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Developmental Activation of Mitochodnrial OPA1 Processing Is Retinoic Acid-Independent

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DEVELOPMENTAL ACTIVATION OF MITOCHONDRIAL OPA1 PROCESSING IS RETINOIC ACID-INDEPENDENT

A Thesis

by

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ABSTRACT

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Mitochondria exist as an organellar network within most eukaryotic organisms, and are critical to biochemical energy production and cellular stress response. Mitochondrial bioenergetic function is directly linked to the complex morphological state of its network, existing in a balance of fusion (interconnected state) or fission (fragmented state). The optic atrophy-1 (OPA1) protein plays a major role in regulating mitochondrial inner membrane fusion, whereas the OMA1 metallopeptidase is highly involved in the degradation of the OPA1 protein, thus contributing to its fragmented state. Cellular stress such as a disruption in mitochondrial membrane potential activates OMA1 cleavage of OPA1. However, our data demonstrates that disruption of membrane potential with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) after cardiac-like differentiation with Retinoic Acid (RA) in H9c2 cardiomyoblast cell lines activates OMA1, suggesting a developmental switch for OPA1 cleavage. To test whether the developmental activation of OMA1 is RA specific, skeletal muscle-like differentiation (with low serum media *lacking* RA) followed by challenge with CCCP revealed robust OPA1 cleavage. These findings demonstrate that very different developmental programs have the ability to activate OPA1 processing, suggesting a broad role for developmental regulation of OPA1 homeostasis through a novel, uncharacterized mechanism.

DEDICATION

This master's study would not have been possible without the support of my family. To my great grandmother Regina Britten who taught me "Damn the torpedo, full speed ahead". To my parents Regina Bryson and Abu-Baker Mohamed, for their endless encouragement. To my husband Mohamed Ezz-Eldin, for his constant love and support. And lastly, to my daughter who has inspired me to be the higher me

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CHAPTER 1

INTRODUCTION

Statement of the Problem

Mitochondria maintain a range of metabolic functions which power life for our cells. Paradoxically, mitochondria also contain the ability to abort the life of cells through apoptosis. It is in this profound contradiction that we find it to be a key organelle to sustaining life. The survival of a cell is dependent upon the inner mitochondrial membrane (IMM), where nutrients are converted into adenosine triphosphate (ATP) to provide energy for the cell, and thus life. The conversion of nutrients into ATP is made possible by a series of redox reactions along the electron transport chain (ETC), where electrons from NADH and $FADH_2$ are oxidized to drive respiration. ETC generates a transmembrane potential (Dy_m) across the IMM, which is used by the F_1F_0 ATP synthase to phosphorylate ADP to ATP.

This study specifically focuses on how disruption of the mitochondrial transmembrane potential may affect the morphological state of the organelle and possibly lead to apoptosis. As structure and function of proteins are interconnected, we focused on the OPA1 protein which resides in the inner membrane of the mitochondria. OPA1 exists in two main isoforms: L- OPA1 and S-OPA1, where both isoforms contribute to the interconnected state of the inner mitochondrial membrane and thus function of the organelle.

Disturbance in the delicate balance between the two isoforms, leads to mitochondrial fragmentation and apoptosis. Understanding the regulators at which OPA1 is regulated by sheds

light on another fascinating mechanism within mitochondrial organelle. Which could possibly leadus closer to understanding cellular development since mitochondrial fusion are involved in cell cycle progression, maintenance of germline stem cells and developmental angiogenesis (Garcia et al., 2020).

Statement of the Purpose

Previously, it has been shown that 143B osteosarcoma cells exhibit OPA1 cleavage when disruption of membrane potential through challenge with CCCP is applied. When we asked if L-OPA1 cleavage would be observed if CCCP was applied to H9c2 cardiomyoblast cells, the results were strikingly different. No L-OPA1 cleavage was observed, leaving room for speculation as to what the difference within the two cells were that would allow for L-OPA1 cleavage in 143B cells, and not H9c2's. Incubation with low serum and RA lead to cardiac-like differentiation within H9c2 cells, which lead to the exploration of; if low serum with RA could activate L-OPA1 cleavage upon CCCP challenge. The results indicated that indeed, L-OPA1 cleavage was observed within the H9c2cells that were differentiated with low serum and RA, but not with 10%FBS alone. Perhaps, there isan developmental activation that must occur within the cells in order for OPA1 cleavage to take place. These results led us to hypothesize if developmental activation of OPA1 cleavage is independent of RA. This investigation will allow us to frame an idea if OPA1 cleavage is an occurrence through cellular differentiation and broader developmental programs. Here, we investigate the possibility of broader developmental programs being at play through confocal imaging, western blot analysis, ImageJ analysis and qRT-PCR gene expression.

CHAPTER II

REVIEW OF THE LITERATURE

Mitochondrial bioenergetics

Embedded within the mitochondrial inner membrane are four large, multisubunit protein complexes that transfer electrons in a stepwise fashion, reducing oxygen and driving the production ATP. From the TCA cycle, electrons from NADH are transferred to Complex I, while electrons from $FADH_2$ enter Complex II. These electrons are then passed to ubiquinone, which is a membrane-associated electron carrier located in the inner membrane space. From ubiquinone, the electrons are passed to Complex III. From Complex III, cytochrome c transfers the electrons to Complex IV, the final electron acceptor, which passes the electrons to molecular oxygen, converting it to H_2O. This sequence of oxidation-reduction reactions catalyzes the pumping of protons (H+) into the intermembrane space from the matrix, generating the transmembrane potential across the inner membrane ($\Delta\psi_m$). This accumulation of protons creates an electrochemical gradient in which the concentration of protons outside the mitochondrial membrane is higher than the inside. This gradient provides the energy to power the F_1F_0-ATP 3 synthase, otherwise known as Complex V. The F_1F_0-ATP synthase is responsible for ATPphosphorylation by combining ADP and inorganic phosphate (Pi) to form ATP, creating metabolic energy that the cell can use (Wai et al. 2016)

OPA1 is a crucial mediator of mitochondrial dynamics

Mitochondrial morphology exists between two states: a fragmented population of vesicular organelles (fission), and an interconnected reticular network (fusion). This morphological balance is highly dynamic, mediated by distinct factors located within the outer and inner mitochondrial membranes. Fission is regulated by dynamin-related protein-1 (DRP1), a cytosolic GTPase that is recruited to the mitochondrial network, where it binds multiple interacting partners (Fis1, Mff, MiD49, MiD51) at the outer membrane. Following mitochondrial recruitment, DRP1 forms a multimeric collar around the organelle, pinching the tubule to divide the organelle (Fig. 1). Together, these factors work to maintain mitochondrial morphology of a balanced fusion and fission network (Gilkerson, De La Torre, and St. Vallier, 2021). Mitochondrial fusion generates an interconnected tubular network, regulated by OPA1 protein in the inner membrane and by mitofusins 1 and 2 in the outer membrane (Mishra et al. 2016).

The OPA1 protein is present within the cell as five isoforms, two long, fusion-active L-OPA1 isoforms and three short, fusion-inactive S-OPA1 isoforms. Recently it has been shown that L-OPA1 canacttocarryoutinnermembranefusioneitherbyhomotypicbindingtoanotherL-OPA1,orby binding tocardiolipin (CL), cooperating together within the mitochondrial inner membrane to establish fusion (Ban et al. 2017). Cardiolipin is a mitochondrial-specific lipid found at high concentrations at the inner membrane, with functions including stabilization of respiratory complexes, regulating cytochrome c release during apoptosis, mitochondrial dynamics, and

mitophagy. The OPA1 gene is comprised of 31 exons, and when mutated, causes autosomal dominant optic atrophy (ADOA), an inherited neurological optic pathology (Baricault et al. 2007). The result of ADOA is the progressive loss of visual activity, centrocoecal scotoma and bilateral temporal atrophy of the optic nerve. Chromosome 3q28-q29 has been mapped as the predominant locus for this disorder (Alexander et al. 2000).

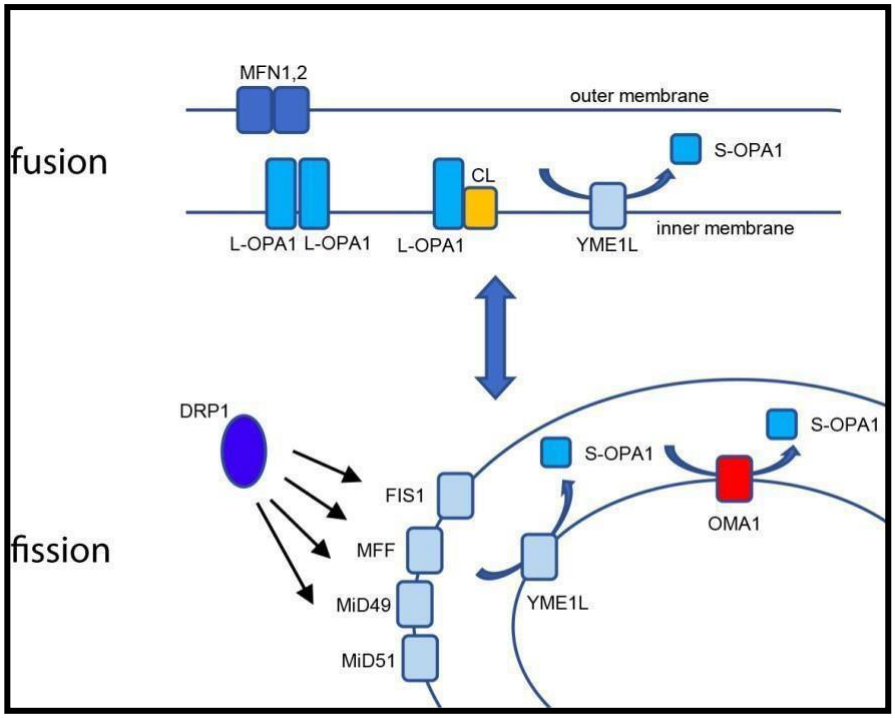

Figure 1. Mitochondrial fusion and fission dynamics. Fusion is mediated by mitofusins 1 and 2 (MFN 1, 2) and OPA1, while fission is carried out by recruitment of DRP1 to the organelle by receptor proteins FIS1, Mff, MiD49, and MiD51. Constitutive and inducible processing of L-OPA1 is mediated by YME1L and OMA1, respectively. From Gilkerson, De La Torre, and St. Vallier, 2021.

In addition to mediating fusion of the mitochondrial inner membrane, OPA1 is directly involved in maintaining the organization of cristae, the structured tubules or shelf-like folds of the mitochondrial inner membrane. Cristae structurally respond to the energetic demands of

the cell; without OPA1, mitochondrial cristae will decrease along with various crista-dynamics such as; shortening, elongation and crista fusion (Hu et al. 2020). OPA1's control in the modification of cristae structure is needed for cellular adaptation to substrate availability and is necessary for cell growth and survival (Patten et al. 2014). OPA1 interacts with the Mitochondrial contact site and Cristae Organizing System (MICOS) to help mediate cristae organization (Stephan et al. 2020). The role of OPA1 in organizing the topology of the inner membrane places it in an integral role to mitochondrial participation in apoptosis and cellular stress response. OPA1 protects cells from apoptosis (Song et al. 2007) by the pro-apoptotic factor cytochrome c being docked and segregated inside the cristate volume (Baricault et al. 2007).

Given the importance of OPA1 to inner membrane fusion, cristae organization, and cellular apoptosis, the balance of OPA1 isoforms is critically important: fusion-active L-OPA1 isoforms will be cleaved to short, fusion-inactive S-OPA1 upon cell stress stimuli. In response to loss of Dy_m, the a and b L-OPA1 isoforms are proteolytically cleaved at the S1and S2 cleavage sites, generating the c, d, and e S-OPA1 isoforms (Griparic et al. 2007; Guillery et al. 2008). This stress-sensitive proteolytic cleavage is mediated by the OMA1 metalloprotease.

OMA1 mediates stress-induced processing of OPA1

Under steady-state conditions, both L- and S-OPA1 isoforms are typically found within mitochondria. The mitochondrial protease Yme1 constitutively produces S-OPA1 isoforms, generating a balance of long and short OPA1 (Griparic et al. 2007). However, Yme1 is not required for inducible proteolysis. Proteolytic cleavage of L-OPA1 in response to loss of Dy_m is caused by the OMA1 metalloprotease: in response to Dy_m uncouplers such as CCCP, OMA1

fully cleaves L-OPA1 isoforms, causing an accumulation of S-OPA1 (Head et al. 2009; Ehses et al. 2009). As such, stress-inducible OMA1 cooperates with constitutive Yme1L to regulate OPA1 balance in mammalian cells (Anand et al. 2014). Insults to mitochondrial homeostasis such as loss of Dy_m or apoptosis lead to activation and self-cleavage of OMA1 involving a HEXXH motif (Zhang, et al. 2014). OMA1 is localized in the inner membrane, with its C- terminal domain is exposed to the inner membrane space (IMS). This placement allows for OMA1 processing of OPA1 since the S1 processing site of OPA1 resides in the IMS (Ehses etal. 2009). The M48 proteolytic domain is IMS-oriented, while the N-terminal Dy_m sensor domain is loosely structured on the matrix side of the inner membrane (Baker et al. 2014).

Previous work in the Gilkerson laboratory used titration of CCCP in cultured 143B cells to demonstrate that a sharp threshold of Dy_m (33% of untreated controls) is required for OPA1-mediated mitochondrial fusion (Jones et al. 2017).

Retinoic acid activates OPA1 processing

To explore whether a similar mitochondrial trans-membrane threshold occurs in more metabolically-demanding cell settings, H9c2 cardiomyoblasts were examined for CCCP-induced OPA1 processing. Surprisingly, the results showed that H9c2s are insensitive to CCCP, with no OPA1 processing observed even after 4 hours of treatment with CCCP. This insensitivity, not previously demonstrated in the literature, is more surprising still due to the full expression of the OMA1 metalloprotease, which does not appear to be activated in response to CCCP in this setting. This led us to speculate whether differentiation could activate CCCP-induced OPA1 processing in H9c2 cells. Growing H9c2 cells in low serum supplemented with retinoic acid (RA) causes cardiac-like differentiation, including increased expression of cardiac-specific genes and cardiomyocyte-like cell morphology (Branco et al.

2015). RA-mediated differentiation restores robust CCCP-induced OPA1 processing in H9c2s (Garcia et al. 2021), providing a novel first demonstration of developmental regulation of OPA1 processing (Figure 2). Here, we explore potential mechanisms behind this intriguing developmental switch.

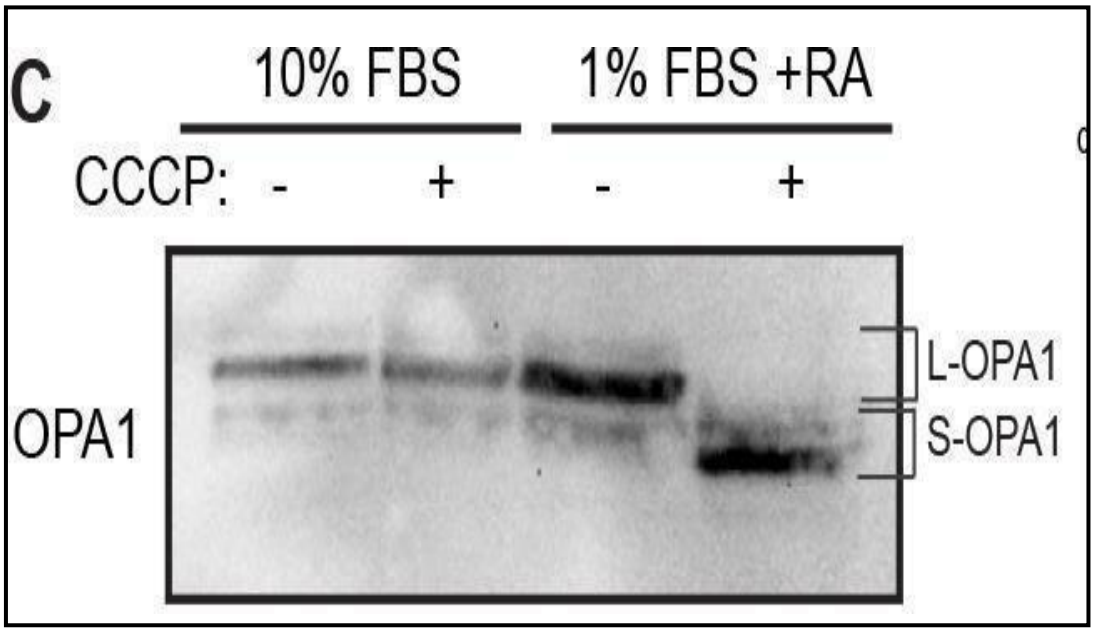

Figure 2: Retinoic acid activates L-OPA1 processing in H9c2 cells.

H9c2 cells incubated with RA show activated of OPA1 processing when challenged with CCCP in comparison with cells incubated with 10%FBS without RA.

CHAPTER III

METHODOLOGY AND FINDINGS

Materials and Methods

Cell culture

H9c2 cardiomyoblasts (ATCC) were passaged at 37 degrees ◦C with 5% CO_2 in high glucose Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FBS) with antibiotic/antimycotic. H9c2 cells were incubated with Retinoic Acid for 5 days, with media changed every day, as per Branco et al. (2015). Retinoic acid contained Vitamin A, an essentialmetabolite for early embryonic development and promotes specification of stem cell neural linage (Zhang et al. 2015). The 10% FBS was diluted to 1% Fetal bovine serum and referred toas low serum. FBS is a growth supplement, containing growth factors that are necessary to support cell growth (ThermoFisher, 2021). H9c2 cells were incubated with low serum for five days, with media changed every day. Retonoic acid, chloramphenicol, and CCCP were purchased from Sigma-Aldrich. Media, FBS, and antibiotic/antimycotic were purchased from Fisher.

Confocal imaging

Cells were seeded to 22 x 22 mm glass coverslips in 6-well culture dishes and incubated with MitoTracker CMXRos, followed by staining with DAPI, and Phalloidin. Thecells weremountedin 50% glycerol in PBS. Samples were visualized on an Olympus FLUOview FV10i confocal microscope.

Western blot

For SDS-PAGE Western blotting, cells were seeded to 100 mm dishes (Corning) and grown inthe indicated media. Cell were lysed by incubating with Laemmli buffer with B-mercaptoethanol (Bio-Rad). Cell lysates were electrophoresed through 6% acrylamide gels in equal volumes and transferred to a PVDF (Bio-Rad) membrane. The PVDF membrane was blocked with 5% nonfat milk in Tris-buffered saline + Tween (TBST). Primary antibody incubations were held overnight at 4 C and secondary goat anti-mouse poly HRP was used to incubate membrane for one hour following primary incubation. The membranes were washed with TBST and developed using WestDura SuperSignal (Thermo,34076). Blots were later visualized with GelDoc XR + Gel Documentation System (Bio-Rad).

ImageJ analysis

ImageJ analysis shown in figures is representative of three independent experiments for reproducibility. OPA1 isoform bands were analyzed from Western blot figures obtained from the three independent experiments. The size of each Long OPA1 isoform was quantified and anaverage of the three trials was obtained and used to run the statistical analysis through One-wayANOVA with post-hoc Tukey HSD Test Calculator. Which allowed for the generation of data being presented with the average mean and standard error.

Gene Expression

Gene expression analysis was performed using reverse transcriptase PCR (qRT-PCR). 10cm dishes were used to incubate the H9c2 cells and processed using QIAGEN RNeasy kitand cDNA prepared by using super-script Reverse Transcriptase. The relative expression was thennormalized to OMA1, OPA1, Bax, Bak and Myog.

Results and Discussion

RA-induced OPA1 processing is chloramphenicol-independent

To explore potential mechanisms of this novel developmental switch, we examined mitochondrial protein synthesis as a potential cooperating mechanism. Battersby's group showed that in cells lacking AFG3L2, OPA1 processing is sensitive to chloramphenicol (CAP) (Richter et al. 2019). CAP binds to the mitochondrial ribosome, thus preventing the synthesis of mtDNA-encoded polypeptides (Storrie and Attardi,1973; Hilander et al. 2018). As H9c2s have decreased AFG3L2 expression relative to control 143B cells, we examined whether the restoration of OPA1 processing in RA-differentiated H9c2s may be sensitive to inhibition of mitochondrial protein synthesis. To explore this mechanistic possibility, we examined whether chloramphenicol (CAP) would attenuate OPA1 processing in RA-treated H9c2s. H9c2 cells were differentiated in RA-containing media, as above, without or with 10 mM CAP, followed by challenge with CCCP (10 mM, 1 hr.,). Lysates of control RA-differentiated showed both L-OPA1 and S-OPA1 isoforms, as expected. In response to CCCP challenge, however, L-OPA1 isoforms were lost in RA-differentiated H9c2s both without and with CAP incubation (Fig. 3) These results demonstrate that chloramphenicol did not turn off OPA1 cleavage, indicating that the RA-mediated activation of OPA1 processing is independent of mitochondrial protein synthesis. Though we don't know exactly how chloramphenicol is unable to reverse OPA1 cleavage, the results provided an interesting insight to the mechanism of OPA1 cleavage induced by treatment of CCCP.

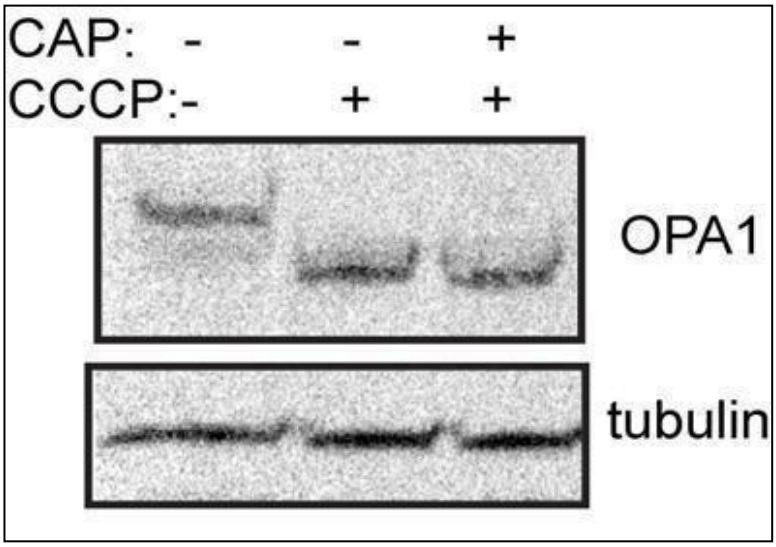

Figure 3: Retinoic acid-induced OPA1 processing is chloramphenicol-independent. OPA1 Western blotting of lysates from RA-differentiated H9c2s without or with 10 micro molar CCCP and/or CAP, n=3 expts.

Growth on low serum lacking RA promotes myocyte-like differentiation of H9c2s

It has been demonstrated that H9c2 cardiomyoblasts can be differentiated by growth in low serum media (1% FBS, as compared with the standard 10%) supplemented with RA. Following growth of H9c2s in RA-containing differentiation media for five days, the cells transition to a more cardiomyocyte-like phenotype, though they are not contractile (Branco et al. 2015). Further, differentiation of H9c2s in low serum media *lacking* RA causes increased expression of skeletal muscle markers, rather than cardiac factors (Branco et al. 2015). This raises a question: is the differentiation-activated OPA1 processing we observe in H9c2s specific to RA, or is OPA1 processing activated by broader developmental programs? To explore whether the induction of OPA1 processing we observe in differentiated H9c2s is RA-dependent, H9c2s were grown in DMEM with 1% FBS lacking RA.

Following five days growth in differentiation media lacking RA, H9c2s were imaged side-by-side with undifferentiated controls by confocal microscopy. To visualize the cells, DAPI (4′,6-diamidino-2-phenylindole) was used to visualize the nuclei of the cells (blue). The nucleus is able to be observed under fluorescence microscopy due to DAPI's high affinity for DNA. MTR (MitoTracker Red) allows for the visualization of the mitochondria due to uptake in response to transmembrane potential. Phalloidin binds to the actin cytoskeleton within the cell, allowing for the visualization of cellular morphology. Coming in a variety of colors, fluorescent green was chosen as the staining color and viewed under fluorescence microscopy as well (ThermoFisher, 2021).

Confocal imaging revealed that cells grown in 1% FBS became elongated with a cardiomyocyte-like morphology, as compare with cells grown in 10% FBS, which retained their blast-type cell morphology (Figure 5). Figure 5 images A-C show the control of H9c2s treated with 10% FBS. Figure A is focuses on the mitochondrial staining with mitotracker red, while figure B focuses on the cytoskeletal staining with phalloidin. Figure C is a merged image of all three stains, revealing the blast-type morphology of the 10% FBS h9c2 treated cells. Conversely, images D and E show the staining of the mitochondria and cytoskeleton with use of mitotracker red and phalloidin however, the merge image depicts a different morphological shape than the control. The 1% FBS H9c2 treated cells took on an elongated shape, indicating differentiation into a skeletal muscle like cell may have occurred. To confirm this, gene expression of cellular differentiation markers was performed within the 10% and 1% FBS treated cells.

Indication of cellular differentiation within H9c2 cells treated with 1% FBS was observed

once confocal imaging revealed a skeletal like morphological state due to the cells elongation (Figure 4). Gene expression analysis was performed through quantitative reverse transcriptase PCR (qRT-PCR) to observe differentiation markers. The gene expression revealed low serum lacking RA decreases gene expression of OMA1, OPA1, Bax, Bak and Myog in H9c2s. Conversely, low-serum with RA had an considerable increase in gene expression, while the 10% FBS was slightly higher than the 1% FBS (Figure 5).

These results indicate differentiation can occur at different levels of gene expression and H9c2 cells grown on low serum media without RA promotes the differentiation of H9c2s into a cardiac like cell. A possible explanation for this outcome could be due to the 10% FBS containing inhibitory effects turn off the growth factors necessary for cell differentiation.

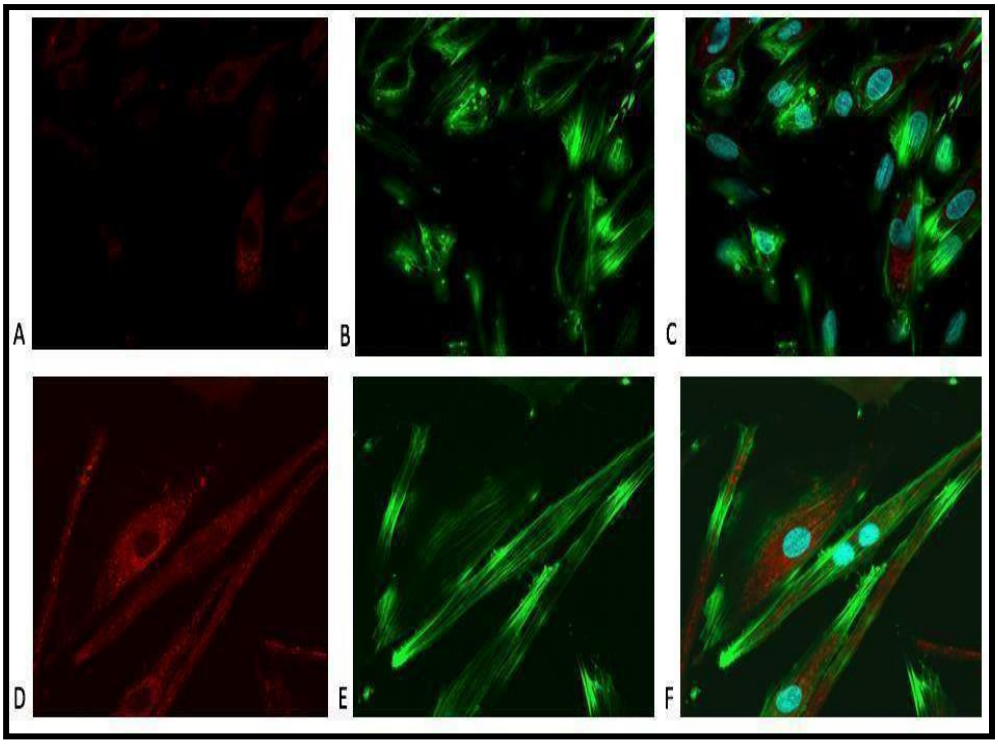

Figure 4: Low serum media elicits myoblast-like differentiation of H9c2 cells. Confocal imaging of H9c2s with 10%FBS (A-C) and 1% FBS (D-F) treated with Mitotracker (Red), Phallodin (Green) and DAPI (Blue).

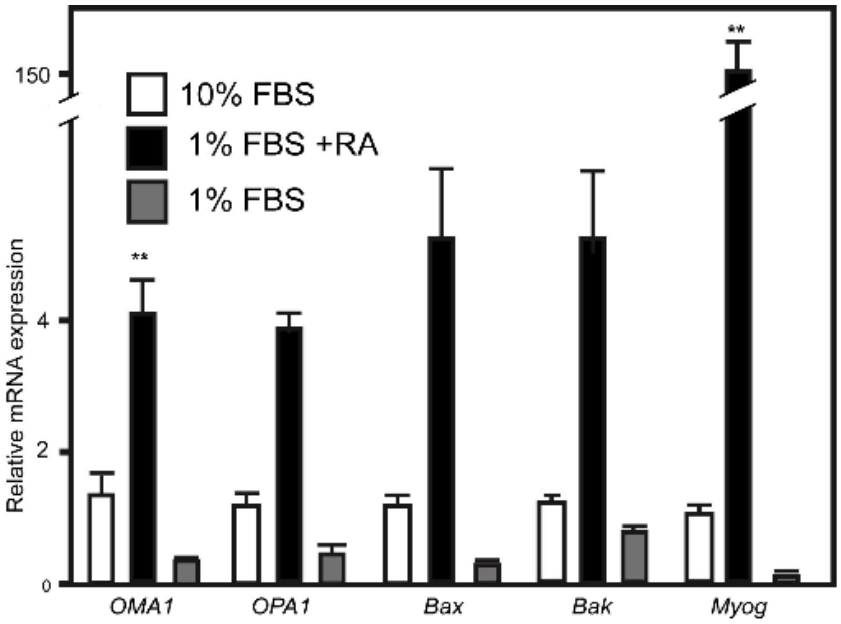

Figure 5. Low serum media elicits skeletal muscle-specific gene expression.

Gene expression of differentiation markers such as Bax, Bak and Myog in addition to OMA1 and OPA1 protein levels were conducted using qRT-PCR for indicated mRNAs ± standard error.

Differentiation of H9c2s in low serum media lacking RA activates OPA1 processing.

To test whether the observed developmental activation of CCCP-induced L-OPA1 processing ~~is~~ requires RA, we performed Western blot analysis of H9c2s in 10% FBS without and with CCCP, RA-differentiated H9c2s without and with 10 micro molar CCCP (1 hr.), and H9c2s differentiated in 1% FBS without and with CCCP. Undifferentiated H9c2s showed L-OPA1 isoforms in both untreated and CCCP-treated lysates, while H9c2s differentiated in 1% FBS with RA show a robust induction of L-OPA1 cleavage under CCCP challenge, consistent with our previous results (Garcia et al. 2021). For H9c2s differentiated in media with 1% FBS *lacking* RA, the results were striking: while differentiated H9c2s have both L- and S-OPA1 isoforms, CCCP challenge induced strong cleavage of L-OPA1, mirroring that seem in RA-differentiated H9c2s under CCCP challenge (Fig. 6A). ImageJ quantitation of OPA1 isoforms

confirmed this: by the quantification of total L-OPA1 present within H9c2 cells incubated with 1% FBS and treated with and without CCCP. A total of six lanes were run; H9c2 cells incubated with 10% FBS, Retinoic acid and 1% FBS. Each test was treated with and without CCCP and the total L-OPA1 percentage was quantified using ImageJ analysis. The results showed a higher reduction in L-OPA1% for cells incubated with 1% FBS and RA in addition to being treated with CCCP. The average amount of Total L-OPA1% throughout the three trials for H9c2s treated with RA and CCCP were; 11.76 while cells treated incubated with 1% FBS and treated with CCCP have an average of 11.34. In comparison to other testing parameters such as the H9c2s incubated in 10% FBS and treated with and without CCCP. An average of 60.00 was observed in cells incubated with 10% FBS, whereas an average of 49.97 was seen in cells treated with CCCP. Furthermore, cells incubated with RA alone revealed an average of 56.01, while cells incubated with 1% FBS revealed an average of 59.27. These results show a higher L-OPA1 percentage in all other cell testing parameters other than the cells that were treated with RA and 1% FBS and treated with CCCP. These results indicate RA is not the only inducer of OPA1 cleavage since the same pattern was observed in cells not treated with RA (Figure 6B).

The L-OPA1 cleavage observed in CCCP challenged H9c2s differentiated without RA indicates that the developmental switch is activated by broader developmental programs than RA alone. Interestingly, the statistical significance was aligned with results reported above. Tukey HSD inference showed, all other testing parameters compared to the H9c2 cells incubated with RA and FBS and treated with CCCP resulted in significance of a p value smaller than 0.01. In contrast to, the other testing parameters that were compared to each other, deemed to be insignificant. The particular significance shown in the treatment parameters which revealed the

highest amount of L-OPA1 cleavage show the statistical significance is attributed to a specific cause. Within this data set, the specific cause is speculated to be due to cellular differentiation by incubation in RA and 1% FBS.

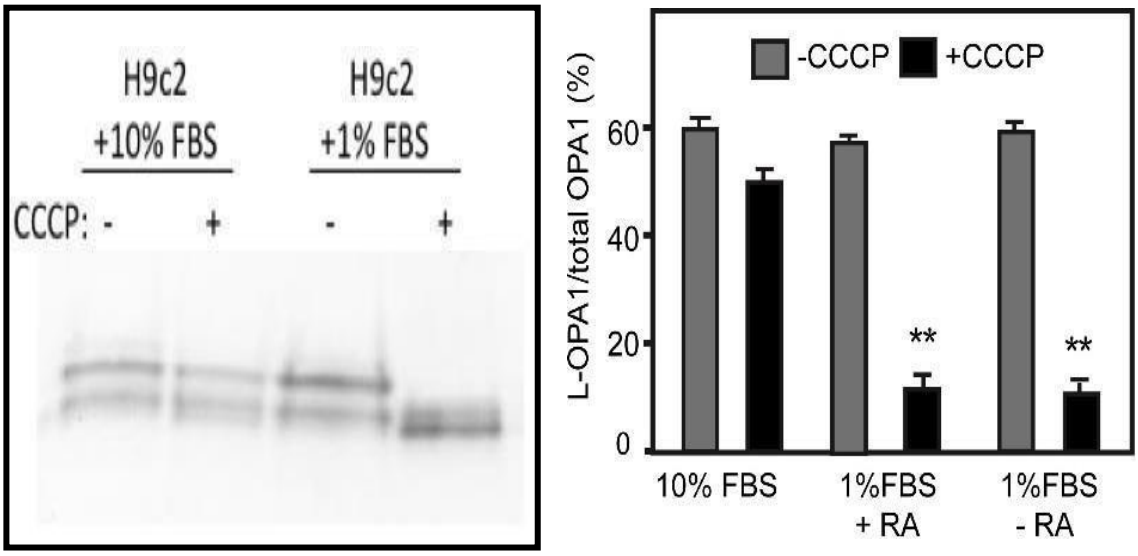

Figure 6: Low serum media lacking retinoic acid activates OPA1 processing.

A.OPA1 Western blotting of lysates from control and low serum (1% FBS) differentiated H9c2s without or with 10 mM CCCP, n=3 expts. B. ImageJ analysis of L-OPA1 levels from Western blots.

CHAPTER IV

SUMMARY AND CONCLUSION

In conclusion, we show that the activation of OPA1 processing is independent of mitochondrial protein synthesis. This was shown by the use of CAP, which inhibits mitochondrial ribosomal protein synthesis, not attenuating L-OPA1 processing when challenged with CCCP. In addition, low serum lacking RA causes differentiation, with distinct gene expression compared to low serum with RA. The relative gene expression analysis revealed significantly lower levels in OMA1, OPA1, Bax, Bak, and Myog. This data indicates, increased gene expression of cardiac markers (Myog) are shown in cells incubated with 1% FBS and RA. Furthermore, developmental induction of OPA1 processing is independent of RA. H9c2 cells incubated with or without RA and challenged with CCCP show L-OPA1 processing. ImageJ quantification of total L-OPA1 amount show, significant decrease in L-OPA1 total within H9c2's incubated with or without RA in low-serum. Demonstrating different developmental programs are at play between the two cells lines. Leading to future investigations of what specific mechanisms play a role in activating OPA1 processing. A key starting point towards this investigation would be to observe OMA1 regulation, as this is the major stress-response for the OPA1 protease. Other future directions would be to look into other cells settings and observe if they are likely to harbor similar developmental switching of OPA1 processing. Future work would include to characterize the induction of OPA1 processing in myocyte-like H9c2s through transmembrane potential (TMRE) analysis and OMA1 degradation activity by western blot

analysis. In addition to investigating the mechanistic switch which activates OMA1 upon cellulardifferentiation in the H9c2 cell line. Perhaps looking into the significant changes occurring within the cell upon differentiation that could contribute to OMA1 activation in OPA1 cleavage.A future question would be to speculate, how does activation of OPA1 processing impact differentiation. We hypothesize: it probably promotes apoptotic priming, as post-mitotic tissues such as cardiac and skeletal muscle have increased apoptosis relative to undifferentiated cells.

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BIOGRAPHICAL SKETCH

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