Introduction

Diabetes is one of the major risk factors for cardiovascular diseases, which is mediated by vascular endothelial dysfunction.

Hypothesis:
We hypothesize that the introduction of environmental factors, such as elevated glucose, urea, cholesterol, and inflammatory cytokines to a healthy patient’s endothelial cells will induce a phenotypic change characteristic of diabetic endothelial cell dysfunction.

Project Aims:
Aim 1: Establish in vitro disease models for diabetic endothelial cell dysfunction.
Aim 2: Develop precision therapeutic strategies for diabetic endothelial cell dysfunction.

Materials and Methods

Blood Collection:
- 62-year-old Caucasian female (AMH04)
  - No diagnosed history of diabetes or cardiovascular disease.
- 68-year-old Caucasian male (CVD07)
  - Diagnosed with diabetes and cardiovascular disease.
- 77-year-old Caucasian male (CVD05)
  - Diagnosed with cardiovascular disease.

Induced Pluripotent Stem Cell-to-Endothelial Cell (iPSC-EC) Differentiation:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood</th>
<th>PBMCs</th>
<th>iPSCs</th>
<th>iPSC-ECs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH04</td>
<td>Blood</td>
<td>PBMCs</td>
<td>iPSCs</td>
<td>iPSC-ECs</td>
</tr>
</tbody>
</table>

Treatment Conditions & Evaluations:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose</th>
<th>Urea</th>
<th>Cholesterol</th>
<th>Nitrate</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 mM</td>
<td>0 mM</td>
<td>0 mM</td>
<td>0 pg/ml</td>
<td>5 days</td>
</tr>
<tr>
<td>Diabetic</td>
<td>25 mM</td>
<td>9 mM</td>
<td>2.5 mM</td>
<td>20 pg/ml</td>
<td>5 days</td>
</tr>
</tbody>
</table>

Evaluation Techniques:

1. Inflammation: Immunofluorescence (IF) & Enzyme-Linked Immunosorbent assay (ELISA)
2. Permeability: Horseradish peroxidase-based permeability assay

Results

1. Inflammation:

- ELISA of AMH04 and CVD07 iPSC-ECs for ICAM-1 and P-sel. Inflammatory cytokine, TNF-α, was used as a positive control. Arrow denotes ICAM-1 localization in the membrane.

2. Permeability:

- Immunofluorescence of AMH04 and CVD07 iPSC-ECs for platelet-selectin (P-sel, red) and nuclei (blue). Inflammatory cytokine, TNF-α, was used as a positive control. Arrow denotes P-sel localization in the membrane.

Discussion

Evaluations:

1. Inflammation:
   - Statistically significant increases in the expression of surface adhesion molecules, such as ICAM-1 and P-sel, after 50 ng/ml TNF-α treatment.
   - Qualitative increase in membrane expression of ICAM-1 and P-sel after treatment with the diabetic condition.
   - CVD07 appeared to have stronger expression of P-sel in the control condition compared to AMH04.
   - Suggests that P-sel expression could be attributed to inflammatory processes within the cell line.

2. Permeability:
   - Statistically significant increase in permeability of the endothelial monolayer for CVD05 after treatment with the diabetic condition.
   - Decreased barrier function and increased endothelial cell permeability, promoting the development of atherosclerotic cardiovascular disease.

Limitations:
The main limitations in this study are the n number of experimental replicates and the number of patient-derived cell lines. This study presents a proof-of-concept, after which, we will expand to other cell lines.

Future Work:
We plan to expand on the EC functional assessments to include thrombosis and oxidative stress. We also seek to translate this model to a high-throughput system. This new system will simultaneously run several pharmacological agents on a patient’s iPSC-EC sample. We expect the application of this model improving the quality of care for diabetic patients through facilitation of pharmacological treatment selection.

Conclusion:
Overall, the diabetic condition treatment resulted in qualitative increases in expression of inflammatory surface proteins. We also see quantitative increases in permeability after treatment with our diabetic condition. We seek to investigate the impact of our diabetic condition on other functional aspects of endothelial cells.

References & Acknowledgements

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