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Autophagy and the Effects of Mutation and Oxidative Stress to Degradation

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Mitochondria are unique organelles capable of providing energy to cells. Chemical interactions and stress cause mutational changes that make some mitochondria dysfunctional. When they become dysfunctional, autophagy occurs, degrading the mitochondria or the non-functional components. Two examples are approached in their effect on autophagy. First protein mitofusin 2 is observed in control environments as well as where it is removed to see the effects it has on mitochondrial autophagy. Oxidative stress is then induced to observe its effects on mitochondria and to compare the results with that of the mitofusin 2 knockout protein.

Key Words:
Autophagy, Mitochondria, mitofusion 2

Introduction

Mitochondria are dynamic organelles that are responsible for aerobic respiration. These are endosymbionts with their original ancestors being bacterial agents which explain their similarity in DNA with bacteria. Mitochondria can be damaged by oxidative stress. This stress type can lead to fragmentation and dysfunction of the mitochondria. These malfunction mitochondria can be degraded by autophagy (2). Autophagy regulates the aged proteins and degrades dysfunctional organelles such as mitochondria. This process occurs when autophagosomes mature by fusing with lysosomes. Upon fusion, the autolysosomes are capable of digestion by releasing acidic hydrolases. Eliminating dysfunctional mitochondria can prevent cell death. However, dysfunction mitochondria can lead to accumulation of waste that can cause aging, neurodegenerative diseases, muscle disorders, and heart disease (1). In recent studies, it has been discovered that mitochondrial dynamic proteins play a role in the normal function of autophagy. Mitofusin 2 was originally thought to cause fusion of autophagosomes and lysosomes. To further study this protein's effects on autophagy, an experimental knockout of this protein was designed to better understand the function. This was seen in the impaired fusion of autophagosome-lysosome as well as cardiac dysfunction (3). Using tert-butyl hydroperoxide (tBHP), the function of autophagy was also tested with induced oxidative stress using H9c2 cardiac myoblast cells.

Recent Progress

To account for the activity and importance of the outer membrane protein mitofusin 2, numerous tests were conducted to better understand its function with autophagy. These tests were conducted using an experimental type heart where the protein was knocked-out as compared to a controlled normal heart that contains the mitofusin 2 protein. When the heart containing the knock-out mitofusin 2 was observed through micrographs of transmission electron microscopy, the area of the mitochondria had increased from the control size of 0.65 μm^2 to 1.05 μm^2 (3).

Also from the transmission electron microscopy, the images showed a large amount of vacuolar structures in the mitofusin 2 knockout that appeared to be autophagosome-like containing a double membrane. This protein was then tested to see if they were in fact autophagosomes accumulating rather than autolysosomes. Using an autophagosome-associated form of an LC3

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protein (LC3-II), which is involved in processing of autophagosome at the molecular level, as a unit to measure, there was an increase of the protein level of the mitofusin 2 knockout by 1.97 fold of that of the control. With the increase in the amount of protein used for processing, it gives rise to the idea that the absence of mitofusin 2 causes an increase in the accumulation of autophagosomes (3).

In order to see if the accumulation of the autophagosomes was due to an overabundant production or due to failed degradation, a protein, p62, used as an autophagic substrate was analyzed in both the control and experimental heart. The analysis showed a 2.09 fold increase in the protein level of p62 in the mitofusin 2 knockout heart as compared to the wild type control. This would show that failed degradation was responsible for the accumulation (3).

The hearts of the control as well as the mitofusin 2 knockout were then subjected to treatment to test the activity of lysosomes. Each heart was given 50nM of LysoTracker™ which serves as an indicator of lysosome. In the protein knockout heart, the surface area had increased by 1.48% as compared to the wild-type at 0.18% which reveals the action of degrading the excess autophagosome in the knockout protein leading to greater density. With this information, it was seen that lysosome formation was not affected by the knockout of mitofusin 2 protein; rather it contributes only to the impairment of degradation of autophagosomes (3).

To see the importance of mitofusin 2 protein in autophagosome-lysosome fusion, cardiomyocytes were transfected with mitofusin 2 shRNA which down regulates the protein but can be mostly restored by mitofusin 2 cDNA. After staining the cultures with LysoTracker™ the scrambled control contained 19.6% more fused autophagosome-lysosomes. Along with the fusion of autophagosome and lysosome, mitofusin 2 was tested for the interaction with RAB7 which is an autophagosome maturation related protein. In the case of starvation where cardiac autophagy is increased, mitofusin 2 and RAB7 were shown to have increased interaction meaning that mitofusin 2 is responsible for mediating the maturation of autophagy (3).

To further investigate mitochondrial dysfunction, oxidative stress was performed in numerous tests to observe its effects. Tert-butyl hydroperoxide (tBHP) was the agent used for the oxidative stress. When subjected to the stress, there were decreased in cellular ATP content, oxygen consumption, and mitochondrial membrane potential. Tests using knockout mitofusin 2 increased the mass of the mitochondria as oxidative stress increases (1). As seen by previous experiments using the mitofusin 2 knockout heart, an accumulation of the light chain 3 II (LC3-II) occurred with the absence of mitofusin 2. With treatment of tBHP on H9c2 cells for 4 hours, the concentration of LC3-II increased with increasing amounts of tBHP. Different effects as well occurred due to concentration and time-dependent treatments of tBHP. Acidic dyes, monodansylcadaverine, acridine orange, and lysotracker red, used to stain mature autophagic vacuoles and lysosomes were used with increasing amounts and duration of oxidative stress. From these tests, decreasing amounts of intensity were shown in the tBHP-treated H9c2 cells therefore showing impairment in autophagic degradation (1).

Discussion

This review shows the importance of mitochondria dynamic protein, mitofusin 2, and its effects on autophagy. During the test with mitofusin 2 it was shown that this protein plays a vital role in regulating cardiac function through the fusion of autophagosomes and lysosomes in the autophagy process. Evidence was provided through transmission electron microscopy where an accumulation of autophogasomes were present in mitofusin 2 deficient hearts. Also contributing to this conclusion was the increased levels of autophagic markers LC3-II and p62 in the protein deficient hearts. Further evidence showed the ability to accumulate both autophagasome and lysosome but was unresponsive in the fusion leading to the idea of impairment in autophagosome degradation. Mitofusin 2 was also shown with the interaction with RAB7 which is a protein involved in the maturation of late autophagosomes. This strong interaction shows the importance that mitofusin 2 plays in the maturation stage of autophagy (2).

Deficiency of mitofusin 2 decreases the rate of respiration. With a reduced mitochondrial respiration, less energy will be produced which could cause an autophagic response to degrade those dysfunctional organelles. The increased capacity of autophagosomes can be characterized by either the over production of autophagosomes or the decrease of clearance due to the inability to degrade. After observation, it was concluded that due to the mitofusin 2, null hearts had the continued capability for the formation of both autophagosomes and lysosomes but had impaired systems of fusion therefore resulting in the decrease of clearance. Therefore mitofusin 2 is responsible for the normal cell function in the heart by clearance of ineffective organelles otherwise causing an accumulation of autophagosomes (2).

Oxidative stress also results in mitochondrial dysfunction and can lead to changes in mitochondrial mass and DNA. Autophagy plays the same role as with mitofusin 2 in eliminating dysfunctional mitochondria. Using tert-Butyl hydroperoxide as the oxidative stress, it selectively inhibits mitochondrial function. The length of time and amount of concentration were responsible for determining the physiological changes. The tested H9c2

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cells were treated with the tBHP which resulted in morphological changes from long filaments to short and round fragments. Mitochondrial activity diminished as seen with increased time and concentration of the tBHP, showing that these organelles are time and concentration-dependent of the induced stress being applied (1).

When an induced stress was applied changes to the mass of the mitochondria and the mitochondrial DNA occurred. This process changed in opposite directions where the mass of the organelle was increased, the copy number of the DNA decreased leading to mitochondrial accumulation due to oxidative stress rather than biogenesis. Also examining the LC3-II levels, results showed an increase with the increase of tBHP concentration. LC3-II, normally used for observing autophagic flux, shows the defective nature of autophagy causing the increase in accumulation of mitochondria (1). However, these studies show two different aspects of obtaining the same result of increased accumulation of autophagasomes. Comparative studies between oxidative stress and protein deficiency should be examined to understand why these differences in the study result in the same outcome.

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