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Review Article

Targeted Mitochondrial Delivery to Hepatocytes: A Review

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Abstract

Defects in mitochondria are responsible for various genetic and acquired diseases. Mitochondrial transplantation, a method that involves introduction of healthy donor mitochondria into cells with dysfunctional mitochondria, could offer a novel approach to treat such diseases. Some studies have demonstrated the therapeutic benefit of mitochondrial transplantation and targeted delivery *in vivo* and *in vitro* within hepatocytes and the liver. This review discusses the issues regarding isolation and delivery of mitochondria to hepatocytes and the liver, and examines the existing literature in order to elucidate the utility and practicality of mitochondrial transplantation in the treatment of liver disease. Studies reviewed demonstrate that mitochondrial uptake could specifically target hepatocytes, address the challenge of non-specific localization of donor mitochondria, and provide evidence of changes in liver function following injection of mitochondria into mouse and rat disease models. While potential benefits and advantages of mitochondrial transplantation are evident, more research is needed to determine the practicality of mitochondrial transplantation for the treatment of genetic and acquired liver diseases.

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Introduction

Mitochondria are the powerhouses of cells, and are different from other organelles in that they have two membranes surrounding their own DNA, RNA, and ribosomes.[1] The latter allow them to produce their own proteins and replicate.[2] They generate energy through the Krebs cycle and the electron transport chain by converting fatty acids and carbohydrates into carbon dioxide and water.[3] They also produce reactive oxygen species (ROS), which are important cell signals for many physiological processes.[4] In addition, mitochondria also play a role in calcium buffering, a process involved in the regulation of ATP production by interaction with metabolic enzymes, such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and isocitrate dehydrogenase.[5] Thus, mitochondria are believed to be essential for all eukaryotic life, especially for mammals.

All mammalian cells, except mature red blood cells, have mitochondria.[6] Defects of mitochondria can lead to various types of diseases, both genetic and acquired. At present, there is no way to replace or repair mitochondria. Mitochondrial transplantation, which involves the introduction of healthy mitochondria into cells with damaged mitochondria, could represent a novel approach to treat diseases that are due to mitochondrial damage. Various studies have explored the applications of mitochondrial transplantation in models including cardiovascular injury,[7–13] respiratory injury,[14–16] neural injury,[17–21] muscle function,[22] renal injury,[23,24] skin,[25] and cancer.[26–28] Few studies, however, have investigated mitochondrial transplantation within the context of genetic and acquired liver disease.

The aim of this review is to discuss the developments and challenges surrounding the preparation, isolation, and delivery of mitochondria into hepatocytes and the liver, and the potential for the treatment of liver disease.

The clinical problem

Diseases that involve mitochondrial dysfunction can be divided into inherited (genetic) and acquired disorders. Besides the differences in transmission and prevalence, a practical distinction between the two lies in the requirement for effects of mitochondrial transplantation to be long-lasting in the former group, while short-term bridging of function may be sufficient in the latter group.

Inherited mitochondrial disorders are a group of diseases caused by mutations in mitochondrial DNA (mtDNA) and/or nuclear DNA which encode mitochondria proteins.[29,30] Nearly every organ and tissue could be affected by mitochondrial disorders, although the neural system and the liver are the most common target organs.[31] Since mtDNA encodes 13 proteins of the respiratory chain, most mutations of mtDNA would result in primary defects of respiratory chain function. There are more than 20 diseases caused by inherited mitochondrial dysfunction, and many of these diseases are either lethal or result in a shortened life span and dysfunctional organs.[30]

Mitochondrial dysfunction could also be caused by acquired damage to mitochondria. The electron transport system, especially the respiratory complexes, are frequent tar-

Keywords: Mitochondria; Hepatocytes; Transplantation; Liver; In vitro techniques.

Abbreviations: ALT, alanine aminotransferase; APAP, acetaminophen; AsG, asialoglycoprotein; AsOR, asialoorosomucoid; AsOR-PL, asialoorosomucoid conjugated to polylysine; AST, aspartate aminotransferase; CCl_4, carbon tetrachloride; DILI, drug-induced liver injury; LLO, listeriolysin O; mtDNA, mitochondrial DNA; NAFLD, nonalcoholic fatty liver disease; ROS, reactive oxygen species; TEM, transmission election microscope.

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gets. Acquired mitochondrial defects are usually caused by toxins, medications, or aging, and are observed in numerous diseases and pathologies including chronic kidney diseases,[32] uremia,[33] cardiac infarctions,[34] cardiac surgery,[35] atherosclerosis,[36] organ transplantations,[37] strokes,[38] spinal cord injury,[39] traumatic brain injury,[40] obesity,[41] diabetes,[42] insulin resistance,[43] as well as age-related disorders such as Alzheimer's and Parkinson's diseases, and various types of cancers.[44]

Generally, cells that are highly metabolically active and those that replicate rapidly require larger numbers of mitochondria and tend to be more susceptible to damage compared to metabolically inactive cells. Therefore, hepatocytes often bear the brunt of damage, and most liver diseases are accompanied with mitochondrial dysfunction.[45–49] Nonalcoholic fatty liver disease (NAFLD), for example, has been described to have structural and molecular changes in hepatic mitochondria. Furthermore, declines in mitochondrial function may contribute to increased mitochondrial cholesterol accumulation, which has been associated with the progression of steatosis to steatohepatitis.[50] Evidence also suggests that the activation of hepatic stellate cells during hepatic fibrosis is associated with mitochondrial dysfunction.[51] Moreover, electron transport chain defects and increased oxidative stress have been reported to contribute to the development of hepatocellular carcinoma.[52]

Preparation and isolation of mitochondria

Among the most important factors that determine whether mitochondrial delivery will be successful are the quality and condition of the mitochondria used. Many different methods have been published about preparation and isolation of mitochondria.[53–57] The most commonly used method includes tissue homogenization followed by several centrifugations at different speeds.[56] There are also many commercial kits for mitochondria isolation, but the protocols of those kits are mainly based on these steps of homogenization and centrifugation. Special isolation buffer is essential for mitochondrial isolation because of the fragility of the membranous structures, and sensitivity to changes in pH, osmolality, temperature, and ionic concentrations.[56,58] Mitochondria isolated in this way are usually contaminated by other organelles, such as lysosomes and cell debris.

To further purify mitochondria, equilibrium density gradient ultracentrifugation is the most widely used technique.[59] However, this method requires special equipment and considerable time for the mitochondria to reach their equilibrium densities. In addition, high centrifugal force during ultracentrifugation may cause damage to mitochondria, and result in variability in the quality, viability, and yield.[60] Banik and Dhar[61] isolated functional mitochondria using paramagnetic iron oxide nanoparticles. This method achieved purification of mitochondria without using centrifugation, but the nanoparticles could not be separated from the mitochondria after isolation. Zischka *et al*.[62] used free-flow zonal electrophoresis to purify mitochondria by a special free-flow apparatus. Farah *et al*.[58] passed isolated mitochondria sequentially through 1.2-μ and 0.8-μ filters to acheive purification. This not only shortened the time required for mitochondria isolation and purification but also resulted in high percentages of intact mitochondria that were shown to have normal function as well.

Cell culture preparations

Pallotti and Lenaz[63] outlined various methods to prepare and isolate mitochondria from cell cultures. Isolation typically involves harvesting cells by centrifugation, a buffer wash, resuspension in solution, homogenization of the suspension with a glass pestle, and centrifugation at various speeds. Various procedures can separate purified mitochondrial fractions, including separation on a sucrose gradient or a "no gradient procedure" that utilizes a mannitol-sucrose buffer instead of isotonic sucrose. Methods to obtain mitoplasts from gradient-purified mitochondria include ones where mitochondria are re-suspended and allowed to swell on ice or where the mitochondria are re-suspended and incubated with digitonin. Frezza *et al*.[56] developed a protocol to isolate mitochondria from mouse embryonic fibroblasts. Isolation in this protocol also involved homogenization of cultured cells as well as centrifugation at different speeds. Compared to other protocols, the investigators employed different speeds in their differential centrifugation steps and used sucrose instead of mannitol as an osmolyte in isolation buffer.

Tissue preparations

An early study demonstrated that centrifugal fractionation of rat liver homogenates prepared in 0.88 M sucrose to separate mitochondria from other cellular components.[53] Since then, many studies have described methods to prepare and isolate mitochondria from tissue. Hovius *et al*.[54] demonstrated that differential and Percoll gradient centrifugations could isolate highly purified and intact mitochondria from rat liver. Renault *et al*.[55] described a protocol to isolate rat mitochondria using homogenization and differential centrifugation; the authors also described protocols to fractionate mitochondria by size and to allow for real-time mitochondrial outer membrane permeabilization measurements.

Frezza *et al*.[56] provided a protocol for preparation and isolation of mitochondria from mouse liver tissue. This protocol is similar to their cell culture preparation and isolation protocol, in that the liver was homogenized and centrifuged at various speeds. The protocol for isolation from mouse skeletal muscle tissue differed in that the minced muscle was incubated with phosphate-buffered saline and trypsin for 30 min, centrifuged at 200×*g* for 10 min at 4°C, then homogenized and centrifuged at various speeds. Additionally, Djafarzadeh and Jakob[57] also used homogenization and differential centrifugation for the isolation of skeletal muscle after trypsinization.

General methods of mitochondrial delivery

The idea of mitochondrial transplantation is based on observations that mitochondria are naturally transferred between cells through various mechanisms, including cell fusion,[64] microvesicles,[65] gap junction,[66] and tunneling nanotube formation[66] (Fig. 1). These mechanisms have been explored in non-hepatic models. Cell fusion involves cells sharing organelles and cytosolic components by coalescence of separate plasma cell membranes (Fig. 1A). Acquistapace *et al*.[67] demonstrated that partial cell fusion and reprogramming could serve as mechanisms to rescue post-mitotic cardiomyocytes *in vitro* using mouse terminally differentiated cardiomyocytes and human multipotent adipose-derived stem cells. Gap junctions between adjacent cells allow for the transfer of small molecules and ions (Fig. 1B). Connexins within gap junctions, such as connexin-43, have been shown to regulate mitochondrial transfer.[64] Tunneling nanotubules are thin membrane channels that allow for the direct intercellular transfer of organelles and membrane vesicles (Fig. 1C).[68]

As part of intercellular communication, cells can also se-

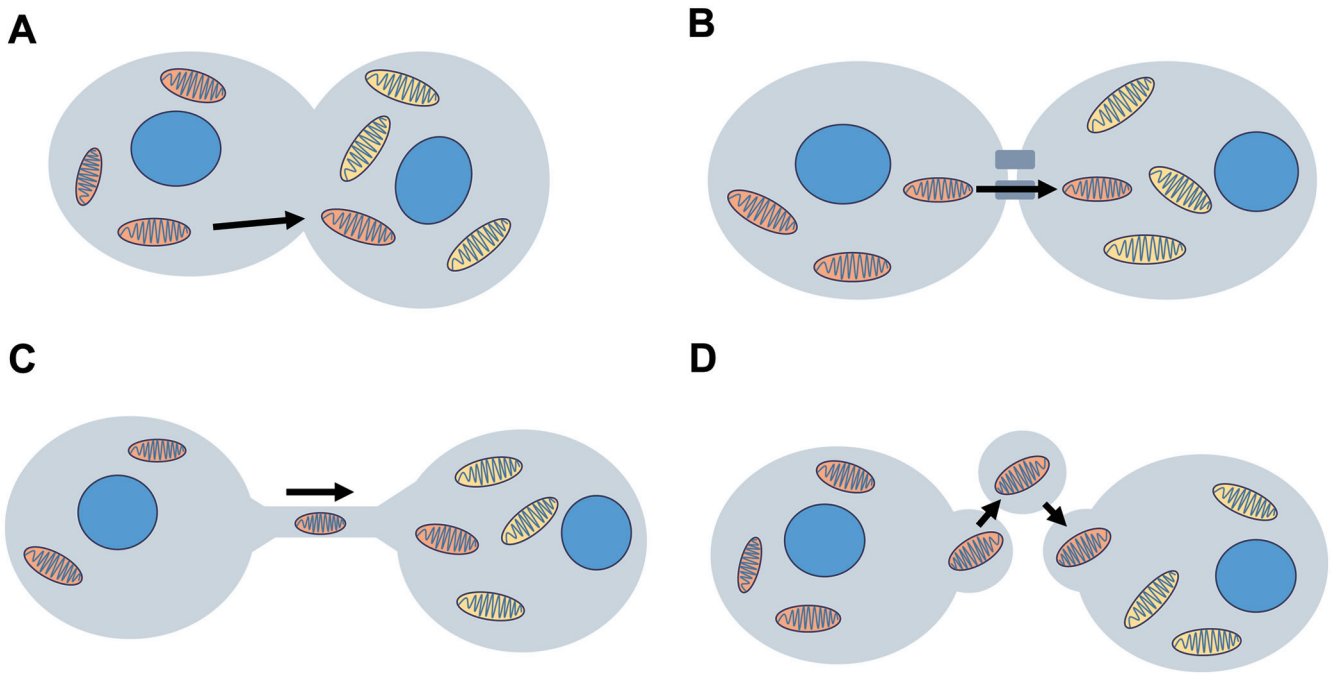

Fig. 1. Mechanisms of mitochondrial transfer. Orange ovals represent donor mitochondria and yellow ovals represent preexisting mitochondria in recipient cells. Donor cells can supply healthy mitochondria to recipient cells with dysfunctional preexisting mitochondria through various mechanisms. (A) Cell fusion. (B) Gap junction. (C) Tunneling nanotubules. (D) Microvesicle transport.

crete extracellular vesicles that vary in shape and size (Fig. 1D). Smaller vesicles may contain mitochondrial fragments that include mitochondrial proteins and mtDNA, while larger particles may contain entire functional mitochondria.[64,69] A commonly used mechanism of mitochondrial delivery involves direct injection.

Detection of donor mitochondria

One of the major challenges in studies on mitochondrial transfer to cells is how to distinguish donor from preexisting mitochondria within recipient cells after transfers. Successful approaches to this problem can allow quantitation, intracellular localization, and survival of donor organelles. A popular method to label and track donor mitochondria is to use plasmid vectors carrying fluorescent proteins and mitochondria targeting sequence.[70] Mitochondria can also be directly stained within cells[71] by several commercial mitochondrial dye kits, such as MitoTracker Red CMXRos or MitoTracker Green FM (Invitrogen-Molecular Probes, Eugene, OR, USA). For very sensitive studies, *in situ* polymerase chain reaction and *in situ* hybridization for amplification of mtDNA have also been used to track mitochondrial delivery.[72]

Targeted delivery of mitochondria to hepatocytes in cell culture

Gupta *et al*.[73] targeted the uptake of mitochondria specifically to hepatocytes by receptor-mediated endocytosis by covalent linking of an asialoglycoprotein (AsG), asialoorosomucoid (AsOR), to polylysine, forming a conjugate, AsOR-PL (Fig. 2). The AsOR serves as a carrier protein that can be recognized and internalized by specific AsG receptors on the plasma membranes of mammalian hepatocytes. The polylysine, a synthetic polycation, allows for coating of negatively charged mitochondria through a non-damaging electrostatic interaction. This conjugate is simply mixed with healthy, functional rat mitochondria as donors. After extensive washing, intracellular uptake of rat mitochondria is assayed by real-time PCR using primers specific for (donor) rat mtDNA to distinguish donor from recipient (human) mtDNA, and is normalized to cellular lactate dehydrogenase gene copy number. For cell culture studies, two human hepatoma cell lines were used: Huh7, AsG receptor (+), and control SK Hep1 cells, AsG receptor (−). Fl-AsOR-PL-mitochondria complexes showed 3,000-fold increases at 1 h, which doubled at 2 h for the Huh7 cells. To determine whether facilitation of endosomal escape could improve the overall mitochondrial delivery, an endosomolytic bacterial protein, listeriolysin O (LLO), was covalently linked to AsOR to form a targetable conjugate, AsOR-LLO. Co-administration with the intention of co-internalization of Fl-AsOR-PL-mitochondria complex and AsOR-LLO increased mtDNA levels in the Huh7 cells to 3-fold over mitochondria complex alone at 2 h. Pre-incubation of an excess of AsOR to compete with mitochondrial complexes decreased DNA levels by 80%, supporting the notion that AsG receptors are involved. No significant levels of donor mtDNA were found in control SK Hep1 AsGR (−) cells under any condition.

Intracellular cytoplasmic localization was demonstrated by immunofluorescence of labelled donor mitochondria and fluorescent endosomal markers. DNA levels peaked at 10 h, but became insignificant by 96 h. However, these recipient cells had a normal complement of mitochondria. To determine the effect of mitochondrial transplantation on cells deficient in mitochondria, cells were exposed to a toxin to destroy mtDNA. Huh7 and SK Hep-1 cells were exposed to toxin until mtDNA was no longer detectable by PCR. These cells, termed mito (−), required special media supplemented with nutrients in order to survive. To determine the effects of mitochondrial transplantation, the mito (−) cells were changed to media without supplements and mitochondrial complexes were added to the media. At regular inter-

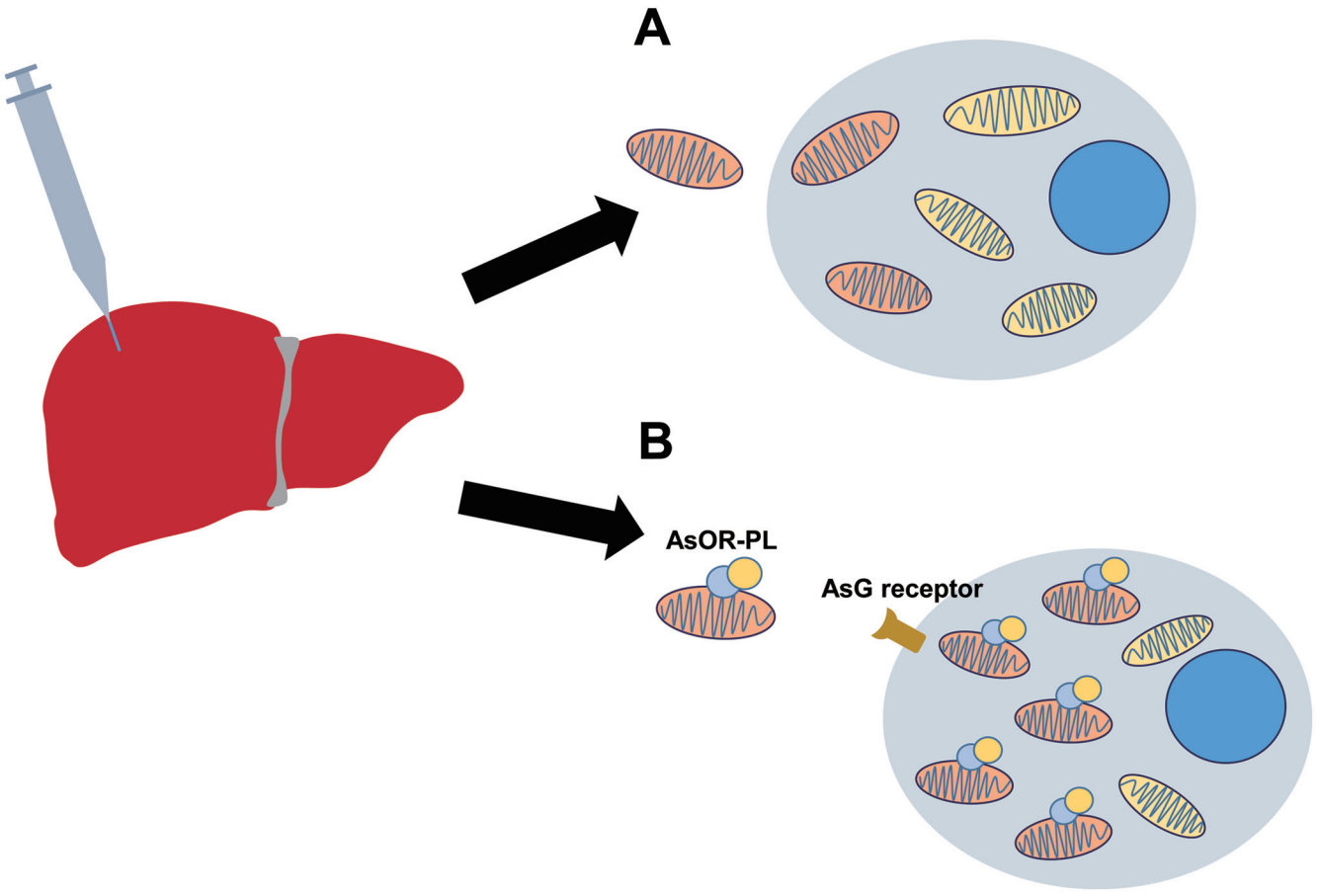

Fig. 2. Targeted delivery of mitochondria in cell culture. Orange ovals represent donor mitochondria and yellow ovals represent preexisting mitochondria in recipient cells. As opposed to traditional methods of direct, non-specific injection of mitochondria (A), Gupta *et al*.[73] targeted the uptake of mitochondria specifically to hepatocytes by preparing AsOR-PL, a conjugate that could be recognized and internalized by AsG receptors, specifically present on mammalian hepatocytes (B).

vals, the cells were assayed for cellular DNA. Cellular DNA levels in controls steadily declined with time. In contrast, cellular DNA of Huh7-mito (−) co-administered complexed mitochondria and AsOR-LLO remained stable through 24 h, and then increased 4-fold by 10 days. Furthermore, 10 days after the co-administration of the complexed mitochondria and AsOR-LLO conjugate, the mito (−) cells had increased oxygen-consumption rates to >90% of the parental hepatocellular carcinoma cells, suggesting a potentially restorative effect in the host cells following mitochondrial delivery. In control SK Hep-1 mito (−) cells, the addition of complexed mitochondria had no effect on either cellular DNA or oxygen consumption. This study demonstrated that mitochondria could be targeted specifically to AsG receptors on human hepatocellular carcinoma cells in culture.[73] However, a problem in these mitochondrial transfer studies is the difficulty in detecting and distinguishing donor for recipient mtDNA.

Animal studies on mitochondrial transplantation to hepatocytes

Table 1 lists recent studies that have investigated the effect of mitochondrial transplantation on the liver function in animal models. Liu *et al*.[72] described a method to specifically deliver mitochondria into the liver. To do this, donor mitochondria were harvested and purified from mouse liver and with PCR primers, as described previously by Gupta *et al*.[73] The formation of the AsOR-PL mitochondrial complexes was found not to alter the mitochondrial morphology, integrity, or oxygen consumption. In addition, a cytochrome c oxidase assay determined that the mitochondrial outer membranes were intact and that the integrity of the membrane were the same before and after complexation. Mitochondria alone and AsOR-PL-mitochondria complexes had nearly identical baseline oxygen consumption rates, and similar patterns of change upon the addition of respiratory transport chain inhibitors indicated that complex formation did not result in any visible damage or detectable decreases in metabolic function. Mitochondria alone, AsOR-PL mitochondrial complexes, and AsOR-PL mitochondrial complexes plus AsOR-LLO were separately infused intravenously by tail vein. Those rats injected with both AsOR-PL mitochondrial complex plus AsOR-LLO were found to have 27.1% of injected mitochondria within the liver by 1 h after the injection, a 3-fold and statistically significant increase over the AsOR-PL mitochondrial complex injected alone. For non-complexed mitochondria alone, only 2.7% of the total dose was found in the liver. For all groups, the fraction of mitochondria delivered to the spleen and lungs did not exceed 2% and 1%, respectively. From 2 h to 24 h after injection, the fold-change of donor mouse DNA in recipient rat livers increased in all groups but remained highest in the AsOR-PL mitochondrial complex plus AsOR-LLO group. Staining also revealed higher intrahepatic localization of donor mitochondria in the complex plus AsOR-LLO group.[72]

Table 1. Animal studies on mitochondrial transplantation to the liver

Study	Model	Mitochondria Source	Route	Results
Lin *et al*., 2013[74]	Partial liver ischemia-re-perfusion model in rats	Donor rat liver	Mitochondria injected into spleen	Reduced elevation of serum ALT, hepatocyte necrosis, and injury
Fu *et al*., 2017[75]	NAFLD model in mice	HepG2 cells transfected with lentiviral vector encoding a fusion protein of green fluorescence protein and mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase	Mitochondria injected into mice tail veins	Reduction in serum transaminase and lipid levels, increased cytochrome oxidase and ATP activity, decreased oxidative injury
Shi *et al*., 2018[76]	APAP-induced liver injury in mice	HepG2 cells transfected with lentiviral vector encoding a fusion protein of green fluorescence protein and mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase	Mitochondria injected into mice tail veins	Reduced oxidation stress and tissue injury, increased liver energy supply
Zhao *et al*., 2020[77]	Persistent CCl_4-induced liver injury model in mice	Liver mitochondria from healthy mice	Mitochondria injected into mice tail veins	Reduced oxidation stress, improved liver surface morphology, enhanced ALT and AST levels
Liu *et al*., 2020[72]	Delivery of targeted mitochondrial complexes in rats	CD-1 mouse liver mitochondria	Mitochondria injected into rat tail veins	27% of total injected mitochondria was found in the liver

NAFLD, non-alcoholic fatty liver disease; CCl_4, carbon tetrachloride; ALT, alanine aminotransferase; ATP, adenosine triphosphate; AST, aspartate aminotransferase.

Lin *et al*.[74] also injected isolated mitochondria into rats with reperfusion injury. Mitochondria were isolated from the non-ischemic liver regions of three donor rats. Donor mitochondria injected into the spleens of recipient rats and recipient livers were harvested for analysis after 45-m ischemia and 240 m of reperfusion. Confocal microscopy revealed that labeled donor mitochondria were distributed among the liver parenchyma after 240 m, though it was not determined when levels of donor mitochondria peaked or for how long they remained in recipient tissue. Regardless, the results demonstrated a reduction of the elevation of serum alanine aminotransferase (ALT), hepatocyte necrosis, and production of ROS in rats that received the allogenic mitochondria.

Fu *et al*.[75] utilized mitochondria to treat NAFLD in mice. Following lentiviral transfection for 48 h, they demonstrated that isolated mitochondria displayed spherical shape, an intact double membrane structure and cristae, and good dispersion using a transmission election microscope (TEM). They injected mice tail veins with green fluorescent protein-tagged mitochondria to assess *in vivo* distribution and found that after 2 h, mitochondria appeared in the liver, lung, brain, muscle, and kidney, and that mitochondria entered the various tissue cells. Following an 8-week, high-fat diet, mice were separated into two therapy groups, one in which the mice were injected with mitochondria once every 3 days for 9 days (therapy group 1) and another in which mice received injections every 3 days for 18 days (therapy group 2), as well as a control fatty liver group in which mice received only saline injections. TEM analysis showed that the fatty liver diet damaged hepatic mitochondrial structure and function, and that the morphology improved 12 h following mitochondrial injection. Additionally, serum aminotransferase and lipid levels were significantly decreased in the therapy groups compared to the control untreated fatty liver mice. Biochemical measurement also demonstrated that cytochrome oxidase activity and ATP were increased in the therapy groups, and that levels of oxidative injury were significantly reduced compared to that in the control untreated fatty liver mice.[75] That study did not assess long-term function or distribution of the transplanted mitochondria. Furthermore, although the transplanted mitochondria provided a protective effect against the high-fat diet, the viability of the isolated mitochondria was unclear beyond the TEM finding of their spherical shape.

The authors conducted another study in which mitochondria isolated from human hepatoma cells were used to treat acetaminophen (APAP)-induced liver injury in mice. Exogenous mitochondria were cultured with mice hepatocytes *in vitro* and were injected into mice tail veins *in vivo*. Mitochondria were tagged with green fluorescent protein to observe the efficiency of mitochondrial transfer and it was found that the mitochondria remained intact following isolation. A live confocal microscopic study demonstrated the fluorescent mitochondria primarily within mouse hepatocytes at 10 m. TEM showed that the mechanism of internalization possibly involved endocytosis, and that the efficiency of mitochondrial transformation into cells was 63.29±9.56%.[76] The status of the mitochondria beyond 10 m after injection was not reported. *In vivo*, injected fluorescent mitochondria were also found in the brain, lung, liver, kidney, and muscle. Flow cytometry showed that the efficiency of mitochondrial transformation was 45.45±0.08%. Twenty-four hours after the APAP-treated mice received exogenous mitochondria, ALT and aspartate aminotransferase (AST) significantly decreased. Histopathological analysis of liver sections found liver damage in the APAP treatment group and reduced hepatotoxicity in the mitochondria treatment group. Mitochondria treatment also caused a 39.9% increase in ATP level and reduced oxidative damage.[76]

In a recent study, this group also investigated the effect of mitochondrial transplantation on carbon tetrachloride (CCl_4)-induced liver injury. In hepatocytes damaged by CCl_4, exogenous mitochondria were found to increase cell viability over 8 h. This viability was not explored beyond 8 h. In an animal model of liver injury, adult male mice

received CCl_4 for 3 weeks and were divided into the following groups: one injected with 0.2 mg/kg mitochondria (mito-low) daily for 7 days, another which was injected with 0.4 mg/kg (mito-high) daily for 7 days, and a control group. Mice injected with fluorescence-labeled mitochondria showed distribution in the liver, lungs, kidney, and heart after 4 h. Fluorescence was strongest in livers impaired by CCl_4, indicating that the injury may increase the amount of mitochondrial entry. Mitochondrial therapy in injured mice improved mitochondrial ultrastructure and also enhanced ALT and AST levels. Furthermore, it reversed the membrane potential decrease caused by CCl_4 injury and significantly increased the levels of respiratory chain-related enzymes. Mitochondrial transplantation also improved liver surface morphology and significantly decreased the size of fibrotic areas. The authors also demonstrated that the mitochondria restored oxidative phosphorylation function, prevented cell proliferation in the setting of injury, and accelerated xenobiotic metabolism transportation, suggesting that mitochondria facilitated the transformation and elimination of CCl_4 and maintained liver protein homeostasis.[77] Although that study only explored the short-term effects of mitochondria transplantation, one strength is that transcriptomic analysis was used to investigate the molecular signal mechanism of mitochondrial therapy. The authors found that CCl_4-induced hepatocyte damage was closely associated with the mitochondrial unfolded protein response pathway, and that activation of this pathway may serve as a protective mechanism to maintain cell homeostasis.[77]

Advantages of mitochondrial transplantation

The mechanisms of non-targeted mitochondrial transfer are nonspecific, resulting in uptake by various cell types.[72] While methods for cell-specific delivery are more complicated than non-targeted delivery, there are also many advantages of targeted delivery to specific cell types. Nonspecific delivery generally results in exposure of mitochondria to cells throughout the body. In contrast, targeted delivery permits mitochondrial transmission only to cells in need, avoiding wasteful delivery elsewhere. This is important, as the supply of purified healthy and functional mitochondria to cells is limited. Specificity can also minimize side effects of delivery beyond the target tissues.

Another potential clinical advantage to transplantation of mitochondria is the fact that mitochondria possess their own DNA and replicate within cells when needed. Because of this property, in theory, introduction of only a small number of healthy donor mitochondria into recipient cells could lead to propagation of those donor organelles to reach the normal complement of mitochondria required by the energy needs of the individual cell. An additional advantage is the possibility of selection of donor mitochondria with particular traits. For example, isolation of mitochondria from sources resistant to certain mitochondrial toxins could be useful in treating individuals with liver failure due to exposure to such toxins.

Implicit in the preparation of donor mitochondria is the possibility of using host cells as a source of donor mitochondria. For example, nucleated blood cells could be propagated and mitochondria harvested as a relatively noninvasive means of obtaining mitochondria identical to those of the host. This could avoid potential immune issues due to differences in antigenicity.

Limitations of mitochondrial transplantation

Most of the studies on mitochondrial delivery have been carried out in non-hepatic models, with relatively few hepatic studies on the potential therapeutic effect of transplanted mitochondria in hepatocytes and the liver. Potential reasons for the lack of hepatic studies include the fact that the liver may not be easily accessible for direct transplantation, there are relatively few animal models of genetic and acquired mitochondrial diseases, and most studies have involved non-targeted delivery because of the difficulty in directing mitochondria to specific cell types. However, from the few studies available, it is apparent that there are potential limitations to the clinical application of mitochondrial transplantation. First, a reliable, standardized source of healthy mitochondria in large numbers are required. There are logistical issues with preservation, maintenance of function, and transportation of the fragile organelles. Because the mitochondria themselves, as well as the delivery systems may introduce foreign antigens, it is possible that adverse immune responses may occur. Finally, while transplantation efficacy has usually been reported for 24 h or somewhat longer periods following mitochondrial injection, long-term effects of mitochondrial transplantation are unkn own.[13,18,20,26,71]

Clinical implications

Elucidating the effectiveness and challenges associated with mitochondrial transfer in hepatocytes and the liver can ultimately help determine whether it would be a feasible therapeutic technique for the treatment of genetic and acquired mitochondrial diseases. While genetic mitochondrial diseases are fortunately rare, genetic mitochondrial mutations are common. One study found that the minimum prevalence rate for mtDNA mutations was 1 in 5,000 adults.[78] In contrast to the low prevalence of genetic mitochondrial disease, acquired mitochondrial dysfunction and disease is common. For example, in NAFLD, increased mitochondrial ROS production and decreased ROS scavenging mechanisms contribute to the disruption of mitochondria homeostasis. This is important because NAFLD is rapidly increasing in prevalence in the USA. It is estimated that between 75 million and 100 million people in the USA have NAFLD and approximately 20–30% of cases progress to nonalcoholic steatohepatitis, which can further progress to cirrhosis, hepatocellular carcinoma, and other complications.[79] Restoration of hepatic mitochondrial function could reduce the morbidity and mortality of the fatty liver epidemic.

Many classes of drugs can also contribute to drug-induced liver injury (DILI) through a variety of mechanisms, including mitochondrial disruption. Drug-induced hepatotoxicity is the principal cause of acute liver failure in the USA. While the incidence of DILI is difficult to assess, some population-based studies predict that the incidence ranges from 13.9 to 19.1 cases per 100,000 people per year.[80] The American DILI Network determined in a prospective study that antimicrobials caused almost half of the cases.[81] Mitochondrial dysfunction resulting from DILI can cause cytolytic hepatitis that can develop into liver failure, as well as steatosis and steatohepatitis, which can progress to cirrhosis.[82] Additionally, APAP-induced liver injury is a significant problem, as that drug is one of the most widely utilized analgesics in the USA.[83] It also accounts for over half of acute liver failures related to overdose, as well as approximately 20% of liver transplantation cases.[84] An important application of mitochondrial transplantation could include treating complications resulting from DILI by restoring mitochondrial function, which could save lives while avoiding the operative risk, expense, and long-term immunosuppression involved in liver transplantation.

Conclusions

In summary, while there is tantalizing evidence of the possibility of transplantation of mitochondria for therapeutic purposes, many questions and hurdles remain. Nevertheless, because the potential benefit is so great, more investigation is warranted to determine whether hepatic mitochondrial transplantation can become a therapeutic reality.

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Conflict of interest

GYW has been the editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2012. The other authors have no conflict of interests related to this publication.

Author contributions

Proposed concept for review and revised the manuscript with critical revisions (GYW), drafted the manuscript (BH and XL).

References

[1] El-Hattab AW, Craigen WJ, Scaglia F. Mitochondrial DNA maintenance defects. Biochim Biophys Acta Mol Basis Dis 2017;1863(6):1539–1555. doi:10.1016/j.bbadis.2017.02.017.
[2] McLean JR, Cohn GL, Brandt IK, Simpson MV. Incorporation of labeled amino acids into the protein of muscle and liver mitochondria. J Biol Chem 1958;233(3):657–663. doi:10.1016/s0021-9258(18)64722-2.
[3] Kim A. A panoramic overview of mitochondria and mitochondrial redox biology. Toxicol Res 2014;30(4):221–234. doi:10.5487/TR.2014.30.4.221.
[4] Gollihue JL, Rabchevsky AG. Prospects for therapeutic mitochondrial transplantation. Mitochondrion 2017;35:70–79. doi:10.1016/j.mito.2017.05.007.
[5] Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: Evidence for a long-term metabolic priming. Proc Natl Acad Sci U S A 1999;96(24):13807–13812. doi:10.1073/pnas.96.24.13807.
[6] McCully JD, Levitsky S, Nido PJ, Cowan DB. Mitochondrial transplantation for therapeutic use. Clin Transl Med 2016;5(1):16. doi:10.1186/s40169-016-0095-4.
[7] McCully JD, Cowan DB, Pacak CA, Toumpoulis IK, Dayalan H, Levitsky S. Injection of isolated mitochondria during early reperfusion for cardioprotection. Am J Physiol Heart Circ Physiol 2009;296(1):H94–H105. doi:10.1152/ajpheart.00567.2008.
[8] Masuzawa A, Black KM, Pacak CA, Ericsson M, Barnett RJ, Drumm C, *et al*. Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2013;304(7):H966–982. doi:10.1152/ajpheart.00883.2012.
[9] Kaza AK, Wamala I, Friehs I, Kuebler JD, Rathod RH, Berra I, *et al*. Myocardial Rescue with Autologous Mitochondrial Transplantation in a Porcine Model of Ischemia/Reperfusion. J Thorac Cardiovasc Surg 2017;153(4):934–943. doi:10.1016/j.jtcvs.2016.10.077.
[10] Blitzer D, Guariento A, Doulamis IP, Shin B, Moskowitzova K, Barbieri GR, *et al*. Delayed Transplantation of Autologous Mitochondria for Cardioprotection in a Porcine Model. Ann Thorac Surg 2020;109(3):711–719. doi:10.1016/j.athoracsur.2019.06.075.
[11] Guariento A, Doulamis IP, Duignan T, Kido T, Regan WL, Saeed MY, *et al*. Mitochondrial transplantation for myocardial protection in ex-situ–perfused hearts donated after circulatory death. J Hear Lung Transplant 2020;39(11):1279–1288. doi:10.1016/j.healun.2020.06.023.
[12] Moskowitzova K, Shin B, Liu K, Ramirez-Barbieri G, Guariento A, Blitzer D, *et al*. Mitochondrial transplantation prolongs cold ischemia time in murine heart transplantation. J Heart Lung Transplant 2019;38(1):92–99. doi:10.1016/j.healun.2018.09.025.
[13] Ali Pour P, Kenney MC, Kheradvar A. Bioenergetics Consequences of Mitochondrial Transplantation in Cardiomyocytes. J Am Heart Assoc 2020;9(7):e014501. doi:10.1161/JAHA.119.014501.
[14] Zhu L, Zhang J, Zhou J, Lu Y, Huang S, Xiao R, *et al*. Mitochondrial transplantation attenuates hypoxic pulmonary hypertension. Oncotarget 2016;7(31):48925–48940. doi:10.18632/oncotarget.10596.
[15] Moskowitzova K, Orfany A, Liu K, Ramirez-Barbieri G, Thedsanamoorthy JK, Yao R, *et al*. Mitochondrial transplantation enhances murine lung viability and recovery after ischemia-reperfusion injury. Am J Physiol Lung Cell Mol Physiol 2020;318(1):L78–88. doi:10.1152/ajplung.00221.2019.
[16] Su Y, Zhu L, Yu X, Cai L, Lu Y, Zhang J, *et al*. Mitochondrial transplantation attenuates airway hyperresponsiveness by inhibition of cholinergic hyperactivity. Theranostics 2016;6(8):1244–1260. doi:10.7150/thno.13804.
[17] Kuo CC, Su HL, Chang TL, Chiang CY, Sheu ML, Cheng FC, *et al*. Prevention of axonal degeneration by perineurium injection of mitochondria in a sciatic nerve crush injury model. Neurosurgery 2017;80(3):475–488. doi:10.1093/neuros/nyw090.
[18] Gollihue JL, Patel SP, Eldahan KC, Cox DH, Donahue RR, Taylor BK, *et al*. Effects of Mitochondrial Transplantation on Bioenergetics, Cellular Incorporation, and Functional Recovery after Spinal Cord Injury. J Neurotrauma 2018;35(15):1800–1818. doi:10.1089/neu.2017.5605.
[19] Fang SY, Roan JN, Lee JS, Chiu MH, Lin MW, Liu CC, *et al*. Transplantation of viable mitochondria attenuates neurologic injury after spinal cord ischemia. J Thorac Cardiovasc Surg 2021;161(5):e337–e347. doi:10.1016/j.jtcvs.2019.10.151.
[20] Zhang Z, Ma Z, Yan C, Pu K, Wu M, Bai J, *et al*. Muscle-derived autologous mitochondrial transplantation: A novel strategy for treating cerebral ischemic injury. Behav Brain Res 2019;356:322–331. doi:10.1016/j.bbr.2018.09.005.
[21] Yan C, Ma Z, Ma H, Li Q, Zhai Q, Jiang T, *et al*. Mitochondrial Transplantation Attenuates Brain Dysfunction in Sepsis by Driving Microglial M2 Polarization. Mol Neurobiol 2020;57(9):3875–3890. doi:10.1007/s12035-020-01994-3.
[22] Orfany A, Arriola CG, Doulamis IP, Guariento A, Ramirez-Barbieri G, Moskowitzova K, *et al*. Mitochondrial transplantation ameliorates acute limb ischemia. J Vasc Surg 2020;71(3):1014–1026. doi:10.1016/j.jvs.2019.03.079.
[23] Jabbari H, Roushandeh AM, Rostami MK, Razavi-Toosi MT, Shokrgozar MA, Jahanian-Najafabadi A, *et al*. Mitochondrial transplantation ameliorates ischemia/reperfusion-induced kidney injury in rat. Biochim Biophys Acta Mol Basis Dis 2020;1866(8):165809. doi:10.1016/j.bbadis.2020.165809.
[24] Doulamis IP, Guariento A, Duignan T, Kido T, Orfany A, Saeed MY, *et al*. Mitochondrial transplantation by intra-arterial injection for acute kidney injury. Am J Physiol Ren Physiol 2020;319(3):F403–413. doi:10.1152/AJPRENAL.00255.2020.
[25] Wu HC, Fan X, Hu CH, Chao YC, Liu CS, Chang JC, *et al*. Comparison of mitochondrial transplantation by using a stamp-type multineedle injector and platelet-rich plasma therapy for hair aging in naturally aging mice. Biomed Pharmacother 2020;130:110520. doi:10.1016/j.biopha.2020.110520.
[26] Chang JC, Chang HS, Wu YC, Cheng WL, Lin TT, Chang HJ, *et al*. Antitumor actions of intratumoral delivery of membrane-fused mitochondria in a mouse model of triple-negative breast cancers. Onco Targets Ther 2020;13:5241–5255. doi:10.2147/OTT.S238143.
[27] Chang JC, Chang HS, Wu YC, Cheng WL, Lin TT, Chang HJ, *et al*. Mitochondrial transplantation regulates antitumour activity, chemoresistance and mitochondrial dynamics in breast cancer. J Exp Clin Cancer Res 2019;38(1):30. doi:10.1186/s13046-019-1028-z.
[28] Sun C, Liu X, Wang B, Wang Z, Liu Y, Di C, *et al*. Endocytosis-mediated mitochondrial transplantation: Transferring normal human astrocytic mitochondria into glioma cells rescues aerobic respiration and enhances radiosensitivity. Theranostics 2019;9(12):3595–3607. doi:10.7150/thno.33100.
[29] Ylikallio E, Suomalainen A. Mechanisms of mitochondrial diseases. Ann Med 2012;44(1):41–59. doi:10.3109/07853890.2011.598547.
[30] Schapira AH. Mitochondrial Diseases. Lancet 2012;379(9828):1825–1834. doi:10.1016/S0140-6736(11)61305-6.
[31] Nunnari J, Suomalainen A. Mitochondria: In sickness and in health. Cell 2012;148(6):1145–1159. doi:10.1016/j.cell.2012.02.035.
[32] Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. Kidney Int 2017;92(5):1051–1057. doi:10.1016/j.kint.2017.05.034.
[33] Popkov VA, Silachev DN, Zalevsky AO, Zorov DB, Plotnikov EY. Mitochondria as a source and a target for uremic toxins. Int J Mol Sci 2019;20(12):3094. doi:10.3390/ijms20123094.
[34] Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura KI, *et al*. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001;88(5):529–535. doi:10.1161/01.RES.88.5.529.
[35] Cherry AD. Mitochondrial Dysfunction in Cardiac Surgery. Anesthesiol Clin 2019;37(4):769–785. doi:10.1016/j.anclin.2019.08.003.
[36] Peng W, Cai G, Xia Y, Chen J, Wu P, Wang Z, *et al*. Mitochondrial Dysfunction in Atherosclerosis. DNA Cell Biol 2019;38(7):597–606. doi:10.1089/dna.2018.4552.
[37] Hassanein T. Mitochondrial dysfunction in liver disease and organ transplantation. Mitochondrion 2004;4(5-6):609–620. doi:10.1016/j.mito.2004.07.015.
[38] Liu F, Lu J, Manaenko A, Tang J, Hu Q. Mitochondria in ischemic stroke: New insight and implications. Aging Dis 2018;9(5):924–937. doi:10.14336/AD.2017.1126.
[39] Kullmann AF, Truschel ST, Wolf-Johnston AS, McDonnell BM, Lynn AM, Kanai AJ, *et al*. Acute spinal cord injury is associated with mitochondrial dysfunction in mouse urothelium. Neurourol Urodyn 2019;38(6):1551–1559. doi:10.1002/nau.24037.
[40] Wang WX, Visavadiya NP, Pandya JD, Nelson PT, Sullivan PG, Springer JE. Mitochondria-associated microRNAs in rat hippocampus following traumatic brain injury. Exp Neurol 2015;265:84–93. doi:10.1016/j.expneurol.2014.12.018.
[41] de Mello AH, Costa AB, Engel JDG, Rezin GT. Mitochondrial dysfunction in

obesity. Life Sci 2018;192:26–32. doi:10.1016/j.lfs.2017.11.019.
[42] Montgomery MK. Mitochondrial dysfunction and diabetes: Is mitochondrial transfer a friend or foe? Biology (Basel) 2019;8(2):33. doi:10.3390/biology8020033.
[43] Gonzalez-Franquesa A, Patti ME. Insulin resistance and mitochondrial dysfunction. Adv Exp Med Biol 2015;982:462–520. doi:10.1007/978-3-319-55330-6_25.
[44] Kudryavtseva AV, Krasnov GS, Dmitriev AA, Alekseev BY, Kardymon OL, Sadritdinova AF, *et al*. Mitochondrial dysfunction and oxidative stress in aging and cancer. Oncotarget 2016;7(29):44879–44905. doi:10.18632/oncotarget.9821.
[45] Peng KY, Watt MJ, Rensen S, Willem Greve J, Huynh K, Jayawardana KS, *et al*. Mitochondrial dysfunction-related lipid changes occur in nonalcoholic fatty liver disease progression. J Lipid Res 2018;59(10):1977–1986. doi:10.1194/jlr.M085613.
[46] Turkseven S, Bolognesi M, Brocca A, Pesce P, Angeli P, Di Pascoli M. Mitochondria-targeted antioxidant mitoquinone attenuates liver inflammation and fibrosis in cirrhotic rats. Am J Physiol Gastrointest Liver Physiol 2020;318(2):G298–304. doi:10.1152/AJPGI.00135.2019.
[47] Ma L, Bi KD, Fan YM, Jiang ZY, Zhang XY, Zhang JW, *et al*. In vitro modulation of mercury-induced rat liver mitochondria dysfunction. Toxicol Res (Camb) 2018;7(6):1135–1143. doi:10.1039/C8TX00060C.
[48] Zavodnik IB, Lapshina EA, Cheshchevik VT, Dremza IK, Kujawa J, Zabrodskaya SV, *et al*. Melatonin and succinate reduce rat liver mitochondrial dysfunction in diabetes. J Physiol Pharmacol 2011;62(4):421–427.
[49] Gao Y, Chu S, Zhang Z, Zuo W, Xia C, Ai Q, *et al*. Early Stage Functions of Mitochondrial Autophagy and Oxidative Stress in Acetaminophen-Induced Liver Injury. J Cell Biochem 2017;118(10):3130–3141. doi:10.1002/jcb.25788.
[50] Simões ICM, Fontes A, Pinton P, Zischka H, Wieckowski MR. Mitochondria in non-alcoholic fatty liver disease. Int J Biochem Cell Biol 2018;95:93–99. doi:10.1016/J.BIOCEL.2017.12.019.
[51] Li X, Zhang W, Cao Q, Wang Z, Zhao M, Xu L, *et al*. Mitochondrial dysfunction in fibrotic diseases. Cell Death Discov 2020;6:68. doi:10.1038/S41420-020-00316-9.
[52] Auger C, Alhasawi A, Contavadoo M, Appanna VD. Dysfunctional mitochondrial bioenergetics and the pathogenesis of hepatic disorders. Front Cell Dev Biol 2015;3:40. doi:10.3389/FCELL.2015.00040.
[53] Hogeboom GH, Schneider WC, Pallade GE. Cytochemical studies of mammalian tissues; isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. J Biol Chem 1948;172(2):619–635. doi:10.1016/S0021-9258(19)52749-1.
[54] Hovius R, Lambrechts H, Nicolay K, de Kruijff B. Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane. BBA Biomembr 1990;1021(2):217–226. doi:10.1016/0005-2736(90)90036-N.
[55] Renault T, Luna-Vargas M, Chipuk J. Mouse Liver Mitochondria Isolation, Size Fractionation, and Real-time MOMP Measurement. Bio Protoc 2016;6(15):e1892. doi:10.21769/bioprotoc.1892.
[56] Frezza C, Cipolat S, Scorrano L. Organelle isolation: Functional mitochondria from mouse liver, muscle and cultured filroblasts. Nat Protoc 2007;2(2):287–295. doi:10.1038/nprot.2006.478.
[57] Djafarzadeh S, Jakob SM. Isolation of intact mitochondria from skeletal muscle by differential centrifugation for high-resolution respirometry measurements. J Vis Exp 2017;121:55251. doi:10.3791/55251.
[58] Farah NK, Liu X, Wu CH, Wu GY. An improved method for preparation of uniform and functional mitochondria from fresh liver. J Clin Transl Hepatol 2019;7(1):46–50. doi:10.14218/JCTH.2018.00064.
[59] Clayton DA, Shadel GS. Purification of mitochondria by sucrose step density gradient centrifugation. Cold Spring Harb Protoc 2014;2014(10):1115–1117. doi:10.1101/pdb.prot080028.
[60] Afanasyeva MA, Ustiugova AS, Golyshev SA, Kopylov AT, Bogolyubova AV, Demin DE, *et al*. Isolation of large amounts of highly pure mitochondria for "omics" studies. Biochem 2018;83(1):76–85. doi:10.1134/S0006297918010108.
[61] Banik B, Dhar S. Centrifugation-free magnetic isolation of functional mitochondria using paramagnetic iron oxide nanoparticles. Curr Protoc Cell Biol 2017;76:25.4.1–25.4.20. doi:10.1002/cpcb.26.
[62] Zischka H, Lichtmannegger J, Jägemann N, Jennen L, Hamöller D, Huber E, *et al*. Isolation of highly pure rat liver mitochondria with the aid of zone-electrophoresis in a free flow device (ZE-FFE). Methods Mol Biol 2008;424:333–348. doi:10.1007/978-1-60327-064-9_26.
[63] Pallotti F, Lenaz G. Isolation and Subfractionation of Mitochondria from Animal Cells and Tissue Culture Lines. Methods Cell Biol 2007;80:3–44. doi:10.1016/S0091-679X(06)80001-4.
[64] Torralba D, Baixauli F, Sánchez-Madrid F. Mitochondria know no boundaries: Mechanisms and functions of intercellular mitochondrial transfer. Front Cell Dev Biol 2016;4:107. doi:10.3389/fcell.2016.00107.
[65] Paliwal S, Chaudhuri R, Agrawal A, Mohanty S. Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. J Biomed Sci 2018;25(1):31. doi:10.1186/s12929-018-0429-1.
[66] Li H, Wang C, He T, Zhao T, Chen YY, Shen YL, *et al*. Mitochondrial transfer from bone marrow mesenchymal stem cells to motor neurons in spinal cord injury rats via gap junction. Theranostics 2019;9(7):2017–2035. doi:10.7150/thno.29400.
[67] Acquistapace A, Bru T, Lesault PF, Figeac F, Coudert AE, Le Coz O, *et al*. Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. Stem Cells 2011;29(5):812–824. doi:10.1002/stem.632.
[68] Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular Highways for Intercellular Organelle Transport. Science 2004;303(5660):1007–1010. doi:10.1126/science.1093133.
[69] Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, *et al*. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun 2015;6:8472. doi:10.1038/ncomms9472.
[70] Gollihue JL, Patel SP, Mashburn C, Eldahan KC, Sullivan PG, Rabchevsky AG. Optimization of mitochondrial isolation techniques for intraspinal transplantation procedures. J Neurosci Methods 2017;287:1–12. doi:10.1016/j.jneumeth.2017.05.023.
[71] Chang JC, Hoel F, Liu KH, Wei YH, Cheng FC, Kuo SJ, *et al*. Peptide-mediated delivery of donor mitochondria improves mitochondrial function and cell viability in human cybrid cells with the MELAS A3243G mutation. Sci Rep 2017;7(1):10710. doi:10.1038/s41598-017-10870-5.
[72] Liu X, Khouri-Farah N, Wu CH, Wu GY. Targeted delivery of mitochondria to the liver in rats. J Gastroenterol Hepatol 2020;35(12):2241–2247. doi:10.1111/jgh.15091.
[73] Gupta N, Wu C, Wu G. Targeted transplantation of mitochondria to hepatocytes. Hepatic Med Evid Res 2016;8:115–134. doi:10.2147/hmer.s116852.
[74] Lin HC, Liu SY, Lai HS, Lai IR. Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats. Shock 2013;39(3):304–310. doi:10.1097/SHK.0B013E318283035F.
[75] Fu A, Shi X, Zhang H, Fu B. Mitotherapy for fatty liver by intravenous administration of exogenous mitochondria in male mice. Front Pharmacol 2017;8:241. doi:10.3389/fphar.2017.00241.
[76] Shi X, Bai H, Zhao M, Li X, Sun X, Jiang H, *et al*. Treatment of acetaminophen-induced liver injury with exogenous mitochondria in mice. Transl Res 2018;196:31–41. doi:10.1016/j.trsl.2018.02.003.
[77] Zhao Z, Hou Y, Zhou W, Keerthiga R, Fu A. Mitochondrial transplantation therapy inhibit carbon tetrachloride-induced liver injury through scavenging free radicals and protecting hepatocytes. Bioeng Transl Med 2020;6(2):e10209. doi:10.1002/btm2.10209.
[78] Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, *et al*. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol 2015;77(5):753–759. doi:10.1002/ANA.24362.
[79] Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, *et al*. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology 2015;62(6):1723–1730. doi:10.1002/HEP.28123.
[80] Katarey D, Verma S. Drug-induced liver injury. Clin Med 2016;16(Suppl 6):s104–s109. doi:10.7861/CLINMEDICINE.16-6-S104.
[81] Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J, *et al*. Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study. Gastroenterology 2015;148(7):1340–1352.e7. doi:10.1053/J.GASTRO.2015.03.006.
[82] Labbe G, Pessayre D, Fromenty B. Drug-induced liver injury through mitochondrial dysfunction: mechanisms and detection during preclinical safety studies. Fundam Clin Pharmacol 2008;22(4):335–353. doi:10.1111/J.1472-8206.2008.00608.X.
[83] Lee WM. Acetaminophen (APAP) hepatotoxicity-Isn't it time for APAP to go away? J Hepatol 2017;67(6):1324–1331. doi:10.1016/J.JHEP.2017.07.005.
[84] Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. J Clin Transl Hepatol 2016;4(2):131–142. doi:10.14218/JCTH.2015.00052.