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Visualizing Hormonal Effects on Cardiomyocyte Hypertrophic Growth Dynamics Using Digital Holographic Microscopy: Does Size Matter?

Biography

Though born in San José, Jacquelyn considers herself a Santa Cruz native. She is tied for the oldest of four siblings with her twin sister. Jacquelyn transferred from Cabrillo College to San José State University, where she earned her bachelor's degree in Biology Systems Physiology. Jacquelyn plans to continue her education at SJSU by earning a M.S. in Biology. When not at school or studying, she can be found waiting tables, attempting to play the guitar, watching scary movies, or at the local karaoke bar.

Visualizing Hormonal Effects on Cardiomyocyte Hypertrophic Growth Dynamics Using Digital Holographic Microscopy: Does Size Matter?

Abstract

Heart disease continues to be the leading cause of death in the United States. Humans are unable to regenerate their heart tissue following an injury. However, neonatal mice are able to regenerate their heart tissue when cardiomyocytes (CMs) proliferate. This regenerative ability is lost approximately one week after birth when proliferating mononucleated CMs become binucleated and can no longer complete the cell-cycle. Recent studies have shown the combined inhibition of thyroid hormone (T3) and norepinephrine (NE) increases CM proliferation, promotes heart regeneration, and reduces cell size *in vivo*. Using digital holographic microscopy, the aim of this study was to (1) validate the novel Halomonitor approach and (2) visualize and quantify the effects of T3 and NE on cardiomyocyte size. CMs were isolated from neonatal rats 1 to 2 days after birth which were then treated with NE and/or T3 in serum-free culture. Live cell imaging was visualized utilizing digital holographic microscopy and changes in CM dynamics and morphology were quantitatively tracked and analyzed using HoloMonitor software. The results from this study validate the HoloMonitor technology by demonstrating that NE induced hypertrophy in CMs after 12 hours. Our results also demonstrate the ability of HaloMonitor technology to differentiate between CMs and non-CMs in living, serum-free culture, while also studying and quantifying their dynamics *in vitro*. Lastly, our data shows that T3 has little effect on CM growth and that NE decreased CM motility.

Introduction

Heart disease plagues the United States and continues to be the leading cause of death for Americans (CDC, 2022). Damage to heart tissue is especially detrimental to one's health due to its inability to regenerate; thus, patients are left with a weaker heart for the rest of their lives. However, this lack of cardiac regenerative ability is not universal to all animals.

Unlike adult mammals, which have a limited capacity to regenerate their hearts after injury, newborn rodents are capable of cardiac tissue regeneration. Such regeneration relies on the proliferation of heart muscle cells called cardiomyocytes (CMs) (Porrello et al, 2011). Mice lose the ability to regenerate their heart tissue within the first week after birth when CMs transition from proliferating mononucleated cells to binucleated cells which are incapable of completing the cell cycle (Soonpa et al, 1996). Following this transition of mononucleated to binucleated CMs, hypertrophy is the cause for increased heart mass.

It is known that inhibition of thyroid hormone (TH) and adrenergic receptor (AR) signaling in postnatal mice CMs causes them to not binucleate and not exit the cell cycle; therefore, the cell can regenerate. Conversely, although the cellular mechanism is unknown, it has been established that the synergistic activation of both TH and AR signaling will prevent cardiomyocyte proliferation *in vivo* (Payumo and Chen et al, 2021). It is known that norepinephrine (NE), a hormone that binds to adrenergic receptors, induces CM hypertrophy. However, little is known about the synergistic activation of TH and AR signaling on cardiomyocyte size. We suspect that T3 and NE signaling activation will promote hypertrophic growth in CMs.

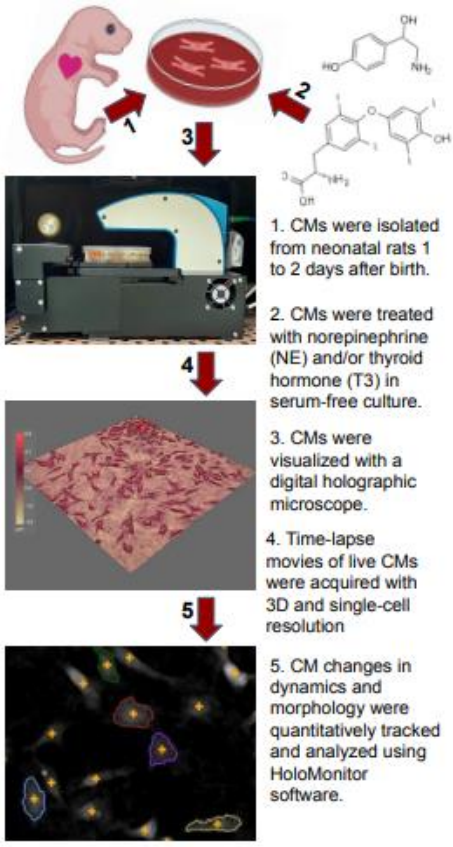
Using digital holographic microscopy, the goal of this research is to quantitatively visualize the hormonal effects of activated T3 and NE signaling in newborn mice CMs (*in vitro*) pertaining to hypertrophic growth dynamics. Specifically, we aim to determine if T3 and NE signaling interactions promote cardiomyocyte hypertrophy.

Because the cellular mechanism of cardiomyocyte proliferation is unknown, this research aims to help answer the question: “Does cardiomyocyte hypertrophy effect proliferation?” Answering this question

will better inform efforts moving forward to identify the mechanism of CM proliferation. This will have a monumental impact on the future of cardiac regenerative medicine.

Methods

Schematic of Experimental Approach:



First, cardiomyocytes were collected from newborn mice 1-2 days after birth. The CMs were then treated with the thyroid hormone triiodothyronine (T3) and norepinephrine (NE) *in vitro* in serum-free culture. The CMs in this study were divided into 4 test groups: (1) control group, receiving no hormone treatment, (2) the T3 treated group, (3) the NE treated group, and (4) the T3 + NE treated group.

After treatment, the CMs were visualized via digital holographic microscopy, HoloMonitor® live cell imaging microscope. Time-lapse movies of live CMs were acquired with 3D and single-cell resolution. CM changes in dynamics and morphology were visually and quantitatively tracked and analyzed using HoloMonitor® software. Significance was determined by Student's t-test and one-way ANOVA.

Results

Cardiomyocytes were able to be distinguished from non-cardiomyocytes (Figure 1) by analyzing optical volume (morphology) and the distance traveled (motility) of cells over the span of 4 hours. CMs were identified by being larger and slower than the non-CMs, which were smaller and had an increase in motility.

Figure 1: Label-free cardiomyocytes are distinguished from non-cardiomyocytes by size and motility.

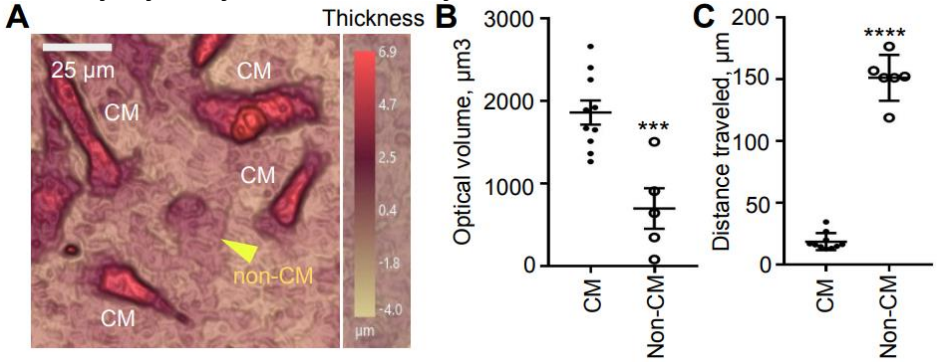


Figure 1. Cardiomyocytes (CM) were distinguished from non-CMs based on cellular morphology and motility. (A) Representative image showing a non-CM (yellow arrowhead) surrounded by CMs (white arrowhead). Non-CMs tend to be smaller. (B) Quantification of optical volume of CMs (n=10) and Non-CMs (n=5) at 4 hours. ***$p<0.001$ (C) Total distance traveled of CMs and Non-CMs at 4 hours after treatment. ****$p<0.0001$ determined by Student's t-test. Segmentation thresholds differ from those used in Figures 2 and 3.

T3 treatment does not have a significant effect on CM size as it is both visually and qualitatively similar to the control. The only significant increase in average optical volume is shown in the NE and NE+T3 treated CMs, with the NE treated having the largest.

Figure 2: Digital holographic microscopy allows real-time monitoring of hypertrophic growth dynamics.

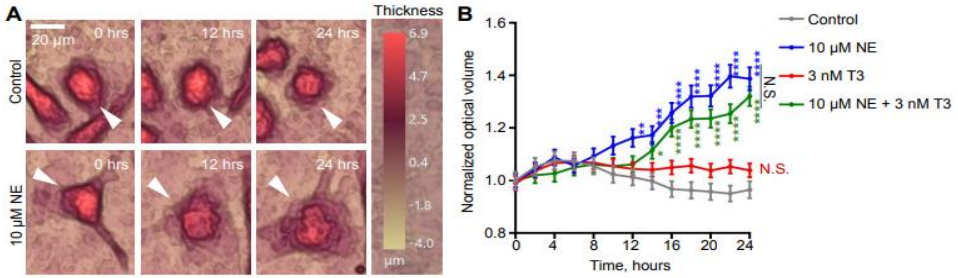


Figure 2. (A) Frames from a time-lapse movie tracking changes in cardiomyocyte (CM) optical volumes over a 24-hour period. Examples of a control CM (top), and a CM treated with norepinephrine (NE) (bottom) are shown. (B) Normalized average optical volumes of CMs in control conditions (gray, n=100), treated with NE (blue. n=100), treated with thyroid hormone (T3) (red, n=100), and treated with NE + T3 (green, n=100). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 determined by one-way ANOVA. All cells normalized to average starting volumes at 0 hours for each respective treatment. Segmentation thresholds were identical to those used in Figure 3.

46-hour end point analysis showed that T3 did not have a significant impact on CM optical volume compared to the control. NE treated CM were visually larger in size than the control, showing that NE does induce CM hypertrophy after 46 hours (expected). The T3 + NE treated CM is visually larger than the control after 46 hours, but on average are not significantly different from the NE treated cells.

Figure 3: Norepinephrine promotes hypertrophic growth while thyroid hormones have little effect after 46 hours.

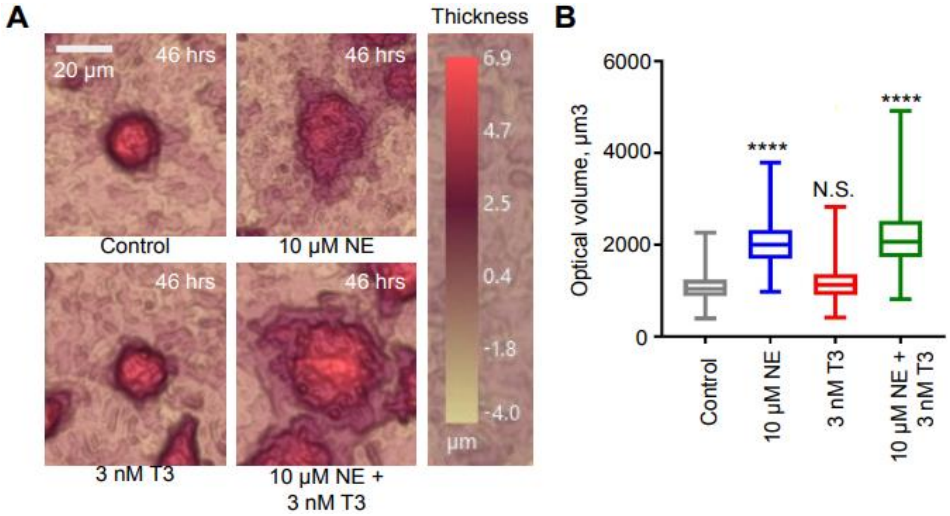


Figure 3. (A) Representative images of cardiomyocytes (CMs) treated with norepinephrine (NE) and/or thyroid hormone (TH) for 46 hours. Control conditions (upper left), after NE treatment (upper right), after T3 treatment (bottom left), and N + T3 treatment (bottom right). (B) Raw optical volumes of CMs after 46 hours in control condition (gray, n=100), after NE treatment (red. n=100), after T3 treatment (blue, n=100), and NE + T3 treatment (green, n=100). ****$p<0.0001$ determined by one-way ANOVA. Segmentation thresholds were identical to those used in Figure 2.

The motility of CMs were tracked over 24 hours. The control and T3 treated CMs did not experience a significant change in motility. However, the motility (average distance traveled) of NE and NE + T3 treated CMs significantly decreased over 24 hours.

Figure 4: An unexpected role for norepinephrine in cardiomyocyte motility regulation.

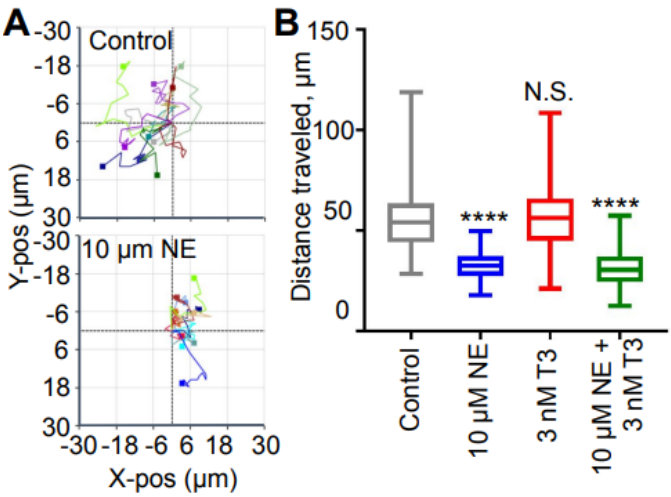


Figure 4. Norepinephrine (NE) decreases in CM motility. (A) Spatial tracking plots of CMs in control conditions (top, n=10) and CMs after treatment of NE (bottom, n=10) over 24 hours. (B) Average distance traveled after 24 hours of CMs in control condition (gray, n=50), NE treatment (blue, n=50), T3 treatment (red, n=50), and NE + T3 treatment (green, n=50).

Discussion

Data obtained from Figure 1 allowed us to distinguish between CM and non-CMs in unfixed living tissue samples in real-time. This was crucial to the study as there appeared to be different types of cells found in cardiac tissue other than CMs.

Data from Figure 2 showed that T3 treatment does not have a significant effect on CM size and that the only significant increase in average optical volume was with the NE-treated CMs. Because we know that NE induces hypertrophy, this data validates the use of the novel HoloMonitor® live cell imaging microscope. The benefits of this technology include 3D visualization and quanti-fication of living cells. Tissue integrity is upheld due to the ability to visualize the cells without

labels or stains/dyes. Additionally, the same sample can be used for different assays and quantifications, thus saving laboratory resources (PHI, n.d.).

The 46-hour end point analysis (Figure 3) allowed us to visually and qualitatively show that the control and T3-treated CMs appear the same in size, indicating that T3 did not have a significant impact on CM optical volume after 46 hours. As expected, visually, NE-treated CMs were larger in size than the control, showing that NE does induce CM hypertrophy. The T3 + NE-treated CM is confirmed to also be visually larger than the control, but not significantly different from the NE-treated cells, indicating that T3 does not have a significant impact on CM hypertrophy.

Motility analysis showed that NE-treated CMs experience a decrease in average distance traveled over 24 hours. This was an unexpected finding; thus, the effect of NE-induced motility inhibition in CMs should be investigated in future studies as both proliferation and migration are required for heart regeneration.

Though this study answered some questions about CM dynamics, more research needs to be done to truly answer the question: Does size matter? It is our hope that future studies will build upon the data obtained from this study to further investigate if/how hormone-induced hypertrophy affects cell cycle activity.

Conclusion

Holographic microscopy is a novel approach to study living, label-free cardiomyocyte (CM) dynamics *in vitro* in real-time and is also able to distinguish CMs from non-CMs. Validation of this technology was achieved by showing that NE induces hypertrophy in CMs over time. We also showed that NE suppressed CM motility, which was unexpected. Lastly, our results also show that T3 treatment had little effect on CM hypertrophic growth dynamics.

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