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Impact of cardiac-specific expression of CD39 on myocardial infarct size in mice

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ABSTRACT

Aims: Prior work suggests that ischemic preconditioning increases the level of CD39 in the heart and contributes to cardiac protection. Therefore, we examined if targeted cardiac expression of CD39 protects against myocardial injury.

Main methods: Mice with cardiac-specific expression of human CD39 (αMHC/hCD39-Tg) were generated, characterized and subjected to left coronary artery ischemia-reperfusion injury and infarct size at 24 h following injury quantified.

Key findings: αMHC/hCD39-Tg mice have increased in cardiac ATPase and ADPase activity compared to WT littermates. The increased activity in αMHC/hCD39-mice was inhibited by the CD39 antagonist sodium polyoxotungstate (POM-1). Measurement of basal cardiac function by echocardiography revealed that αMHC/hCD39-Tg mice have a lower resting heart rate and increased stroke volume. In response to myocardial ischemia, systolic and diastolic function was better preserved in αMHC/hCD39-Tg compared to WT mice. Comparison of Tau also revealed preserved cardiac relaxation during ischemia in αMHC/hCD39-Tg hearts. Assessment of myocardial infarct size in response to 60 min of ischemia and 24 h of reperfusion demonstrated a significant reduction in infarct size in αMHC/hCD39-Tg hearts. Analysis of isolated cardiomyocytes revealed no basal difference in calcium transients between WT and αMHC/hCD39-Tg cardiomyocytes. However, in response to isoproterenol stimulation, there was a trend toward lower calcium transients in αMHC/hCD39 cardiomyocytes suggesting less calcium accumulation in response to metabolic stress.

Significance: Cardiac-specific expression of CD39 reduces myocardial dysfunction and infarct size following ischemia-reperfusion injury. Increasing nucleotidase expression in the heart may be a novel approach to protect the heart from ischemic injury.

1. Introduction

With myocardial injury, nucleotides (ATP, UTP, ADP, UDP) are released into the extracellular space where they can activate specific receptors that contribute to myocardial injury. These extracellular nucleotides are hydrolyzed by nucleotidases, thereby limiting the magnitude and duration of signaling. Ectonucleotidase diphosphohydolase-1 (CD39) is one such nucleotidase that hydrolyzes extracellular ATP and ADP to AMP. We have previously demonstrated that global overexpression of CD39 conveys protection against myocardial ischemia-reperfusion injury [1]. A prior study also has suggested that ischemic preconditioning increases the expression of CD39 in the heart and that CD39 activity is required for the protection conveyed by ischemic preconditioning [2]. However, recent work has demonstrated that with myocardial ischemia-reperfusion injury, CD39 expression and activity is lost on the cardiac vasculature [3]. Therefore, the specific aim of this study was to determine if cardiac-specific overexpression

Abbreviations: αMHC, alpha myosin heavy chain; CD39, Ectonucleoside triphosphate diphosphohydrolase-1; POM-1, sodium polyoxotungstate; Tg, Transgenic; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate.

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of CD39 protects against myocardial ischemia–reperfusion injury. Here we demonstrate that cardiac-specific nucleotidase expression protects the heart from ischemia-reperfusion injury.

2. Materials and methods

2.1. Mice

The investigation conformed to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and was approved by the Vanderbilt University and The Ohio State University Institutional Animal Care and Use Committees. Mice with overexpression of human CD39 were generated by directional cloning of the human CD39 cDNA into the αMHC-construct using BamHI and SalI. Linearized transgene plasmid was prepared by low melting agarose (LMA) gel separation and purified with the UltraClean Kit (MO BIO Laboratories, Inc., 12224-250). All animals were purchased from Taconic Farm, and housed on individually ventilated cage (IVC) rack (Lab Products Inc.). Female mice C57BL/6 at 3–5 weeks old served as embryo donors. Donor mice were superovulated and mated with C57BL/6 stud male mice; the oocytes were harvested and cultured as described prior to microinjection [4]. ICR female mice aged 6–10 weeks old in estrus were mated with vasectomized male ICR mice to induce pseudopregnacy. The pseudo-pregnant female mice served as recipients to carry embryos to term [5]. Microinjected embryos were surgically transferred into the oviduct of recipients. After embryos transfer (ET) surgery, the recipients were housed on a conventional static rack throughout the gestation and nursing period. All animal experiments were performed in a Specific Pathogen Free (SPF) barrier animal facility. The resulting PCR-positive αMHC/hCD39-Tg mice were backcrossed for more than ten generations in a C57BL/6 background. Male mice, ten-fifteen weeks old (25–30 g), αMHC/hCD39-Tg or wild-type (WT) littermate control mice were used in the protocols.

2.2. Nucleotidase activity

Nucleotidase activity was measured as previously published [1].

2.3. Immunoblot analysis

To determine the level and specificity of CD39 expression in transgenic mice, hearts were excised, the aorta cannulated, perfused with ice-cold saline. The atria and left ventricle were separated and snap frozen for storage at −80 °C until homogenized for immunoblot analysis as previously described [1,6]. Twenty to fifty micrograms of protein was loaded on acrylamide gels (Bio-Rad) separated by electrophoresis on a 7.5% polyacrylamide gel and transferred using mini-protean electrophoresis system (Bio-Rad). The specific antibodies used for immunoblot analysis of human CD39 expression were CD39: (Abcam; #108248) and GAPDH (Cell Signaling Technology; #2118). Membranes

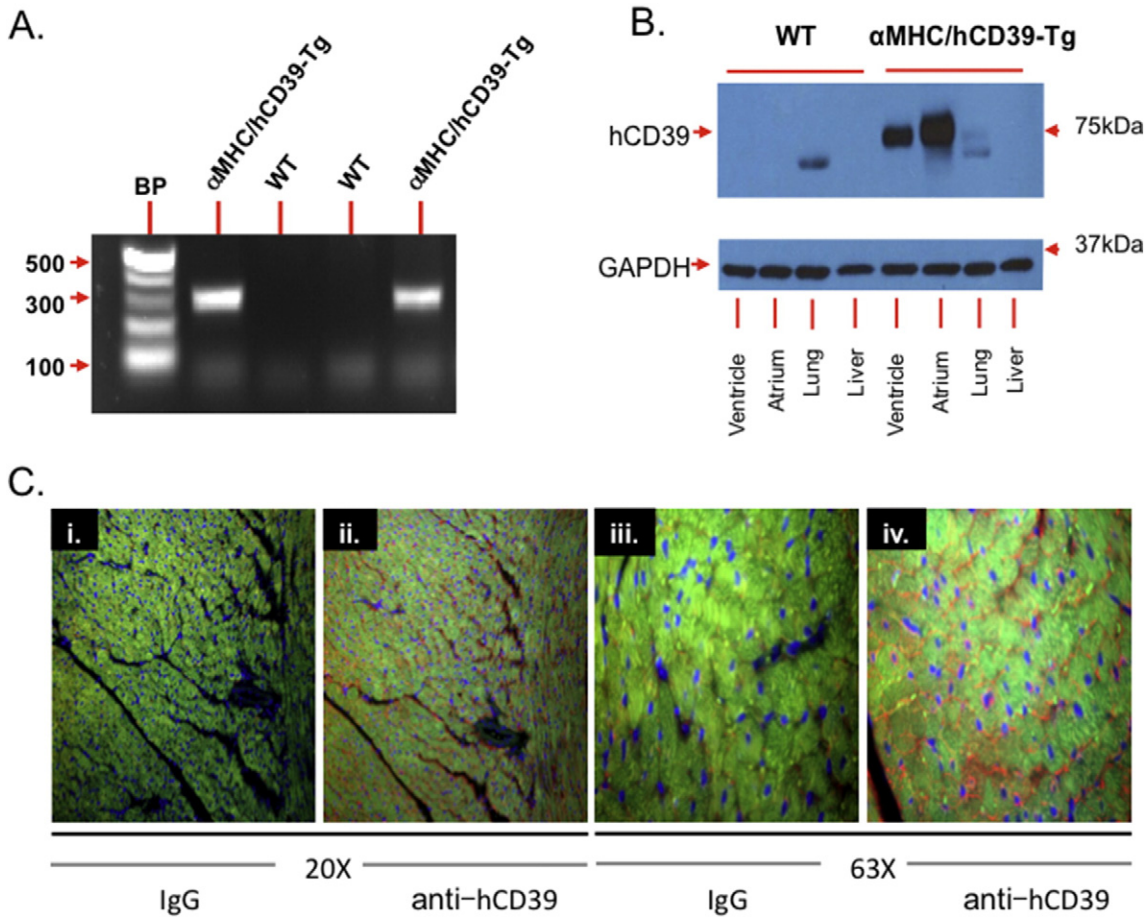

Fig. 1. Cardiac-specific expression of hCD39 in mice. A, PCR confirmation of αMHC/hCD39 transgene insertion in mice. Primers specific for the αMHC promoter and the hCD39 transgene were used to screen and genotype mice for the presence of the αMHC/hCD39 transgene. A specific 300 bp product was obtained only in mice with the αMHC/hCD39 transgene. B, Immunoblot analysis of WT and αMHC/hCD39 left ventricle (ventricle), left atrium (atrium), lung and liver for human CD39 expression and GAPDH expression. C, Immunohistochemistry of αMHC/hCD39 left ventricular samples for the expression of human CD39 (i. 20 IgG control, ii. 20× anti-human CD39, iii. 63× IgG control, iv. 63× anti-human CD39).