. The reverse transcription–polymerase chain reaction (RT- PCR) with selected primers was used for amplification of ANF, collagen-I (Col-I), OPN, and GAPDH mRNA. ANF primers used were 5'-CTCTGAGAGACGGCAGTGCT-3' (forward) and 5'-ACGGAGAGGGTGAGACGTAT-3' (reverse). Col-I primers used were 5'-AAACCCGAGGTATGCTTGATCTGTA-3' (forward) and 5'-GTCCCTCGACTCCTACATCTTCTGA-3' (reverse). OPN primers used were 5'-ATGAGATTGGCAGTGATTTGCTT-3' (forward) and 5'-TTAGTTGACCTCAGAAGATGCACTCT-3' (reverse). GAPDH primers used were 5'-ATGTTCCAGTATGACTCCACTCACG-3' (forward) and 5'-GAAGACACCAGTAGACTCCACGACA-3' (reverse). ANF, Col-I, and OPN sequences were amplified in a thermal cycler (Perkin-Elmer) for optimal cycles. The quality of RNA preparation and cDNA synthesis was verified by amplifying DNA coding GAPDH, a housekeeping protein, under the same conditions. RT-PCR products were visualized on 2% agarose gels with ethidium bromide. Signals were digitized and evaluated with an optical scanner (GT-9500, Seiko) with density measured with the use of an NIH image program in the public domain (Research Services Branch, NIH).

RESULTS

In this study, we demonstrated that:

1. Administration of AII induced the development of hypertension, cardiac hypertrophy, and cardiac fibrosis in WT mice.
2. Chronic loss of OPN by gene targeting abolished the development of not cardiac hypertrophy but cardiac fibrosis in mice with AII–induced hypertension
3. On the other hand, Ep abolished the development of both cardiac hypertrophy and cardiac fibrosis in mice with AII–induced hypertension.

4. When cardiac function was measured by echocardiography, WT mice developed prominent concentric cardiac hypertrophy and diastolic dysfunction but not systolic dysfunction after AII infusion. Ep inhibited the progression of both concentric cardiac hypertrophy and diastolic dysfunction. However, chronic loss of OPN by gene targeting leads to significant systolic dysfunction after AII infusion.

These results suggest that OPN has a pivotal role in the development of AII induced cardiac fibrosis and remodeling. Moreover, the effect of Ep on the prevention of not cardiac hypertrophy but fibrosis might partially be mediated through the inhibition of OPN.

DISCUSSION

OPN and Cardiac Fibrosis Increasing evidences suggested that OPN has a pivotal role in the progression of cardiac fibrosis. First, it had been reported that AII upregulates expression of OPN mRNA in cardiac fibroblasts and that monoclonal antibody directed against OPN completely blocks the mitogenic effect of AII on cultured rat cardiac fibroblasts, and blocks AII induction of cardiac fibroblast collagen gel contraction.[5] These findings suggest OPN may be an important mediator of AII induced cardiac fibrosis. Moreover, it had been reported that the collagen accumulation in heart after AMI was markedly decreased in OPN deficient mice as compared with that in WT mice. OPN has also been shown to interact with fibronectin and collagen, suggesting its possible role in matrix organization and/or stability in other model. These results were consistent with our results that the chronic depletion of OPN by gene targeting markedly prevented the progression of cardiac perivascular fibrosis by AII infusion.Furthermore, some reports demonstrated the underlying mechanism by which OPN regulate the myocardial fibrosis. In cardiac fibroblast model, it had been reported that monoclonal antibody against β_3 integrin blocked both AII and OPN-induced collagen gel contraction, suggesting that OPN acts via an integrin-dependent pathway.[5] More recently, it had been reported that OPN regulate the collagen accumulation by modulating IL-1ß-stimulated increases in MMP-2 and -9 activity via the PKC-zeta. Thus, it was conceivable that OPN regulate the myocardial fibrosis in AII induced cardiac remodeling through the involvement of integrin, IL-1ß, and MMPs.

OPN and Cardiac Hypertrophy Recent reports also showed that the expression of OPN in heart was elevated in LVH and LV failure.[10] Graf et al. demonstrated that the expression of OPN was upregulated with the development of LVH and that the cardiomyocyte was a major source of OPN expression in the heart.[18] On the other hand, Singh et al. reported that the expression of OPN in LVH heart was markedly increased only with heart failure and that a major source of OPN expression was nonmyocytes in the interstitium and perivascular space.[10] A possible explanation for differences in findings between these reports may relate to the markedly different time periods studied. Taken together, it seems reasonable to think that OPN have a pivotal role in the process of development of cardiac hypertrophy and/or subsequent remodeling. Moreover, these results suggest that the source of OPN may be myocytes early in LV hypertrophy but shifts to interstitial cells in late hypertrophy with the development of heart failure. However, it is not known whether the elevated OPN expression is a cause or result of cardiac hypertrophy and/or subsequent remodeling. In this study, we clearly demonstrated that the chronic depletion of OPN by gene targeting did not inhibit but rather progress cardiac hypertrophy in some parameter, and deteriorated cardiac systolic function by AII infusion. These results suggested that OPN may have an important role in not preventing cardiac hypertrophy but compensating AII induced cardiac hypertrophy and remodeling.

OPN and Cardiac Remodeling and Underlying Mechanism The precise mechanism by which OPN deficiency affects the cardiac remodeling after AII infusion was not known. However, we have some speculations to explain it. Cardiac remodeling involves the production and destruction of extracellular matrix proteins, cell proliferation and migration, and apoptotic and necrotic cell death. Cardiac fibroblasts are crucially involved in these processes, producing growth factors and cytokines that act as autocrine and paracrine factors, as well as extracellular matrix proteins and proteinases. Moreover, the

interactions between cardiac fibroblasts and cardiomyocytes are thought to be essential for the progression of cardiac remodeling.

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1．International Symposium: Cardiomyopathy and Heart Failure・Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy　2003年10月17日

2．第7回日本心不全学会学術総会・ Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy　2003年10月23日

3．第7回日本心血管内分泌代謝学会学術総会・ Osteopontin is Essential for Cardiac Fibrosis and Remodeling in Angiotensin II-Induced Cardiac Hypertrophy　2003年11月25日

2004年3月1日