論文の内容の要旨

論文題目　**The ATP-binding Cassette Transporter BCRP1/ABCG2 is Essential for Cardiac Repair after Myocardial Infarction**

和訳　　**ABC トランスポーターBCRP1/ABCG2 は心筋梗塞後の組織修復に非常に重要な役割を果たしている**

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Background:

Myocardial infarction (MI) is the most common cause of cardiac morbidity and mortality, and left ventricular remodeling after MI is still a serious problem even in the era in which emergent cardiac catheterization is available world-wide. Ventricular remodeling is linked to heart failure progression and is associated with poor prognosis following MI. Accordingly, it is of critical importance to develop therapeutic strategies that will effectively inhibit the development of ventricular remodeling and failure after MI.

BCRP1/ABCG2 is a member of the ATP-binding cassette transporter family originally

identified by its ability to confer drug resistance in tumor cells by active efflux of multiple drugs. This protein has been shown to be expressed in various normal organs, and has been suggested to regulate several tissue defense mechanisms. Previous studies have suggested that BCRP1/ABCG2 may have functions in tissue defense in the diseased heart. However, the physiological significance of its expression in cardiac injury has not yet been fully elucidated. This study was performed to clarify the impact of BCRP1/ABCG2 expression on cardiac repair after MI.

Methods and Results:

First, I confirmed the expression of BCRP1/ABCG2 mRNA in murine heart by reverse transcription polymerase chain reaction (PCR). Immunohistochemistry showed that BCRP1/ABCG2 was mainly expressed in endothelial cells of microvessels in the heart.

To clarify the impact of BCRP1/ABCG2 expression on cardiac repair after MI, I induced MI in 8- to 12-week-old wild-type (WT) and Bcrp1/Abcg2 knock-out (KO) mice by ligating the left anterior descending artery. The survival rate up to 28 days after MI was significantly lower in KO mice than in WT mice (KO 28.3% versus WT 74.5%, $p<0.0001$) mainly due to cardiac rupture in 4 to 6 days after MI. Echocardiography showed that dilatation of the left ventricle (LV), thickening of the non-infarcted area, and ejection fraction were worse in KO mice than in WT mice at 28 days after MI. Hemodynamic measurements by the micro pressure transducers through the right carotid artery demonstrated that LV function was more deteriorated in KO mice than those in WT mice at 28 days after MI. Heart weight to body weight ratio was greater in KO mice than in WT mice, and infarct size at 28 days after MI, assessed by sirius red staining, was significantly larger in KO mice

than in WT mice, although initial area at risk and initial infarct size did not differ between the 2 groups. Myocyte cross-sectional area (CSA) and collagen volume fraction (CVF) in the non-infarcted area at 28 days after MI, assessed by hematoxilin-eosin (H&E) and sirius red staining, respectively, were greater in KO mice than in WT mice. These results indicated that ventricular remodeling after MI was exaggerated in KO mice compared with WT mice.

As angiogenesis and recruitment of macrophages and myofibroblats play an important role in would healing process, capillary, macrophages and myofibroblasts density in the peri-infarction area were assessed by immunohistochemistry with anti-CD31 antibody, anti-Mac-3 antibody and anti-α-smooth muscle actin antibody, respectively. At 5 days after MI, capillary, macrophages and myofibroblasts density in the peri-infarction area were significantly reduced in KO mice compared with WT mice. I also assessed cytokine mRNA expression in the peri-infarction area by real time PCR. At 5 days after MI, gene expression of pro-inflammatory, angiogenesis-related and fibrosis-related cytokines was comparable or higher in KO mice than in WT mice, whereas cytokine mRNA expression levels at baseline were comparable between WT and KO mice.

To assess the impact of BCRP1/ABCG2 expression in microvascular endothelial cells of the heart, in vitro experiments with human microvascular endothelial cells from the heart (HMVEC-Cs) were performed. MTS assay showed that pharmacological inhibition of BCRP1/ABCG2 with fumitremorgin C (FTC) resulted in impaired survival of human microvascular endothelial cells from the heart under oxidative stress, although BCRP1/ABCG2 inhibition did not alter the expression pattern of ICAM-1, VCAM-1 and eNOS even under oxidative stress. Flow

cytometry analysis demonstrated that protoporphyrin Ⅸ concentration was higher in HMVEC-Cs with FTC than in those without FTC, which might exaggerate oxidative stress in HMVEC-Cs.

Discussion:

In the present study, I found that BCRP1/ABCG2 is expressed mainly in the endothelial cells of microvessels in the heart. I also demonstrated that genetic disruption of BCRP1/ABCG2 deteriorated mortality and cardiac remodeling after MI. In KO mice, angiogenesis and recruitment of macrophages and myofibroblasts were impaired. In vitro experiments showed that BCRP1/ABCG2 played an important role in survival of microvascular endothelial cells of the heart under oxidative stress possibly by preventing from accumulation of intra-cellular protoporphyrin Ⅸ.

In this study, I found that BCRP1/ABCG2 is essential for microvascular endothelial cell survival under oxidative stress, which is important for angiogenesis in damaged tissues. This result may explain why angiogenesis was impaired in KO mice, although the expression levels of angiogenesis-related cytokines were higher in KO mice. Previous studies suggested the mechanisms by which BCRP1/ABCG2 expression protect cell death. BCRP1/ABCG2 has been demonstrated to efflux protoporphyrin Ⅸ. The regulation of porphyrins and heme within a cell is important because the accumulation of heme within cell can ultimately lead to the accumulation of iron and the production of cell-damaging reactive oxygen species by the Fenton reaction. This may explain why an inhibition of BCRP1/ABCG2 lead to impaired survival of microvascular endothelial cells under oxidative stress, but not under normal condition.

Impaired angiogenesis might lead to delayed cardiac repair after MI. I also found that macrophage recruitment was impaired in KO mice at 5 days after MI, although the expression levels of pro-inflammatory cytokines were comparable or higher in KO mice than in WT mice. The previous study showed that loss of BCRP1/ABCG2 does not affect hematopoiesis. In addition, my in vitro experiments showed that BCRP1/ABCG2 had no effect on expression of adhesion molecules such as ICAM-1 and VCAM-1 in microvascular endothelial cells. Reduced capillary density, therefore, might lead to the impaired macrophage recruitment from blood stream. As macrophages play an important role in clearance of necrotic cardiomyocytes, impaired macrophage recruitment might lead to impaired cardiac healing and also lead to fragility of myocardium.

In the present study, the number of myofibroblasts was reduced in KO mice compared with WT mice at 5 days after MI. As myofibroblasts play a pivotal role in strengthening wound in healing process, impaired recruitment of myofibroblasts may explain why cardiac rupture was more often observed in KO mice in 4 to 6 days after MI. However, my data showed that transforming growth factor β 1 (TGF-β 1), a cytokine which has been shown to induce differentiation of fibroblasts into myofibroblasts, was highly expressed in KO mice. Although these results appear discrepant, impaired cytokine balance by impaired angiogenesis may explain this discrepancy because TGF-β is a pleiotropic and multifunctional cytokine, known to exert diverse and often contradictory cellular effects on all cell types.

In the previous study, a PPARγ agonist has been shown to regulate Bcrp1/Abcg2 expression positively. In addition, a PPARγ agonist has been demonstrated to ameliorate

ventricular remodeling after MI in mice. These results suggest that a PPAR gamma agonist may improve cardiac healing, in part, through up-regulation of BCRP1/ABCG2 expression. Together with these results, my findings suggest that up-regulation of BCRP1/ABCG2 might be a promising strategy for treatment of MI.

Conclusions:

I demonstrated that BCRP1/ABCG2 plays a pivotal role in cardiac repair after MI via protection of microvascular endothelial cells. My results suggest that BCRP1/ABCG2 may be of interest for a therapeutic target to improve clinical outcomes after MI.