Donor tissue-specific exosome profiling enables noninvasive monitoring of acute rejection in mouse allogeneic heart transplantation

Andreas Habertheuer, MD, PhD,[a] Laxminarayana Korutla, PhD,[a] Susan Rostami, BS,[a] Sanjana Reddy, MS,[a] Priti Lal, MD,[b] Ali Naji, MD, PhD,[a] and Prashanth Vallabhajosyula, MD, MS[a]

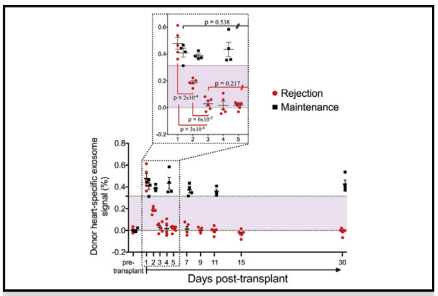
ABSTRACT

Objective: In heart transplantation, there is a critical need for development of biomarkers to noninvasively monitor cardiac allografts for immunologic rejection or injury. Exosomes are tissue-specific nanovesicles released into circulation by many cell types. Their profiles are dynamic, reflecting conditional changes imposed on their tissue counterparts. We proposed that a transplanted heart releases donor-specific exosomes into the recipient's circulation that are conditionally altered during immunologic rejection. We investigated this novel concept in a rodent heterotopic heart transplantation model.

Materials and Methods: Full major histocompatibility mismatch (BALB/c [H2-K^d] into C57BL/6 [H2-K^b]) heterotopic heart transplantation was performed in 2 study arms: Rejection (n = 64) and Maintenance (n = 28). In the Rejection arm, immunocompetent recipients fully rejected the donor heart, whereas in the Maintenance arm, immunodeficient recipients (C57BL/6 $Prkdc^{SCID}$) accepted the allograft. Recipient plasma exosomes were isolated and a donor heart-specific exosome signal was characterized on the nanoparticle detector for time-specific profile changes using anti-H2-K^d antibody quantum dot.

Results: In the Maintenance arm, allografts were viable throughout follow-up of 30 days, with histology confirming absence of rejection or injury. Time course analysis (days 1, 2, 3, 4, 5, 7, 9, 11, 15, and 30) showed that total plasma exosome concentration ($P = .157$) and donor heart exosome signal ($P = .538$) was similar between time points. In the Rejection arm, allografts were universally rejected (median, day 11). Total plasma exosome quantity and size distribution were similar between follow-up time points ($P = .278$). Donor heart exosome signals peaked on day 1, but significantly decreased by day 2 ($P = 2 \times 10^{-4}$) and day 3 ($P = 3.3 \times 10^{-6}$), when histology showed grade 0R rejection. The receiver operating characteristic curve for a binary separation of the 2 study arms (Maintenance vs Rejection) demonstrated that a donor heart exosome signal threshold < 0.3146 was 91.4% sensitive and 95.8% specific for diagnosis of early acute rejection.

Conclusions: Transplant heart exosome profiling enables noninvasive monitoring of early acute rejection with high accuracy. Translation of this concept to clinical settings might enable development of a novel biomarker platform for allograft monitoring in transplantation diagnostics. (J Thorac Cardiovasc Surg 2018;■:1-11)



Acute rejection leads to distinct changes in the recipient's plasma donor heart-specific exosome pool.

Central Message

Circulating donor-specific exosome profiles in recipient blood herald early acute rejection of cardiac allograft.

Perspective

In heart transplantation, there is a critical need for accurate, noninvasive biomarkers to monitor for immunologic rejection because current clinical standards are based on routine cardiac allograft biopsy. We propose that in an animal of cardiac allotransplantation, donor heart-specific exosome profiling from recipient plasma serves as a noninvasive biomarker for diagnosis of early rejection.

See Editorial Commentary page XXX.

BS

From the [a]Departments of Surgery and [b]Pathology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pa.

Supported by an American Association for Thoracic Surgery Junior Faculty Grant.

Read at the 97th Annual Meeting of The American Association for Thoracic Surgery, Boston, Massachusetts, April 29-May 3, 2017.

Received for publication May 24, 2017; revisions received Nov 14, 2017; accepted for publication Dec 7, 2017.

Address for reprints: Prashanth Vallabhajosyula, MD, MS, Division of Cardiovascular Surgery, 6 Silverstein, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104 (E-mail: Prashanth.vallabhajosyula@uphs.upenn.edu).

0022-5223/$36.00

https://doi.org/10.1016/j.jtcvs.2017.12.125

Abbreviations and Acronyms

ANOVA = analysis of variance
AUC = area under the curve
ddcfDNA = donor-derived cell free DNA
EMB = endomyocardial biopsy
IgG = Immunoglobulin G
ISHLT = International Society for Heart and Lung Transplantation
MHC-1 = major histocompatibility complex-1
ROC = receiver operation characteristics

Scanning this QR code will take you to a supplemental video. To view the AATS Annual Meeting Webcast, see the URL next to the webcast thumbnail.

Heart transplantation remains the only curative therapy in patients with end-stage heart failure. Even with major medical advances, immunologic rejection and immunosuppressive regimen-related complications account for the majority of morbidity and mortality in patients undergoing transplant. A recent report by the International Society for Heart and Lung Transplantation (ISHLT) shows that the incidence of rejection events in the first year posttransplant is 25%.[1] The current gold standard of allograft surveillance comprises empiric surveillance and histology-guided endomyocardial biopsy (EMB), which is associated with procedural complications and can consume time and resources from patient and health system standpoints.[2,3] The frequency of surveillance biopsies varies among centers, with routine biopsies typically being performed weekly within the first month posttransplant and at increasing intervals thereafter. ISHLT reports that a heart transplant patient undergoes 17 EMBs on average during the first 2 years posttransplantation.[1] At our institution, a typical heart transplant recipient undergoes 20 surveillance EMBs during the first 2 years posttransplant, and that is if there are no rejection episodes.

Exosomes are bilayered, membrane-bound nanoparticles (30-200 nm) arising from endosomal compartments called multivesicular bodies. They are secreted by many tissue types into bodily fluids, including blood and urine.[4-6] Along with surface marker profiles that are identical to their tissue counterparts, exosomes carry stable proteomic and RNA signatures that are condition specific. Exosomes are being extensively studied for their diagnostic potential, but similar to other quantitative assays based on circulating free proteins and nucleic acids, whole plasma exosome analysis is also associated with a high noise-to-signal ratio because many tissue types contribute to the total plasma exosome pool.[4] We recently proposed that characterization of tissue-specific exosomes from bodily fluids would improve diagnostic accuracy by reducing the noise-to-signal ratio and therefore serve as a better biomarker. In the context of transplantation, we hypothesized that transplant tissue-specific exosome profiling from recipient circulation would serve as an accurate biomarker platform. To characterize transplant tissue exosomes, we took advantage of 2 concepts: transplanted tissues release distinct donor major histocompatibility complex-1- (MHC-1) specific exosomes into a recipient's circulation, and iatrogenic donor–recipient MHC mismatch introduced from allotransplantation allows for donor-specific exosome profiling from recipient blood. In the context of heart transplantation, we proposed that donor heart-specific exosome profiling would enable noninvasive monitoring of immunologic rejection. We report our investigation of this concept in a heterotopic heart transplantation model.

MATERIALS AND METHODS

Mice

All experiments were conducted in accordance with approved protocols through the University of Pennsylvania Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. C57BL/6 (MHC H2-K^b) immunocompetent wild type and C57BL/6 immunodeficient ($Prkdc^{SCID}$) mice (MHC H2-K^b) served as recipients. BALB/c (MHC H2-K^d) mice served as heart donors. All animals were purchased from The Jackson Laboratory (Bar Harbor, Maine).

Study Design

The 2 study groups were Rejection and Maintenance arms. In the Rejection arm, full MHC mismatch (BALB/c into C57BL/6) heart transplants were performed, resulting in acute rejection with allograft asystole by day 9 to 12 (median, day 11). Recipients were killed for plasma exosome analysis and allograft histology on days 1, 2, 3, 4, 5, 7, 9, 11, 15, and 30. Along with naïve pretransplant recipients, at least 5 transplants were performed for each time point (n = 64 total). In the Maintenance arm, full MHC mismatch heart transplant was performed into C57BL/6 immunodeficient recipients, and because these animals lack T and B cells, they accepted the allograft long-term. Recipients were killed on days pretransplant, 1, 2, 4, 7, 11, and 30 (4 transplants per time point; n = 28 total), and recipient plasma exosome and donor heart histologic analysis was performed. Schematic of the study design is shown in Figure 1.

Heterotopic Heart Transplantation and Posttransplant Monitoring

Animals were anesthetized with ketamine and xylazine, and donors received 200 U heparin after which the heart was explanted. Each recipient's abdominal aorta and inferior vena cava was exposed, and the donor heart pulmonary artery to vena cava and donor aorta to recipient aorta anastomoses were performed to complete the allograft implantation. The heterotopic heart transplantation procedure is available in Video 1. Allograft