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MITOCHONDRIAL BIOGENESIS IN LIVER CELLS DUE TO STRESS

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Abstract - *Mitochondria are the double membrane organelle that provides energy in the form of ATP through the electron transport chain and its one of the major features was stress response and its cellular adaptability to those stress are the fundamental role in cellular survival by activating mitochondrial biogenesis which is the process of growth and division of mitochondrial mass and integrity from preceding mitochondria. This was regulated by various cellular signals; PGC-1 alpha is the master regulator of mitochondrial biogenesis that activates various transcriptional factors of NRF-1 and NRF-2 genes which encode for mitochondrial protein. The multiplex signaling network enables the mitochondria to sense internal or external changes caused by different stress and involved in the alterations of its bioenergetics, oxidative, thermogenic, and apoptotic responses appropriately and triggers mitochondrial biogenesis that helps to re-establish its network and consequently improve its functions. In this paper, we summarized that some of the stress factors such as oxidative stress, drug-induced stress by paracetamol and toxic substances stress can trigger mitochondrial biogenesis in liver cells and also discussed that the relationship between mitochondrial function, longevity, and inhibition of senescence. To evaluate the biogenesis in mitochondria various parameters should be analyzed; for mitochondrial number and volume by transmission electron microscope and fluorescent microscope, PCR to assess mtDNA, immunoblotting for mitochondrial protein, and radio-labeling were used. A better knowledge of the process and pathways involved in mitochondrial biogenesis might be a strategy to improve preventive and therapeutic targets for many diseases and disorders.*

Key Words: Mitochondrial biogenesis, Oxidative stress, acetaminophen, Hexavalent chromium

1. INTRODUCTION

The liver is one of the largest and richest organs in the body regarding the number and density of mitochondria, and also highly regenerative [1]. Usually, it is located in the right upper hand of the abdominal cavity underneath the diaphragm. Liver cells can be divided into four major types such as hepatocytes, hepatic stellate cells, kupffer cells, and liver sinusoidal endothelial cells these are the cells which are responsible for maintaining the functions and shape of the liver [2]. The liver is an organ that involves an active metabolic process for indispensable functions such as the breakdown of fats, proteins, and carbohydrates, production, and excretion of bile, activation on enzymes, balancing energy metabolism and storing excess glucose into glycogen, plasma protein synthesis, detoxification, and purification of blood. Mostly the liver sustains immune tolerance under physiological conditions. Under stress conditions, immune tolerance was discontinued by various processes such as hypoxia-reoxygenation, production of overactive kupffer cells, oxidative stress, toxic substances, drug-induced stress, acidity and alkalinity, viral infection, excess production of stress hormones and sympathetic nerve activation, and the influx of gut-derived lipopolysaccharides and norepinephrine thus causes the induction of liver inflammation [3]. The liver cells can fight back by activating diverse factors such as creating an immune defense mechanism, activating proteins that are responsible for blood clotting, storing glycogen from excess glucose. Mitochondria are the important organelle that provides energy in the form of ATP for the intracellular metabolism. Hepatocytes are the cells which have rich in mitochondria approximately 500 to 4000 per cell and it has solitary features of mitochondria when compared to other organs mitochondria. The significance of mitochondria in liver cells is to maintain hepatic metabolism, reactive oxygen species homeostasis, cell survival, and death regulation [1]. Mitochondrial biogenesis is the growth and division of preceding mitochondria thus increases in mitochondrial mass and integrity which was regulated by numerous factors such as PGC-1 alpha [(peroxisome proliferators-activated receptor)-gamma coactivator-1 alpha], AMPK (AMP-activated protein kinase), CaMKIV, Nitric oxide, SIRT1 (sirtuin 1), TORC, calcineurin, RIP140, and Sin3A. Mitochondrial biogenesis was vastly influenced by environmental stresses such as oxidative stress, exercise, caloric restriction, low temperature, cell division, cell renewal, and cellular differentiation [4]. Cellular adaptability to various stresses is a fundamental role in survival. Mitochondria are one of the important features of stress responses in all cells [5]. The multiplex signaling network enables the mitochondria to sense internal or external changes caused by different stress and alters their bioenergetic, oxidative, thermogenic and apoptotic responses appropriately, and triggers mitochondrial biogenesis that helps to re-establish its network and consequently improve its functions [6]. Therefore the specifications of mitochondria may help narrow the therapeutic options for many metabolic and aging disorders.

2. SIGNIFICANCE OF LIVER CELLS IN METABOLISM

The liver plays a vital role in metabolism and also regulating the levels of glucose, lipids as well as energy metabolism. Liver metabolism can be classified into three major groups; carbohydrate metabolism, lipid metabolism, and protein metabolism

Carbohydrate metabolism involves several processes such as Glycolysis, Glucogenesis, Glyconeogenesis, and Glycogenolysis. In glycolysis, the glucose is oxidized to generate ATP for energy metabolism. In glycogenesis, the liver transforms the excess glucose into glycogen. In glyconeogenesis, when the glycogen storage is reduced in the liver then it synthesis glucose from amino acids, glycerol, and lactate to increase glycogen storage in the body. Glycogenolysis is the process of breakdown glycogen into glucose-1-phosphate which takes place in hepatocytes and myocytes [7].

Lipid metabolism in hepatocytes can be compiled by three processes are, 1) the addition of lipids, including uptake of lipids and fatty acids and fatty acids synthesis, 2) lipid utilization includes fatty acid degradation, beta-oxidation, and secretion of the low-density protein, 3) storage of lipids includes triglyceride synthesis and the formation of lipid [8]

Protein metabolism is also known as protein synthesis. The key role of the liver is to regulate the metabolism of amino acids and proteins and also involves four important functions in protein metabolism; 1)plasma proteins involves the formation of clotting factors, albumin, thyroid-binding globulin and these are the proteins essential for the maintenance of transportation of hormones, homeostasis, oncotic pressure and acute phase productions, 2) amino acid interconversion process involves the initial synthesis of keto acid and then transferred to various stages of transamination to the keto acid by replacing keto oxygen. The liver has a high capacity to synthesis non-essential amino acid and this leads to regulate diverse homeostatic functions, 3) deamination of amino acids involves transamination of amino acids, the keto acid products of amino acid deamination oxidized to produce energy through ATP, and 4) urea synthesis occurs in hepatocytes and that involves Interconversion and degradation of amino acids result in ammonia production. Ammonia removal occurs chiefly through urea synthesis and subsequent excretion by the kidneys (for ammonia excretion) [9].

3. MITOCHONDRIAL BIOGENESIS

Mitochondrial biogenesis is the growth and division of preceding mitochondria thus increases in mitochondrial mass and integrity which was regulated by numerous factors such as PGC-1 alpha [(peroxisome proliferators-activated receptor)-gamma coactivator-1 alpha belongs to the family of transcriptional co-activators (PGC-1 alpha, PGC- beta, PPR) which plays a vital role in regulating mitochondrial biogenesis and cellular energy metabolism in many organs. In the liver PGC-1 alpha present at low but it regulates most of the metabolic pathways such as gluconeogenesis, ketogenesis, fatty acid oxidation, and heme biosynthesis [1]. Mitochondrial biogenesis was largely influenced by various environmental stresses such as oxidative stress, exercise, caloric restriction, low temperature, fasting or energy deprivation, cell division, cell renewal, and cellular differentiation [4]. Under stress conditions, PGC-1 alpha is stimulated by both transcriptional (CREB – cAMP response element-binding protein) and post-transductional (AMPK – AMP-activated protein kinase induced phosphorylation and SIRT1 mediated deacetylation). Regulation of mitochondrial biogenesis is one of the processes developed by cells to protect the mitochondrial integrity from the various effects of mitochondrial lesions. Following the activation of PGC-1 alpha, different nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) were also activated that indicates the increasing expression of mitochondrial proteins. AMP-activated kinase that links metabolism to mitochondrial function and metabolism to stabilize mitochondrial membrane potential for the survival of hepatocytes. In the liver, the activation of AMPK decreases gluconeogenesis and fatty acid synthesis to increase fatty acid oxidation and mitochondrial biogenesis [1]. These are the types of stress that can induce mitochondrial biogenesis

3.1 Oxidative stress

It is caused by an imbalance between the production of free radicals and the accumulation of reactive oxygen species which are the byproducts of the respiratory chain which involves cellular signaling from mitochondria to the nucleus. This state of imbalance was triggered by various internal or external stresses such as UV light, heavy metals, certain chemicals, ionizing radiations, and pollutants which leads to an increase or decrease in the production of ROS in the cell to causes cellular damage or tissue damage [10]. The recent study demonstrates that the non-fatal concentration of hydrogen peroxide and buthionine sulphoximine decreases the intracellular glutathione and increases the mitochondrial mass and mtDNA copy number in the human cell [11]. Another study shows that oxidative stress-induced lipopolysaccharides increase the nuclear genes of NRF-1 and mtTFA which was responsible for inducing mitochondrial biogenesis [12]. In affected cells, oxidative stress plays two important roles; mitochondrial proliferation and supply energy for cell survival and is also involved in cellular damage mechanism and protein synthesis. On another hand, it produces excess ROS to cause oxidative damage to the cell and initiate apoptotic activity. Hence it indicates that mitochondrial biogenesis depends on the level of oxidative stress, intracellular antioxidant property, and quality of mitochondria [12].

3.2 Drug induced stress by paracetamol

Paracetamol is also known as acetaminophen which is commonly used as therapeutic does. The Induction of mitochondrial biogenesis by acetaminophen protects against hepatotoxicity in the liver. This study was demonstrated by using mice were treated with APAP dose and sacrificed between 0 and 96 hours and used for several assays. At the initial stage, APAP causes mitochondrial dysfunction and decrement in mitochondrial biogenesis and also in ETC activity. But after 12hrs both the electron transport chain and mitochondrial DNA gradually increase which indicates mitochondrial biogenesis. This evidence shows that reactive metabolite of APAP binds to mitochondrial protein leads to alteration in mitochondrial morphology [13].

3.3 Toxic substances

Hexavalent chromium is a prevalent environmental pollutant extensively used in many industries for electroplating, welding, and chromate plating which causes a threat to human health. Many researchers have shown that mitochondrial biogenesis can be activated by oxidative stress or inflammatory stress. However, Hexavalent chromium induced mitochondrial biogenesis was uncertain. The minute concentration of Hexavalent chromium can induce mitochondrial biogenesis by increasing mitochondrial DNA copy number and mitochondrial mass. Expression of genes related to mitochondrial function complex I and V, mRNA levels of antioxidant enzymes including SOD 1 and SOD 2 (superoxide dismutase 1 and 2) were upregulated with less concentration of hexavalent chromium and also a key transcriptional regulator of mitochondrial biogenesis PGC-alpha, NRF-2 and mitochondrial transcriptional factor A (TFAM) were upregulated. But at the same time, huge concentration could be lethal and also inhibits mitochondrial biogenesis, expression of regulatory factors and antioxidants were highly down regulated [14].

4. METHODS TO ASSESS BIOGENESIS IN MITOCHONDRIA

To determine the mitochondrial biogenesis in the cell various parameters should be analyzed.

4.1 Microscopic methods

Fluorescent microscope is the most common method to assess biogenesis in mitochondria. Some dyes can enter into mitochondria and fluoresce that can be observed under the fluorescent microscope. It shows the relative area which was occupied by mitochondria. These are the fluorescent dyes used in mitochondrial biogenesis are 10-n-Nonyl-Acridine for inner mitochondrial membrane, Rhodamine123 dye, and JC-1for mitochondrial membrane potential.

4.2 Transmission electron microscope

The most preferred method for the evidence of biogenesis is Transmission Electron Microscope (TEM) followed by morphometry which was used to measure relative or absolute area occupied by mitochondria, mitochondrial pathology, extracellular morphology, and general cell. This includes various steps such as fixation, dehydration, embedding, sectioning, and staining

4.3 Mitochondrial DNA determination

The mitochondrion has its genome and each has approximately 2 to 10 copies of DNA that involve in many activities. The measurement of mtDNA is directly proportional to the number of mitochondria in the cell. Traditionally, southern blot analysis was used to evaluate the mitochondrial DNA but now newer and rapid methods have used Real-time PCR to quantify the mitochondrial DNA and then compare it to genomic DNA as a marker. In real-time PCR, NADH dehydrogenase subunit I is an enzyme present in mitochondria that encodes mitochondrial protein. So the measurement of this enzyme also indicates the mitochondrial number.

4.4 Immunoblotting assay

It is also called western blotting which is mainly used to evaluate the mitochondrial protein. Increased protein level also suggests that more mitochondria are present. Increased PGC-1 alpha, NRF-1, NRF-2, and mtTFA protein levels also indicate that the gene program of biogenesis has turned on. Transcripts or proteins of cytochrome c oxidase should be uplifted if biogenesis of mitochondria has existed, this can be analyzed by either northern blot for transcripts or southern blot for protein quantification. These are the indirect measures of mitochondrial biogenesis.

4.5 Radio-labeling

It is mainly used to evaluate the translation rates of mtDNA encoded proteins by incorporating amino acid into the mitochondrial protein using an in-vivo model to assess mitochondrial biogenesis. Leucine is the most frequently used amino acid. The major drawback of using radio labeling is the expensive and large dosage required since the dilution in the whole body must be taken into an account [15].

5. RELATIONS BETWEEN MITOCHONDRIAL FUNCTION, INHIBITION OF SENESCENCE AND LONGEVITY

Mitochondria, which play a vital role in energy homeostasis and reactive oxygen species (ROS) metabolism that, requires lifetime control and constant regeneration. The main function of mitochondrial metabolism is to maintain mitochondrial DNA, transcription, and translation. In the entire organism, aging is a degenerative process that is involved in the deterioration of cellular functions [17]. The acquisition of reactive oxygen species during age could lead

to a significant level of oxidative stress which causes DNA damages. The excess production of ROS and DNA damage triggers the cells to inhibit the cell cycle and halt the proliferation of damaged cells by inducing senescence. However, If the damage is mild it can undergo a repair mechanism and regenerate normally but when the degree of damage is high, it may lose its regenerative capacity thus undergo apoptosis or senescence [16]. PGC-1 alpha is the master regulator of mitochondrial biogenesis that could significantly activate nuclear transcriptional factors (NRF-1 and NRF-2). The significant role of NRF-2 or ARE (antioxidant response element) is to control the expression of the antioxidant gene and this was regulated by various signaling pathways. In this review, we hypothesized that the activation of NRF-2 could inhibit senescence and extends longevity [17].

CONCLUSIONS

The liver is one of the major organs has rich in mitochondria that regulate several processes of cellular homeostasis, reactive oxygen species, cellular survival, and death regulations. However, the major feature of mitochondria that enables to sense the alterations in the cell and get adapted to those stress triggers mitochondrial biogenesis which is regulated by the master regulator PGC-1 alpha. Some of the key factors of biogenesis were oxidative stress by using a nonlethal concentration of hydrogen peroxide to induce mitochondrial biogenesis; drug-induced stress by the less concentration of acetaminophen can also induce mitochondrial biogenesis. Some of the toxic substances like Hexavalent chromium which was a known threat to humans can also trigger mitochondrial biogenesis in very minute quantity. On the other hand, larger concentrations can also trigger various tissue damages and mitochondrial dysfunction in the cell. Therefore, a better understanding of the process and pathways involved in mitochondrial biogenesis might be a strategy to develop various therapeutic targets for diseases and disorders.

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