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Protecting donor livers during normothermic machine perfusion with stem cell EVs

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Two rapidly growing areas in transplantation research are the use of normothermic machine perfusion (NMP) of donor organs and the therapeutic use of stem cell-derived extracellular vesicles (EVs) to improve graft viability. In this issue of *Transplantation*, Rigo et al describe the first study to combine these 2 exciting fields in an innovative approach to recondition injured donor livers before transplantation [1].

The utilization of extended criteria donor (ECD) livers is increasing in an attempt to keep up with the growing gap between liver supply and demand. Thus, to improve outcomes of transplanted ECD livers, graft optimization or repair strategies are being evaluated. One such strategy is the application of NMP to better preserve donor livers as well as to recondition ECD livers. Unlike cold storage (CS) or hypothermic machine perfusion, NMP restores physiological conditions by continuously supplying oxygen and nutrients at normothermic (37°C) temperature during *ex situ* liver preservation. The goal here is to minimize (or avoid) ischemia and hypothermia and provide a means to assess organ viability prior to transplantation. For example, Krangwa et al recently demonstrated that elevated D-dimer (a marker of fibrinolysis) concentration during liver NMP correlated with ischemia-reperfusion injury and may serve as a predictor of poor graft function [2]. Preclinical experiments indicate numerous benefits of NMP over CS, and initial clinical trials demonstrate safety and feasibility [3]. Future studies will determine if NMP might someday replace CS, but the real excitement lies with the use of NMP as a platform for reconditioning ECD livers to expand the donor organ pool.

Use of NMP not only enables assessment of organ viability but also provides a novel platform upon which marginal donor organs could be therapeutically reconditioned for successful transplantation. One such therapy currently under heavy research is the application of mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs). MSCs are known to modulate immune responses, which has led to a large number of clinical trials using MSCs for cell therapy in many fields including liver disease [4] and solid organ transplantation [5]. Many studies are showing that MSCs exert most of their therapeutic function in a paracrine manner via release of EVs (ie MSC-EVs). EVs are a family of small

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membrane vesicles that differ in size, biogenesis and molecular content. Regarding mechanism of action, EVs exert their therapeutic effect by delivering various proteins, microRNA, mRNA, and sometimes genomic DNA into target tissues. Thus, EVs can exert different functions since EV cargo is related to their parent cell lineage and metabolic state during EV production. Because EVs can be released by many tissues after organ transplantation, EVs are also becoming viewed as a new category of biomarkers in transplantation [6]. For example, plasma EVs may contain specific mRNA transcripts that serve as a biomarker to predict antibody-mediated rejection after kidney transplantation [7]. Alternatively, many groups are studying the therapeutic functions of MSC-EVs in animal models of organ injury. Here, the impact of MSC-EVs on liver disease has been studied in a number of models; all showing that administration of MSC-EVs is protective [8].

Very few studies in this area have applied MSC-EVs during NMP of donor organs, none of which included livers. For example, our laboratory recently demonstrated that MSC-EVs protect donation after circulatory death (DCD) lungs during ex vivo lung perfusion [9]. Another study by Gregorini et al showed that MSC-EVs protected DCD kidneys in the setting of hypothermic machine perfusion [10]. Thus, the current study by Rigo et al is quite novel in that it is the first to describe NMP-directed MSC-EV therapy in injured livers [1]. In this case, EVs were isolated from human liver stem-like cells (HLSCs) and administered to livers 15 minutes after initiation of NMP. The injury model used in this study was quite different from a clinical transplant setting in that healthy rat livers experienced ongoing hypoxic injury during 4 hours of NMP by maintaining suboptimal oxygen delivery via low hematocrit. The authors show that this model results in limited but progressive injury as characterized by increasing levels of cytolysis enzymes (AST, ALT and LDH) in the perfusate and areas of necrosis and apoptosis in liver sections while bile production was maintained. Further results indicated that inclusion of HLSC-EVs (only a single dose was tested) in the perfusate significantly reduced histological damage, apoptosis, and levels of AST and LDH compared to NMP without HLSC-EVs. In addition, using immunofluorescence, the authors were able to demonstrate uptake (engraftment) of HLSC-EVs by hepatocytes after 4 hours of NMP. These results provided proof-of-concept that HLSC-EV treatment effectively attenuated liver injury during hypoxic NMP.

Several limitations are inherent in this study. First, the authors did not perform liver transplantation to determine if protection during NMP results in improved outcomes after transplant. Future studies will entail transplantation after NMP, but because NMP allows the assessment of donor liver viability, it is reasonable to predict that improved function during NMP will lead to better outcomes after transplant. Second, little mechanistic insight was gained from this study except that it was shown that EV treatment limited hypoxia-dependent responses as shown by reduced RNA expression of HIF-1 α and TGF- α 1. The determination of internal contents of EVs with studies like RNA sequencing may help define the mechanistic pathways affected by EV treatment. Third, the study did not evaluate a dose response for EVs, and it would have been more informative if a longer time of perfusion was evaluated. Another remaining question is whether HLSC-derived EVs provide comparable or superior protection versus EVs derived from other types of stem cells. Despite these limitations, this study demonstrated proof-of-concept that delivery of HLSC-EVs by NMP can recondition injured donor livers before transplantation. This warrants further

investigation into this promising strategy to increase the utilization of ECD livers through a pretransplantation viability assessment and reconditioning protocol.

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Abbreviations

CS	cold storage
DCD	donation after circulatory death
ECD	extended criteria donor
EV	extracellular vesicle
HLSC	human liver stem-like cell
MSC	mesenchymal stem cell
NMP	normothermic machine perfusion

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