ImmunoTools *special* Award 2023

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Unraveling the Circular RNA enigma: Illuminating its role in Diabetic Foot Ulcer pathogenesis and mitochondrial dynamics

BACKGROUND: Diabetes has emerged as a growing global burden. 20% of diabetic patients are more prone to impaired wound healing responses, most notably in the form of Diabetic Foot Ulcer (DFU). In DFU, the wound healing process is impaired, commonly resulting in non-healing ulcers. Since DFU's molecular pathogenesis remains poorly understood, this poses a significant challenge for the development of effective treatments.

Circular RNA (circRNA) is a novel type of RNA in which the 3' and 5' ends are covalently linked through a process known as back-splicing. Emerging data indicates that circRNAs possess the capacity to modulate gene expression, regulate transcription, and hold therapeutic potential. Additionally, mitochondria dysfunction has been identified as a pivotal mechanism in diabetes pathogenesis, attributed to the generation of abnormal reactive oxygen species. Hence, understanding the underlying molecular mechanism may offer insights into reactivating the healing program in DFU.

AIM: The objective of this study is to uncover the role of circRNA in human skin wound healing, with a particular focus on DFU, and to reveal its contribution to mitochondrial dynamics.

RESEARCH PLAN: We have collected wound-edge biopsies from DFU patients as well as surgical wounds of healthy volunteers. Fibroblasts and keratinocytes will be isolated from these samples, followed by real-time RT-PCR to unravel circRNA abundance. Moreover, to determine the circRNA action mode, its subcellular localization will be revealed by separating cell lysates into nuclear, cytoplasmic, and mitochondrial fractions. Western blotting will confirm the purity of each fraction, after which real-time RT-PCR will be employed in each subcellular compartment to assess circRNA expression.

To gain a deeper understanding of circRNAs biological function, *in vitro* experiments will be conducted. The circRNA will either be knocked down using siRNAs or overexpressed with plasmid vectors and introduced to skin cells, specifically Human Dermal adult Fibroblasts (HDFa), through transfection. To reveal the impact of modified circRNA expression on cellular processes in wound healing, cells will be analysed regarding their migration, proliferation, and colony formation ability. In

addition, cells will be assessed for mitochondrial function based on basal respiration, oxygen consumption rate and ATP production.

To comprehend circRNA regulation in DFU, HDFa cells will be treated with a panel of growth factors and cytokines critical for wound repair and homeostasis. These include IL-1, IL-6, IL-22, IFN-γ, TNF-α, TGF-β, EGF, FGF, IGF, GM-CSF, KGF. Subsequently, RNA will be extracted and the circRNA expression will be determined by real-time RT-PCR, to unravel the mechanisms governing differential circRNA expression during DFU and wound healing. Additionally, the cell response to these stimuli in terms of inflammation and differentiation will be evaluated.

Furthermore, to identify the genes and signaling pathways regulated by the DFU-related circRNA, transcriptome microarray analysis will be performed in skin cells with circRNA knockdown or overexpression, followed by ingenuity pathway analysis.

Finally, the therapeutic potential of circRNA will be explored using a human *ex vivo* - or a mouse *in vivo* wound model. Briefly, after generating wounds on the skin followed by circRNA treatment, wound healing will be monitored over the course of 7 days.

The ImmunoTools reagents will allow a comprehensive investigation of circRNA regulation during normal skin wound healing and DFU pathogenesis. Investigating the role of circRNA represents a promising area for pharmaceutical intervention. The insight gained from this project will deepen our understanding on DFU and unravel new drug targets for clinical studies, benefiting diabetic patients worldwide suffering from DFU or other hard-to-heal wounds.