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Studies to elucidate the location and function of STAT5A/pyruvate dehydrogenase complex in adipocytes

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by

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Abstract

Adipocytes are insulin-sensitive cells that store lipids and have important endocrine functions. STAT5, a transcription factor member of the JAK-STAT pathway is expressed highly within adipocytes and plays important roles in adipocyte differentiation and function. Disturbances to the JAK-STAT pathway can lead to a variety of pathogeneses, including type 2 diabetes mellitus. STAT5 is activated by phosphorylation of a conserved tyrosine residue in response to cell stimulation by hormones or cytokines. Growth hormone (GH) is a potent STAT5 activator that can regulate adipocyte function. Studies in our laboratory suggested that STAT5 might have a mitochondrial function based on its association with a subunit of pyruvate dehydrogenase complex (PDC), which is known to localize and function within mitochondria. Hence, we assessed STAT5 expression in the mitochondria of 3T3-L1 adipocytes and hypothesized that it may regulate mitochondrial biogenesis. We found that GH-induced activation of STAT5A did not modulate mitochondrial biogenesis significantly. However, using immunofluorescence methods, we observed that one of the PDC components, PDC-E2, is present within fat cell nuclei where it also associates with tyrosine phosphorylated STAT5. These highly novel studies demonstrate that PDC interacts with STAT5, and we hypothesize that this association is important in the modulation of histone acetylation or transcriptional regulation of STAT5 target genes.

Introduction

Adipocytes have long been thought to solely function as energy storage depots. However, it is now known that these cells play a major role in the regulation of glucose metabolism and exhibit important endocrine functions. Disturbances that affect adipocytes can lead to obesity and other metabolic disorders, such as type 2 diabetes mellitus (T2DM). Much research has focused on studying signaling proteins and transcription factors that regulate adipocyte gene expression in an effort to understand the pathogenesis of metabolic diseases.

The Stephens laboratory has focused on the STAT (signal transducer and activator of transcription) family of transcription factors that are involved in the JAK-STAT pathway. This family consists of seven members: STAT1, 2, 3, 4, 5A, 5B, and 6. In adipocytes, STAT5A and STAT5B can be activated by growth hormone (GH), prolactin (PRL), or oncostatin M (OSM)[1, 2, 3]. In response to receptor stimulation by cytokines or hormones, JAK kinases phosphorylate receptor tyrosine residues, allowing for STAT recruitment and phosphorylation. Upon tyrosine phosphorylation the STATs dimerize, which allows them to translocate into the nucleus to regulate expression of target genes. In adipocytes, STAT5 is known to regulate the expression of genes involved in adipogenesis, glucose and lipid metabolism, and insulin sensitivity[4, 5].

Although the role of STAT5 as a nuclear transcription factor is well studied and clearly important in regulating adipocyte development and function, only a small portion of total STAT5 translocates to the nucleus upon activation. Drs. Stephens and Richard hypothesized that identifying novel STAT5-interacting proteins would provide insight into additional extra-nuclear functions of STAT5. Using a non-biased proteomics-based approach, they observed that STAT5A associates with the E2 subunit of the pyruvate dehydrogenase complex (PDC) and several other mitochondrial proteins. PDC is a mitochondrial matrix protein that converts

pyruvate into acetyl-CoA. Data from our lab using subcellular fractionation and transmission electron microscopy (TEM) indicates that STAT5A can also localize within mitochondria in adipocytes. Previous research shows that STAT5 can interact with PDC-E2 and can also translocate into the mitochondria of B and T lymphocyte cells to bind to the mitochondrial D-loop oligonucleotide, which contains a putative STAT5 binding site[6]. Although our current data clearly indicates a strong STAT5-PDC-E2 interaction, it is not clear if this association or STAT5 itself contributes to mitochondrial function.

Mitochondria play a major role in non-shivering thermogenesis in brown adipose tissue by producing UCP-1. However, mitochondria in white adipose tissue have not been extensively studied. Previous studies have shown that mitochondrial dysfunction and down-regulation of mitochondrial biogenesis increase the risk for certain metabolic diseases, such as T2DM and cardiovascular disease[7]. However, the role of STAT5 in regulation mitochondrial biogenesis or function in adipocytes has not been previously studied. Given that STAT5 is a transcription factor and mice without GH receptors have increased levels of key regulators of mitogenesis[8], we hypothesized that STAT5 could regulate genes that are involved in mitochondrial biogenesis and morphology.

Recently, researchers have demonstrated that PDC translocates from the mitochondria into the nucleus in mammalian carcinoma, fibroblast, and epithelial cell lines[9]. They also show that nuclear PDC is able to produce acetyl-CoA that is used as a substrate for histone acetylation[9]. However, PDC's presence within adipocyte nuclei and its ability to regulate STAT5 target gene expression in fat cells has not been previously studied. Using immunocytochemistry techniques, our data also indicate that PDC is present within the adipocyte nucleus and interacts with phosphorylated STAT5. Given that our previous research using subcellular fractionation

and immunoprecipitation suggests that STAT5A potentially interacts with PDC-E2 in 3T3-L1 adipocytes, we hypothesize that nuclear PDC interacts with STAT5 to modulate gene expression through acetylation of histones or other nuclear proteins.

Our novel results demonstrate that STAT5 can translocate into and localize in adipocyte mitochondria. We also observe moderate increases in mitochondrial copy number in response to GH treatment, but there is no regulation of mitochondrial biogenesis markers, except for PGC1α. However, these results were not consistently replicated, so we shifted our focus to the STAT5-PDC-E2 interaction. We also observed STAT5 binding with PDC-E2 within the nucleus of adipocytes. These data provide new insight into the possible functions of STAT5 and PDC within the mitochondria and nuclei in adipocytes and have paradigm-shifting implications that can change the way researchers think about JAK-STAT signaling.

Materials and Methods

Cell Culture

For DNA and mRNA isolations, mature 3T3-L1 adipocytes in 12-well plates were serum-deprived with 5% calf serum in Dulbecco's Modified Eagle Medium (DMEM) for 24 hours. After serum deprivation, adipocytes were treated with 10 nM growth hormone (GH) or the vehicle ($NaHCO_3$) for 48 hours. Adipocytes were treated with 1 μM rosiglitazone (Rosi) and DMSO (CTL) as a positive and negative control, respectively.

Immunogold Labeling and Transmission Emission Microscopy

Fully differentiated 3T3-L1 adipocytes were treated with 10 nM GH for 15 mins. The cells were fixed, scraped, and embedded in LR White resin. Ultra-thin sections (90 nm) were cut and put on carbon-coated nickel grids. Immunogold labeling was performed on the specimens. An anti-STAT5A primary antibody and a gold-conjugated anti-rabbit IgG antibody were used.

The stained sections were then contrast stained with uranyl acetate and lead citrate to visualize the mitochondrial structures. The specimen was then observed using a JEM-1400 transmission electron microscope. Ying Xiao in the Socolofsky Microscopy Center at LSU performed these steps.

Genomic DNA isolation

Genomic DNA was isolated using Qiagen DNeasy Blood and Tissue Kit or the Quick-gDNA MiniPrep Kit (Zymo Research). For the Qiagen kit, cells were scraped in 200 µL of phosphate buffered saline (PBS) buffer. Cells were lysed and gDNA was isolated according to the manufacturer's standard protocol. For the Zymo Research kit, cells were lysed in 500 µL of Genomic Lysis Buffer (Zymo Research) and transferred into the spin column. DNA isolation proceeded according to the standard protocol and stored at -80°C.

Total mRNA isolation

Total mRNA was isolated using the Qiagen RNeasy Mini Kit. Treated cells were lysed in 350 µL of RLT Buffer (Qiagen) and transferred into the Qiagen spin columns. mRNA isolation was followed according to standard protocol and stored at -20°C.

cDNA synthesis

cDNA was synthesized from total mRNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). 1.5 µg of RNA was added to each reaction. The reverse transcription was performed using the following thermal cycler conditions: 25°C for 10 mins, 37°C for 120 mins, 85°C for 5 mins, and hold at 4°C. The cDNA was stored at -20°C until needed for quantitative PCR (qPCR).

Quantitative PCR

Quantitative PCR was performed using the SYBR Premix Ex Taq (Tli RNase H Plus) with ROX Plus (Takara). Primers for COX1, LPL, cyclophilin A, cyclophilin B, TBP, Nrf1, Tfam1, Nos3, and PGC1α were ordered from Integrated DNA Technologies (IDT). 4 ng of gDNA or cDNA was added for each reaction and was run on Applied Biosystems 7900 Sequence Detection System.

Fluorescence Analysis

Mature 3T3-L1 adipocytes were seeded on a 96-well glass-bottom plate and allowed to adhere overnight. Cells were serum-deprived overnight and treated with 10 nM GH and vehicle ($NaHCO_3$). Cells were treated with 1 μM rosiglitazone and DMSO as a positive and negative control, respectively. Cells were stained with 150 nM MitoTracker Red CMXRos and counterstained with DAPI. Fluorescence was measured on Molecular Devices FlexStation at 561 nm.

Immunoprecipitation and Western Blotting

Fully differentiated 3T3-L1 adipocytes were treated with 5 nM GH. Control cells were untreated. Monolayers were carefully collected in immunoprecipitation (IP) buffer and subcellular fractionation was performed on the samples to obtain cytoplasmic and nuclear fractions. Anti-STAT5A and anti-PDC-E2 antibodies (Santa Cruz) were used for immunoprecipitation reactions (300 μg protein). STAT5A and PDC-E2 proteins were western blotted.

Immunofluorescence

Mature 3T3-L1 adipocytes were seeded and allowed to adhere to either 6-well glass-bottom plates or coverslips in 12-well plates overnight. The cells were serum-deprived overnight and treated with 5nM GH or $NaHCO_3$ for studies involving STAT5-pY and PDC-E2 interaction.

Cells were stained with MitoTracker Red CMXRos (300 nM in 0.3% BSA, Molecular Probes) for 15 mins in a 37°C incubator, fixed with 3.7% *p*-formaldehyde for 15 mins, and blocked with 10% BSA for 30 mins. The cells were incubated with STAT5-pY rabbit polyclonal (Millipore) or PDC-E2 mouse monoclonal primary antibody (Santa Cruz) overnight. Cells were incubated with Dylight 488-conjugated goat anti-mouse secondary antibody (Thermo Scientific) or AlexaFluor 594-conjugated goat anti-rabbit secondary antibody (Molecular Probes) for 1 hr. DAPI (0.2 μg/mL, Thermo Scientific) was added for 5 mins to counterstain the nuclei. Cells were imaged on a Leica TCS SP5 laser scanning confocal microscope on a 63x oil immersion objective. The lasers for wavelengths 405 nm (DAPI), 488 nm (green) and 561 nm (MitoTracker, red) were set at 30% intensity. The laser for wavelength 591 nm (AlexaFluor, red) was set at 80% intensity.

Results

STAT5 localization within mitochondria in 3T3-L1 mature adipocytes

To test if STAT5A is present within the mitochondria of adipocytes, immunogold labeling and transmission electron microscopy was performed on mature 3T3-L1 adipocytes. STAT5A was found to be localized within the mitochondria (red arrows) and the cytoplasm (blue arrows) (Fig. 1A). Fluorescence microscopy was used to further demonstrate that activated STAT5 resides within adipocyte mitochondria. Phosphorylated STAT5 was labeled with Dylight 488 antibodies, which fluoresces green (Fig. 1B). Mitochondria were stained with MitoTracker, which is a fluorescent red dye that accumulates specifically in mitochondria based upon its membrane potential (Fig. 1C). When the images are merged together, orange-yellow coloration in the extra-nuclear space indicates colocalization of STAT5-pY with mitochondria (Fig. 1D). The presence of phosphorylated STAT5 (green) within the nucleus (blue) is consistent with its

nuclear translocation to modulate gene transcription (Fig. 1D and E). Furthermore, subcellular fractionation combined with immunoprecipitation using the anti-STAT5A antibody revealed the presence of STAT5A within the mitochondrial fraction (data not shown). The combination of data from these multiple techniques demonstrates that STAT5 is present within the mitochondria of 3T3-L1 adipocytes.

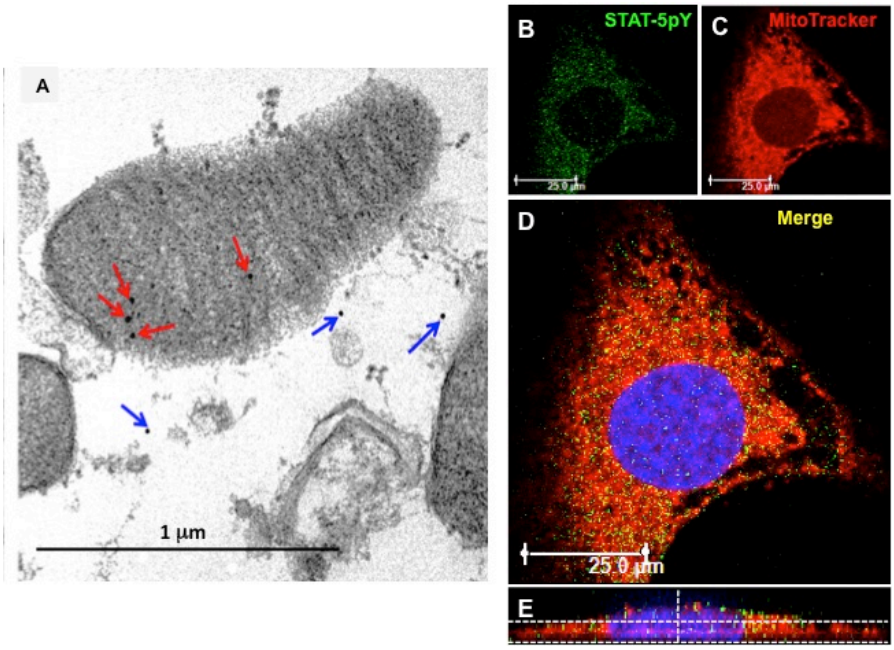

Figure 1. Immunogold labeling, electron microscopy, and fluorescence microscopy demonstrate that STAT5A resides in the mitochondria of adipocytes.
Left: Fully differentiated 3T3-L1 adipocytes were labeled according to standard immunogold labeling procedures. An anti-STAT5A primary antibody was used and the secondary antibody used was a gold-conjugated anti-rabbit IgG antibody. Red arrows indicate gold labeling of STAT5A in the mitochondria and blue arrows show labeling of STAT5A in the cytosol (A). **Right**: Mature 3T3-L1 adipocytes were treated with 5 nM GH and stained with MitoTracker CMXRos (red; C). STAT5-pY was labeled with a Dylight 488 conjugated antibody (green; B). The red and green channels were merged and demonstrated localization of STAT5 with mitochondria (yellow; D). Orthogonal z-sectioning shows STAT5 within the nucleus (green; E) and colocalization of STAT5 and the mitochondria (yellow; E).

Role of STAT5 on the regulation of mitochondrial copy number and mitogenesis

Mitochondrial biogenesis is regulated through the transcription of both nuclear and mitochondrial DNA[10]. Since STAT5 is found inside the mitochondria of GH-treated adipocytes, we hypothesized that STAT5 activation may regulate mitochondrial biogenesis and copy number. To test this hypothesis, mature 3T3-L1 cells were treated with vehicle or GH. Cells

treated with DMSO and rosiglitazone (Rosi) were used a negative and positive control, respectively. DMSO was used as the vehicle treatment for comparison against Rosi-treated cells. The cells were stained with MitoTracker Red CMXRos and total fluorescence for each well was measured. In response to Rosi treatment, mitochondrial staining significantly increased by 18.7% compared to DMSO-treated cells. In GH-treated cells, mitochondrial staining modestly increased by 8.4% relative to vehicle-treated cells, but the increase was not statistically significant ($p = 0.0625$) (Fig. 2). This modest increase in MitoTracker fluorescence suggests that GH may increase mitochondrial copy number. However, since MitoTracker is dependent upon mitochondrial membrane potential and mitochondrial fluorescence is only an indicator of mitochondrial copy number, we examined if GH treatment directly affects mitochondrial copy number.

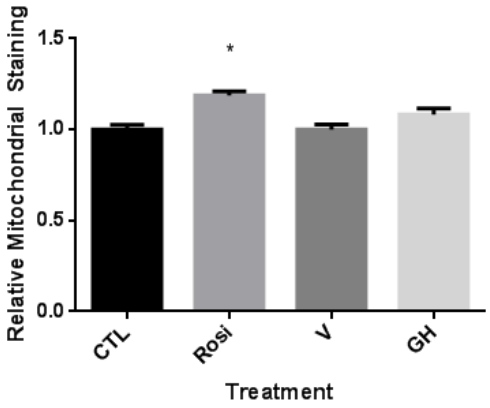

Figure 2. Relative mitochondrial fluorescence increases in response to GH treatment.
Fully differentiated 3T3-L1 cells were seeded onto a 96-well plate and were serum-deprived overnight. Adipocytes were treated with 10 nM growth hormone (GH) for 24 hours and were stained with MitoTracker Red CMXRos. Cells were fixed, nuclei were counterstained with DAPI, and fluorescence at 561 nm was measured. The fluorescence for the GH-treated cells was normalized to the vehicle-treated cells (V) and rosiglitazone-treated cells (Rosi) was normalized to the control (CTL, DMSO); * $p < 0.05$.

To test the modulation of mitochondrial copy number, total genomic DNA from mature 3T3-L1 adipocytes treated with vehicle or GH was extracted and the ratios of COX1 (cytochrome c oxidase subunit I, a mitochondrial-encoded gene) to LPL (lipoprotein lipase, a nuclear-encoded gene) were calculated (Fig. 3A). Mitochondrial copy number significantly

increased about 23.8% in GH-treated cells relative to vehicle-treated cells. Although mitochondrial copy number increased with rosiglitazone treatment, the increase was not statistically significant. These results demonstrate that there is an increase of mitochondrial copy number due GH treatment.

In order to test if this increase is due to mitochondrial biogenesis, mRNA from adipocytes was extracted, reverse transcribed, and assessed for known markers that regulate mitochondrial biogenesis. These regulators include Nrf1 (nuclear respiratory factor 1), Tfam (mitochondrial transcription factor A), Nos3 (nitric oxide synthase 3), and PGC1α (peroxisome proliferator-activated receptor gamma co-activator 1-alpha)[11]. Cells treated with the positive control, Rosi, increased expression of Tfam. Treatment with GH did not regulate any of these mitochondrial biogenesis markers, except for a modest down-regulation of PGC1α relative to the vehicle treatment ($p = 0.0783$) (Fig. 3B). This suggests that GH-induced STAT5 does not modulate transcription of these mitochondrial biogenesis markers. When mitochondrial copy number experiments were repeated several more times, we were unable to obtain compelling data and consistent results (data not shown), so we shifted our focus to the interaction of STAT5 and PDC-E2.

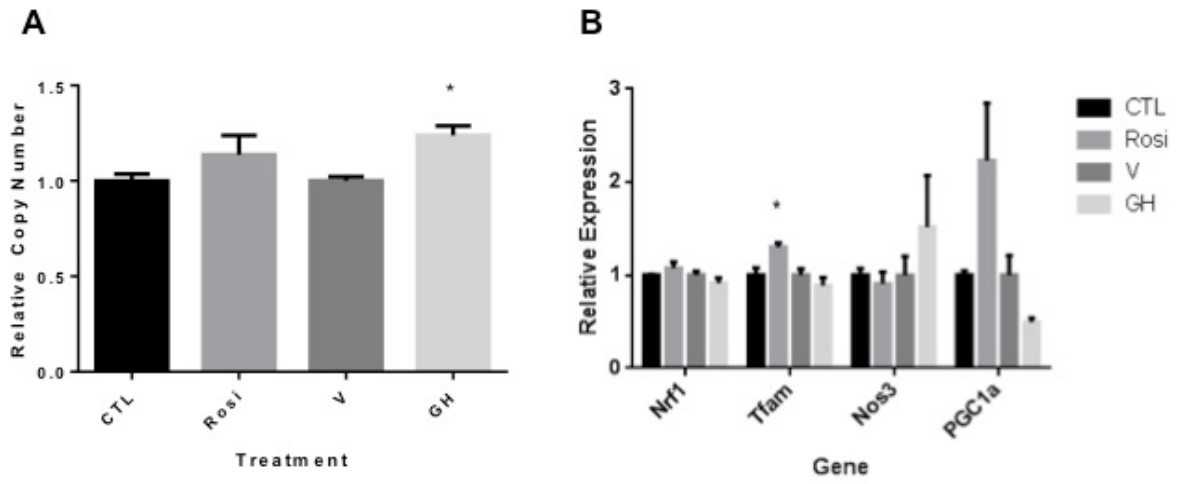

Figure 3. Relative mitochondrial copy number increases in response to growth hormone (GH) treatment and PGC1α is down-regulated in response to GH treatment.
A) Genomic DNA from treated cells was extracted and probed with primers for COX1 (cytochrome c oxidase subunit 1) and LPL (lipoprotein lipase) during quantitative PCR (qPCR). COX1:LPL ratios were calculated for each. Rosiglitazone-treated (Rosi) cells were normalized to control-treated (CTL) cells and GH-treated cells were normalized to vehicle-treated (V) cells; * $p < 0.05$. B) Total mRNA was extracted and probed with primers for Nrf1, Tfam, Nos3, and PGC1α during RT-qPCR. These genes were normalized to the geometric average of cyclophilin A, cyclophilin B, and TATA binding protein. Rosiglitazone-treated (Rosi) cells were normalized to control-treated (CTL) cells and GH-treated cells were normalized to vehicle-treated (V) cells; * $p < 0.05$. Although not significant, PGC1α was down-regulated in a GH-dependent manner.

STAT5 and PDC-E2 interactions

Previous mass spectrometry screening results in our lab suggested that PDC-E2 interacts with STAT5A. To confirm this potential interaction, 3T3-L1 adipocytes were exposed to GH for 20 minutes to activate STAT5 and were fractionated into cytoplasmic and nuclear fractions. Each fraction was subjected to immunoprecipitation (IP) with a STAT5 or PDC-E2 antibody followed by western blotting with the same antibodies. The presence of bands in the "Nuc +" fraction in adipocytes treated with GH indicates that the STAT5A and PDC-E2 interaction occurs in the nuclear fraction. However, the nuclear fraction was contaminated with mitochondria, so it is not known from this data alone if the interaction occurs within the nucleus or mitochondria (Fig. 4). STAT5 and PDC-E2 also interacts in human adipocytes *in vitro* and in mouse adipose tissue *in vivo* (data not shown).

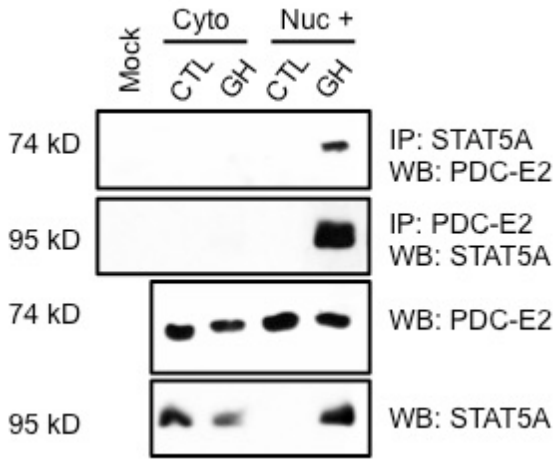

Figure 4. PDC-E2 interacts with STAT5A in a GH-dependent manner in murine adipocytes.
Fully differentiated 3T3-L1 adipocytes were treated with 5 nM GH for 20 minutes. Control (CTL) cells were untreated. Monolayers were collected and subjected to subcellular fractionation to separate into cytoplasmic and nuclear fractions. Immunoprecipitation (IP) reactions were conducted with 300 µg of protein. Mock samples contained antibodies but no extract. PDC-E2 and STAT5A proteins were detected using western blotting.

To determine if PDC-E2 is present in the nuclei of adipocytes, untreated 3T3-L1 adipocytes were stained with MitoTracker Red CMXRos (red; Fig. 5B, left). The cells were fixed, blocked, and incubated with an anti-PDC-E2 mouse antibody. A Dylight 488-conjugated anti-mouse secondary antibody was used (green; Fig. 5A, left). The nuclei were counterstained with DAPI (blue). The red and green channels were merged (Fig. 5C, left) and orthogonal sections were obtained using the z-stack feature (Fig. 5D, left). PDC-E2 staining (green) was found to be within (not on top of) the nucleus (blue). Immunofluorescence using STAT5-pY and PDC-E2 antibodies was also used to further confirm the location of the STAT5 and PDC interaction within the cells. Mature 3T3-L1 adipocytes were serum-deprived overnight and were treated with growth hormone. The cells were fixed, blocked, and incubated with anti-PDC-E2 mouse antibody and anti-STAT5pY rabbit antibody. A Dylight 488-conjugated anti-mouse antibody (green; Fig. 5A, right) and an AlexaFluor 594-conjugated anti-rabbit antibody (red; Fig. 5B, right) were used as secondary antibodies. When the two channels were merged, the yellow-orange staining within the cytoplasm indicates that PDC-E2 interacts with STAT5-pY (Fig. 5C, right). White staining within the nucleus clearly indicates nuclear colocalization of PDC-E2 and

STAT5-pY following GH-stimulation (Fig. 5C, right). Orthogonal sectioning of the cell also indicates white coloration due to the yellow-orange and blue (Fig. 5D, right).

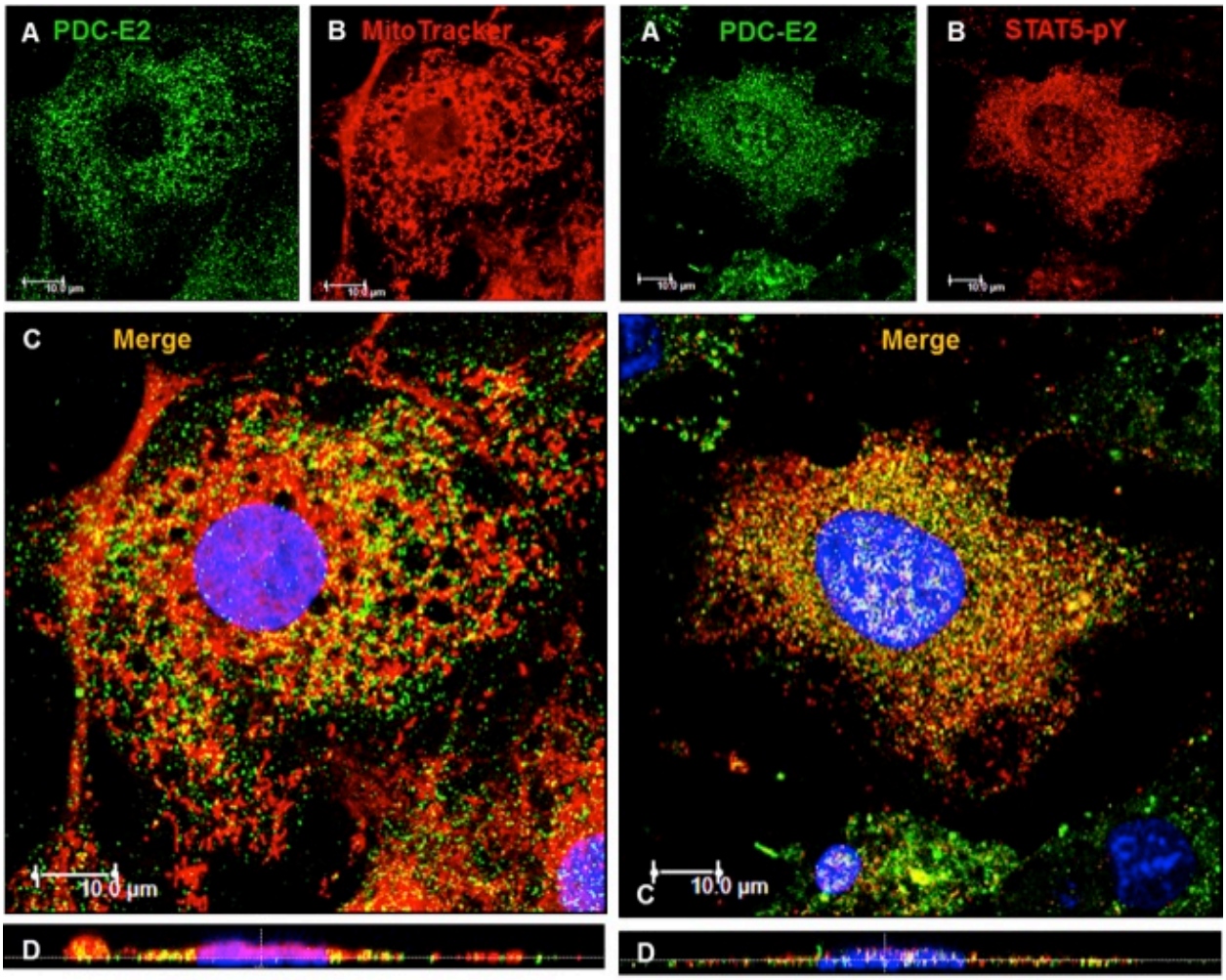

Figure 5. PDC-E2 localizes within mitochondria and nuclei of adipocytes and interacts with STAT5-pY. **Left**: Mature 3T3-L1 adipocytes were stained with MitoTracker Red CMXRos (red; B). PDC-E2 was labeled using anti-PDC-E2 mouse primary antibody and Dylight 488-conjugated anti-mouse secondary antibody (green; A). Nuclei were counterstained with DAPI (blue). Red and green channels were merged (C). Orthogonal sectioning using the z-stack feature was used to indicate PDC-E2 within the nucleus (D). **Right**: Cells were serum-deprived overnight and treated with 5 nM GH for 20 minutes. PDC-E2 was labeled using anti-PDC-E2 mouse antibody and a Dylight 488-conjugated anti-mouse secondary antibody (green; A). STAT5-pY was labeled using anti-STAT5-pY rabbit antibody and a AlexaFluor 594-conjugated anti-rabbit secondary antibody (red; B). Nuclei were counterstained with DAPI (blue). Red and green channels were merged (C). Orthogonal sectioning using the z-stack feature (15 sections) was used to show STAT5 and PDC-E2 interaction inside and outside the nucleus (D).

Discussion

Results from transmission electron microscopy and fluorescence microscopy clearly show that STAT5 is present within the mitochondria of 3T3-L1 adipocytes. Previous studies

indicate that STAT5 can translocate into the mitochondria of the LSTRA murine leukemic T cell line[6]. These results suggest that STAT5 also is present within the mitochondria of other cell types and may have a mitochondrial function, such as regulation of mitochondrial gene expression. We observed modest increases in mitochondrial staining and copy number. However, GH-induced STAT5 activation did not significantly regulate key nuclear-encoded markers for mitochondrial biogenesis. There may be factors other than mitochondrial biogenesis that affect mitochondrial copy number and staining, such as mitochondrial fusion or fission, which use dynamin proteins to regulate mitochondrial replication[12]. There is only a small subset of genes encoded by the mitochondrial genome[13]. If we had stronger, positive results with the ability of GH to regulate mitochondrial copy number, we would examine the GH-modulated gene expression of these mitochondrial encoded genes and the dependence on STAT5 using knockdown experiments. We observed a noticeable (although not statistically significant) down-regulation in PGC1α expression in response to GH treatment, and reduced PGC1α expression in adipose tissue is associated with mitochondrial dysfunction and the development of obesity and insulin resistance in mice models[14]. Subsequent experiments with mitochondrial copy number and biogenesis did not produce consistent and compelling results, so we determined that GH-induced STAT5 activity does not significantly contribute to the regulation of mitochondrial biogenesis and copy number.

We have shifted our focus to STAT5 and PDC interactions with the recent publication showing that nuclear PDC regulates histone acetylation through the acetyl-CoA production in other cell lines[9]. These new data made us revisit one of our previous projects. Results from a non-biased mass spectrometry-based screening experiment indicated that STAT5 interacts with PDC-E2. This interaction was validated in mouse (Figure 4) and human adipocytes and in mouse

adipose tissue using immunoprecipitation and western blotting techniques (data not shown). However, the subcellular location of the STAT5A-PDC-E2 association was unclear due to mitochondrial contamination in the nuclear fraction. Our studies using immunofluorescence microscopy demonstrate the colocalization of STAT5 and PDC-E2 inside the adipocyte nucleus. We plan to perform more experiments to elucidate the function and relevance of the nuclear interaction between STAT5 and PDC in adipocytes. We have also observed that STAT5A interacts with the other subunits of PDC (data not shown), so we will also test the function of the STAT5 interaction with these PDC subunits in the future.

To our knowledge, there is no previous research examining the role of mitochondrial STAT5 in adipocytes. Non-biased screening using mass spectrometry produced the novel observations that STAT5 interacts with PDC-E2. Our novel results show that STAT5 can translocate and localize in the mitochondria in adipocytes. Although the functions of mitochondrial STAT5 have not been elucidated in this study, we will continue to explore the possible roles of STAT5 in the adipocyte mitochondria in the future. Current studies in our lab plan to elucidate the role of the STAT5 and PDC interaction on gene regulation in adipocytes and its possibility to regulate adipogenesis.

Conclusion and Future Directions

Adipocytes and mitochondria play a major role in the glucose and lipid homeostasis and pathogenesis of disease. We identified a novel interaction between STAT5 and PDC, which led us to study STAT5 in adipocyte mitochondria. In this study, the potential roles of GH-induced STAT5A activation on the regulation of mitochondrial biogenesis and mitochondrial copy number in 3T3-L1 adipocytes have been examined. We demonstrated that STAT5A is present within the adipocyte mitochondria and there was a modest increase in mitochondrial staining and

copy number due to GH treatment. However, there is no regulation of mitochondrial biogenesis markers. While this study does not offer a conclusive answer to the potential function of mitochondrial STAT5, it does confirm the presence of STAT5 within adipocyte mitochondria and that GH treatment may modulate mitochondrial copy number. This novel finding is important because it enhances our understanding of the JAK-STAT signaling pathway by demonstrating that STAT proteins can translocate into the mitochondria in addition to the nucleus. When studying STAT5 in the future, we must consider how mitochondrial STAT5 may affect the experimental outcome and the physiological response of adipocytes.

The recent publication about nuclear PDC regulating histone acetylation has shifted our focus to the interaction between STAT5 and PDC. Previous studies in our lab using subcellular fractionation and immunoprecipitation suggested the potential interaction of STAT5 and PDC-E2. In this study, we demonstrate that PDC-E2 is present within the adipocyte nuclei and we confirmed its interaction with STAT5 in the nucleus. This result is further validated using immunofluorescence. We have also recently observed that PDC-E2 can bind to DNA containing STAT5 binding sites and this binding may be regulated through growth hormone (data not shown). This is important because it shows that nuclear PDC may interact with STAT5 to promote nuclear protein and/or histone acetylation, which may demonstrate a novel function of nuclear PDC in regulating STAT5-target gene expression.

Although we were not able to determine the function of the STAT5 and PDC interaction during the course of this honors thesis, we will perform more experiments to elucidate the function and relevance of nuclear PDC and STAT5 interaction in adipocytes. We plan to determine the domains of STAT5 that are necessary for PDC binding and the ability of PDC to modulate histone acetylation of STAT5 target genes following GH stimulation. We will also

look at the potential role of the STAT5/PDC interaction on adipocyte processes, such as adipogenesis and lipolysis, and we will examine chromatin binding of PDC to STAT5-binding sites. These future studies promise to bring new insight to the modulation of STAT5 signaling by PDC. Since STAT5 and PDC both play important roles in adipose tissue development and function, our studies will possibly generate novel insights into the pathogenesis and prevention of obesity-related metabolic dysfunction.

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