THE ROLE OF CYTOSKELETAL PROTEIN FLIGHTLESS I (FLII) IN DIABETIC WOUND HEALING

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Discipline of Paediatrics

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INTRODUCTORY STATEMENT

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Nadira Ruzehaji

23rd of January 2013
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ABSTRACT

Skin lesions and ulcerations are common and severe complications of diabetes. A significant proportion of these wounds fail to respond to conventional treatment, hence amputation is a feared outcome of diabetes. Overexpression of Flightless (Flii) inhibits wound healing and ablation of Flii using specific neutralising monoclonal antibodies (FnAb) enhances cellular proliferation and migration. It was therefore hypothesized that decreasing Flii expression in diabetic wounds would create a permissive environment for cellular proliferation, enhanced neovascularization, and improved healing outcomes. The aim of this study was to determine whether genetic Flii gene knockdown or treatment with FnAb were effective in improving diabetic wound repair. A mouse model of diabetes was used in which type 1 diabetes was induced using streptozotocin. Diabetes was subsequently induced in low (Flii\(^{+/}\)), normal (WT) and high (Flii\(^{Tg/Tg}\)) mice. Full-thickness dorsal wounds were created and it was found that these wounds healed more rapidly when Flii gene expression was decreased. Further studies revealed that this improved healing was accompanied by a robust pro-angiogenic response with significantly elevated von Willebrand factor and VEGF positive endothelial cell infiltration. In a separate study, wounds in WT diabetic mice were injected intradermally with FnAb and here too improved healing was observed with significantly increased rate of re-epithelialisation compared with placebo control. We investigated the angiogenic response of FnAb both in vitro and in vivo. FnAb enhanced capillary tube formation in human umbilical vein endothelial cells (HUVEC) and promoted formation of functional neovasculature in vivo. Mice with reduced Flii also showed increased numbers of mature blood vessels using an in vivo Matrigel plug assay with increased recruitment of \(\alpha\)-SMA positive cells and improved tight junction aiding cell to cell attachments. In conclusion, reducing Flii levels in wounds either genetically or using neutralising
antibodies promotes wound healing in diabetic mice by enhancing epithelialisation and improving angiogenic processes. Manipulating Flightless I may therefore be a potential approach for therapeutic intervention in the treatment of the diabetic foot.
PUBLICATIONS ARISING FROM WORK IN THIS THESIS


**NATIONAL AND INTERNATIONAL SCIENTIFIC MEETING ABSTRACTS**


AWARDS ARISING FROM WORK PRESENTED IN THIS THESIS

2009 AUGU/RC Heddle Award

The University of Adelaide

2009 Australian Federation of University Women

Brenda Nettle Award

2009 Postgraduate Travelling Fellowship

The University of Adelaide

2011 Health Sciences Faculty Finalist

The University of Adelaide Three Minute Thesis Competition

2011 Postgraduate Research Conference

The University of Adelaide

People’s Choice Award
2011 Freemasons Foundation

Trevor Prescott Memorial Award

2011 Young Investigator Award 2011

2012 Best Oral Award

Australian Society for Medical Research

2012 The Adelaide Research & Innovation Prize

Project with most commercial potential

2012 Best Oral Presentation

AWTRS conference, Sydney, Australia
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>αSMA</td>
<td>Alpha smooth muscle actin</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>EM</td>
<td>Electron microscopy</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>Flii</td>
<td>Flightless I</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Matrix metalloproteinase 9</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
</tbody>
</table>
PDGF Platelet-derived growth factor
PCR Polymerase chain reaction
RNA Ribonucleic acid
STZ Streptozotocin
TGF Transforming growth factor
TIMP Tissue inhibitor of metalloproteinase
TNF-α Tumour necrosis factor alpha
VEGF Vascular endothelial growth factor
vWF von Willebrand factor