Cardiac mitochondrial transplantation: the force awakens

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***TEXT**:*

"Without the midi-chlorians, life could not exist, and we would have no knowledge of the Force (...)"

[Star Wars: The Phantom Menace]

Mitochondria are intracellular organelles responsible for energy production. Maintaining a healthy mitochondrial mass is critical for the longevity of postmitotic cells, such as cardiomyocytes. The heart has the highest energy demand of any organ in the body. Under physiological conditions, cardiomyocyte´s mitochondria have to produce more than 5 Kg of ATP daily. To meet these massive energetic demands, mitochondria account for 30% of cardiomyocyte volume. Abnormalities in cardiomyocyte´s mitochondrial function have thus a significant deleterious impact on heart function. Primary mitochondrial diseases result in complex phenotypes that many times include a cardiac dysfunction, mainly in the form of dilated or hypertrophic cardiomyopathy.(1-3) Besides rare genetic diseases, mitochondrial dysfunction also plays an important role in the pathogenesis of "common" diseases. Damage to mitochondria is a well-recognized factor contributing to irreversible cardiac loss in ischemia/reperfusion injury (myocardial infarction).(4,5) Heart failure, either with reduced or preserved ejection fraction, is another condition that is frequently associated with cardiomyocyte´s mitochondrial dysfunction,(6) regardless of the primary cause of the disease (i.e. ischemic origin, pressure overload, etc).(7) Genetically-induced imbalanced mitochondrial dynamics leading to fragmentation of these organelles is associated with an overt dilated cardiomyopathy phenotype in mice.(8) Finally, cumulative evidence shows that the leading mechanism of anthracycline-induced cardiotoxicity is an irreversible damage to cardiomyocyte´s mitochondria.(9-11) Unlike other organelles, mitochondria originate only from pre-existing peers through binary fission; cells cannot generate new mitochondria via any other route. In addition,

cardiomyocytes´ proliferative capacity in adult hearts is negligible. Thus, heart diseases resulting in irreversible mitochondrial defects are currently only amenable to palliative treatments.

In recent years, it has been shown that mitochondrial transfer from a healthy (donor) cell to a damaged (recipient) cell occurs physiologically as a mechanism for increasing cell fitness, eventually by rescuing respiration defects or regulating signalling.(12-15) Cell-to-cell mitochondrial transfer occurs not only as a means to incorporate competent mitochondria into a dysfunctional cell, but also as a means to expel dysfunctional mitochondria (through extracellular vesicles, EVs) for subsequent external mitophagy by the recipient cell.(16) A very recent landmark study has demonstrated that cardiomyocytes outsource mitophagy to a network of cardiac-resident macrophages to maintain heart homeostasis.(17)

After the recognition of the cell-to-cell mitochondrial transfer phenomena physiologically occurring in different organs, the next question was whether this could be artificially manipulated for therapeutic purposes. The concept of mitochondrial artificial transfer (also called mitochondrial transplant) began to evolve. McCully´s lab in Harvard was pioneer in testing the feasibility of mitochondrial transplantation into hearts after experimental ischemia reperfusion injury.(18-20) Fresh isolated mitochondria from skeletal muscle were directly injected (naked) in the heart one day after experimental infarction, with apparently promising results in terms of rescuing ischemic myocardium in the border zone. The same group reported in the pig model of myocardial infarction that intracoronary injection of fresh mitochondria isolated from the own pig skeletal muscle was able to reduce infarct size and improve cardiac function.(21) Transplanted (naked) mitochondria have been shown to enter cardiomyocytes and integrate within the host mitochondria network.(22) Intramyocardial injection of naked mitochondria has

even been performed in pediatric patients undergoing cardiac surgery without apparent side effects.(23) After these early experiences, several groups have reported successful mitochondrial transplantation in different organs.(24-26) Several strategies have been developed to increase the efficiency of naked mitochondrial transplantation (27-29). However, despite most of these studies reported acceptable mitochondrial transfer efficiency into target organs/cells, as well as metabolic and functional benefits, there are concerns about these methodologies(30,31): what is the viability of naked mitochondria after aggressive isolation protocols? How much time can injected mitochondria survive in a hostile environment (interstitial space of the target organ) before entering the target cell? is the mechanism responsible for naked mitochondria internalization the same for all target cell-types? etc.

In the present issue of the Journal, Ikeda et al.(32) present a novel strategy for artificial cardiac mitochondrial transplant that could overcome some of the shortcomings of the previous approaches. Authors exploited the well-known abilities of EVs to efficiently transfer their cargo into different cells.(33) In their elegant study, Ikeda et al(32) demonstrate through an impressive set of in vitro experiments that human induced pluripotent stem cells-derived cardiomyocytes (iCM) secrete EVs that are rich in functional mitochondria. In their study, iCM-derived EVs efficiently transferred their cargo (mitochondria and other material) into recipient cardiomyocytes in vitro. Medium rich in iCM-derived EVs improved mitochondrial function of cardiomyocytes subjected to simulated ischemia. Isolated (naked) mitochondria did not show the same beneficial effects on cardiomyocytes. Finally, in a mouse model of ischemia/reperfusion injury, direct intramyocardial injection of iCM-derived EVs resulted in cardioprotection.

The cardioprotective effects of EVs is not entirely new since, among others, Vicencio et al reported 5 years ago that exosomes isolated from human plasma exerted

cardioprotection in experimental ischemia/reperfusion by a pathway involving cardiomyocyte´s TLR4.(34) Whether the same mechanisms are involved in the transfer of mitochondria contained in iCM-derived EVs is unknown. The present study by Ikeda et al.(32) is a very important piece for the field, but still there are several open questions that need to be addressed before this approach can be considered translational. First, while most of the evidence of the work suggests that the benefits observed were secondary to efficient mitochondrial transfer, the fact that non-mitochondrial cargo of iCM-derived EVs also rescued mitochondrial function in recipient cells leaves the door open for a mitochondria-independent mechanism. Second, the composition of the EVs was uncontrolled since it was a spontaneous phenomenon upon culture iCMs in certain conditions. Whether the mitochondrial composition of EVs change upon different conditions is unknown. Third, it is unknown if other cell types also pack functional mitochondria in their EVs and whether they exert the same cardioprotective effect.

Several considerations should be done related to the translational potential of this approach. The cardiac disease chosen by Ikeda et al.,(32) myocardial infarction (ischemia/reperfusion injury), is valid as a proof of concept in mice but extremely hard to translate to the clinics. In the acute setting, the possibility of implementing advanced therapies, such as mitochondrial transplant, is extremely challenging. This would require heterologous material ready to use, with the additional ethical and logistic complications. The chronic heart failure and anthracycline-induced cardiotoxicity settings seem more appropriate disease models. Unfortunately, it is unknown if this novel methodology based on iCM-derived EVs will be beneficial in these disease contexts. The route of administration is a relevant aspect when considering a therapy in humans. Direct myocardial injection of EVs containing mitochondria is an aggressive approach unlikely to be widely implemented. Intracoronary administration of naked mitochondria has been

reported to result in their engraftment in cardiomyocytes in the pig model,(21) so it seems an approach worth testing for the iCM-derived EVs methodology. Finally, before considering this advanced therapy in humans, it is imperative to perform robust preclinical trials with relevant endpoints.(35,36)

In summary, the present paper(32) demonstrates that human induced pluripotent stem cells-derived cardiomyocyte release mitochondria-rich EVs that have the potential to release their cargo into recipient damaged cardiomyocytes. This novel strategy for mitochondrial transplant theoretically overcome some limitations of previous methodologies, such as naked mitochondria injection. The possibility of finding an efficient way of transplanting healthy mitochondria into failing hearts to restore myocardial energy production will represent a revolution in the treatment of myocardial diseases. For all groups working in this fascinating field, may the force be with you.

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Carlos Galán-Arriola did the Central illustration.

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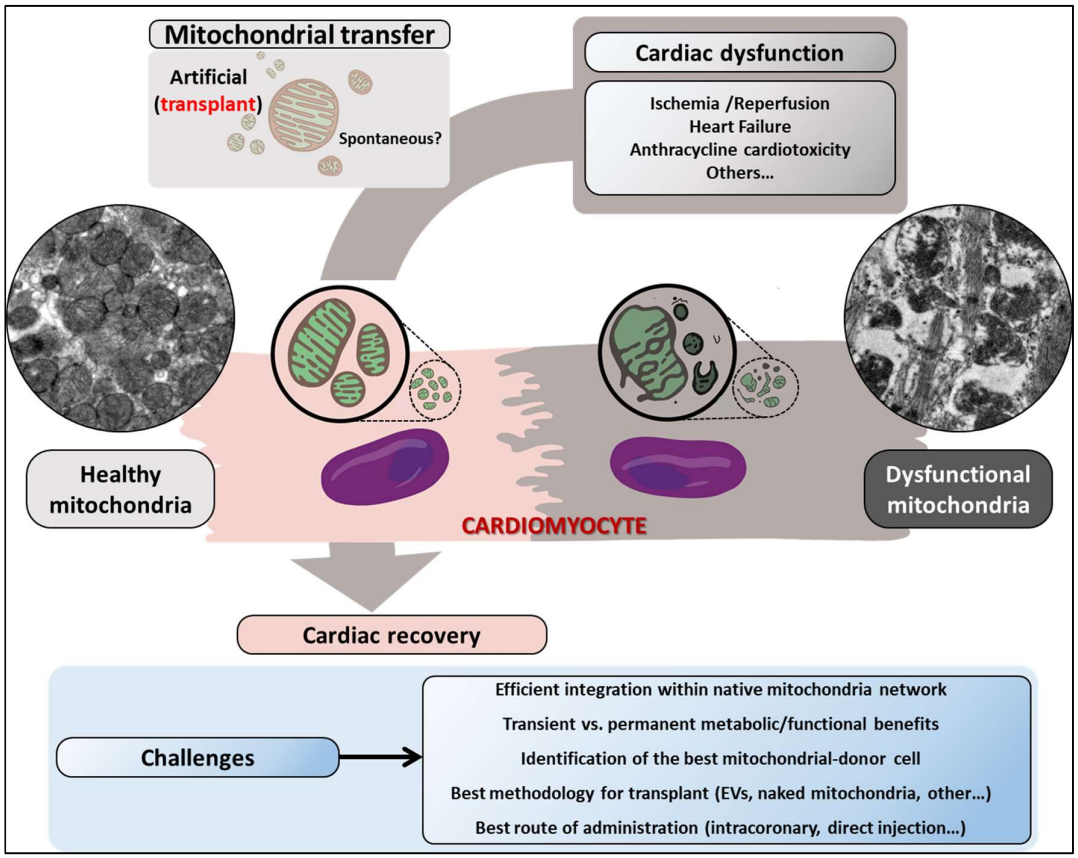
CENTRAL ILLUSTRATION. Opportunities and challenges for cardiac mitochondrial transplantation.



Mitochondrial transplant is the artificial transfer of healthy mitochondria into cells with dysfunctional native organelles. This revolutionary approach would open a window of opportunity for treating many myocardial diseases in which mitochondria dysfunction plays a main role. Before this potential therapy can be considered translational, several challenges should be solved. Besides the challenges represented in the Figure, it is imperative to test this intervention in relevant large animal models. Pigs models of anthracycline-induced cardiotoxicity(11) and dilated cardiomyopathy secondary to hibernated myocardium(37) have been developed and seem ideally suited for this endeavor. Transmission electron microscopy mitochondria shown in the Figure correspond to a healthy pig heart (left) and from a pig with terminal heart failure

secondary to anthracycline-induced cardiotoxicity from the Translational Laboratory for Cardiovascular Imaging and Therapy at the CNIC.