Title

CRIPSR/Cas9 Technology: Hypoimmunogenic Pluripotent Stem Cells Evade Immune Rejection in Fully Immunocompetent Allogeneic Recipients

Journal

The Journal of Heart and Lung Transplantation, 40(4)

ISSN

1053-2498

Authors

Hu, X
Deuse, T
Gravina, A
et al.

Publication Date

2021-04-01

DOI

10.1016/j.healun.2021.01.1806

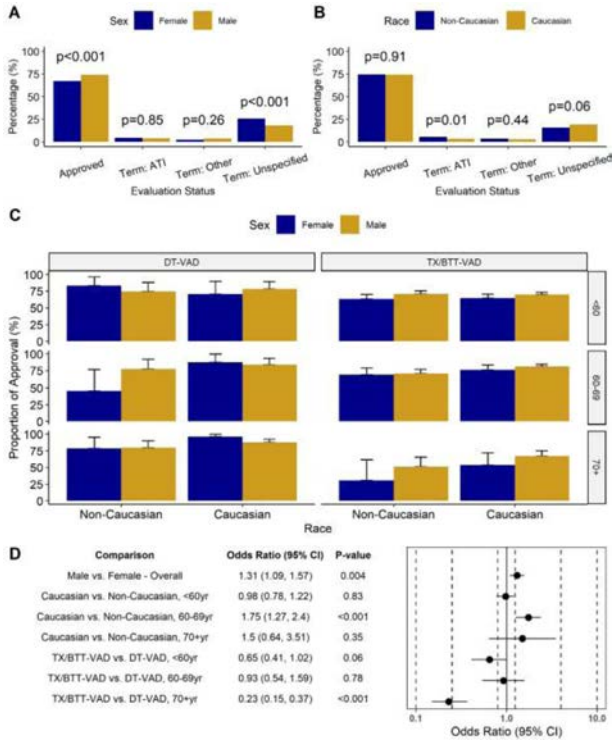
Peer reviewed

those with advanced heart failure (HF). Racial disparities in disease management also exist.

Methods: In a retrospective review of patients referred for advanced HF therapy at our center, evaluation status was classified as approved for evaluation or as terminated due to access to insurance, other or unspecified reasons. Race and gender distributions were compared to statewide proportions adjusted for incidence of HF and by evaluation status. A multivariable model of the proportion approved, accounting for gender, age (<60, 60-69, 70+), race, program (destination VAD, transplant/bridge to transplant VAD), and interactions, was constructed.

Results: Of 2893 referred patients, 2085 (72%) were approved for evaluation, 138 (5%) terminated due to insurance access, 108 (4%) other, and 560 (19%) unspecified. Three in 4 referrals were men, indicating a bias relative to statewide data (74% vs. 48%; p<0.001). Moreover, the proportion of men approved was 7% greater than women (95% CI: 3% - 11%; Fig. 1A). The racial composition was 25% African American and 73% Caucasian. Relative to statewide data, representation of African Americans was 5% greater (25% vs. 20%; <0.001) and comparable for Caucasians (73% vs. 74%; p = 0.55) with no differences between Caucasians vs. non-Caucasians approved (Fig. 1B). Accounting for variables in Fig. 1C, significant differences in the odds of acceptance were found by gender, race for 60-69 year-olds, and program for 70+ year-olds (Fig. 1D).

Conclusion: In this cohort, incoming referral bias toward males was further skewed at entry into the evaluation process for advanced HF therapy. Incoming referral bias toward African Americans is suggested. In 60-69 year-olds, approval for evaluation is skewed toward Caucasians. Understanding this data may help ensure equitable access to advanced HF therapies.



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Extracellular Vesicles Surface Protein Profile as Biomarkers to Characterize Allograft Rejection in Heart Transplanted Patients

C. Castellani,[1] J. Burello,[2] M. Fedrigo,[1] A. Burrello,[3] S. Bolis,[2] D. DI Silvestre,[4] F. Tona,[1] T. Bottio,[1] V. Biemmi,[2] G. Toscano,[5] G. Gerosa,[1] G. Thiene,[1] C. Basso,[1] S.L. Longnus,[6] G. Vassalli,[7] A. Angelini,[1] and L. Barile.[2] [1]University of Padua, Padova, Italy; [2]Cardiocentro Ticino Foundation Lugano, Lugano, Switzerland; [3]University of Bologna, Bologna, Italy; [4]ITB-CNR, Milano, Italy; [5]Azienda Ospedaliera of Padua, Padova, Italy; [6]University Hospital of Bern, Bern, Switzerland; and the [7]Università Svizzera Italiana and Cardiocentro Ticino Foundation, Lugano, Switzerland.

Purpose: Circulating extracellular vesicles (EVs) are raising considerable interest as a non-invasive diagnostic tool.We aimed to investigate differences in plasma-derived EV surface protein profiles as a biomarker to be used in combination with endomyocardial biopsies (EMBs) for the diagnosis of allograft rejection.

Methods: Plasma was collected from 90 patients (53 training cohort, 37 validation cohort) before EMB. EV concentration was assessed by nanoparticle tracking analysis. EV surface antigens were measured using a multiplex flow cytometry assay composed of 37 fluorescently labeled capture bead populations coated with specific antibodies directed against respective EV surface epitopes. According to differential EV-markers expression two diagnostic model were built and validated through supervised learning algorithms (linear discriminant analysis-LDA- and random forest model- RF)

Results: The concentration of EVs was significantly increased and their diameter decreased in patients undergoing rejection as compared with negative ones. The trend was highly significant for both antibody-mediated rejection and acute cellular rejection ($p < 0.001$). Among EV surface markers, CD3, CD2, ROR1, SSEA-4, human leukocyte antigen (HLA)-I, and CD41b were identified as discriminants between controls and acute cellular rejection, whereas HLA-II, CD326, CD19, CD25, CD20, ROR1, SSEA-4, HLA-I, and CD41b discriminated controls from patients with antibody-mediated rejection. Receiver operating characteristics curves confirmed a reliable diagnostic performance for each single marker (area under the curve range, 0.727-0.939). According to differential EV-marker expression, a diagnostic model was built and validated in an external cohort of patients. Our model was able to distinguish patients undergoing rejection from those without rejection. The accuracy at validation in an independent external cohort reached 86.5%. Its application for patient management has the potential to reduce the number of EMBs. Further studies in a higher number of patients are required to validate this approach for clinical purposes

Conclusion: Circulating EVs are highly promising as a new tool to characterize cardiac allograft rejection and to be complementary to EMB monitoring

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CRIPSR/Cas9 Technology: Hypoimmunogenic Pluripotent Stem Cells Evade Immune Rejection in Fully Immunocompetent Allogeneic Recipients

X. Hu,[1] T. Deuse,[1] A. Gravina,[1] D. Wang,[2] G. Tediashvili,[1] H. Reichenspurner,[2] M.M. Davis,[3] L.L. Lanier,[4] and S. Schrepfer.[1] [1]Department of Surgery, Division of Cardiothoracic Surgery, Transplant and Stem Cell Immunobiology-Lab, UCSF, San Francisco, CA; [2]Department of Cardiovascular Surgery, University Heart Center Hamburg, Hamburg, Germany; [3]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA; and the [4]Dept of Microbiology and Immunology and the Parker Institute for Cancer Immunotherapy, UCSF, San Francisco, CA.

Purpose: Induced pluripotent stem cells (iPSCs) are promising candidates for regenerative medicine. To overcome the allogeneic immune barrier, we created hypo-immune iPSCs using CRISPR/Cas9 editing strategies and demonstrate long-term survival of allografts without the need of immunosuppression.

Methods: Hypoimmunogenic mouse and human iPSCs were generated via CRISPR/Cas9 technology by knocking out major histocompatibility complex (MHC) class I and class II and overexpression of the “don’t eat me” signal CD47. Unmodified and hypoimmunogenic mouse and human iPSCs were transplanted into allogeneic BALB/c mice or humanized mice, respectively. Cellular and antibody responses as well as innate immune responses were analyzed and *in vivo* bioluminescence imaging was performed to assess cell survival.

Results: After allogeneic transplantation, hypoimmunogenic mouse and human cells showed a significantly decreased immune response in ELISPOT assays, comparable to the level of syngeneic transplants. No donor-specific antibodies were detected in mice and humanized mice receiving hypoimmunogenic cells demonstrating a lack of immune recognition.

According to the 'missing-self' hypothesis, lack of target cell MHC class I expression facilitates NK cell-mediated cytotoxicity. Our data show the existence and functionality of the immune checkpoint signal regulatory protein-α (SIRPα) in innate immune cells which is modulated by CD47 overexpression. The lack of immune activation after transplantation of hypoimmunogenic iPSCs resulted in graft survival of transplanted mouse and human iPSCs in all allogeneic recipients with an observation period of 50 days.

Conclusion: Hypoimmunogenic iPSCs might serve as an unlimited cell source for the generation of universally-compatible "off-the-shelf" cell grafts or tissues in future clinical applications without the need of immunosuppression.

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Reconditioning of Donation after Cardiac Death Hearts by Ex-Vivo Machine Perfusion with a Novel HTK-N Preservation Solution

L. Saemann,[1] S. Korkmaz,[1] F. Hoorn,[1] P. Zhou,[1] Q. Ding,[1] Y. Guo,[1] G. Veres,[2] S. Loganathan,[1] M. Brune,[3] F. Wenzel,[4] M. Karck,[1] and G. Szabó.[2] [1]Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany; [2]Department of Cardiac Surgery, University of Halle, Halle (Saale), Germany; [3]Department of Medicine I and Clinical Chemistry, University of Heidelberg, Heidelberg, Germany; and the [4]Faculty Medical and Life Sciences, Furtwangen University, Villingen-Schwenningen, Germany.

Purpose: Hearts donated from cardiac death donors (DCD) can be successfully maintained by ex-vivo machine perfusion (EVMP) with blood. Donor heart function will be deteriorated depending on the warm ischemia time (WIT). However, the reconditioning capability of machine perfusion with a novel crystalloid preservation solution histidine-tryptophane-ketoglutarate-N (HTK-N) has not been investigated.

Methods: In a pig model of DCD, cardiac death was induced by termination of ventilation, followed by harvesting of donor hearts. After a total WIT of 30 min, hearts underwent either 4 h of EVMP with warm donor blood (DCD-B) in a beating state or cold EVMP with oxygenated HTK-N (DCD-HTK-N), both followed by reperfusion in Langendorff fashion, or were directly undertaken reperfusion (DCD) (N=8/group). During reperfusion, hemodynamic function was measured by a left ventricular (LV) balloon catheter. Oxidative and nitrosative stress as well as necrosis pathways and morphology of LV-tissue samples were analyzed by 4-hydroxy-2-nonenal (HNE), nitrotyrosine (NT), poly(adenosine diphosphate-ribose) polymerase (PARP) and hematoxylin-eosin (HE) staining.

Results: After 60 min of reperfusion, DCD-HTK-N hearts showed significantly improved end-systolic pressure (ESP = 172±10 mmHg, p=0.02) and rate of pressure increasement (dp/dt_{max} = 2161±214 mmHg/s, p=0.005) compared to immediately reperfused DCD-hearts (ESP = 132±5 mmHg, dp/dt_{max} = 1240±167 mmHg/s) at 20 mL of balloon volume. dp/dt_{max} and rate of pressure decreasement (dp/dt_{min} = -1501±228, p=0.005) were also significantly improved after EVMP with HTK-N compared to blood perfusion (dp/dt_{max} = 1177±156, dp/dt_{min} = - 637±79). After HTK-N perfusion, oxidative and nitrosative stress as well as necrosis were significantly decreased compared to direct reperfusion (HNE: p=0.005, NT: p=0.002, PARP: p=0.019) or blood perfusion (HNE: p<0.001, NT: p=0.004, PARP: p=0.028). HE staining showed a significantly elevated level of cell swelling in DCD-B hearts (527±25 μm^2) compared to DCD-HTK-N hearts (420±15 μm^2, p=0.008).

Conclusion: EVMP with HTK-N is able to recondition systolic function of DCD-hearts and leads to improved diastolic donor heart function compared to EVMP with blood by reducing oxidative stress, nitrosative stress as well as preventing cardiomyocytes from morphologic change.

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The Effect of Reperfusion on Cerebral Activity in Donors after Circulatory Death Following Normothermic Regional Perfusion

F.F. Dalsgaard,[1] N. Moeslund,[1] M. Pedersen,[2] E.Q. Montvilas,[3] and H. Eiskjær.[4] [1]Department of Cardiology, Research Unit, Aarhus University Hospital, Clinical Institute, Aarhus, Denmark; [2]Comparative Medicine Lab, Aarhus University Hospital, Clinical Institute, Aarhus, Denmark; [3]Department of Neurophysiology, Aarhus University Hospital, Aarhus, Denmark; and the [4]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark.

Purpose: To investigate brain activity and intracerebral physiological parameters following no-touch and normothermic regional perfusion (NRP) in donors after circulatory death.

Methods: The study comprised 16 female pigs of 85 kg. Following anesthesia, five burr holes were placed in the skull targeting the parasagittal cortex bilaterally. To assess cortical brain activity two needles were used to record electroencephalography (EEG) and somatosensory evoked potentials (SSEP). Two probes measuring intracranial pressure and cerebral blood perfusion (CBF), temperature, and oxygen were inserted. Additionally, a microdialysis catheter for metabolic monitoring of lactate, pyruvate, glycerol, and glucose was inserted. The animals were cannulated for the heart-lung machine and baseline measurements were performed before withdrawal from life support. Before NRP, the animals were randomized into two groups: Clamp or non-clamp of the aortic arch vessels. After mechanical asystole, a no-touch period of 8 minutes was observed before the institution of NRP. The animals were weaned from NRP after 30 minutes and subsequently monitored for 3 hours after weaning.

Results: In the clamp group, flatline EEG and no-SSEPs were recorded after weaning in all pigs. In the non-clamp group, SSEPs were observed after weaning in 6/8 pigs. In all pigs, a peculiar EEG activity of periodic 4-5 Hz complexes, amplitude up to 300 μV, with a repetition interval of one in every 8-60 seconds was recorded. In two pigs, continuous EEG activity was observed alongside the periodic complexes. Additionally, spontaneous respiratory movements were observed in 6/8 pigs in the non-clamp group during a brief pause of ventilation. Preliminary analysis of intracranial measurements of CBF, oxygen, and temperature indicates successful occlusion of the arch vessels in the clamp group and as such cessation of cerebral perfusion. All results await final statistical analysis.

Conclusion: Some degree of cerebral function returns following circulatory death and resuscitation with NRP and cerebral reperfusion. We observed continuous EEG activity alongside periodic complexes resembling the so-called Nu-complexes thought to originate in the hippocampus. In addition, SSEP response and negative apnea tests suggest that brain stem function returns following NRP with preserved cerebral perfusion.

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CA125 Predicts Right Heart Hemodynamic and Function and Identifies Distinct Congestion Phenotypes: Guidance for Therapy

Y. Peled,[1] R. Beigel,[1] E. Ram,[1] M. Arad,[1] R. Klempfner,[1] J. Patel,[2] and E. Raanani.[1] [1]Sheba Medical Center and Tel Aviv University, Ramat Gan, Israel; and the [2]Cedars-Sinai Heart institute and David Geffen School of Medicine, Los Angeles, CA.

Purpose: Elevated carbohydrate antigen 125 (CA125) correlates with serosal effusions and has previously been thought to be associated with the clinical severity of heart failure (HF) and fluid congestion. We assessed CA125 as a predictor of hemodynamics and investigated the additive prognostic benefit of B-type natriuretic peptide (BNP).

Methods: Between 2015-2019, 235 patients hospitalized for HF were prospectively assessed for CA125 and BNP serum levels simultaneously with echocardiography and right heart study.

Results: CA125 correlated positively with intracardiac pressures and inversely with cardiac index (CI). The strongest correlation was for right atrial pressure (RAP) (ρ = 0.82, p<0.001). CA125 >42 U/mL had a sensitivity of 95.8% and specificity of 95.3% for the prediction of RAP ≥11 mmHg. The predictive accuracy of CA125 vs BNP was superior for RAP (AUC 0.99 vs 0.84, p<0.001) and right ventricular (RV) pressure (AUC 0.99 vs 0.82, p<0.001), but BNP was better for the prediction of pulmonary capillary wedge pressure (AUC 0.95 vs 0.82, p<0.001) and CI (AUC 0.92 vs 0.84, p=0.03). Elevated CA125 was independently associated with increased right heart pressures, while BNP with left-sided filling pressures and lower CI (p<0.05). Combining CA125 and BNP values delineated 4