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There is great concern with an increase in the number of Americans who are overweight and obese. Fat cells or adipocytes play a central role in obesity. These cells are metabolically active and play a fundamental role in energy allocation and storage. The adipocyte functions as the energy storage cell by storing excess energy in the forms of triglycerides in lipid vesicles within the cell. The morphology of mitochondria is a dynamic process that varies from cell type to cell type and in response to a variety of signals and conditions (Wilson-Fritch, 2002; Wilson-Fritch, 2004). The morphology of mitochondria in the cell often reflects the functions of that type of cell. In my thesis I characterize the changes in mitochondrial morphology and actin during adipogenesis. In this thesis I found that mitochondria undergo a radical change in morphology during the first two days of adipogenesis. In the pre-adipocyte cell mitochondria assume a reticular morphology that is distributed uniformly throughout the cell. After stimulation of differentiation this reticular morphology fragments. The fragmented mitochondrial morphology persists throughout adipocyte differentiation and is the form of the mitochondria present in the mature adipocyte. These results suggest that the reorganization of mitochondrial morphology is established early during adipogenesis and may play a role in the functions of fully differentiated adipocytes.

ANALYSIS OF MITOCHONDRIA MORPHOLOGY DYNAMICS DURING ADIPOGENESIS

By

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Obesity is reaching epidemic levels in modern America. Approximately 25% of Americans are obese and more than 50% are overweight. This increase in the obesity population is unclear. The net result of obesity is the accumulation of excess white adipose tissue (Gregoire, 2001). The simplest explanation to the cause of obesity is when more energy is taken in than is used (Chiu, 2004). One possible explanation to the dramatic increase in the number of overweight individuals is a heterogenous chain of causality which includes different biological factors. Understanding the cellular and molecular processes associated with obesity will lead to a more comprehensive understanding of this disease and further the development of therapies to treat diseases related to obesity (Gregoire, 2001).

Obese people have the ability to loose weight but some of these people find it difficult to sustain the weight loss (Webber, 2003). This observation suggests that obesity is far more heterogeneous than previously believed. Obesity is a complex genetic disorder that is associated with a number of different cellular processes, such as uncoupling protein polymorphisms, hormones, and mutations of the leptin gene (Webber, 2003). Polymorphisms of uncoupling proteins 2 and 3 (UCP-2 and -3) are associated with obesity by having low rates of energy expenditures (Webber, 2003). UCP-3 is likely to play a role in fat oxidation verses energy expenditures (Webber, 2003). Low rates of

fat oxidation can cause an individual to be susceptible to weight gain. One major cause of obesity is mutations of specific genes. Mutation in leptin causes early onset obesity, which is attributed to hyperphagia (Montague, 1997). Mutations in mitofusin 2 (Mfn2), in mitochondria fusion, causes an individual to become obese by causing reduced glucose oxidation and membrane potential (Chen and Chan, 2005).

Glucocorticoids, leptin and insulin are hormones that control appetite. Mutations of these proteins will cause an individual to consume more food and the consequence of more food consumption is more adipocytes produced in order to store excess energy. Leptin is one example of a protein hormone that regulates body weight and metabolism. Leptin level also increased in expression during terminal differentiation (Gregoire, 1998). Leptin proteins are in adipocytes which controls hunger. Leptin also affects the metabolic affects of insulin which include glucose transport, glycogen synthease, lipogenesis, and protein synthesis (Muller, 1996). Other ways that leptin helps with metabolism by increasing insulin-stimulated utilization of glucose and stimulates lipolysis (Siegrist-Kaiser, 1997). When the leptin gene is not expressed, increased food intake occurs because the gene is not regulating hunger (Webber, 2003).

Adipocytes

Adipocytes are lipid filled storage cells which play important roles in energy homeostasis. When an organism consumes more food than needed for its current metabolic needs, lipogenesis occurs, converting this energy into long term storage molecules in lipovaccules within adipocyte (Webber, 2003). The stored energy can than

be used later during fasting and other situation of need. Adipocytes also play a significant role in energy homeostasis, by releasing adipocyte-derived signaling molecules that act at distant sites to regulate energy homeostasis (Walczak and Tontonoz, 2002). There are two types of adipocytes in mammals; white adipose tissue (WAT), which stores excess energy as triglycerides in lipid droplets, and brown adipose tissue (BAT) which utilizes lipids to generate heat (Tong and Hotmisligil, 2001). White adipose tissue is white because they lack the iron that brown adipose tissue contains. In my experiment I will be using white adipose tissue to look at the morphology of the actin and tubulin filaments and mitochondrial morphology.

Adipogenesis

The differentiation of adipocytes called adipogenesis begins with a population of undifferentiated mesenchymal cells that receive a specific set of adipogenic and mitogenic signals from genes (Guo, 2000; Smas, 1995; Wilson-Fritch, 2002; Tong and Hotamisligil, 2001). These signals instruct the mesenchymal cells to undergo an immediate growth arrest. This growth arrest involves the expression of two transcription factors, C/EBP alpha and PPAR gamma (Gregoire, 1998).

After growth arrest, undifferentiated mesenchymal cells receive adipogenic and mitogenic signals in order to continue in the differentiation process (Gregoire, 1998). During growth arrest and clonal expression, the cell shows changes in gene expression. Genes such as lipoprotein lipase (LPLs), C/EBP alpha, and PPAR gamma are increased in expression during this stage of adipogenesis (Gregoire, 1998). Preadipocyte factor 1

(Pref-1) shows a dramatic decrease in expression (Gregoire, 1998). The shape of the cell goes from a fibroblastic, flat shape to a spherical, rounded shaped cell. Changes in the extracellular matrix, cytoskeleton and morphology are shown during early gene expression. Late gene expression and terminal differentiation show an increase in mRNA levels to form proteins that allow for lipid metabolism (Gregoire, 1998).

Mitochondria

The centerpiece of cellular metabolism is the mitochondria. The mitochondria contain the molecular machinery that governs many distinct metabolic processes by which chemical energy in the form of lipids, carbohydrates, and proteins are converted to ATP. Mitochondria are cellular organelles that contain two distinct membranes (Meeusen and Nummari, 2005). The inner membrane of the mitochondria encloses the mitochondrial matrix where the Krebs cycle and several other bioenergetic pathways that contribute to mitochondrial energy metabolism including pyruvate oxidation, tricarboxylic acid (TCA) cycle, fatty acid beta oxidation, and oxidative phosphorylation (Goldenthal and Marin-Garcia, 2004). Embedded within the mitochondrial inner membrane are protein complexes of electron transport which establishes a proton gradient used by the F_0F_1 ATPase, to generate ATP. The outer membrane consists of surface proteins such as transmembrane and fusion proteins and interacts with the cytoskeleton. In addition to production of ATP, mitochondria also perform a number of different tasks including detoxification of environmental poisons and the regulation of cellular homeostasis through a complex and largely undescribed signaling network.

These signaling networks allow the mitochondria to respond to a variety of challenges including environmental stress and alteration of energy source (Goldenthal and Marin-Garcia, 2004).

For instance, mitochondria respond to environmental stimuli such as high calorie diets and cold temperature by altering their numbers in a process called mitochondria biogenesis (Wu, 1999). Mitochondria biogenesis can be also stimulated by drugs such as thiazolidenedione which increase the levels of several proteins that are involved with mitochondria biogenesis (Wilson-Fritch, 2002). Central to mitochondrial biogenesis is the PGC-1 gene which encodes a transcriptional co-activator that triggers mitochondria replication by stimulating mitochondrial DNA replication and biogenesis during stress and environmental stimuli (Wu, 1999). PGC-1 is also activated by increases in cAMP levels caused by norepinepherine receptors which are activated by the sympathetic nervous system when there is a change in an outside environment (Wu, 1999).

In addition to the number of mitochondria within a cell, mitochondrial morphology or the organization and distribution of mitochondria within the cell also contributes to the functions of this organelle in the cell. Depending on cell type and other factors mitochondria assume a variety of different morphologies ranging from a tubular network to fragmented (Santel and Fuller, 2000). Central to the regulation of mitochondrial morphology are the opposing processes of mitochondrial fusion and mitochondrial fission which are coordinated to optimize cellular and mitochondrial function. Mitochondrial fission is controlled by a family of small enzymes of the Mfn1 family (Sentel, 2003). The morphology of mitochondrial is also regulated by the

absence of the protein endophilin B1 (Karbowski, 2004). Endophilin B1 is an enzyme (fatty acyl transferase, related to GTPase) which is required for maintenance of mitochondrial morphology (Karbowski, 2004). In the study preformed by Karbowski *et al*, they showed that endophilin B1 affected mitochondria by regulating dynamic stability of the mitochondrial networks in mammalian cells and helps form the outer membrane bound structures resembling those found in neuronal terminals after inactivation of endophilin B1.

In yeast cells mitochondrial fusion forms tubular networks allowing energy to be distributed evenly among the cell and other mitochondrial processes such as cellular apoptosis to happen efficiently (Fritz, 2003). Three proteins are involved in mitochondria fusion, Fzo1, Mgm1, and Ugo1 (Meeusen and Nunnari, 2005). Fzo1, a member of the large conserved groups of GTPase, has a critical role in the fusion of the two membranes (Westermann, 2002). Studies have shown that mutated yeast cells which do not contain the Fzo1 gene, have fragmented mitochondria from the lack of fusion (Westermann, 2002). The same results occur when looking at both Ugo1 and Mgm1 (Sesaki and Jensen, 2001; Sesaki, 2003).

Mitochondrial fusion is important in the inheritance and maintenance of the mitochondrial genome and cellular roles because fusion of the membranes allows the unification of mitochondrial compartments (Westermann, 2002). The mechanism behind fusion of the two mitochondrial membranes is rather complex and involves the fusion of both inner and outer mitochondrial membranes. Furthermore, mitochondrial fusion must

be complex since the process is coordinated with the antagonistic process of fission to govern proper mitochondrial function within the cell (Westermann, 2002).

Specific Aims: Characterization of the changes in mitochondrial morphology during adipogenesis.

In this thesis, I will characterize the changes in mitochondrial morphology during the process of adipogensis. During adipogenesis the cell undergoes radical changes: a quiescent, undifferentiated cell can differentiate into cells that are fully functional and metabolically active. Mitochondria are the source of cellular energy and alter their morphology under a variety of different conditions including different cell type, alteration of carbon source, point in the cell cycle and viral infection (Santel and Fuller, 2000). Presumably, this alteration of mitochondrial morphology is a response, partly due to alteration in energy needs of the cell. I hypothesize that during adipocyte differentiation, the number and distribution of mitochondria will increase because the differentiated adipocyte is a metabolically active and responsive cell. To determine whether there is a mitochondrial morphology change during adipogenesis and whether there is a correlation between mitochondrial morphology and function during adipocyte differentiation I will perform the following experiment:

I will use 3T3-L1 preadipocytes to characterize the changes in mitochondrial morphology during adipocyte differentiation. The 3T3-L1 cell line was chosen for this experiment because when it undergoes growth arrest and hormonal stimulation, a programmed line of differentiation is activated which causes a large lipid vaccule to form

(Wilson-Fitch, 2002). Additionally, these cells become sensitive to insulin, express GLUT4, and shows insulin-induced activation of glucose uptake in primary preadipocytes (Wilson-Fitch, 2002).

The major physical difference between preadiopcytes and adipocytes is the shape of the cells (Gregoire, 2001). When preadipocytes differentiate into adipocytes, it must change from its fibroblastic shape to a spherical shape (Gregorie, 2001). In order to determine whether or not there are morphological changes in the mitochondria and actin during differentiation, I will use immunoinfluorescence techniques. The immunoinfluorescence techniques will include two different types of stains. One of the stains will include phalloidin which is a toxin derived from the death cap mushrooms. Phalloidin only stains the f-actin fibers in cells. We evaluated the changes in mitochondrial morphology using an anti-cytochrome c, which labels the cytochome c protein in the electron transport chain.

CHAPTER II

METHODS

Cell Culture, Cell Plating and Feeding of M.D.I. cocktail

We used the 3T3-L1 cell line (Hajra, 2000; Parton, 2002; Wade, 2005). 3T3-L1 preadipocytes undergo adipogenesis spontaneously when left in calf-serum culture media over a period of time, but when exposed to a solution of methylisobutylxanthine, dexamethasone, and insulin (MDI) the process accelerates (Gregoire, 1998). The cells were fed 10% CS-Dulbecco's Modified Eagle Media every other day up to day 4 (up to 90% confluence) after plating. On the seventh day, the cells were treated with MDI media (methylisobutylxanthine [MIX], dexamethasone [DEX], and Insulin [I]). Two days after the cells were treated with MDI, cells were treated with a media consisting of DMEM, insulin, and fetal bovine serum (FBS). Two days after the cells were treated with DMEM and insulin, the cells were fed 10% FBS-DMEM media.

One of the experimental treatments was using MIX treatment only. The cells were fed with a 10% CS-DMEM every day up to Day four (up to 90% confluence) after

plating. On the seventh day, the cells were treated with MIX alone. Two days after the cells were treated with MIX, cells were treated with media consisting of DMEM, insulin and 10% fetal bovine serum (FBS). Two days after the cells were treated with DMEM and insulin, the cells were fed with 10% FBS-DMDM media for the remainder of the experiment duration.

Another experimental treatment was using DEX treatment only. The cells were fed with a 10% CS-DMEM every day up to day 4 (up to 90% confluence) after plating. On the seventh day, the cells were treated with DEX alone. Two days after the cells were treated with DEX, cells were treated with media consisting of Dulbecco's Modified Eagle Media (DMEM), insulin and fetal bovine serum (FBS). Two days after the cells were treated with DMEM and insulin, the cells were fed with 10% FBS-DMDM media for the remainder of the experiment duration.

The last experiment treatment was using insulin treatment only. The cells were fed with a 10% CS-DMEM every day up to day 4 (up to 90% confluence) after plating. On the seventh day, the cells were treated with insulin alone. Two days after the cells were treated with insulin, cells were treated with media consisting of Dulbecco's Modified Eagle Media (DMEM), insulin and fetal bovine serum (FBS). Two days after the cells were treated with DMEM and insulin, the cells were fed with 10% FBS-DMDM media for the remainder of the experiment duration.

Cell Staining and Fixation

Over the seven day period of adipogenesis, cover slips containing differentiating cells were taken and washed in 1X Phosphate Buffered Saline (PBS). The cells were fixed for 20 minutes at room temperature in a 4% paraformaldehyde solution in 1X PBS. After fixation the cells were washed in 1X PBS and incubated in an incubation solution of PBT (1X PBS, 1% Bovine serum, 1% goat serum, and 0.1% Triton-X) for 20 minutes at room temperature. The cells were then washed and a solution with incubation mix the primary antibody was added. To examine mitochondrial organization and distribution within the differentiating adipocytes I used the mouse monoclonal anti-cytochrome c 7H8.2C12 antibody (BD PharMingen) and an Goat Anti-mouse secondary antibody conjugated to CY3 (Jackson labs, catalogue #115-165-003)). I examined filamentous actin in these cells using Alexia488 Phalloidin (Molecular Probes) at a 1:500 dilution.

The cells were incubated with the primary antibody at 37°C for one hour. The reason for incubating at 37°C is because that is optimal temperature. After incubation the cells were washed three times with 1X PBS. The cells were then immediately incubated in a secondary antibody solution consisting of PBT and a 1:2000 dilution of the secondary antibody. Again, the cells were incubated at 37°C for 40 minutes. After this incubation, the coverslips with the cells were washed three times in 1X PBS with the final wash, containing 1:5000 dilution of Hoescht (Molecular Probes) to stain the nuclei of each cell. PBS was added to rinse out BSA in the washing solution. After the cells were rinsed, the cells were mounted in Difco anti-fade mounting media (Fischer Scientific).

CHAPTER III

RESULTS

During differentiation of a preadipocyte to a fully differentiated adipoctye cell, the overall cellular morphology undergoes radical changes (Figure 1). To initiate adipodgenesis we treated our 3T3-L1 preadipocytes with the MDI cocktail. 3T3-L1 cells possess a flat fibroblastic cellular morphology. After MDI treatment, the 3t3-L1 cells begin to round up and by day five after MDI treatment begin to express lipid vacuoles, the characteristic feature of adipocytes. *In vitro* the transition from preadipocyte to fully differentiated adipocyte takes seven days. For my thesis, I wanted to observe the mitochondrial morphology changes during this process using specific antibodies to mitochondrial proteins. I also wanted to correlate any changes in mitochondrial morphology with changes in the actin cytoskeleton.

To do these experiments, I differentiated preadipocyte 3T3-L1 cells using a standard protocol and collected samples at specific time points during the seven days of differentiation. I have found that mitochondria morphology does undergo a great radical change when the cells were treated with MDI media. In the preadipocyte control, Day 0 samples, the mitochondria possessed a tubular in morphology (see the arrow in the first frame of Figure 2). In samples from Day 2 after MDI treatment, the mitochondria have fragmented and appear clustered around the nucleus (see the arrow in the middle frame of Figure 2). This fragmented mitochondrial organization remained throughout

adipogenesis and can be seen in the fully differentiated adipocyte (see last frame of Figure 2).

Since mitochondrial morphology depends on interaction with the cytoskeleton (Boldogh, 1998), I wanted to look at the changes in actin cytoskeleton during adipogeneis to determine whether these could be correlated with the observed changes in mitochondrial morphology. Actin morphology does change during adipogenesis. Day 0, my preadipocyte control show actin appeared as stress fibers throughout the cell (first frame of Figure 3). Day 2, I saw a change in actin, where the stress fibers were reduced (middle frame of Figure 3). Day 7 demonstrated that actin is only found cortically around the cell perimeter and in a perinuclear formation. My results show that during adipogenesis, the F-actin was reorganized from stress fibers, scattered throughout the cell in a pre adipocyte to the cell cortex and in a cap around the nucleus in a differentiated adipocyte (last frame of Figure 3).

I also wished to determine whether specific component of our treatment were sufficient enough to induce changes in mitochondrial morphology. The identification of a specific component within the differentiation mix responsible for the changes observed above would help elucidate the mechanism responsible for the observed reorganization of the mitochondria that occurs during adipogenesis. When only M, D, or I was used alone, none of the single treatments resulted in 90% differentiation and although some cells did differentiate and these cells looked identical to adipocyte differentiated by a complete MDI cocktail treatment. Therefore it was impossible to made a definitive conclusion between the single treatment and the changes in mitochondrial morphology that I

observed and no distinction could be made in the role of the M, D or I in the alteration of mitochondrial morphology.

In summary, the rearrangement of mitochondrial morphology and actin show that the changes in these two cellular components occur simultaneously starting with MDI treatment but are not coordinated (Figure 4). By day 2 after MDI treatment, differentiating adipocytes show both mitochondria fragmentation and loss of actin stress fibers. However unlike mitochondria which remain fragmented consistently through differentiation, actin continues to change throughout adipogenesis. By Day 5 we observed further increase in the cortical actin and reduction of stress fibers and by Day 7 in fully differentiated adipocytes, almost all of the filamentous actin could be found cortically and concentrated in a cap around the nucleus. During adipogenesis, a significant difference in mitochondrial morphology is seen within two days after induction, suggesting that the changes in mitochondrial morphology are an early event proceeding obvious markers of differentiation such as lipid vacuole formation.

Figure 1: A schematic of adipocyte development. Starting from Day 0, the preadipocyte is flat and looks fibroblastic shaped. A preadipocyte control was taken before MDI treatment. MDI was applied to the preadipocytes at Day 0. Two days after MDI treatment, Day 2; the mitochondria fragment and there is a slow rearrangement of f-actin. Five days later, Day 5; the actin become cortically around the cell and lipid vacuoles (LV) begin to appear. By Day 7, the cell is a round, sphere shaped cell with an increase in size and larger lipid vacuoles (LV). The nucleus is labeled (N).

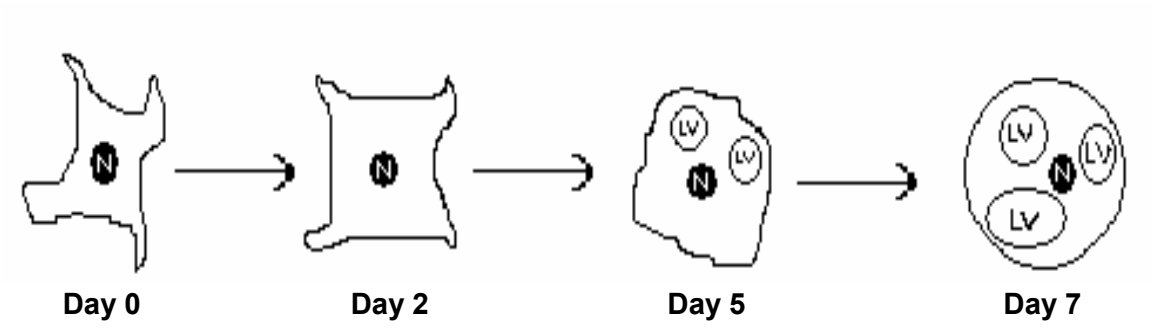


Figure 2: Mitochondrial morphology dynamics during adipogenesis. Differentiating adipodyctes were fixed in 4% formaldehyde solution and labeled with a primary antibody to anti-cytochrome antibody and then with a secondary antibody Goat anti-mouse antibody conjugated to CY3. At Day 0, the preadipocyte controls, the mitochondrion has a tubular and reticular morphology. At Day 2, there is a change in mitochondrial morphology where the mitochondria appear fragmented and clustered around the nucleus. Fragmented mitochondria persist though the differentiation to the mature adipocyte (Day7) and cluster around the nucleus and lipid vacuoles.

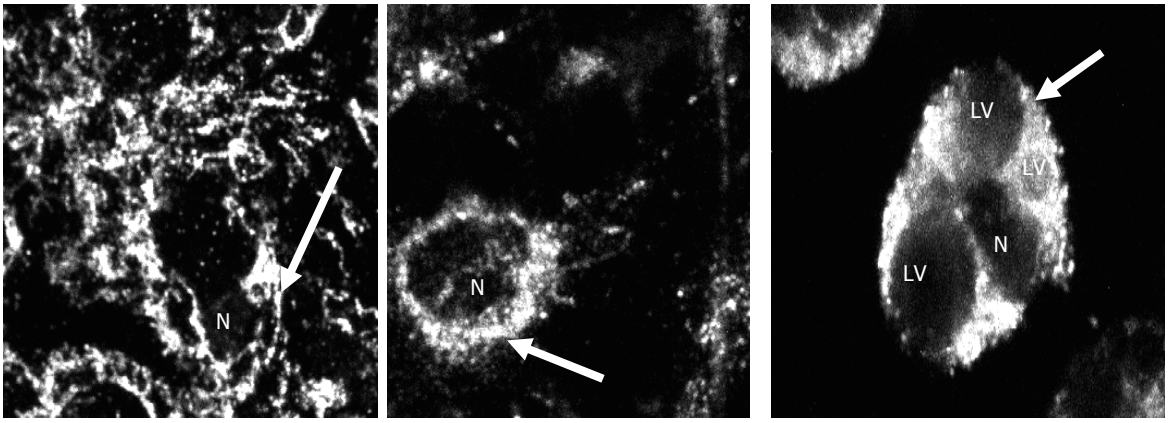


Figure 3: Actin cytoskeleton dynamic during adipogenesis. Samples collected at specific time points, fixed in 4% formaldehyde solution and labeled with Phalloidin Alexia 488 to visualize the f-actin within the cell. At Day 0, the preadipocyte control, the actin appears as stress fibers throughout the cell. At Day 2 changes in actin cytoskeleton appear with a reduction in the stress fibers. At Day 7, the fully differentiated adipocyte, actin is found cortically around the cell periphery and in a perinuclear formation.

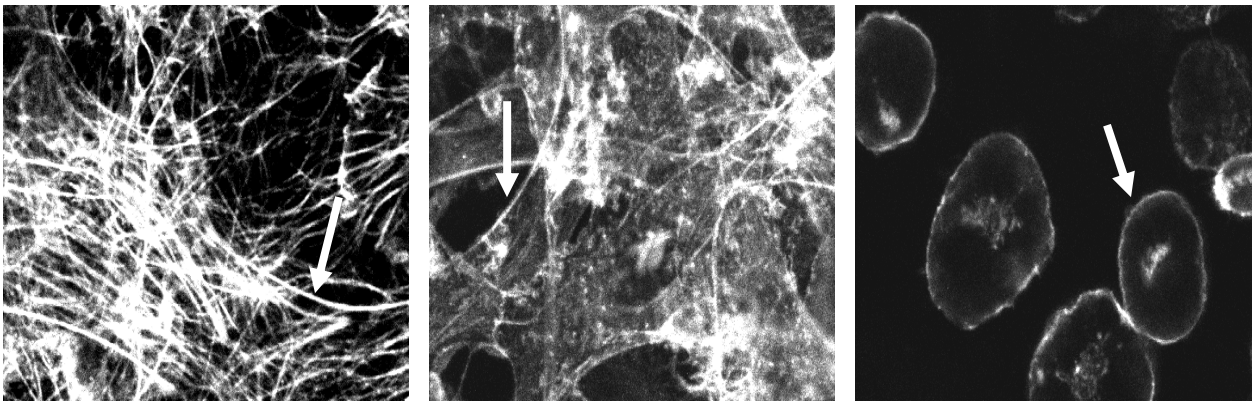
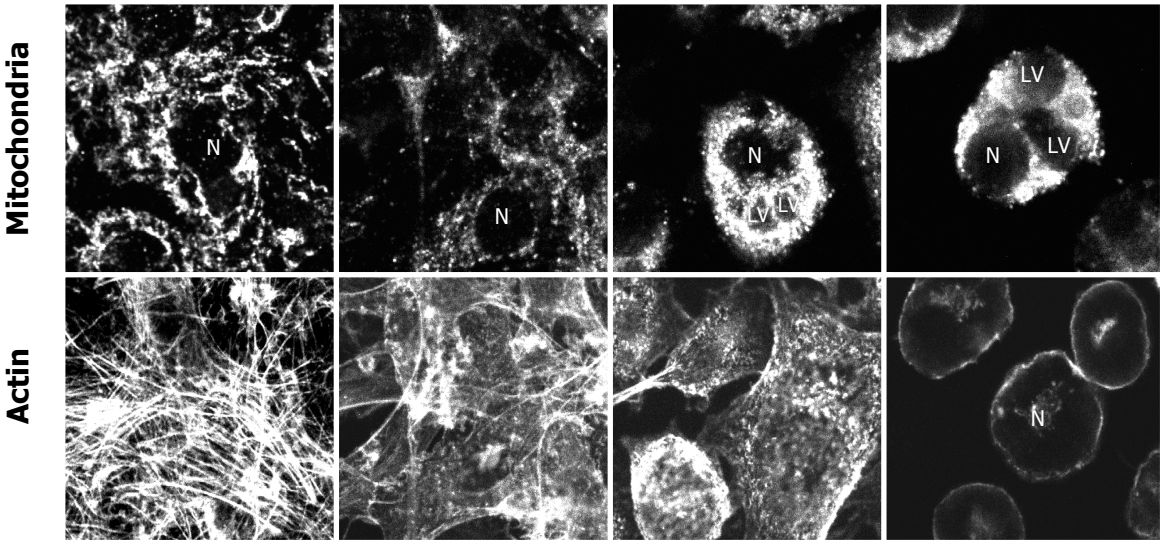


Figure 4: Summary of my results comparing changes in mitochondria morphology verses changes in actin morphology. Changes in mitochondrial morphology during adipogenesis were not fully coordinated with changes in actin cytoskeleton morphology. Samples were collected at four time points during differentiation: Day 0- pre-adipocyte control; Day 2- two days after MDI induction of differentiation; Day 5- five days after MDI induction of differentiation and Day 7- seven days after MDI induction of differentiation, these are fully differentiated adipocytes. Cells were fixed in 4% formaldehyde solution treated with Phalloidin Alexia 488 to label the f-actin of these cells and anti-cytochrome c was used to label the cytochrome c proteins in the mitochondria. These slides were viewed under a confocal microscope to take image shots of the areas of interest in the cell.



CHAPTER IV

DISCUSSION

In this thesis I have characterized the changes that occur in mitochondrial morphology and the actin cytoskeleton during adipogenesis. During adipocyte differentiation the mitochondrion undergoes radical reorganization transitioning from a reticular, tubular organization to a punctate fragmented morphology. During adipogenesis the actin also changes. The actin cytoskeleton transitions from bundles of filamentous actin in the form of stress fibers found throughout the cytoplasm of the predipocyte to a cortical deposition of actin and a nuclear cap of F-actin in the mature adipocyte.

In order to examine which specific component of my induction cocktail resulted in changes in mitochondrial morphology or actin distribution I used single components of the MDI cocktail. To do this I attempted to induce adipogenesis using only one M, D, or I of the cocktail at a time. When only M, D, or I component was used singly to induce adipogenesis of 3T3-L1 predipocytes, I only observed very low levels (<5%) of adipocyte differentiation. I can conclude that a coordinated and near complete differentiation of preadipocytes to mature adipocyte requires at least 2 out of the 3 treatments together. Furthermore, when I observed adipocyte differentiation, the mature adipocytes had fragmented mitochondria and an actin distribution identical to those differentiated by a complete MDI cocktail. These results suggest that the change of mitochondrial morphology are not due to a single component but are part of the program

of adipocyte differentiation. We used MDI to induce adipogenesis however there are other ways to do this (Mukherjee, 2000; Janke, 2002). It is possible that all of these treatments converge on a common trigger of differentiation and that downstream of this are changes in mitochondrial morphology and actin.

Signaling pathways and regulation of specific genes are required for differentiation to occur. It is well known that MDI is needed to allow for preadipocyte to undergo differentiation to develop into mature adipocytes (Gregoire, 2001; Pedersen, 2001; Guo, 2000; Patel, 1999; Hamm 2001). When only M, D, or I was used, the results were lower in cell number, frequency of activation and slower differentiation. The cells that contained these specific treatments also resulted in less rearrangement of filaments and mitochondria.

When using the MDI cocktail to induce adipogenesis, a set of specific genes involved in adipogenesis were activated. One important mediator of adipocyte differentiation is PPAR gamma (Walczak and Tontonoz, 2002). PPAR gamma is a transcription factor that regulates genes involved in lipid metabolism (Walczak and Tontonoz, 2002). The formation of the fat vacuoles confirms that PPAR gamma is turned on to regulate lipid metabolism. PPAR gamma also has the ability to regulate the rates of lipid uptake and efflux, which can help explain how some people are susceptible to atherosclerosis, the narrowing of arteries due to fat plaque build up (Walczak and Tontonoz, 2002). The differentiation of white adipose tissue requires specific genes.

C/EBP alpha (CCAAT enhancer binding protein alpha) is an important transcription factor for white adipocyte differentiation (Linhart, 2001).

The fragmentation of the mitochondria suggests that a fission program might be activated during the early stages of adipocyte differentiation. Mitochondria fusion and fission are opposite and coordinated processes, which allow for rapid change of mitochondrial organization. Fusion is the process where mitochondria are joined together. There are two specific proteins that are involved in mitochondrial fusion, Mitofusin (Mfn) and OPA1. Mfn and OPA1 are two specific proteins that are needed for mitochondrial fusion (Chen, 2005). In a future study, I would observe the intensities of these two proteins. I would specifically look for an increase in the Drp1 and Fis1 and/or a decrease in Mfn and OPA1 The importance of mitochondrial fusion is to exchange mtDNA, resistance to apoptosis, and plays a role in regulating mitochondrial metabolism (Westermann, 2002, Meeusen and Nunnari, 2005, McBride, 2006).

Fission is the process where mitochondria are broken down into smaller rod shaped mitochondria. In a future study, I would observe, specifically Fission1 (Fis1) and Dynamin related protein 1 (Drp1) the MDI treatment. The hFis protein has a direct link to mitochondrial fission but not apoptosis (Alirol, 2006). Specific gene expressions with the different treatments could show which specific genes are turned on or inhibited when the experimental treatments are applied. The results I obtained show that mitochondria are broken apart. There could be a possibility that Fission1 (Fis1) and Dynamin related protein 1 (Drp1) are increased during adipogenesis. Mitochondrial fission has been

shown as a prerequisite for apoptosis and plays a role in regulating mitochondrial metabolism (Meeusen and Nunnari, 2005; McBride, 2006). These two mechanisms are the basis for all mitochondrial morphology and change of mitochondria in cells (Meeusen and Nunnari, 2005).

Changes in actin distribution during adipogeneis were observed, I would desire to see how specific proteins have an effect on changes in actin distribution. I would specifically look at the ADAM 12 protein since this protein is on the surface of cells and interact with actin cytoskeleton. ADAM 12 assists in rearrangement of actin filaments during adipogenesis (Kawaguchi, 2003). A future study in using ADAM 12 proteins would be to knock out ADAM 12 by using RNAi and observe how the actin cytoskeleton rearranges during adipogenesis. While looking at the actin rearrangement, I can link it to the question to whether or not the change in mitochondrial morphology during adipogenesis is directly related to the changes seen in the actin cytoskeleton.

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APPENDIX A

List of Terms

BAT = brown adipose tissue

C/EBP = CCAAT/enhancer binding protein

DEX = dexamethasone

DMEM = Dulbecco's Modified Eagle Media

Drp1 = Dynamin Related Protein 1

FBS = Fetal Bovine Serum

Fis1 = Fission 1

Mfn = Mitofusin

MIX = methylisobutylxanthine

PBS = Phosphate Buffered Saline

PGC = PPAR gamma co-activators

PPAR = peroxisome proliferator-activated receptors

UCP = uncoupling protein

WAT = white adipose tissue