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Review

Prospects for therapeutic mitochondrial transplantation

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ABSTRACT

Mitochondrial dysfunction has been implicated in a multitude of diseases and pathological conditions- the organelles that are essential for life can also be major players in contributing to cell death and disease. Because mitochondria are so well established in our existence, being present in all cell types except for red blood cells and having the responsibility of providing most of our energy needs for survival, then dysfunctional mitochondria can elicit devastating cellular pathologies that can be widespread across the entire organism. As such, the field of "mitochondrial medicine" is emerging in which disease states are being targeted therapeutically at the level of the mitochondrion, including specific antioxidants, bioenergetic substrate additions, and membrane uncoupling agents. New and compelling research investigating novel techniques for mitochondrial transplantation to replace damaged or dysfunctional mitochondria with exogenous healthy mitochondria has shown promising results, including tissue sparing accompanied by increased energy production and decreased oxidative damage. Various experimental techniques have been attempted and each has been challenged to accomplish successful transplantation. The purpose of this review is to present the history of mitochondrial transplantation, the different techniques used for both *in vitro* and *in vivo* delivery, along with caveats and pitfalls that have been discovered along the way. Results from such pioneering studies are promising and could be the next big wave of "mitochondrial medicine" once technical hurdles are overcome.

1. Introduction to mitochondria

Mitochondria are located in the cell cytoplasm and often referred to as the powerhouse of the cell, as they produce most of the cell's energy in the form of adenosine triphosphate (ATP). However, they are also referred to as the nuclear power plant of the cell as they can efficiently make ATP while simultaneously producing a small but manageable amount of destructive oxidative agents known as reactive oxygen species (ROS). When mitochondria are damaged they can become extremely reactive and damaging to themselves and surrounding mitochondria, to the effect that it can cause cell death. To this end, while mitochondria are essential for life, they are also important factors in unleashing numerous pathways that can lead to both apoptosis and necrosis.

It is theorized that mitochondria were once bacteria that became engulfed by a cell and then utilized for its respiratory capabilities. Evidence of this theory lies in the fact that mitochondria have two lipid membranes and their own circular DNA (Nass and Nass, 1963), which allows them to make their own proteins (Mc et al., 1958). As evolution progressed, cells became more dependent on mitochondria for their energy supply, even integrating the coding for key mitochondrial proteins within the nuclear DNA. Mitochondrial DNA (mtDNA) only encodes for 13 mitochondrial proteins and needs the cell's nuclear DNA to provide the rest of the necessary proteins. Mitochondria are inherited maternally (Hutchison et al., 1974), meaning that each offspring will have mtDNA identical to their mother's, with no input from the father's mtDNA. It is thus possible to track familial lineage using the maternal mitochondria. This also implicates maternal inheritance of mtDNA mutations and disorders. However, the amount of mutated mtDNA in an egg can vary, known as heteroplasmy (Lightowlers et al., 1997), resulting in differing phenotypes making inheritance patterns that may not be obvious. Further, in an attempt to bypass inheritance of mtDNA mutations, mitochondrial replacement has been attempted in fertilization techniques to replace defective mtDNA with healthy mtDNA from a

Abbreviations: AIF, apoptosis inducing factor; ATP, adenosine triphosphate; CAP, chloramphenicol; CTVT, canine transmissible venereal tumor; DAMPs, damage-associated molecular patterns; ETC, electron transport chain; ETS, electron transport system; GSH, glutathione; IMM, inner mitochondrial membrane; ROS, reactive oxygen species; MnSOD, manganese superoxide dismutase; mBMSC, mouse bone marrow derived stromal cells; mtDNA, Mitochondrial DNA; MERRF, myoclonic epilepsy with ragged-red fibers; MPTP, mitochondrial permeability transition pore; NMDA, *N*-methyl-D-aspartate; O_2^-, superoxides; OGD, oxygen glucose deprivation; PCR, polymerase chain reaction; PKC, protein kinase C; RNS, reactive nitrogen species; SCI, spinal cord injury; TBI, traumatic brain injury; TMRE, Tetramethylrhodamine Ethyl Ester; TUNEL, TdT-mediated dUTP nick-end labeling

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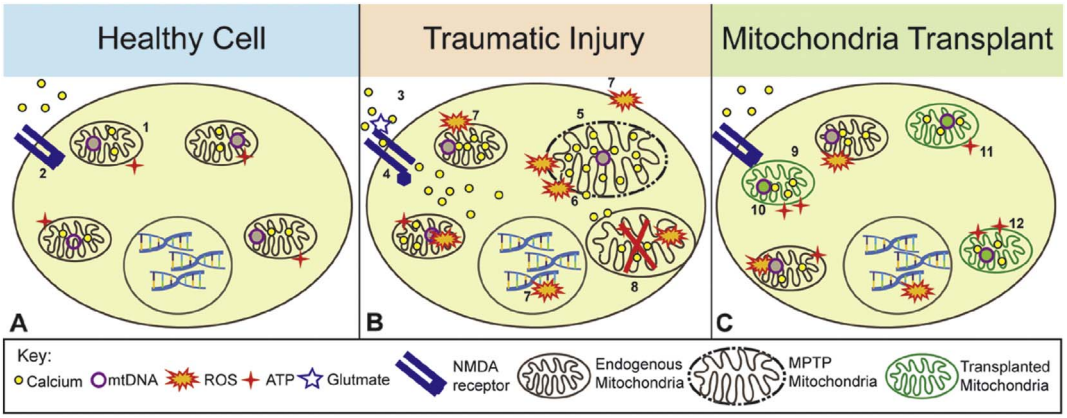

Fig. 1. Schematic depiction of how mitochondrial transplantation after injury may promote cell survival. **A**. During normal cellular function, mitochondria sequester and store calcium in their matrix and produce ATP (**1**) while NMDA receptors remain closed (**2**). **B**. After traumatic injury, the cell is subjected to glutamate excitotoxicity mediated by activated NMDA receptors (**3**) which allow massive calcium influx into cells (**4**) and subsequent uptake into mitochondria. This causes a loss of membrane potential across the inner mitochondrial membrane leading to mitochondrial permeability transition pore (MPTP) formation and subsequent swelling of mitochondria (**5**). Consequent bursting of the outer mitochondrial membrane then releases calcium, ROS, and apoptotic factors (**6**). ROS damages membrane lipids, mtDNA, electron transport chain proteins, and nuclear DNA (**7**), which leads to death of adjacent mitochondria (**8**). **C**. After healthy mitochondria, depicted in green, are transplanted into damaged cells they can increase antioxidants to combat ROS release (**9**) and provide new sources of mtDNA (**10**). Further, these mitochondria increase ATP production (**11**) and overall calcium buffering capacity (**12**) in the compromised cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

donor (Cohen et al., 1998; Cohen et al., 1997).

1.1. Mitochondrial function

1.1.1. Oxidative phosphorylation

Mitochondria provide for most of the cell's energy needs in the form of ATP (see Fig. 1A-1). In the process of oxidative phosphorylation mitochondria use substrates, namely NADH and FADH produced by the Kreb's cycle in the mitochondrial matrix, to power the electron transport system (ETS) located in the inner mitochondrial membrane (IMM). NADH donates an electron to complex I of the electron transport chain (ETC), an exchange that results in hydrogen atoms being pumped against a concentration gradient from the matrix into the inner membrane space. The electron then passes across the other complexes of the ETC in a series of oxidation/reduction reactions, each time releasing energy and pumping hydrogen from the matrix to the inner membrane space (for comprehensive review see Nicholls and Ferguson, 2013). This creates a membrane potential (between 140 and 180 mV) across the inner mitochondrial membrane, which is used by complex V to form ATP from ADP with simultaneous passage of hydrogen back into the mitochondrial matrix (Reid et al., 1966). When the electron reaches complex IV, it is released back into the matrix where it joins with oxygen. The oxygen is held at complex IV until enough electrons are present to allow it to form two water molecules. During oxidative phosphorylation, some ROS are created but are in small amounts that can be counteracted by endogenous antioxidant mechanisms within the mitochondria (see Cheeseman and Slater, 1993, Droge, 2002).

1.1.2. Endogenous antioxidant systems

During oxidative phosphorylation, electron flow leads to a small amount of ROS production, namely in the form of superoxide radicals. Electrons released from the ETC can reduce oxygen molecules to superoxides (O_2^-). Manganese superoxide dismutase (MnSOD) localized within the mitochondria (Weisiger and Fridovich, 1973) can oxidize superoxides into less reactive hydrogen peroxide (McCord and Fridovich, 1969). Hydrogen peroxide, however, can undergo the Fenton reaction, creating hydroxyl radicals (OH^-) that can damage lipids, proteins, and nucleic acids (Winterbourn, 1995). Alternatively, glutathione (GSH) peroxidase can convert hydrogen peroxide into water and is present in the intermembrane space of mitochondria (Arai et al., 1999). Damage to mitochondria can increase the endogenous antioxidant systems activities. For example, complex I deficiencies increase the expression of MnSOD which turns superoxide into hydrogen peroxide in cultured fibroblasts (Pitkanen and Robinson, 1996).

1.1.3. Calcium buffering

The mitochondrial matrix holds a negative charge of around − 240 mV, (see Michelakis, 2008) allowing the mitochondria to actively sequester positively charged calcium ions into their matrix (see Fig. 1A-1). This is important as calcium is involved in various intracellular signaling pathways. Under normal conditions, concentration of cytoplasmic calcium is 0.1 μM, extracellular calcium concentration is 1 mM, favoring calcium movement into the cytoplasm, (see Bianchi et al., 2004). The inner mitochondrial membrane has a potential of − 180 mV, favoring calcium influx into the matrix through the calcium uniporter (Bygrave and Ash, 1977), with equilibrium being reached when the calcium concentration within the mitochondrial matrix reaches 10^6 higher than the cytosol (Bianchi et al., 2004). Once inside the matrix, energy is required to export calcium back out of the matrix to overcome the electrochemical force driving calcium influx. It is found that the energy released from ATP hydrolysis (34.1 kJ/mol) is sufficient to allow movement of 1 mol of calcium (33 kJ) across the inner mitochondrial membrane and into the intermembrane space (Bianchi et al., 2004). Importantly, calcium is required for the activation of different metabolic enzymes such as pyruvate dehydrogenase, α-ketoglutaratedehydrogenase, and isocitrate dehydrogenase. This calcium influx into the mitochondrial matrix leads to increased metabolism through interaction with these metabolic enzymes, increasing ATP production for the cell (Jouaville et al., 1999).

1.2. Mitochondrial dysfunction

Mitochondrial dysfunction is implicated in many disease states such as Parkinson's Disease (Mizuno et al., 1989; Winklhofer and Haass, 2010), Alzheimer's Disease (Swerdlow and Khan, 2004), muscular dystrophy (Onopiuk et al., 2009), macular degeneration (Burdon, 1995), and aging-related neurodegeneration (Lin and Beal, 2006), among many others. While the dysfunction may be caused by a genetic defect of the mtDNA in certain disease states, it can also be caused by damage to the ETS of otherwise healthy mitochondria. Such mitochondria dysfunction is also involved in pathologies seen after many traumatic insults including cardiac infarctions (Braunwald and Kloner, 1985; Ide et al., 2001), stroke (Bolanos et al., 2009; Krajewski et al., 1999; Sims and Muyderman, 2010), traumatic brain injury (TBI)