Role of IGF2BP2 and HMGA2 in adipocyte differentiation and Type 2 Diabetes

Samuel I. Ares
Diedra M. Wrighting
David Altshuler
Adipose tissue is necessary for proper glucose regulation, which protects against T2D

- **Normal Glucose Homeostasis**
  - Glucose enters the blood stream
  - Pancreatic β-cells secrete insulin
  - Adipocytes secrete hormones that increase insulin sensitivity, repress food intake and glucose production

- **Type 2 Diabetes**

<table>
<thead>
<tr>
<th>Elevated glucose levels in blood</th>
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<tbody>
<tr>
<td>Insulin Resistance</td>
</tr>
<tr>
<td>Impaired insulin secretion</td>
</tr>
<tr>
<td>Over production of glucose</td>
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</tbody>
</table>

Rosen & Spiegelman, Nature 2006
Identify what is the potential role of genes in GWAS loci in adipocytes to understand association with T2D.

Gene Mutations:
- PPARγ
- HMGA2
- IGF2BP2

Consequences:
- Impaired adipose tissue formation/function
- Elevated triglycerides
- Insulin resistance
- Glucose dysregulation
- Type 2 Diabetes

Impaired adipose tissue formation/function
Aims

1. To identify the optimal method for manipulating gene expression in Human preadipocytes
2. Differentiate genetically altered Human preadipocytes into adipocytes
Gene Overexpression

- **Nucleofection**: ~70% efficiency but low survival rate

- Cell Survival decreased in presence of plasmid
Gene Knockdown

Accell Delivery Media

- Enhances siRNA, enabling passage through cell membrane

![Diagram of Gene Knockdown Process]

1. Internalization
2. Formation of RISC complex
3. Formation of siRNA/mRNA complex
4. Gene silencing

Standard Accell delivery protocol:

- Adherent cells
- Plate cells in growth media
- Replace with Accell delivery mix
- Assay for knockdown

Timeline:

- Hrs -24, 0, 72, 96+
Experiment: Assess genes knockdown. Accell delivery protocol is staggered with preadipocyte differentiation

- Delivery efficiency was <1%
- Oil Red O Stains lipid droplets in cells red
- Lipid absorption was uninhibited.
Experiment: Assess effect of FGF/Serum On Accell mediated gene knockdown

- Delivery efficiency was ~23%
- siRNA Delivery Efficiency increases in absence of serum and FGF

siNon-Target Control

![Graph](image)

Media Supplement Conditions: FGF (F) and Serum (S)
Absence of FGF/Serum decreases cell survival rate

**siNon-Target Control Cell Survival**

**siCyclophilin B Control Cell Survival**

<table>
<thead>
<tr>
<th>Media Supplement Conditions: FGF (F) and Serum (S)</th>
<th>Viable Cells</th>
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<tbody>
<tr>
<td>+F+S</td>
<td>90</td>
</tr>
<tr>
<td>-F+S</td>
<td>30</td>
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<tr>
<td>+F-S</td>
<td>50</td>
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<td>-F-S</td>
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<td>+F-S</td>
<td>60</td>
</tr>
<tr>
<td>-F-S</td>
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</table>
Two possibilities: FGF/serum interfere with knockdown or Cyclophilin B expression is FGF/serum dependent

- Western Blot

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<tr>
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<th>siNon-Target</th>
<th>siCyclophilin B</th>
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<tbody>
<tr>
<td>FGF</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-Cyclophilin B</td>
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<td>Anti-β Actin</td>
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- Quantitative PCR on No si treated Samples

**FGF(F) and Serum (S) Treatments**
Conclusion

• Nucleofection yields greater efficiency than Lipofectamine treatment but higher cell mortality

• Accell mediated knockdown may not be suitable to assess differentiation of knockdown preadipocytes

• Use viral vectors knockdown and overexpress genes
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