ChGn-2 Plays a Cardioprotective Role in Heart Failure Caused by Acute Pressure Overload

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学 位 論 文 の 内 容 要 旨

ChGn-2 Plays a Cardioprotective Role in Heart Failure Caused by Acute Pressure Overload

ChGn-2は、急性圧過負荷によって引き起こされる心不全に対して心臓保護的に働く

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A. Background

The extracellular matrix (ECM) of the heart provides structural support and regulates cytokine and growth factor activities. Cardiac ECM, which consists of collagens, glycoproteins, proteoglycans, and glycosaminoglycans, considerably changes in heart disease in a quantitative and qualitative way.

Chondroitin sulfate (CS), which consists of N-acetylgalactosamine (GalNAc) and glucuronic acid (GluUA), is one of the most abundant glycosaminoglycans (GAGs) in the heart. The synthesis of CS chains is accomplished by the collaboration of a number of glycosylation enzymes. Chondroitin sulfate N-acetylgalactosaminyltransferase (ChGn)-2 is a critical enzyme that elongate CS chains. ChGn-2 deletion has been demonstrated to diminish and shorten the CS chains in mouse tissues, which was beneficial in the prevention of atherosclerosis by lowering the accumulation of oxidized LDL.

Recently, it has been reported that excess CS-GAGs in the heart worsen the heart failure by retaining inflammatory cytokines at an advanced stage; nevertheless, it remains unclear whether and the mechanism by which CS-GAGs are causally involved in the progression of heart failure. In this study, we revealed another facet of CS in heart failure by exploring the role of ChGn-2 in heart failure caused by acute pressure overload.

B. Methods

For in vivo studies, mice with target deletion of ChGn-2 (ChGn-$2^{-/-}$) on C57BL/6 genetic background were generated. ChGn-$2^{-/-}$ and wild-type (WT) mice at the age of 8 weeks old were subjected to transverse aortic constriction (TAC) procedure to induce acute pressure overload and heart failure. Echocardiography was performed at day 0, 3, 7, and 14 after the procedure to assess the left ventricular (LV) systolic functions. After 2 weeks of TAC procedure, mice were sacrificed and the hearts were collected. The heart was then subjected to histological analysis, and RNA/protein isolation.

For in vitro studies, conditioned mediums were prepared from primary cardiac human cardiac fibroblasts (HCFs) and primary mouse cardiac fibroblasts (MCFs) isolated from either WT or ChGn-$2^{-/-}$ mice in the presence or absence of cyclic

mechanical stretch. Cardiomyocytes (H9C2 cell lines and neonatal rat cardiomyocytes (NRCs)) were then treated with control or stretched CFs-derived conditioned medium to assess the cardioprotective effect of CS-GAGs secreted from the CFs.

C. Results

1. Loss of ChGn-2 exacerbates the cardiac dysfunction due to acute pressure overload

Acute pressure overload generated by TAC considerably enhanced CS-GAGs accumulation in WT mouse heart, which was significantly diminished in ChGn-2$^{-/-}$ mouse heart. Acute pressure overload continuously impaired the left ventricular (LV) systolic function in WT mice. Notably, marked deterioration in LV systolic function was detected in ChGn-2$^{-/-}$ mice as early as 3 days after TAC, and the worsened LV systolic function persisted during the observation period. Cardiac hypertrophy was enhanced in ChGn-2$^{-/-}$ mouse comparing to that in WT mouse 2 weeks after TAC, while the cardiac fibrosis was not different between these mice. Furthermore, cardiomyocyte apoptosis was also deteriorated in the heart of ChGn-2$^{-/-}$ mouse comparing to that in WT mouse 2 weeks after TAC. These data strongly suggest that CS-GAGs elongated by ChGn-2 might play a cardioprotective role in the acute phase of heart failure.

2. GAGs derived from cardiac fibroblasts (CFs) protect cardiomyocytes from death

CFs are subjected to excess mechanical stretch in an overloaded heart, hence we exposed HCFs and MCFs to cyclic mechanical stretch in vitro. In both HCFs and MCFs, mechanical stretch significantly increased the ChGn-2 expression and GAGs production. ChGn-2$^{-/-}$ MCFs failed to produce more GAGs after stretch stimuli, indicating a critical role of ChGn-2 in the stretch-induced GAGs production in CFs.

To explore the role of GAGs derived from CFs in cardiomyocyte apoptosis, H9C2 cells and neonatal rat cardiomyocytes (NRCs) were cultured in the conditioned medium (CM) prepared from HCFs or MCFs, and apoptosis was induced by doxorubicin (DOX)-treatment. Apoptosis was significantly reduced in both H9C2 cells and NRCs only when cultured in CMs derived from the stretched HCFs or MCFs. These data suggest that stretch stimulated the production and/or secretion of cardioprotective factors in CFs.

Notably, the cardioprotective effects of CMs derived from stretched CFs were completely abolished when GAGs were degraded by chondroitinase ABC (ChABC). Moreover, the cardioprotective effects of CM derived from stretched MCFs were disappeared in ChGn-2$^{-/-}$ MCFs. In addition, overexpression of ChGn-2 in HCFs or MCFs enhanced the cardioprotective effects of their CM even in the absence of the stretch stimuli. These findings strongly suggest that CFs produce more GAGs through increased ChGn-2 expression in response to stretch stimuli, and these GAGs protect cardiomyocyte from death in the overloaded heart.

3. CS-GAGs protect cardiomyocyte from death through interaction with CD44

To further analyze the cardioprotective role of CS-GAGs, we examined the effects of purified chondroitin sulfate A (CS-A), one of the major GAGs produced by CFs, in cardiomyocyte apoptosis. Treatment with CS-A reduced DOX-induced apoptosis in H9C2 cells and NRCs, in association with preserved Akt activity. The cardioprotective effect of CS-A was abolished when the PI3K/Akt pathway was inhibited by LY294002, indicating a crucial role of PI3K/Akt pathway in the CS-A-mediated cardioprotection.

CD44, a cell surface glycoprotein, has been reported to interact with CS-GAGs. Also, it has been reported that CD44 enhances cell survival and proliferation by activating the PI3K/Akt pathway. We therefore examined an involvement of CD44 in the CS-A-mediated cardioprotection. CD44 blockade using an anti-CD44 antibody abrogated the cardioprotective effect of CS-A in H9C2 cells and NRCs. CD44 blockade also abolished the

preservation of Akt activity in cells treated with CS-A. These data strongly suggest that CS-A directly protect cardiomyocytes from death through CD44-PI3K/AKT axis.

4. CS-GAGs prevent cardiomyocyte death by activating IGF-1

We found that stretch stimuli substantially increased IGF-1 expression in HCFs, and that stretched HCF-CM showed higher IGF-1 concentration than in control HCF-CM. Because CS-GAGs are known to interact with various growth factors, we explored a possible interaction between CS-GAGs and IGF-1. We first analyzed whether CS-A binds to IGF-1. Cell culture plates were coated with CS-A, followed by incubation with IGF-1. After washing out the unbound IGF-1, H9C2 cells were plated onto the plates, and the effect of IGF-1 (bound to CS-A coating) was analyzed. CS-A coating caused significant retention of IGF-1, which induced ANP and BNP expression, activated Akt, and inhibited the apoptosis in H9C2 cells. Notably, digestion of CS-A using ChABC abolished the retention of IGF-1.

We then analyzed whether CS-A affects IGF-1 biological activity. In H9C2 cells, addition of IGF-1 into the culture medium strongly activated Akt and prevented DOX-induced apoptosis. The cardioprotective effects of IGF-1 were enhanced by the addition of CS-A in a synergistic manner, indicating that the interaction with CS-GAGs enhances IGF-1 cardioprotective function. These findings indicate that CS-GAGs show cardioprotective effects through dual pathways: a direct pathway via CD44 and an indirect pathway involving IGF-1 binding and activation.

D. Discussion

In this study, we discovered a cardioprotective role of ChGn-2 and CS-GAGs using the acute pressure overload model. Our findings provide mechanistic insights into a facet of the CFs-cardiomyocyte interaction under pathological condition. Through mechanical strain, increased workload stimulates ChGn-2 expression in CFs, resulting in increased synthesis of CS-GAGs. At the same time, mechanical stretch enhances the expression of cardioprotective growth factors such IGF-1 in CFs. These CS-GAGs and IGF-1 protect cardiomyocytes synergistically, and thus prevent heart failure at least in an acute phase.

Previous study showed a detrimental role of CS-GAGs in the development of heart failure. The authors demonstrated the excess accumulation of CS-GAGs in human failing heart, and showed that treatment with rhASB (C4S-specific sulfatase) improved the LV systolic function in rats after TAC. They also demonstrated that CS-GAGs binds to TNF-α, and potentiates TNF-α-induced inflammation. Therefore, excess accumulation of CS-GAGs appeared to play an unfavorable role in chronic heart failure in an advanced stage. In contrast, our data showed that insufficient CS-GAGs production (due to loss of ChGn-2) deteriorated LV systolic function as early as 3 days after TAC in mice. These data strongly suggest that a sufficient amount of CS-GAGs is required for cardiomyocytes to resist acute injury stress. Increased CS-GAGs production in CFs subjected to mechanical stretch is probably an adaptive response to resist acute pressure overload stress; however prolonged CS-GAGs overproduction may lead to maladaptation. Because of the biphasic effects CS-GAGs in cardiac function and remodeling in heart failure, careful consideration and stage-dependent approaches are required when applying CS-GAGs-targeted therapy for heart failure.

論文審査の結果の要旨

受付番号	甲 第3196号	氏　名	ANDREAS HARYONO
論文題目 Title of Dissertation	ChGn-2 Plays a Cardioprotective Role in Heart Failure Caused by Acute Pressure Overload ChGn-2は、急性圧過負荷によって引き起こされる心不全に対して 心臓保護的に働く		
審査委員 Examiner	主　査 Chief Examiner 小川 渉 副　査 Vice-examiner 鈴木 敬 副　査 Vice-examiner 堀江 裕之		

神戸大学大学院医学(系)研究科（博士課程）

（要旨は１，０００字～２，０００字程度）

Chondroitin sulfate (CS) is one of the most abundant glycosaminoglycans in the heart. Chondroitin sulfate N-acetylgalactosaminyltransferase (ChGn)-2 is a critical enzyme that elongate CS chains. In this study, the candidate investigated the role of ChGn-2 in heart failure caused by acute pressure overload.

Acute pressure overload generated by TAC considerably enhanced CS-GAGs accumulation in WT mouse heart, which was significantly diminished in ChGn-2$^{-/-}$ mouse heart. Marked deterioration in LV systolic function and cardiac hypertrophy were detected in ChGn-2$^{-/-}$ mice after TAC, suggesting that CS-GAGs elongated by ChGn-2 play a cardioprotective role in the acute phase of heart failure.

The candidate found that mechanical stretch stimulated the production and/or secretion of cardioprotective factors in cardiac fibroblasts. The cardioprotective effects of CM derived from stretched MCFs were disappeared in ChGn-2$^{-/-}$ MCFs. Overexpression of ChGn-2 in HCFs or MCFs enhanced the cardioprotective effects of their CM even in the absence of the stretch stimuli. These findings suggest that CFs produce more GAGs through increased ChGn-2 expression in response to stretch stimuli, and these GAGs protect cardiomyocyte from death in the overloaded heart.

To further analyze the cardioprotective role of CS-GAGs, the candidate examined the effects of purified chondroitin sulfate A (CS-A), one of the major GAGs produced by CFs, in cardiomyocyte apoptosis. Treatment with CS-A reduced DOX-induced apoptosis in H9C2 cells and NRCs, in association with preserved Akt activity. The cardioprotective effect of CS-A was abolished when the PI3K/Akt pathway was inhibited by LY294002, indicating a crucial role of PI3K/Akt pathway in the CS-A-mediated cardioprotection. The candidate examined an involvement of CD44 in the CS-A-mediated cardioprotection. CD44 blockade using an anti-CD44 antibody abrogated the cardioprotective effect of CS-A in H9C2 cells and NRCs. CD44 blockade also abolished the preservation of Akt activity in cells treated with CS-A. These data suggest that CS-A directly protect cardiomyocytes from death through CD44-PI3K/AKT axis.

The candidate found that stretch stimuli substantially increased IGF-1 expression in HCFs, and that stretched HCF-CM showed higher IGF-1 concentration than in control HCF-CM. Cell culture plates were coated with CS-A, followed by incubation with IGF-1. After washing out the unbound IGF-1, H9C2 cells were plated onto the plates, and the effect of IGF-1 (bound to CS-A coating) was analyzed. CS-A coating caused significant retention of IGF-1, which induced ANP and BNP expression, activated Akt, and inhibited the apoptosis in H9C2 cells. Digestion of CS-A using ChABC abolished the retention of IGF-1. In H9C2 cells, addition of IGF-1 into the culture medium strongly activated Akt and prevented DOX-induced apoptosis. The cardioprotective effects of IGF-1 were enhanced by the addition of CS-A in a synergistic manner, indicating that the interaction with CS-GAGs enhances IGF-1 cardioprotective function.

The candidate, having completed studies on the role of ChGn-2 in cardio protection and having advanced the field of knowledge in the area of the molecular mechanism of cardio protection in heart failure, is hereby recognized as having qualified for the degree of Ph.D.(Medicine)