ER Stress and ATF6alpha potently induce S-Phase in Old Mouse Beta Cells Cultured Ex-Vivo in High Glucose

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ER STRESS AND ATF6α POTENTLY INDUCE S-PHASE IN OLD MOUSE β-CELLS CULTURED EX VIVO IN HIGH GLUCOSE

A Master’s Thesis Presented

By

Jarin Tyler Snyder

Submitted to the Faculty of the

University of Massachusetts Graduate School of Biomedical Sciences, Worcester

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in Biomedical Sciences

December 11th, 2020
Dedication

Thank you to my wife, Arielle, for taking amazing care of me, Javiahn, and Eddy while I labored to produce this thesis.

Thank you to my mom, who instilled in me a love for learning from a young age.

Thank you to GG, Auntie Didi, and Suzie, who provide me an endless source of inspiration in my fight against aging-associated disease.

Thank you to Laura and Rohit for your amazing mentorship. Never before have I felt so close to making an impact on aging-associated disease as I have under your wings!

Thank you to Brian Gablaski, Christine Darko, Rachel Stamateris, Daniel Harrington, Dave Harlan and everyone else at the Diabetes Center of Excellence for being there to help.

Thank you to my thesis committee for donating their time during a global pandemic to give this thesis a thorough review.

Thank you to Dean Lane, Assistant Dean Thompson, and Brian Lewis, whose advice and encouragement ultimately pushed me to complete this thesis.

Finally, thank you to the State of Massachusetts, for ensuring that a foster kid like me had the opportunity to go to college and find his passion in life. We must continue to fight until no promising young mind is wasted to poverty.

In memory of my cousin,

Isaiah Snyder

June 4th, 1986-December 6th, 2020

Thank you!
Acknowledgements

Laura Alonso and Rohit Sharma provided mentoring during the conception, execution, and recording of this thesis. Rohit Sharma instructed how to perform islet isolation and assisted with bile duct cannulation. Laura Alonso edited and approved this manuscript. Aged mice were donated by the Keaney and Swain labs. This work was supported by NIH/NIDDK: R01DK114686, R01 DK113300, NIH/NIGMS: R25GM113686, the American Diabetes Association grant #1-18-IBS-233 in collaboration with the Order of the Amaranth, and the George F. and Sybil H. Fuller Foundation.
Abstract

Aging is associated with a loss of proliferation of the insulin-secreting beta cell, a possible contributing factor to the greatly increased rate of type-2 diabetes in the elderly. A landmark study from our lab previously illustrated that mild endoplasmic reticulum (ER) stress drives beta cell proliferation specifically through ATF6α, one arm of the tripartite Unfolded Protein Response (UPR). It is unknown if old beta cells differ from young beta cells in UPR signaling or proliferative response to ER stress or ATF6α activation. To investigate, young and old mouse islets were cultured \textit{ex vivo} in high glucose, and beta cell proliferation was quantified by BrdU incorporation after treatment with low dose thapsigargin or activation of overexpressed ATF6α. In addition, levels of UPR signaling were compared by semi-quantitative \textit{Xbp1} splicing assay. Interestingly, although old beta cells displayed reduced proliferation in glucose compared to young beta cells, their proliferative response to low-dose thapsigargin and ATF6α activation were nearly identical, and no difference was found in \textit{Xbp1} splicing under high glucose or high ER stress conditions. These results suggest that the aged mouse beta cell does not have impaired UPR-responsive proliferation or aberrant UPR signaling when cultured \textit{ex vivo}. 
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Chapter 1. Introduction

Type-2 diabetes is an age-associated chronic disease, with a peak incidence of 25% in the US population aged ≥65 (Fishman et al., 2014). Lifestyle interventions targeting people with prediabetes delay onset and reduce incidence of disease (Diabetes Prevention Program Research Group, 2009), but most people pass through the prediabetes phase without being diagnosed, and multiple barriers limit implementation of successful lifestyle changes. Treatments targeting aging as a risk factor for diabetes are lacking. As an epidemic of obesity coincides with population aging in the coming decades, the incidence of diabetes is expected to increase around the world (Charvat et al., 2015). Complications of diabetes including cardiovascular disease, renal disease, blindness and neuropathy are more frequent and severe in the elderly (Huang et al., 2014), underscoring the need for better prevention and treatment of diabetes in this growing population.

Aging is associated with an increase in insulin resistance that may or may not be compensated by increased insulin production (Liu et al., 2014). Progression to diabetes is thus regulated by the ability of the pancreatic beta cell to increase insulin production in order to adapt to increased insulin demand. In non-diabetic obese individuals, insulin resistance is increased, but beta cell mass and insulin secretion are also increased to sufficiently maintain glucose control (Linneman et al., 2014). This suggests that healthy beta cells can proliferate to adapt to increased insulin demand. In late-stage diabetes, beta cell mass is lost (Chen et al., 2017), highlighting beta cell regeneration as a therapeutic goal for both type-1 and type-2 diabetes patients. However, many studies have concluded that in aged humans and mice adaptive beta cell proliferation is severely reduced or entirely
lost (Perl et al., 2010; Kushner, 2013). Indeed, while immature mice exposed to high-fat diet can successfully adapt by increasing beta cell mass through beta cell proliferation, adult mice have limited capacity to increase beta cell mass or proliferation and develop diabetes (Tschen et al., 2009). Not only does a loss of beta cell adaptive proliferation predispose the elderly to diabetes, it also poses a hurdle to overcome in devising novel therapies to prevent and treat diabetes through beta cell regeneration. However, the exact mechanisms that cause beta cells to cease proliferating with age remain controversial.

The age-associated loss of beta cell proliferation is hypothesized to be due to cell-cycle arrest in beta cells that are either quiescent, terminally differentiated, or senescent in the aged islet (Kushner et al., 2013). Although these cell states all feature cell cycle arrest, they are physiologically distinct. Quiescence is a normal physiological state in which beta cells are non-proliferative but are poised to proliferate when induced by pro-mitogenic signals. Evidence suggests beta cell quiescence is regulated by a glucose-sensing mechanism, and the length of beta cell quiescence increases with age (Salpeter et al., 2010). Terminal differentiation is a normal developmental program that results in an irreversible block on cell division. It has been suggested that a loss of proliferation via age-associated CDK inhibitor p16\textsuperscript{INK4a} upregulation is a developmental program that boosts insulin secretion (Helman, et al., 2016). Similarly, senescence is a state of irreversible cell-cycle arrest featuring upregulated p16 that is usually a pathological stress response to potentially oncogenic stimuli such as DNA damage (Campisi & Fagagna, 2007). Senescent cells negatively impact insulin secretion both by cell-intrinsic restriction of regenerative capacity as well as through paracrine effects known as the “Senescence Associated
Secretory Phenotype” (SASP) (Coppe et al., 2008; Aguayo-Mazzucato et al., 2019). Senescent cells accumulate in the islet with age and in diabetes, and high fat diet or free fatty-acids induce senescence in beta cells via p16 (Sone & Kagawa, 2005; Krishnamurthy et al., 2006; Pascoe et al., 2012; Aguayo-Mazzucato et al., 2019). Interestingly, loss of p16 is not sufficient to restore the proliferative response to glucose in mouse islets (Moreno-Asso et al. 2013), suggesting that unknown factors are responsible for the age-associated loss of glucose-responsive beta cell proliferation.

Some evidence suggests that aged beta cells are mostly unresponsive to signals that promote proliferation in young beta cells, indicative of a permanent cell-cycle arrest typical of senescence or terminal differentiation (Tschen et al., 2009) while other studies suggest that proliferation can still be induced in old beta cells through specific stimuli, such as transplantation into the youthful microenvironment (Chen et al., 2009; Almaca et al., 2014) or partial beta cell ablation (Stolovich-Rain et al., 2012). In order to increase the relevance to the real-world population and improve diabetes treatment, future studies on mechanisms that promote proliferation in young beta cells should also be investigated in old beta cells to test the age-dependence of their effect.

The unfolded protein response (UPR) acts to maintain protein folding capacity and is critical to beta cell function due to the increased protein folding in the endoplasmic reticulum that is necessary to produce and secrete enough insulin to maintain glucose homeostasis (Wang & Kaufman, 2016). A previous study from our lab showed that activation of the ER-stress sensing transcription factor Atf6α is necessary and sufficient for hyperglycemia-induced proliferation in beta cells from humans or mice (Sharma et al.,
However, these experiments were done using young mice and no comparisons
between old and young human or mouse islets were made.

Using similar methods, this study interrogated whether old mouse beta cells
respond similarly to young cells by upregulating proliferation in response to mild ER stress
or ATF6 activation. I also began to probe for differences in ER stress responses between
young and old mouse beta cells. This is an important question because impaired ER stress
sensing or aberrant UPR expression with age might explain the reduction of
hyperglycemia-induced proliferation in old beta cells. Indeed, a loss of proteostasis is
commonly considered a hallmark of aging (Lopez-Otin et al., 2013). Conversely, if
activation of ATF6α induces proliferation of the old beta cell, this pathway might represent
a novel target to promote regeneration of aged beta cells. More broadly, these experiments
will illuminate the relationship between ER stress and cell-cycle status in old beta cells,
opening the door to future studies aimed toward regeneration of beta cells in order to
prevent and treat age-associated diabetes.
Chapter 2. Methods

Mice. All mouse procedures were approved by the UMass Medical School Institutional Animal Care and Use Committee. “Young” (10-14 weeks) and “old” (10 months-25 months) C57BL/6J mice from either sex were collected from a variety of backgrounds, including WT mice bred in our colony, “WT-like” Adf6-flox or Grp78-flox C57BL/6J mice lacking Cre expression, aged breeders, as well as spare C57BL/6J mice donated from the colonies of other labs raised under similar conditions at UMass Medical School. Since the islet mass of old mice is larger compared to that of young mice, for each replicate islets from 2 young mice were combined to provide sufficient material to match with each old mouse.

Mouse islet isolation, dispersion, and culture. Islets were isolated using collagenase P and density gradient centrifugation, handpicked, cultured, and dispersed in 0.05% trypsin as previously described (Sharma et al., 2019). Dispersed cells were plated in 500 uL islet complete media (ICM: RPMI containing 10% FBS (Atlanta Biologics), penicillin/streptomycin, and 5 mM glucose) on uncoated glass coverslips for immunostaining (50 IE) or directly onto Nunc™-treated polystyrene 24-well plates (ThermoFisher) for RNA/Protein extraction (100 IE). 16-24 hrs later the media was replaced with ICM containing 15mM glucose simultaneously with the addition of experimental treatments. Thapsigargin (Sigma-Aldrich) was delivered at 20 nM for mild UPR (Figure 1) or 1 μM for decompensated UPR (Figure 3). Ad-ATF6:DHFR (Shoulders et al., 2013) was added at MOI=10, and cells were treated with 10 μM TMP or DMSO control. All samples exposed to experimental treatments were matched with DMSO-treated
control samples from the same pool of dispersed cells. Dispersed islet cells were then cultured for 72 hrs. To allow quantification of S-phase entry, coverslip-containing wells were treated with 10μg/mL BrdU (Sigma-Aldrich) for the last 24 hrs.

**Immunostaining, microscopy, and cell counting.** Immuno-staining for mouse insulin and BrdU incorporation were executed as previously described (Sharma *et al.*, 2019) using primary antibodies from Abcam (BrdU: #ab6326, insulin: #ab7842), DyLight secondary antibodies from Jackson ImmunoResearch Laboratories, Inc, and DAPI (Sigma-Aldrich). Fluorescent microscopy was performed on a Nikon fluorescent microscope. Images were blinded and the number of Ins+ and BrdU+Ins+ cells were manually counted. At least 500 cells were counted for each sample. Data are reported as the percent of Ins+ cells that were BrdU+Ins+.

**Cell Lysis, RNA extraction, cDNA synthesis** Cells were washed 3x in cold PBS before lysis. Cells were lysed and RNA extracted using the RNA/Protein Purification Plus Kit (NorgenBiotek) per the manufacturer’s instructions. 300-600ng cDNA was synthesized using the SuperScript IV VILO Master Mix (ThermoFisher) per the manufacturer’s instructions.

**Semi-quantitative gel-based PCR assay for Xbp1 splicing** The assay was performed as previously described (Sharma *et al.*, 2015). Gel electrophoresis was performed on 2% agarose gel. Bands were quantified using Image J.
**Statistical Analysis** Data analysis was performed using GraphPad Prism 8. For all experiments, the interaction between age and the response to treatment was investigated using the Repeated Measures Two-Way ANOVA followed by Sidak’s multiple comparisons testing. The α-level was set to .05. Data are presented as individual data points or mean with standard error; the number of replicates is included in each figure.
Chapter 3. Results

Although it is known that aged beta cells have impaired glucose-responsive proliferation (Moreno-Asso et al., 2013), it is unknown if aged beta cells can be induced to proliferate by the UPR as previously illustrated for young beta cells (Sharma et al., 2015). To compare the effect of increasing ER stress on beta cell-proliferation, young and old mouse beta cells were cultured in high glucose media and treated with low-dose thapsigargin (20 nM) or equivalent DMSO as control. Thapsigargin functions to trigger the UPR by blocking activity of the sarco/endoplasmic reticulum Ca2+ ATPase (SERCA), preventing calcium uptake into the ER necessary for proper protein folding (Oslowski & Urano, 2011) Beta cell proliferation was measured by BrdU incorporation, which is a reliable marker for beta cell S-phase entry (Sharma et al., 2019). As expected, old beta cells had significantly reduced BrdU incorporation compared to young beta cells when cultured in 15mM glucose media (Figure 1, p=0.0003). However, low-dose thapsigargin significantly increased BrdU incorporation in both young and old beta cells (Figure 1, p=0.0092 and p=0.0048 respectively). Although old beta cells stimulated with thapsigargin had significantly reduced proliferation compared to young beta cells under identical conditions(Figure 1, p=0.0003), the magnitude of the pro-proliferative effect of thapsigargin appeared nearly identical between young and old subjects. As such, there was no interaction detected between age and response to treatment (Figure 1). In addition, unaccounted for subject-specific effects were not significant. Thus, although aged beta cells have an impaired proliferative response to glucose, they do not appear to have impaired UPR-induced proliferation, at least in this ex vivo model system.
Atf6α was previously identified as the UPR component both necessary and sufficient for ER stress-induced proliferation (Sharma et al., 2015). To determine if Atf6α activation could rescue the impaired glucose-responsive proliferation of the aged beta cell, stress-independent activation of Atf6α was achieved using the ATF6α-dihydrofolate reductase (DHFR) fusion system previously developed by Shoulders et al. (Shoulders et al., 2013). Binding of the DHFR inhibitor trimethoprim (TMP) to the DHFR-domain dose-dependently stabilizes its’ folding structure, so that in the absence of TMP the protein remains unfolded and is rapidly degraded (Shoulders et al., 2013). Young and old mouse beta cells were infected with Ad-ATF6α-DHFR and cells were exposed to 10μM TMP or DMSO for 72 hrs. As in the previous thapsigargin assay, Repeated Measures Two-Way ANOVA showed that the main effects of age and TMP treatment were significant, but not their interaction or subject-specific effects (Figure 2). In this experiment, the difference in proliferation between young and old beta cells did not reach the predetermined P threshold of <0.05 to be considered statistically significant. This likely reflects a failure to reject a false null hypothesis due to the consistent downward trend observed in the proliferation of old cells over several experiments (Figure 1 & 2) and the well-supported nature of age-associated beta cell proliferative decline in the literature. Nevertheless, the readiness of old beta cells to proliferate is promising for the future of beta cell regeneration. Promisingly, stabilization of ATF6α through treatment of Ad-ATF6α:DHFR-transduced young and old mouse beta cells with TMP caused a dramatic and significant increase in BrdU incorporation (Figure 2, p=0.0002). Like the previously noted response to thapsigargin, the magnitude of the change in proliferation of young and old beta cells exposed to TMP were
remarkably similar. Young and old beta cells displayed on average 7.8% and 7.5% greater BrdU incorporation respectively when exposed to TMP, reflecting a roughly 3-fold increase in beta cell proliferation compared to DMSO-treated controls grown in high glucose. This data confirmed that Atf6α-mediated beta cell proliferation is not inhibited by aging, at least in this ex vivo tissue mouse model system.

It is unknown whether the stress response of mouse islet cells exposed to high dose thapsigargin is affected by aging. Since a loss of proteostasis is a hallmark of aging (Lopez-Otin et al., 2013) I hypothesized that aging islets may have dysfunctional UPR signaling which could contribute to impaired glucose-responsive proliferation. To assess the UPR state of young and old beta cells exposed to mild/adaptive or severe/decompensating ER stress, Xbp1 splicing was measured as a readout for IRE1 activity. In addition to ATF6 and PERK, IRE1 is the third component of the tripartite UPR that consists of transluminal proteins that communicate protein folding stress in the endoplasmic reticulum to other organelles (Reviewed in Sharma, Snyder, & Alonso, 2019). Through its cytoplasmic domain, IRE1 splices Xbp1 into sXbp1, which encodes a transcription factor that can induce unique subsets of UPR genes both independently and through heterodimerization with ATF6α (Shoulders et al., 2013). 15mM glucose mildly induced Xbp1 splicing whereas high dose thapsigargin (1μM Tg) greatly increased splicing (Figure 3) in young and old beta cells. However, there was no obvious impact of aging on Xbp1 splicing under high glucose or high-thapsigargin conditions (Figure 3). Total Xbp1 mRNA was only significantly upregulated by thapsigargin in old beta cells, but a relatively large variance in the old beta cells combined with small sample size confound any extrapolation from this result.
Although a lack of large differences in *Xbp1* splicing or expression may suggest that the burden of ER stress or the level of UPR signaling is unaffected by age in the mouse islet, further exploration of the UPR and ER stress loads in young and old beta cells is required. A series of technical errors, resource and time constraints prevented a more satisfactory investigation of RNA/protein changes in the beta cell with age, ER stress, and/or ATF6α activation. Future experiments will investigate if there are age-associated changes in expression of direct ATF6-targets such as *Grp78* and *calreticulin* (Shoulders *et al.*, 2013).
<table>
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(D)

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<th>95.00% CI of diff.</th>
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<th>Adjusted p-Value</th>
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<tr>
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<td>-0.03303 to -0.004929</td>
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<td>-0.03267 to -0.006381</td>
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<td>0.0048</td>
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Figure 3.1: The effect of age and low-dose thapsigargin on the proliferation of mouse beta cells cultured in high glucose (15mM) media. (A) Representative immunofluorescent images of dispersed islets from young or old mice cultured in high glucose on glass coverslips, treated with 20nM thapsigargin or DMSO for 72 hrs. Cells were incubated with BrdU for the last 24 hrs then fixed in PFA before immunofluorescence labeling. (B) Graph shows mean, SEM, and individual values for each group. Y=Young (10-14 weeks, N=7) O=Old (10 months+, N=8) Tg= 20 nM thapsigargin. (C) Repeated Measures Two-Way ANOVA investigated the interaction between aging and the proliferative response to low-dose thapsigargin in high glucose. The main effects of age and treatment, but not their interaction or subject-specific effects, were highly significant. (D) Post-hoc Sidak’s multiple comparisons test suggested that although low-dose thapsigargin significantly induced proliferation in both young and old mouse beta cells, proliferation was significantly reduced in older beta cells when compared to young beta cells across treatments.
Ad-ATF6:DHFR

(A) 

DMSO

TMP

Young

Old

Insulin

DAPI

BrdU

(B)

(C)

\[ \text{BrdU}^{+}\text{Ins}^{+}/\text{Ins}^{+} \]

\[ \text{BrdU}^{+}\text{Ins}^{+}/\text{Ins}^{+} \]

**p=0.0625**

Young

Old

Ad-ATF6:DHFR
<table>
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<th>% of total variation</th>
<th>p-value</th>
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(D) Sidak’s multiple comparisons test

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<tr>
<td>DMSO</td>
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<td>TMP</td>
<td>0.03476</td>
<td>0.001592 to 0.06794</td>
<td>*</td>
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<tr>
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<tr>
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<td>-0.1117 to -0.03887</td>
<td>***</td>
<td>0.0004</td>
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Figure 3.2: The effect of stabilization of ATF6α:DHFR with TMP on the proliferation of young or old mouse beta cells transduced with the Ad-ATF6:DHFR construct. (A) Representative immunofluorescent images of dispersed islets isolated from young (10-14 weeks) or old (10+ months) mice, cultured in high glucose on glass coverslips, transduced with Ad-ATF6:DHFR at MOI=10 and treated with DMSO or TMP for 72 hrs to stabilize the ATF6:DHFR protein and activate the ATF6α transcription factor. Cells were incubated with BrdU for the last 24 hrs then fixed in PFA before immunostaining for insulin and BrdU. (B) Graph shows mean, SEM, and individual values for each group. N=7 for all groups. Y=Young O=Old TMP=Trimethoprim. (C) Repeated Measures Two-Way ANOVA investigated the interaction between aging and the proliferative response to the stabilization of ATF6:DHFR in high glucose. The main effects of age and treatment, but not their interaction or subject-specific effects, were highly significant. (D) Post-hoc Sidak’s multiple comparisons test suggested that although activation of ATF6α significantly induced proliferation in both young and old mouse beta cells, proliferation was reduced when comparing old beta cells treated with TMP to young beta cells under the same conditions. In this experiment, the frequency of proliferation between young and old beta cells in the absence of TMP was not significantly different.
**Source of Variation** | % of total variation | p-value | Significant?
--- | --- | --- | ---
Age x Treatment | 0.008234 | 0.9378 | ns
Age | 0.004034 | 0.9542 | ns
Treatment | 85.77 | 0.0002 | ***
Subject | 6.757 | 0.5465 | ns

**Sidak's multiple comparisons test**

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<th>Significant?</th>
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<tr>
<td>Young</td>
<td>-4.410</td>
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<td>Old</td>
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<td>**</td>
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</table>
Source of Variation | % of total variation | p-value | Significant?
--- | --- | --- | ---
Age x Treatment | 0.008234 | 0.9378 | ns
Age | 0.004034 | 0.9542 | ns
Treatment | 85.77 | 0.0002 | ***
Subject | 6.757 | 0.5465 | ns

Sidak’s multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Adjusted p-value
--- | --- | --- | --- | ---
Young - Old | DMSO | 0.1171 | -0.2090 to 0.4432 | ns | 0.6123
 | Tg | -0.04602 | -0.3721 to 0.2801 | ns | 0.9243
DMSO – Tg1uM | Young | -0.2003 | -0.5280 to 0.1274 | ns | 0.2265
 | Old | -0.3634 | -0.6911 to -0.03572 | * | 0.0333

Figure 3.3: Semi-quantitative gel-based assay for Xbp1 splicing to assess UPR induction in response to high-dose thapsigargin in young and old mouse beta cells in high glucose. Tg = 1uM thapsigargin Y = Young (10-14 weeks) O = Old (10=months) (A) One of two imaged gels, showing bands for spliced and unspliced Xbp1 compared to Actin for the first two experiments. (B) Graph shows mean, SEM, and individual spliced Xbp1/Total Xbp1 values, as quantified using ImageJ, for each group. N=4 for all groups. (C) Similar, except Total Xbp1/Actin is shown on the y-axis. (B) & (C) Repeated Measures Two-Way ANOVA investigated the interaction between aging and UPR induction in response to high-dose thapsigargin. The effect of high-dose thapsigargin was highly significant, but the effect of age, the interaction of age and response to treatment, or subject-specific effects were not significant. Post-hoc Sidak’s multiple comparisons test confirmed no significant difference between young and old beta- cells in sXbp1/unspliced Xbp1 or Total Xbp1/Actin in high glucose conditions with or without high-dose thapsigargin. These data suggest that 1uM thapsigargin significantly increased Xbp1 splicing in young and old beta cells.
Chapter 4. Discussion

Collectively, the previously described experiments suggest that, at least ex vivo, old mouse beta cells are induced into S-phase by mild ER stress or activation of ATF6α similarly to young beta cells. This is promising evidence that old beta cells are capable of regeneration. However, several caveats are necessary. First, I did not test if TMP itself can induce proliferation in the absence of ATF6:DHFR. Future experiments should properly control for this. Secondly, more experiments are necessary to clarify the role of ATF6α in beta cell proliferation in vivo. Accumulating evidence suggests that the effects of beta cell aging are not cell-intrinsic, but rather are the result of interaction with the aged extracellular environment. For example, transplantation of old islets to young hosts (Chen et al., 2009; Almaca et al., 2014) is sufficient to restore the proliferation of aged mouse beta cells. Future experiments should explore how the aged microenvironment impacts the UPR and UPR-induced proliferation in vivo.

Interestingly, the magnitude of the roughly 3-fold increase in beta cell proliferation in young and old beta cells when ATF6 was activated (Figure 2) is similar to that observed by Yuval Dor’s group through their studies of aged islets in vivo. They showed a 3-fold induction of beta cell proliferation during islet regeneration after beta cell ablation via specific doxycycline-induced diphtheria toxin expression (Stolovich-Rain et al., 2012). The remarkable similarity of this effect could suggest our ex vivo model faithfully models the dynamics of beta cell regeneration in vivo.

As previously noted, mice for this study were resourcefully collected from a variety of sources, including spare mice from our colony and other labs at UMass Medical
School. Although this might have increased experimental variability due to slight differences in life histories and genetic background between subjects, potentially increasing the risk of accepting a false null hypothesis, it proved to be a cost-effective approach to generate interesting preliminary data describing a generalizable biological phenomenon while gaining skills in islet isolation and handling. The precision of future studies can be improved by ensuring that living conditions are controlled throughout the lifespan of the mice studied. This could be through breeding and aging mice under controlled conditions “in-house” or by purchasing aged mice from commercial breeders, such as the aged C57BL/6J mice available from The Jackson Laboratory.

The age-dependence of the proliferative response to ER stress and ATF6 should be investigated in other model systems. Although in their landmark paper Sharma et al. did include islets from human donors spanning a wide range of ages, no direct comparison of proliferation between young and old donors was made. However, it is difficult to control for extraneous variables when utilizing human cadaver tissues. The rat beta cell also appears to undergo severe loss of proliferation with age (Gu et al., 2012) and is therefore a promising alternative model system to investigate the age-dependence of UPR-induced proliferation.

Although UPR activity according to Xbp1 splicing did not appear to differ between young and old beta cells, more precise techniques such as qPCR may be necessary to detect differences. Interestingly, a recent study utilizing single-cell RNA sequencing discovered that aged non-diabetic cynomolgus monkey beta cells display a pattern of aberrantly increased UPR expression, which was suggested to inhibit glucose-induced
insulin secretion (Li et al., 2020). It will be important to determine if proliferation is affected by this chronic UPR observed in the monkey islet.

Furthermore, although I observed that activation of ATF6α increased mouse beta cell proliferation as measured by BrdU incorporation at 72 hrs, it is unclear how long UPR-induced proliferation lasts, whether any compensatory cell cycle-arrest is induced, or how the functional state of these newly created daughter cells may differ from mother cells. Accumulating evidence suggests that aged postmitotic beta cells actually have improved function compared to younger, immature beta cells (Avrahami et al., 2015; Helman et al., 2016; Puri et al., 2018). Future studies will be necessary to determine if modulation of ATF6α is a therapeutic goal in treating diabetes.
References


