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.


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. The latter allow them to produce their own proteins and replicate. They generate energy through the Krebs cycle and the electron transport chain by converting fatty acids and carbohydrates into carbon dioxide and water. They also produce reactive oxygen species (ROS), which are important cell signals for many physiological processes. 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. Thus, mitochondria are believed to be essential for all eukaryotic life, especially for mammals.

All mammalian cells, except mature red blood cells, have mitochondria. 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, 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. Nearly every organ and tissue could be affected by mitochondrial disorders, although the neural system and the liver are the most common target organs. 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.

Mitochondrial dysfunction could also be caused by acquired damage to mitochondria. The electron transport system, especially the respiratory complexes, are frequent tar-
gets. Acquired mitochondrial defects are usually caused by toxins, medications, or aging, and are observed in numerous diseases and pathologies including chronic kidney diseases, uremia, cardiac infarctions, cardiac surgery, atherosclerosis, organ transplantations, strokes, spinal cord injury, traumatic brain injury, obesity, diabetes, insulin resistance, as well as age-related disorders such as Alzheimer’s and Parkinson’s diseases, and various types of cancers.

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. Non-alcoholic 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. Evidence also suggests that the activation of hepatic stellate cells during hepatic fibrosis is associated with mitochondrial dysfunction. Moreover, electron transport chain defects and increased oxidative stress have been reported to contribute to the development of hepaticcellular carcinoma.

**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. The most commonly used method includes tissue homogenization followed by several centrifugations at different speeds. There are also many commercial kits for mitochondrial isolation, but the protocols of those kits often bear the brunt of damage, and most liver diseases are accompanied with mitochondrial dysfunction. Mitochondria isolated in this way are usually contaminated by other organelles and cytosolic components by coalescence of plasma cell membranes. Acquistapace et al. 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, such as lysosomes and cell debris.

To further purify mitochondria, equilibrium density gradient ultracentrifugation is the most widely used technique. 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. Banik and Dhar 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. used free-flow zonal electrophoresis to purify mitochondria by a special free-flow apparatus. Farah et al. isolated mitochondria sequentially through 1.2-µ and 0.8-µ filters to achieve 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 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. developed a protocol to isolate mitochondria from 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. Since then, many studies have described methods to prepare and isolate mitochondria from tissue. Hovius et al. demonstrated that differential and Percoll gradient centrifugations can isolate highly purified and intact mitochondria from rat liver. Renault et al. 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. 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 200xg for 10 min at 4°C, then homogenized and centrifuged at various speeds. Additionally, Djaferzadeh and Jakob 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, microvesicles, gap junctions, and tunneling nanotube formation (Fig. 1). These mechanisms have been explored in non-hepatic models. Cell fusion involves cells sharing organelles and cytolic components by coalescence of separate plasma cell membranes (Fig. 1A). Acquistapace et al. 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. Tunneling nanotubules are thin membrane channels that allow for the direct intercellular transfer of organelles and membrane vesicles (Fig. 1C). As part of intercellular communication, cells can also se-
cretes 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. 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. Mitochondria can also be directly stained within cells 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.

**Targeted delivery of mitochondria to hepatocytes in cell culture**

Gupta *et al.* 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-

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**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 nanotubes. (D) Microvesicle transport.
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. 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. 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. 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.
and production of ROS in rats that received the allogenic rum alanine aminotransferase (ALT), hepatocyte necrosis, the results demonstrated a reduction of the elevation of se-

for how long they remained in recipient tissue. Regardless, among the liver parenchyma after 240 m, though it was not revealed that labeled donor mitochondria were distributed

recipient livers were harvested for analysis after 45-m is-

chondria injected into the spleens of recipient rats and

non-ischemic liver regions of three donor rats. Donor mi-

were isolated from the

following mitochondrial injection. Additionally, serum ami-

increased in the therapy groups, and that levels of oxidative

injury were significantly reduced compared to that in the control untreated fatty liver mice. That study did not as-

cess 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. Exog-

enaus mitochondria were cultured with mice hepatocytes in vitro and were injected into mice tail veins in vivo. Mitochon-

dria were tagged with green fluorescent protein to observe the efficiency of mitochondrial transfer and it was found that the mitochondria remained intact following isola-

tion. A live confocal microscopic study demonstrated the flu-

orescent mitochondria primarily within mouse hepatocytes at 10 m. TEM showed that the mechanism of internalization possibly involved endocytosis, and that the efficiency of mi-

tochondrial transformation into cells was 63.29±9.56%. The status of the mitochondria beyond 10 m after injection was not reported. In vivo, injected fluorescent mitochondria were also found in the brain, 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. Mitochon-

dria treatment also caused a 39.9% increase in ATP activity, decreased oxidative injury.

In a recent study, this group also investigated the ef-

fect of mitochondrial transplantation on carbon tetrachlo-
ide (CCL\textsubscript{4})-induced liver injury. In hepatocytes damaged by CCL\textsubscript{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

### Table 1. Animal studies on mitochondrial transplantation to the liver

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<td>Reduction in serum transaminase and lipid levels, increased cytochrome oxidase and ATP activity, decreased oxidative injury</td>
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<td>Shi et al., 2018\textsuperscript{76}</td>
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<td>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</td>
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<td>Zhao et al., 2020\textsuperscript{77}</td>
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<tr>
<td>Liu et al., 2020\textsuperscript{72}</td>
<td>Delivery of targeted mitochondrial complexes in rats</td>
<td>CD-1 mouse liver mitochondria</td>
<td>Mitochondria injected into rat tail veins</td>
<td>27% of total injected mitochondria was found in the liver</td>
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NAFLD, non-alcoholic fatty liver disease; CCL\textsubscript{4}, carbon tetrachloride; ALT, alanine aminotransferase; ATP, adenosine triphosphate; AST, aspartate aminotransferase.
received CCl₄ 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 CCl₄, 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 CCl₄ 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 CCl₄ and maintained liver protein homeostasis. Although that study only explored the short-term effects of mitochondrial transplantation, the strength is that the transcriptomic analysis was used to investigate the molecular signal mechanism of mitochondrial therapy. The authors found that CCl₄-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.

Advantages of mitochondrial transplantation

The mechanisms of non-targeted mitochondrial transfer are nonspecific, resulting in uptake by various cell types. 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 tissue.

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 antigenticity.

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, the low prevalence of genetic and acquired 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 unknown.

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. In contrast, to 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.

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. The American DILI Network determined in a prospective study that antimicrobials caused almost half of the cases. 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. Additionally, APAP-induced liver injury is a significant problem, as that drug is one of the most widely utilized analgesics in the USA. It also accounts for over half of acute liver failures related to overdose, as well as approximately 20% of liver transplantation cases. An important application of mitochondrial transplantation could include treating complications resulting from injury by restoring mitochondrial function, which could save lives while avoiding the operative risk, expense, and long-term immunosuppression involved in liver transplantation.
Heinlein B.D. et al: Mitochondrial transplantation in hepatocytes

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).

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