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THE ROLES OF MICRORNAS IN SKIN WOUND HEALING

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The Roles of MicroRNAs in Skin Wound Healing

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Dedicated to my parents

献给我的父母

ABSTRACT

Skin is an essential biological barrier of the human body, and wound healing is the fundamental physiological process to keep its integrity. Chronic non-healing wounds are growing socioeconomic and health concerns, which longs for more understanding of their pathophysiology to discover effective treatments. In this thesis, we focused on how microRNAs (miR) work together with their target protein-coding genes to regulate the complex wound healing process, and by exploring the roles they play in chronic wounds we aimed to discover potential therapeutic targets.

In paper I, a distinct up-regulation of miR-31 in human acute wounds was identified from profiling analysis. We discovered miR-31 as a pivotal regulator in promoting keratinocyte proliferation and migration by targeting EMP1 during wound healing, emphasizing its importance in re-epithelialization.

In paper II, miR-34 family, as a famous tumour suppressor, popped out amidst the top upregulated microRNAs in venous ulcer. *In vitro*, miR-34a and miR-34c enhanced inflammatory response of epidermal keratinocytes via targeting LGR4 and positively regulating NF-κB signalling pathway. *In vivo*, mouse model of either miR-34 local overexpression or Lgr4 knockout displayed impaired wound healing with excessive inflammation and suppressed cell growth. These suggest that miR-34 play a pathological role in chronic wounds by contributing to the excessive inflammation.

In paper III, in continuity with our previous report that miR-132 displays anti-inflammatory and pro-proliferative roles in keratinocytes, we studied the function of miR-132 in another major skin resident cell type fibroblasts. By both overexpression and inhibition, miR-132 was proved to facilitate migration of primary human dermal fibroblasts, through targeting RASA1 and regulating Ras signalling. Since fibroblasts derived from chronic wounds are non-migratory, our study suggests the miR-132-RASA1-Ras axis with potential therapeutic impact.

In paper IV, we tested the therapeutic potential of microRNAs, taken miR-132 as an example. A significant downregulation of miR-132 was revealed in diabetic foot ulcer. Intradermal injection of liposome-encapsulated miR-132 mimics effectively accelerated wound healing. Moreover, *ex vivo* human model exhibited ameliorated re-epithelialization upon miR-132 topical application, denoting that local treatment of miR-132 deserves further evaluation in a clinical trial as a potential target for treating chronic wounds.

Conclusively, this thesis investigated the crucial functions of miR-31, miR-34 and miR-132 in different phases of normal skin wound healing process and in chronic wounds, and pointed out a promising potential of microRNA-based therapy in treating chronic wounds.

LIST OF SCIENTIFIC PAPERS

MicroRNA-31 Promotes Skin Wound Healing by Enhancing Keratinocyte Proliferation and Migration

Dongqing Li, Xi Li, Aoxue Wang, Florian Meisgen, Andor Pivarcsi, Enikö Sonkoly, Mona Ståhle and Ning Xu Landén

J Invest Dermatol. 2015 Jun;135(6):1676-1685.

MicroRNA-34 Family Enhances Wound Inflammation by Targeting LGR4

Jianmin Wu*, Xi Li*, Dongqing Li, Xiaolin Ren, Yijuan Li, Eva K. Herter, Mengyao Qian, Maria A. Toma, Anna-Maria Wintler, Irène Gallais Sérézal, Ola Rollmane, Mona Ståhle, Jakob D. Wikstrom, Xiyun Ye, Ning Xu Landén

Manuscript

MicroRNA-132 Promotes Fibroblast Migration via Regulating RAS P21 Protein Activator 1 in Skin Wound Healing

Xi Li, Dongqing Li, Jakob D. Wikstrom, Andor Pivarcsi, Enikö Sonkoly, Mona Ståhle, Ning Xu Landén

Sci Rep. 2017 Aug 10;7(1):7797.

MicroRNA-132 with Therapeutic Potential in Chronic Wound

Xi Li*, Dongqing Li*, Aoxue Wang, Tongbin Chu, Warangkana, Lohcharoenkal, Xiaowei Zheng, Jacob Grünler, Sampath Narayanan, Sofie Eliasson, Eva K. Herter, Yang Wang, Yannan Ma, Marcus Ehrström, Liv Eidsmo, Andor Pivarcsi, Enikö Sonkoly, Sergiu-Bogdan Catrina, Mona Ståhle, Ning Xu Landén

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MicroRNA-132 Enhances Transition from Inflammation to Proliferation during Wound Healing
Dongqing Li, Aoxue Wang, Xi Liu, Florian Meisgen, Jacob Grünler, Ileana R. Botusan, Sampath Narayanan, Erdem Erikci, Xi Li, Lennart Blomqvist, Lei Du, Andor Pivarcsi, Enikö Sonkoly, Kamal Chowdhury, Sergiu-Bogdan Catrina, Mona Ståhle, Ning Xu Landén
J Clin Invest. 2015 Aug 3;125(8):3008-26.

Psoriasis Skin Inflammation-induced MicroRNA-26b Targets NCEH1 in Underlying Subcutaneous Adipose Tissue
Louisa Cheung, Rachel M. Fisher, Natalia Kuzmina, Dongqing Li, Xi Li, Olivera Werngren, Lennart Blomqvist, Mona Ståhle, Ning Xu Landén
J Invest Dermatol. 2016 Mar;136(3):640-648.

Comparison of MicroRNAome of Human Normal and Chronic Wounds Reveals MicroRNA-17~92 as Critical for Wound repair
Dongqing Li, Hongmei Peng, Le Qu, Jakob D. Wikstrom, Aoxue Wang, Tongbin Chu, Eva K. Herter, Xi Li, Xinling Bi, Queping Liu, Irène Gallais Sérézal, Ola Rollman, Warangkana Lohcharoenkal, Lorenzo Pasquali, Jacob Grünler, Yang Wang, Yannan Ma, Andor Pivarcsi, Enikö Sonkoly, Sergiu-Bogdan Catrina, Changchun Xiao, Mona Ståhle, Qing-Sheng Mi, Li Zhou, Ning Xu Landén
Submitted

WAKMAR2, A Long Non-coding RNA Downregulated in Human Chronic Wounds, Modulates Keratinocyte Motility and Production of Inflammatory Chemokines
Eva K. Herter, Dongqing Li, Maria A. Toma, Manika Vij, Xi Li, Dani Visscher, Aoxue Wang, Tongbin Chu, Pehr Sommar, Lennart Blomqvist, David Berglund, Mona Ståhle, Jakob D. Wikstrom, Ning Xu Landén
Accepted by J Invest Dermatol.

Human Skin Long Non-coding RNA WAKMAR1 Regulates Wound Healing by Enhancing Keratinocyte Migration
Dongqing Li, Lara Kular, Manika Vij, Eva K. Herter, Xi Li, Aoxue Wang, Tongbin Chu, Eleni Liapi, Lennart Blomqvist, Pehr Sommar, David Berglund, Jakob D. Wikstrom, Maja Jagodic, Ning Xu Landén
Submitted

Circular RNA Hsa_circ_0084443 Plays a Pathological Role in Diabetic Foot Ulcer by Regulating Keratinocyte Migration and Proliferation
Aoxue Wang, Maria A. Toma, Jingxin Ma, Dongqing Li, Tongbin Chu, Jing Wang, Xi Li, Ning Xu Landén
Submitted

CONTENTS

LIST OF ABBREVIATIONS

3'-UTR	three prime untranslated region
ADSC	adipose-derived stem cells
AGEs	accumulated glycation end products
Ago	argonaute
AU	arterial ulcer
BMSC	bone marrow-derived stem cells
CCL	CC chemokine ligand
CXCL	CXC chemokine ligand
DAMPs	damage-associated molecular patterns
DFU	diabetic foot ulcer
DMEM	Dulbecco's Modified Eagle's Medium
DU	diabetic ulcer
EC	endothelial cells
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EMP1	epithelial membrane protein 1
ENCODE	Encyclopedia of DNA Elements
ESC	epidermal stem cells
FANTOM	Functional ANnoTation Of the Mammalian genome
FBS	fetal bovine serum
FGF	fibroblast growth factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GO	gene ontology
GSEA	gene set enrichment analysis
GSK-3β	glycogen synthase kinase 3 beta
H&E	hematoxylin and eosin
HDF	human dermal fibroblasts
HEKa	human adult epidermal keratinocytes
HIF	hypoxia-inducible factor
HRP	horseradish peroxidase
ICAM	intercellular adhesion molecule
IF	immunofluorescence
IFN	interferon
IGF	insulin-like growth factors
IHC	immunohistochemistry
IL	interleukin
iPSC	induced pluripotent stem cells
ISH	in situ hybridization
KGF	keratinocyte growth factor
KO	knock out

LCM	laser capture microdissection
LE	leading edge
LepR	leptin receptor
LGR4	leucine rich repeat containing G protein-coupled receptor 4
LPS	lipopolysaccharides
MACS	magnetic-activated cell sorting
MBS	miRNA binding sites
miRNA	microRNA
MMP	matrix metalloproteinase
mRNA	messenger RNA
MSC	mesenchymal stem cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NO	nitric oxide
NPWT	negative-pressure wound therapy
NW	normal wound
PAMPs	pathogen-associated molecular patterns
PDGF	platelet-derived growth factor
pri-miRNAs	primary microRNA
PU	pressure ulcer
PVD	peripheral vascular diseases
qRT-PCR	quantitative real-time polymerase chain reaction
RASA1	RAS p21 protein activator 1
RISC	RNA-induced silencing complex
ROS	reactive oxygen species
RT	reverse transcription
SELE	e-selectin
cSCC	cutaneous squamous cell carcinoma
T2DM	type 2 diabetes mellitus
TGF-β	transforming growth factor β
TLR	Toll-like receptor
TNF	tumor necrosis factor
UV	ultraviolet
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
WT	wild type
VU	venous ulcer
α-SMA	alpha smooth muscle actin

1 BACKGROUND

1.1 THE SKIN

Skin is the largest organ of the human body, serving as the first line of defense against the external environment. The skin not only serves as a physical and biochemical barrier against mechanical, chemical, radical injury and microorganisms, but also perform other functions such as sensory recognition, water protection and thermoregulation.

From the outside in, human skin is composed of three distinct anatomical compartments: epidermis, dermis, and hypodermis (Fig 1). In addition, skin contains cutaneous appendages including hair follicles, eccrine, apocrine and sebaceous glands and nails.

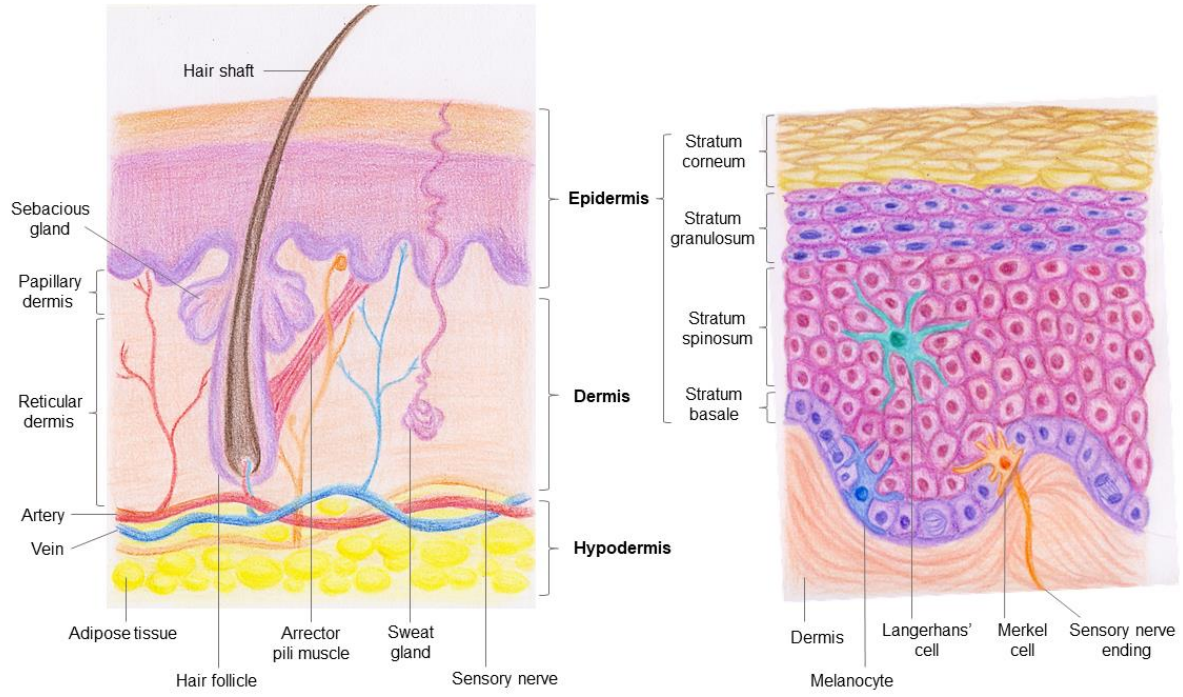


Fig 1. Skin anatomy.

1.1.1 Epidermis

The epidermis of human skin is typically 0.05 - 0.1mm in thickness, consisting of stratified squamous epithelium that is devoid of blood supply. It continuously renews itself and constitutes different layers of cell (Fig 1). Stratum basale is the lowermost layer of epidermis formed by one

single layer of keratinocytes that majorly express keratin proteins 5 and 14. These cells remain attached to the basement membrane via hemidesmosomes at the interface of dermis and epidermis. Basal keratinocytes actively proliferate and differentiate while moving outwards to build spinous layer (stratum spinosum). Spinous keratinocytes primarily express keratin 1 and 10, and remain connected by tight intercellular junctions called desmosomes. Further differentiation of suprabasal cells leads to formation of the next superficial layer of epidermis known as granular layer (stratum granulosum). In this layer, the cells become more flattened and elongated in appearance, have increased protein synthesis and lipogenesis, and express protein markers such as filaggrin, loricrin and involucrin. In the epidermis of the palms of the hands and soles of the feet, there is an additional layer called stratum lucidium located between the stratum granulosum and next outermost layer of stratum corneum. In stratum corneum, keratinocytes undergo terminal differentiation to form corneocytes or dead cells. These cells gradually loose their nuclei and organelles, secrete lamellar bodies, form cornified cell envelope, and eventually shed from the skin surface by process of desquamation (Blanpain and Fuchs, 2009, Fuchs, 2016, J.A. McGrath, 2010, Menon, 2002).

The cellular composition of epidermis mainly consists of > 90% keratinocytes. Additionally, it also contains other cell types (Fig 1) such as melanocytes, Merkel cells and immune cells including Langerhans' cells and T cells. Melanocytes reside in the basal layer, and produce melanin which is transported to nearby keratinocytes to induce pigmentation and protect skin against UV radiation. Langerhans' cells are antigen-presenting immune cells of the skin, with dendrites piercing through keratinocytes' intercellular junctions to scout any penetrating antigens. The epidermis also hosts nerve-ending Merkel cells, which are essential for light-touch sensation and discrimination of shapes and texture (Di Meglio et al., 2011, J.A. McGrath, 2010).

1.1.2 Dermis

The dermis is separated from the epidermis by the basement membrane, which is majorly composed of collagen IV, VII and laminins. The dermis contains mainly extracellular matrix (ECM), e.g. collagens, elastic fibers and proteoglycans, providing essential structure, strength and elasticity to the skin. The dermis could be anatomically divided into the upper papillary dermis (stratum papillare) and the deeper reticular dermis (stratum reticulare), which are different in ECM composition: the papillary dermis is characterized by thin, poorly organized collagen fiber bundles which are primarily collagen I and III, in contrast to the thick, directionally oriented matrix networks in the reticular dermis (J.A. McGrath, 2010, Sorrell and Caplan, 2004). Dermal papillae

are the superficial ridge-like structures of the papillary dermis, which form the interactive surface between the epithelium and mesenchyme.

Fibroblast is the major cell type synthesizing connective tissues in the dermis. Interestingly, fibroblasts present in papillary and reticular dermis have distinct gene expression profiles, such as for the genes related to the Wnt pathway, ECM components and immunoregulation, indicating they are a heterogeneous group of cells with different biological functions. For example, it has been known that papillary fibroblasts exhibit an anti-inflammatory phenotype, yet reticular fibroblasts are primed to respond to injury and infection. In the lower dermis, there are also preadipocytes, which are a subpopulation of fibroblasts with the capacity to differentiate into adipocytes (Lynch and Watt, 2018, Philippeos et al., 2018).

In addition, the dermis comprises of a wide range of other cell types and structures such as sebaceous glands (e.g., sebocytes), blood vessels (e.g., endothelial cells, smooth muscle cells), eccrine and apocrine sweat glands, lymphatic vessels, as well as tissue-resident immune cells. As there are no blood and lymphoid vessels in the epidermis, those in the dermis are important in providing nourishment and clearing waste. The dermis also harbors neurons and nerve endings that mediate touch and heat (J.A. McGrath, 2010, Lynch and Watt, 2018).

1.1.3 Hypodermis

Hypodermis lies below dermis and is also called subcutaneous tissue. It is mainly composed of loose connective tissue and lobules of fat, accounting for approximately 80% of fat in human body (Lee et al., 2013). Subcutaneous fat tissue acts as padding cushion, energy reservoir and as thermo-regulator. The major cell types in hypodermis include adipocytes, pre-adipocytes, fibroblasts, macrophages and monocytes. In addition, adipose-derived stem cells stand for an abundant and accessible type of adult stem cells that can differentiate into multiple cell lineages and then further exert diverse influences (Mizuno, 2009).

1.2 SKIN WOUND HEALING

Wound healing is a fundamental and complex physiological process to keep the skin barrier intact. Wound healing is usually characterized as four sequential, albeit overlapping phases (Fig 2), i.e. hemostasis (0 – few hours after injury), inflammation phase (1 – 3 days), proliferation phase (4 – 21 days) and remodelling phase (21 days – 1 year).

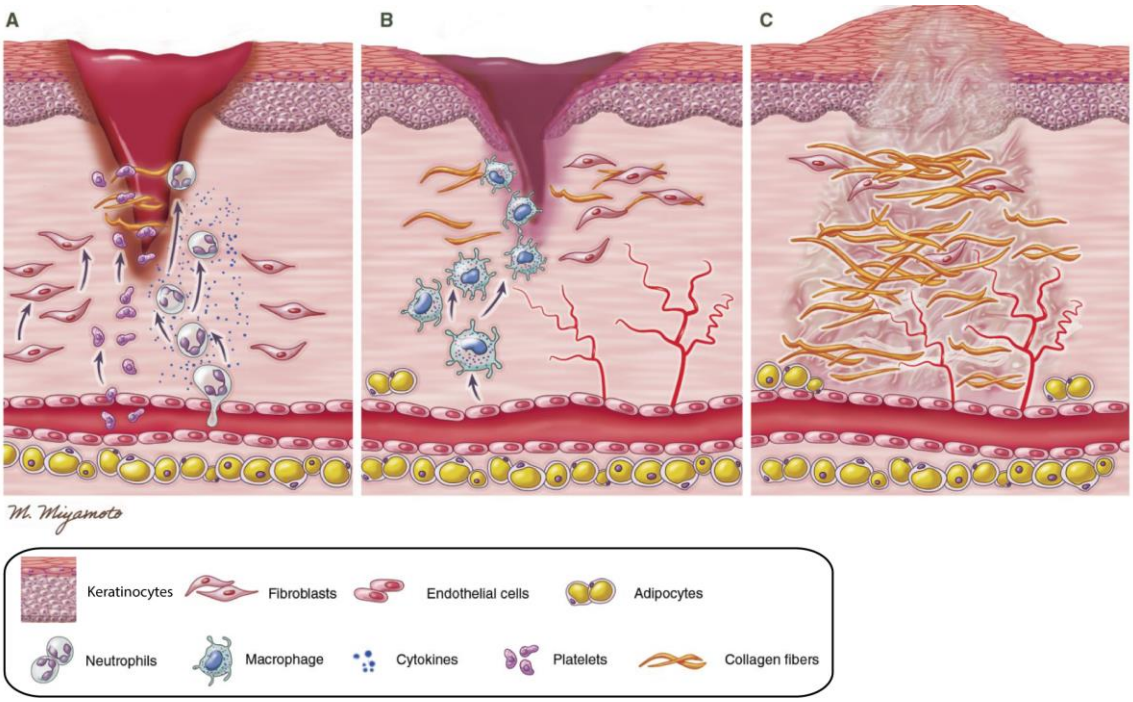


Fig 2. Simplified schematic representation of acute wound healing process: (A) Hemostasis and early inflammation phase. (B) Late inflammation phase and proliferation phase. (C) Remodelling phase. *Reproduced and modified with permission from Foster et al., 2018. Copyright American Society for Clinical Investigation.*

1.2.1 Hemostasis phase

Immediately following injury, the wound healing process initiates when platelets get exposed to ECM which triggers the coagulation cascade. A provisional clot (eschar), containing blood, fibrin, fibronectin and vitronectin, is formed within minutes to hours to rapidly stop bleeding and rapidly plug the defect (Reinke and Sorg, 2012). Simultaneously, the disrupted keratinocytes initiate release of pre-stored interleukin-1 (IL-1) as an early damage alert signal (Barrientos et al., 2008, Di Meglio et al., 2011). Activated platelets also produce a wide range of cytokines and growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor β (TGF-β) (Diegelmann and Evans, 2004). Together, cytokines

and chemokines play critical roles in the following healing process, for example CXCL1 (GRO-α), CXCL5, CXCL8 (IL-8) are important chemoattractant for neutrophils; and CCL2, CCL3, CXCL10 attract monocytes/ macrophages. Therefore, the formation of provisional clot serves as a scaffold for the migration of these cells and keratinocytes (Reinke and Sorg, 2012).

1.2.2 Inflammation phase

In the early inflammatory phase, neutrophils are the primary cell type recruited to the wound site from the circulation. Stimulated by the chemokines released from the injured skin, blood vessel endothelial cells (EC) start to express adhesion molecules, e.g. vascular cell adhesion molecule (VCAM1), intercellular adhesion molecule 1 (ICAM1) and e-selectin (SELE), enhancing adhesion of neutrophils to the blood vessel wall and their extravasation. Once outside the circulation, neutrophils follow the gradient of chemokines and migrate to the injured area (Landen et al., 2016, Vestweber, 2015). Upon entering the wound, neutrophils exert phagocytosis to remove bacteria, foreign material and damaged tissue debris; secrete antimicrobial substances, protease and inflammatory cytokines, e.g. TNF-α, IL-1β, IL-6 (Eming et al., 2007, Reinke and Sorg, 2012). Neutrophils constitute nearly 50% of cells in the early stages of the wound (Ridiandries et al., 2018), and are normally present in the wound for 2-5 days unless infection occurs (Wilgus et al., 2013).

In the late inflammatory phase, monocytes are recruited to the wound site from the circulation in response to CCL2, CCL3, and CCL5 secreted by neutrophils and later by themselves. Together with the skin-resident macrophages, they become the dominant inflammatory cell types in the wound for approximately two weeks post-injury (Ridiandries et al., 2018), and play an important role in shifting the inflammatory phase to the following proliferative phase, which is largely due to their impressive phenotype plasticity. Upon recognition of pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and inflammatory signals (e.g. IFN-γ, TNF-α), macrophages polarize to a pro-inflammatory M1 phenotype (the classically activated macrophages), which produce cytokines and chemokines to attract more leukocytes, exert phagocytosis, promote Th1-Th17-type response and play host defense role (Snyder et al., 2016). Later on, M1 macrophages transit to the M2 phenotype (alternatively activated macrophages) with anti-inflammatory functions, which is key for inflammation resolution (Landen et al., 2016). M2 macrophages induce neutrophil apoptosis and ingest them (Meszaros et al., 1999, 2000), produce growth factors, e.g. vascular endothelial growth factor (VEGF) and TGF-β, to promote angiogenesis, re-epithelialization, fibroblast migration and matrix deposition (Landen et al., 2016, Snyder et al., 2016).

Other cell types that assist the process of inflammation include the resident mast cells, which release granules containing histamine, enzymes and other active amines, that can promote mononuclear cell leakage from surrounding blood vessels, wound fluid accumulation and inflammation signs, i.e., redness, heat, swelling and pain (Diegelmann and Evans, 2004). The adaptive immune system, including B and T cells, also involves in a later but more specific role, not only in the inflammation phase but subsequently in remodelling as well, which still needs more exploration (Landen et al., 2016). Moreover, dermal fibroblasts can also interact with these immune cells and regulate their infiltration, behavior and apoptosis by changing survival factors and chemokines gradient, to adjust inflammation levels (Landen et al., 2016, Smith et al., 1997).

1.2.3 Proliferation phase

Re-epithelialization initiates already in a few hours to one day post skin injury. The process is stimulated by nitric oxide (NO) and growth factors, such as EGF family (including EGF, TGF-α, HB-EGF), keratinocyte growth factor (KGF), FGF, IGF-1 and nerve growth factor (NGF), produced by multiple cell types in the wound (Diegelmann and Evans, 2004, Martin, 1997). Wound-edge keratinocytes alter their gene expression profiles in order to invade through fibrin clot and migrate on granulation tissue towards the wound center, which forms a non-proliferative migrating leading edge (LE) (Aragona et al., 2017). In the proliferative hub surrounding the wound-edge, epidermal progenitors rapidly divide asymmetrically, while activated stem cells from interfollicular epidermis, hair follicle and infundibulum give rise to new progenitors that expand along LE to rebuild normal multi-cell-layered epidermis (Aragona et al., 2017, Lau et al., 2009).

During this phase, the provisional clot inserted in the wound bed is replaced by a newly-formed granulation tissue. Activated by PDGF, bFGF and TGF-β, dermal fibroblasts in the wound neighbourhood and circulating fibrocytes (a group of bone marrow-derived mesenchymal progenitors) start to proliferate and migrate along the connective tissue matrix into the provisional clot (Martin, 1997, Mori et al., 2005). Around a week after wounding, fibroblasts fully invade into the fibrin clot, produce proteases, such as matrix metalloproteinases (MMPs), to degrade the provisional matrix while synthesizing new ECM. A portion of fibroblasts differentiate into myofibroblasts, expressing α smooth muscle actin (α-SMA) to establish a contractile force pulling the wound margins together (Martin, 1997).

To supply the healing wound with oxygen and nutrients, it is important to form new blood vessels, i.e., neovascularization/ angiogenesis, which is induced by local hypoxia, low pH due to

high metabolic activity, growth factors (e.g. PDGF, bFGF, VEGF), hypoxia-inducible factor (HIF) and TGF-β in the wounds (Diegelmann and Evans, 2004, Martin, 1997). The activated ECs sprouted from the existing blood vessels (angiogenesis), and endothelial progenitors migrate towards the required site (vasculogenesis), proliferate and differentiate to form new capillaries, arteries and venules (Li et al., 2003, Reinke and Sorg, 2012).

1.2.4 Remodelling phase

The final stage of wound healing is the remodelling phase, also called the maturation phase, which lasts for months to years depending on the size and type of the wound. At the end of the proliferation phase, angiogenesis and proliferation cease. The provisional matrix (mainly fibrin, fibronectin, proteoglycans and collagen III) are gradually degraded by MMPs; meanwhile more collagen I is being produced by the residual fibroblasts to reconstruct a tough scar tissue with tensile strength comparable (maximum 80%) to the unwounded skin (Demidova-Rice et al., 2012). Excessive cells either leave the wound or are subjected to apoptosis, such as recruited immune cells and myofibroblasts. Nerve regeneration progresses. The number and flow of new blood vessels diminish and regress when healing is complete (Reinke and Sorg, 2012). In most cases, although wounds get repaired, appendages fail to regenerate once lost.

1.3 CHRONIC WOUNDS

Chronic wounds, or hard-to-heal wounds, are defined as cutaneous wounds failing to heal within 6 weeks (Tricco et al., 2015). They are cutaneous defects which fail to proceed through an orderly and timely tissue repair to retrieve complete structure and integral function (Eming et al., 2014). The major causes underlying chronic wounds are vascular insufficiency (venous/arterial ulcer, VU/AU), diabetes mellitus (diabetic ulcer, DU) and local mechanical pressure (pressure ulcer, PU). More than 90% of chronic wounds are categorized as VU, DU or PU (Mustoe et al., 2006). Systemic factors, e.g. advanced age, malnutrition and compromised immune status, also sow the seeds for impaired healing (Eming et al., 2014). Chronic wounds result in a heavy health care burden, growing socioeconomic loss, and decreased patients' quality of life, high amputation rate and even mortality. It has been estimated in developed countries, approximately 1-2% of populations will experience hard-to-heal wound (Gottrup, 2004), which already consumes 2-4% of their health care budget in developed countries (Frykberg and Banks, 2015, Gottrup et al., 2001, Richmond et al., 2013).

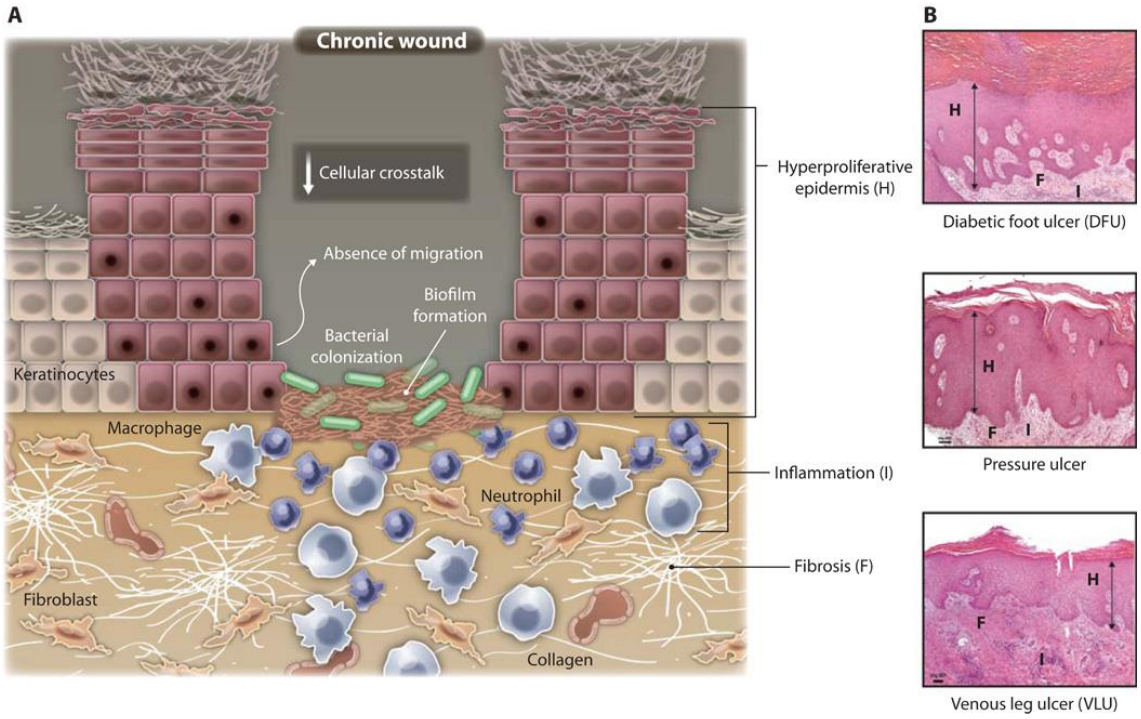


Fig 3. Illustrations of molecular and cellular mechanisms that are impaired in chronic wounds. *Reproduced with permission from Eming et al., 2014. Copy Right American Association for the Advancement of Science*

1.3.1 Venous ulcer and diabetic ulcer

VU constitutes ~80% of all non-healing leg ulcers (Medina et al., 2005), with a rising incidence up to 3 to 4% among the elderly (Eming et al., 2014), due to various reasons that lead to venous hypertension and chronic venous insufficiency. VUs are clinically characterized as superficial painless cutaneous defects with fibrous granulation tissue and cloudy proteolytic exudates mostly on lower extremities, where the whole limb may exhibits edema, surrounding varicose veins, eczema and hemosiderin hyperpigmentation (Medina et al., 2005, Valencia et al., 2001). Edema plays a major role in VU, which causes lower tissue oxygen level (Mustoe et al., 2006). Fibrotic cuffs wrapping capillaries also contribute to the pathogenesis by interfering with oxygen and nutrition exchange, inducing endothelial damage, lipodermatosclerosis, and entrapping leukocytes (Browse and Burnand, 1982, Valencia et al., 2001).

Diabetes is an escalating global burden, with 422 million diabetic patients worldwide (Organization, 2016). Up to 20% of them suffer from impaired wound healing, and the most common form is diabetic foot ulcer (DFU) (Guo and Dipietro, 2010). Among all kinds of chronic wounds, DFU is the most notorious one – most difficult to heal even after multiple treatments (Amin and Doupis, 2016), which accounts for >80% lower leg amputations (Frykberg and Banks, 2015). DFUs are often located on more distal extremities like calf and feet, with necrotic base and irregular margins (Medina et al., 2005). Under diabetes milieu, prolonged exposure to hyperglycemia leads to increased glycation of proteins and accumulated glycation end products (AGEs), which disturb numerous cellular responses, such as suppressing bFGF, EGFR signalling (Medina et al., 2005). Upon injuries, diabetic patients exhibit reduced capacity of recruiting immune cells, which leads to delayed inflammatory response, increasing the risk of infection (Medina et al., 2005). Pathological macrovascular and microvascular changes and peripheral neuropathy in diabetic patients are important pathogenetic risk factors for development of DFU (Amin and Doupis, 2016, Cavanagh et al., 2005).

1.3.2 Pathophysiology of chronic wounds

Chronic wound abnormalities are commonly featured as persistent inflammation, presence of infection and biofilm, hyper-proliferative but non-migratory keratinocytes, senescent fibroblasts, impaired angiogenesis, excessive proteolytic enzymes and reactive oxygen species (ROS) (Fig 3) (Diegelmann and Evans, 2004, Eming et al., 2014, Martin and Nunan, 2015). Nevertheless, the molecular mechanisms behind the impairment of wound healing are still poorly understood. This may be partially due to the complexity of the normal wound healing process,

difficulty capture early pathophysiological events and lack of animal models recapitulating non-healing wounds. Future investigations are required to reveal the complicated and dynamically changing microenvironment of chronic wounds, and there might be sharing pathological mechanisms between different etiologies and as well unique factors/ pathways influencing individual subtypes of chronic wounds.

1.3.2.1 Hypoxia, ischemia and reperfusion

Most chronic wounds (VU, AU, DU) are associated with peripheral vascular diseases (PVD) with insufficient delivery of oxygen and nutrients. Local pressure-induced ischemia is also implicated in development of PU (Sen, 2009). Additionally, hypoxia is intensified by increased energy consumption during healing, ROS, infection, pain and anxiety etc (Sen, 2009). Ischemia-reperfusion occurs repetitively, leading to cytotoxic tissue damage, for instance, damage of mitochondria oxidative function, cell swelling, leukocytes trapping, overproduced ROS, and microvascular dysfunction (Mustoe et al., 2006). Insufficient capillary growth has been observed in both DU and VU (Eming et al., 2014), which further exacerbates the hypoxic situation in chronic wounds.

1.3.2.2 Microbiome and infection

Bacteria exist in all open wounds, but are normally limited to contamination or colonization, sometimes local infection, but rarely develop into spreading infection (cellulitis or even septicemia) (Edwards and Harding, 2004). Polymicrobial presence has been studied in chronic wounds and the bioburden, including the microbial diversity, microbial load, and the presence of pathogenic microorganisms, has been reported to play a pivotal role in impaired wound healing even without clinical infection (Misic et al., 2014). The most prevalent bacteria present in chronic wound are *Staphylococcus, Pseudomonas,* and *Corynebacterium*. In chronic wounds, bacteria often aggregate in a self-secreted extracellular polysaccharide biofilm, which protects them from host immune response or treatment with antibiotics (Zhao et al., 2013). Bacterial products and antibacterial immune reactions damage tissue and even lead to necrosis (Landen et al., 2016). Failure to clear these foreign bodies and the sustained presence of high bacterial load result in a sustained influx of pro-inflammatory cells and increased inflammation (Eming et al., 2014).

1.3.2.3 Persistent inflammation

In chronic wounds, the orderly efficient healing process is disturbed, and wounds are stuck in a chronic inflammatory state (Eming et al., 2009). Comparing the exudate from chronic wounds with healthy wounds, the level of mediators characterizes for persistent inflammation, such as cytokines and S100 family members, were found to be elevated (Beidler et al., 2009, Eming et al., 2010). The excessive cellular infiltration involving neutrophils, pro-inflammatory M1 macrophages and prolonged presence of T cells with low CD4+/CD8+ ratio further strengthens this observation (Loots et al., 1998). These cells produce proteases degrading ECM, growth factors, clotting factors, complements and immunoglobulin, and also release free oxygen radicals further damaging tissue (Dovi et al., 2004). Impairment of neutrophils phagocytosis by macrophages has been observed in DFU, which prolongs inflammation and delays healing (Khanna et al., 2010). Moreover, defection of M1 to M2 macrophage phenotype transition has been implied in the pathogenesis of chronic wounds, as M2 macrophages are important to remove neutrophils, resolve inflammation and initiate the following proliferative phase (Landen et al., 2016).

1.3.2.4 Ageing and senescence

Advanced age is an obvious common feature that most chronic wounds patients share, regardless of their diverse etiologies. Changes of hormones with ageing, particularly estrogens and androgens, are principle contributing factors in compromised wound healing despite of chronological ageing (Eming et al., 2014, Wilkinson and Hardman, 2017). Reduced number and function of stem cells/ progenitors, as well as irreversible cell aging with limited proliferative potential and altered response to stress also make the elderly prone to the development of chronic wounds(Makrantonaki et al., 2017). Moreover, cells at wound site enter into a replicative premature senescence due to constant exposure to inflammation and stress (Mustoe et al., 2006). This leads to a senesce-associated secretory phenotype which alter the microenvironment and impact neighbouring cells (Makrantonaki et al., 2017). For example, prematurely aged fibroblasts have diminished migratory capacity and are unresponsive to growth factors. They secret proteolytic MMPs, which inhibit proliferation, angiogenesis, and induce uncontrolled tissue degradation (Jun and Lau, 2010, Martin and Nunan, 2015, Medina et al., 2005). Senescent keratinocytes also produce anti-angiogenic factor maspin, impairing wound healing (Nickoloff et al., 2004).

1.3.2.5 Other pathological factors

In the wound-edge of skin ulcers, hyper-proliferative keratinocytes demand frequent cycling of epidermal progenitor/ stem cells, which could cause fast reduction or even depletion of local stem cell populations (Eming et al., 2014). A variety of other risk factors were also reported to linked to impaired wound healing, for instance, obesity, stress, alcoholism, smoking, disease such as jaundice and uremia, and medications such as glucocorticoid steroids and chemotherapy (Guo and Dipietro, 2010, Jozic et al., 2017).

1.3.3 Current treatments for chronic wounds

Conventional treatments for chronic wounds include non-specific and specific interventions. Thorough assessment of vascular state, drug usage, patients' general health and nutrition status is fundamental for design of suitable treatment strategies. Wound bed preparation and extensive surgical debridement are used to remove exudate debris and devitalized wound-edge tissues to achieve a well-vascularized bed (Pang et al., 2017) in order to initiate secondary healing (Tsourdi et al., 2013). It is critical to eliminate infection with antiseptics and antibiotics, based on the results of pathogen identification (Cavanagh et al., 2005, O'Toole, 2010, Valencia et al., 2001). It is imperative to minimize skin irritation or friction and protect wounds from further contaminations and maintain proper moisture and temperature conditions. To implement this, different types of wound dressings have been developed, such as semipermeable films, hydrogels, cellulose and collagens etc. (Jones et al., 2018, O'Toole, 2010, Pang et al., 2017). Moreover, various kinds of skin grafting, e.g. allogenic or autologous skin flap, tissue-engineered skin equivalents, are commonly used to ease the pain and assist in healing process. However, their associated limitations in form of reduced vascularization, poor mechanical integrity and immune rejection impedes their extensive clinical utility (O'Toole, 2010, Stone et al., 2017, Tsourdi et al., 2013).

Specific assessments and treatments are crucial in targeting primary etiology. For example, compression therapy is routinely used for VU to reverse edema. This is a cornerstone and demonstrates the largest effect on successful healing rate in most VU treatment (Valencia et al., 2001). VU and AU benefit from laser treatment and blood vessel surgery, which addresses circulation problem with less recurrence. For DU, metabolic and glycemic control as well as offloading are helpful, and surgical interventions in use include tenotomy, tendon lengthening, reconstruction, or removal of bony prominences, which may cause secondary ulceration (Cavanagh et al., 2005).

1.3.4 Progress of chronic wound research

The efforts aiming to cure chronic wounds are on-going. Local delivery of growth factors, including bFGF, GM-CSF, EGF, and KGF-1, has not achieved satisfying outcomes in clinical trials; only rhPDGF-BB entered clinical use but with limited effects (Barrientos et al., 2014, Barrientos et al., 2008, Makrantonaki et al., 2017, Pang et al., 2017). This may be due to the reduced expression or responsiveness of growth factor receptors on the wound-edge cells and rapid degradation of these growth factors in the proteolytic wound environment (Barrientos et al., 2008, Pang et al., 2017).

In recent years, a myriad of advanced nanotechnologies have been developed for targeting specific problems of non-healing wounds. Various nanoparticle, polymer, lipid and peptide based therapeutic intervention strategies are being designed to address the problems associated with chronic wounds, such as nanomaterials with intrinsic beneficial properties for wound healing, or delivery vehicles/ scaffolds for other therapeutic agents. However, a series of challenges surround nanoparticle-based therapeutics due to their elusive physicochemical properties, biological behaviours, immune toxicity and difficulty in purification (Hamdan et al., 2017).

Therapies with live cells, e.g., keratinocytes, fibroblasts, platelets and bioengineered skin equivalent, have been used in clinical practice, which exhibit promising effects in some VU and DU cases (Brem et al., 2003, Falanga et al., 1998, Stone et al., 2017, You and Han, 2014), which still needs to be tested and validated. Continuous efforts have been made for the development of stem cell-based treatments by using, e.g., mesenchymal stem cells (MSC) (Cao et al., 2017), bone marrow-derived stem cells (BMSC), adipose-derived stem cells (ADSC), epidermal stem cells (ESC) and induced pluripotent stem cells (iPSC), which have shown promising results in animal wound models but with limited success in clinical trials (Duscher et al., 2016, Pang et al., 2017). Stem cell-based treatments face problems, such as low stem cell yield, high cost, poor cell delivery and survival, and impaired *in vivo* cellular function (Duscher et al., 2016).

Recently, use of commensals or engineered microorganisms provides an alternative therapeutic approach towards non-healing wounds. Commensal bacteria could compete with pathogenic bacteria, produce antimicrobial substances, inactivate toxins/ metabolites, and modulate host immune response. *In vivo* and *in vitro* studies have demonstrated that topical application of specific strains or certain bacteria extracts, such as *S. epidermis*, lactobacilli and bifidobacteria, may improve wound healing. However, further research is required to learn more about the underlying mechanisms involving gut–brain–skin axis and probiotic–host interactions, and to explore their effects in patients with complicated background (Lukic et al., 2017).

The complicated landscape of chronic wounds represents a major obstacle in finding new efficient therapies. Overall, these current capabilities are economically demanding and require special expertise to implement and achieve benefits in chronic wound treatment modalities. Moreover, efficient knowledge of molecular pathology of chronic wounds can help us in better designing of advanced technologies with the potential to get clinically translated.

1.4 MICRORNAS

As stated in the central dogma of molecular biology, genomic DNA is transcribed to RNA, and RNA is translated into protein. However, the Encyclopedia of DNA Elements (ENCODE) and Functional ANnoTation Of the Mammalian genome (FANTOM) projects have revealed that only ~2% of our DNA encode protein, ~97 – 98% of transcription results in non-protein-coding RNAs (Esteller, 2011). Many of these non-protein-coding RNAs have been shown to play important regulatory roles in the cells. This thesis focuses on one group of short non-coding RNAs, i.e. microRNAs.

1.4.1 MicroRNA biogenesis

MiRNAs are single-stranded non-protein coding RNA with around 22 nucleotides on average (19 – 25 nt), which are highly conserved post-transcriptional gene regulators (Bartel, 2004). Most miRNA genes are transcribed by RNA polymerase II to form primary precursors (pri-miRNAs), which are subjected to the cleavage by nuclear RNase III Drosha-DGCR8 to shorter hairpin-shaped precursors (pre-miRNAs, approximately 60 nt) and exported from the nucleus to the cytoplasm (Ameres and Zamore, 2013). An alternative pathway was reported that some intronic microRNA precursors can be processed by RNA splicing bypassing the cleavage of Drosha (Ruby et al., 2007). In the cytoplasm, pre-miRNAs are further processed into short RNA duplexes (miRNA:miRNA*) by another RNase III enzyme DICER, and then separated into single strands to form mature miRNAs (Bartel, 2004, Ha and Kim, 2014) (Fig 4). Some miRNAs are transcribed from their own transcription unit with independent promoters. About 40% miRNAs are derived from the introns and 10% from the exons of other coding or non-coding transcripts, sharing transcriptional regulation with the host genes (Monteys et al., 2010, Ozsolak et al., 2008). MiRNA genes are often clustered together in the genome, which are transcribed as a multi-cistronic primary transcript and then further processed; even though, the expression pattern of these miRNAs may differ within the cluster due to complicated post-transcriptional regulation (Bartel, 2004, Ha and Kim, 2014, Zhao and Srivastava, 2007).

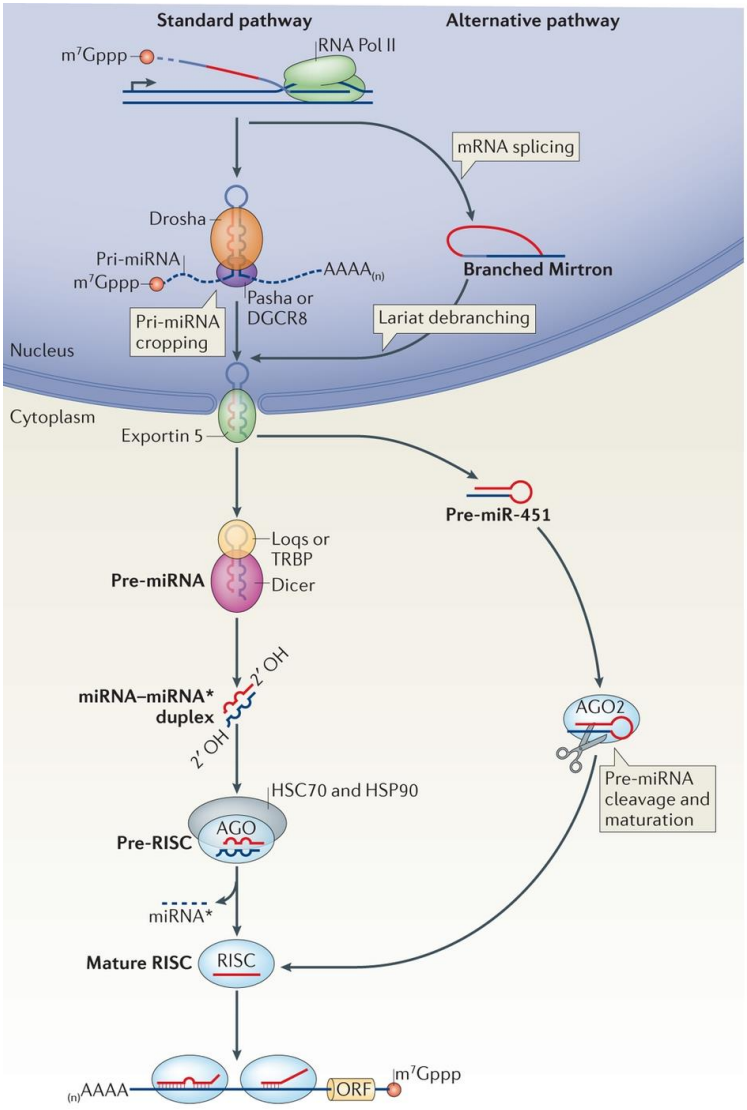


Fig 4. Illustration of microRNA biogenesis. *Reproduced with permission from Ameres et al., 2013. Copyright Springer Nature.*

1.4.2 The action modes of microRNAs

To mediate their biological function, mature miRNAs are loaded to the argonaute (AGO) protein to form the RNA-induced silencing complex (RISC), and bring this enzyme complex to the target messenger RNA (mRNA) (Ameres and Zamore, 2013). The targeting process is mainly mediated by the pairing between the miRNA seed region (the 2nd-7th nucleotides from the 5' end of a miRNA) and the miRNA binding sites (MBS) at the 3' untranslated region (UTR) of target mRNAs. Additionally, the supplementary/compensatory base-pairing between the rest part of a miRNA and the MBS is responsible for defining the specificity and efficiency of miRNA-mediated regulation (Bartel, 2009, Jonas and Izaurralde, 2015). As a result, miRNAs down-

regulate the expression of target genes by repressing translation, reducing stability or degrading their mRNAs. Moreover, the RISC can also silence target gene at the genomic level by formation of heterochromatin or via DNA elimination (Pratt and MacRae, 2009). A single miRNA usually has dozens and even hundreds of targets, which often form a signalling network, exerting the biological functions of this miRNA by fine-tuning the protein-coding genes (Bartel, 2009). MiRNAs have been demonstrated to play important roles in almost all the biological and physiological processes investigated to date. In line with this, aberrant miRNA expression and function have been identified in many diseases. Therefore, modulation of these disease-related miRNAs has been shown to be beneficial in several clinical trials, thus making microRNA a favourable candidate to be explored for novel therapy (van Rooij and Kauppinen, 2014).

1.4.3 MicroRNAs in the skin

MiRNAs have been identified as important regulators in the skin (Mancini et al., 2014, Mannucci et al., 2017, Schneider, 2012). Using mice models with skin-specific knock-out of Dicer or Dgcr8, miRNAs have been shown to be essential for epidermal and hair-follicle development, maintenance and function (Andl et al., 2006, Yi et al., 2006, Yi et al., 2009). Several miRNAs are highly expressed in the skin, suggesting their potential functional roles in skin morphogenesis (Yi et al., 2006). For example, miR-203 as a skin-specific miRNA promotes keratinocyte differentiation and restricts cell proliferation, and it defines a molecular boundary between proliferative basal progenitors and differentiating suprabasal cells (Sonkoly et al., 2007, Sonkoly et al., 2010b, Yi et al., 2008). Inhibition of miR-34a and miR-34c restores keratinocyte cell cycle progression (Antonini et al., 2010). Among the highest expressed miRNA in skin stem cells, miR-125b serves as a repressor of stem cell differentiation (Zhang, 2011), and miR-205 controls neonatal expansion of stem cells and is indispensable for postnatal epidermal homeostasis (Teta et al., 2012, Wang et al., 2013). In terms of dermal homoeostasis, miR-145 is highly expressed in fibroblasts, and its inhibition enhances reprogramming of dermal fibroblasts to induced pluripotent stem cells (Barta et al., 2016).

The roles of microRNAs have also been studied in different skin disorders. Our colleagues were the first to demonstrate differentially expressed miRNAs in psoriasis (Sonkoly et al., 2007), and further studied the functions of the top regulated ones. For instance, higher expression of miR-21 in psoriasis may contribute to skin inflammation by inhibiting T cell apoptosis (Meisgen et al., 2012). Up-regulation of miR-31 in psoriasis contributes to skin inflammation by regulating communication between immune cells and keratinocytes (Xu et al., 2013). Also up-regulated in psoriasis, miR-146a suppresses TLR2-induced as well as IL-17-mediated inflammatory

responses in keratinocytes (Meisgen et al., 2014, Srivastava et al., 2017). In atopic dermatitis, miR-155 is overexpressed and increases T-cell proliferative responses (Sonkoly et al., 2010a). In skin cancer, miR-125b is top down-regulated in cutaneous squamous cell carcinoma (cSCC) and functions as a tumour suppressor (Xu et al., 2012). Whereas miR-31 is overexpressed in cSCC and enhances tumour cell motility and colony formation ability. (Wang A. et al., 2014). MiR-203 acts as a tumour suppressor in both cSCC and melanoma (Lohcharoenkal et al., 2018, Lohcharoenkal et al., 2016).

1.5 MICRORNAS IN SKIN WOUND HEALING

Emerging studies have shown that miRNAs play key roles in all phases of normal skin wound healing and also involved in the pathogenesis of chronic wounds (Herter and Landen, 2017, Li and Landen, 2017).

1.5.1 MiRNAs in normal skin wound healing

1.5.1.1 Inflammation phase

Several miRNAs have been found to regulate wound inflammation. For instance, miR-146a functions as a negative regulator of inflammatory response in keratinocytes and macrophages (Hou et al., 2009, Srivastava et al., 2017, Taganov et al., 2006, Xu, 2012). Its expression is upregulated by activation of Toll-like receptors (TLR2-5) (Taganov et al., 2006). As a negative feedback loop, miR-146a inhibits TLR-2-induced inflammation by targeting several key factors within the NF-κB signalling pathway such as IL-1 receptor-associated kinases 1 (IRAK1), IRAK2, and tumour necrosis factor receptor-associated factor 6 (TRAF6) (Meisgen et al., 2014). The level of miR-146a was found decreased in the inflammation phase of human skin wound healing (Li et al., 2015) and also in the wound of diabetic mice with increased NF-κB signalling (Xu, 2012).

A mouse model lacking miR-155 expression exhibits reduced inflammatory cells recruitment and faster healing with improved regenerated architecture (van Solingen et al., 2014, Yang et al., 2014). In a rat model, miR-155 inhibition shows faster healing of acute wounds, with reduced inflammation (Ye et al., 2017). MiR-23b has recently been shown to promote cutaneous wound healing *in vivo* via inhibition of the inflammatory responses (Li, 2018). MiR-21 has been studied in multiple aspects regarding inflammation in wounds. MiR-21 has been identified as an important regulator for resolving inflammation in wounds, which inhibits LPS-induced inflammatory response and increases IL-10 production in macrophages (Das et al., 2014, Recchiuti et al., 2011). In another study, it has been shown that overexpression of miR-21 in wounded rat model significantly improves the healing rate owing to activation of AKT/PI3K signalling pathway by targeting PTEN. (Han et al., 2017).

1.5.1.2 Proliferation phase

Besides its anti-inflammatory role, miR-21 also facilitates migration of keratinocytes and fibroblasts (Madhyastha et al., 2012, Yang et al., 2011), and promotes proliferation and fibrogenesis of fibroblasts from hypertrophic scars or keloid (Li et al., 2016, Liu et al., 2014, Zhu et al., 2014). Interestingly, both inhibition (Wang et al., 2012, Yang et al., 2011) and overexpression (Pastar et al., 2012) of miR-21 delayed wound healing *in vivo* by impairing re-epithelialization and granulation tissue formation. A parallel study have shown that miR-21 KO mice exhibits decreased healing rate possibly due to the targeting of Smad7-Smad2/3-Elastin pathway (Li et al., 2018). Additionally, knock-in or intradermal injection of miR-21 in aged mice significantly benefits wound healing by improving age-associated skin wound defects (Long et al., 2018).

There're several microRNAs reported as multiple players involved in diverse cellular functions. MiR-130a delays re-epithelialization in human *ex vivo* skin wound model by targeting early growth response factor 3 (EGR3) and leptin receptor (LepR) in primary human keratinocytes (Pastar et al., 2012). It also promotes angiogenesis by targeting mesenchyme homeobox 2 (MEOX2) and homeobox A5 (HOXA5) in endothelial cells (Chen and Gorski, 2008). MiR-210 expression is induced by hypoxia through stabilizing HIF-1α, which results in downregulation of the cell-cycle regulatory protein E2F3 in keratinocytes and impairs re-epithelialization (Biswas et al., 2010). Hypoxia-induced miR-210 also promotes angiogenesis by inhibiting the receptor tyrosine kinase ligand Ephrin-A3 (Fasanaro et al., 2008). Moreover, as an "ischemic memory", increased miR-210 expression attenuates keratinocytes proliferation. Accordingly, local inhibition of miR-210 improved ischemic wound closure (Ghatak et al., 2016). In another independent study, miR-219-5p is also induced by hypoxia in keratinocytes, and its target TMEM98 could inhibit inflammatory response while enhance cell proliferation and migration (Tang and Ran, 2018). In addition to its anti-inflammatory role, local overexpression of miR-155 accelerates *in vivo* wound healing by targeting MMP2 to promote keratinocytes migration (Yang et al., 2017).

For re-epithelialization specifically, miR-17-3p promotes keratinocyte proliferation and migration (Yan et al., 2017). An acute upregulation of miR-200c were reported at the early phase of wound healing in aged skin, and miR-200c inhibits keratinocyte migration and delays re-epithelialization of human *ex vivo* wounds (Aunin et al., 2017). MiR-203 shows a strong expression in the hyperplastic suprabasal epidermis surrounding the wound but is almost completely absent in the migratory front, and in terms of function miR-203 inhibits keratinocytes proliferation and migration, contributing to re-epithelialization (Deppe et al., 2016, Viticchie et al., 2012).

As for angiogenesis, overexpression of miR-148b promotes endothelial cell migration, proliferation and angiogenesis by targeting TGFB2 and SMAD2 (Miscianinov et al., 2018). Down-regulation of miR-200b by hypoxia was reported in endothelial cells, which induces angiogenesis to accelerate wound healing by targeting ETS-1, GATA2 and VEGFR2 (Chan et al., 2011, Chan et al., 2012). A synthetic inhibitor of microRNA-92a positively regulates angiogenesis and wound healing in both db/db mice and normal pigs (Gallant-Behm et al., 2018). Furthermore, inhibition of miR-92a improves wound healing in diabetic mice by targeting Itga5 and Sirt1 to enhance cell growth and angiogenesis (Lucas et al., 2017).

1.5.1.3 Remodelling phase

MiR-29 family is important regulator in fibroblasts of multiple organs associated with TGF-β1-directed fibrogenesis (Cushing et al., 2011, Jafarinejad-Farsangi et al., 2015, Maurer et al., 2010, van Rooij et al., 2008, Yang et al., 2013). In a previous report, *in vivo* local delivery of miR-29b improves wound remodelling as depicted by reduced contraction, increased collagen III/I ratios(Guo et al., 2018, Monaghan et al., 2014), and reduces excessive scar formation owing to suppression of TGF-β1-Smad-CTGF signalling pathway (Guo et al., 2017). MiR-98 has been proved to directly target Col1α1 gene, leading to declined viability and increased apoptosis of fibroblasts (Bi et al., 2017). MiR-185 also play as an inhibitor of fibroblast growth, through directly targeting TGF-β1 and Col-1 genes (Xiao et al., 2017). In a rat wound healing model, miR-149 ameliorates arrangement of collagen bundles and attenuates inflammatory cell infiltration (Lang et al., 2017). MiR-203 increases epidermal stem cell differentiation to myofibroblasts by targeting Hes1, and inhibition of miR-203 accelerates wound closure and reduces scar formation accompanied with increased collagen reassignment (Zhou et al., 2018). In addition, miR-1908 has been found to increase fibroblast proliferation and production of TGF-β1, IL-1α, TNF-α and type I collagen, and miR-1908 inhibitor reduces scar formation in burn wounds(Xie et al., 2016).

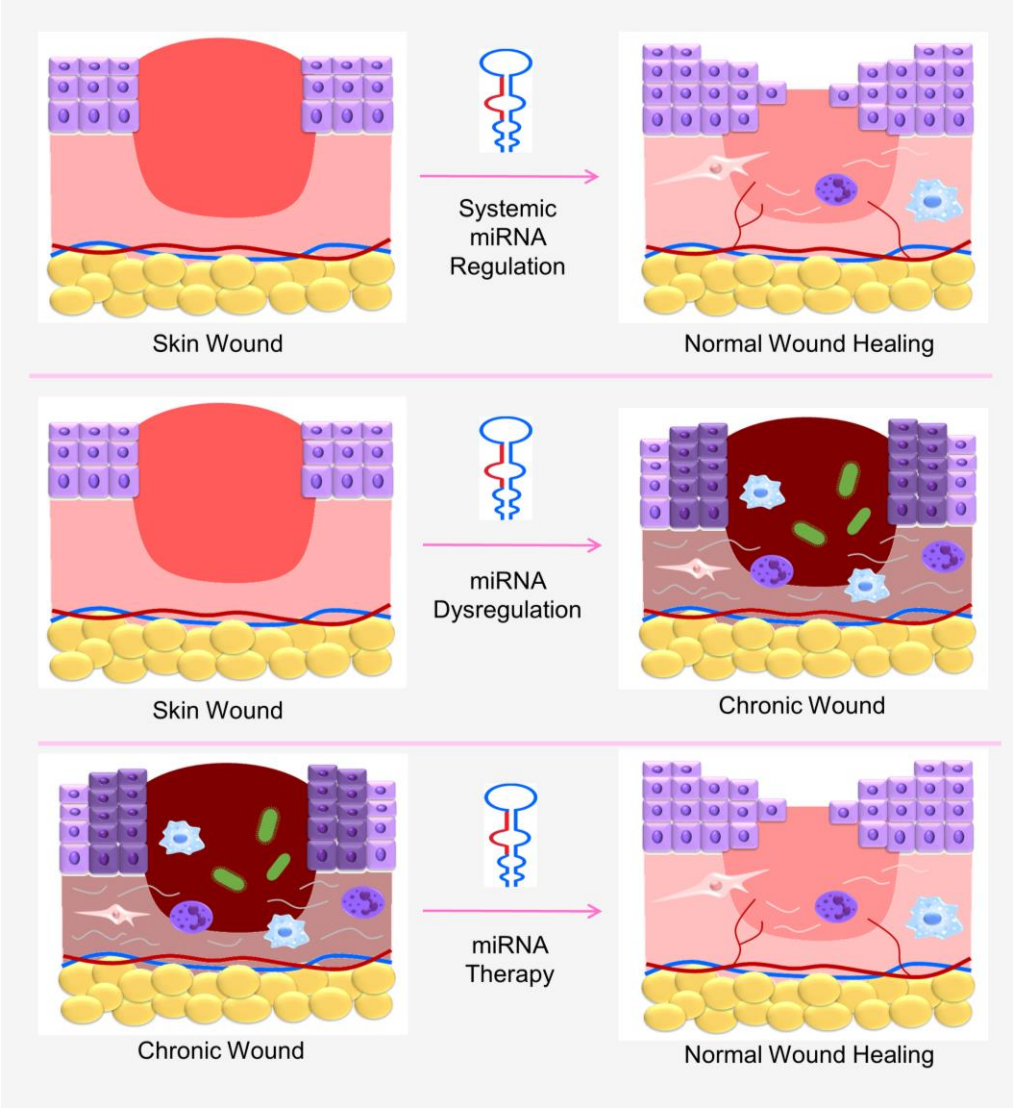


Fig 5. Schematic illustration to demonstrate our research outline.

1.5.2 MiRNAs in chronic wounds

Aberrant expression and function of several miRNAs have been reported in chronic wounds. In mouse diabetic model, miR-26a is found upregulated in the wounds compared with wild-type mice, and inhibition of miR-26a accelerated wound closure by inducing angiogenesis and increasing granulation tissue formation (Icli et al., 2016). Increased expression of miR-15b in diabetic mouse wounds is associated with impaired angiogenic response (Xu et al., 2014). MiR-27b rescues impaired the functions of bone marrow-derived angiogenic cells and improves wound healing (Wang J. M. et al., 2014). TNF-α neutralization leads to miR-200b downregulation, which supports wound closure by improving angiogenesis in diabetic mice (Chan et al., 2012).

A study comparing the plasma miRNA profile in type 2 diabetic (T2DM) patients with and without chronic wounds identified 41 deregulated miRNAs, among which decreased miR-191 and miR-200b levels were validated. Furthermore, the authors showed that miR-191 secreted by endothelial cells or platelets was either taken up by dermal endothelial cells to reduce angiogenesis, or taken by fibroblasts to inhibit migration, thereby causing delay in wound repair (Dangwal et al., 2015). In another study it was shown that hematopoietic stem cells of diabetic mice differentiate less into macrophages, where decreased let-7d-3p directly upregulates Dnmt1, leading to impaired wound healing (Yan et al., 2018). A recent study reported miR-129 and miR-335, which are significantly downregulated in the serum and skin tissues of diabetic patients, and are known to improve diabetic wound healing by targeting Sp1 to repress MMP9 expression (Wang et al., 2018).

MiR-146a has been found decreased in diabetic mouse wounds (Xu, 2012), and application of miR-146a conjugated with cerium oxide nanoparticles improved wound healing of diabetic mice without impairing biomechanical properties of the healed skin (Zgheib et al., 2018). Additionally, miR-296-5p is also found significantly decreased in amputated lower limb tissues of diabetic patients compared with the adjacent normal tissue, and overexpression of miR-296-5p inhibits β-cells proliferation by targeting sodium-glucose transporter 2 and increases the healing rate of diabetic wounds both *in vivo* and *in vitro* (Liu X. et al., 2018). In primary fibroblast derived from diabetic foot ulcers in comparison to nondiabetic foot fibroblasts, integrative analysis of miRNA and mRNA paired expression profiling reveals inhibition of cell growth and motility, as well as enhancement of cell differentiation and senescence, where induced miR-21-5p, miR-34a-5p and miR-145-5p play important roles (Liang et al., 2016).

In a porcine biofilm-infected wound model, miR-146a and miR-106b are induced by biofilm at wound-edge, which impairs epidermal barrier function (Roy et al., 2014). Experiments with miR-142$^{-/-}$ mice indicates that miR-142 family is indispensable for the clearance of S. *aureus* from wound sites to prevent infection (Tanaka et al., 2017). In *S. aureus*-infected wild-type mice wounds, treatment with miR-223 KO neutrophils or anti-miR-223 markedly rescues the healing process from the chronic, slow healing wounds, with prolonged neutrophil activation and enhanced clearance of bacteria (de Kerckhove et al., 2018). *S. aureus*, the prevalent pathogen in DFUs, were found to induce miR-15b-5p expression in both acute wounds and DFUs, and miR-15b-5p represses DNA repair and inflammatory response (Ramirez et al., 2018).

Negative-pressure wound therapy (NPWT) is one of the mostly used and effective methods in treating chronic wounds, where miR-195 is revealed markedly upregulated 7 days after NPWT together with increased microvessel density in granulation tissue (Liu Y. et al., 2018).

MiR-29a expression is suppressed in DFU, but treatment of mesenchymal stem cells (MSCs) has been shown to improve diabetic wound healing via increasing miR-29b expression which in turn leads to decreased expression of MMP9, thereby improving collagen I content in diabetic wounds (Xu et al., 2017). Bone marrow-derived mesenchymal stem cells (BM-MSC) treated diabetic mice wounds exhibit lower miR-155 expression and faster wound healing (Jiang L., 2016). Another report suggested that molecular modification of MSCs by inhibiting miR-205 improves their therapeutic effects on diabetic wounds by relieving its target VEGF expression (Zhu et al., 2017).

Together, these studies indicate that miRNAs play important roles in the pathophysiology of chronic wounds, which makes them promising therapeutic and diagnostic entities.

2 AIMS

In this thesis, we aimed to explore the roles of several important microRNAs and the gene networks regulated by them in the human skin wound healing process and in chronic non-healing wounds.

The objectives of this research were

- To understand the expression and function of miR-31 in epidermal keratinocytes during normal skin wound healing (paper I);
- To determine the functional role of miR-34 family in keratinocytes of normal wounds and in venous ulcers (paper II);
- To identify the function of miR-132 in dermal fibroblasts during skin wound healing (paper III);
- To explore the therapeutic potential of miR-132 in diabetic ulcers (paper IV).

3 MATERIALS AND METHODS

Patients and healthy donors

In this study, patients with non-healing ulcers, including venous ulcer patients (VU) and diabetic foot ulcer patients (DFU), that persisted for more than 6 weeks were enrolled. Exclusion criteria were apparent soft tissue infection in need of systemic antibiotics, under systemic antibiotics 24 hours prior to biopsy, as well as immune-compromised patients. Tissue samples were taken using a 4-mm biopsy punch at the non-healing edges of chronic wounds (Fig 6A). In addition, we recruited healthy donors who have no diabetes, skin disease, unstable heart disease, infections, bleeding disorder, immune suppression, and any on-going medical treatments. We created full-thickness excisional wounds using a 4-mm biopsy punch on the skin of each healthy donor, and the excised skin was saved as an intact skin control. The wound-edge tissue was collected using a 6-mm biopsy punch one and / or seven days later (Fig 6B). Local lidocaine injection was used for anaesthesia while sampling. All donors have signed a written informed consent for the collection and use of clinical samples. The study was approved by the Stockholm Regional Ethics Committee and conducted according to the Declaration of Helsinki Principles.

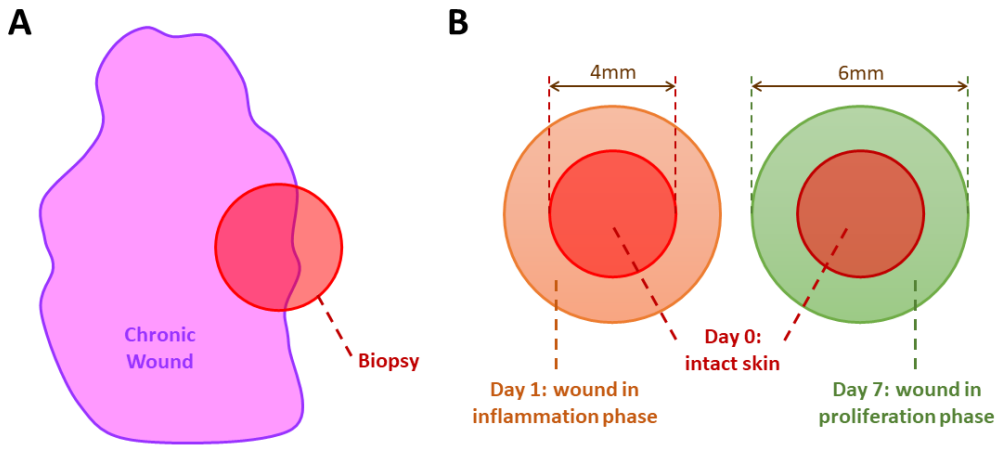


Fig 6. Schematic diagram of biopsy collection from chronic non-healing wound-edges (A) and from healthy volunteers (B).

Human *ex vivo* wound model

We collected human skin that was discarded from abdominal reduction surgeries. All the skin donors were provided informed consent for the study. The study was approved by the Stockholm Regional Ethics Committee and conducted according to the Declaration of Helsinki Principles. A superficial wound was created using a 2-mm biopsy punch on the skin, then the wound was excised using a 6-mm biopsy punch, and subsequently transferred to a 12-well cell culture plate to let the tissue attach to the plate. Next, Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FBS) and antibiotics (penicillin 100 units/L and streptomycin 100 µg/ml; ThermoFisher Scientific, Carlsbad, CA) were added around the skin tissue, and let its epidermal surface exposed in the air to create a liquid-air interface (Fig 7). We topically treated the wounds with 0.1 nmol mirVana hsa-miR-132-3p mimic or miRNA negative control (ThermoFisher Scientific) packed in transfection reagent MaxSuppressor™ *In Vivo* RNA-LANCEr II (Bioo Scientific, Austin, TX) and then dissolved in 30% pluronic F- 127 gel (Sigma-Aldrich, St Louis, MO). Wound samples were collected 3 and 5 days after injury for gene expression and histological analysis.

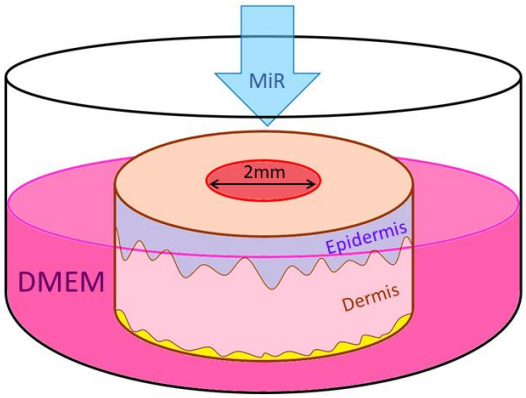


Fig 7. Schematic diagram of human *ex vivo* wound model.

Mouse in vivo wound model

Male wildtype C57BL/6N mice (WT mice) and diabetic BKS(D)-Lepr^{db}/JOrlRj mice (db/db mice) on the C57Bl/6J background were obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice were caged individually for one week, handled daily, before experiments. The hair on the back was shaved with an electric clipper followed by a depilatory cream and then washed with phosphate-buffered saline (PBS, 1X) one day before wounding. General anaesthesia was

performed with 3% isoflurane (Abbott, Chicago, IL, USA) and two full-thickness wounds were made on the dorsum using a 4-mm biopsy punch. Indicated dose of miRNA mimics or negative control (ThermoFisher Scientific) were mixed with MaxSuppressor™ *In Vivo* RNA-LANCEr II (Bioo Scientific) according to manufacturer's instruction in a total volume of 100µl for each wound. The mixtures were injected intradermally into the wound-edges of mice immediately after wounding. After the surgical procedure, mice received intraperitoneal buprenorphine (0.03 mg/kg) for relief of possible pain and distress. The size of wound area was photographed every other day until euthanized. The wound area was measured using ImageJ 1.32 software (National Institutes of Health) and expressed as percentage of the original wound size. The mice were sacrificed at the specified time points after injury, and wound-edge tissues, intact skin and internal organs were collected for histology, gene expression and protein analysis.

Laser capture microdissection (LCM)

Human skin or wound tissues were embedded in Tissue-Tek (Thermo Scientific) and snap-frozen; mice tissues were fixed with formalin and then embedded in paraffin. Tissues were sectioned in 10-µm thickness and stained with hematoxylin. Laser capture microdissection was performed with Leica LMD7000 (Leica, Bernried, Germany). RNA from microdissected tissue was purified using miRNAeasy mini kit or miRNAeasy FFPE kit (Qiagen, Hilde, Germany).

Magnetic-activated cell sorting (MACS)

Fresh human skin or wound samples were washed with cold PBS (1X) for 2–3 times and then incubated in 5 U/mL dispase (ThermoFisher Scientific) overnight at 4°C. Epidermis was separated from dermis using forceps. Epidermis was diced using scissors and then digested in Trypsin/EDTA Solution (ThermoFisher Scientific) for 15 minutes at 37°C. From the epidermal cell suspension, CD45- and CD45+ cells were separated using CD45 microbeads with MACS MS magnetic columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Dermis was incubated in the enzyme mix from the whole skin dissociation kit (Miltenyi Biotec) for 3 hours and further processed by Medicon tissue disruptor (BD Biosciences, San Diego, CA). Dermal cell suspension was sorted sequentially through CD90, CD14 and CD3 microbeads with MACS MS magnetic columns according to manufacturer’s instructions (Miltenyi Biotec).

Cell culture, transfections and treatments

Human dermal fibroblasts (HDFs) were isolated from adult human skin obtained from abdominal reduction plastic surgery. Briefly, after dispase digestion, dermis was cut into small pieces and washed with PBS (1X). After dermal tissue attached to cell culture plate of 12-well plate, DMEM medium supplemented with 10% FBS and 1% penicillin streptomycin (100 units/mL Penicillin and 100 µg/mL Streptomycin, ThermoFisher Scientific) was added into the plate. After sufficient fibroblasts grew out from the dermal tissue which usually takes a week, the dermis pieces were removed and the cells were cultured to be confluent. The third to tenth passages of HDFs were used in this study.

Human adult epidermal keratinocytes (HEKa) were purchased from Cascade Biologics and cultured in EpiLife serum-free keratinocyte growth medium supplemented with Human Keratinocyte Growth Supplement at a final Ca^{2+} concentration of 0.06 mM and with penicillin streptomycin (ThermoFisher Scientific) at 37°C in 5% CO_2. The fourth-passage of keratinocytes were used in this study.

HDFs or HEKa at 60% confluence were transfected with 20 nM mimics of a specific miRNA or miRNA negative control (ThermoFisher Scientific); 20 or 40 nM miRNA miRCURY LNA Power inhibitor or negative control A (Exiqon, Hilde, Germany); 20 or 30 nM Silencer select predesigned siRNA for specific gene or siRNA negative control (ThermoFisher Scientific) for 24 or 48 hours using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA).

In some experiments, cells were treated with TGF-β1, TGF-β2 (10 ng/mL, Immuno tools), SB431542 (15 µM, Tocris), rh-TNF-α (50 ng/mL, R&D Systems, Minneapolis, MN), or Calycuin A (50 nM, Cell signalling).

RNA extraction

Skin and wound biopsies from human or mice were homogenized in liquid nitrogen using a Mikro-Dismembrator U (B. Braun Biotech Inc.) or using Tissue Lyser LT (Qiagen) prior to RNA extraction. Total RNA was extracted from tissues using the miRNeasy Mini kit (Qiagen) and from cells using Trizol Reagent (ThermoFisher Scientific). RNA quantity and quality were determined using Nanodrop 2000 or Nanodrop one (ThermoFisher Scientific) and Agilent2200 Bioanalyzer chip (Agilent Technologies, Stockholm Sweden).

Transcriptomic profiling and analysis

Gene expression profiling of human or mice samples were performed using Affymetrix human HuGene-2_1-st, Clariom™ S or mouse Clariom™ D assays (ThermoFisher Scientific) at the microarray core facility of Karolinska Institute. Gene ontology analysis was performed according to the Database for Annotation, Visualization and Integrated Discovery (DAVID v6.8) (Huang da et al., 2009a, 2009b). Gene set enrichment analysis (GSEA) was performed using public software from Broad Institute (Mootha et al., 2003, Subramanian et al., 2005). The data have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number (Edgar et al., 2002).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was reverse transcribed using specific miRNA reverse transcription (RT) primers or miRNA RT primer pool with MicroRNA Reverse Transcription Kit (ThermoFisher Scientific) for miRNA detection, or using RevertAid First Strand cDNA Sythesis Kit (ThermoFisher Scientific) for mRNA detection. Expression of miR-132, miR-31, miR-34a-5p and miR-34c-5p was determined by Taqman miRNA assays and normalized to RNU48 in human and U6 in mice. Expression of primary precursors of miR-132 or miR-31, RASA1, MKI67, EMP1, TNF, IL1B, IL8, CXCL1, CXCL5, CCL2, CCL20, LGR4, Tnf, Il1a, Il1b, Il6, Cxcl1, Cxcl5, Cxcl10, Ccl2 and Lgr4 was detected by TaqMan based pre-designed qPCR assays (ThermoFisher Scientific or Integrated DNA Technologies, Coralville, IA). Gene expression was normalized based on the values of 18S rRNA or GAPDH in humans, and Gapdh or Actb in mice. Gene expression was detected by QuantStudio™ 7 Flex Real-Time PCR System (ThermoFisher Scientific), and relative expression was calculated using ΔΔCt.

Histology, immunohistochemistry (IHC), and immunofluorescence (IF) staining

Tissues were paraffin-embedded and sectioned in 6μm thickness by microtome. After deparaffinization and rehydration, hematoxylin and eosin (H&E) staining were performed. The length and area of newly-formed epithelial tongue in wounds were quantified using Image J (National Institutes of Health).

IHC were performed to detect Gr-1 (1:100, BD Biosciences), Krt5 (1:2000, BioLegend, San Diego, CA), LGR4 (1:200, Sigma), CD45 (1:200, Abcam) and PCNA (1:20000, Abcam), which

were detected using specific first antibodies and the horseradish peroxidase (HRP)-conjugated secondary antibodies (DakoCytomation, Glostrup, Denmark). Second antibodies were visualized using VectaStain ABC Kit and AEC or DAB Peroxidase Substrate Kit (Vector Laboratories, Burlingame, CA).

IF were performed to detect Ki-67 (1:400, Cell Signaling Technology), α-SMA (1:200, Abcam, Cambridge, UK) and EMP1 (1:400, Abcam). Primary antibodies were detected with Alexa Fluor 564-conjugated goat anti-rabbit IgG (H+L) (ThermoFisher Scientific) and visualized with a fluorescence microscope (Zeiss, Goettingen, Germany). DAPI (4',6-diamidino-2-phenylindole, ThermoFisher Scientific) was used for nuclear staining.

Matched IgG isotype controls were included as negative control for each staining. All slides were evaluated by at least two independent observers unaware of the identity of the samples, and multiple sections were evaluated for each sample.

In situ hybridization (ISH)

ISH was performed on formalin-fixed paraffin-embedded sections. Briefly, after deparaffinization, sections were treated with proteinase K (2 µg/mL) for 5 minutes at 37 °C and prehybridized for 30 minutes at 50/ 49°C for miR-132/ miR-31 respectively. Hybridization was performed for 2 hours at 50/ 49°C with a miR-specific or scramble digoxigenin (DIG)-labeled miRCURY locked nucleic acid probe (25nM) (Exiqon, Vedbaek, Denmark). Next, the slides were washed with 2X Saline-Sodium Citrate buffer (SSC) followed by incubation at 55/ 67°C (for miR-132/miR-31 respectively) with 0.1X SCC buffer. The sections were incubated with alkaline phosphatase (AP) - conjugated sheep anti-DIG Fab fragments (1:1000, Roche, Mannheim, Germany) for one hour at room temperature. The probe was visualized by adding BM purple AP substrate according to the manufacturer's instructions (Roche).

Protein detection

Cell lysates prepared with radioimmunoprecipitation assay (RIPA) buffer (ThermoFisher Scientific) were resolved on 4-20% gradient gels and transferred onto nitrocellulose membrane (Bio-Rad). Western blotting was performed for EMP1 (1:1000, Abcam), LGR4 (1:500, Santa Cruz Biotechnology, Dallas, Texas), p-p65 Ser468 (1:1000, Cell Signaling Technology, Danvers, MA), p-p65 Ser536 (1:1000, Cell Signaling Technology), p65 (1:1000, Cell Signaling

Technology), p-GSK-3β (1:1000, Cell Signaling Technology), GSK-3 (1:5000, BD Transduction Laboratories) and β-actin (1:20000, Sigma). Immune complexes were detected by Amersham™ ECL™ Prime Western Blotting Detection Reagent according to the manufacturer's protocol (GE Healthcare, Buckinghamshire, UK).

Conditioned medium from cultured keratinocytes was collected, and protein levels of IL-8 and CXCL5 were measured by enzyme-linked immunosorbent assay (ELISA) (BioLegend, San Diego, CA) according to the manufacturer's instructions.

Neutrophil chemotaxis assay

Whole blood was collected by venipuncture from healthy volunteers in 0.2% EDTA-anticoagulated tubes. Erythrocytes were removed using dextran sedimentation (1:1 mixture of blood with 6% dextran/0.9% NaCl) followed by hypotonic lysis using ddH_2O. Neutrophils were isolated from the resulting cell suspension using Ficoll-Histopaque (Sigma, St. Louis, MO) density centrifugation. Purified neutrophils were suspended in EpiLife medium. Using a 3µm polyethylene terephthalate membrane cell culture insert (BD Biosciences), $3x10^5$ neutrophils were added to the inner chamber, while the outer chamber contained conditioned medium from treated or control keratinocytes. After incubation for 1.5 hours at 37 °C in 5% CO_2, the migrated neutrophils in the outer chamber were counted using CountBright™ absolute counting beads (ThermoFisher Scientific) in a FACScan flow cytometer (Beckman Coulter CyAn ADP).

Analysis of cell growth

CyQUANT® Cell Proliferation Assay was performed according to the manufacturer's instructions (Invitrogen, Karlsruhe, Germany).

EdU (5-ethynyl-2'-deoxyuridine) cell cycle assay was performed using Click-It™ EdU Imaging Kit (ThermoFisher Scientific) performed according to the manufacturer's instructions and analyzed on a FACScan flow cytometer (Beckman Coulter CyAn ADP).

For colony formation assays, HEKa were seeded into 12-well plate (8000 cells/well) 24 hours after transfection. After 7-10 days, the cells were stained with 0.1% crystal violet (Sigma-Aldrich, Saint Louis, MO) and photographed. Methanol was used to dissolve crystal violet and absorbance value was measured at 540 nm.

Analysis of cell migration

For scratch assay, when transfected HDFs were grown to full confluence in collagen-coated 6-well plate, a scratch was made manually with a 10 µL pipette tip. The cells were incubated with DMEM medium supplemented with mitomycin C (10 µg/mL, Sigma-Aldrich) and photographed under microscope at the indicated time points. The wound areas were measured using Image J (National Institutes of Health). Healing rate = 100% - percentage of the initial wound area size. We also evaluate cell migration using IncuCyte® live cell analysis system. Transfected HEKa were grown to full confluence in ImageLock 96-well plate (Essen BioScience, Ann Arbor, MI) and scratch wound was made using an Essen® 96-pin WoundMaker™ (Essen BioScience). The assay plates were incubated in the IncuCyte® live cell analysis system (Essen BioScience), and the IncuCyte® ZOOM software was set to scan the plates every 2 hours and analyze cell migration.

Transwell migration assay was performed using the Corning® Matrigel® Chamber 8 µm (BD Falcon). $1x10^5$ transfected cells in growth-factor-free medium were placed into the inner chamber of the insert. Medium containing growth factor was added into the outer chamber. 24 hours later, the cells migrated through the membrane were stained with 0.1% crystal violet and counted under microscope.

Luciferase reporter assay

Luciferase reporter plasmids (SwitchGear Genomics, Menlo Park, CA.) were designed to contain either wildtype 3'-UTR of predicted miRNA target gene or mutation of the predicted target sites. HEKa were co-transfected with 0.1 µg of the luciferase reporter plasmids with 2 pmol miRNA or control mimics using Lipofectamine™ 2000 in a 96-well plate. Luciferase assays were performed 48 hours after transfection using the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

Statistics

Statistical significance was determined by two tailed Student's t-test or Wilcoxon Matched Pairs Signed Rank Test or Mann-Whitney U Test. Differences between groups were computed using two-way repeated-measures ANOVA. The correlation between the expressions of different

genes in the same sample was made using Pearson's correlation test. For all statistical tests, P values < 0.05 were considered to be statistically significant.

4 RESULTS AND DISCUSSION

4.1 MICRORNA-31 PROMOTES RE-EPITHELIALIZATION BY TARGETING EMP1

We have performed a miRNA profiling and identified miR-31 as one of the top upregulated miRNAs in the human wounds at the inflammatory phase compared to the intact skin (Li et al., 2015). Interestingly, increased miR-31 expression has also been found in psoriasis (Morhenn et al., 2013, Xu et al., 2013) and in cutaneous squamous cell carcinoma (Bruegger et al., 2013b, Wang A. et al., 2014), both of which are skin diseases with keratinocyte hyper-proliferation. Moreover, miR-31 has been reported to regulate keratinocyte differentiation (Peng et al., 2012) and hair growth (Mardaryev et al., 2010). In this study, we aimed to understand the role of miR-31 in human skin wound healing.

4.1.1 MiR-31 expression is up-regulated in wound-edge keratinocytes

In human *in vivo* skin wound model (Fig 6B), we found that the levels of both primary transcript and mature form of miR-31 were gradually increased with wound healing, which was shown by qRT-PCR analysis (Fig 8 A-B; Paper I, Fig 1a-c). Moreover, we separated epidermal and dermal compartments of the skin and wound-edges using LCM, and we found that miR-31 was majorly up-regulated in the epidermal keratinocytes during wound repair (Fig 8C), which was further confirmed by miR-31 in situ hybridization assay (Fig 8D; Paper I, Fig 1d-e). Therefore, in the following study, we focused on the role of miR-31 in keratinocytes.

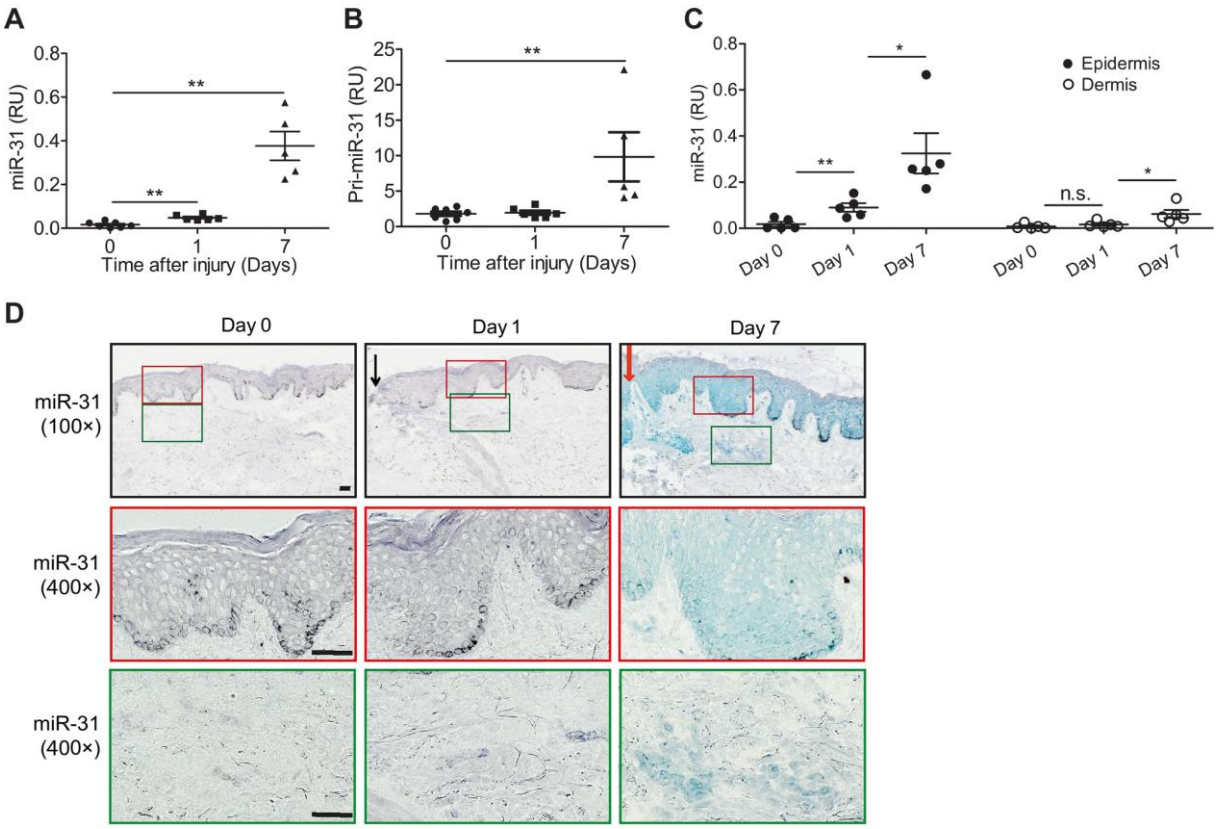


Fig 8. The expression of mature (A) and primary transcript of miR-31 (B) in human wound biopsies detected by qRT-PCR. (C) MiR-31 expression in the epidermis and dermis of human wounds separated by LCM. (D) In situ hybridization of miR-31 in human skin (Day 0) and wounds (Day 1 and Day 7 after injury). *Reproduced with permission from Li et al., 2015. Copyright The Society of Investigative Dermatology and Elsevier.*

4.1.2 MiR-31 promotes keratinocyte proliferation and migration

Either double-stranded miR-31 mimics (pre-miR-31) or miR-31 specific inhibitors (anti-miR-31) were transfected to human primary keratinocytes to overexpress or knock-down miR-31 respectively. To study the impact of miR-31 on keratinocyte growth, we analysed the transfected keratinocytes with CyQUANT cell proliferation assay (Fig 9A; Paper I, Fig 2a), detection of proliferation marker Ki-67 expression (Fig 9B; Paper I, Fig 2b), colony formation assay (Fig 9C; Paper I, Fig 2d-e) and EdU cell cycle assay (Fig 9D; Paper I, Fig 2c). And we found that miR-31 promoted proliferation and long-term growth of keratinocytes. Moreover, we performed scratch wound healing assay (Fig 9E-F; Paper I, Fig 3a-b) and transwell migration assay (Fig 9G-H; Paper I, Fig 3c-d), both showing that miR-31 enhanced keratinocyte motility.

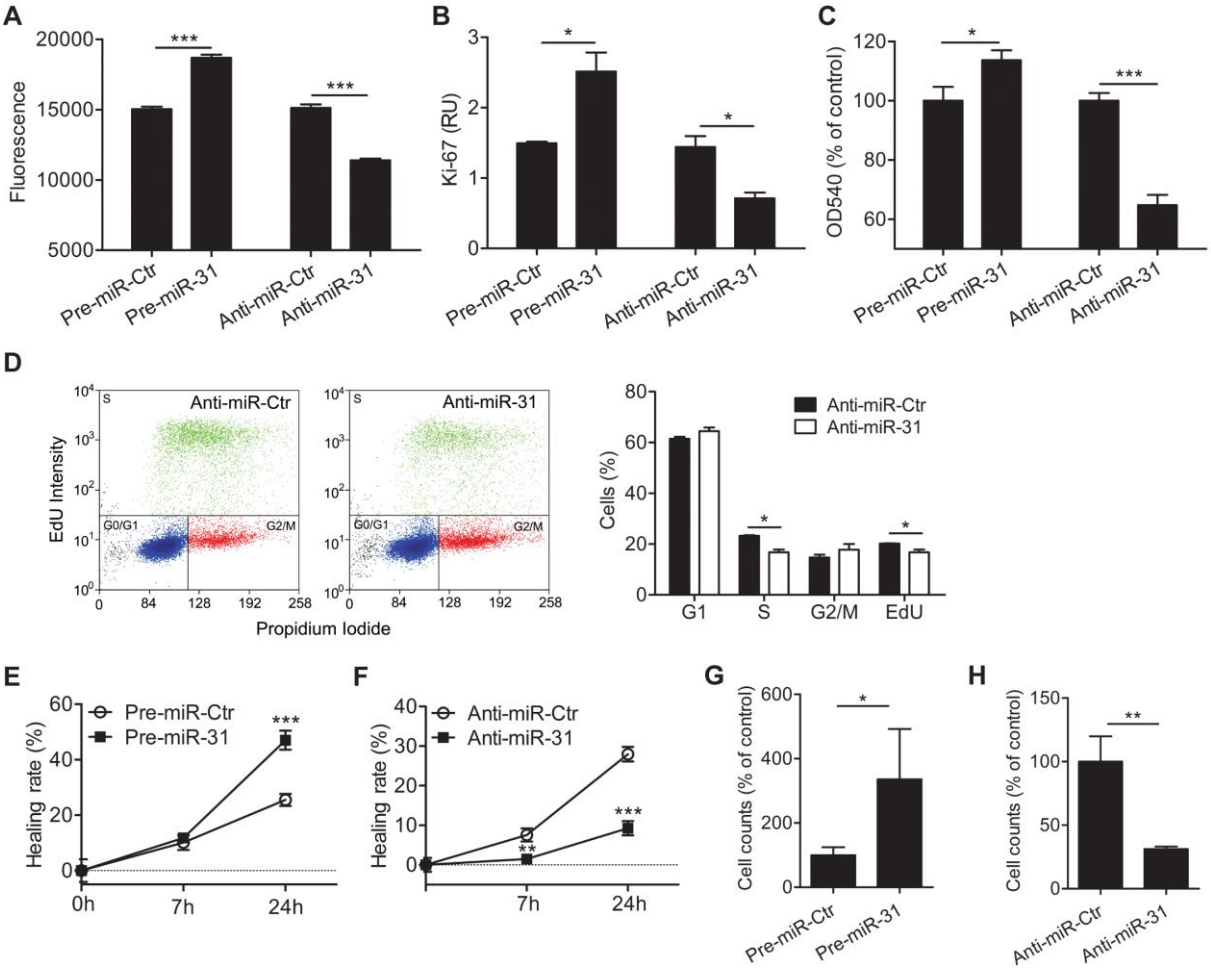


Fig 9. In HEKa transfected with miR-31mimics (pre-miR-31) or inhibitors (anti-miR-31), the following assays were performed: (A) CyQUANT cell proliferation assay. (B) Expression of proliferation marker Ki-67 detected by qRT-PCR. (C) Quantification of colony formation assay. (D) EdU cell cycle assay. (E-F) Scratch assay. (G-H) Transwell migration assay. *Reproduced with permission from Li et al., 2015. Copyright The Society of Investigative Dermatology and Elsevier.*

4.1.3 EMP1 is an important target of miR-31

Epithelial membrane protein 1 (EMP1) was previously identified as a direct target of miR-31 in oesophageal squamous cell carcinoma (Zhang et al., 2011), and here we confirmed this finding in HEKa using 3'UTR luciferase reporter assay (Fig 10A; Paper I, Fig 4a-c). Moreover, we showed that the expression of EMP1 was negatively correlated with the level of miR-31 in human keratinocytes (Fig 10B; Paper I, Fig 4d-e) and also during skin wound healing (Fig 10C-D; Paper I, Fig 4f-h). Furthermore, we found that silencing of EMP1 mimicked the effects of miR-31 overexpression in keratinocytes, i.e. enhancement of cell proliferation and migration (Fig 10E-G; Paper I, Fig 5), implying that EMP1 is a critical target mediating the functions of miR-31 in

keratinocytes. TGF-β1 was previously identified as an inducer of miR-31 expression in keratinocytes by our group (Xu et al., 2013), and here we discovered that TGF-β2 also induced miR-31 expression, repressing EMP1 expression (Paper I, Fig 6a-g).

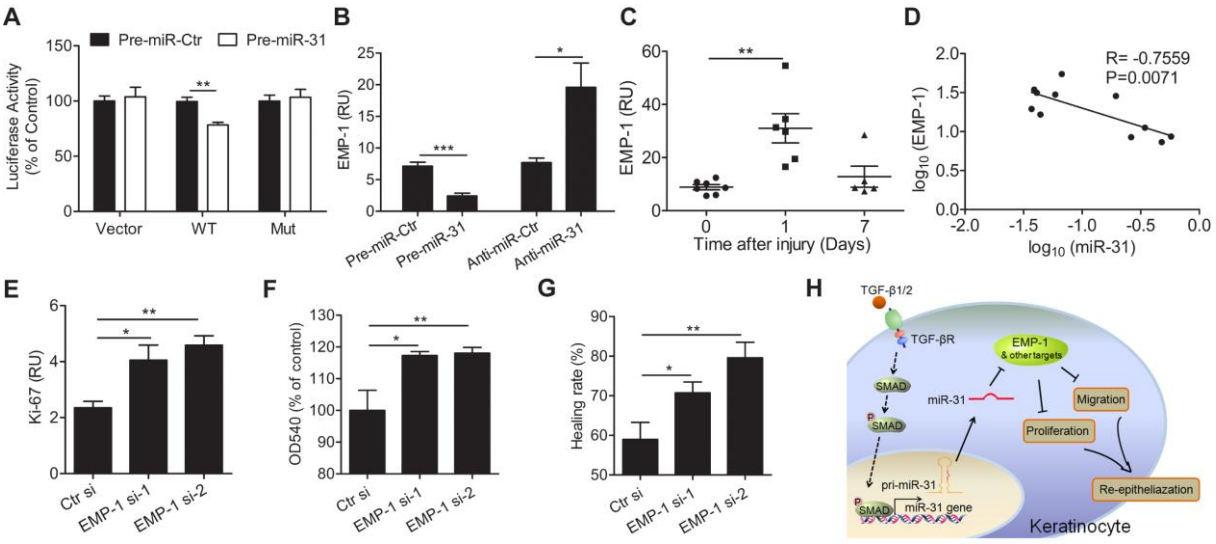


Fig 10. (A) Luciferase reporter plasmids containing wild-type (WT) or mutant (Mut) 3'-UTR of EMP1 were transfected into HEKa together with pre-miR-31 or control, and luciferase activity was measured. (B) Expression of EMP1 mRNA in keratinocytes overexpressing or lacking miR-31. (C) Expression of EMP1 mRNA in human wounds detected by qRT-PCR. (D) Spearman-correlation of EMP1 with miR-31 expression in human wounds. QRT-PCR of proliferation marker Ki-67 (E), colony formation assay (F), and scratch migration assay (G) were performed in HEKa with EMP1 expression silencing. (H) Schematic summary of the results in section 4.1. *Reproduced with permission from Li et al., 2015. Copyright The Society of Investigative Dermatology and Elsevier.*

4.1.4 Discussion

In this study, we characterized the dynamic expression pattern of miR-31 during normal human skin wound healing process. The basal level of miR-31 is low in the intact skin, while upon injury its expression gradually increases from day 1 (inflammatory phase) through day 7 (proliferative phase). Comparing this with its primary transcript (pri-miR-31), which is only upregulated in day-7 wounds, we assume that the change of miR-31 level during wound healing results from different regulatory mechanisms: the early increase of mature miR-31 is likely due to faster processing of the existing pri-miR-31, yet its later and stronger upregulation requires enhanced transcription of miR-31 gene.

To understand how the increased miR-31 expression affects skin wound healing, we performed different functional assays in human primary keratinocytes with miR-31 overexpression or inhibition. Our results reveal that miR-31 promotes both the proliferation and migration of keratinocytes, which are fundamental cellular events during the proliferative phase. Thereby, the high expression of miR-31 in keratinocytes in the proliferative phase may be important for the re-epithelialization process.

In addition to our findings, miR-31 has been known as an important regulator in other physiological processes of the skin, i.e., miR-31 regulates epidermal keratinocyte differentiation by enhancing Notch signalling (Peng et al., 2012); and miR-31 is upregulated in the anagen phase of hair cycle, while inhibition of miR-31 accelerates anagen development (Mardaryev et al., 2010). Moreover, miR-31 expression has also been found upregulated in several skin diseases, such as in psoriasis miR-31 promotes cytokine and chemokine production of keratinocytes (Morhenn et al., 2013, Xu et al., 2013); while in cutaneous squamous cell carcinoma miR-31 facilitates the motility and colony-forming ability of cancer cells (Bruegger et al., 2013a, Wang A. et al., 2014). Taken together, miR-31 is an important regulator in epidermal keratinocytes under both physiological and pathological conditions.

To understand the underlying molecular mechanism of miR-31-mediated regulation, we aimed to identify its direct targets. By combining bioinformatic and experimental strategies, here we demonstrate that EMP1 is targeted by miR-31 in keratinocytes. EMP1 has been mainly recognized as a tumour suppressor previously, as it inhibits growth and motility of different types of cancer cells (Sun G. et al., 2014, Sun G. G. et al., 2014a, Sun G. G. et al., 2014b, Sun G. G. et al., 2014c, Zhang et al., 2011). Here we show that EMP1 also suppresses growth and migration of normal human keratinocytes. In the wound-edge tissues from the inflammatory to the proliferative phases, we observed a negative-correlated expression pattern between miR-31 and EMP1, implying that miR-31 may down-regulate EMP1 expression in human wounds *in vivo*. However, the expression of both miR-31 and EMP1 is activated in day-1 wound compared to the intact skin, indicating that EMP1 expression is induced by other unknown factors at the early phase of skin wound healing, which warrants further investigation.

In conclusion, our study proposes that during normal skin wound healing, the increase of miR-31 level by TGF-β1 and TGF-β2 enhances proliferation and migration of keratinocytes, and miR-31 exerts these functions partially by targeting EMP1, which collectively facilitate re-epithelialization (Fig H; Paper I, Fig 6h). Our result is confirmed by a recent report using miR-31 loss-of-function mouse models (Shi et al., 2018), demonstrating miR-31 as an important molecule regulating keratinocyte function to improve wound healing.

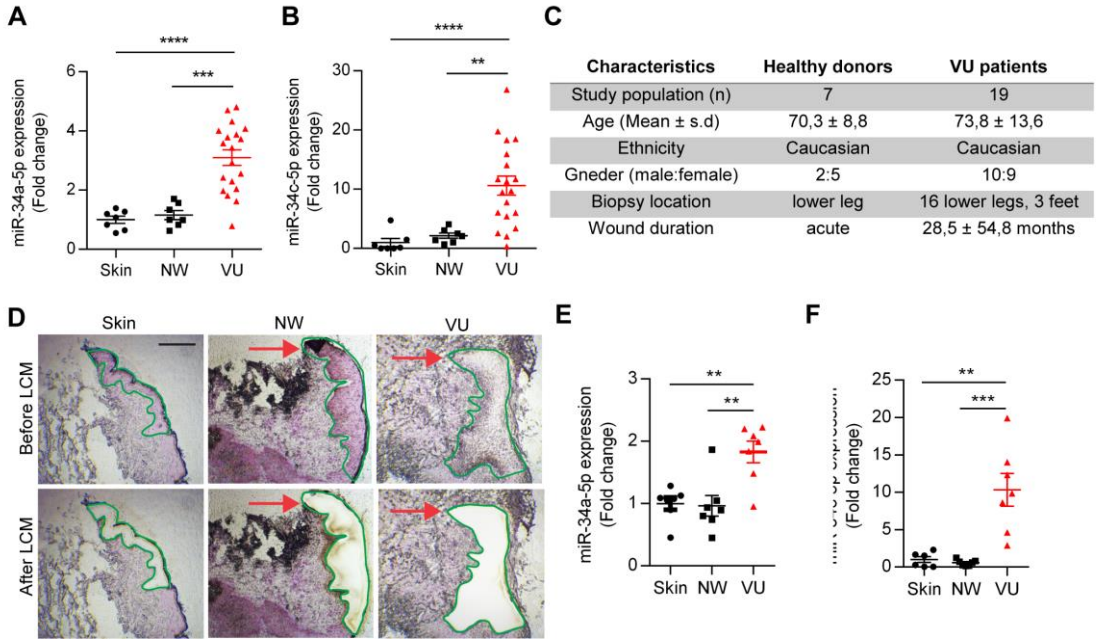
4.2 MICRORNA-34 FAMILY ENHANCES WOUND INFLAMMATION BY TARGETING LGR4

As a major cell type in epidermis, keratinocyte is not only indispensable for re-epithelialization, but also important in the immune response of the skin. When skin gets hurt, keratinocytes are the first to capture signals of physical trauma or other external stimuli, and start innate immune response by producing cytokines and chemokines (Chen and DiPietro, 2017, Strbo et al., 2014). The chronic non-healing wound has been characterized by excessive and prolonged inflammation, which impedes wound healing and causes additional tissue damage. However, largely still awaits to be explored about the role of keratinocytes in the chronic inflammation.

MicroRNA-34 family has been well known for their tumour suppressive functions, especially inhibition of cancer cell mitosis and migration. Moreover, miR-34a is the first microRNA entering clinical trial (ClinicalTrials.gov Identifier: NCT01829971 and NCT02862145) (Beg et al., 2017). Tumour and wound healing share various similar mechanisms (Sundaram et al., 2018), thus miR-34 raised our interest.

4.2.1 Increased miR-34a and miR-34c expression in the wound-edge keratinocytes of human venous ulcers

Our recent RNA-sequencing study identified miR-34a and miR-34c among the top up-regulated miRNAs in VUs compared with normal wounds (NW) or intact skin from healthy donors (unpublished data). MiRNA-34 family comprises three homologues, miR-34a, b and c, but only miR-34a-5p and miR-34c-5p, but not miR-34b, are detected in human skin and wound tissues by RNA-sequencing analysis (Paper II, Fig. S1). For validation, 19 VU samples were collected and compared with normal wounds or skin tissues from 7 healthy volunteers (Fig 11A; Paper II, Table 1). Both miR-34a and miR-34c were validated higher expressed in VU compared with either normal wounds or intact skin by qRT-PCR (Fig 11B-C; Paper II, Fig 1A-B). Besides, in the LCM isolated wound-edge epidermis, which are mainly composed of keratinocytes, more miR-34 expression was detected in the VUs than the skin or normal wounds (Fig 11D-F; Paper II, Fig 1C-E).



Characteristics	Healthy donors	VU patients
Study population (n)	7	19
Age (Mean ± s.d)	70,3 ± 8,8	73,8 ± 13,6
Ethnicity	Caucasian	Caucasian
Gneder (male:female)	2:5	10:9
Biopsy location	lower leg	16 lower legs, 3 feet
Wound duration	acute	28,5 ± 54,8 months

Fig 11. (A) Characteristics of healthy donors and VU patients. QRT-PCR analysis of miR-34a (B) and miR-34c (C) in the skin and wound biopsies. (D-F) QRT-PCR analysis of miR-34 expression in LCM separated epidermis.

4.2.2 MiR-34 enhances keratinocyte inflammatory response and impedes wound healing

Transcriptomic analysis of HEKa overexpressing miR-34a or miR-34c suggests that these miRNAs not only play a role in cell proliferation, but also in innate immune response and inflammation (Paper II, Fig 2). We show that in human primary keratinocytes, miR-34a and miR-34c promoted the production of several cytokines and chemokines, for instance TNF and CXCL5 (Fig 12A; Paper II, Fig 3A-B).

In wild-type mice (Fig 12B; Paper II, Fig 4A), liposome-encapsulated miR-34a mimics were intradermally injected around the wounds once after injury, which led to a transient overexpression of miR-34a (Fig 12C; Paper II, Fig 4B). Interestingly, local overexpression of miR-34a delayed wound closure macroscopically (Fig 12D, F; Paper II, Fig 4C-D) and impaired formation of new epidermal tongue microscopically (Fig 12E, G; Paper II, Fig 4E-F). Elevated cytokine and chemokine expression was observed in miR-34-treated wounds, indicating enhanced local inflammation (Fig 12I; Paper II, Fig 4G).

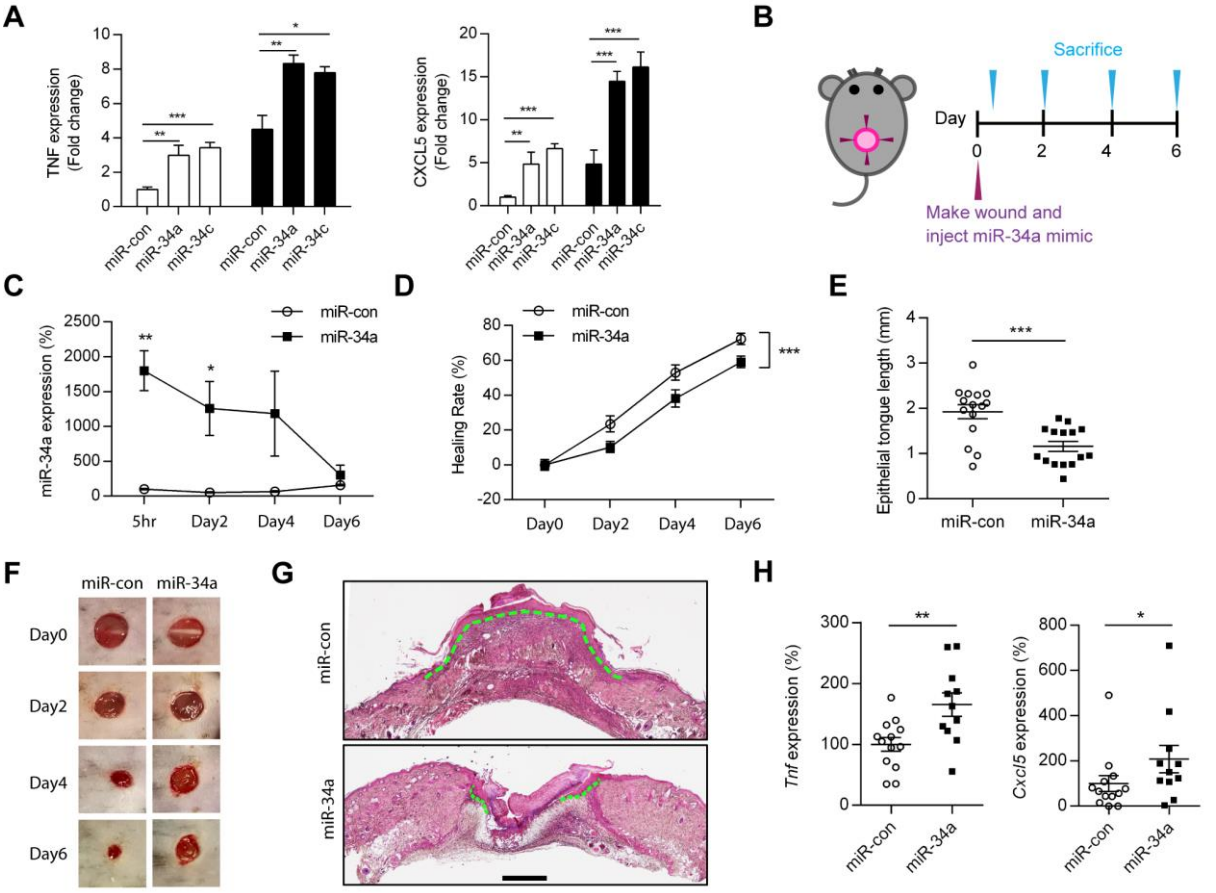


Fig 12. (A) QRT-PCR analysis of TNF and in HEKa overexpressing miR-34. (B) Experimental setup of mouse *in vivo* wound model. (C) QRT-PCR of miR-34a in treated mice wounds. (D) Quantification of wound closure. (E) Measurement of the length of the newly-formed epithelial tongue. (F) Representative pictures of mice wounds. (G) Representative pictures of H&E stained wound tissues. (H) QRT-PCR analysis of Tnf and Cxcl5 in mice wounds.

4.2.3 LGR4 is a target mediating the biological function of miR-34 in keratinocytes

Combining eleven different miRNA target algorithms and microarray of HEKa overexpressing miR-34a or miR-34c, five genes were commonly predicted as targets of miR-34. After checking the expression of these five genes in human skin and wound samples, we found that the level of LGR4 was negatively correlated with miR-34 expression *in vivo* (Fig 13A; Paper II, Fig 5A-C, Fig S5), suggesting that LGR4 may be a miR-34 target in human skin wounds. This was experimentally verified by 3'UTR luciferase reporter assay in human primary keratinocytes (Fig 13B; Paper II, Fig 5D-E, I). Accordingly, we showed that overexpression of miR-34 supressed LGR4 expression (Fig 13C; Paper II, Fig 5F-H).

Silencing of LGR4 in human keratinocytes results in increased IL-8 and CXCL5 production, which phenocopied the pro-inflammatory function of miR-34a and miR-34c (Fig 13D; Paper II, Fig 6A-E). Furthermore, while miR-34 inhibitor reduces cytokine and chemokines production, additional silencing of LGR4 could rescue this anti-inflammatory effect (Paper II, Fig 6F), indicating that miR-34a/c exert their pro-inflammatory function through targeting LGR4. Interestingly, we found that wound closure is slower in Lgr4 knock-out mice compared with wild-type mice (Fig 13E-F; Paper II, Fig 7A-C). Inflammatory molecular signature (such as increased tnf and Cxcl5 expression) and more infiltrated immune cells (Fig 13G-H; Paper II, Fig 7E-F) were detected in the wounds of Lgr4 KO mice compared with WT mice, which is similar as the mice wounds with miR-34a overexpression (Paper II, Fig 4).

Mechanistically, previous studies have reported that LGR4 inhibits GSK-3β serine 9 (Ser9) phosphorylation, thus increasing the proportion of the unphosphorylated active GSK-3β (Luo et al., 2016, Zhang et al., 2003); and GSK-3β phosphorylats p65 at Ser468, which negatively regulates NF-κB activity (Buss et al., 2004). And our findings link these two pieces of evidences, showing that miR-34-LGR4 axis regulates GSK-3β-induced p65 Ser468 phosphorylation, thus impacting the activity of NF-κB signal that is a central signalling pathway controlling cellular inflammatory response (Fig 13I; Paper II, Fig 8).

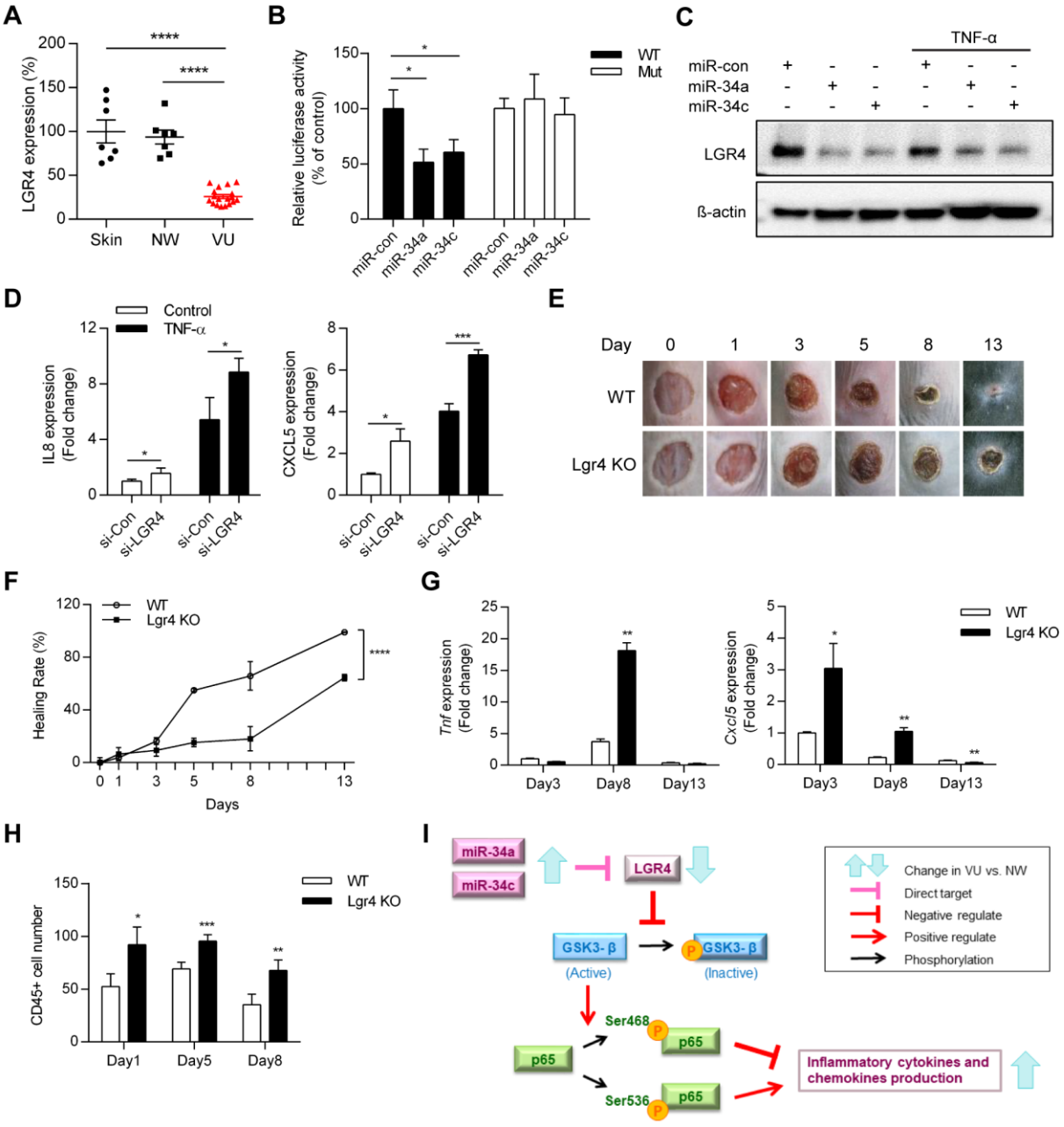


Fig 13. (A) The expression of LGR4 in VUs was detected by qRT-PCR. (B) Luciferase assay in human keratinocytes co-transfected wild-type or mutant LGR4 3'UTR luciferase reporters with miR-34 mimics. (C) Western blotting analysis of LGR4 in keratinocytes overexpressing miR-34. (D) QRT-PCR of IL8 and CXCL5 in HEKa with LGR4 silencing. (E) Representative pictures of WT and Lgr4 KO mice wounds. (F) Wound healing rate of WT or Lgr4 KO mice. (G) MRNA expression of Tnf and Cxcl5 in WT and Lgr4 KO mice wounds. (H) Quantification of immunohistochemistry staining of CD45+ immune cells in mice wounds. (I) Schematic summary of the results in section 4.2.

4.2.4 Discussion

MiR-34 family members are well-known tumour suppressor in a wide range of cancer types (Wang et al., 2015, Wong et al., 2011). Several *in vitro* studies have shown that overexpression of miR-34a reduces cell proliferation, migration, invasion, stemness while increasing apoptosis and senescence in various cancer cell lines (Bader, 2012, Maroof et al., 2014). In various animal models, treatment with miR-34a mimics have been shown to inhibit the growth of primary tumours, block metastasis, and improve survival (Adams et al., 2016, Bader, 2012, Moles, 2017). Nonetheless, a clinical trial of miR-34 treatment in cancer patients has been terminated recently, because of severe immune-related adverse events (Beg et al., 2017), which exposes an urgent need to understand the role of miR-34 in immune response. To this end, miR-34a has been shown to inhibit B cell development (Rao et al., 2010), facilitate dendritic cell differentiation (Hashimi et al., 2009), inhibit macrophages efferocytosis (McCubbrey et al., 2016), impair neutrophils migration but enhance their production of TNF (Cao et al., 2015, Shikama et al., 2016). Our study reveals that miR-34a and miR34c also increase the production of pro-inflammatory cytokines and chemokines from epidermal keratinocytes, enhancing the capacity of keratinocytes to recruit and activate immune cells in skin wounds, which conforms to the excessive inflammation of VUs where miR-34 is overexpressed.

We identified LGR4 as a direct target of both miR-34a and miR-34c, which is a leucine-rich repeat-containing G-protein coupled receptor. It has been reported that LGR4 induces keratinocyte proliferation (Jin et al., 2008, Kato et al., 2007, Wang et al., 2010), and loss of LGR4 results in partially impaired hair follicle development (Mohri et al., 2008). In addition, in macrophages LGR4 was reported to play an anti-inflammatory role (Du et al., 2013), and to enhance anti-inflammatory M2 polarization (Tan et al., 2018). Here we show that loss of LGR4 enhances inflammatory response and impairs wound re-epithelization.

Our study identified miR-34a and miR-34c among the top up-regulated miRNAs in VU compared to intact skin or acute wounds from healthy donors, which function to exacerbate keratinocyte inflammatory response and delayed wound closure by targeting LGR4, implying their pathological role in chronic wounds.

4.3 MICRORNA-132 FACILITATES FIBROBLAST MIGRATION BY TARGETING RASA1

In addition to epidermal keratinocytes, dermal fibroblasts also play an essential role in skin wound healing, such as to form granulation tissue, contract wound, regulate angiogenesis, assist re-epithelialization, produce extracellular matrix and reconstruct new dermis. Primary fibroblasts derived from the non-healing edges of chronic wounds display impaired cellular functions, including reduced proliferative and migratory capacities, decreased secretion and response to various growth factors, and excessive production of proteinases.

In our previous study (Li et al., 2015), microRNA-132 (miR-132) was identified as a top up-regulated miRNA in the inflammatory phase of human acute wounds compared with the intact skin from the same donor. We show that miR-132 impedes inflammatory response but boosts cell proliferation in primary epidermal keratinocytes by directly targeting HB-EGF. Local inhibition of miR-132 delays wound closure in mouse *in vivo* and human *ex vivo* wound models (Li et al., 2015). Following this research line, we further investigated the function of miR-132 in human dermal fibroblasts (HDFs).

4.3.1 The expression pattern of miR-132 during human normal skin wound healing

To reveal the expression pattern of miR-132 during human skin wound healing, we collected intact skin and acute wounds at the inflammatory phase (1 day after injury) and at the proliferative phase (7 days after wounding) from healthy volunteers (Fig 6; Paper III, Fig 1A). Using laser capture microdissection, we found that miR-132 expression was increased in both epidermal and dermal compartments of wound-edges compared to the intact skin (Fig 14A; Paper III, Fig 1B). Next, using MACS magnetic cell separation, we isolated several cell types from the skin and wounds, i.e. epidermal CD45- cells (mainly keratinocytes) and CD45+ cells (leukocytes), dermal CD90+ cells (fibroblasts), CD14+ cells (macrophages), and CD3+ cells (T cells). Interestingly, miR-132 was found highly expressed in human dermal CD90+ HDFs (Fig 14B; Paper III, Fig 1C) and its level was upregulated by skin injury (Fig 14C; Paper III, Fig 1D). In addition, we found TGF-β1 increased miR-132 transcription in HDFs, which effect could be specifically blocked by TGF-β receptor inhibitor (Paper III, Fig 1E-G).

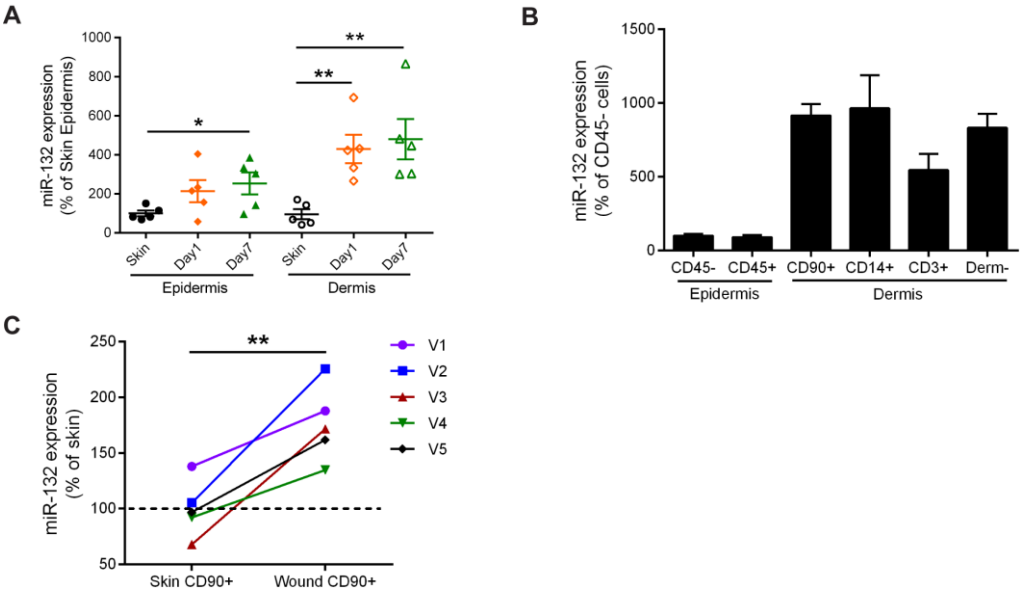


Fig 14. QRT-PCR of miR-132 in LCM separated human epidermis and dermis from human skin and wounds (A), in different cell populations isolated from human skin (B), in CD90+ cells isolated from the skin and acute wounds (C). *Reproduced with permission from Li et al., 2017. Copyright Springer Nature Publishing AG.*

4.3.2 MiR-132 promotes HDF migration by targeting RASA1

To reveal the role of miR-132 in dermal fibroblasts, microarray analysis was performed in HDFs transfected with miR-132 mimics. Gene ontology (GO) and gene set enrichment analysis (GSEA) analysis of microarray data suggested that miR-132 may have an impact on cell motility (Fig 15A-C; Paper III, Fig 2). Using both scratch wound healing assay (Fig 15D; Paper III, Fig 3A) and transwell migration assay in HDF transfected miR-132 mimics or inhibitors, we demonstrated that miR-132 promoted fibroblast migration (Fig 15E; Paper III, Fig 3C). (Paper III, Fig 3 B, D),

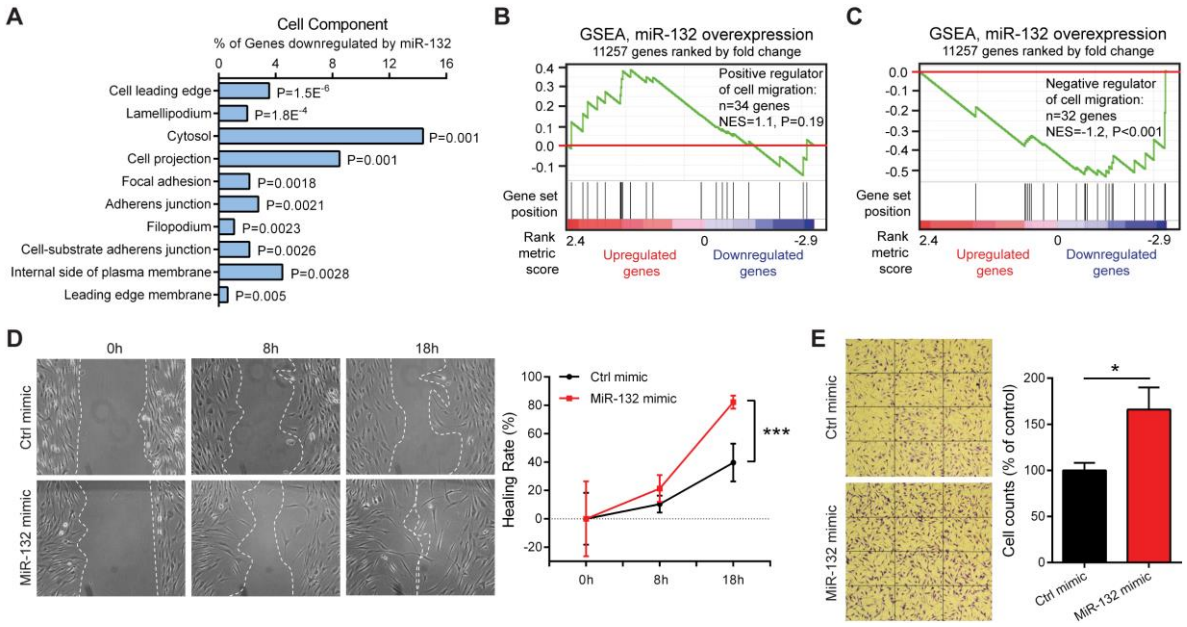


Fig. 15. (A) The top10 gene ontology of cell component terms for the genes down-regulated by miR-132 in fibroblasts. GSEA evaluated enrichment within the microarray data for the genes reported to accelerate (B) or impair (C) cell migration. Scratch assays (D) and transwell migration assays (E) for HDFs transfected with miR-132 mimics. *Reproduced with permission from Li et al., 2017. Copyright Springer Nature Publishing AG.*

In the transcriptomic analysis, we found that RAS signalling pathway was regulated by miR-132 (Fig 16A; Paper III, Fig 4A-B). Among the RAS related genes, we noticed that RASA1 (RAS p21 protein activator 1) was one of the top down-regulated genes upon miR-132 overexpression (Paper III, Fig 4C). RASA1 has been previously reported as a direct target of miR-132 in 293T cells and mouse dorsal root ganglion neurons (Anand et al., 2010, Hancock et al., 2014), and here we found miR-132 also supressed RASA1 expression in HDFs (Fig 16B; Paper III, Fig 4C-D), Moreover, silencing of RASA1 promotes HDF migration (Fig 16C-D; Paper III, Fig 5A-C), phenocopying miR-132 overexpression.

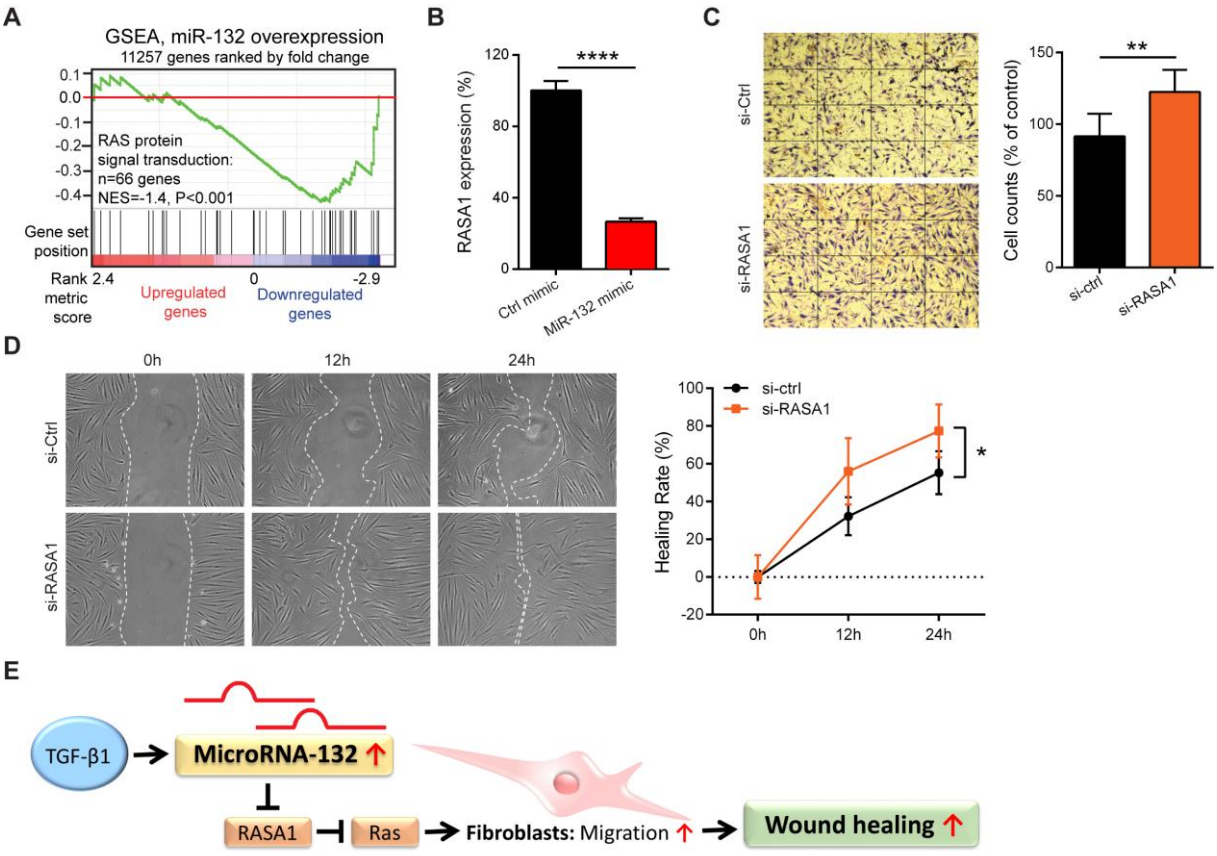


Fig 16. (A) GSEA for RAS-related genes within the microarray data. (B) Expression of RASA1 in HDFs overexpressing miR-132. Transwell migration assay (C) and scratch assay (D) for HDFs with RASA1 silencing. (E) Schematic summary of the results in section 4.3. *Reproduced with permission from Li et al., 2017. Copyright Springer Nature Publishing AG.*

4.3.3 Discussion

Together with our previous study (Li et al., 2015), we show that miR-132 expression is up-regulated in both wound-edge keratinocytes and dermal fibroblasts during normal skin wound healing (Fig 14; Paper III Fig 1A-D). During this study, we established MACS magnetic cell isolation protocol with limited quantity of human samples. Using this methodology, more detailed gene expression profiles could be studied in different cell populations from normal wound and chronic wound samples, which might be beneficial to identify key etiological factors and further to design treatment by correcting them.

Transcriptomic analysis of fibroblasts overexpressing miR-132 leads us to focus on the effect of miR-132 on cell migration. Using several functional assays, we demonstrate that miR-132 enhances the motility of HDFs. Moreover, we find that RAS signalling is regulated by miR-132

in HDFs and among the genes involved in this signalling pathway RASA1 is a direct target of miR-132. RAS is a small GTPase switching between an active GTP-bound and an inactive GDP-bound state, and RASA1 is one of the critical RAS GTPase activating proteins (RasGAP) which negatively regulates RAS activity by increasing the rate of GTP hydrolysis (Pamonsinlapatham et al., 2009, Rajalingam et al., 2007). Stimulated by tyrosine kinase or growth factor receptors, RAS gets activated and acts through its effectors, for instance RAF, PI3K, MEKK signalling cascades, thus controlling various basic cellular functions (Rajalingam et al., 2007). To our interest, RAS signalling has been shown to regulate the motility of fibroblasts and keratinocytes during skin wound healing (Ehrenreiter et al., 2005). Moreover, RASA1 interacts with p190 RhoGAP to regulate directed movement of fibroblasts (Kulkarni et al., 2000). It also inhibits cell migration by competing with Rab21 to bind integrins and regulating receptor trafficking (Mai et al., 2011). In accordance with this, we show that silencing of RASA1 increases fibroblast migration, which phenocopies the effects of miR-132, suggesting that the RASA1-RAS signal axis is critical in mediating the pro-migratory effect of miR-132 in fibroblasts.

In addition, miR-132 has been found highly expressed and play functional roles in some other cell types involved in skin wound healing (Fig 14B-C; Paper III, Fig 1B-C). Besides keratinocytes, the anti-inflammatory effects of miR-132 has also been reported in monocytes and macrophages (Essandoh et al., 2016, Li et al., 2015, Liu et al., 2015, Nahid et al., 2013). Moreover, miR-132 is up-regulated in neutrophils after their extravasation and infiltration into the skin (Larsen et al., 2013). MiR-132 has also been shown to enhance endothelial cell proliferation and improve vascularization (Anand et al., 2010, Devalliere et al., 2014, Lei et al., 2015, Mulik et al., 2012, Westenskow et al., 2013). Together with our findings showing that miR-132 promotes HDF migration, we propose that miR-132 may be a promising candidate to be used in wound treatment, which idea was tested in the paper IV.

4.4 MICRORNA-132 IMPROVES WOUND HEALING OF DIABETIC MOUSE MODEL AND HUMAN *EX VIVO* MODEL

Chronic wound is a growing health and economic problem worldwide. Diabetic foot ulcer (DFU) is the most difficult type of chronic wounds to treat, and it is associated with high amputation rate and mortality. Our previous studies revealed that in epidermal keratinocytes miR-132 has pro-proliferative and anti-inflammatory function (Li et al., 2015), and in dermal fibroblasts miR-132 plays a pro-migratory role (Paper III). Based on these findings, we further explored the therapeutic potential of miR-132 in chronic wounds, in particular DFU.

4.4.1 The expression of miR-132 is decreased in human diabetic foot ulcers and in diabetic mouse wounds

To study DFU, we have been collecting wound-edge biopsies of DFU from patients with type 2 diabetes mellitus and also acute wounds and intact skin from healthy volunteers (Fig 17A-B; Paper IV, Fig 1a, Table 1). MiR-132 expression is found significantly reduced in the DFU, especially in the epidermal compartment, compared to the normal skin wounds, shown by qRT-PCR (Fig 17C; Paper IV, Fig 1b) and *in situ* hybridization (Fig 17D; Paper IV, Fig 1c). Similarly, lower miR-132 expression was observed in wounds of leptin receptor-deficient (db/db) mice, which is a commonly used diabetic model, compared to the wild-type mice (Fig 17E; Paper IV, Fig 1d).

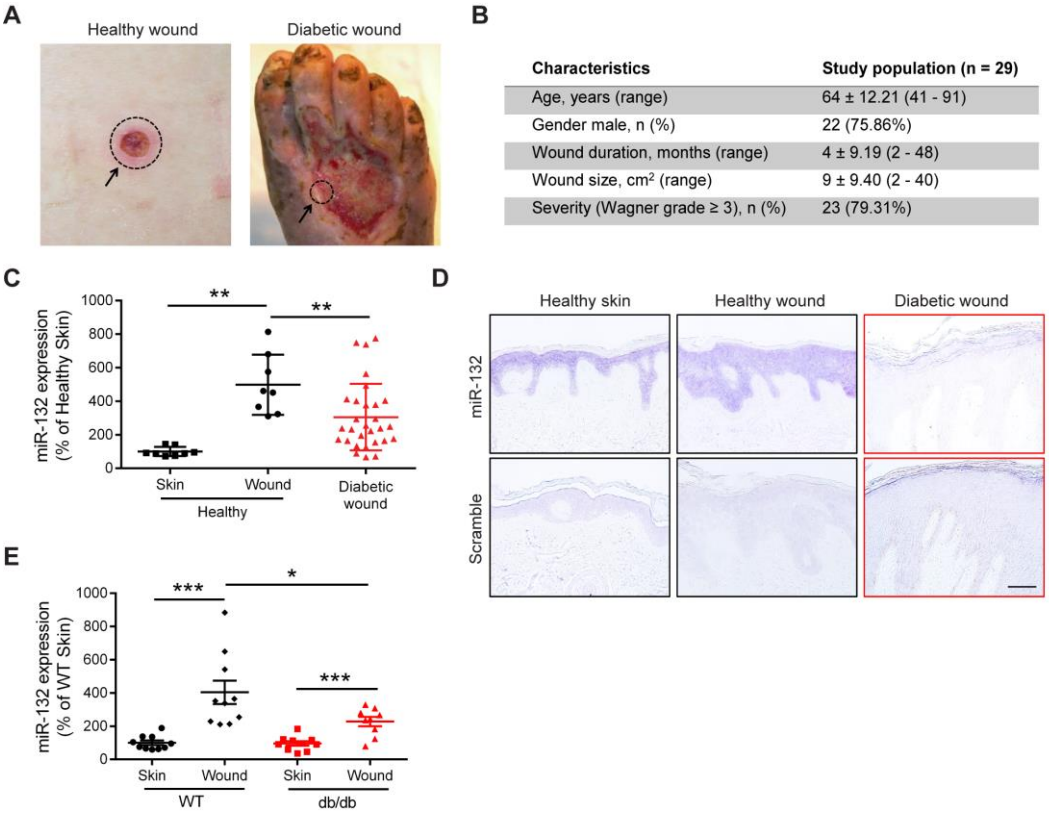


Fig 17. (A) Sample collection. (B) Characteristics of DFU patients. Analysis of miR-132 expression in human skin and wounds of healthy donors and DFUs by qRT-PCR (C) and *in situ* hybridization (D). (E) QRT-PCR of miR-132 in skin and wound of WT or db/db mice. *Reproduced with permission from Li et al., 2017. Copyright The Society of Investigative Dermatology and Elsevier.*

4.4.2 MiR-132 promotes wound healing of diabetic mice

Full-thickness wounds were made on the dorsum of db/db mice using a 4 mm biopsy punch (Fig 18A; Paper IV, Fig 2c), and then intradermal injection of miR-132 mimics around the wounds overexpressed miR-132 successfully (Fig 18B; Paper IV, Fig 2d-e). Local injection of miR-132 mimics accelerated wound healing of db/db diabetic mice, which was assessed by both macroscopic (Fig 18C-D; Paper IV, Fig 3a-b) and histomorphometry analysis (Fig 18E-G; Paper IV, Fig 3c-e). MiR-132 overexpression increased wound-edge keratinocyte proliferation (Fig 18H-I; Paper IV, Fig 3f-g) and reduced local Cxcl1 expression and neutrophil infiltration (Fig 18J-L; Paper IV, Fig 3h-j).

Transcriptomic analysis was performed in the mice wounds treated by either control or miR-132 mimics, showing that the miR-132 may negatively regulates several central inflammatory

signalling pathways, for example NF-κB signalling, TNF signalling, NOD-like receptor signalling and Toll-like receptor signalling pathways (Paper IV, Fig 4).

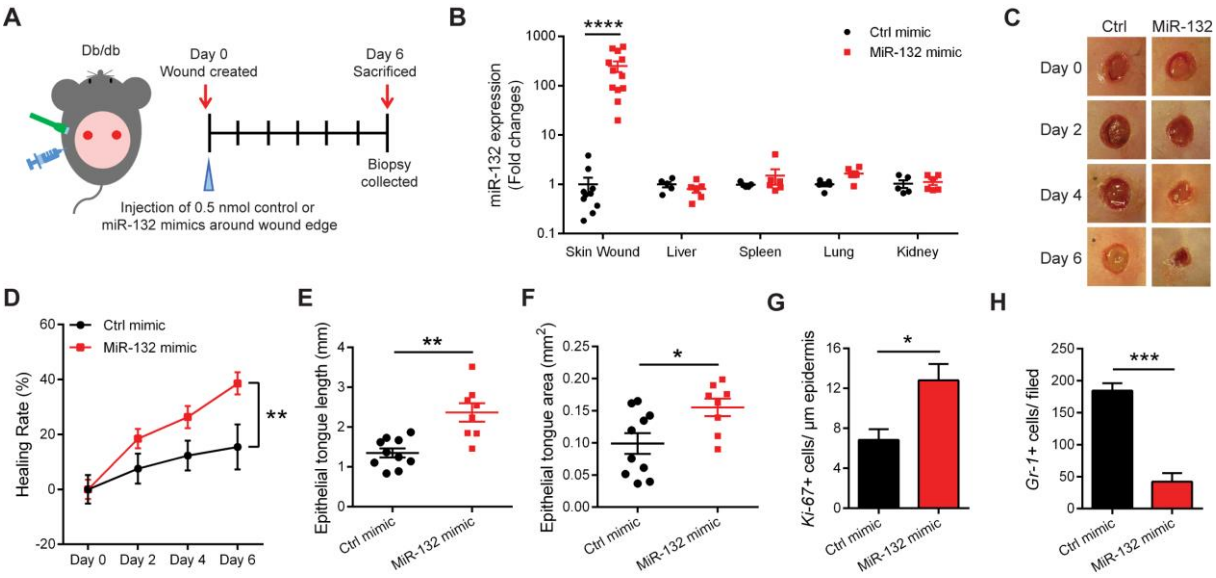


Fig 18. (A) Experimental setup. (B) Expression analysis of miR-132 in mouse wound and internal organs. (C) Representative pictures of wounds. (D) Wound closure is characterized as healing rate = 100% - the percentage of the initial wound area size (n = 12 wounds/ group). Quantification of the length (E) and area (F) of newly-formed epithelial tongue. Count of Ki-67 (G) and Gr-1 positive cells (H), detected by immunohistochemistry, in wound-edge epidermis. *Reproduced with permission from Li et al., 2017. Copyright The Society of Investigative Dermatology and Elsevier.*

4.4.3 MiR-132 accelerates re-epithelialization of human *ex vivo* wounds

We further tested the effect of topical application of miR-132 on re-epithelization of human *ex vivo* wound. Liposome-formulated miR-132 mimics were mixed with pluronic F-127 gel, which is a commonly used thermos-reversible vehicle gel, and then the mixture was topically applied on human *ex vivo* wounds right after wounding (Fig 19A; Paper IV, Fig 5a). Three days later, *ex vivo* wounds were confirmed for miR-132 overexpression (Fig 19B; Paper IV, Fig 5b). Five days after wounding, we found that the miR-132-treated wounds were completely re-epithelialized, but not the control-treated group (Fig 19C-D; Paper IV, Fig 5c-d). Increased number of Ki-67+ keratinocytes was observed in the wound-edge epidermis of the miR-132 treated wounds compared with the control group (Fig 19E; Paper IV, Fig 5e-f).

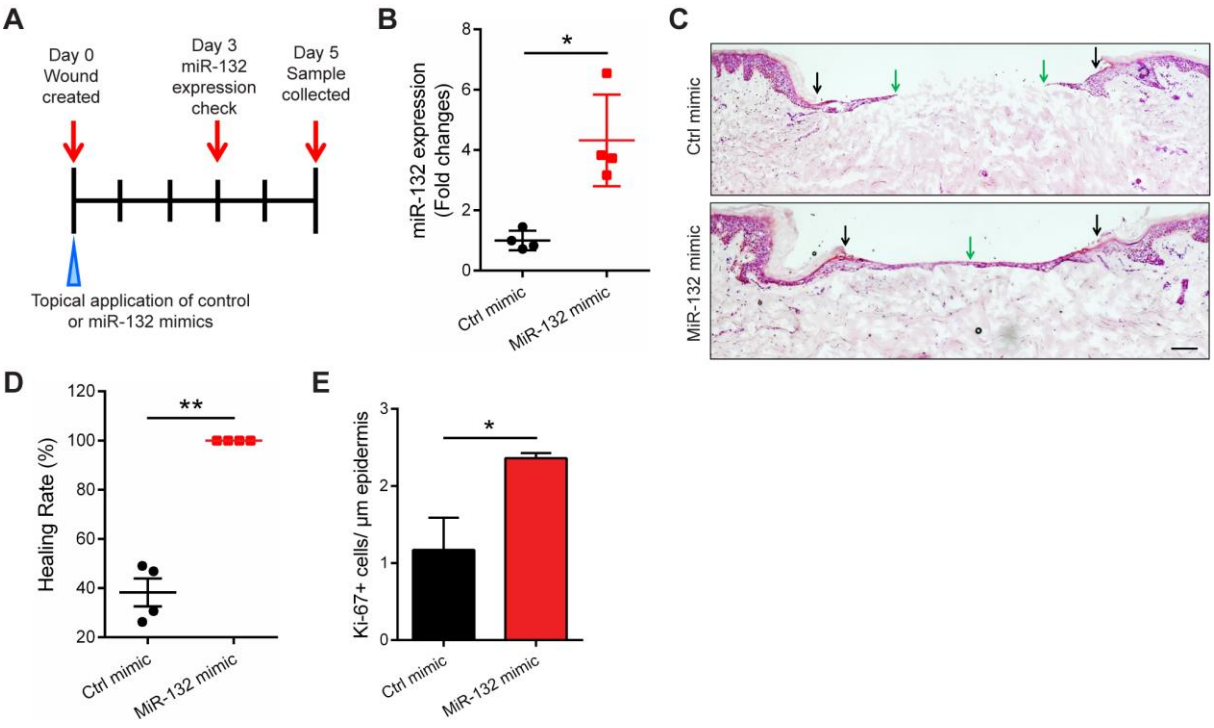


Fig 19. (A) Experimental setup. (B) QRT-PCR analysis of miR-132 in the treated *ex vivo* wounds. (C) Representative pictures of H&E stained wounds. (D) Re-epithelization is characterized as healing rate. (E) Count of Ki-67 positive cells, detected by immunohistochemistry, in wound-edge epidermis. *Reproduced with permission from Li et al., 2017. Copyright The Society of Investigative Dermatology and Elsevier.*

4.4.4 Discussion

In this study, we found a lower miR-132 expression in wound-edges of DFU. As TGF-β positively regulates miR-132 expression (Li et al., 2015), we hypothesize that the reduced level of miR-132 in DFU may result from lack of TGF-β signalling there (Jude et al., 2002).

We have previously shown that lack of miR-132 delays wound closure in WT mice with increased local inflammation and decreased cell growth (Li et al., 2015). Here we show that local administration of miR-132 accelerates wound healing in diabetic mice with suppressed inflammatory response and improved re-epithelialization. Transcriptomic analysis of miR-132-treated mice wounds (Paper IV Fig 4) reveals an anti-inflammatory molecular signature, confirming our previous results showing that miR-132 represses the activity of the NF-kB signalling (Li et al., 2015). As DFU is featured with prolonged and excessive inflammation, our findings suggest that miR-132 may be used to treat chronic wounds by repressing inflammation.

Contraction mainly contributes to the wound closure in rodents, but not in humans (Volk and Bohling, 2013). We have previously shown that miR-132 does not affect mouse dermal fibroblast contraction (Li et al., 2015). And here we found that there was no obvious difference of myofibroblast presence in the dermis between the miR-132 mimics-treated group and the control group (Paper IV Figure S6). In addition, we did not observe any obvious difference in hair cycle between the miR-132 and the control-treated groups (Paper IV Fig 3k). These data demonstrate that the accelerated wound healing in the miR-132-treated group is not due to the impacts of miR-132 on contraction or hair follicle cycling.

Dysregulation of microRNA exists in most human diseases, therefore modulation of their expression opens new gates to invent treatments. A few miRNAs have marched into clinical trials, e.g. miR-34 for solid tumours, miR-103/107 for diabetes, and miR-122 for Hepatitis C virus infection. Nevertheless, shortages and challenges remain, such as the instability of designed molecules, difficulty of localized delivery, lack of biocompatible vehicles and hard-to-predict adverse effects (Chakraborty et al., 2017, Rupaimoole and Slack, 2017). Wound healing is a good niche to test miRNA-based therapy owing to the obvious advantages of easy access of the application site for topical administration and possibility for phenotypic evaluation. After intradermal injection of miR-132, its increased expression was not observed in the inner organs (Fig 18B; Paper IV Fig 2d, S4), suggesting the local administration is specific. Besides, no changes were discovered in body weight, blood glucose, or morphology and histology of internal organs between the miR-132-treated group versus the control group (Paper IV Fig S3, S5), indicating low systemic toxicity. LCM of the injected mouse wounds reveals efficient overexpression of miR-132 in both epidermis and dermis (Paper IV Fig 2e), implying its effects in multiple cellular types within the wounds.

Together, we identify miR-132 as a downregulated miRNA in DFU and local treatment with miR-132 mimics promotes wound closure in diabetic mice and re-epithelization of human *ex vivo* wounds by regulating multiple inflammation-related signalling pathways.

5 CONCLUSION

Skin is an imperative physical and immunological barrier of our body. Any disruption in skin integrity needs to be amended properly and efficiently in order to avoid manifestation of secondary complications. Wound healing is a complicated biological process involving interaction between different cell types and biomolecules, in order to restore barrier properties of the skin. Often this cascade of well-coordinated healing events gets delayed leading to formation of chronic non-healing wounds. Hence, we found it interesting to explore the contribution of different epidermal, dermal and immune cells in the skin, towards its healing ability, and how it gets altered in chronic wounds, with the major focus on microRNA mediated interactions. We believe the knowledge in this direction still has many caveats that require better understanding of the pathophysiology in order to design effective treatments.

In this direction, we first studied the expression of miRNA in normal human skin wound healing process and in chronic non-healing wounds. Using miRNA profiling (Li et al., 2015), miR-31 and miR-132 were identified as top up-regulated miRNAs in the inflammatory phase of wound healing. The expression of miR-31 was mainly confined to epidermal keratinocytes and was found to reach maximum during the proliferation phase (Paper I). Whereas miR-132 was gradually up-regulated in the inflammatory and proliferative phases, especially in epidermal keratinocytes and dermal fibroblasts of wounds. Additionally, miR-132 was also widely expressed in other immune cells in both epidermis and dermis (Paper III). Furthermore, we found an obvious down-regulation of miR-132 expression in diabetic ulcers, the most difficult type of chronic wound to treat, compared with normal wounds from healthy donors (Paper IV). On the other hand, miR-34 family exhibited increased expression in the epidermis of venous ulcer, the most common type of chronic wound, compared with either acute wounds or the intact skin (Paper II).

Secondly, we explored the functions of miRNAs during different phases and in different cell types of skin wound healing. In the inflammatory phase, miR-132 was previously shown to be anti-inflammatory but pro-proliferative regulator of keratinocytes by targeting HB-EGF and activating EGFR, ERK, and STAT3 signalling (Li et al., 2015). In the proliferation phase, miR-31 was found to promote keratinocyte proliferation and migration by targeting EMP1, thereby to facilitate re-epithelialization (Paper I). And miR-132 improved the migratory ability of dermal fibroblasts via targeting RASA1 and regulating Ras signalling, which benefits granulation tissue formation (Paper III). Since chronic wounds are non-epithelialized, excessively inflamed and fibroblasts derived from chronic wounds are non-migratory, our study suggests that miR-31 and miR-132 have potential therapeutic importance for chronic wounds (Paper I, III).

Third, we aimed to understand how the dysregulation of microRNAs contributes to chronic wounds. MiR-34 family was identified among the top up-regulated non-coding RNAs in VU, which enhanced inflammatory response of epidermal keratinocytes via targeting LGR4 and raising the activity of NF-κB signalling (Paper IV), suggesting the dysregulation of miR-34 plays a pathological role in excessive inflammation of VU (Paper II). Similarly, we found the level of miR-132 was decreased in DFU (Paper IV), and lack of miR-132 impeded wound healing (Li et al., 2015).

Lastly, we examined the therapeutic potential of miRNA in chronic wounds. Based on previous findings and availability of animal models, we chose to test miR-132 in diabetic wounds. Interestingly, local overexpression of miR-132 accelerated wound closure in diabetic mice, where inflammatory cells and molecular signatures were remarkably reduced (Paper IV). Moreover, *ex vivo* human model also demonstrated accelerated re-epithelialization upon miR-132 topical application, indicating that local treatment of miR-132 may be further evaluated in a clinical trial as a potential treatment for chronic wounds (Paper IV).

Altogether, we have selected three top regulated genes from microRNA profiling of normal wounds from healthy volunteers and of chronic wounds from patients (data not shown in this thesis). Their functional significance and mechanistic insights have been explored in normal wound healing and chronic wounds: miR-31 has been studied in keratinocytes where we accented its importance for re-epithelialization of normal wound healing, miR-34 is one of the top upregulated microRNAs in venous ulcer with pro-inflammatory roles in keratinocytes and impaired mouse wound healing, and miR-132 has been explored in dermal fibroblasts with promising therapeutic potential in diabetic ulcers. In conclusion, this thesis highlights the crucial roles of miRNAs in keratinocytes and fibroblasts during skin wound healing, and demonstrates their dysregulation contributes to the pathogenesis of chronic wounds. Correction of these dysregulated miRNAs indicates a novel strategy to treat the chronic wounds such as diabetic ulcer and venous ulcer.

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7 REFERENCES

A Multicenter Phase I Study of MRX34, MicroRNA miR-RX34 Liposomal Injection.

Pharmacodynamics Study of MRX34, MicroRNA Liposomal Injection in Melanoma Patients With Biopsy Accessible Lesions.

Adams BD, Parsons C, Slack FJ. The tumor-suppressive and potential therapeutic functions of miR-34a in epithelial carcinomas. Expert Opin Ther Targets 2016;20(6):737-53.

Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013;14(8):475-88.

Amin N, Doupis J. Diabetic foot disease: From the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. World journal of diabetes 2016;7(7):153-64.

Anand S, Majeti BK, Acevedo LM, Murphy EA, Mukthavaram R, Scheppke L, et al. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nature Medicine 2010;16(8):909-U109.

Andl T, Murchison EP, Liu F, Zhang Y, Yunta-Gonzalez M, Tobias JW, et al. The miRNA-processing enzyme dicer is essential for the morphogenesis and maintenance of hair follicles. Curr Biol 2006;16(10):1041-9.

Antonini D, Russo MT, De Rosa L, Gorrese M, Del Vecchio L, Missero C. Transcriptional repression of miR-34 family contributes to p63-mediated cell cycle progression in epidermal cells. The Journal of investigative dermatology 2010;130(5):1249-57.

Aragona M, Dekoninck S, Rulands S, Lenglez S, Mascre G, Simons BD, et al. Defining stem cell dynamics and migration during wound healing in mouse skin epidermis. Nat Commun 2017;8:14684.

Aunin E, Broadley D, Ahmed MI, Mardaryev AN, Botchkareva NV. Exploring a Role for Regulatory miRNAs In Wound Healing during Ageing:Involvement of miR-200c in wound repair. Sci Rep 2017;7(1):3257.

Bader AG. miR-34 - a microRNA replacement therapy is headed to the clinic. Front Genet 2012;3:120.

Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2014;22(5):569-78.

Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2008;16(5):585-601.

Barta T, Peskova L, Collin J, Montaner D, Neganova I, Armstrong L, et al. Brief Report: Inhibition of miR-145 Enhances Reprogramming of Human Dermal Fibroblasts to Induced Pluripotent Stem Cells. Stem Cells 2016;34(1):246-51.

Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116(2):281-97.

Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136(2):215-33.

Beg MS, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. Invest New Drugs 2017;35(2):180-8.

Beidler SK, Douillet CD, Berndt DF, Keagy BA, Rich PB, Marston WA. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. J Vasc Surg 2009;49(4):1013-20.

Bi S, Chai L, Yuan X, Cao C, Li S. MicroRNA-98 inhibits the cell proliferation of human hypertrophic scar fibroblasts via targeting Col1A1. Biol Res 2017;50(1):22.

Biswas S, Roy S, Banerjee J, Hussain SR, Khanna S, Meenakshisundaram G, et al. Hypoxia inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds. Proc Natl Acad Sci U S A 2010;107(15):6976-81.

Blanpain C, Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol 2009;10(3):207-17.

Brem H, Young J, Tomic-Canic M, Isaacs C, Ehrlich HP. Clinical efficacy and mechanism of bilayered living human skin equivalent (HSE) in treatment of diabetic foot ulcers. Surgical technology international 2003;11:23-31.

Browse NL, Burnand KG. The cause of venous ulceration. Lancet (London, England) 1982;2(8292):243-5.

Bruegger C, Kempf W, Spoerri I, Arnold AW, Itin PH, Burger B. MicroRNA expression differs in cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. Experimental dermatology 2013a;22(6):426-8.

Bruegger C, Kempf W, Spoerri I, Arnold AW, Itin PH, Burger B. MicroRNA expression differs in cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. Experimental dermatology 2013b;22(6):426-8.

Buss H, Dorrie A, Schmitz ML, Frank R, Livingstone M, Resch K, et al. Phosphorylation of serine 468 by GSK-3beta negatively regulates basal p65 NF-kappaB activity. J Biol Chem 2004;279(48):49571-4.

Cao MW, Shikama Y, Anzai M, Kimura J. Impaired Neutrophil Migration Resulting from Mir-34a and Mir-155 Overexpressed in Neutrophils from Myelodysplastic Syndrome Patients. Blood 2015;126(23).

Cao Y, Gang X, Sun C, Wang G. Mesenchymal Stem Cells Improve Healing of Diabetic Foot Ulcer. Journal of Diabetes Research 2017;2017:10.

Cavanagh PR, Lipsky BA, Bradbury AW, Botek G. Treatment for diabetic foot ulcers. Lancet (London, England) 2005;366(9498):1725-35.

Chakraborty C, Sharma AR, Sharma G, Doss CGP, Lee SS. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. Mol Ther Nucleic Acids 2017;8:132-43.

Chan YC, Khanna S, Roy S, Sen CK. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. J Biol Chem 2011;286(3):2047-56.

Chan YC, Roy S, Khanna S, Sen CK. Downregulation of endothelial microRNA-200b supports cutaneous wound angiogenesis by desilencing GATA binding protein 2 and vascular endothelial growth factor receptor 2. Arterioscler Thromb Vasc Biol 2012;32(6):1372-82.

Chen L, DiPietro LA. Toll-Like Receptor Function in Acute Wounds. Advances in wound care 2017;6(10):344-55.

Chen Y, Gorski DH. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. Blood 2008;111(3):1217-26.

Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. Am J Respir Cell Mol Biol 2011;45(2):287-94.

Dangwal S, Stratmann B, Bang C, Lorenzen JM, Kumarswamy R, Fiedler J, et al. Impairment of Wound Healing in Patients With Type 2 Diabetes Mellitus Influences Circulating MicroRNA Patterns via Inflammatory Cytokines. Arterioscler Thromb Vasc Biol 2015;35(6):1480-8.

Das A, Ganesh K, Khanna S, Sen CK, Roy S. Engulfment of apoptotic cells by macrophages: a role of microRNA-21 in the resolution of wound inflammation. Journal of immunology (Baltimore, Md : 1950) 2014;192(3):1120-9.

de Kerckhove M, Tanaka K, Umehara T, Okamoto M, Kanematsu S, Hayashi H, et al. Targeting miR-223 in neutrophils enhances the clearance of Staphylococcus aureus in infected wounds. EMBO Mol Med 2018;10(10).

Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Advances in skin & wound care 2012;25(7):304-14.

Deppe J, Steinritz D, Santovito D, Egea V, Schmidt A, Weber C, et al. Upregulation of miR-203 and miR-210 affect growth and differentiation of keratinocytes after exposure to sulfur mustard in normoxia and hypoxia. Toxicol Lett 2016;244:81-7.

Devalliere J, Chang WG, Andrejecsk JW, Abrahimi P, Cheng CJ, Jane-wit D, et al. Sustained delivery of proangiogenic microRNA-132 by nanoparticle transfection improves endothelial cell transplantation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2014;28(2):908-22.

Di Meglio P, Perera GK, Nestle FO. The multitasking organ: recent insights into skin immune function. Immunity 2011;35(6):857-69.

Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Frontiers in bioscience : a journal and virtual library 2004;9:283-9.

Dovi JV, Szpaderska AM, DiPietro LA. Neutrophil function in the healing wound: adding insult to injury? Thrombosis and haemostasis 2004;92(2):275-80.

Du B, Luo W, Li R, Tan B, Han H, Lu X, et al. Lgr4/Gpr48 negatively regulates TLR2/4-associated pattern recognition and innate immunity by targeting CD14 expression. J Biol Chem 2013;288(21):15131-41.

Duscher D, Barrera J, Wong VW, Maan ZN, Whittam AJ, Januszyk M, et al. Stem Cells in Wound Healing: The Future of Regenerative Medicine? A Mini-Review. Gerontology 2016;62(2):216-25.

Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30(1):207-10.

Edwards R, Harding KG. Bacteria and wound healing. Current opinion in infectious diseases 2004;17(2):91-6.

Ehrenreiter K, Piazzolla D, Velamoor V, Sobczak I, Small JV, Takeda J, et al. Raf-1 regulates Rho signaling and cell migration. J Cell Biol 2005;168(6):955-64.

Eming SA, Hammerschmidt M, Krieg T, Roers A. Interrelation of immunity and tissue repair or regeneration. Semin Cell Dev Biol 2009;20(5):517-27.

Eming SA, Koch M, Krieger A, Brachvogel B, Kreft S, Bruckner-Tuderman L, et al. Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. J Proteome Res 2010;9(9):4758-66.

Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. The Journal of investigative dermatology 2007;127(3):514-25.

Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Science translational medicine 2014;6(265):265sr6.

Essandoh K, Li YT, Huo JZ, Fan GC. MiRNA-Mediated Macrophage Polarization and its Potential Role in the Regulation of Inflammatory Response. Shock 2016;46(2):122-31.

Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011;12(12):861-74.

Falanga V, Margolis D, Alvarez O, Auletta M, Maggiacomo F, Altman M, et al. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. Arch Dermatol 1998;134(3):293-300.

Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 2008;283(23):15878-83.

Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Advances in wound care 2015;4(9):560-82.

Fuchs E. Epithelial Skin Biology: Three Decades of Developmental Biology, a Hundred Questions Answered and a Thousand New Ones to Address. Curr Top Dev Biol 2016;116:357-74.

Gallant-Behm CL, Piper J, Dickinson BA, Dalby CM, Pestano LA, Jackson AL. A synthetic microRNA-92a inhibitor (MRG-110) accelerates angiogenesis and wound healing in diabetic and nondiabetic wounds. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2018;26(4):311-23.

Ghatak S, Li J, Chan YC, Gnyawali SC, Steen E, Yung BC, et al. AntihypoxamiR functionalized gramicidin lipid nanoparticles rescue against ischemic memory improving cutaneous wound healing. Nanomedicine 2016;12(7):1827-31.

Gottrup F. A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. American journal of surgery 2004;187(5A):38S-43S.

Gottrup F, Holstein P, Jorgensen B, Lohmann M, Karlsmar T. A new concept of a multidisciplinary wound healing center and a national expert function of wound healing. Archives of surgery (Chicago, Ill : 1960) 2001;136(7):765-72.

Guo J, Lin Q, Shao Y, Rong L, Zhang D. miR-29b promotes skin wound healing and reduces excessive scar formation by inhibition of the TGF-beta1/Smad/CTGF signaling pathway. Can J Physiol Pharmacol 2017;95(4):437-42.

Guo L, Huang X, Liang P, Zhang P, Zhang M, Ren L, et al. Role of XIST/miR-29a/LIN28A pathway in denatured dermis and human skin fibroblasts (HSFs) after thermal injury. J Cell Biochem 2018;119(2):1463-74.

Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89(3):219-29.

Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15(8):509-24.

Hamdan S, Pastar I, Drakulich S, Dikici E, Tomic-Canic M, Deo S, et al. Nanotechnology-Driven Therapeutic Interventions in Wound Healing: Potential Uses and Applications. ACS Cent Sci 2017;3(3):163-75.

Han Z, Chen Y, Zhang Y, Wei A, Zhou J, Li Q, et al. MiR-21/PTEN Axis Promotes Skin Wound Healing by Dendritic Cells Enhancement. J Cell Biochem 2017;118(10):3511-9.

Hancock ML, Preitner N, Quan J, Flanagan JG. MicroRNA-132 is enriched in developing axons, locally regulates Rasa1 mRNA, and promotes axon extension. J Neurosci 2014;34(1):66-78.

Hashimi ST, Fulcher JA, Chang MH, Gov L, Wang S, Lee B. MicroRNA profiling identifies miR-34a and miR-21 and their target genes JAG1 and WNT1 in the coordinate regulation of dendritic cell differentiation. Blood 2009;114(2):404-14.

Herter EK, Xu Landen N. Non-Coding RNAs: New Players in Skin Wound Healing. Advances in wound care 2017;6(3):93-107.

Hou J, Wang P, Lin L, Liu X, Ma F, An H, et al. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. Journal of immunology (Baltimore, Md : 1950) 2009;183(3):2150-8.

Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009a;37(1):1-13.

Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009b;4(1):44-57.

Icli B, Nabzdyk CS, Lujan-Hernandez J, Cahill M, Auster ME, Wara AK, et al. Regulation of impaired angiogenesis in diabetic dermal wound healing by microRNA-26a. J Mol Cell Cardiol 2016;91:151-9.

J.A. McGrath JU. Anatomy and Organization of Human Skin. Rook's Textbook of Dermatology. 1: Blackwell Publishing Ltd.; 2010.

Jafarinejad-Farsangi S, Farazmand A, Mahmoudi M, Gharibdoost F, Karimizadeh E, Noorbakhsh F, et al. MicroRNA-29a induces apoptosis via increasing the Bax:Bcl-2 ratio in dermal fibroblasts of patients with systemic sclerosis. Autoimmunity 2015;48(6):369-78.

Jiang L. ZZ-M, Gao F., et.al. Bone marrow mesenchymal stem cells accelerate wound healing in diabetic mice via inhibiting the expression of microRNA-155 to up-regulate Sirt1 in endothelial cells. Int J Clin Exp Pathol 2016;9:2980–8.

Jin C, Yin FR, Lin M, Li HX, Wang ZL, Weng JS, et al. GPR48 regulates epithelial cell proliferation and migration by activating EGFR during eyelid development. Invest Ophth Vis Sci 2008;49(10):4245-53.

Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 2015;16(7):421-33.

Jones RE, Foster DS, Longaker MT. Management of Chronic Wounds-2018. JAMA 2018;320(14):1481-2.

Jozic I, Vukelic S, Stojadinovic O, Liang L, Ramirez HA, Pastar I, et al. Stress Signals, Mediated by Membranous Glucocorticoid Receptor, Activate PLC/PKC/GSK-3beta/beta-catenin Pathway to Inhibit Wound Closure. The Journal of investigative dermatology 2017;137(5):1144-54.

Jude EB, Blakytny R, Bulmer J, Boulton AJ, Ferguson MW. Transforming growth factor-beta 1, 2, 3 and receptor type I and II in diabetic foot ulcers. Diabet Med 2002;19(6):440-7.

Jun JI, Lau LF. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nature cell biology 2010;12(7):676-85.

Kato S, Mohri Y, Matsuo T, Ogawa E, Umezawa A, Okuyama R, et al. Eye-open at birth phenotype with reduced keratinocyte motility in LGR4 null mice. FEBS Lett 2007;581(24):4685-90.

Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PloS one 2010;5(3):e9539.

Kulkarni SV, Gish G, van der Geer P, Henkemeyer M, Pawson T. Role of p120 Ras-GAP in directed cell movement. J Cell Biol 2000;149(2):457-70.

Landen NX, Li D, Stahle M. Transition from inflammation to proliferation: a critical step during wound healing. Cellular and molecular life sciences : CMLS 2016;73(20):3861-85.

Lang H, Zhao F, Zhang T, Liu X, Wang Z, Wang R, et al. MicroRNA-149 contributes to scarless wound healing by attenuating inflammatory response. Mol Med Rep 2017;16(2):2156-62.

Larsen MT, Hother C, Hager M, Pedersen CC, Theilgaard-Monch K, Borregaard N, et al. MicroRNA Profiling in Human Neutrophils during Bone Marrow Granulopoiesis and In Vivo Exudation. PloS one 2013;8(3).

Lau K, Paus R, Tiede S, Day P, Bayat A. Exploring the role of stem cells in cutaneous wound healing. Experimental dermatology 2009;18(11):921-33.

Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med 2013;34(1):1-11.

Lei Z, van Mil A, Brandt MM, Grundmann S, Hoefer I, Smits M, et al. MicroRNA-132/212 family enhances arteriogenesis after hindlimb ischaemia through modulation of the Ras-MAPK pathway. J Cell Mol Med 2015;19(8):1994-2005.

Li D, Landen NX. MicroRNAs in skin wound healing. Eur J Dermatol 2017;27(S1):12-4.

Li D, Wang A, Liu X, Meisgen F, Grunler J, Botusan IR, et al. MicroRNA-132 enhances transition from inflammation to proliferation during wound healing. J Clin Invest 2015;125(8):3008-26.

Li G, Zhou R, Zhang Q, Jiang B, Wu Q, Wang C. Fibroproliferative effect of microRNA-21 in hypertrophic scar derived fibroblasts. Experimental cell research 2016;345(1):93-9.

Li HH, X.; Zuo, K.; Li, L.; Liu, J.; Yuan, X.; Shen, Y.; Shao, M.; Pang, D.; Chu, Y.; Zhao, B. miR-23b promotes cutaneous wound healing through inhibition of the inflammatory responses by targeting ASK1. Acta Biochim Biophys Sin (Shanghai) 2018;50(11):1104-13.

Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microscopy research and technique 2003;60(1):107-14.

Li X, Guo L, Liu Y, Su Y, Xie Y, Du J, et al. MicroRNA-21 promotes wound healing via the Smad7-Smad2/3-Elastin pathway. Experimental cell research 2018;362(2):245-51.

Liang L, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, et al. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2016;24(6):943-53.

Liu F, Li Y, Jiang R, Nie C, Zeng ZG, Zhao N, et al. miR-132 inhibits lipopolysaccharide-induced inflammation in alveolar macrophages by the cholinergic anti-inflammatory pathway. Experimental Lung Research 2015;41(5):261-9.

Liu X, Wang Y, Zhang X, Zhang X, Guo J, Zhou J, et al. MicroRNA-296-5p promotes healing of diabetic wound by targeting sodium-glucose transporter 2 (SGLT2). Diabetes Metab Res Rev 2018:e3104.

Liu Y, Tang N, Cao K, Wang S, Tang S, Su H, et al. Negative-Pressure Wound Therapy Promotes Wound Healing by Enhancing Angiogenesis Through Suppression of NLRX1 via miR-195 Upregulation. Int J Low Extrem Wounds 2018;17(3):144-50.

Liu Y, Wang X, Yang D, Xiao Z, Chen X. MicroRNA-21 affects proliferation and apoptosis by regulating expression of PTEN in human keloid fibroblasts. Plastic and reconstructive surgery 2014;134(4):561e-73e.

Lohcharoenkal W, Das Mahapatra K, Pasquali L, Crudden C, Kular L, Akkaya Ulum YZ, et al. Genome-Wide Screen for MicroRNAs Reveals a Role for miR-203 in Melanoma Metastasis. The Journal of investigative dermatology 2018;138(4):882-92.

Lohcharoenkal W, Harada M, Loven J, Meisgen F, Landen NX, Zhang L, et al. MicroRNA-203 Inversely Correlates with Differentiation Grade, Targets c-MYC, and Functions as a Tumor Suppressor in cSCC. The Journal of investigative dermatology 2016;136(12):2485-94.

Long S, Zhao N, Ge L, Wang G, Ran X, Wang J, et al. MiR-21 ameliorates age-associated skin wound healing defects in mice. J Gene Med 2018;20(6):e3022.

Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. The Journal of investigative dermatology 1998;111(5):850-7.

Lucas T, Schafer F, Muller P, Eming SA, Heckel A, Dimmeler S. Light-inducible antimiR-92a as a therapeutic strategy to promote skin repair in healing-impaired diabetic mice. Nat Commun 2017;8:15162.

Lukic J, Chen V, Strahinic I, Begovic J, Lev-Tov H, Davis SC, et al. Probiotics or pro-healers: the role of beneficial bacteria in tissue repair. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2017;25(6):912-22.

Luo J, Yang ZF, Ma Y, Yue ZY, Lin HY, Qui GJ, et al. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. Nature Medicine 2016;22(5):539-46.

Lynch MD, Watt FM. Fibroblast heterogeneity: implications for human disease. J Clin Invest 2018;128(1):26-35.

Madhyastha R, Madhyastha H, Nakajima Y, Omura S, Maruyama M. MicroRNA signature in diabetic wound healing: promotive role of miR-21 in fibroblast migration. Int Wound J 2012;9(4):355-61.

Mai A, Veltel S, Pellinen T, Padzik A, Coffey E, Marjomaki V, et al. Competitive binding of Rab21 and p120RasGAP to integrins regulates receptor traffic and migration. J Cell Biol 2011;194(2):291-306.

Makrantonaki E, Wlaschek M, Scharffetter-Kochanek K. Pathogenesis of wound healing disorders in the elderly. Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG 2017;15(3):255-75.

Mancini M, Lena AM, Saintigny G, Mahe C, Di Daniele N, Melino G, et al. MicroRNAs in human skin ageing. Ageing Res Rev 2014;17:9-15.

Mannucci C, Casciaro M, Minciullo PL, Calapai G, Navarra M, Gangemi S. Involvement of microRNAs in skin disorders: A literature review. Allergy Asthma Proc 2017;38(1):9-15.

Mardaryev AN, Ahmed MI, Vlahov NV, Fessing MY, Gill JH, Sharov AA, et al. Micro-RNA-31 controls hair cycle-associated changes in gene expression programs of the skin and hair follicle. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2010;24(10):3869-81.

Maroof H, Salajegheh A, Smith RA, Lam AK. MicroRNA-34 family, mechanisms of action in cancer: a review. Curr Cancer Drug Targets 2014;14(8):737-51.

Martin P. Wound healing--aiming for perfect skin regeneration. Science (New York, NY) 1997;276(5309):75-81.

Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. The British journal of dermatology 2015;173(2):370-8.

Maurer B, Stanczyk J, Jungel A, Akhmetshina A, Trenkmann M, Brock M, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis Rheum 2010;62(6):1733-43.

McCubbrey AL, Nelson JD, Stolberg VR, Blakely PK, McCloskey L, Janssen WJ, et al. MicroRNA-34a Negatively Regulates Efferocytosis by Tissue Macrophages in Part via SIRT1. Journal of immunology (Baltimore, Md : 1950) 2016;196(3):1366-75.

Medina A, Scott PG, Ghahary A, Tredget EE. Pathophysiology of chronic nonhealing wounds. The Journal of burn care & rehabilitation 2005;26(4):306-19.

Meisgen F, Xu Landen N, Wang A, Rethi B, Bouez C, Zuccolo M, et al. MiR-146a negatively regulates TLR2-induced inflammatory responses in keratinocytes. The Journal of investigative dermatology 2014;134(7):1931-40.

Meisgen F, Xu N, Wei T, Janson PC, Obad S, Broom O, et al. MiR-21 is up-regulated in psoriasis and suppresses T cell apoptosis. Experimental dermatology 2012;21(4):312-4.

Menon GK. New insights into skin structure: scratching the surface. Adv Drug Deliv Rev 2002;54 Suppl 1:S3-17.

Meszaros AJ, Reichner JS, Albina JE. Macrophage phagocytosis of wound neutrophils. Journal of leukocyte biology 1999;65(1):35-42.

Meszaros AJ, Reichner JS, Albina JE. Macrophage-induced neutrophil apoptosis. Journal of immunology (Baltimore, Md : 1950) 2000;165(1):435-41.

Miscianinov V, Martello A, Rose L, Parish E, Cathcart B, Mitic T, et al. MicroRNA-148b Targets the TGF-beta Pathway to Regulate Angiogenesis and Endothelial-to-Mesenchymal Transition during Skin Wound Healing. Mol Ther 2018;26(8):1996-2007.

Misic AM, Gardner SE, Grice EA. The Wound Microbiome: Modern Approaches to Examining the Role of Microorganisms in Impaired Chronic Wound Healing. Advances in wound care 2014;3(7):502-10.

Mizuno H. Adipose-derived stem cells for tissue repair and regeneration: ten years of research and a literature review. J Nippon Med Sch 2009;76(2):56-66.

Mohri Y, Kato S, Umezawa A, Okuyama R, Nishimori K. Impaired hair placode formation with reduced expression of hair follicle-related genes in mice lacking Lgr4. Dev Dyn 2008;237(8):2235-42.

Moles R. MicroRNAs-based Therapy: A Novel and Promising Strategy for Cancer Treatment. Microrna 2017;6(2):102-9.

Monaghan M, Browne S, Schenke-Layland K, Pandit A. A collagen-based scaffold delivering exogenous microrna-29B to modulate extracellular matrix remodeling. Mol Ther 2014;22(4):786-96.

Monteys AM, Spengler RM, Wan J, Tecedor L, Lennox KA, Xing Y, et al. Structure and activity of putative intronic miRNA promoters. RNA 2010;16(3):495-505.

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34(3):267-73.

Morhenn VB, Nelson TE, Gruol DL. The rate of wound healing is increased in psoriasis. Journal of dermatological science 2013;72(2):87-92.

Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S. Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. Experimental cell research 2005;304(1):81-90.

Mulik S, Xu J, Reddy PB, Rajasagi NK, Gimenez F, Sharma S, et al. Role of miR-132 in angiogenesis after ocular infection with herpes simplex virus. The American journal of pathology 2012;181(2):525-34.

Mustoe TA, O'Shaughnessy K, Kloeters O. Chronic wound pathogenesis and current treatment strategies: A unifying hypothesis. Plastic and reconstructive surgery 2006;117(7):35s-41s.

Nahid MA, Yao B, Dominguez-Gutierrez PR, Kesavalu L, Satoh M, Chan EKL. Regulation of TLR2-Mediated Tolerance and Cross-Tolerance through IRAK4 Modulation by miR-132 and miR-212. Journal of Immunology 2013;190(3):1250-63.

Nickoloff BJ, Lingen MW, Chang BD, Shen M, Swift M, Curry J, et al. Tumor suppressor maspin is up-regulated during keratinocyte senescence, exerting a paracrine antiangiogenic activity. Cancer research 2004;64(9):2956-61.

O'Toole EAM, J.E. Wound healing. Rook's Textbook of Dermatology. 1. 8 ed: Blackwell Publishing Ltd.; 2010.

Organization WH. Global report on diabetes. Geneva2016.

Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, et al. Chromatin structure analyses identify miRNA promoters. Genes Dev 2008;22(22):3172-83.

Pamonsinlapatham P, Hadj-Slimane R, Lepelletier Y, Allain B, Toccafondi M, Garbay C, et al. P120-Ras GTPase activating protein (RasGAP): A multi-interacting protein in downstream signaling. Biochimie 2009;91(3):320-8.

Pang C, Ibrahim A, Bulstrode NW, Ferretti P. An overview of the therapeutic potential of regenerative medicine in cutaneous wound healing. Int Wound J 2017.

Pastar I, Khan AA, Stojadinovic O, Lebrun EA, Medina MC, Brem H, et al. Induction of specific microRNAs inhibits cutaneous wound healing. J Biol Chem 2012;287(35):29324-35.

Peng H, Kaplan N, Hamanaka RB, Katsnelson J, Blatt H, Yang W, et al. microRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. Proc Natl Acad Sci U S A 2012;109(35):14030-4.

Philippeos C, Telerman SB, Oules B, Pisco AO, Shaw TJ, Elgueta R, et al. Spatial and Single-Cell Transcriptional Profiling Identifies Functionally Distinct Human Dermal Fibroblast Subpopulations. The Journal of investigative dermatology 2018;138(4):811-25.

Pratt AJ, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. J Biol Chem 2009;284(27):17897-901.

Rajalingam K, Schreck R, Rapp UR, Albert S. Ras oncogenes and their downstream targets. Biochim Biophys Acta 2007;1773(8):1177-95.

Ramirez HA, Pastar I, Jozic I, Stojadinovic O, Stone RC, Ojeh N, et al. Staphylococcus aureus Triggers Induction of miR-15B-5P to Diminish DNA Repair and Deregulate Inflammatory Response in Diabetic Foot Ulcers. The Journal of investigative dermatology 2018;138(5):1187-96.

Rao DS, O'Connell RM, Chaudhuri AA, Garcia-Flores Y, Geiger TL, Baltimore D. MicroRNA-34a perturbs B lymphocyte development by repressing the forkhead box transcription factor Foxp1. Immunity 2010;33(1):48-59.

Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN. MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2011;25(2):544-60.

Reinke JM, Sorg H. Wound repair and regeneration. European surgical research Europaische chirurgische Forschung Recherches chirurgicales europeennes 2012;49(1):35-43.

Richmond NA, Maderal AD, Vivas AC. Evidence-based management of common chronic lower extremity ulcers. Dermatologic therapy 2013;26(3):187-96.

Ridiandries A, Tan JTM, Bursill CA. The Role of Chemokines in Wound Healing. Int J Mol Sci 2018;19(10).

Roy S, Elgharably H, Sinha M, Ganesh K, Chaney S, Mann E, et al. Mixed-species biofilm compromises wound healing by disrupting epidermal barrier function. J Pathol 2014;233(4):331-43.

Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. Nature 2007;448(7149):83-6.

Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017;16(3):203-22.

Schneider MR. MicroRNAs as novel players in skin development, homeostasis and disease. Brit J Dermatol 2012;166(1):22-8.

Sen CK. Wound healing essentials: let there be oxygen. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2009;17(1):1-18.

Shi J, Ma X, Su Y, Song Y, Tian Y, Yuan S, et al. MiR-31 Mediates Inflammatory Signaling to Promote Re-Epithelialization during Skin Wound Healing. The Journal of investigative dermatology 2018;138(10):2253-63.

Shikama Y, Cao M, Ono T, Feng X, Noji H, Kimura H, et al. Reduction of c-Fos via Overexpression of miR-34a Results in Enhancement of TNF- Production by LPS in Neutrophils from Myelodysplastic Syndrome Patients. PloS one 2016;11(8):e0158527.

Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells - Synthesis of chemokines and regulation of inflammation. American Journal of Pathology 1997;151(2):317-22.

Snyder RJ, Lantis J, Kirsner RS, Shah V, Molyneaux M, Carter MJ. Macrophages: A review of their role in wound healing and their therapeutic use. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2016;24(4):613-29.

Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. The Journal of allergy and clinical immunology 2010a;126(3):581-9 e1-20.

Sonkoly E, Wei T, Janson PC, Saaf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PloS one 2007;2(7):e610.

Sonkoly E, Wei T, Pavez Lorie E, Suzuki H, Kato M, Torma H, et al. Protein kinase C-dependent upregulation of miR-203 induces the differentiation of human keratinocytes. The Journal of investigative dermatology 2010b;130(1):124-34.

Sorrell JM, Caplan AI. Fibroblast heterogeneity: more than skin deep. J Cell Sci 2004;117(Pt 5):667-75.

Srivastava A, Nikamo P, Lohcharoenkal W, Li D, Meisgen F, Xu Landen N, et al. MicroRNA-146a suppresses IL-17-mediated skin inflammation and is genetically associated with psoriasis. The Journal of allergy and clinical immunology 2017;139(2):550-61.

Stone RC, Stojadinovic O, Rosa AM, Ramirez HA, Badiavas E, Blumenberg M, et al. A bioengineered living cell construct activates an acute wound healing response in venous leg ulcers. Science translational medicine 2017;9(371).

Strbo N, Yin N, Stojadinovic O. Innate and Adaptive Immune Responses in Wound Epithelialization. Advances in wound care 2014;3(7):492-501.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102(43):15545-50.

Sun G, Zhao G, Lu Y, Wang Y, Yang C. Association of EMP1 with gastric carcinoma invasion, survival and prognosis. Int J Oncol 2014;45(3):1091-8.

Sun GG, Lu YF, Fu ZZ, Cheng YJ, Hu WN. EMP1 inhibits nasopharyngeal cancer cell growth and metastasis through induction apoptosis and angiogenesis. Tumour Biol 2014a;35(4):3185-93.

Sun GG, Wang YD, Cui DW, Cheng YJ, Hu WN. Epithelial membrane protein 1 negatively regulates cell growth and metastasis in colorectal carcinoma. World J Gastroenterol 2014b;20(14):4001-10.

Sun GG, Wang YD, Lu YF, Hu WN. EMP1, a member of a new family of antiproliferative genes in breast carcinoma. Tumour Biol 2014c;35(4):3347-54.

Sundaram GM, Quah S, Sampath P. Cancer: the dark side of wound healing. FEBS J 2018.

Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 2006;103(33):12481-6.

Tan B, Shi X, Zhang J, Qin J, Zhang N, Ren H, et al. Inhibition of Rspo-Lgr4 facilitates checkpoint blockade therapy by switching macrophage polarization. Cancer research 2018.

Tanaka K, Kim SE, Yano H, Matsumoto G, Ohuchida R, Ishikura Y, et al. MiR-142 Is Required for Staphylococcus aureus Clearance at Skin Wound Sites via Small GTPase-Mediated Regulation of the Neutrophil Actin Cytoskeleton. The Journal of investigative dermatology 2017;137(4):931-40.

Tang Q, Ran H. MicroRNA-219-5p inhibits wound healing by targeting TMEM98 in keratinocytes under normoxia and hypoxia condition. Eur Rev Med Pharmacol Sci 2018;22(19):6205-11.

Teta M, Choi YS, Okegbe T, Wong G, Tam OH, Chong MM, et al. Inducible deletion of epidermal Dicer and Drosha reveals multiple functions for miRNAs in postnatal skin. Development 2012;139(8):1405-16.

Tricco AC, Antony J, Vafaei A, Khan PA, Harrington A, Cogo E, et al. Seeking effective interventions to treat complex wounds: an overview of systematic reviews. BMC medicine 2015;13:89.

Tsourdi E, Barthel A, Rietzsch H, Reichel A, Bornstein SR. Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. Biomed Res Int 2013;2013:385641.

Valencia IC, Falabella A, Kirsner RS, Eaglstein WH. Chronic venous insufficiency and venous leg ulceration. Journal of the American Academy of Dermatology 2001;44(3):401-21; quiz 22-4.

van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. EMBO Mol Med 2014;6(7):851-64.

van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 2008;105(35):13027-32.

van Solingen C, Araldi E, Chamorro-Jorganes A, Fernandez-Hernando C, Suarez Y. Improved repair of dermal wounds in mice lacking microRNA-155. J Cell Mol Med 2014;18(6):1104-12.

Wang A, Landen NX, Meisgen F, Lohcharoenkal W, Stahle M, Sonkoly E, et al. MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. PloS one 2014;9(7):e103206.

Wang D, Zhang Z, O'Loughlin E, Wang L, Fan X, Lai EC, et al. MicroRNA-205 controls neonatal expansion of skin stem cells by modulating the PI(3)K pathway. Nature cell biology 2013;15(10):1153-63.

Wang JM, Tao J, Chen DD, Cai JJ, Irani K, Wang Q, et al. MicroRNA miR-27b rescues bone marrow-derived angiogenic cell function and accelerates wound healing in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol 2014;34(1):99-109.

Wang L, Yu J, Xu J, Zheng C, Li X, Du J. The analysis of microRNA-34 family expression in human cancer studies comparing cancer tissues with corresponding pericarcinous tissues. Gene 2015;554(1):1-8.

Wang T, Feng Y, Sun H, Zhang L, Hao L, Shi C, et al. miR-21 regulates skin wound healing by targeting multiple aspects of the healing process. The American journal of pathology 2012;181(6):1911-20.

Wang W, Yang C, Wang XY, Zhou LY, Lao GJ, Liu D, et al. MicroRNA-129 and -335 Promote Diabetic Wound Healing by Inhibiting Sp1-Mediated MMP-9 Expression. Diabetes 2018;67(8):1627-38.

Wang Z, Jin C, Li H, Li C, Hou Q, Liu M, et al. GPR48-Induced keratinocyte proliferation occurs through HB-EGF mediated EGFR transactivation. FEBS Lett 2010;584(18):4057-62.

Westenskow PD, Kurihara T, Aguilar E, Scheppke EL, Moreno SK, Wittgrove C, et al. Ras pathway inhibition prevents neovascularization by repressing endothelial cell sprouting. J Clin Invest 2013;123(11):4900-8.

Vestweber D. How leukocytes cross the vascular endothelium. Nature reviews Immunology 2015;15(11):692-704.

Wilgus TA, Roy S, McDaniel JC. Neutrophils and Wound Repair: Positive Actions and Negative Reactions. Advances in wound care 2013;2(7):379-88.

Wilkinson HN, Hardman MJ. The role of estrogen in cutaneous ageing and repair. Maturitas 2017;103:60-4.

Viticchie G, Lena AM, Cianfarani F, Odorisio T, Annicchiarico-Petruzzelli M, Melino G, et al. MicroRNA-203 contributes to skin re-epithelialization. Cell Death Dis 2012;3:e435.

Volk SW, Bohling MW. Comparative wound healing--are the small animal veterinarian's clinical patients an improved translational model for human wound healing research? Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2013;21(3):372-81.

Wong MY, Yu Y, Walsh WR, Yang JL. microRNA-34 family and treatment of cancers with mutant or wild-type p53 (Review). Int J Oncol 2011;38(5):1189-95.

Xiao K, Luo X, Wang X, Gao Z. MicroRNA185 regulates transforming growth factorbeta1 and collagen1 in hypertrophic scar fibroblasts. Mol Med Rep 2017;15(4):1489-96.

Xie C, Shi K, Zhang X, Zhao J, Yu J. MiR-1908 promotes scar formation post-burn wound healing by suppressing Ski-mediated inflammation and fibroblast proliferation. Cell Tissue Res 2016;366(2):371-80.

Xu J, Zgheib C, Hodges MM, Caskey RC, Hu J, Liechty KW. Mesenchymal stem cells correct impaired diabetic wound healing by decreasing ECM proteolysis. Physiol Genomics 2017;49(10):541-8.

Xu J, Zgheib C, Hu J, Wu W, Zhang L, Liechty KW. The role of microRNA-15b in the impaired angiogenesis in diabetic wounds. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2014;22(5):671-7.

Xu JW, W.; Zhang, L.; Dorset-Martin, W.; Morris, M. W.; Mitchell, M. E.; Liechty, K. W. The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. Diabetes 2012;61(11):2906-12.

Xu N, Meisgen F, Butler LM, Han G, Wang XJ, Soderberg-Naucler C, et al. MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40. Journal of immunology (Baltimore, Md : 1950) 2013;190(2):678-88.

Xu N, Zhang L, Meisgen F, Harada M, Heilborn J, Homey B, et al. MicroRNA-125b down-regulates matrix metallopeptidase 13 and inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion. J Biol Chem 2012;287(35):29899-908.

Yan H, Song K, Zhang G. MicroRNA-17-3p promotes keratinocyte cells growth and metastasis via targeting MYOT and regulating Notch1/NF-kappaB pathways. Pharmazie 2017;72(9):543-9.

Yan J, Tie G, Wang S, Tutto A, DeMarco N, Khair L, et al. Diabetes impairs wound healing by Dnmt1-dependent dysregulation of hematopoietic stem cells differentiation towards macrophages. Nat Commun 2018;9(1):33.

Yang L, Zheng Z, Zhou Q, Bai X, Fan L, Yang C, et al. miR-155 promotes cutaneous wound healing through enhanced keratinocytes migration by MMP-2. J Mol Histol 2017;48(2):147-55.

Yang LL, Liu JQ, Bai XZ, Fan L, Han F, Jia WB, et al. Acute downregulation of miR-155 at wound sites leads to a reduced fibrosis through attenuating inflammatory response. Biochem Biophys Res Commun 2014;453(1):153-9.

Yang T, Liang Y, Lin Q, Liu J, Luo F, Li X, et al. miR-29 mediates TGFbeta1-induced extracellular matrix synthesis through activation of PI3K-AKT pathway in human lung fibroblasts. J Cell Biochem 2013;114(6):1336-42.

Yang X, Wang J, Guo SL, Fan KJ, Li J, Wang YL, et al. miR-21 promotes keratinocyte migration and re-epithelialization during wound healing. Int J Biol Sci 2011;7(5):685-90.

Ye J, Kang Y, Sun X, Ni P, Wu M, Lu S. MicroRNA-155 Inhibition Promoted Wound Healing in Diabetic Rats. Int J Low Extrem Wounds 2017;16(2):74-84.

Yi R, O'Carroll D, Pasolli HA, Zhang Z, Dietrich FS, Tarakhovsky A, et al. Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. Nat Genet 2006;38(3):356-62.

Yi R, Pasolli HA, Landthaler M, Hafner M, Ojo T, Sheridan R, et al. DGCR8-dependent microRNA biogenesis is essential for skin development. Proc Natl Acad Sci U S A 2009;106(2):498-502.

Yi R, Poy MN, Stoffel M, Fuchs E. A skin microRNA promotes differentiation by repressing 'stemness'. Nature 2008;452(7184):225-9.

You HJ, Han SK. Cell therapy for wound healing. J Korean Med Sci 2014;29(3):311-9.

Zgheib C, Hilton SA, Dewberry LC, Hodges MM, Ghatak S, Xu J, et al. Use of Cerium Oxide Nanoparticles Conjugated with MicroRNA-146a to Correct the Diabetic Wound Healing Impairment. J Am Coll Surg 2018.

Zhang F, Phiel CJ, Spece L, Gurvich N, Klein PS. Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3. J Biol Chem 2003;278(35):33067-77.

Zhang LS, N.; Polak, L.; Fuchs, E. Specific microRNAs are preferentially expressed by skin stem cells to balance self-renewal and early lineage commitment. Cell Stem Cell 2011;8(3):294-308.

Zhang T, Wang Q, Zhao D, Cui Y, Cao B, Guo L, et al. The oncogenetic role of microRNA-31 as a potential biomarker in oesophageal squamous cell carcinoma. Clin Sci (Lond) 2011;121(10):437-47.

Zhao G, Usui ML, Lippman SI, James GA, Stewart PS, Fleckman P, et al. Biofilms and Inflammation in Chronic Wounds. Advances in wound care 2013;2(7):389-99.

Zhao Y, Srivastava D. A developmental view of microRNA function. Trends Biochem Sci 2007;32(4):189-97.

Zhou Z, Shu B, Xu Y, Liu J, Wang P, Chen L, et al. microRNA-203 Modulates Wound Healing and Scar Formation via Suppressing Hes1 Expression in Epidermal Stem Cells. Cell Physiol Biochem 2018;49(6):2333-47.

Zhu HY, Li C, Bai WD, Su LL, Liu JQ, Li Y, et al. MicroRNA-21 regulates hTERT via PTEN in hypertrophic scar fibroblasts. PloS one 2014;9(5):e97114.

Zhu L, Wang G, Fischbach S, Xiao X. Suppression of microRNA-205-5p in human mesenchymal stem cells improves their therapeutic potential in treating diabetic foot disease. Oncotarget 2017;8(32):52294-303.