PVLSI PIONEER VALLEY LIFE SCIENCES INSTITUTE
A Baystate Medical Center ▪ UMass Amherst Research Partnership

Final Scientific/Technical Report to DOE

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Awarded To: Pioneer Valley Life Sciences Institute

Project Title: Pioneer Valley Life Sciences Institute Program in Endocrinology and Metabolism

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Specific Aim I- To determine the role of TGFβ superfamily ligands in regulating β-cell function, proliferation, and survival (Drs. Schneyer and Brown)

We first determined which TGFβ superfamily ligands were produced in pancreatic islets fo C57BL6 mice and tissue surrounding islets (islet-depleted pancreatic tissue). We discovered that myostatin was among the most highly expressed TGFβ family ligands. We next compared this expression to islet depleted tissue (IDF), that is, the pancreatic tissue remaining after islets are removed. Most ligands were expressed at equal levels in the two fractions whereas myostatin, and its antagonist follistatin like-3 (FSTL3) were both expressed at much higher levels in islets compared to non-islet tissue. This is the first description of myostatin expression in islets suggesting that myostatin may play important roles in islet function that is regulated by FSTL3. We also localized activin A and B proteins to α-cells in islets. We compared the mouse expression pattern to rat islets and found that the overall ligand expression pattern was different and no myostatin was detectable in rat islets. This suggests differences in the prevalence of TGFβ family of ligands among species which may affect regulation of islet function in these species. Nevertheless, in all species examined so far, activins A and B, and the antagonist FSTL3 are all expressed in islets.

We also explored the function of these growth factors in islet biology. Islets were treated with ligands in low glucose followed by high glucose. We found that in mice, no effect of ligand addition was detectable but in rats, activin, myostatin and TGFβ all increased glucose-stimulated insulin release. This suggests that either mouse islets are functionally distinct from rat islets with respect to TGFβ family actions, or that production of endogenous ligands within islets in culture of mouse islets is obfuscating the effect of exogenous ligands. We found that treating mouse and human islets with activin A or B increased proliferation of α-cells. This effect of activins was also observed using an α-cell line. These findings suggest that one role of activin is to regulate α-cell proliferation.

It was recently reported that upon complete depletion of β-cells, α-cells can transdifferentiate to β-cells. If this transdifferentiation process also occurs under more physiological conditions, our results with activin suggest that FSTL3 from β-cells regulates activin (and possibly myostatin) bioactivity in α-cells which ultimately regulates α-cell proliferation and transdifferentiation into β-cells. This would explain the phenotype of FSTL3 KO mouse that we reported. In these mice we observed β-cell hyperplasia and larger islets consistent with loss of repression of activins and/or myostatin. More importantly, this research points to antagonists of FSTL3 as potential new therapies for diabetes in which β-cells are lost, since they may increase new β-cell formation.

The following publications from 2011 contain the details of the data summarized above:

Brown M, Bonomi L, Kimura F, Ungerleider N, Schneyer A. Follistatin And Follistatin Like-3 Differentially Regulate Adiposity And Glucose Homeostasis. Obesity (Silver Spring) 2011 Oct;19(10):1940-9.

Brown M, Kimura F, Bonomi L, Ungerleider N, Schneyer A. Differential synthesis and action of TGFb superfamily ligands in mouse and rat islets. Islets. 2011.Nov/Dec;3(6):1-9.

Specific Aim I- Aim 2- Myoblast physiology (Drs. Schwartz and Yadava)

We tested the feasibility of using respiratory activity and extracellular acidification rates of myoblasts to decipher the state of insulin resistance in cultured myoblasts. This was carried out using a 24-well Extracellular Flux (XF) Analyzer developed by Seahorse Bioscience (Billerica, MA). Rates of respiration (i.e. oxygen consumption) and glycolysis (i.e. acidification due to lactate release) were measured simultaneously using myoblasts 10-60,000/well in replicates. Briefly, the respiratory capacity was monitored in the presence of uncoupler FCCP, and glycolysis capacity was monitored in the presence of oligomycin in the presence and absence of FCCP. Next, conditions were optimized to measure the fatty acids oxidation (FAO) rates by myoblasts using first C2C12 cells and then primary human myoblasts. While in the C2C12 myoblasts we consistently observed an increase in FAO rate (32±9%), the results were inconsistent in the primary human myoblasts. Increase in respiration rate following addition of the 200μM palmitate complexed with bovine serum albumin (BSA) was taken as FAO rate, and its sensitivity to the carnitine palmitoyltransferase 1 (CPT1) inhibitor etomoxir was tested. The etomoxir sensitive FAO was similar (22±5.20%) in the absence and presence of palmitate. Our

measurements did not show any acute change in respiration rates by glucose addition in the presence of insulin. However, there was a significant increase in the acidification rate when insulin was added with glucose compared to glucose alone. This suggests that extracellular acidification rate can permit determination of insulin sensitivity in small samples.

These assays were used to study the role of metabolism in Facioscapulohumeral Muscular Dystrophy (FSHD), a genetic degenerative disease primarily affecting voluntary facial muscles. A comparison of FSHD myoblasts with normal myoblasts did not show any evidence of impairments in respiratory activity, mitochondrial mass or reactive oxygen species production. However, when we measured the insulin induced acidification rate in the presence of glucose, it was relatively higher (not significant) in the FSHD myoblasts. These data suggest that impaired insulin sensitivity of myoblasts is not an underlying factor in FSHD.

We have characterized a mouse model of partial Complex I deficiency with variable penetrance (20-60%) in different tissues and primary cells. In these mice, a mild insulin resistance appears at ~7 month of age. Early phase of glucose clearance is also affected. This is in agreement with our data from primary pancreatic β cells that show that Complex I function may be more critical in the acute phase of insulin secretion. However, it can have progressive influence on muscle pathophysiology with age.

Our accomplishments are aligned with the goals and objectives of the project. We plan to publish the results with additional data from ongoing work in near future.