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MITOCHONDRIAL-TARGETED DRUG DELIVERY

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ABSTRACT

Mitochondrion is an important organelle in the cell, playing critical roles in various biological processes, such as calcium homoeostasis, cellular stress response, cellular metabolism and cellular apoptosis. Thus, mitochondrion is also a promising therapeutic target for solving current clinical issues and preventing various human diseases such as diabetes, Alzheimer's disease, cardiovascular disorders, and cancer. In this review, firstly, we aimed at the function and influence of mitochondria in diseases. Furthermore, the targeting strategies to mitochondrion based on the highly negative potential of mitochondrial membrane and mitochondrial import proteins were also discussed for developing more efficient treatments for mitochondrial diseases.

Keywords: Mitochondrion, drug delivery, nanoparticle, cationic ligand

1. INTRODUCTION

Mitochondrion regarded as a hallmark and the cellular powerhouses of eukaryotic cells provide most cellular energy in the form of ATP via oxidative phosphorylation. Mitochondrion consists of four parts: the outer mitochondrial membrane, the intermembrane space, the inner mitochondrial membrane and the mitochondrial matrix **[1]**. Mitochondrion contains their own DNA, which could regulate oxidative phosphorylation and protein assembly. It is reported that most of mitochondrial proteins are encoded by nuclear gene and transported from cytosol

where they are synthesized to mitochondrion by specific targeting signals **[2]**.

Mitochondrion also plays critical roles in various biological processes of cells, such as calcium homoeostasis, cellular stress response, cellular metabolism and cellular apoptosis **[3]**. The defects or dysfunctions of mitochondrion are associated with four diseases including diabetes, Alzheimer's disease, cardiovascular disorders, and cancer **[4-9]**. Due to its important effect on cellular processes, mitochondrion has become an important target for treating diseases and developing drug candidate. The objective of this review is to briefly introduce the relationship between mitochondrion and four diseases (diabetes, Alzheimer's disease, cardiovascular disorders, and cancer) and outline the current design strategies of mitochondrial targeted therapeutic systems.

2. MITOCHONDRIAL DYSFYNCTION AND DISEASES

The double membrane structured mitochondrion occupies a pivotal position in the biology of higher eukaryotic cells, which regulate cell survival and death via unique biological action. Many diseases, such as diabetes, Alzheimer's disease, cardiovascular disorders, and cancer **[10-15]**, were found to be related to mitochondrial dysfunction, which might be caused by genetic mutations of mitochondrial proteins and oxidative stress.

2.1. Diabetes

Plenty of studies had shown that insulin resistance, a pathological condition in which cells failed to respond to the hormone insulin, preceded the onset of type 2 diabetes (T_2DM), which is a highly prevalent disease rapidly developing as one of the biggest global health challenges **[16-18]**. Many mechanisms have recently emerged as potential causes of insulin resistance and/or diabetes progression. Recently, mitochondrial dysfunction is considered to be a key factor in the pathogenesis of T_2DM **[19]**. The muscle mitochondrion in insulin-resistant exhibited impaired oxidative phosphorylation with a high production of reactive oxygen species (ROS) **[20]**, which would cause lipid accumulation, reduce glucose consumption and alter insulin signaling.

2.2. Alzheimer's disease

Alzheimer's disease (AD) is a chronic and age-related progressive

neurodegenerative disease that usually starts slowly and gradually worsens over time **[21, 22]**. AD is characterized by impairment of cognitive function and severe memory loss **[23]**. The most important hypotheses to explain the causes of AD are amyloid hypothesis and tau hypothesis. The former is relate to the deposition of extracellular amyloid β-peptide (Aβ), while the latter is corresponding to the formation of intracellular tangles composed of phosphorylated Tau protein **[24]**. Recent study revealed that metabolic dysfunction especially mitochondrial dysfunction is an early and causative event in Aβ-induced pathology and a promising target for intervention **[25]**. AD is a complex, multifactorial disease and mitochondrial dysfunction has proved to be a common pathological sign. The mechanism of mitochondrial dysfunction in AD is unclear, but a research team found that improvement of mitochondrial proteostasis to resist specific protein stresses can reduce amyloid plaque formation **[26]**.

2.3. Cardiovascular disorders

Mitochondrion regulates the catabolic and anabolic metabolism, cellular response, sulphur metabolism, calcium homeostasis, and initiation of caspase-dependent apoptosis **[27-31]**. A substantial number of evidences indicated that mitochondrial dysfunction was closely associated with the pathogenesis of multiple cardiovascular disorders **[32]**. And many studies proved that within the cardiovascular environment, the oxidative stress induced by mitochondrial dysfunction accelerated the development of cardiovascular disease **[33]**. Therefore, protecting mitochondrial function from oxidative stress damage would be an effective strategy to treat cardiovascular disorders. Adlam V J *et al* constructed a mitochondria-targeted antioxidant named Mito Q, which consists with two part: a lipophilic triphenylphosphonium (TPP) and a ubiquinol antioxidant. The result showed that administration of a mitochondrial-targeted antioxidant (Mito Q) protected heart muscle function, prevented myocardial cell death, and improved the respiratory control ratio in rats subject to ischemia/reperfusion injury **[34]**.

2.4. Cancer

Mitochondrial function and structure integrity are the keys to the effective

production of cell energy and cell survival. The role of mitochondrion in cancer was described in 1931 by Otto Warburg. Under hypoxia conditions, cancer cells would undergo a metabolic rearrangement, which favor metabolism via glycolysis rather than more efficient oxidative phosphorylation pathway **[35]**. Mitochondrial metabolic rearrangement is related to many characteristics of cancer cells including increased ROS production, decreased oxidative phosphorylation, insensitivity to antigrowth signals and impaired apoptosis **[36-40]**. In order to further understand the relationship between mitochondrial function with cancer, many tumor suppressor genes are being investigated. P53 protein is known as the "genome guardian" and it can inhibit tumor development through regulating the cell cycle, controlling apoptosis, and enhancing genome stability. Due to mitochondrial dysfunction, many different types of cancer show a high incidence of p53 mutations, which resulted in the expression of mutant p53 proteins. Besides, it had been reported that mutations in p53 in tumor cells affect mitophagy and convert the main cellular energy metabolism into glycolysis **[41]**.

3. MITOCHONDRIAL TARGETED THERAPEUTIC STRATEFIES

3.1. Mitochondrial targeted cationic ligands

According to the oxidative phosphorylation, the electrons produced by the citric acid cycle in the mitochondrial matrix can propagate from one protein complex to another protein in the mitochondrial inner membrane. During electron transport, the participating protein complexes push protons from the matrix out to the mitochondria intermembrane space thereby forming a concentration gradient of protons **[42]** and forming a transmembrane electric potential up to approximately 150 mV (negative inside) **[43]**. Actually in most of the cancer types, mitochondrion of cancer cells exhibit significantly increased transmembrane potential when compared with normal cells **[44]**. Given that, many cationic ligands prefer to accumulate in the mitochondrial of cancer cells via the electrostatic interaction with the negative mitochondrial membrane. Herein, we briefly describe the mitochondrial targeted cationic ligands.

3.1.1 Triphenylphosphonium

Triphenylphosphonium (TPP), a

well-known mitochondrial targeting moiety, contains three benzene rings to increase the molecular surface area and a phosphorus atom to provide positive charge **[45] (Figure 1)**. Previous research reported that the accumulation of TPP in the mitochondrial matrix can be 100～1000 folds higher when compared to cytosol **[46]**. Due to the efficient mitochondrial targeted capacity, many studies had been utilized TPP and its derivatives as mitochondrial targeted ligands. For example, TPP was conjugated with glucolipid-like conjugates (CSOSA) to produce mitochondria-targeted micelles for loading with weakly acidic celastrol (CTPP-CSOSA/Cela). CTPP-CSOSA/Cela selectively accumulated to mitochondrial and released weakly acidic celastrol via the weaker mitochondrial alkaline pH, which could enhance ROS levels, decrease mitochondria membrane potential, release cytochrome C into cytoplasm, and finally promoting tumor cellular apoptosis **[47]**.

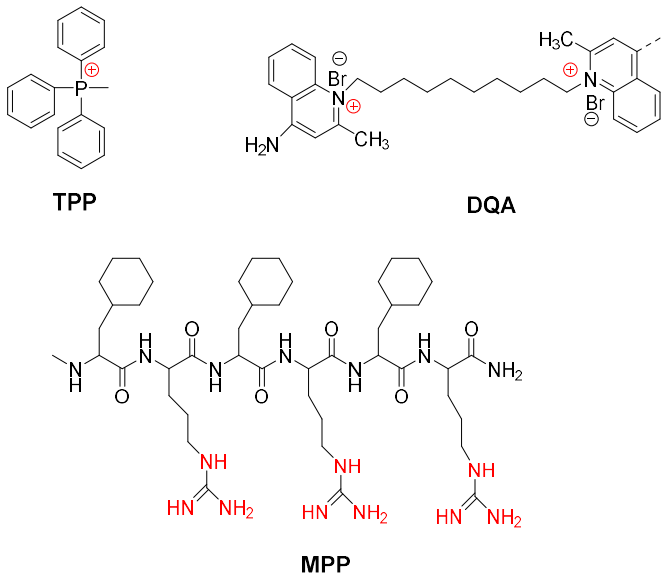

Figure 1: The structures of mitochondrial targeted cationic ligands

3.1.2 Dequalinium

Dequalinium (DQA), contains two positively charged centers of quaternary ammonium salt and long fatty chains **(Figure 1)**, can penetrate the lipid bilayer of cells and accumulate in the mitochondrion, which has been used as mitochondrial targeting cationic ligand. The liposome modified with DQA-phospholipid derivative (DEPE-PEG_{2000}-DQA) could be used to load resveratrol for mitochondrial targeting delivery. By destroying

mitochondrial membrane potential and releasing cytochrome C, the mitochondrial targeting resveratrol liposomes significantly upregulated the apoptosis of the cancer cell, which had a better antitumor efficacy **[48]**.

3.1.3 Mitochondrial penetrating peptide

Mitochondrial penetrating peptide (MPP) is a short peptide, which contain arginine (R) and lysine (L) to provide positive charge, and phenylalanine (F) and cyclohexylalanine (Fx) to provide lipophilic residue **[49]**. With minimal toxicity, MPP exhibits efficiently mitochondrial accumulation in human cancer cells **[50]**. Loading with Antimycin A, a hydrophobic and mitochondrial electron transporter inhibitor, the liposome functionalized with MPP exhibited greater mitochondrial accumulation and enhanced anticancer therapy **[51]**.

3.2 Mitochondrial import protein

Most of proteins in mitochondrion are encoded by nuclear genes and synthesized on cytosolic ribosomes, called precursor proteins. With the help of the mitochondrial targeting signals and the sorting signals, these proteins can correctly transport into mitochondrion **[52, 53]**. There were two ways for protein transport into mitochondrion including the pre-sequence pathway and the carrier pathway. The precursor proteins contain amino-terminal pre-sequence acting as mitochondrial targeting signal peptide (MTS), directing proteins that synthesized in cytosolic into mitochondrion **[54, 55]**. MTS peptides display a positive charge, and they can form amphiphilic α-helices **[56]**. Most of precursor proteins are imported to mitochondrion by the entry gate, which is the translocase of the mitochondrial outer membrane (TOM complex). Tom 40 occupies the central component of TOM. After crossing the TOM complex, precursor proteins are sorted by the TIM 23 complex in order to correctly import **[57]**. The TIM 23 complex consists of Tim 17, Tim 21, Tim 23, and Tim 50 **[58, 59]**. Once inside the matrix, the chaperone heat shock protein 70 (mt Hsp70) process as MTS, which drive precursor proteins move into matrix **[60]**. Dysfunction of these proteins may induce cell death and cause diseases. It had been reported that a lipid derivative conjugated with MTS could deliver certain types of proteins to mitochondrion **[61]**, indicating that it is likely to deliver drugs to mitochondrion via the mitochondrial

protein import chain.

4. MITOCHONDRIAL TARGETED NANO-DELIVERY SYSTEMS

4.1 Mitochondrial targeted nanotubes

Single wall carbon nanotubes (SWNTs) can be thought of as cutouts from a two-dimensional hexagonal lattice of carbon atoms rolled up along one of the Bravais lattice vectors of the hexagonal lattice to form a hollow cylinder **[62]**. Due to their unique physiochemical properties, SWNTs are broadly used for various biomedical applications such as drug delivery and photothermal therapy. Chen *et al*. found that SWNTs functionalized with phospholipid-polyethylene glycol could localize exclusively in mitochondrion of tumor cells **[63]**. In addition, using mitochondrial targeting SWNTs could selectively accumulate in mitochondrion and enhance the effect of photothermal therapy after laser irradiation to destroy mitochondrion for inducing apoptosis, which prove to be a promising selective local treatment modality with minimizing adverse side effects **[64]**.

4.2 Mitochondrial targeted nanoparticle with charge transversal function

It had been reported that positively charged nanoparticles can easily enter into cells and target to mitochondrion through the enhanced affinity to the negatively charged cell membrane and mitochondrial membrane **[65]**. However, positive charged nanoparticles are usually rapidly bound by plasma proteins and quickly cleared during circulation, which cannot achieve the expected effect **[66]**. To resolve the above conflict, many delivery systems with charge transversal function had been exploited and applied to target mitochondrion. Due to the different pH between blood or body fluids (pH 7.4), tumor microenvironment (pH 6.5-7.0), and endosome/lysosomes (pH 4.0-6.0), Mo *et al*. reported a novel mitochondria-targeted nanocarrier system based on zwitterionic oligopeptide liposomes (HHG2C18-L) with multistage pH responses **(Figure 2)**. HHG2C18 had two amino acid groups (histidine and glutamic acid) and one pH-sensitive group (hexa-hydro-benzoic amide), which acts as a hydrophilic block. And two stearyl alkane chains act as hydrophobic blocks, whose structure is similar to natural phospholipids. The first stage of pH response is caused by the mildly acidic tumor extracellular

microenvironment, in which the surface charge of HHG2C18-L becomes to positive. After entering the endosome/lysosome, a subsequent stage of pH response occurs. In this process, due to the acidic of the endosome/lysosome, the proton sponge effect and the pH-sensitive linker increase the positive charge of HHG2C18-L. In the end, with the high positive charge, HHG2C18-L has the ability to target mitochondria through electrostatic interaction. This intelligent liposome provides a meaningful nanoplatform for improving mitochondrial targeting efficiency and therapeutic effect **[67]**.

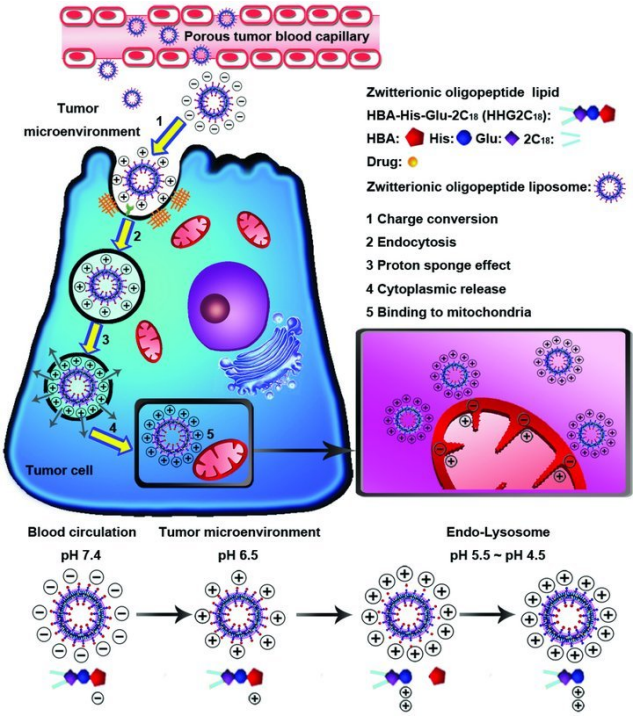

Figure 2: Schematic illustration liposomes (HHG2C18 -Lipo) with multistage pH response [67]

CONCLUSIONS AND FUTURE PERSPECTIVES

Mitochondrion has attracted attention as a targeted organelle for the treatment of diseases. Plenty of studies reveal that mitochondrial targeted therapy improves the treatment efficacy, reduces side effect and toxicity on normal tissues. This review summarized the influence of mitochondrial dysfunction on representative diseases and mitochondrial targeted therapeutic strategies. Despite researchers have conducted a large number of mitochondrial targeted studies and achieved many positive results, the mitochondrial targeted therapy in clinic is a relatively new concept. No

uniform standard of mitochondrial targeted agents is established, and most of them are limited to cell and animal studies. Therefore, more researches should be carried on for further exploring mitochondrial relative pathways and mechanisms, optimizing of mitochondrial targeted preparations and launching clinical studies, thereby providing effective therapies for the patients who suffer from mitochondrial diseases.

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