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

NADIRA RUZEHAJI

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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 α-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

Ruzehaji, N., Grose, R., et al. (2012). "Cytoskeletal protein Flightless (Flii) is elevated in chronic and acute human wounds and wound fluid: neutralizing its activity in chronic but not acute wound fluid improves cellular proliferation." *European Journal of Dermatology*; accepted for publication in July 2012.

Ruzehaji, N., Mills, S., et al. (2013). "The Action of Flightless I and Toll-Like Receptors during Wound Healing in Diabetic Wounds." *Journal of BioMed Research International*; accepted for publication in January 2013.

Ruzehaji, N., Kopecki, Z., Appleby, SL., Bonder, CS., Fitridge, R., Cowin, AJ. (2013) "Attenuation of Flightless I improves healing in a murine model of type 1 diabetes through increased angiogenesis in the wounds". *Diabetes*; manuscript submitted in January 2013.

Ruzehaji, N., Mills, S., et al. (2013). "The Expression of Flightless I and Toll-Like Receptors in Human Diabetic Wounds". *International Wound Journal*; manuscript submitted in January 2013

McInnes, W., **Ruzehaji, N**., Wright, N., Cowin, AJ., Fitridge, R. (2013) "Successful treatment of venous stasis ulcers with larvae aided wound debridement". *Journal of Wound Care*; manuscript submitted in January 2013.

Lei, N., Franken, L., **Ruzehaji, N.,** et al. (2012). "Flightless, secreted through a late endosome/lysosome pathway, binds LPS and dampens cytokine secretion." *Journal of Cell Science*; accepted for publication in June 2012.

Cowin, A. J., Lei, N., Franken, L., **Ruzehaji, N.,** et al. (2012) "Lysosomal secretion of Flightless upon injury has the potential to alter inflammation." *Journal of Communicative & Integrative Biology;* accepted for publication in August 2012.

Ruzehaji, N., & Cowin, AJ. (2011): Textbook: Chapter 2: "The Inhibitory Factors that Influence Wound Healing." In: Wound Management for the Advanced Practitioner. Eds Asimus, Swanson, McGuinness, Publisher: IP Communications.

NATIONAL AND INTERNATIONAL SCIENTIFIC MEETING ABSTRACTS

Ruzehaji N, Kopecki Z, Appleby SL, Bonder CS, Fitridge R, Cowin AJ (2012). "Ablation of Flightless protein improves healing in a murine model of type 1 diabetes through increased angiogenesis in the wounds." Australian Society for Medical Research, South Australian Scientific Meeting, Adelaide, Australia.

Ruzehaji, N., Wallace, H., et al (2012). Neutralization of Flightless I (Flii) using Flii-specific monoclonal antibodies accelerates impaired healing in diabetic wounds through improved cell proliferation." Postgraduate Conference, The University of Adelaide, Adelaide, Australia.

Ruzehaji N, Kopecki Z, Appleby SL, Bonder CS, Fitridge R, Cowin AJ (2012). "Ablation of Flightless protein improves healing in a murine model of type 1 diabetes through increased angiogenesis in the wounds." The 3nd Meeting of the Australasian Wound & Tissue Repair Society, Sydney, Australia.

Ruzehaji N, Wallace H, Stacey M, Krumbiegel D, Zola H, Fitridge R, Cowin AJ "Neutralization of Flightless I (Flii) using Flii-specific monoclonal antibodies accelerates impaired healing in diabetic wounds through improved cell proliferation." The 15th International Congress of Endocrinology, Florence, Italy.

Ruzehaji N, Wallace H, Stacey M, Krumbiegel D, Zola H, Fitridge R, Cowin AJ (2010). "Neutralization of Flightless I (Flii) in Chronic and Acute Wound Fluid using Flii-specific Monoclonal Antibodies Improves Cell Proliferation." The 2nd Meeting of the Australasian Wound & Tissue Repair Society, Perth, Western Australia.

Ruzehaji N, Wallace H, Stacey M, Krumbiegel D, Zola H, Fitridge R, Cowin AJ (2009). "Extracellular Function of the Actin-Remodelling Protein Flightless I May Be important In Acute Wound Responses." 5th Joint Meeting of the European Tissue Repair Society and the Wound Healing Society, Limoges, France.

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

LIST OF ABBREVIATION

αSMA	Alpha smooth muscle actin
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
EC	Endothelial cells
EM	Electron microscopy
EGF	Epidermal growth factor
ECM	Extracellular matrix
FGF	Fibroblast growth factor
Flii	Flightless I
GFR	Glomerular filtration rate
H&E	Haematoxylin and Eosin
IgG	Immunoglobulin
IL	Interleukin
MMP-9	Matrix metalloproteinase 9
mRNA	Messenger ribonucleic acid

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