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UNDERSTANDING THE ROLE OF REGULATORY RNAs IN HUMAN SKIN WOUND HEALING

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UNDERSTANDING THE ROLE OF REGULATORY RNAs IN HUMAN SKIN WOUND HEALING

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my beloved parents!

致我亲爱的父母!

Popular science summary of the thesis

As the human body's largest organ, the skin covers the surface area. We can consider the skin as our shell. It protects the body from extreme temperature, bacterial infection, and mechanical stress. If the skin is wounded, it has to be repaired on time; otherwise, the whole body may be affected. As the dominant cell type in the epidermis, keratinocytes can recruit immune cells and participate in re-epithelialization, which are essential for wound closure. In the past ten years, more noncoding RNAs have been identified, and they cannot be translated into proteins. Compared to protein-coding genes, noncoding RNAs have cell type and context specificity, which may serve as therapeutic targets. In this thesis, we mainly focus on three long noncoding RNAs (lncRNAs) (**Paper I, III, IV**) and microRNAs (miRNAs) (**Paper II**) and characterize their biological functions as well as the underlying mechanism in human skin wound healing.

Paper I identified wound and keratinocyte migration-associated lncRNA 1 (WAKMAR1). WAKMAR1 promoted keratinocyte migration and re-epithelialization of human *ex vivo* tissue, and its deficiency in chronic wounds may contribute to delayed healing. Mechanistically, E2F Transcription Factor 1 (E2F1) was a hub gene that regulates the WAKMAR1-associated gene network. Furthermore, WAKMAR1 decreased methylation at the E2F1 promoter via its interaction with DNA methyltransferases (DNMTs), which enhanced E2F1 expression. This study demonstrates WAKMAR1 as a molecule of importance for wound healing.

Paper II comprehensively analyzed small and long RNAs in normal wounds and venous ulcers (VUs). We identified 17 VU-relevant miRNAs, and their targets were associated with the pathology of VUs. Bioinformatics analysis showed that the upregulated miRNAs in VU were predicted to promote inflammatory response but impair cell proliferation. However, the downregulated miRNAs might be needed for cell proliferation and migration. Moreover, we confirmed the combined effects of miR-34a-5p, miR-424-5p, and miR-516-5p upregulated in VU. The combination of miR-34a-5p and miR-424-5p impaired keratinocyte proliferation and migration, but that of miR-34a-5p and miR-516b-5p promoted the expression of the pro-inflammatory Chemokine (C-C Motif) Ligand 20 (CCL20). This study identifies VU-relevant miRNAs and demonstrates that their abnormal expression may contribute to the pathogenesis of nonhealing wounds.

Paper III identified a skin-specific lncRNA HOXC13-AS that was downregulated during wound healing. We analyzed our single-cell RNA sequencing data in the human skin and found that HOXC13-AS was highly expressed in differentiated keratinocytes. Moreover, HOXC13-AS was inhibited by the epidermal growth factor receptor (EGFR) signaling pathway but

gradually increased during keratinocyte differentiation. We confirmed that HOXC13-AS promoted keratinocyte differentiation using differentiation models *in vitro* and organotypic epidermis. Mechanistically, the interaction between HOXC13-AS and COPI Coat Complex Subunit Alpha (COPA) interfered with the retrograde transport from the Golgi to the endoplasmic reticulum (ER) and thus promoted ER stress and keratinocyte differentiation. This study demonstrates that HOXC13-AS is essential for epidermal differentiation.

Paper IV identified lncRNA SNHG26 as essential for the transition from the inflammatory to proliferative phases during skin wound healing. SNHG26 was upregulated during wound healing, and its deficiency in mice showed delayed re-epithelialization. We performed single-cell RNA sequencing analysis in Snhg26 deficient mice and observed decreased migratory but increased inflammatory keratinocyte progenitors in the wound edge. Moreover, we confirmed that SNHG26 enhanced cell proliferation and migration but inhibited inflammatory response in human keratinocytes and *ex vivo* wounds. Mechanistically, SNHG26 interacted with Interleukin Enhancer Binding Factor 2 (ILF2) and guided it from the inflammatory genomic loci to the Laminin Subunit Beta 3 (LAMB3) genomic locus, which facilitates the inflammatory-to-proliferative state transition of keratinocyte progenitors. This study provides compelling evidence for SNHG26 being a crucial regulator for human skin wound healing.

Abstract

Human skin wound healing is characterized by four phases in a timely manner, including hemostasis, inflammation, proliferation, and remodeling. Various cell types are involved in the biological process. Keratinocytes that constitute around 95% of epidermal cells recruit immune cells by secreting pro-inflammatory cytokines/chemokines and undergo re-epithelialization in the proliferation phase. Ribonucleic acids (RNAs) without protein-coding capacity, defined as noncoding RNAs, consist of the majority of transcription output, are indispensable for multiple biological processes and are critical during disease contexts. Due to their cell and context specificity, noncoding RNAs present a therapeutic potential. However, revealing their underlying mechanism in the skin wound healing is the prerequisite. In this thesis, we identified and comprehensively characterized the role of long noncoding RNAs (lncRNAs) (**Paper I, III, IV**) and microRNAs (miRNAs) (**Paper II**) in human skin wound healing.

Paper I identified Wound And Keratinocyte Migration-Associated lncRNA 1 (WAKMAR1), and it was upregulated during wound healing but deficient in nonhealing wounds. WAKMAR1 silencing inhibited keratinocyte migration and re-epithelialization of human *ex vivo* wounds, whereas its overexpression promoted cell migration. Moreover, we revealed that the WAKMAR1-regulated network composed of pro-migratory genes was driven by E2F Transcription Factor 1 (E2F1). Further mechanistic investigation showed that WAKMAR1 enhanced E2F1 expression by hijacking DNA methyltransferases (DNMTs) and reducing methylation at the E2F1 promoter. This study demonstrates that WAKMAR1 is essential for keratinocyte migration and re-epithelialization of human *ex vivo* wounds, and its deficiency may be associated with delayed healing.

Paper II aimed to identify clinically relevant miRNAs and develop an open database for future studies in skin wound healing. We performed the comprehensive and integrative small and long RNA sequencing analysis in human skin, normal wounds collected at different healing phases, and venous ulcers (VUs). We found 17 VU-relevant miRNAs, whose targets were overrepresented in the VU-specific signature. The upregulated miRNAs in VU were predicted to promote inflammatory response but impair cell proliferation, but the downregulated miRNAs might be needed for cell proliferation and migration. We tested the combined effects of miR-34a-5p, miR-424-5p, and miR-516-5p upregulated in VU. Simultaneous overexpression of miR-34a-5p and miR-424-5p had stronger inhibitory effects on keratinocyte proliferation and migration, whereas the combination of miR-34a-5p and miR-516b-5p promoted the expression of the pro-inflammatory Chemokine (C-C Motif) Ligand 20 (CCL20).

Overall, our study identifies VU-relevant miRNAs and demonstrates that their abnormal expression may contribute to the pathogenesis of nonhealing wounds.

Paper III investigated the role of the HOXC13 Antisense RNA (HOXC13-AS) in epidermal differentiation. LncRNA HOXC13-AS was specifically expressed in human skin and downregulated in the early phases of wound healing. We analyzed our single-cell RNA sequencing in the human skin and found that HOXC13-AS was highly expressed in the differentiated keratinocytes. Furthermore, we showed that HOXC13-AS was decreased by the epidermal growth factor receptor (EGFR) signaling pathway but gradually increased during keratinocyte differentiation. Transcriptomic analysis and functional assays indicated that HOXC13-AS promoted keratinocyte differentiation using differentiation models *in vitro* and organotypic epidermis. Mechanistically, we revealed that HOXC13-AS physically interacted with COPI Coat Complex Subunit Alpha (COPA) which is essential for the retrograde transport from the Golgi to the endoplasmic reticulum (ER). HOXC13-AS hijacked COPA, which interfered with the retrograde transport, promoting ER stress and keratinocyte differentiation. Rescue assays confirmed that the role of HOXC13-AS in keratinocyte differentiation was dependent on COPA. Overall, this study demonstrates HOXC13-AS as a molecule of importance for epidermal differentiation.

Paper IV focused on lncRNA SNHG26, which plays a key role in the transition from inflammation to proliferation during wound healing. SNHG26 was upregulated during wound healing, and Snhg26 knockout mice showed delayed re-epithelialization. By single-cell RNA sequencing analysis, we found decreased migratory but increased inflammatory keratinocyte progenitors in the wound edge of Snhg26 deficient mice. Moreover, we confirmed that SNHG26 enhanced cell proliferation and migration but inhibited inflammatory response in human keratinocytes and *ex vivo* wounds. Mechanistically, we demonstrated that Interleukin Enhancer Binding Factor 2 (ILF2) physically interacted with SNHG26 using RNA pulldown and RNA immunoprecipitation (RIP). Chromatin immunoprecipitation (ChIP) and chromatin isolation by RNA purification (ChIRP) sequencing showed that SNHG26 guided ILF2 from the inflammatory genomic loci to the Laminin Subunit Beta 3 (LAMB3) genomic locus, switching the gene network and facilitating the inflammatory-to-proliferative state transition of keratinocyte progenitors. This study provides compelling evidence for SNHG26 being a crucial regulator for human skin wound healing.

List of scientific papers

I. **Human skin long noncoding RNA WAKMAR1 regulates wound healing by enhancing keratinocyte migration**
Dongqing Li, Lara Kular, Manika Vij, Eva K. Herter, Xi Li, Aoxue Wang, Tongbin Chu, Maria A. Toma, **Letian Zhang**, Eleni Liapi, Ana Mota, Lennart Blomqvist, Irène Gallais Sérézal, Ola Rollman, Jakob D. Wikstrom, Magda Bienko, David Berglund, Mona Ståhle, Pehr Sommar, Maja Jagodic, Ning Xu Landén
Proc Natl Acad Sci U S A. 2019 May 7;116(19):9443-9452.

II. **Integrative small and long RNA omics analysis of human healing and nonhealing wounds discovers cooperating microRNAs as therapeutic targets**
Zhuang Liu*, **Letian Zhang***, Maria A. Toma, Dongqing Li, Xiaowei Bian, Irena Pastar, Marjana Tomic-Canic, Pehr Sommar†, Ning Xu Landén†
Elife. 2022 Aug 12;11: e80322.

III. **Human skin specific long noncoding RNA HOXC13-AS regulates epidermal differentiation by interfering with Golgi-ER retrograde transport**
Letian Zhang, Minna Piipponen, Zhuang Liu, Dongqing Li, Xiaowei Bian, Guanglin Niu, Jennifer Geara, Maria A. Toma, Pehr Sommar, Ning Xu Landén
Submitted

IV. **The lncRNA SNHG26 drives the inflammatory-to-proliferative state transition of keratinocyte progenitor cells during wound healing**
Dongqing Li†, Zhuang Liu*, **Letian Zhang***, Xiaowei Bian, Jianmin Wu, Li Li, Ling Pan,Yunting Xiao, Jiating Wang, Xiya Zhang, Wang Wang, Maria A. Toma, Minna Piipponen, Yuping Lai, Pehr Sommar, Ning Xu Landén†
Submitted

*,†,#Authors contributed equally

Scientific papers not included in this thesis

The injury-induced circular RNA circGLIS3 activates dermal fibroblasts to promote wound healing
Maria A. Toma, Qizhang Wang, Dongqing Li, Yunting Xiao, Guanglin Niu, Jennifer Geara, Manika Vij, Minna Piipponen, Zhuang Liu, **Letian Zhang**, Xiaowei Bian, Aoxue Wang, Pehr Sommar, Ning Xu Landen
bioRxiv

Circular RNA signatures of human healing and non-healing wounds
Maria A. Toma*, Zhuang Liu*†, Qizhang Wang, **Letian Zhang**, Dongqing Li, Pehr Sommar, Ning Xu Landén†
J Invest Dermatol. 2022 Oct;142(10):2793-2804.e26.

Single-Cell Analysis Reveals Major Histocompatibility Complex II–Expressing Keratinocytes in Pressure Ulcers with Worse Healing Outcomes
Dongqing Li*, Shangli Cheng*, Yu Pei†, Pehr Sommar†, Jaanika Kärner†, Eva K. Herter, Maria A. Toma, **Letian Zhang**, Kim Pham, Yuen Ting Cheung, Zhuang Liu, Xingqi Chen, Liv Eidsmo, Qiaolin Deng#, Ning Xu Landén#
J Invest Dermatol. 2022 Mar;142(3 Pt A):705-716.

Contents

List of abbreviations

ASOs	Antisense oligonucleotides
BSA	Bovine serum albumin
CCL20	Chemokine (C-C Motif) ligand 20
ChIP	Chromatin immunoprecipitation
ChIRP	Chromatin isolation by RNA purification
COPA	COPI coat complex subunit alpha
COPI	Coatomer protein complex I
CXCR3	C-X-C motif chemokine receptor 3
DAPI	4', 6-diamidino-2-phenylindole
DFUs	Diabetic foot ulcers
DNMTs	DNA methyltransferases
E2F1	E2F transcription factor 1
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
FGF2	Fibroblast growth factor 2
FLG	Filaggrin
FIRRE	Functional intergenic repeating RNA element
FISH	Fluorescent in situ hybridization
GO	Gene ontology
GSEA	Gene set enrichment analysis
HB-EGF	Heparin-binding EGF-like growth factor
hnRNP	Heterogeneous nuclear ribonucleoprotein
HOXC13-AS	HOXC13 Antisense RNA
HS	Hypertrophic scar
IF	Immunofluorescence
IGF	Insulin growth factor
IL-1α	Interleukin one alpha

IL-1β	Interleukin one beta
IL-6	Interleukin 6
ILF2	Interleukin enhancer binding factor 2
IVL	Involucrin
kDa	Kilodaltons
KGF	Keratinocyte growth factor
KO	Knockout
KRT1	Keratin 1
KRT5	Keratin 5
KRT10	Keratin 10
KRT14	Keratin 14
LAMB3	Laminin subunit beta 3
LCM	Laser capture microdissection
LncRNA	Long noncoding RNA
LOR	Loricrin
MACS	Magnetic activation cell sorting
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
miRNAs	MicroRNAs
MMPs	Matrix metalloproteinases
mRNAs	messenger RNAs
NEAT1	Nuclear enriched abundant transcript 1
NF-κB	Nuclear factor kappa B
NORAD	Noncoding RNA activated by DNA damage
nt	Nucleotide
NW1	One-day wound edges from healthy volunteers
NW7	Seven-day wound edges from healthy volunteers
PBS	Phosphate-Buffered Saline
PCA	Principal component analysis
PDGF	Platelet-derived growth factor
Pol II	Polymerase II
PRC2	Polycomb repressive complex 2

PUs	Pressure ulcers
qRT-PCR	Quantitative reverse transcription PCR
RIP	RNA immunoprecipitation
RNA	Ribonucleic acid
rRNA	ribosomal RNA
SC	Stratum corneum
siRNA	Small interfering RNA
TF	Transcription factor
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
UPR	Unfolded protein response
VEGF-A	Vascular endothelial growth factor A
VUs	Venous ulcers
WAKMAR1	Wound and keratinocyte migration-associated lncRNA 1
WGCNA	Weighted gene co-expression network analysis
WT	Wild type
XIST	X-inactive specific transcript

1 Literature review

1.1 The human skin

As the human body's largest organ, the skin covers a surface area of around $2m^2$ and accounts for approximately 20% of adult body weight (Bragazzi et al., 2019). The skin is a barrier protecting the body from dehydration and the external environment's biological, physical, and chemical stress (Kanitakis, 2002). It is one source of vitamin D (Mostafa and Hegazy, 2015) and plays the role of thermal regulation and sensations. The skin comprises three layers, i.e., epidermis, dermis, and subcutaneous tissue, along with vital appendages, including hair follicles, nails, sweat and sebaceous glands (**Fig. 1**).

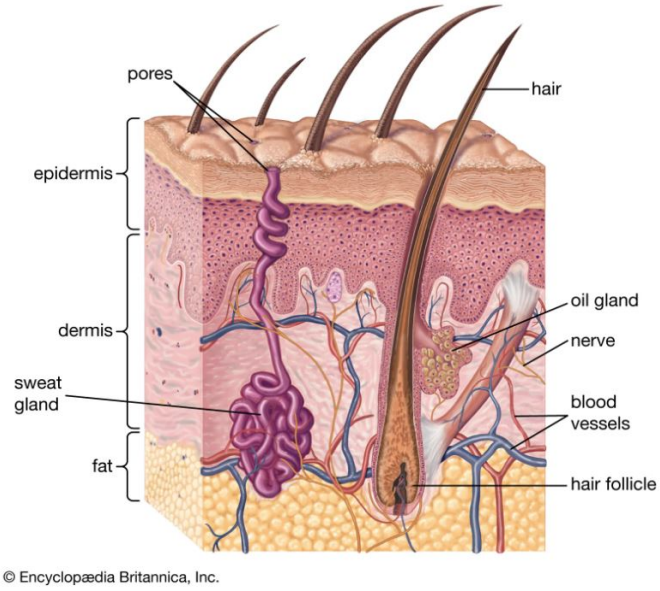

Figure 1 *Anatomy of the human skin. By courtesy of Encyclopædia Britannica, Inc., copyright 2017; used with permission.*

1.2 The epidermis

The epidermis is the outermost layer of the skin, composed of multiple stratified, squamous epithelium layers. It contains multiple cell types, including keratinocytes, melanocytes, Merkel cells, and Langerhans cells. Among these cell types, keratinocytes make up around 95% of the epidermis. Based on the cell morphology and keratinocyte differentiation states, the epidermis can be further divided into the basal layer, spinous layer, granular layer, clear/translucent layer (only found on the palms and soles), and cornified layer (Kolarsick et al., 2011; Losquadro, 2017) (**Fig. 2**).

The basal layer (also called stratum basale) is the innermost layer of the epidermis. It is a single layer of column-shaped keratinocytes with a long axis perpendicular to the dermis and attaches

to the basement membrane by hemidesmosomes (Kolarsick et al., 2011). Basal keratinocytes marked with keratin 5 and 14 (KRT5/14) are mitotically active and push outwards to refill epidermal cells in the suprabasal layers (Chu, 2012; Moll et al., 2008). Melanocytes reside in the stratum basale and transfer melanin into numerous keratinocytes, which are vital for determining the skin color and photoprotection of skin cells (Cichorek et al., 2013). Merkel cells also situate in the layer with large numbers in touch-sensitive areas, including fingertips and tips. They act as mechanical transducers that convert signals into sensory neurons (Maksimovic et al., 2014).

The spinous layer (stratum spinosum) consists of 5-10 layers of early differentiated keratinocytes with a polyhedral shape. The spinous cells express differentiation marker genes KRT1 and KRT10 and contact neighboring cells by desmosomes (Chu, 2012; Moll et al., 2008), which appear as spines, named for the spinous layer. Langerhans cells are found in the layer, where they play a role in antigen presentation and participate in the immune system of the skin (Chomiczewska et al., 2009).

The granular layer (stratum granulosum) varies in thickness in proportion to that of the overlying cornified layer, ranging from one to thirty cell layers (Kolarsick et al., 2011). This layer is formed by flattened cells containing keratohyalin granules and lamellar bodies. Filaggrin (FLG) is the main component of keratohyalin granules, which aggregates the keratin intermediate filaments into tight bundles (Eckhart et al., 2013). Moreover, lipid lamellae and enzymes in lamellar bodies are extruded into the extracellular place between the granular layer and the cornified layer, and the enzymes from lamellar bodies modify the intercellular lipids (Eckhart et al., 2013). The granular keratinocytes also express differentiation marker genes such as Involucrin (IVL) and Loricrin (LOR) (Nithya et al., 2015; Watt, 1983).

As the outermost layer of the epidermis, the cornified layer (stratum corneum, SC) comprises corneocytes, which are dead, big, and flattened keratinocytes at the terminal differentiation state (Matsui and Amagai, 2015). Corneocytes tightly contact each other by corneodesmosomes, which are connected to the cornified envelope, where keratins are still the main components and play an essential role in the mechanical resistance of the SC (Eckhart et al., 2013). Ultimately, the corneocytes are shed from the uppermost layers of the SC, undergoing desquamation. The entire differentiation process is counterbalanced by desquamation and regeneration to maintain epidermal homeostasis (Candi et al., 2005). The SC is essential for many barrier tasks, preventing water loss, alleviating exogenous oxidants, protecting against invading microbes, and protecting other cells and structures beneath from the detrimental effect of ultraviolet radiation (Del Rosso and Levin, 2011).

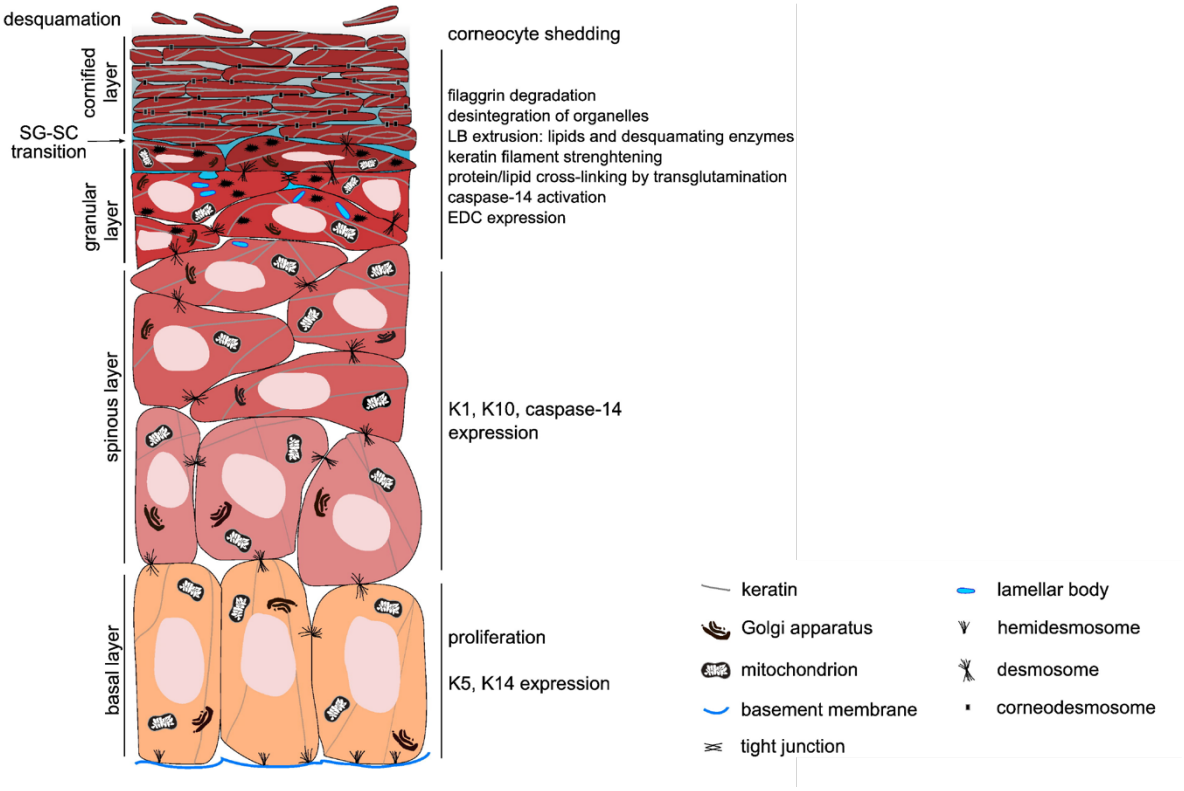

Figure 2 *The process of keratinocyte differentiation. Adapted from Eckhart et al., 2013; used with permission.*

1.3 The dermis

The dermis lies between the epidermis and the subcutaneous tissue and provides skin pliability, elasticity, and tensile strength (Kolarsick et al., 2011). It comprises extracellular matrix (ECM) proteins, multiple resident cell types, and numerous appendages (Xue et al., 2022). The dermis can be divided into the upper papillary and the underlying reticular dermis. The papillary dermis contains thin and poorly organized collagen bundles with a significant amount of collagen type III. Various cells with a high density situate in the area (Meigel et al., 1977; Sorrell and Caplan, 2009). The reticular dermis comprises thicker and well-organized collagen bundles enriched in collagen type I. Different cell types sparsely locate in this layer (Stunova and Vistejnova, 2018; Watt and Fujiwara, 2011).

Fibroblasts are the primary cell type in the dermis and contribute to collagen production (Sorrell and Caplan, 2004). A recent single-cell RNA sequencing result finds at least four distinct fibroblast clusters in adult human skin (Philippeos et al., 2018). Those cells play different roles in cell proliferation, cell differentiation, producing ECM and cytokines/growth factors (Xue et al., 2022).

The subcutaneous tissue lies beneath the dermis. It contains fat, superficial fascia, blood vessels, and nerves. This layer connects the skin with deeper tissue like bone and muscle

(Losquadro, 2017). In addition to its essential role in energy-relevant processes, current studies demonstrate that adipose tissue contributes to skin wound healing and immune response (Chen et al., 2019; Rippa et al., 2019). Moreover, adipose-derived stem cells have been widely used for tissue repair and regeneration in the past several years (Feisst et al., 2015; Trevor et al., 2020).

1.4 Normal wound healing process

In healthy people, burns, traumatic injuries, or surgical wounds can usually heal in a timely mode (Li et al., 2007). The normal wound healing process has been characterized as four sequential but overlapping phases, i.e., hemostasis, inflammation, proliferation, and remodeling **(Fig. 3)**.

1.4.1 The hemostasis

The hemostasis phase occurs immediately after injury and lasts for a few hours. The platelets enter wounds from the traumatized blood vessels and play a central role in forming a fibrin clot and coagulation. During homeostasis, anti-thrombotic agents and prostacyclin secreted from the neighboring endothelial cells prevent platelet attachment and aggregation (Golebiewska and Poole, 2015). Upon wounding, vasoconstrictors released by injured cells contribute to reflexive contracture of the smooth muscle, which are crucial for rapidly and temporally reducing bleeding. Subsequent coagulation is required to resolve to bleed eventually (Strecker-McGraw et al., 2007). In order to form the platelet plug, platelets bind to the thrombogenic subendothelial matrix through their G protein-coupled receptors and activate the inside-out signaling pathway, which contribute to platelet activation and enhanced attachments of platelets to each other and the matrix (Pradhan et al., 2017; Rodrigues et al., 2019). A secondary hemostasis plug or the thrombus is formed through the crosslink between fibrin and factor XIII and subsequent binding to the aggregated platelet. The fibrin clot serves as a provisional matrix for infiltrating cells. Platelets also produce several growth factors, i.e., platelet-derived growth factor (PDGF) (Eriksson et al., 1992), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), and insulin growth factor (IGF) (Pool, 1977; Rodrigues et al., 2019), which are essential for activating fibroblasts, endothelial cells, and macrophages (Reinke and Sorg, 2012).

1.4.2 The inflammatory phase

In the early inflammatory phase, neutrophils are recruited from blood vessels to the wound areas by injury-related signals, such as the activated supplement system, the degranulation of platelets, and bacterial products (Grose and Werner, 2004). Neutrophils account for 50% of all

cells in the wound on the first day after the injury. They release toxic granules, mainly composed of proteases, perform phagocytosis, and generate neutrophil extracellular traps to kill bacteria and remove necrotic tissues (Rodrigues et al., 2019). Neutrophils also secret chemoattractants, including interleukin one alpha (IL-1α), interleukin one beta (IL-1β), and tumor necrosis factor alpha (TNF-α), to activate local fibroblasts and keratinocytes and recruit macrophages (Eming et al., 2007; Hubner et al., 1996; Reinke and Sorg, 2012).

In the late phase of inflammation, approximately three days after injury, if the wound does not get infected, macrophages predominate at the wound site, which play a role in phagocytosis of pathogens and secretion of growth factors and cytokines, crucial for the following steps of wound repair (Reinke and Sorg, 2012; Tziotzios et al., 2012). In the early stage, pro-inflammatory macrophages that express TNF-α, interleukins (IL)-6, and IL-1β, engulf and kill pathogens in phagosomes (Rodrigues et al., 2019). The M1 macrophages also secret matrix metalloproteinases (MMPs) to facilitate the digestion of the extracellular matrix and aid cell migration. In turn, the digested fragments of the extracellular matrix promote inflammation (Sorokin, 2010). Moreover, the macrophages contribute to eliminating expended neutrophils at around three days post-injury. When the inflammation is resolved, macrophages transit into an anti-inflammatory M2 phenotype, and the M2 macrophages play a critical role in the proliferative phase of wound healing (Rodrigues et al., 2019).

1.4.3 The proliferative phase

In about 3-10 days after injury, wound healing enters the proliferative phase. It is characterized by the formation of granulation tissue, restoration of the vascular system, and re-epithelialization. In order to form granulation tissue, fibroblasts migrate into the wound edge, producing the ECM, mainly in the form of collagen. This granulation tissue replaces the provisional matrix forming at the hemostasis phase. Some fibroblasts differentiate into myofibroblasts, which play a significant role in wound contraction (Gurtner et al., 2008).

The angiogenesis process is initiated by endothelial cells activated by the vascular endothelial growth factor A (VEGF-A) and fibroblast growth factor 2 (FGF2) (Turksen, 2019). The endothelial cells undergo a sprouting process. They proliferate and migrate into the wounds, forming new blood vessels essential to provide nutrition and oxygen for tissue repair (Li et al., 2007; Reinke and Sorg, 2012). Interestingly, endothelial cells play different biological roles regulated by the Notch pathway and its effectors (e.g., Delta-like 4 and Jagged 1) during angiogenesis (Rodrigues et al., 2019). Tip cells act as leader cells that extend towards an

increasing VEGF-A gradient, whereas stalk cells follow the tip cells to keep the integrity of the existing vasculature (Gerhardt et al., 2003).

The re-epithelialization proceeds when the wound-edge keratinocytes and the epithelial stem cells from nearby hair follicles or sweat glands proliferate and migrate to the wound bed, restoring the broken epidermis (Lau et al., 2009; Martin, 1997). Upon injury, keratinocytes are modulated by integrin signaling and several growth factors. Suprabasal keratinocytes express integrin that results in upregulated Erk-MAPK signaling and production of pro-inflammatory cytokines, which are vital for cell hyperproliferation and immune response (Hobbs et al., 2004). Furthermore, integrin deficiency in keratinocytes delays wound healing (Grose et al., 2002; Reynolds et al., 2008). Migratory keratinocytes are activated by the epidermal growth factor family such as epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), and transforming growth factor alpha (TGF-α), as well as fibroblast growth factors, including FGF2 and keratinocyte growth factor (KGF), which also enhance the expression of keratins 6, 16 and 17 (Freedberg et al., 2001; Werner et al., 1994). Those keratins are critical in keratinocyte motility (Pastar et al., 2014). Additionally, IL-1, IL-6, and TNF-α contribute to cell migration (Barrientos et al., 2008). In the later phases of wound healing, keratinocytes reduce the expression of basal cell markers, including KRT5 and KRT14, which is caused by TGF- β produced from activated fibroblasts (Pastar et al., 2014).

1.4.4 The remodeling phase

The remodeling phase is the final stage of wound healing, which could last for one year or even longer. During this phase, all the activated angiogenic and wound metabolic processes gradually decline and then stop (Reinke and Sorg, 2012). In ECM, the collagen III synthesized at the proliferative phase converts to collagen I, the major ECM component before injury (Cañedo-Dorantes and Cañedo-Ayala, 2019; Gonzalez et al., 2016). Macrophages participate in the phase by eliminating dead cells and ECM debris. Macrophages do not eliminate excessive fibroblasts in time, which may cause hypertrophic scars (HSs) (Wernig et al., 2017). During remodeling, endothelial cells become apoptotic and quiescent, forming stable and well-perfused blood vessels. Endothelial cells express C-X-C motif chemokine receptor 3 (CXCR3) in the phase, which decreases the formation of endothelial tubes (Bodnar et al., 2006). CXCR3 deficient mice show HSs, suggesting its critical role in remodeling (Yates et al., 2010).

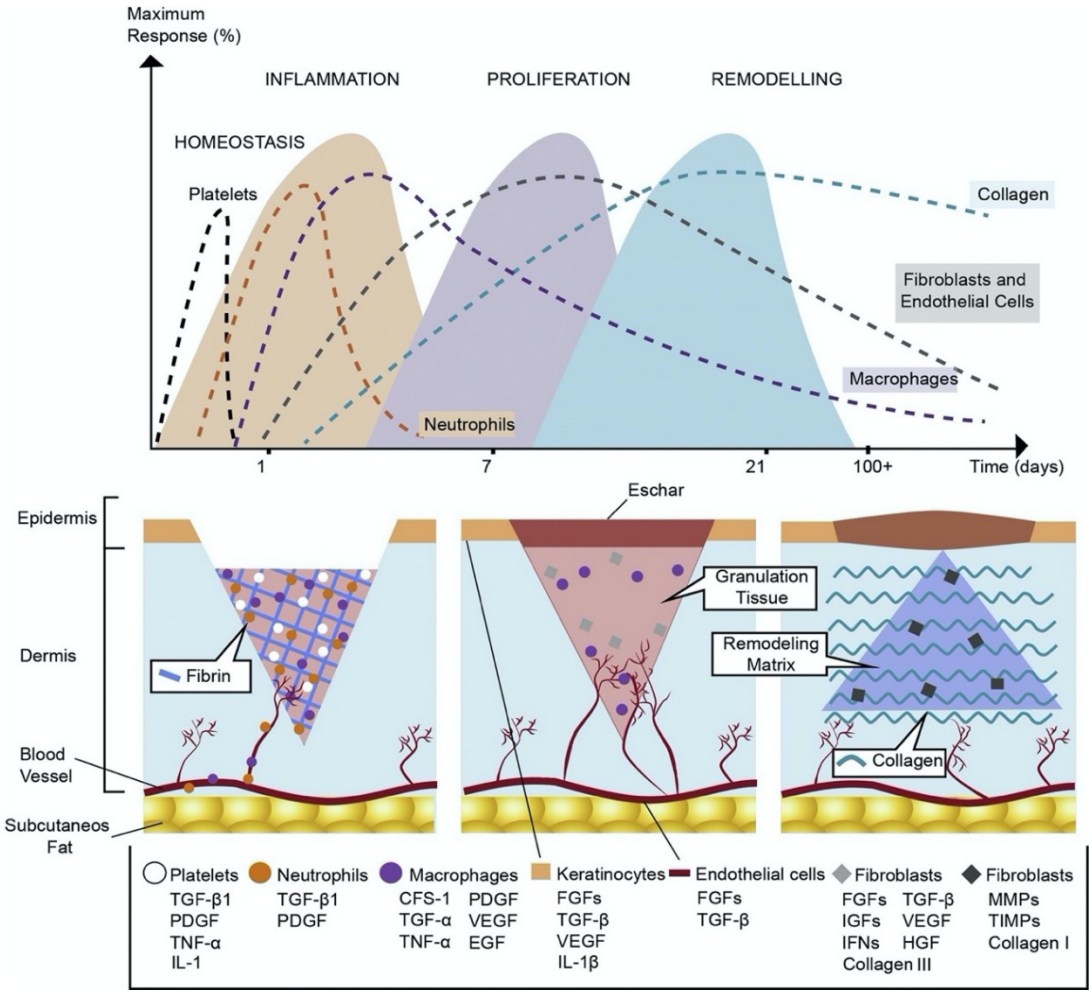

Figure 3 *Schematic diagram of the normal wound healing process. Adapted from Zomer and Trentin, 2018; used with permission.*

1.5 Chronic wounds

1.5.1 Etiology and epidemiology

Chronic wounds are a global problem and are defined as barrier defects of extended duration (more than six weeks) or frequent recurrence (Fonder et al., 2008; Singh et al., 2004). Older people suffering from blood supply problems, immunological deficiency, metabolic diseases, or chronic mechanical stress are susceptible to chronic wounds.

Pressure ulcers (PUs) are caused by insufficient blood supply due to prolonged biomechanical force, which often happen to the elderly, wheelchair-bound or bedridden, and malnutrition (Dealey et al., 2015; Grey et al., 2006). The prevalence of PUs ranged from 0.02% in the community, 4.0% in nursing homes, to 5.8% in hospitals, based on a 2019 study in Portugal (Falanga et al., 2022; Lopes et al., 2020). A study in Spain showed a similar community prevalence, and a study in China reported a similar nursing home prevalence (Diaz-Herrera et al., 2021; Falanga et al., 2022; Wei et al., 2021). In Switzerland, they assessed 51 nursing homes, and the prevalence ranged from 0 to 19% (Courvoisier et al., 2018). In addition to

residence, the prevalence of PUs is also affected by patients' characteristics and age (Anthony et al., 2019; Courvoisier et al., 2018; Falanga et al., 2022; Mervis and Phillips, 2019).

Venous ulcers (VUs) are the most common type of chronic wounds after PUs (Falanga et al., 2022), which mainly occur in the low extremity of the body and result from chronic venous diseases and venous hypertension (Fonder et al., 2008). The prevalence of VUs is around 1.5 to 3 cases per 1,000 population (Homs-Romero et al., 2021). Women and the elderly are susceptible to VUs (Falanga et al., 2022; Margolis et al., 2002; Nelson and Adderley, 2016).

Due to its association with diabetes, diabetic foot ulcers (DFUs) may be the best-studied type of chronic wound (Falanga et al., 2022). Diabetic patients have a 15%-20% risk of developing DFUs during their lifetime (Adiewere et al., 2018; Gottrup and Apelqvist, 2012; Nolan et al., 2011). Because of the lack of effective treatment, the five-year mortality rate of DFUs and ischemic ulcers is even higher than that of some cancer types (Eming et al., 2014).

1.5.2 Pathophysiology

The pathophysiology of chronic wounds is very complex, and the underlying mechanism is unclear. Healing of chronic wounds might be disturbed at any phase, showing a nonlinear organization of the phases instead of fitting the 'linear' paradigm of sequential but overlapping stages of normal wound healing (Falanga et al., 2022). Aberrations in many factors reviewed in normal wound healing likely result in chronic wounds. Persistent inflammation (Loots et al., 1998; Sindrilaru et al., 2011), pathogenic bacteria infection, senescence, and proteases are also implicated in the pathogenesis of chronic wounds (Gushiken et al., 2021).

Inflammation is prolonged in nonhealing wounds, mainly because of the stalled transition from the inflammatory to the proliferative phase (Eming et al., 2009; Eming et al., 2014). The increased number of infiltrating neutrophils and macrophages results in delayed healing in chronic wounds (Loots et al., 1998; Sindrilaru et al., 2011). Besides, the production and activation of proinflammatory cytokines, e.g., TNF-α and IL-1β, further prolong the inflammatory stage (Barrientos et al., 2008). Those cytokines contribute to increased MMPs that continuously lead to the degradation of local ECM and then delay cell migration (Barrientos et al., 2008). Inflammasome, a multiprotein complex derived from the innate immune system, is also involved in chronic wounds. It has a role in the secretion and activation of IL-1β (Feldmeyer et al., 2007; Stojadinovic et al., 2013). Moreover, excessive bacterial load results in the accumulation of proinflammatory cells and thus prolongs inflammation and impairs wound healing (Eming et al., 2014; Frank et al., 2009; Pastar et al., 2013; Roche et al., 2012).

During the inflammatory phase, microbes are expected to be eliminated by the immune response. However, pathogenic bacteria form biofilms that enhance their resistance to the host immune response, leading to chronic inflammation (Pastar et al., 2013). It becomes clear that biofilm helps establish polymicrobial communities resulting in their resistance to antibiotics that eliminate a specific bacterial population (Falanga et al., 2022). It has been shown that premature aging in fibroblasts from VUs decreases the capacity for migration (Wall et al., 2008). Senescent fibroblasts and keratinocytes likely have a role in antifibrotics by expressing MMPs 2, 3, and 9 (Jun and Lau, 2010). Moreover, senescent keratinocytes secrete the anti-angiogenic factor maspin, which delays wound repair (Nickoloff et al., 2004). Deregulation of proteases and their inhibitors also results in chronic wounds. MMPs such as collagenase and gelatinases A and B are increased in the fluids of chronic wounds (Eming et al., 2010; Yager et al., 1996). Excessive serine proteases break down fibronectin and multiple growth factors, impairing the proper wound healing process (Buchstein et al., 2009; Eming et al., 2014; Lauer et al., 2000; Wlaschek et al., 1997).

1.5.3 Management

Efficient treatments that can cure chronic wounds are currently lacking, which can be partially attributed to the insufficient knowledge of chronic wound pathology, high development costs, and poor design of clinical trials (Maderal et al., 2012). However, new wound dressings, devices, drugs, and surgical and biological approaches have substantially improved the management of chronic wounds (Falanga et al., 2022).

Several types of wound dressing have been applied to chronic wounds: moisture-retentive dressings (also called occlusive dressings) contribute to the formation of granulation tissue and promote the healing of chronic wounds (Eaglstein and Falanga, 1997; Obagi et al., 2019); foams are used for fluid absorption; hydrocolloids aid antibacterial protection and painless debridement (Falanga, 1988). A systemic drug Pentoxifylline has been used to promote wound healing of VUs (Falanga et al., 1999; Jull et al., 2002). Moreover, surgical debridement that removes necrotic tissue can help to accelerate the healing of chronic wounds (Falanga et al., 2008). Negative pressure is another valuable treatment for chronic wounds. The device removes chronic wound fluid that impairs cell proliferation *in vitro* and delays wound healing *in vivo* (Bucalo et al., 1993; Katz et al., 1991; Langer et al., 2015; Seidel et al., 2020). As antibiotic-resistant bacteria present in chronic wounds, antimicrobial approaches without antibiotics are investigated. Oral probiotic supplementation has been shown to improve the healing of DFUs, which may be mediated by the gut–skin axis (Mohseni et al., 2018).

Advanced therapeutic approaches are used for the treatment of chronic wounds. The U.S. Food and Drug Administration (FDA) has approved the application of recombinant PDGF to treat DFUs (Falanga, 2005; Steed, 1995), but its therapeutic efficiency on other types of chronic wounds is unclear. Bioengineered or 'artificial' skin consisting of living cells has also been used to promote wound healing (Falanga et al., 1998; Falanga and Sabolinski, 1999; MacNeil, 2007; Veves et al., 2001).

1.6 Non-coding RNAs

Human beings are estimated to express 20,000 protein-coding genes, constituting less than 2% of the genome. In 2012, the Encyclopedia of DNA Elements project (Consortium, 2004) uncovered that 80% of the human genome could be transcribed, but the majority of transcription output are RNAs without protein-coding capacity, i.e., non-coding RNAs (Fatica and Bozzoni, 2014; Mercer et al., 2009). This review focuses on two types of non-coding RNAs, i.e., microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) and characterizes their roles in skin wound healing.

1.7 MicroRNAs

MicroRNAs are single-strand non-coding RNAs with approximately 22 nucleotides (nts) in length (Bartel, 2018). In the canonical biogenesis pathway, miRNA genes are transcribed to primary precursors (pri-miRNAs) by RNA polymerase II (Pol II), which are cleaved by a microprocessor composed of an RNase III enzyme Drosha, and an RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8), forming pre-miRNA hairpins with approximately 70 nts in length. The pre-miRNAs are transported by an exportin 5/RanGTP complex to the cytoplasm. They are further processed by another RNase III enzyme Dicer into miRNA duplexes. Transactivation-responsive RNA-binding protein (TRBP) facilitates cleavage of some miRNAs and contributes to the subsequent assembly of the RNA-induced silencing complex (Lin and Gregory, 2015). Only the guide strand retains in the silencing complex and binds to the 3' untranslated region (UTR) of target mRNAs, which leads to the repression of translation or degradation of the mRNA targets (Bushati and Cohen, 2007; Gebert and MacRae, 2019; Li et al., 2022) (**Fig. 4**). As most miRNAs are only partially complementary to their mRNA targets, individual miRNAs can regulate multiple targets, and sometimes these targets are involved in a common signaling pathway (Inui et al., 2010). Furthermore, one mRNA can be bound and regulated by several miRNAs that may exhibit synergistic effects.

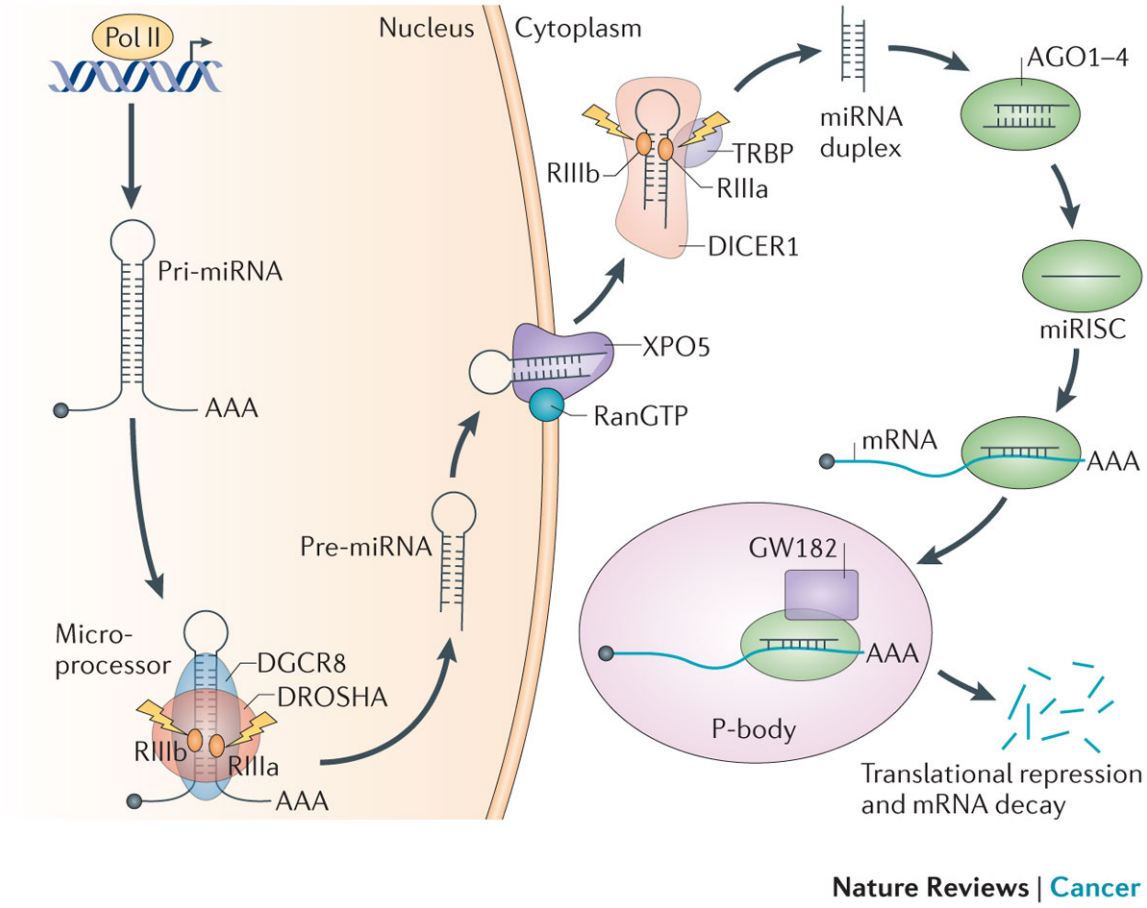

Figure 4 *Schematic diagram of miRNA biogenesis. Adapted from Lin and Gregory, 2015; used with permission.*

1.7.1 MiRNAs in skin wound healing

The expression and function of miRNAs have been studied in the different phases of skin wound healing. Dysregulated miRNAs are implicated in the persistent inflammation, a hallmark signature of chronic wounds. MiR-146a-5p has been identified as a negative feedback regulator of the innate immune response in keratinocytes. It is induced by the NF-κB-dependent signaling and inhibits excess inflammatory response (Hou et al., 2009; Meisgen et al., 2014; Taganov et al., 2006). The dysregulated miR-146a-5p expression has been reported in the mouse diabetic wound model, and correcting its expression can improve wound healing (Xu et al., 2012).

Moreover, our study has shown that miR-132-3p induced by TGF-β1 and TGF-β2 plays a critical role in promoting the transition from the inflammatory to the subsequent phase of wound healing. By increasing the activity of STAT3 and ERK pathways, miR-132-3p enhances cell growth. It inhibits the nuclear factor kappa B (NF-κB) pathway by suppressing the secretion of chemokines and the recruitment of immune cells. Accordingly, the miR-132-3p knockout mouse model exhibits delayed wound healing (Li et al., 2015b). MiR-34a-5p and miR-34c-5p are reported to be upregulated in epidermal cells of VUs compared to those of

normal wounds or the skin. They enhance inflammation by targeting LGR4 and mediating the NF-κB signaling pathway (Wu et al., 2020). Moreover, we found reduced expression of miR-19a/b-3p and miR-20a-5p in the wound-edge keratinocytes of several types of chronic wounds, i.e., VUs, DFUs, and PUs. MiR-19a/b-3p and miR-20a-5p can repress keratinocyte inflammatory response, and their loss may contribute to the sustained inflammation in chronic wounds (Li et al., 2020a).

Several miRNAs have been reported to regulate the biological processes in the proliferative phase. MiR-21-5p can promote keratinocyte and fibroblast migration targeting TIMP3 and PTEN, respectively (Madhyastha et al., 2012; Wang et al., 2012; Yang et al., 2011). Its downregulation results in delayed re-epithelialization. Additionally, miR-31-5p is upregulated in inflammatory and proliferative phases of skin wound healing compared to intact skin (Li et al., 2015a). Overexpression of miR-31-5p promotes keratinocyte migration and proliferation, and its epidermal deficiency results in delayed wound healing in mice (Shi et al., 2018). In contrast, let-7-5p and miR-200b/c-3p are downregulated in mouse wound healing. Overexpression of let-7-5p impairs cell migration in *vitro* and re-epithelialization in mice by targeting IGF2BP2 (Wu et al., 2017). MiR-200b/c-3p represses RAC1 and thus inhibits keratinocyte migration and re-epithelialization in mice (Tang et al., 2020). Angiogenesis-relevant miRNAs have been studied in wound healing as well. MiR-148b-3p is a critical regulator in TGF-β signaling crucial for endothelial cell homeostasis. Overexpression of miR-148b-3p promotes migration, proliferation, and angiogenesis of endothelial cells. It also accelerates wound healing and vascularization in mice (Miscianinov et al., 2018). MiR-200b-3p and miR-15b-5p are upregulated in diabetic wounds and inhibit angiogenesis by targeting VEGF and VEGF-R2, respectively. Knockdown of both miRNAs promotes wound healing and vascularization in mice (Pizzino et al., 2018).

Pathological scarring, e.g., HSs and keloids, may appear after wound repair due to the continuous growth of granulation tissue. MiR-21-5p has been identified as an HS biomarker, promoting the expression of fibrosis markers by targeting Smad7 (Zhou et al., 2015). Moreover, the knockdown of miR-21-5p inhibits HS growth in a nude mice model (Li et al., 2016). MiR-29a-3p is downregulated in fibroblasts isolated from keloid compared to those from healthy skin. Overexpression of miR-29a-3p decreases the expression of collagen I and III, whereas miR-29a-3p silencing increases the expression (Zhang et al., 2016).

1.7.2 Current status of miRNA-based wound treatment

Due to the unmet needs of wound treatment and the critical roles of miRNAs in skin wound healing, the development of miRNA-based therapy has attracted broad interest from biotech companies and pharmaceutical industries. The first siRNA drug was approved in 2018 (Ozcan et al., 2015), which paved the way for miRNA therapeutics development. Although there are no FDA-proved miRNA drugs for medical intervention, several drug candidates associated with accelerating wound healing undergo early-phase clinical trials. For example, the company miRagen performs a second phase one trial with MRG-110, a synthetic miR-92a inhibitor (Hanna et al., 2019). In the preclinical study, MRG-110 has been shown to increase granulation tissue formation and accelerate wound healing in a diabetic mouse model and a porcine wound model. MRG-110 exhibited a better therapeutic effect than the rhPDGF-BB in db/db mice model (Gallant-Behm et al., 2018). Also, miR-29 (MRG-201) is undergoing a phase one trial to treat keloid and reduce scar tissue formation (Hanna et al., 2019).

Despite a few miRNAs under clinical trials, challenging obstacles remain in the development of miRNA-based treatments for wound healing. Many miRNAs have been investigated *in vitro* and in animal models, but their role in human skin wound healing remains unclear. Although animal models provide an indispensable resource for understanding molecular and cellular functions of miRNAs, they cannot replace studies of human wounds. The mechanisms identified based on animal models, predominantly rodents, are only sometimes relevant to human wounds (Falanga et al., 2022). Healing of rodent wounds mainly relies on contraction instead of re-epithelialization. Therefore, more human wound data are needed to identify the most pathogenesis-relevant miRNAs in chronic wounds. In addition, the miRNA targetome has yet to be systematically investigated. Most studies focus on only one miRNA and one of its targets without considering the miRNA-target networks. To this end, a comprehensive and integrative analysis of miRNAs and their targets is needed in chronic wounds.

1.8 Long non-coding RNAs

Long non-coding RNAs (lncRNAs) are a group of RNAs that are longer than 200 nts and have no protein-coding capacity. Much like messenger RNAs (mRNAs), most lncRNAs are transcribed by RNA Pol II and often have 5'-end m^7G caps and 3'-end poly (A) tails (Guttman et al., 2009). Despite the absence of a translated open reading frame, lncRNAs exhibit similar biochemical features and length as mRNAs (Quinn and Chang, 2016; Statello et al., 2021). LncRNAs also differ from mRNAs: lncRNAs have fewer but longer exons (Cabili et al., 2011; Derrien et al., 2012); they are lowly expressed with poorer sequence conservation among

mammal species (Quinn and Chang, 2016; Ulitsky and Bartel, 2013); they exhibit higher tissue and cell type specificity.

1.8.1 Biogenesis of lncRNAs

Compared to mRNAs, lncRNAs are preferably localized in the nucleus. Their transcription mode may result in the nuclear location to some extent. Many lncRNAs are transcribed by phosphorylation-dysregulated Pol II (Schlackow et al., 2017). Those lncRNAs are weakly co-transcriptionally spliced, and their transcription termination is irregulated by polyadenylation signals (Statello et al., 2021). They are accumulated on chromatin and further degraded by the RNA exosome. U1 small nuclear RNA binding sites are observed on some chromatin-localized lncRNAs that are tethered on chromatin by recruiting U1 small nuclear ribonucleoprotein to Pol II during transcription (Yin et al., 2020). A lack of Pol II-associated elongation factor SPT6 also contributes to the accumulation of some lncRNAs on chromatin in the form of DNA damage-associated R-loops (Nojima et al., 2018). These lncRNAs are more transcribed because of the relocation of histone H3 trimethylated at Lys36 (H3K36me3) to their loci upon SPT6 loss. Weaker internal splicing signals, alternative polyadenylation signals, and nuclear retention elements within lncRNAs coordinately regulate the nuclear localization of lncRNAs (Statello et al., 2021).

Cytoplasmic lncRNAs share the processing and export pathways of mRNAs. They tend to undergo the export pathway of nuclear RNA export factor 1 due to fewer exons compared to mRNAs (Zuckerman et al., 2020). In the cytoplasm, RNA-binding proteins likely guide various lncRNAs to different organelles or specific areas within the cytoplasm. LncRNAs have been found in ribosomes and mitochondria (Statello et al., 2021).

1.8.2 Gene regulation by lncRNAs

Although 30,000 - 60,000 lncRNAs have been identified in humans (Hon et al., 2017; Iyer et al., 2015), controversy remains that lncRNAs are either functional or junk. Hundreds of papers state that emerging lncRNAs play a vital role in multiple biological processes. By contrast, others view most lncRNAs are transcriptional noise, and few lncRNAs act as essential regulators in human physiology and pathology (Ponting and Haerty, 2022). Recent studies demonstrate that transcription activity and DNA elements inside the lncRNA locus may play a role rather than the lncRNA itself (Kopp and Mendell, 2018). The minority of lncRNAs confer the regulatory function solely dependent on their RNA sequence. Nevertheless, those functional lncRNAs that consist of possibly thousands of molecules become a major gene class (Kopp and Mendell, 2018). According to their action modes, lncRNAs can be broadly

classified into two groups: *cis*-acting lncRNAs regulate the expression and chromatin state of neighboring genes, while *trans*-acting lncRNAs modulate the expression of genes distant from their transcription sites (Kopp and Mendell, 2018).

1.8.2.1 Cis-acting lncRNAs

The *in cis* actions of lncRNA include but are not limited to: lncRNA transcripts recruit other regulatory factors, e.g., polycomb repressive complex 2 (PRC2), to the locus of neighboring genes and regulate the expression of neighboring genes (Cerase et al., 2015); the process of transcription and splicing of the lncRNA regulates the expression of neighboring genes, independent of its transcript (Engreitz et al., 2016); the regulation only relies on the DNA element within the promoter or locus, separate of encoded DNA and lncRNA production process (Groff et al., 2016) (**Fig. 5**).

Sequence-dependent lncRNA regulation *in cis*

LncRNAs play critical roles in chromatin silencing and activation *in cis*. The best-known example is the lncRNA X-inactive specific transcript (XIST). During embryonic development, XIST causes the silencing of almost all genes in one of two X chromosomes in the cells of female mammals (Kopp and Mendell, 2018). Distal regions across the X chromosome are identified by XIST by exploiting the three-dimensional conformation of the X chromosome, which allows the silencing domain of XIST to spread across the entire chromosome (Engreitz et al., 2013; Statello et al., 2021).

Transcription or splicing of lncRNA loci confers regulatory effects

In some cases, the activity of lncRNA loci regulates the expression of neighboring genes, independent of its sequence. Engreitz and colleagues show that the prematurely terminal transcription or mutation of the first 5' splice site of lncRNA Blustr decreased the expression of the neighboring gene Sfmbt (Engreitz et al., 2016). However, the depletion of downstream exons and introns of Blustr did not influence the Sfmbt level, which suggests it is irregulated by specific sequences of Blustr. Indeed, the transcription or splicing process of Blustr plays a vital role in changing the chromatin states of the Sfmbt promoter and promoting its subsequent transcription (Kopp and Mendell, 2018). In another example, the prematurely transcriptional termination of Upperhand (Uph) decreases the expression of the neighboring gene Hand2. However, inserting the tdTomato coding sequence at the Uph locus and deleting the mature Uph transcript do not affect Hand2 expression (Anderson et al., 2016).

Functional DNA elements within lncRNA loci

Linc-21 is transcribed close to CDKN1A and was first reported to act as a *trans*-acting lncRNA and play a role via p53 (Huarte et al., 2010). Recent studies showed that its broad effects are likely attributed to the changed level of Cdkn1a-encoded protein p21 (Dimitrova et al., 2014). Genetic evidence showed that the genomic locus of linc-21 contains gene-regulatory elements that modulate the expression of neighboring genes even without the lncRNA expression (Groff et al., 2016). Additionally, the lncRNA Bendr locus functions as an enhancer-like element, independent of the transcription or sequence of Bendr (Engreitz et al., 2016). Depletion of the promoter of Bendr inhibits the expression of its neighboring gene Bend4, whereas the premature transcriptional termination of Bendr by inserting poly A signal into the first intron does not affect the Bend4 level.

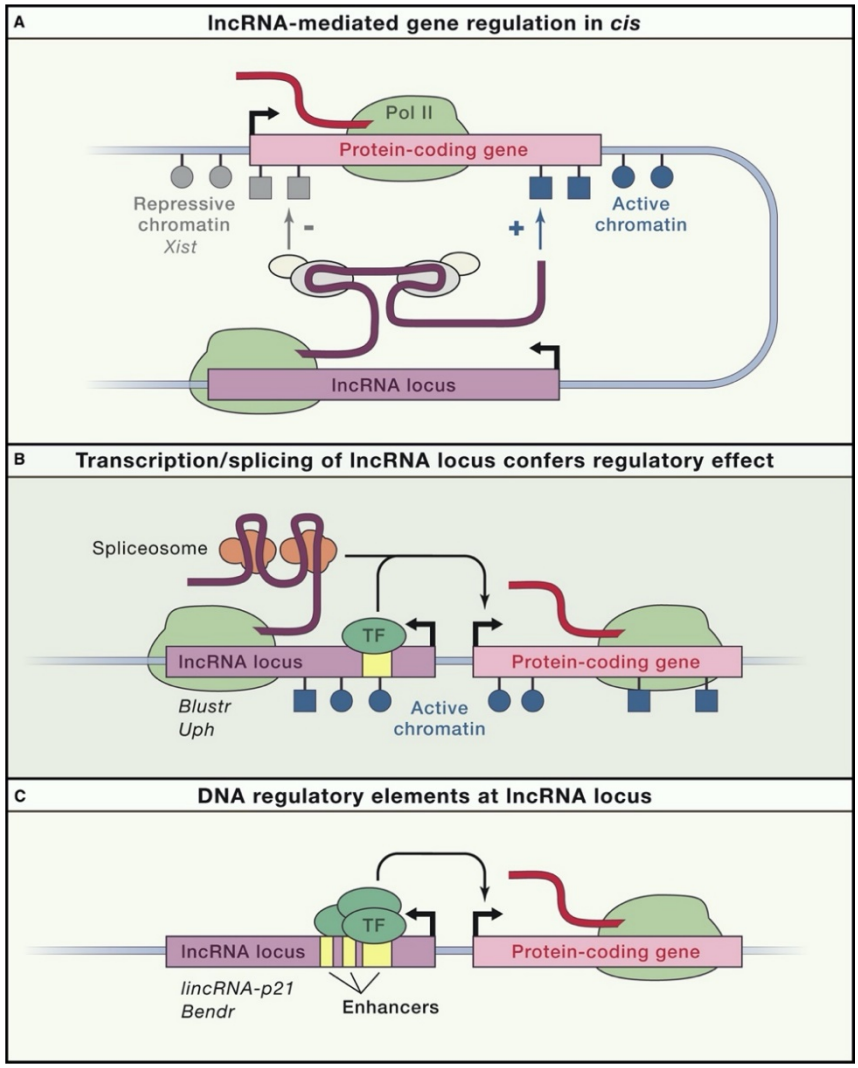

Figure 5 *Functions of the cis-acting lncRNAs. Adapted from Kopp and Mendell, 2018; used with permission.*

1.8.2.2 *Trans*-acting lncRNAs

Trans-acting lncRNAs play a role by mediating gene expression and chromatin states at areas distant from their transcription site, affecting nuclear structure and organization, and interacting with RNA or protein molecules (Kopp and Mendell, 2018) (**Fig. 6**).

Gene regulation by lncRNAs *in trans*

Trans-acting lncRNAs can modulate the chromatin states and affect the binding of transcription factors (TFs). For example, lncRNA HOTAIR regulates the expression of HOXD genes by recruiting PRC2 to the distant HOXD locus and changing the chromatin state (Chu et al., 2011; Rinn et al., 2007). Additionally, lincRNA-EPS interacts with the heterogeneous nuclear ribonucleoprotein (hnRNP), which results in a repressive effect on the targets of lincRNA-EPS (Kopp and Mendell, 2018). The targets of *trans*-acting lncRNAs are not limited to Pol II-transcribed genes. LncRNA SLERT enhances pre-rRNA transcription by interacting with and sequestering DDX21 from RNA polymerase I-binding loci, which relieves the repressive effect of DDX21 on transcription (Xing et al., 2017).

Organization of nuclear architecture by lncRNAs

The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was reported to localize in nuclear speckles containing splicing machinery components (Hutchinson et al., 2007). The recruitment of MALAT1 to speckles is mediated by its interactions with multiple splicing-associated proteins (Bernard et al., 2010). MALAT1 functions as a scaffold that enhances the relocation of nuclear speckles to nascent transcripts (Engreitz et al., 2014; West et al., 2014). The nuclear enriched abundant transcript 1 (NEAT1) localizes in paraspeckles that contain proteins involved in RNA production (Hutchinson et al., 2007). NEAT1 is required to form and maintain the nuclear compartments by interacting with paraspeckle proteins (West et al., 2014). Like MALAT1, NEAT1 facilitates the positioning of paraspeckles at active gene loci (West et al., 2014). Functional intergenic repeating RNA element (Firre) is transcribed from the X chromosome and remains at this transcription site and five unlinked autosomal loci. The trans-chromosomal interactions are mediated by Firre and hnRNP U, and the knockdown of Firre impairs the inter-chromosomal interactions, indicating the critical role of lncRNAs in chromatin localization (Hacisuleyman et al., 2014).

LncRNAs serve as decoy molecules regulating the interacting proteins and RNAs

Trans-acting lncRNAs can also serve as decoy molecules to interfere with the functions of proteins and other RNA molecules (Lee et al., 2016; Noh et al., 2018; Rashid et al., 2016; Tichon et al., 2016). RNA-binding proteins PUMILIO1 and PUMILIO2 regulate mRNA decay and translation. The noncoding RNA activated by DNA damage (NORAD) contains 15 PUMILIO response elements (PRE) that are specific binding sites for PUMILIOs. Thus, NORAD serves as a negative regulator of PUMILIOs, which interferes with the binding of the proteins to their mRNA targets (Lee et al., 2016; Tichon et al., 2016). LncRNAs can also regulate other RNA molecules through base-pairing interactions. For example, the cerebellar degeneration-related protein 1 antisense RNA (CDR1as) binds miR-7 via its over 70 conserved seed matches for miR-7, which results in the de-repression of miR-7 targets. On the other hand, CDR1as contains a perfectly complementary binding site for miR-671, which leads to the degradation of CDR1as (Hansen et al., 2013; Hansen et al., 2011; Memczak et al., 2013).

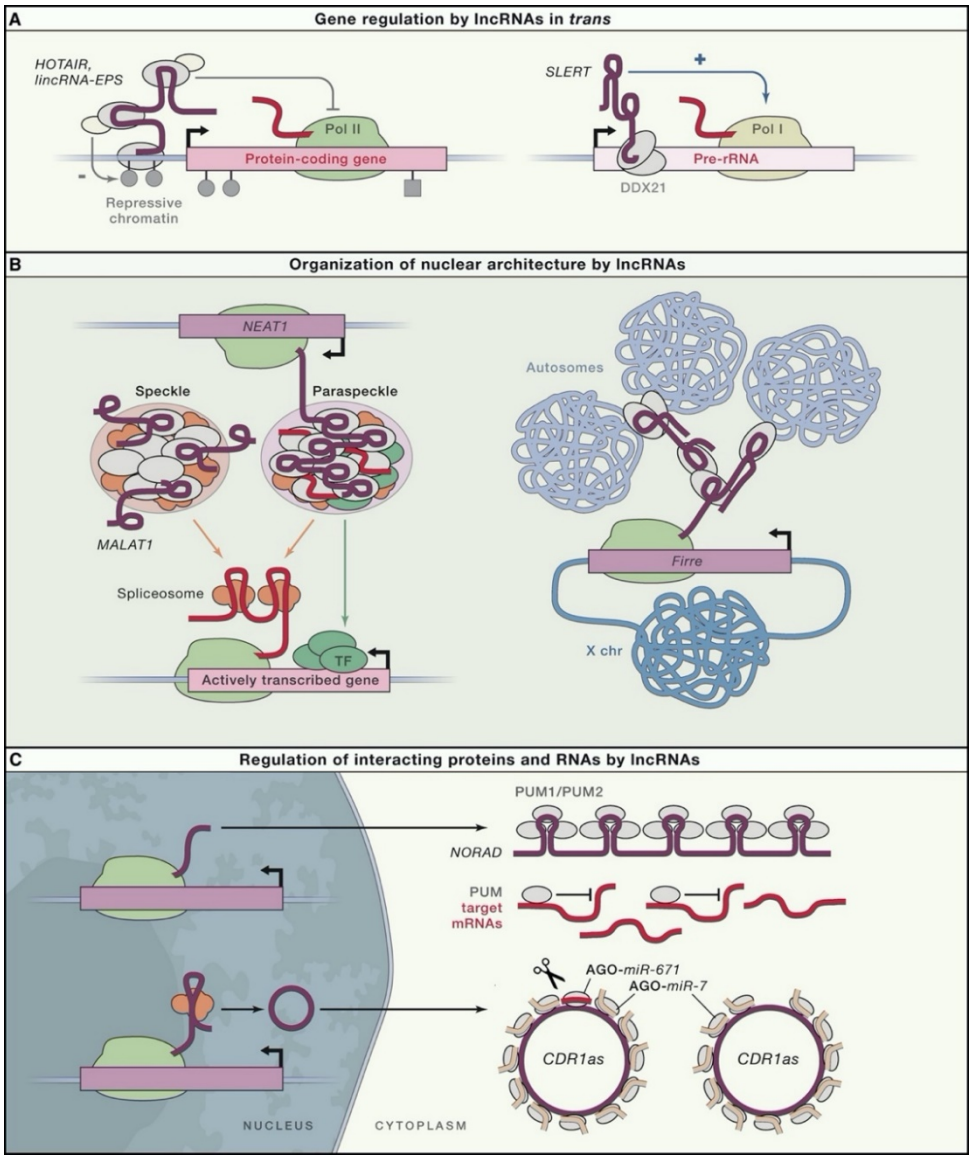

Figure 6 *Functions of trans-acting lncRNAs. Adapted from Kopp and Mendell, 2018; used with permission.*

1.8.3 LncRNAs in skin wound healing

The role of lncRNAs in skin wound healing is mostly unclear, and only a few lncRNAs have been studied in the wound repair process. For example, the lncRNA ANRIL (antisense non-coding RNA in the INK4 locus) has been shown to promote wound healing by regulating the expression of Prox1, which is vital for lymphangiogenesis (He et al., 2019). Mechanistically, ANRIL acts as a competing RNA to sequester miR-181a from Prox1 (He et al., 2019). Additionally, lncRNA GAS5 is upregulated in cells and tissues from diabetic wounds. It promotes macrophage polarization toward an M1 phenotype, and its downregulation enhances the healing of diabetic wounds (Hu et al., 2020).

Our group has recently characterized two novel lncRNAs that regulate keratinocyte migration in wound repair, termed WAKMAR1 and WAKMAR2 (Wound And Keratinocyte Migration-Associated lncRNA 1 and 2). The role of WAKMAR1 in human skin wound healing is characterized in the result part of the thesis. WAKMAR2 is RNA polymerase II-transcribed and polyadenylated lncRNA induced by TGFβ in keratinocytes and presents in both the nucleus and cytoplasm. WAKMAR2 promotes keratinocyte migration and re-epithelialization in a human *ex vivo* wound model and inhibits the inflammatory response of keratinocytes. A lack of the lncRNA may contribute to the pathology of chronic wounds (Herter et al., 2019).

1.8.4 LncRNAs in epidermal differentiation

LncRNAs also play essential roles in regulating epidermal homeostasis. For example, anti-differentiation non-coding RNA (ANCR) is downregulated in differentiated keratinocytes, adipocytes, and osteoblasts (Cai et al., 2019; Kretz et al., 2012). Deletion of ANCR causes upregulation of the differentiation genes in keratinocyte progenitors. It is required to maintain the undifferentiated state of progenitor cells (Kretz et al., 2012). Conversely, another lncRNA, i.e., terminal differentiation-induced non-coding RNA (TINCR), is highly expressed in differentiated keratinocytes. Its downregulation in the organotypic epidermal tissue reduces the expression of the differentiation-related marker genes, thereby disrupting epidermal barrier formation (Kretz et al., 2013). Besides, TINCR was shown to interact with differentiation-related mRNAs and Staufen1 protein in the cytoplasm, increasing the stability of these mRNAs. Furthermore, both ANCR and TINCR are the upstream regulators of MAF and MAFB, which are the essential transcription factors for keratinocyte differentiation, making up a regulatory network for epidermal barrier formation (Lopez-Pajares et al., 2015). Recently, Panatta and colleagues identified lncRNA UC291, which is upregulated during keratinocyte differentiation and regulates epithelial differentiation by interacting with ACTL6A protein to modulate chromatin remodeling activity (Panatta et al., 2020). Additionally, LINC00941 was found to

control human epidermal homeostasis via repressing SPRR5, a novel positive regulator of keratinocyte differentiation (Ziegler et al., 2019).

2 Research aims

This thesis aims to understand the role of long noncoding RNAs and microRNAs in human skin wound healing and epidermal differentiation.

The objectives of the individual studies are to:

- Study the role of a long noncoding RNA WAKMAR1 (Wound And Keratinocyte Migration-Associated lncRNA 1) in human skin wound healing (**Paper I**)

- Identify the functional importance of clinically relevant miRNAs in normal wounds and nonhealing venous ulcers (**Paper II**)

- Characterize the functional importance of a skin-specific long noncoding RNA HOXC13-AS in epidermal differentiation (**Paper III**)

- Investigate the functional significance of a long noncoding RNA SNHG26 in human skin wound repair (**Paper IV**)

3 Methods

Ethical considerations

The collection of the human biopsies has been performed in collaboration with medical doctors at Karolinska University Hospital and the Second Hospital of Dalian Medical University (Dalian, China). Written informed consent was obtained from all donors to collect and use clinical samples. All the experiments have been approved by the Stockholm Regional Ethics Committee (Document number: 2016/1621-31/2 and 2019-02335) and the Ethics Committee of the Second Hospital of Dalian Medical University and conducted according to the Declaration of Helsinki's principles.

Healthy volunteers were given local anesthesia to relieve the pain during surgeries. Superficial skin samples were taken using 4-6 mm punches, a minimally invasive sampling technique. The wounds were then stitched to facilitate healing. For chronic wound patients, biopsy collection is a routine procedure at the clinic for diagnosis, which results in a little discomfort and no risk of complication.

In order to protect the privacy of donors, all samples were coded. Only responsible doctors have access to information that links analysis results to individuals. We only have access to clinical information, including sex, age, wound position, and medical history, which is needed to interpret our results. The samples were analyzed immediately after collection, and the remnant was discarded following biological waste instructions. Participation in the project is entirely voluntary. The donors can withdraw their consent and stop participating in the projects without further explanation, and the samples will be discarded.

All the animal experiments have been performed according to animal ethics approval (Document number: 11854-2020). When planning and performing animal experiments, we follow the three Rs principle (Refinement, Reduction, and Replacement). General anesthesia and painkillers are utilized during and after surgeries to alleviate the pain. We scrutinize the status of animals and implement the humane endpoints strictly, under the close supervision of veterinarians. We use a minimal number of animals, which allows us to obtain confident results. Also, to further reduce the usage of animals, we collect multiple samples from each animal. However, animals are still an irreplaceable resource for studying wound healing *in vivo*.

Human wound samples

As normal wounds and chronic wounds were collected from Caucasian and Asian donors, chronic wounds were always compared to normal wounds from healthy volunteers of the same ethnicity (**Paper I–IV**). Three wounds were created using 4 mm biopsy punches, and wound-edge tissues were collected with 6 mm punches one day, seven days, and thirty days later, representing all phases of human skin wound healing (i.e., hemostasis/inflammation, proliferation, and remodeling).

Patients with chronic wounds, despite conventional therapy, persisted for more than two months were recruited. Patients with soft tissue infection, taking systemic antibiotics one day prior to biopsy, and immunocompromised patients were excluded from our studies. A 4 mm biopsy punch was used at the non-healing edges of chronic wounds. Healthy donors with diabetes, skin disease, unstable heart disease, infections, bleeding disorder, immune suppression, and any ongoing medical treatments were excluded.

To investigate the cell- or epidermis-specific expression of noncoding RNAs, we isolated specific cell populations using Laser Capture Microdissection (LCM) (**Paper I, II**) and Magnetic Activation Cell Sorting (MACS) (**Paper I-III**). For LCM, snap-frozen wound biopsies were cut into 8 μm sections after being embedded with Tissue-Tek O.C.T. Compound (ThermoFisher Scientific). The sections were stained with hematoxylin, and LCM was performed with Leica LMD7000 (Leica) to separate the epidermis. Using MACS, the CD45- epidermal cells (mainly keratinocytes) and CD90+ dermal cells (dermal fibroblasts) were isolated from the skin and wounds. After washing with Phosphate-Buffered Saline (PBS), the fresh skin and wound tissues were incubated in dispase II (5 U/ml, ThermoFisher Scientific) at 4°C overnight, and the epidermis was separated from the dermis the next day. After the digestion in 0.025% trypsin/ethylenediaminetetraacetic acid (EDTA) solution at 37°C for 15 min, single epidermal cells were loaded into magnetic columns together with CD45 microbeads (Miltenyi Biotec), and CD45− epidermal cells were collected. The dermis was incubated with an enzyme mix from a human Whole Skin Dissociation Kit (Miltenyi Biotec) at 37°C for 3 hours and processed using a Medicon tissue disruptor (BD Biosciences). The dermal cell suspension was incubated with CD90 microbeads, and CD90+ dermal cells were isolated using magnetic columns.

Cell culture, treatment, and transfection

Human adult epidermal keratinocytes were cultured in EpiLife medium supplemented with 10% Human Keratinocyte Growth Supplement (HKGS) and 1% Penicillin and Streptomycin

at 37°C in 5% CO2 (All from ThermoFisher Scientific) (**Paper I-IV**). We cultured adult dermal fibroblasts in Medium 106 supplemented with Low Serum Growth Supplement (LSGS) and 1% Penicillin and Streptomycin (All from ThermoFisher Scientific) (**Paper II**). We changed the culture medium every other day and subcultured cells when the culture was around 80% confluent. Briefly, the cells were washed with warm PBS two times, followed by incubation with 0.025% trypsin/EDTA at 37°C for 3 to 4 min. When 90% of the cells detached, we neutralized the trypsin with the Defined Trypsin Inhibitor (ThermoFisher Scientific). The cells were harvested by centrifuge and re-suspended with fresh culture medium. All the experiments were performed when cells were in passage 4.

To study the mechanism regulating the expression of the lncRNAs, we treated keratinocytes with cytokines and chemokines for multiple time points (**Paper I, III, IV**). To study the biological function of lncRNAs and miRNAs, keratinocytes or fibroblasts were transfected with the small interfering RNA (siRNA), pcDNA 3.1 (+) vectors, antisense oligonucleotides (ASOs), and miRNA mimics or inhibitors using Lipofectamine™ 3000 (ThermoFisher Scientific) (**Paper I-IV**). To identify the function of HOXC13-AS in keratinocyte differentiation, we increased calcium concentration to 1.5 mM in EpiLife medium (**Paper III**).

RNA extraction and quantitative reverse transcription PCR (qRT-PCR)

Prior to RNA extraction, the skin and wound biopsies were homogenized using TissueLyser LT (Qiagen). Total RNA was isolated from human tissues using a miRNeasy Mini kit (Qiagen) and cells using the TRIzol™ Reagent (ThermoFisher Scientific). RNA quantity was measured by NanoDrop One (ThermoFisher Scientific). The same amounts of RNA were used for reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) (**Paper I-IV**). The reverse transcription for miRNAs was performed using TaqMan® Advanced miRNA cDNA Synthesis Kit (ThermoFisher Scientific) (**Paper II**). Gene expression was determined by TaqMan expression assays (ThermoFisher Scientific) or by SYBR Green expression assays (ThermoFisher Scientific) and normalized based on the values of the housekeeping gene B2M, GAPDH, and 18S (for mRNAs and lncRNAs) or U48 (for miRNAs) using the delta-delta Ct-method.

Transcriptomic profiling

Gene expression microarrays were performed using human Clariom™ S assays (ThermoFisher Scientific) following the standard protocol at the core facility for Bioinformatics and Expression Analysis (BEA) at Karolinska Institute (**Paper I–IV**). Data were analyzed and visualized in Transcriptome Analysis Console (TAC). We performed gene

set enrichment analysis (GSEA) using public software (Subramanian et al., 2005) and Gene Ontology (GO) analysis by MetaCore software (Thomson Reuters). Heatmaps were plotted by the Multiple Experiment Viewer software.

For **bulk long RNA-sequencing** (**Paper II-IV**), we removed ribosomal RNAs (rRNAs) using the Epicentre Ribo-zero® rRNA Removal Kit (Epicentre). The NEB Next® UltraTM Directional RNA Library Prep Kit for Illumina® was used to prepare strand-specific total-transcriptome RNA sequencing libraries for the whole biopsies. The isolated cell RNA-seq libraries were prepared using the Ovation® SoLo® RNA-Seq library preparation kit (Tecan). All prepared libraries were sequenced on the Illumina Hiseq 4000 platform (Illumina). Raw sequencing reads were filtered using Trimmomatic v0.36 software (Bolger et al., 2014). We aligned the filtered reads to the human reference genome (GRCh38.p12) and the comprehensive gene annotation file (GENCODEv31) using STAR v2.7.1a (Dobin et al., 2013). Unique mapped fragments to exons were used to quantify gene expression by the Subread package (Liao et al., 2014), followed by the differential expression analysis using DESeq2 (Love et al., 2014).

For **paper II**, the **small RNA sequencing** libraries were prepared using NEB Next® Multiplex Small RNA Library Prep Set for Illumina® (NEB) and sequenced on an Illumina Hiseq 2500 platform (Illumina) using single-end 50bp reads. Raw small RNA sequencing data was assessed using FastQC v0.11.8 for quality control. We cleaned low-quality reads and removed sequencing adaptors and redundancies using mapper.pl module in the miRDeep2 v0.1.3 package (Friedlander et al., 2012; Mackowiak, 2011) before mapping reads to the human reference genome (hg38) by the software Bowtie v1.2.2 (Langmead et al., 2009). Known miRNAs were identified with the miRDeep2.pl module and compared to miRNAs in miRBase release 22.1 (Kozomara et al., 2019). The mapped read counts were acquired from the quantifier.pl module output. The TPM method (transcript per million = mapped read count/total reads * 10^6) was used to normalize the raw counts of miRNAs (Zhou et al., 2010). Differential expression analysis was performed using DESeq2.

For **paper III-IV**, **single-cell RNA sequencing** was performed. Epidermal and dermal cell suspension were obtained using the MACS protocol mentioned in the thesis. Red blood cells and dead cells were removed using Red Blood Cell Removal Solution (Miltenyi Biotec) and Dead Cell Removal Kit (Miltenyi Biotec). We mixed an equal number of epidermal and dermal cells, loaded them onto each channel of the Single Cell chip (10x Genomics), and then performed droplet encapsulation on the Chromium Controller. Following the manufacturer's protocol, we prepared sequencing libraries using the library construction kit (10x Genomics)

and performed sequencing on Illumina NovaSeq 6000 S4 v1.5. Raw sequencing data were processed with the cellranger pipeline (10X Genomics, version 5.0.1), including demultiplexing, aligning to the hg38 reference genome, barcode counting, and unique molecular identifier (UMI) quantification.

We used the Seurat R package (version 4.0.6) (Hao et al., 2021) to remove the genes expressed in less than ten cells and cells expressing less than 500 genes, less than 1000 UMI counts, or more than 20% mitochondrial genes. The data were normalized with sctransform (Hafemeister and Satija, 2019). The top 4000 variable genes were used to generate principal component analysis (PCA) using RunPCA, and the first 40 principal components (PCs) were performed to remove potential confounding factors by the Harmony package (Korsunsky et al., 2019). We used the RunUMAP function with the first 40 harmonies to generate UMAP plots. The clusters were identified using the FindNeighbors and FindClusters functions with a resolution of 0.8.

Cell functional assays

To study cell migration and proliferation (**Paper I-IV**), we used the IncuCyte live cell imaging system. Briefly, human primary keratinocytes were plated in Essen ImageLock 96-well plates (Sartorius) at 15,000 cells per well for migration assay. When cells were confluent, they were pretreated with Mitomycin C (5 μg /ml) (sigma) for two hours and scratched with the Essen wound maker. For cell proliferation, cells were plated in 12-well plates at 20,000 cells per well. The cells were imaged every two hours in IncuCyte (Sartorius), and the images were processed using the IncuCyte software (Sartorius).

To study cell differentiation (**Paper III**), we used suspension-induced keratinocyte differentiation and organotypic epidermis models. Keratinocytes were suspended at 10^5 cells/mL concentration in the EpiLife medium containing 1.45% methylcellulose (Sigma-Aldrich). We diluted the methylcellulose with PBS and collected the cells by centrifugation at different time points after cell suspension. For the organotypic epidermis, we cultured keratinocytes in CnT-PR medium (CELLnTEC) and placed 1.55×10^5 cells onto 0.47 cm^2 inserts with 0.4 μm pore size (Nunc). Six inserts were put into a 60 mm cell culture dish filled with the CnT-PR medium. Two days later, the medium was changed to a CnT-PR-3D medium (CELLnTEC). After 24 hours, we removed the medium inside the inserts and left the 3.2 ml 3D medium in the dish. The surface of the inserts was dry for the following days of the 3D culture. The medium outside was changed every other day. Inserts were collected on day 12 after airlifting, followed by fixation and embedding.

Immunofluorescence (IF)

The attached cells were first fixed and permeabilized with 4% paraformaldehyde and 0.1% Triton X-100, respectively, and the paraffin-embedded tissue sections were deparaffinized and rehydrated. We blocked the nonspecific binding sites on the cells and tissue sections using 5% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature and incubated cells and sections with primary antibodies at 4℃ overnight. On the following day, the cells and sections were washed three times with PBS, followed by incubation with the secondary antibodies for one hour in the dark at room temperature. Subsequently, the secondary antibodies were removed by washing with PBS three times, and the samples were mounted with the ProLong™ Diamond Antifade Mountant with DAPI or 4', 6-diamidino-2-phenylindole (ThermoFisher Scientific). The staining was visualized by Zeiss LSM800 confocal microscopy (Carl Zeiss) at Biomedicum Imaging Core (BIC) at Karolinska Institute and analyzed with ImageJ software (National Institutes of Health). Information regarding antibody dilutions is shown in the papers (**Paper I-IV**).

Fluorescent in situ hybridization (FISH)

In order to visualize the expression of lncRNAs in cells and tissue sections, the probes were designed for RNAscope assays (ACD). Cells cultured in chamber slides were fixed in 4% paraformaldehyde for 15 minutes, followed by sequential dehydration with ethanol of different concentrations. After blocking with hydrogen peroxide for 10 minutes and digesting with Protease III for 10 minutes at room temperature, cells were incubated with probes at 40 degrees for 2 hours. The signals were amplified using RNAscope® Multiplex Fluorescent Detection Kit v2 (ACD). Fresh frozen tissue sections were fixed in cold 4% formaldehyde for 15 minutes, followed by blocking with Hydrogen Peroxide for 10 minutes and digesting with RNAscope® Protease IV for 30 minutes at room temperature. After the probe incubation and sequential signal amplification, the cells and tissue sections were mounted with ProLong™ Diamond Antifade Mountant with DAPI (ThermoFisher Scientific). The results were visualized with LSM800 confocal microscope (Carl Zeiss) (**Paper I, III, and IV**).

Western blot

The attached cells were scraped in cold radioimmunoprecipitation assay (RIPA) buffer complemented with the protease and phosphatase inhibitors. After incubation at 4 degrees for 30 min, cell lysis was centrifuged, and the supernatant was collected for subsequent western blot. The protein concentration was determined by the bicinchoninic acid assay (BCA) assay. The same amount of total proteins was loaded in TGX™ precast protein gels (Bio-Rad),

followed by nitrocellulose membrane transfer. Blots were probed with the primary antibodies at 4 degrees overnight. On the following day, the blots were incubated with horseradish peroxidase (HRP) conjugated secondary antibodies at room temperature for 1 hour. β-actin or GAPDH levels were used as an internal control. ImageJ and Image Lab (Bio-Rad) were used to quantify proteins. Details about antibody dilutions can be found in **paper III**.

RNA pulldown

Full-length lncRNAs were transcribed *in vitro* and labeled using the T7 MEGAscript kit and Pierce RNA 3′ End Desthiobiotinylation Kit (ThermoFisher Scientific). The pulldown assay was performed using the Pierce Magnetic RNA-Protein Pull-Down Kit (ThermoFisher Scientific) according to the manufacturer's instructions. Desthiobiotinylated HOXC13-AS, magnetic beads, and keratinocyte protein lysates were mixed. The beads were washed several times and boiled in SDS buffer at 96 degrees for 10 minutes, and the eluted RNA-bound proteins were collected. The proteins were separated in the TGX^{TM} gels (Bio-Rad), followed by silver staining. Protein bands at around 150 kilodaltons (kDa) or 45 kDa were excised and analyzed by mass spectrometry at the Proteomics Biomedicum core facility, Karolinska Institute (**Paper III, IV**).

RNA immunoprecipitation (RIP)

We performed RIP using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore). Human primary keratinocytes were first lysed in RIP lysis buffer. Magnetic beads were incubated with around 5 µg of the antibody for 30 minutes at room temperature. The beads were washed several times with RIP wash buffer, and the cell lysate was incubated with the beads-antibody complex at 4 degrees overnight. IgG from the same species was used as a negative control. The RNA-protein-antibody complex was purified by magnetic separation. The immunoprecipitated RNA was extracted and analyzed using qRT-PCR (**Paper III, IV**).

Human *ex vivo* wound model

For **paper I** and **IV**, the human skin obtained from abdominal reduction surgeries was used for the *ex vivo* wound model. A 2 mm excisional wound was first made on the skin and excised using an 8 mm biopsy punch. The excised tissue was cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotics. GapmeRs were mixed with 30% F-127 gel (Sigma-Aldrich) and topically treated on the wounds immediately after wounding and every other day.

Chromatin Immunoprecipitation (ChIP)

For **paper I** and **IV**, when cells reached 90% confluence, the cells were cross-linked with 1% methanol-free formaldehyde (ThermoFisher Scientific) for 10 min at room temperature, followed by quenching using 0.125 M glycine. MAGnify™ Chromatin Immunoprecipitation System (ThermoFisher Scientific) was used to perform ChIP. Briefly, cells were lysed in the cell lysis buffer supplemented with protease inhibitors, and the cell lysate was sonicated using Bioruptor UCD-200 (Diagenode) to generate 100-500 bp chromatin fragments. The chromatin fragments were incubated with Dynabeads® coupled antibodies at 4 degrees for 2 hours. Beads were washed, and the chromatin was reversely cross-linked using Reverse Crosslinking Buffer. The genomic DNA was isolated using the reagents provided in the kit.

Chromatin Isolation by RNA purification (ChIRP)

For **Paper IV**, the ChIRP was performed as previously described (Chu et al., 2012). The probes with BiotinTEG labeling at the 3' end were designed by Biosearch Technologies (www.biosearchtech.com) and generated from Eurofins Scientific. Cells were cross-linked and quenched, and the nuclei were isolated using Swelling buffer. The nuclei were lysed in the Nuclear Lysis Buffer and sonicated using Bioruptor for 3 hours at 4 degrees. The chromatin was incubated with the probes for 4 hours at 37 degrees, and the RNA-chromatin complex was captured with Dynabeads MyOne Streptavidin C1 magnetic beads (ThermoFisher Scientific). The chromatin was reversely cross-linked with Proteinase K (20 mg/ml), and DNA was purified using Phenol: chloroform: isoamyl alcohol.

In vivo wound mouse model

For **paper IV**, The Snhg26 knockout mice and their control littermates were generated by Cyagen Biosciences Company. The C57BL/6J wild-type mice were purchased from Charles River. 8-weeks old mice were caged individually one week before *in vivo* wound experiments. Dorsal hairs were shaved with clippers, followed by a depilatory cream. On the same day, general anesthesia was performed with 3% isoflurane. Two 4-mm full-thickness wounds extending through the panniculus carnosus were made on the dorsum on each side of the midline using a biopsy punch. During the first two days, the mice received Intraperitoneal injection of buprenorphine (0.03 mg/kg) twice a day. Wound areas were photographed every day. The size of wound areas was calculated with ImageJ.

Statistics

GraphPad Prism Version 7 (Dotmatics) was used to perform data analysis. Statistical significance among the two groups was determined by a two-tailed Student's t-test (parametric test) or Mann-Whitney Test (non-parametric test). The significance among multiple groups was determined by one-way ANOVA. Pearson's correlation test was performed on log2-transformed data. The data were considered significant when $P < 0.05$.

4 Results and discussion

4.1 Long noncoding RNA WAKMAR1 promotes keratinocyte migration and re-epithelialization during human skin wound healing

Rationale

WAKMAR1 (also called LOC105372576) was an annotated but uncharacterized lncRNA. By comprehensive analysis of published microarray data, we found WAKMAR1 was a top-downregulated lncRNA in DFUs compared to the intact skin (Ramirez et al., 2018). Additionally, WAKMAR1 is mainly expressed in the skin compared to the other 26 human tissues (Fagerberg et al., 2014), which prompted us to investigate the function and mechanism of this lncRNA underlying human skin wound healing.

Main findings

WAKMAR1 is downregulated in wound-edge keratinocytes of human chronic wounds.

To explore the expression mode of WAKMAR1 in normal and chronic wounds, we collected one-day (NW1) and seven-day (NW7) wound edges from healthy volunteers, representing inflammatory and proliferative phases of normal wound healing, respectively. Wound edges from patients with VUs or DFUs were also collected. We demonstrated that WAKMAR1 was upregulated in NW1 and NW7 compared to the intact skin, whereas its expression significantly decreased in VUs and DFUs compared to NW7 by qRT-PCR analysis (**Fig. 7A**, **B**). Furthermore, using LCM to isolate the epidermis, we confirmed that WAKMAR1 was specifically reduced in the epidermis of VUs compared to the skin and NW7 (**Fig. 7C**). In order to investigate the mechanism regulating WAKMAR1 expression, primary keratinocytes were treated with a panel of wound healing-related cytokines and chemokines. Our qRT-PCR results showed TGF-β superfamily (TGF-β1, TGF-β2, TGF-β3, and BMP-2) led to the upregulation of WAKMAR1 (**Fig. 7D**). Importantly, co-treatment of TGF-β2 and BMP-2 showed a stronger synergistic effect on increasing WAKMAR1 expression, which was mitigated by SB431542 and/or DMH1 that inhibit TGF-β type I receptor and BMP type I receptors, respectively (Hao et al., 2010) (Inman et al., 2002) (**Fig**. **7E**). Moreover, silencing SMAD1, SMAD3, or SMAD4 significantly decreased WAKMAR1 expression induced by TGF-β2 and BMP-2 (**Fig**. **7F**).

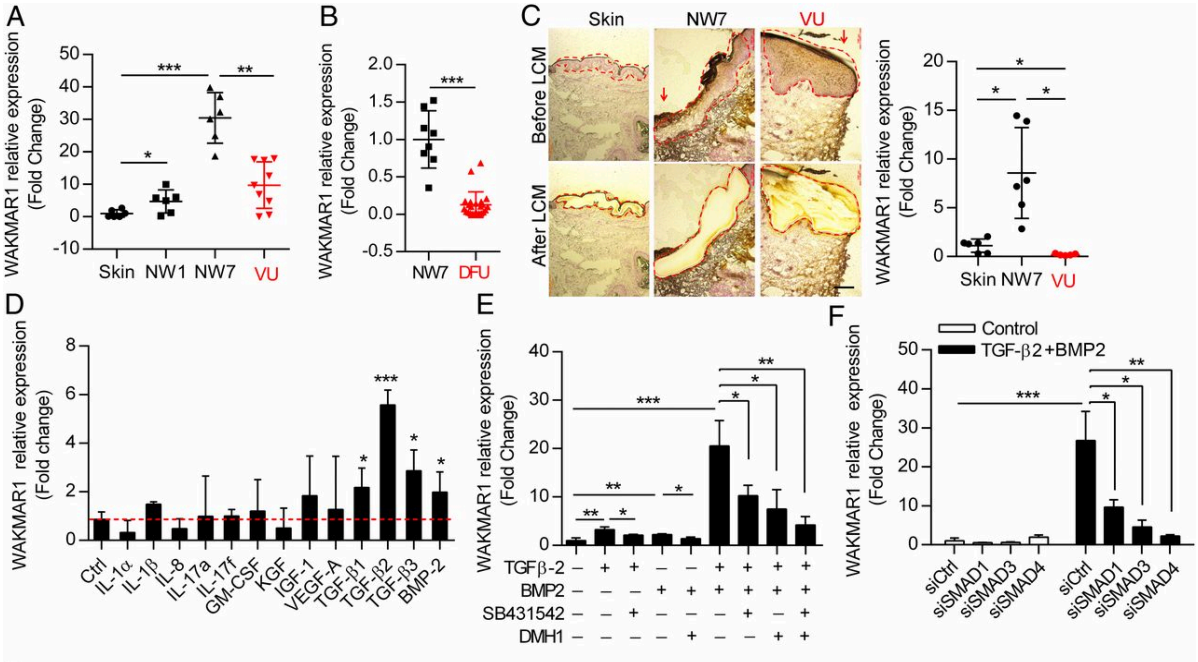

Figure 7 *WAKMAR1 is downregulated in wound-edge keratinocytes of human chronic wounds and activated by TGFβ signaling. qRT-PCR of WAKMAR1 in the skin, normal wounds, and venous ulcers* ***A****, as well as diabetic foot ulcers* ***B****.* ***C*** *qRT-PCR of WAKMAR1 in the epidermis isolated from the skin, and wound edges of normal wounds and venous ulcers.* ***D*** *qRT-PCR of WAKMAR1 in keratinocytes treated with cytokines or growth factors for 24h. qRT-PCR of WAKMAR1 in keratinocytes treated with growth factors and/or inhibitors* ***E*** *or transfected with SMAD1-, SMAD3-, and SMAD4-specific siRNAs, followed by treatments of growth factors* ***F****.*

WAKMAR1 is required for keratinocyte migration and re-epithelialization of human ex vivo wounds.

Due to the altered expression of WAKMAR1 in chronic wounds compared to NW7, we hypothesized that it might play a role in keratinocyte migration that is impaired in nonhealing wounds. To this end, we transfected primary keratinocytes with WAKMAR1-specific GapmeRs, and confirmed its downregulation by qRT-PCR analysis (**Fig. 8A**). Using the scratch and transwell assay, we showed the migration rate and capacity were significantly reduced upon WAKMAR1 knockdown (**Fig. 8B**). Furthermore, we increased WAKMAR1 expression using a CRISPR/SAM system that promoted its endogenous transcription (Joung et al., 2017) (**Fig. 8C**). Overexpression of WAKMAR1 increased migration rate of keratinocytes (**Fig. 8D**). To study the function in a more physiological state, we used a human *ex vivo* model. In brief, a wound was generated on the excised human skin that was cultured for 4-7 days (Stojadinovic and Tomic-Canic, 2013). WAKMAR1-specific GapmeRs were applied on the wounds on the first day and every other day. We confirmed WAKMAR1 was significantly downregulated in the epidermis but not the dermis of *ex vivo* wounds (**Fig. 8E**). WAKMAR1 silencing resulted in delayed re-epithelialization analyzed by quantifying newly formed epidermis (**Paper I**, Fig. 3J).

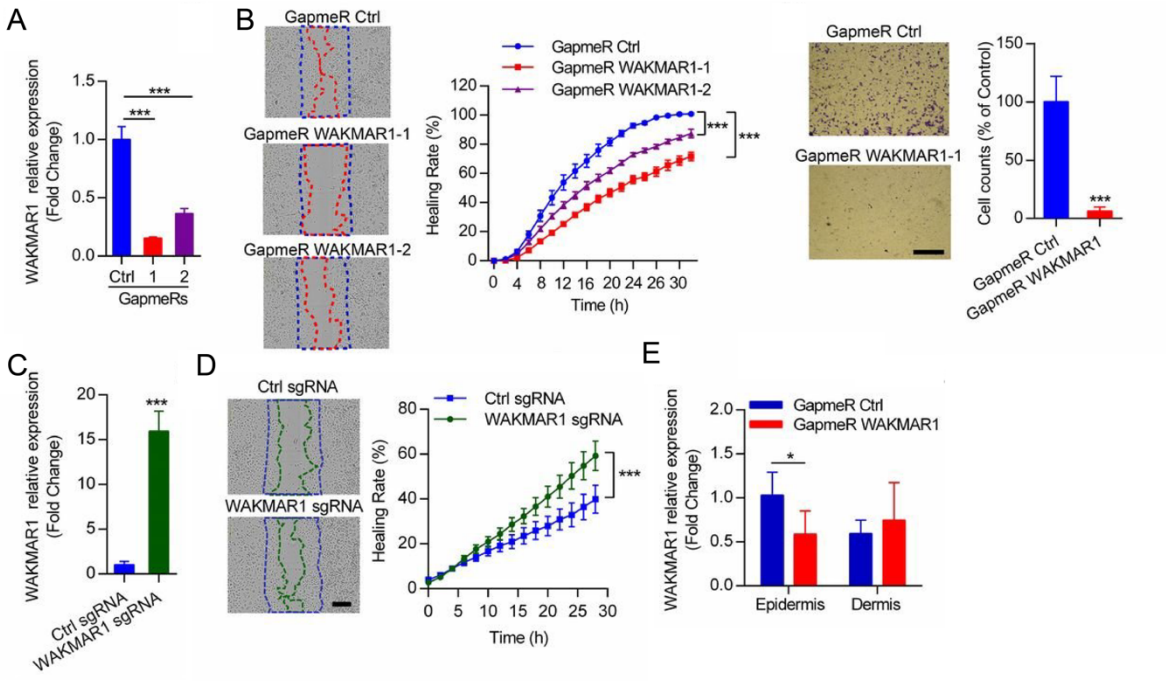

Figure 8 *WAKMAR1 is required for keratinocyte migration and re-epithelialization of human ex vivo wounds.* ***A*** *qRT-PCR of WAKMAR1 in keratinocytes transfected with WAKMAR1-specific GapmeRs.* ***B*** *Scratch wound (left) and transwell migration assay (right) of keratinocytes after WAKMAR1 knockdown. qRT-PCR of WAKMAR1* ***C*** *and scratch wound assay* ***D*** *in keratinocytes transfected with CRISPR/Cas9-SAM plasmids.* ***E*** *qRT-PCR of WAKMAR1 in LCM-isolated epidermis and dermis of human ex vivo wounds treated with CRISPR/Cas9-SAM plasmids.*

WAKMAR1 regulates a gene network associated with keratinocyte migration, where E2F1 acts as an upstream regulator.

To investigate the mechanism underlying the promigratory function of WAKMAR1, we performed a transcriptomic analysis in keratinocytes with WAKMAR1 knockdown. Cell migration-associated genes were significantly enriched for downregulated genes upon WAKMAR1 knockdown by GSEA (**Fig. 9A**). Using STRING protein network analysis (von Mering et al., 2007), we identified several hub migration regulators that were controlled by WAKMAR1 (**Fig. 9B**). Expression changes of the hub genes (CDK6, HMMR, E2F1, KIF11, and FOS) were further validated in keratinocytes and human *ex vivo* wounds after WAKMAR1 silencing or overexpression. We demonstrated that they were downregulated upon WAKMAR1 knockdown, whereas WAKMAR1 overexpression enhanced their levels (**Paper I**, Fig. 4C-E). Moreover, the knockdown of the genes delayed cell migration, which is consistent with the phenotype observed by WAKMAR1 downregulation (**Paper I**, Fig. 4F).

To identify if the WAKMAR1-regulated gene network was governed in a coordinated manner by common TFs, we performed MetaCore TF analysis, which showed that E2F1 was the top WAKMAR1-regulated TF (**Fig. 9C**). In line with it, GSEA demonstrated that E2F1-regulated genes were significantly enriched for downregulated genes upon WAKMAR1 knockdown

(**Fig. 9D**). Importantly, silencing of E2F1 abolished the pro-migratory effect caused by WAKMAR1 overexpression (**Fig. 9E**). The results suggested that E2F1 mediated the biological function of WAKMAR1. In order to study the physiological role of E2F1 in wound healing, we explored its expression pattern in NWs and chronic wounds. Similar to WAKMAR1, E2F1 was downregulated in VUs and DFUs compared to NW7 (**Paper I**, Fig. 5E-G). In addition, there was a significantly positive correlation between E2F1 and WAKMAR1 expression during wound healing (**Paper I**, Fig. 5H-J).

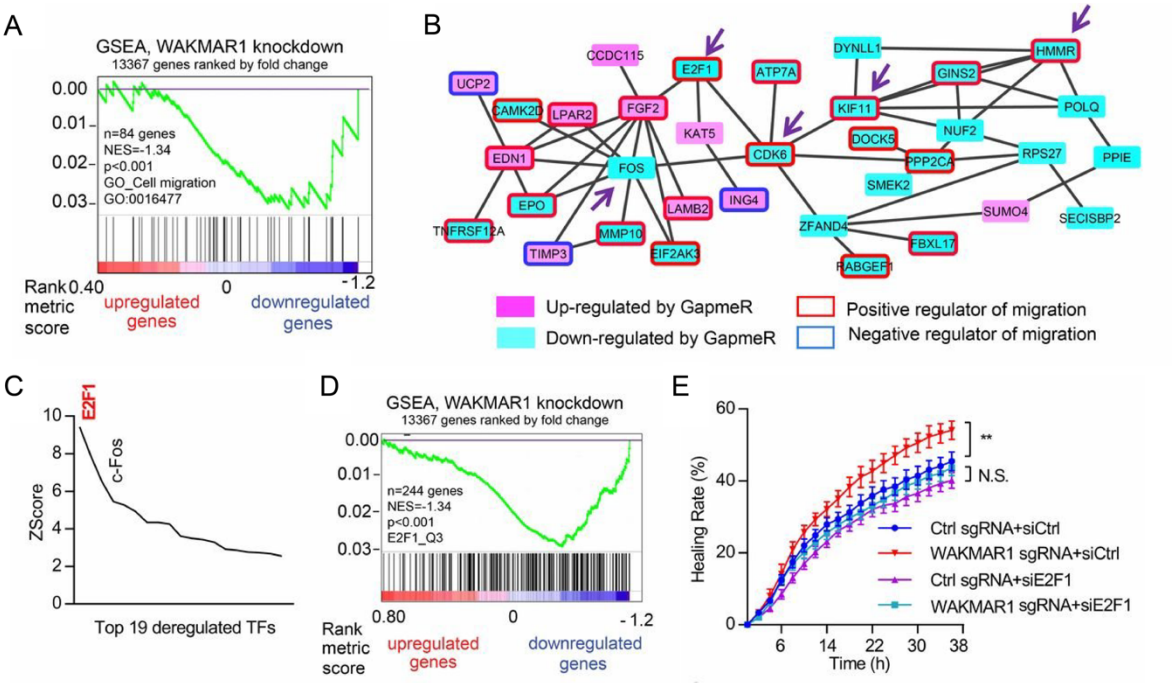

Figure 9 *WAKMAR1 regulates a gene network associated with keratinocyte migration, where E2F1 acts as an upstream regulator.* ***A*** *GSEA for the cell migration-related genes using microarray data in keratinocytes with WAKMAR1 knockdown.* ***B*** *STRING protein network analysis within deregulated genes after WAKMAR1 knockdown.* ***C*** *MetaCore TF analysis using WAKMAR1-regulated genes.* ***D*** *GSEA for E2F1-target genes using microarray data in keratinocytes with WAKMAR1 knockdown.* ***E*** *Scratch wound assay of keratinocytes upon WAKMAR1 overexpression and/or E2F1 silencing.*

WAKMAR1 regulates DNA methylation at the E2F1 promoter and thus influences its expression.

WAKMAR1 was mainly localized in the nucleus (**Paper I**, Fig. 2G, 3E), which suggests that WAKMAR1 may play a role *in cis* that regulates the expression of neighboring genes. Moreover, we showed that E2F1 was the top downregulated gene located in the same chromosome as WAKMAR1 (**Fig. 10A**). This prompted us to investigate how WAKMAR1 regulates E2F1 expression. We found that knockdown of WAKMAR1 led to high methylation levels at CpG1 and CpG5 regions at the E2F1 promoter, whereas WAKMAR1 overexpression inhibited them by analyzed by methyl-sensitive restriction enzyme (MSRE) qPCR (**Fig. 10B**, **C**). We hypothesized that WAKMAR1 might regulate DNA methylation via DNA

methyltransferases (DNMTs). The RIP results revealed that WAKMAR1 physically interacted with DNMT1, DNMT3A, and DNMT3B. Furthermore, we performed ChIP-qPCR and demonstrated DNMT1 occupancy at CpG1 and CpG5 regions was increased upon WAKMAR1 knockdown, whereas it was decreased after WAKMAR1 overexpression (**Fig. 10D**).

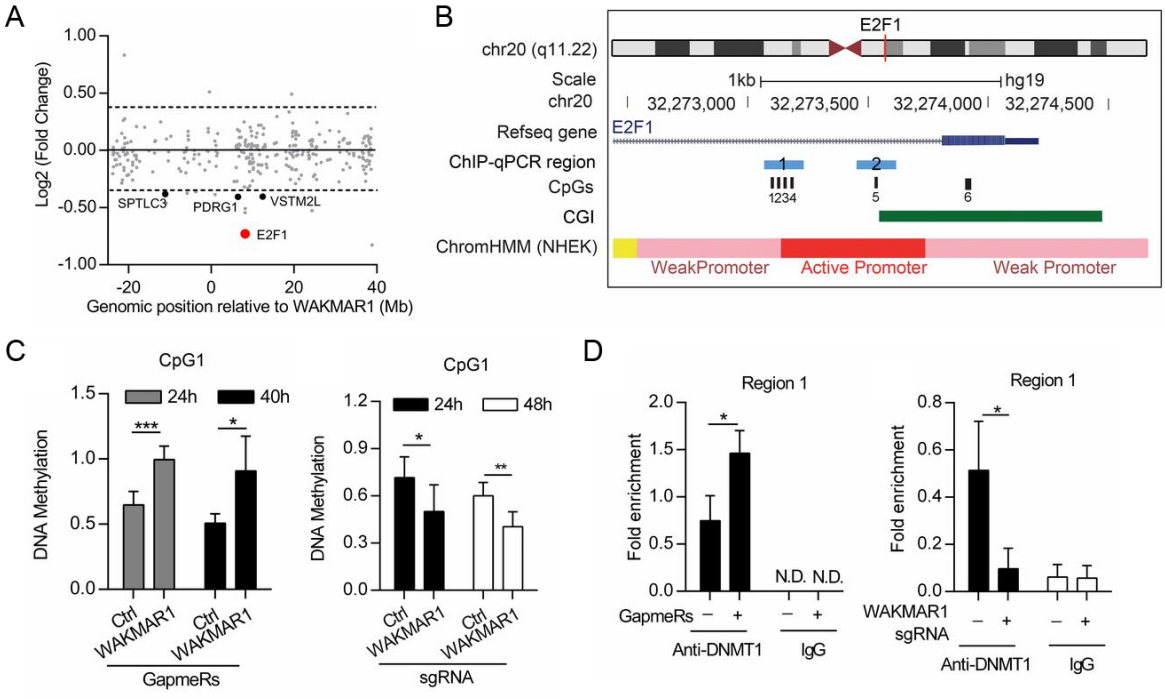

Figure 10 *WAKMAR1 regulates DNA methylation at the E2F1 promoter and thus influences its expression.* ***A*** *Expression change of genes localized at Chr20 in keratinocytes with WAKMAR1 knockdown.* ***B*** *CpG sites and regions analyzed by ChIP-qPCR at the E2F1 promoter.* ***C*** *MSRE-qPCR analysis of DNA methylation at CpG1 in keratinocytes upon WAKMAR1 knockdown (left) or overexpression (right).* ***D*** *ChIP-qPCR of region 1 at the E2F1 promoter in keratinocytes after WAKMAR1 knockdown (left) or overexpression (right) using DNMT1 antibody or IgG.*

Discussion

E2F1 knockout exhibits delayed migration and impaired proliferation in keratinocytes, and wound healing is delayed in E2F1-deficient mice characterized by reduced re-epithelialization (D'Souza et al., 2002). Our study revealed its clinical relevance to DFUs and VUs, where E2F1 was significantly downregulated compared to NW7. By the STRING network analysis and GSEA, we confirmed that E2F1 was a hub gene downregulated upon WAKMAR1 knockdown, and its regulated genes were enriched for downregulated genes after WAKMAR1 silencing. E2F1 silencing could entirely abolish the enhanced keratinocyte migration caused by WAKMAR1 overexpression. Our data suggest that WAKMAR1 regulates keratinocyte migration via E2F1.

LncRNAs play critical roles in epigenetic regulation. They interact with chromatin modifier proteins, act as regulators of DNA methylation, and influence chromatin architecture (Morlando and Fatica, 2018). Around 70-80% of CpG sites with DNA methylation are found in mammalian genomes (Li and Zhang, 2014). DNA methylation is mainly found in CpG dinucleotides, where the fifth carbon of cytosines (5-methylcytosine, 5mC) is methylated. Promoter and first-exon regions containing DNA methylation lead to repressive transcription of the related genes. DNMT3A and DNMT3B result in the establishment of DNA methylation, and DNMT1 is critical for methylation maintenance (Greenberg and Bourc'his, 2019). LncRNAs DACOR1 and ecCEBPA have been shown to interact directly with DNMT1. DACOR1 contributes to the re-localization of DNMT1 and thus regulates the expression of specific genes. By contrast, ecCEBPA inhibits DNMT1 by sequestering DNMT1 from its functional loci.

In this study, we have identified WAKMAR1 as an epigenetic regulator, modulating methylation levels at the E2F1 promoter by interacting with DNMTs during skin wound healing. To provide more insights into the mechanism, we demonstrated the increased methylation level at CpG1 and CpG5 at the E2F1 promoter upon WAKMAR1 knockdown, but the level decreased after WAKMAR1 overexpression. Furthermore, we performed RIP, and WAKMAR1 was specifically enriched in precipitants purified with DNMT3A, DNMT3B, and DNMT1 antibodies. This result demonstrated that WAKMAR1 could physically interact with DNMT3A, DNMT3B, and DNMT1. Importantly, our ChIP-qPCR analysis showed that WAKMAR1 silencing increased the occupancy of DNMT1 at the E2F1 promoter, whereas overexpression of WAKMAR1 decreased it, suggesting that WAKMAR1 sequesters DNMTs from E2F1 promoter and thus inhibits the DNA methylation and promotes E2F1 transcription.

4.2 Comprehensive small and long RNA omics analysis of normal wounds and venous ulcers identifies clinically relevant microRNAs

Rationale

MiRNAs hold great potential as therapeutic entities. However, most existing knowledge regarding skin wound healing comes from animal wounds or *in vitro* studies, which is challenging to fully simulate the human disease complexity, and the mechanism identified in the animal models could fail to extrapolate to humans. In this project, we aimed to profile both miRNA and mRNA expression in the same human normal wounds and venous ulcer biopsies and identify the core miRNA regulators that control the gene regulatory network critical for human skin wound healing.

Main findings

Dysregulated miRNAs and mRNAs in venous ulcers.

To establish a systematic and comprehensive gene regulatory network, we performed small and long RNA sequencing in the same skin and wound tissues from age- and gender-matched healthy donors and VU patients. A differential expression analysis identified 32 miRNAs and 424 mRNAs significantly dysregulated in VUs compared to the skin, NW1, and NW7 (**Paper II**, Fig. 1c-e). We also performed weighted gene co-expression network analysis (WGCNA), revealing 13 miRNA expression modules across these tissue samples. In particular, miRNA modules 8 and 12 (m8 and m12) were upregulated, whereas m3, m7, and m9 modules were downregulated in VUs (**Fig. 11A**). In parallel, we performed WGCNA for the long RNA-seq data and found that mRNA module 9 (M9), M10, M11, and M12 were upregulated while M5 was downregulated in VUs (**Fig.11B**). We also showed upregulated genes in VUs were related to the extracellular matrix organization, cell adhesion, inflammation, and adaptive immunity whereas downregulated genes were involved in RNA processing, protein production as well as cell mitosis (**Paper II**, Fig. 2f).

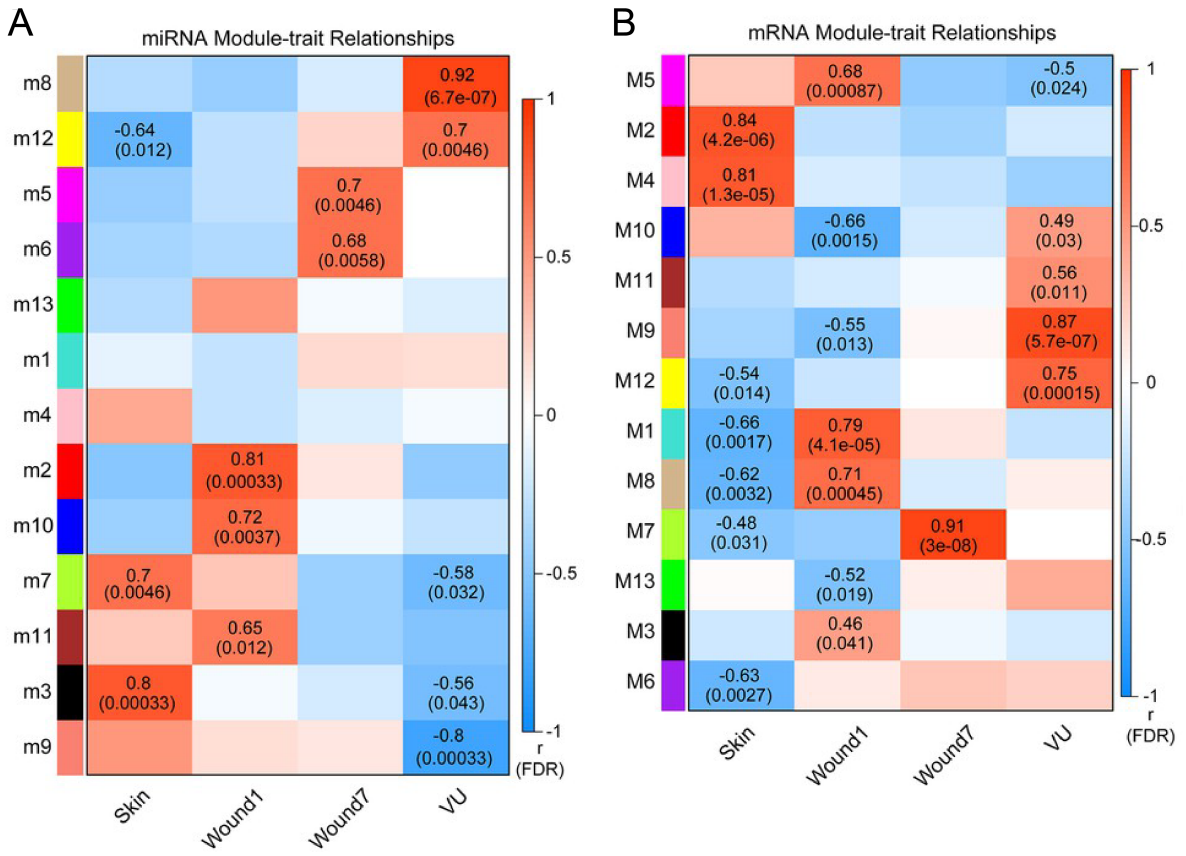

Figure 11 *Weighted gene co-expression network analysis of miRNAs and mRNAs in human wound healing. Heatmap shows Pearson's correlations between miRNA module eigengenes and sample groups* ***A****, and mRNA module eigengenes and sample groups* ***B****. The positive and negative correlations are indicated in red and blue, respectively.*

The miRNAs contribute to the gene regulatory network in venous ulcers.

To understand how miRNAs contribute to dysregulated mRNAs in VUs, we performed a correlation analysis between VU-associated miRNA and mRNA modules (**Paper II**, Fig. 3d). Among the negatively correlated miRNA and mRNA modules, we discovered that the predicted targets of downregulated m9 miRNAs were enriched for upregulated M9 mRNA module, while the predicted targets of upregulated miRNAs and m8 miRNAs were enriched for the downregulated mRNAs and M5 mRNA module, suggesting that miRNAs have a significant contribution to the abnormal gene expression in VUs (**Fig. 12A**). Furthermore, we identified a shortlist of miRNAs with their targets significantly enriched for the VU-associated mRNA modules, downregulated mRNAs, and upregulated mRNAs in VUs. The predicted targets of two VU-related downregulated miRNAs (miR-144-3p and miR-218-5p) and five m9 miRNAs (miR-205-5p, miR-211-5p, miR-506-3p, miR-509-3p, and miR-96-5p) were enriched for the upregulated M9 mRNAs, whereas the targets of three VU-related upregulated miRNAs (miR-450-5p, miR-512-3p, and miR-516b-5p) and seven m8 miRNAs (miR-424-5p, miR-34a-5p, miR-34c-5p, miR-516a-5p, miR-517a-3p, miR-517b-3p, and miR-7704) were enriched for

M5 mRNAs and downregulated mRNAs in VUs (**Fig. 12B**). We propose that these miRs may contribute to chronic wound pathology because both their expression and targets are specifically correlated with the phenotypic trait, VU.

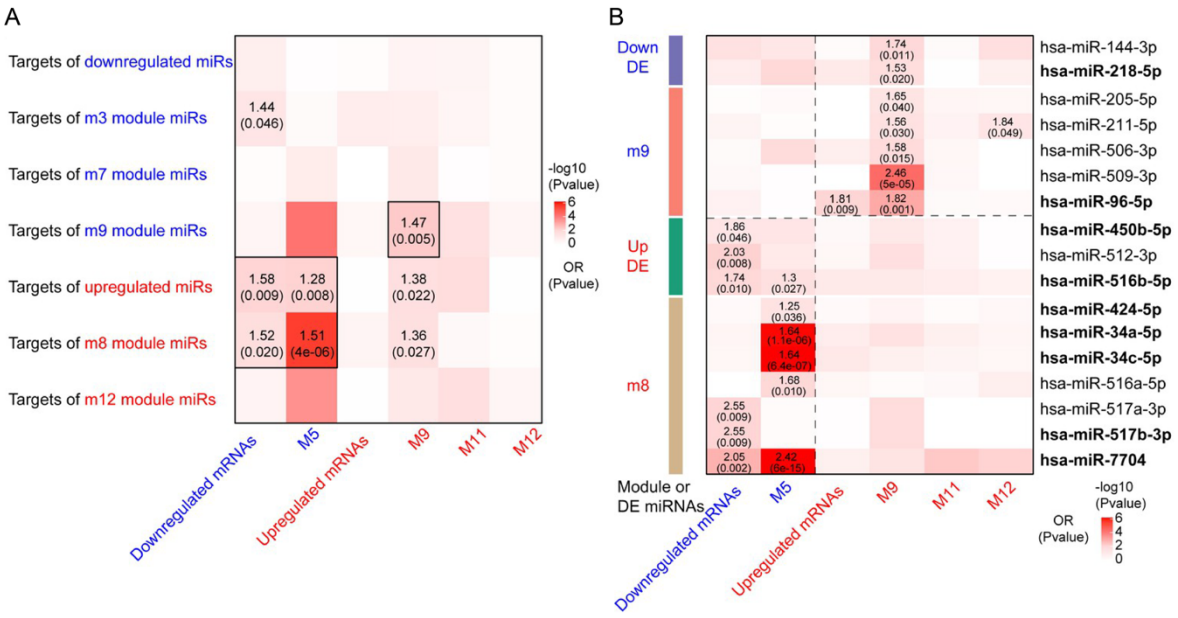

Figure 12 *The miRNAs contribute to the gene regulatory network in venous ulcers. Heatmaps show the enrichment analysis of deregulated miRNAs and miRNAs in VU-associated modules* ***A*** *and the individual candidate miRNAs* ***B*** *in deregulated mRNAs and mRNAs in VU-associated mRNA modules.*

Experimental validation of miRNA expression and cooperativity of miRNAs involved in venous ulcers.

To validate our findings in silico, I first validated the expression of nine differentially expressed miRNAs in a bigger cohort by qRT-PCR. Consistent with RNA sequencing data, miR-149-5p, miR-218-5p and miR-96-5p were downregulated, whereas miR-7704, miR-424-5p, miR-31-3p, miR-450b-5p, miR-516b-5p and miR-517b-3p were upregulated in VUs (**Paper II**, Fig. 5a-j). We previously characterized the upregulation of miR-34a/c-5p in VUs (Wu et al., 2020). To confirm the targets of the miRNAs, we performed microarray analyses in keratinocytes or fibroblasts transfected with miRNA mimics. Also, we re-analyzed the published microarray data of keratinocytes with miR-34a/c-5p overexpression (Wu et al., 2020). We found that the predicted targets of these miRNAs were significantly downregulated compared to non-targeted mRNAs (**Paper II**, Fig. 6a-j). Importantly, the experimentally validated targets of miR-34a/c-5p and miR-7704 were significantly enriched for the M5 mRNA module, whereas the targets of miR-218-5p were enriched for the upregulated mRNAs and the M9 module in VUs, suggesting their potential involvement in chronic wound pathology (**Paper II**, Fig. 6k).

Additionally, functional annotations were performed using the targets regulated by VU-associated miRNAs (**Paper II**, Fig. 8a). The upregulated miRNAs in VU (miR-34a/c-5p, miR-

424-5p, miR-450-5p, miR-7704, and miR-516-5p) were predicted to enhance inflammation but impair proliferation, whereas the downregulated miRNAs (miR-218-5p and miR-96-5p) were needed to regulate cell growth (**Fig. 13A**). This prompted us to further validate the cooperative role of miRNAs in cell migration, proliferation, and immune response. To this end, we co-transfected miR-34a-5p and miR-424-5p mimics in primary keratinocytes and investigated cell growth by detecting cell proliferation marker Ki67. The combination caused a stronger inhibitory effect on keratinocyte proliferation than the single miRNA overexpression (**Fig. 13B**, **Paper II**, Fig. 9b). In line with it, the cooperatively repressive effect of miR-34a-5p and miR-424-5p on cell growth was confirmed in a live cell imaging system (**Paper II**, Fig. 9c). Moreover, simultaneous overexpression of miR-34a-5p and miR-424-5p inhibited cell migration, whereas knockdown of miR-34a-5p or miR-424-5p promoted it (**Paper II**, Fig. 9d-g). By bioinformatics analysis, miR-34a/c-5p and miR-516b-5p jointly regulated a set of genes associated with an inflammatory response, indicating that they may cooperate in impacting stronger on the response (**Paper II,** Fig.8e, f). We demonstrated the higher expression of inflammatory cytokine CCL20 upon simultaneous overexpression of miR-34-5p and miR-516b-5p compared to overexpression of the individual miRNAs by qRT-PCR (**Fig. 13C**).

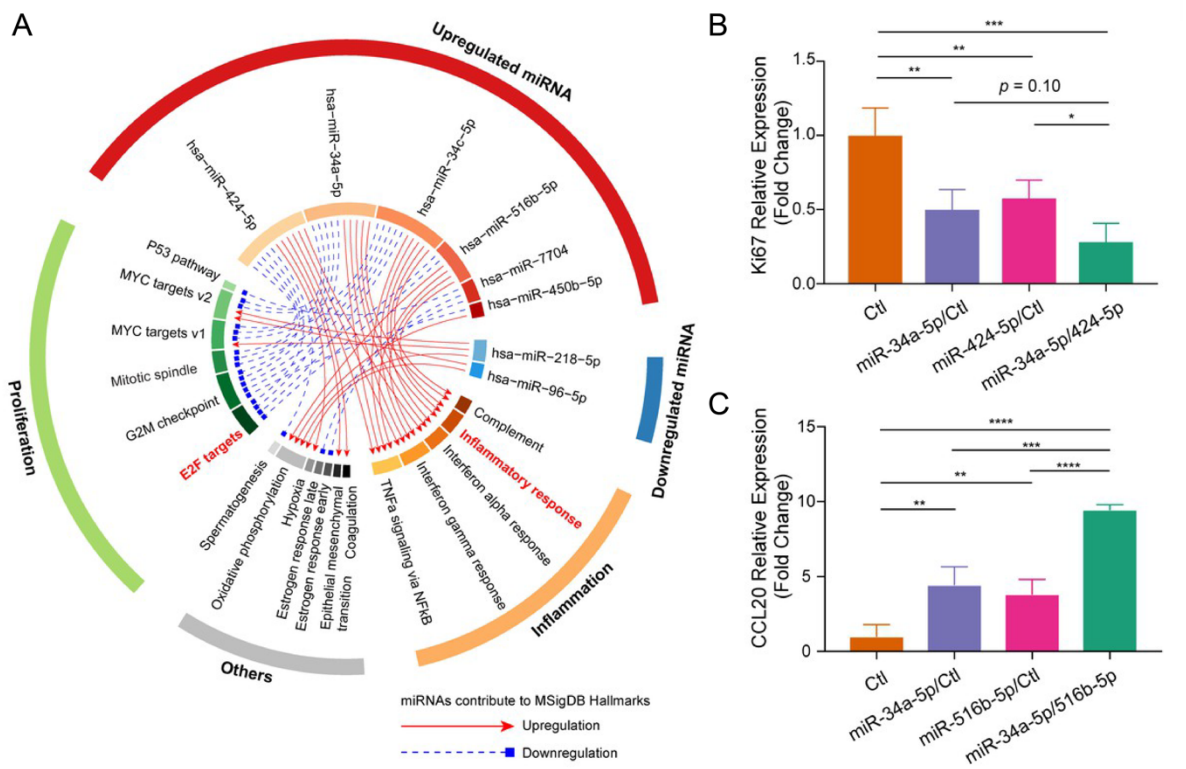

Figure 13 *Cooperativity of the miRNAs involved in venous ulcers.* ***A*** *Molecular Signatures Database hallmarks analysis using genes up- or downregulated by the miRNAs.* ***B*** *qRT-PCR of Ki67 in keratinocytes with miR-34a-5p and/or miR-424-5p overexpression.* ***C*** *qRT-PCR of CCL20 in keratinocytes with miR-34a-5p and/or miR-516b-5p overexpression.*

Discussion

In this study, we revealed the temporal expression changes of miRNAs and mRNAs in normal skin wound healing and compared them to VU-relevant signatures. In addition to identifying differentially expressed miRNAs and mRNAs, we performed WGCNA to arrange them into multiple expression modules according to their co-expression patterns. In line with previous studies, skin hemostasis and barrier functions-related genes were downregulated in the wounds; genes associated with immune response, RNA processing, and protein production were enriched for NW1, and genes involved in cell mitosis were enriched for NW7. Furthermore, we identified TFs driving these biological processes. For example, NFKB1 and RELA play a vital role in immune functions which act as the upstream regulators for M1 mRNAs (Liu et al., 2017). E2F1 is required to enhance cell growth that regulates many genes enriched for M7 mRNAs (Ertosun et al., 2016).

In addition, our study revealed that downregulated genes in VUs were associated with RNA processing and protein production, as well as cell mitosis, whereas upregulated genes in VUs were related to the extracellular matrix organization, cell adhesion, inflammation, and adaptive immunity. These findings were consistent with the previous observation in patients with VUs (Blumberg et al., 2012) (Pappas et al., 1999) (Stone et al., 2020). We demonstrated that the predicted targets of downregulated m9 miRNAs were enriched for the upregulated M9 mRNAs in VU. The m9 miRNAs might be driven by TFs, including KLF4, KLF5, GATA3, and GRHL2, as their binding sites are enriched for this miRNA module. Previous studies have shown the downregulation of GATA3 and KLF4 in VU (Stojadinovic et al., 2008) (Stojadinovic et al., 2014).

Moreover, we performed functional annotations for the targets regulated by VU-relevant miRNAs to identify the cooperation between the miRNAs. MiRNA cooperativity can contribute to synergistic repression on target genes, increasing the specificity and efficacy of treatment (Lai et al., 2019). We analyzed miRNA annotated functions and their expression changes, suggesting that the upregulated miRNAs in VU (miR-34a/c-5p, miR-424-5p, miR-450-5p, miR-7704, and miR-516-5p) enhance inflammation but inhibit proliferation, whereas miR-218-5p and miR-96-5p required for proliferation are downregulated in VU. Furthermore, we experimentally validated the combined effect of miR-34a-5p and miR-424-5p on cell proliferation and migration and that of miR-34a-5p and miR-516b-5p on the immune response. The combination of miR-34a-5p and miR-424-5p had a stronger repressive effect on cell proliferation and migration compared to overexpression of the individual miRNAs.

Simultaneous overexpression of miR-34a-5p and miR-516b-5p produced more CCL20 mRNAs compared to single overexpression of miR-34a-5p or miR-516b-5p.

Overall, we performed a comprehensive and integrative analysis of miRNA and mRNA profiling in normal wounds and venous ulcers and thus identified VU-relevant miRNAs by analyzing the expression of their targets in VU. Additionally, we developed a compendium (https://www.xulandenlab.com/humanwounds-mirna-mrna), which is an open resource and valuable for future research in human skin wound healing.

4.3 Investigating the role of long noncoding RNA HOXC13-AS in epidermal differentiation

Rationale

LncRNAs constitute important layers of epigenetic regulation. However, the role of lncRNAs in skin wound healing is mostly unclear. We identified HOXC13-AS as a skin-specific lncRNA, and its expression was significantly downregulated during human skin wound healing. In the study, we aimed to reveal the biological function and underlying mechanism of HOXC13-AS.

Main findings

HOXC13-AS is downregulated in wounds due to high growth factors and decreased differentiation.

We performed RNA-seq analysis in full-thickness skin and wound biopsies, as well as CD45 negative epidermal cells (enriched with keratinocytes) isolated from the skin and day-7 wounds of healthy donors. Among 19 lncRNAs deregulated in wound tissues and epidermal cells, HOXC13-AS was the top skin-specific lncRNA. It was significantly downregulated in wound-edge epidermal cells compared to the skin (**Paper III**, Fig.1b, c). Its decreased expression in wound healing was further confirmed in a larger cohort by qRT-PCR and FISH analysis (**Paper III**, Fig. 1g, i, j, k). Using single-cell RNA sequencing analysis in human skin, we demonstrated that HOXC13-AS was specifically expressed in granular cells (differentiated keratinocytes) (**Paper III**, Fig. 2a, b, c). Furthermore, we performed a correlation analysis between HOXC13-AS and other expressed genes in the granular cells, and the top 50 HOXC13-AS-correlated genes were enriched for biological processes related to skin development and keratinocyte differentiation, suggesting HOXC13-AS is involved in the processes (**Paper III**, Fig. 2d).

To study signaling pathways regulating HOXC13-AS expression, I treated keratinocytes with a panel of growth factors and cytokines highly relevant to wound healing (Barrientos et al., 2008). EGF and HB-EGF, the growth factors in the EGFR pathway, inhibited HOXC13-AS expression by qRT-PCR analysis (**Paper III**, Fig. 3a). To test if EGF affects HOXC13-AS transcription or stability, we captured and purified nascent HOXC13-AS after EGF treatment for 8h. The decreased expression of the newly transcribed HOXC13-AS was confirmed by qRT-PCR analysis (**Fig. 14A**). EGF did not influence HOXC13-AS degradation in keratinocytes treated with EGF and Actinomycin D, a chemical blocking gene transcription

(**Fig. 14B**). The results indicate EGF may inhibit HOXC13-AS expression through reducing its transcription. Moreover, blocking EGFR signaling with its inhibitor AG-1478 (Kolev et al., 2008) increased HOXC13-AS expression in a dose-dependent manner (**Paper III**, Fig. 3d). As EGFR signaling is a negative regulator in epidermal cell differentiation (Kolev et al., 2008), we then examined the expression of HOXC13-AS in a calcium-induced keratinocyte differentiation model, where HOXC13-AS expression gradually increased over time (**Fig. 14C**). Besides, we have shown that genes involved in epidermal cell differentiation were downregulated during wound repair (Liu et al., 2022). Our data suggest that the expression of HOXC13-AS decreases in the wounds, likely due to high growth signaling and decreased differentiation.

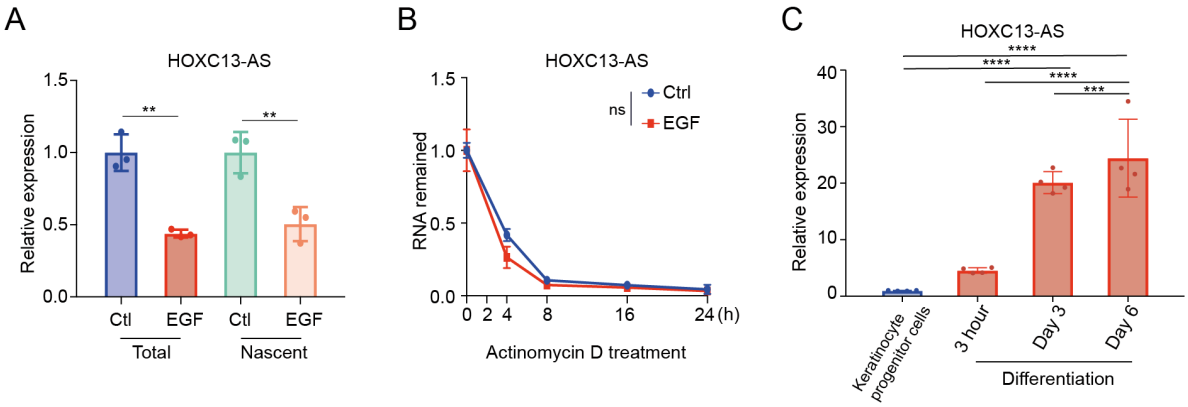

Figure 14 *HOXC13-AS is downregulated in wounds due to high growth factors and decreased differentiation.* ***A*** *qRT-PCR analysis of total and nascent HOXC13-AS in keratinocytes after EGF treatment for 8 hours.* ***B*** *qRT-PCR analysis of HOXC13-AS in keratinocytes treated with EGF and then actinomycin D for 0-24 hours.* ***C*** *qRT-PCR analysis of HOXC13-AS in keratinocyte progenitors and differentiated keratinocytes induced with calcium.*

HOXC13-AS promotes keratinocyte differentiation.

To understand the biological function of HOXC13-AS, we inhibited its expression using siRNA pools and overexpressed it with a HOXC13-AS expression vector. The efficiency of knockdown and overexpression was confirmed by qRT-PCR and FISH analysis (**Paper III**, Fig. 4a-c). Moreover, neither HOXC13-AS knockdown nor overexpression affected HOXC13 expression (**Paper III**, Supplementary Fig. 3). We then performed microarray analysis in differentiated keratinocytes induced by calcium. GO analysis revealed that the biological processes related to epidermis development and keratinocyte differentiation were enriched for the downregulated genes, whereas leukocyte chemotaxis was enriched for the upregulated genes in differentiated keratinocytes with HOXC13-AS knockdown (**Fig. 15A**). To evaluate the role of HOXC13-AS in a more physiological state, we performed correlation analyses using RNA sequencing data of human wound tissues and isolated epidermal cells. There was a positive correlation between HOXC13-AS and differentiation-related genes but a negative

correlation between HOXC13-AS and inflammation-related genes (**Fig. 15B**). In addition, I assessed HOXC13-AS function in the suspension-induced differentiation model and organotypic epidermis, where differentiation markers KRT10, FLG, and IVL were downregulated after HOXC13-AS knockdown, whereas they were upregulated upon HOXC13-AS overexpression, suggesting HOXC13-AS promotes keratinocyte differentiation (**Paper III**, Fig. 5, Supplementary Fig. 5).

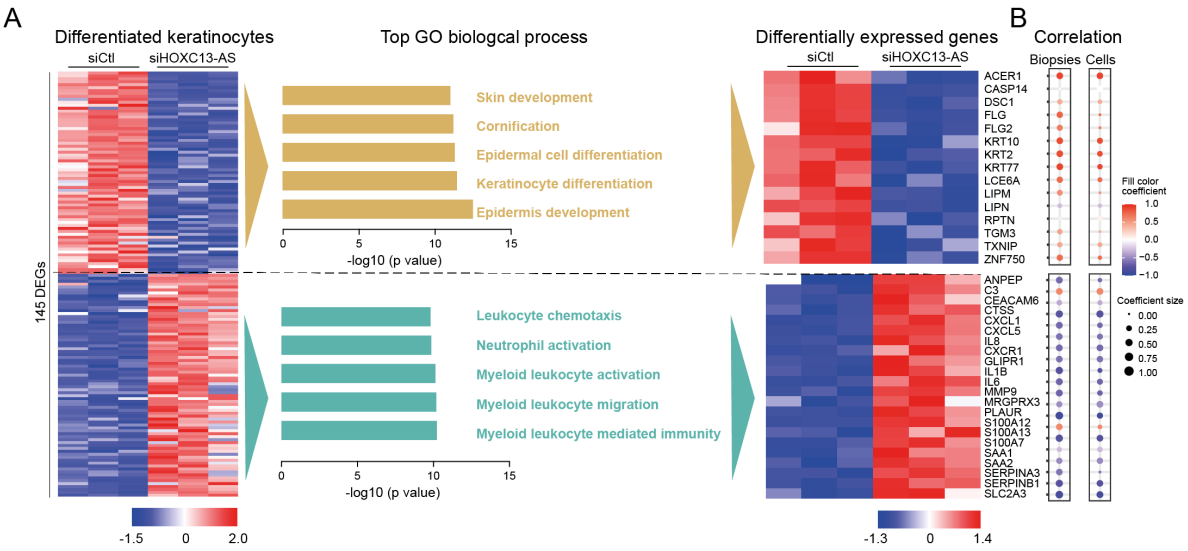

Figure 15 *HOXC13-AS regulates keratinocyte differentiation and inflammatory response.* ***A*** *Heatmap (left) shows the differentially expressed genes (DEGs) in differentiated keratinocytes with HOXC13-AS knockdown. GO analysis using DEGs is shown in the middle. The DEGs related to the GO terms are shown in the right heatmap.* ***B*** *Correlation analysis of HOXC13-AS and genes using RNA-seq of biopsies and epidermal cells.*

HOXC13-AS interacts with COPA protein and interferes with the Golgi-ER retrograde transport.

We demonstrated that HOXC13-AS was mainly localized in the cytoplasm analyzed by FISH and fractionation assays, suggesting HOXC13-AS may act *in trans* (**Paper III**, Fig. 4a, b, 6a). To explore the underlying molecular mechanism of HOXC13-AS, we performed *in vitro* biotinylated RNA synthesis, followed by RNA pulldown. We then analyzed the protein partners co-precipitated with biotinylated HOXC13-AS using gel electrophoresis, followed by silver staining. Compared to the poly $(A)_{25}$ RNA pull-down fraction, a more intensive band at around 150 kDa was observed in the HOXC13-AS pull-down fraction, so the areas were excised for mass spectrometry analysis (**Paper III**, Fig. 6c). We found COPI Coat Complex Subunit Alpha (COPA), eukaryotic translation initiation factor 3 subunit A (EIF3A) and High-density lipoprotein binding protein (VIGLN) more enriched for HOXC13-AS pull-down fraction (**Paper III**, Fig. 6d). Next, we assessed if their function is associated with keratinocyte differentiation where HOXC13-AS plays a vital role. Knockdown of COPA significantly

upregulated the expression of differentiation markers, including KRT10, FLG, and IVL (**Fig. 16A**, **Paper III**, Fig. 6e, g, h). Western blot confirmed more COPA proteins in the HOXC13-AS pull-down fraction (**Fig. 16B**). Moreover, the specific interaction between HOXC13-AS and COPA was further validated using RIP, followed by qRT-PCR (**Fig. 16C**, **Paper III**, Fig. 6l). These results suggest HOXC13-AS may regulate keratinocyte differentiation via its interaction with COPA.

COPA constitutes a part of coatomer protein complex I (COPI) that is indispensable for retrograde transport from the Golgi to the endoplasmic reticulum (ER). Next, we evaluated if HOXC13-AS is also involved in the transport. To this end, we treated cells with brefeldin A (BFA), which blocks the ER-to-Golgi transport, and then examined the redistribution area of the Golgi marker GM130, representing the Golgi-to-ER transport (**Paper III**, Fig. 7a, b). We found that HOXC13-AS knockdown increased, but HOXC13-AS overexpression decreased GM130 retrograde transport (**Paper III**, Fig. 7c, d). A previous study has shown that COPA deficiency leads to ER stress that triggers the unfolded protein response (UPR) (Watkin et al., 2015). We confirmed the upregulation of ER stress genes (sXBP1, tXBP1, ATF4, CHOP, BIP, and EDEM) upon COPA silencing (**Fig. 16D**). Notably, we demonstrated that knockdown of HOXC13-AS decreased the expression of ER stress markers, whereas HOXC13-AS overexpression enhanced their expression (**Fig. 16E**, **F**). Importantly, co-depletion of HOXC13-AS and COPA rescued the repressive effects of HOXC13-AS knockdown on keratinocyte differentiation, ER stress, and GM130 retrograde transport, suggesting the function of HOXC13-AS is dependent on COPA (**Fig. 16G**, **H**; **Paper III**, Fig. 8d).

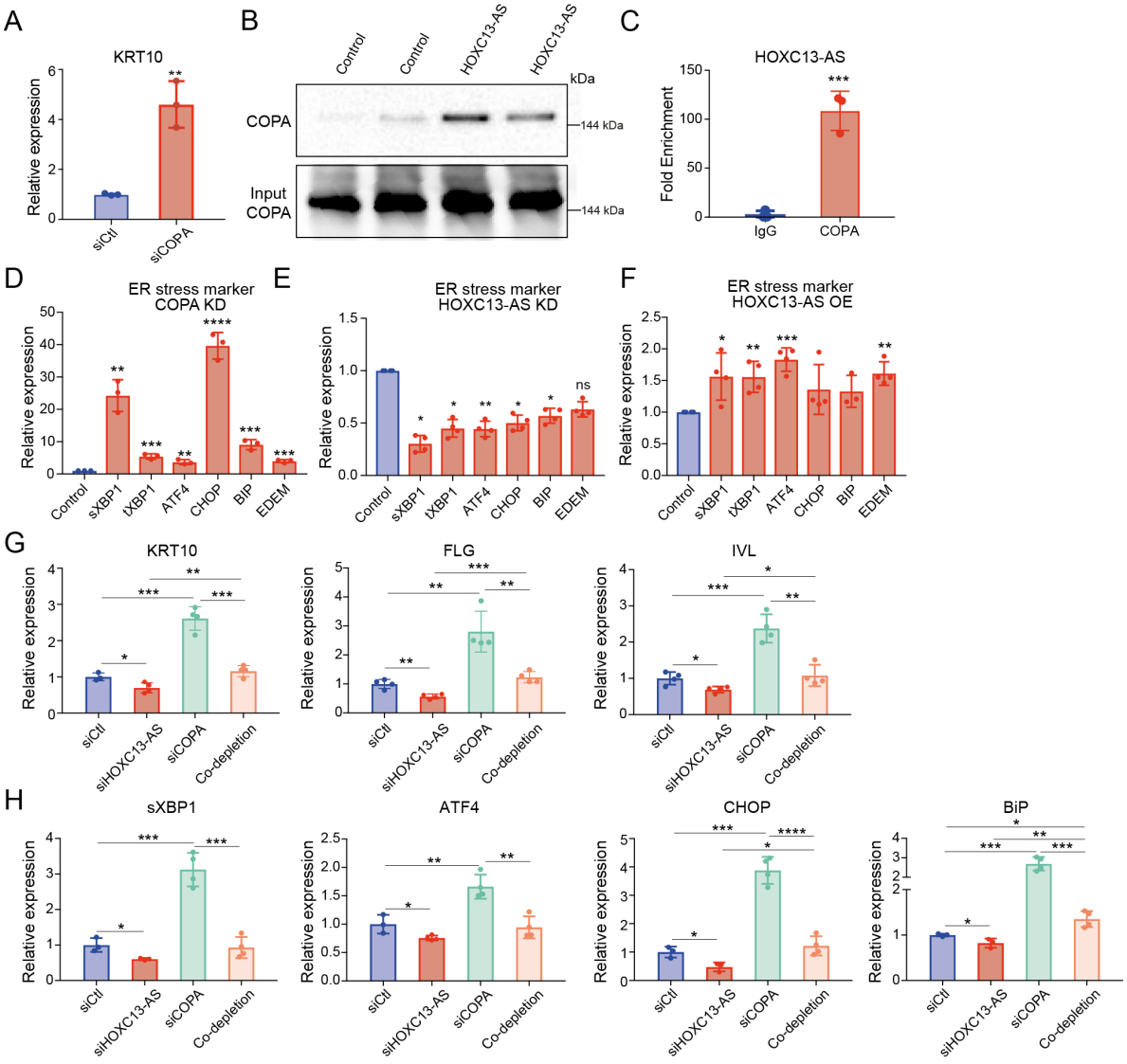

Figure 16 *HOXC13-AS interacts with COPA protein and interferes with Golgi-ER retrograde transport.* ***A*** *qRT-PCR of KRT10 in differentiated keratinocytes after COPA knockdown.* ***B*** *Western blot of COPA after HOXC13-AS or control RNA pulldown.* ***C*** *qRT-PCR analysis of HOXC13-AS retrieved from RIP using COPA antibody or IgG. qRT-PCR analysis of ER stress markers in differentiated keratinocytes after COPA knockdown* ***D****, HOXC13-AS knockdown* ***E****, or HOXC13-AS overexpression* ***F****. qRT-PCR analysis of differentiation markers* ***G****, and ER stress markers* ***H*** *in differentiated keratinocytes with individual HOXC13-AS or COPA knockdown, or co-depletion of HOXC13-AS and COPA.*

Discussion

By analyzing RNA sequencing data of wound tissues and isolated epidermal cells, we identified a recently evolved and non-conserved human lncRNA HOXC13-AS, which plays a vital role in keratinocyte differentiation. In previous studies, HOXC13-AS has been identified as a biomarker in various cancer types (Gao et al., 2019; Li et al., 2020b; Li et al., 2019; Liu et al., 2019; Wang et al., 2021; Xiong et al., 2020; Zhou et al., 2019). To the best of our knowledge, our study first revealed the physiological function of HOXC13-AS, particularly in the skin and keratinocytes, where HOXC13-AS was mainly expressed. A lack of more physiological studies of HOXC13-AS also suggests its low expression in other tissues.

HOXC13-AS acts as an oncogene, enhancing cancer cell proliferation (Li et al., 2020b). However, HOXC13-AS knockdown did not influence keratinocyte migration or proliferation in our study.

Although a previous study has demonstrated that physiological ER stress is activated during keratinocyte differentiation, the mechanism is still unknown (Sugiura et al., 2009). In the study, we discovered HOXC13-AS hijacked COPA that interfered with the Golgi-to-ER retrograde transport and thus promoted ER stress and keratinocyte differentiation. COPA constitutes a part of COPI and mediates the retrograde transport of cargo proteins from the Golgi to the ER and within cis-Golgi compartments (Beck et al., 2009). COPA mutation leads to impaired retrograde transport and thus causes ER stress, as well as UPR and NF-κB pathway activation (Todd et al., 2008). We demonstrated that COPA knockdown enhanced ER stress and promoted keratinocyte differentiation in *vitro*. As we did not find the expression change of COPA across different epidermal layers in human skin, the increased ER stress during keratinocyte differentiation may be attributed to highly expressed HOXC13-AS in the suprabasal layer.

4.4 Exploring the function of long noncoding RNA SNHG26 in skin wound healing

Rationale

The transition from the inflammatory phase to the subsequent proliferative phase is critical for wound healing. Failure of this phase transition results in non-healing wounds. Understanding the underlying mechanism may help develop novel treatments for wound healing. This study identified a conserved long noncoding RNA SNHG26, upregulated in the inflammatory and proliferative phases. We aimed to reveal the role of SNHG26 during this phase transition.

Main findings

LncRNA expression and function analysis in skin wound healing.

By RNA-seq analysis, we identified 342 lncRNAs with significantly (one-way ANOVA, $p \leq 0.005$) altered expression in the skin, NW1 and NW7. Among these lncRNAs, 20 lncRNAs were conserved across humans and rodents (**Paper IV**, Fig. 1A). Next, we focused on these evolutionarily conserved lncRNAs and performed siRNA screenings to investigate their roles in the cell growth, migration, and inflammatory response in human primary keratinocytes. SNHG26 stood out from the 20 lncRNAs, as its downregulation significantly inhibited cell proliferation and cell migration, as well as enhanced production of the inflammatory cytokines (**Paper IV**, Fig. 1B). SNHG26 was upregulated in the inflammatory and proliferative phases, suggesting the pro-proliferative, pro-migratory, but anti-inflammatory roles during skin wound healing (**Fig. 17A**).

SNHG26 is encoded on chromosome 7 in humans and on chromosome 5 in mice (**Paper IV**, Fig. 1C). The gene is located between the TOMM7 and FAM126A coding genes in both humans and mice, suggesting interspecies syntenic conservation (**Paper IV**, Supplementary Fig. 1). Similar to the expression of SNHG26 in human wound repair, it was transiently upregulated in the early phases of mouse wound healing (**Fig. 17B**). Importantly, we found that SNHG26 predominantly increased in wound-edge basal keratinocytes analyzed by FISH (**Fig. 17C**). We also demonstrated that SNHG26 was nuclear located and polyadenylated noncoding RNA with a length of 3100 nts (**Paper IV**, Fig. 1H-L).

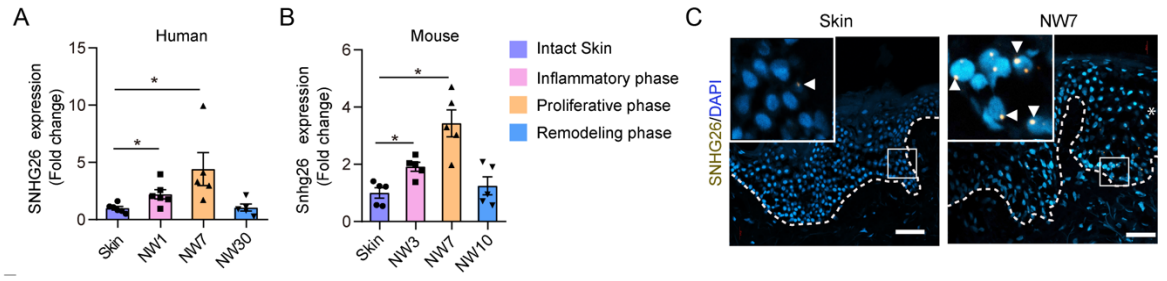

Figure 17 *LncRNA expression in skin wound healing. qRT-PCR of SNHG26 in human skin and wounds* ***A****, and Snhg26 in mouse skin and wounds* ***B****.* ***C*** *FISH of SNHG26 in human skin and day 7 acute wounds.*

Single-cell transcriptomic analysis of impaired wound repair in Snhg26-KO mice.

To study the role of SNHG26 in wound healing *in vivo*, we generated Snhg26 knockout (KO) mice. The Snhg26-KO mice showed an overall normal phenotype despite a little thinner epidermis than that of the wild-type (WT) littermate controls (**Paper IV**, Supplementary Fig. 2). Importantly, Snhg26 KO significantly reduced the healing rate and the area of the epithelial tongue (**Fig. 18A**; **Paper IV**, Fig. 2F). In line with it, intradermal injection of SNHG26 antisense oligonucleotides (ASOs) at the wound edges of the WT mice delayed wound healing (**Paper IV**, Fig. 2B). To unravel the mechanism underlying delayed wounds, we isolated epidermal cells from the skin and wounds of WT and KO mice for transcriptomic analysis. The biological processes related to the skin development and epidermis development were enriched for downregulated genes in the skin and wound of SNHG26 KO mice, whereas the inflammatory response was enriched for the upregulated genes in the wound of the KO mice (**Paper IV**, Fig. 2C, Supplementary Fig. 2). Additionally, increased macrophage presence (CD68+) was found at the wound-bed dermis in the Snhg26-KO mice analyzed by IF staining (**Paper IV**, Fig. 2H).

Next, we performed single-cell RNA sequencing analysis in the skin and wounds from WT and KO mice. We identified 23 cell clusters, including keratinocytes (C1-C6), hair follicle cells (C7-C10), melanocytes (C11), fibroblasts (C12-C14), sebaceous gland cells (C15), vascular endothelial cells (C16-C17), muscle cells (C18-C19), macrophages (C20), T helper cells (Th cells, C21), γδ T cells (C22), and Langerhans cells (C23) (**Paper IV**, Fig. 3A). We first compared the cell composition and discovered decreased C2_Basal stem and progenitors 2 (Bas2) and C3_Bas3 cells but increased C4_Bas4 cells in the wounds of KO mice (**Fig. 18B**). To understand functional roles of the 3 subclusters, we performed functional annotations using their marker genes. Mitosis-related genes (e.g., Pcna and Mki67) were highly expressed in C2_Bas2, predicting that it is a proliferative cell cluster. Glycolysis is a hallmark for epithelial

cell migration and re-epithelialization during wound healing (Konieczny et al., 2022), and keratinocyte activation markers Krt6b and Krt16 (Cheng et al., 2013) were enriched for C3_Bas3. Moreover, the enrichment of multiple inflammation signals (e.g., the TFs Fosl1 and Rel) indicates C4_Bas4 as an inflammatory cell cluster (Hannemann et al., 2019; Hilliard et al., 2002) (**Fig. 18C**; **Paper IV,** Figure S4, S5A, Table S5, and S6). In line with it, the three progenitor clusters represented unique functional features when we compared combination scores using overall expression of genes associated with the biological processes, including proliferation, migration, and inflammation (**Fig. 18D**). Our data suggest fewer proliferative or migratory keratinocytes but more inflammatory keratinocytes in the wound edge of the Snhg26-KO mice than in those of the WT mice.

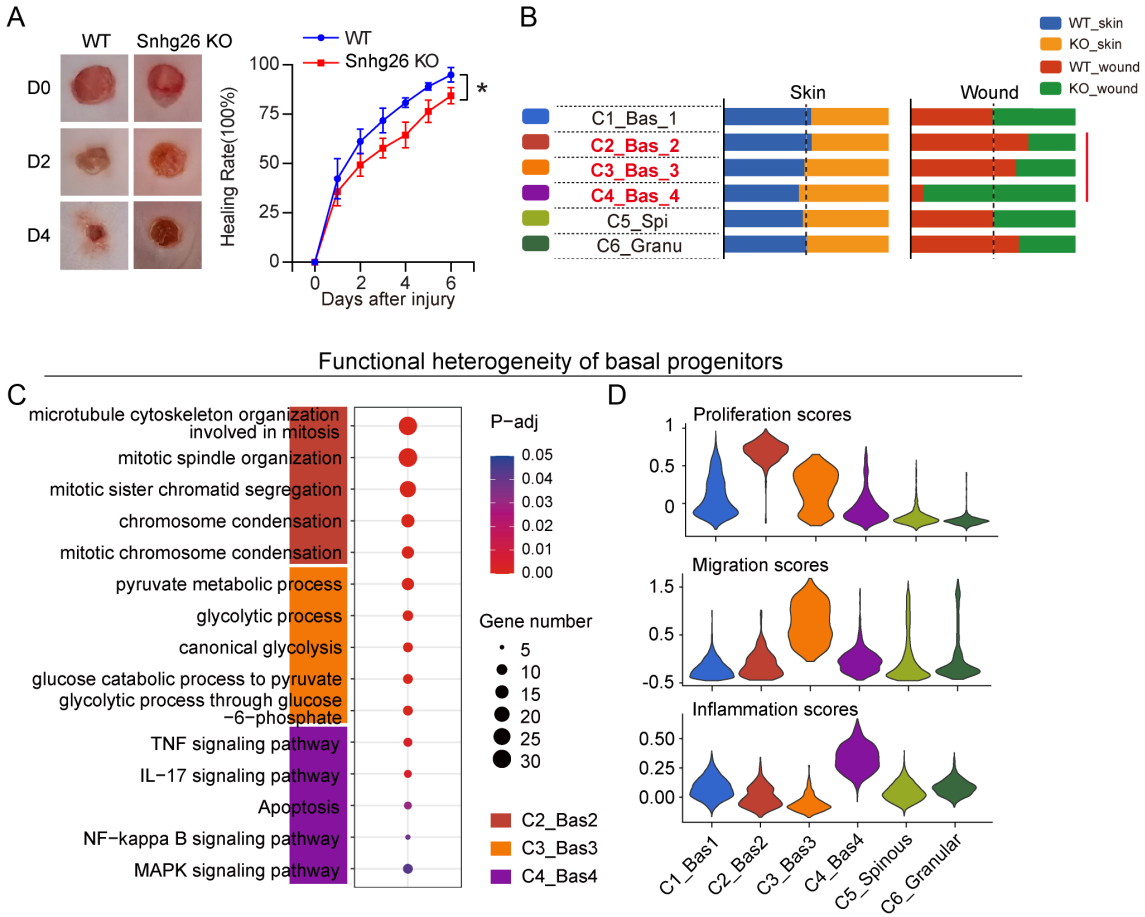

Figure 18 *Single-cell transcriptomic analysis of impaired wound repair in Snhg26-KO mice.* ***A*** *Representative wound images and healing rates of Snhg26-KO mice compared to those of WT mice.* ***B*** *Comparison of cell composition of the skin and wound between the KO and WT mice.* ***C*** *GO and KEGG analysis of the 200 most highly expressed DEGs in the three progenitor clusters.* ***D*** *The proliferation, migration, and inflammatory scores were plotted for each keratinocyte cluster.*

SNHG26 inhibits the inflammatory response in human keratinocytes and promotes re-epithelization in human ex vivo wounds.

To understand the function of SNHG26 in human wound healing, we inhibited SNHG26 expression using SNHG26 antisense oligos in human keratinocytes and triggered the inflammatory response with TNFα. We then performed microarray analysis, and GO results showed that the biological processes related to I-κB/NF-κB signaling and cytokine-mediated signaling pathway were enriched for the upregulated genes, whereas the mitosis-associated genes were enriched for the downregulated genes after SNHG26 knockdown (**Paper IV**, Fig. 4A). We further confirmed the upregulation of cytokines (e.g., IL6, IL8, and CCL20) after SNHG26 knockdown in mRNA levels. On the contrary, overexpression of SNHG26 inhibited TNFα-induced cytokine expression (**Paper IV**, Fig. 4B, C). Using scratch wound assays and cell proliferation assays, we confirmed SNHG26 silencing impaired keratinocyte migration and proliferation (**Paper IV**, Fig. 4D, E). Moreover, we showed that downregulation of SNHG26 impaired re-epithelialization of human *ex vivo* wounds, indicating the physiological relevance of SNHG26 to human skin wound healing (**Fig. 19 A-D**).

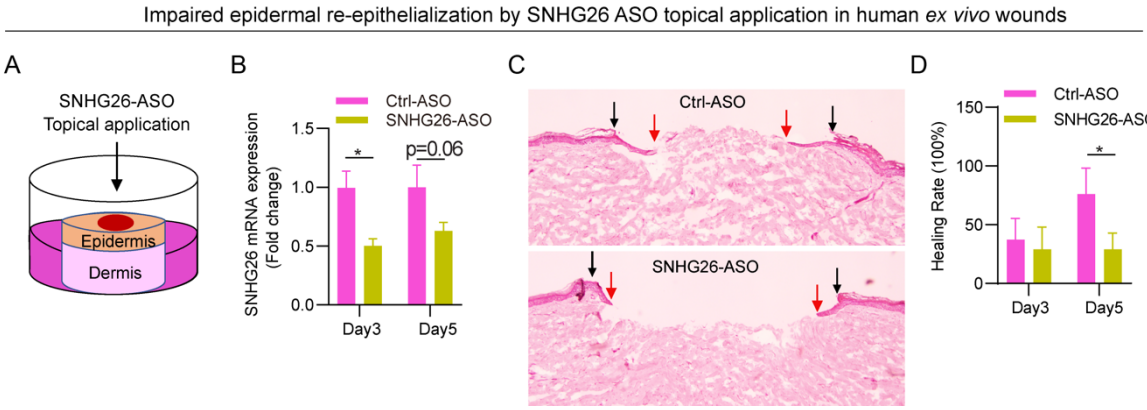

Figure 19 *SNHG26 knockdown impaired re-epithelialization of human ex vivo wounds.* ***A*** *Schematic illustration showing the topical application of SNHG26-ASOs to human ex vivo wounds.* ***B*** *qRT–PCR of SNHG26 expression in human ex vivo wounds with SNHG26 knockdown.* ***C*** *Representative photographs showing hematoxylin and eosin staining of ex vivo wounds. Black arrows indicate the initial wound edges, and red arrows indicate newly formed epidermis in the front of the tongue (day 5).* ***D*** *Quantification of wound re-epithelialization.*

SNHG26 guides ILF2 protein from the inflammatory genomic loci to LAMB3 genomic locus.

To investigate the mechanism underlying the essential biological functions of SNHG26, we first aimed to identify its protein partner and performed SNHG26 RNA pulldown, followed by gel electrophoresis and Coomassie staining. A stronger band at around 45 kDa was observed in the SNHG26 pull-down fraction than the negative control pull-down fraction, which was

enriched with interleukin enhancer binding factor 2 (ILF2) protein analyzed by mass spectrometry (**Paper IV**, Fig. 5A, B). Furthermore, RIP analysis revealed that SNHG26 was co-precipitated with the ILF2 protein using an ILF2-specific antibody (**Paper IV**, Fig. 5D, E). Our results suggest SNHG26 physically binds to ILF2.

ILF2 is a transcription regulator for mitotic and inflammatory genes (Guan et al., 2008). To understand if SNHG26 regulates the inflammatory response mediated by ILF2, we performed ChIP sequencing and found that the occupancy of ILF2 at the promoter region of JUN was increased after SNHG26 silencing, which was validated by ILF2 ChIP–qPCR (**Fig. 20A, B**). As a master transcription factor, Jun/AP1 regulates inflammatory gene expression in the epidermis (Guinea-Viniegra et al., 2009). Notably, by the scRNA-seq data analysis, we found that Jun expression was increased, and its regulated genes were significantly enriched for the upregulated genes in the wound-edge basal keratinocytes of Snhg26-KO wounds (**Paper IV**, Fig. 5K, L).

As SHNG26 regulates cell migration and inflammatory response, we hypothesized that SNHG26 might facilitate ILF2 to pro-migratory gene loci during the transition from the inflammatory to the proliferative phase. To this end, we performed Chromatin Isolation by RNA Purification (ChIRP) that captures the SNHG26-chromatin complex using anti-SNHG26 biotinylated oligos, and the DNA was purified for sequencing (**Paper IV**, Fig. 6A, B). We found high occupancy of SNHG26 at LAMB3 genomic locus (**Fig. 20C**). Importantly, we showed that SNHG26 knockdown significantly inhibited ILF2 binding to the LAMB3 locus analyzed by ILF2 ChIP-qPCR (**Fig. 20D**), suggesting that SNHG26 facilitates ILF2 protein to the LAMB3 locus by interacting with chromatin. LAMB3 is required for keratinocyte proliferation and migration (De Rosa et al., 2019). Consistent with the previous result, we showed that LAMB3 knockdown significantly inhibited keratinocyte migration and proliferation (**Paper IV**, Fig. 6F-J). In line with it, scRNA-seq analysis confirmed Lamb3 was significantly downregulated in the wound-edge basal keratinocytes in Snhg26-KO mice, compared to those in WT mice (**Paper IV**, Fig. 6M).

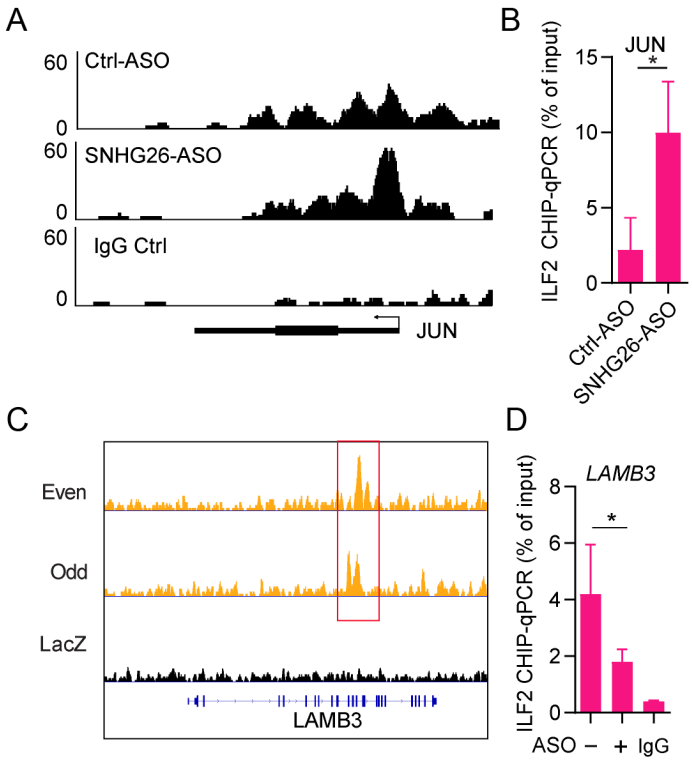

Figure 20 *SNHG26 guides ILF2 protein from the inflammatory genomic loci to LAMB3 genomic locus.* ***A*** *Genome browser tracks of ILF2 ChIP-seq signal intensity showing increased enrichment of ILF2 at the promoter regions of JUN after SNHG26 knockdown.* ***B*** *ChIP–qPCR analysis of ILF2 at the promoter of JUN in keratinocyte progenitors after SNHG26 knockdown.* ***C*** *Genome browser tracks of SNHG26 CHIRP-seq signal intensity showing SNHG26 occupancy at the LAMB3 locus.* ***D*** *ChIP–qPCR analysis of ILF2 at the promoter of LAMB3 in keratinocyte progenitors after SNHG26 knockdown.*

Discussion

The failed transition from the inflammatory to the proliferative phase in skin wound healing is implicated in various types of wound complications, including chronic wounds and hypertrophic or keloid scars (Eming et al., 2017; Landen et al., 2016; MacLeod and Mansbridge, 2016; Toulon et al., 2009). To understand the underlying mechanism facilitating the transition, we focused on lncRNAs which act as epigenetic regulators in various pathological and physiological contexts (Reddy et al., 2014; Shan et al., 2017; Wan et al., 2013; Xing et al., 2014). By analyzing lncRNA expression in human skin and wounds and performing functional screenings, we discovered that SNHG26 plays pro-proliferative, pro-migratory, and anti-inflammatory roles. We further explored the function of SNHG26 *in vivo* using a genetically engineered-KO mouse model. In addition to delayed re-epithelialization observed in wounds of Snhg26-KO mice, single-cell RNA seq analysis revealed fewer proliferative or migratory but more inflammatory basal keratinocytes in the wound edge of the Snhg26-KO mice than that of the WT mice.

Mechanistically, SNHG26 physically binds to the ILF2 protein, which forms a heterodimer complex with ILF3 (Guan et al., 2008; Nourreddine et al., 2020) and plays a vital role in maintaining embryonic stem cell pluripotency and regulates the expression of cell cycle-associated genes (Ye et al., 2017). In addition, this complex sequesters mitotic mRNAs from Staufen-mediated mRNA decay (Nourreddine et al., 2020). Moreover, the ILF2/ILF3 complex is reported to interact with the promoters of multiple inflammatory genes (e.g., IL2 and IL13) and immediate early genes (e.g., EGR1, FOS, and JUN) to modulate their transcription (Kiesler et al., 2010; Tsai et al., 2021; Wu et al., 2019). In this study, we showed SNHG26 interacted with ILF2 and guided it from the inflammatory genomic loci to the LAMB3 locus, which switches cell states of keratinocyte progenitors to facilitate cell proliferation and migration. In summary, we identified a conserved lncRNA SNHG26 as a vital regulator in basal keratinocytes' transition from the inflammatory to the proliferative state during skin wound healing.

5 Concluding remarks

A comprehensive understanding of skin wound healing is challenging due to the high cellular diversity and complexity implicated in the process (Wilkinson and Hardman, 2020). Although animal models provide an indispensable resource for exploring molecular and cellular functions in wound healing, their relevance to humans raises questions. Noncoding RNAs, including long noncoding RNAs and microRNAs, exhibit tissue- and condition-specific expression patterns and play vital roles in gene regulation. Unraveling the functions of noncoding RNAs in human skin wound healing facilitates their potential therapeutic applications. In our studies, we performed high-throughput small and long RNA sequencing in human wound tissues and isolated epidermal cells, and several long noncoding RNAs and microRNAs were identified in wound repair.

In **Paper I**, we identified a lncRNA WAKMAR1, which was upregulated during human skin wound healing but deficient in non-healing wounds. By treating primary keratinocytes with a panel of growth factors and cytokines, we demonstrated that WAKMAR1 expression was induced by TGF-β signaling. WAKMAR1 silencing inhibited keratinocyte migration and re-epithelialization of human *ex vivo* wounds, whereas its overexpression promoted cell migration. Furthermore, GO analysis revealed that migration-related genes were significantly enriched for downregulated genes after WAKMAR1 knockdown. Using the STRING network analysis, E2F1 was identified as a hub gene among the WAKMAR1-regulated genes. Moreover, GSEA suggested that E2F1-regulated genes were enriched for downregulated genes after WAKMAR1 silencing. The pro-migratory effect observed after WAKMAR1 overexpression was abolished by E2F1 silencing. We further showed that WAKMAR1 knockdown increased DNA methylation at the E2F1 promoter, whereas WAKMAR1 overexpression decreased it. Consistent with it, WAKMAR1 knockdown increased the binding of DNMT1 at the E2F1 promoter, whereas WAKMAR1 overexpression decreased it. Overall, WAKMAR1 interacts with DNMTs and thus modulates the methylation levels at the E2F1 promoter, which results in the altered expression of E2F1.

In **Paper II**, we analyzed paired miRNA and mRNA expression profiling in normal wounds and venous ulcers. We identified 32 miRNAs and 424 mRNAs dysregulated in VU compared to the intact skin and NWs. By WGCNA, 13 distinct miRNA and 13 mRNA modules were identified. Notably, m3, m7, and m9 miRNA modules were downregulated, but m8 and m12 were upregulated in VU. The M5 mRNA module was downregulated, but M9, M10, M11, and M12 were upregulated in VU. We next performed correlation analyses between VU-associated

miRNA and mRNA modules. Further enrichment analysis showed that predicted targets of the VU-associated miRNAs were enriched for VU-related mRNAs. Moreover, we found 17 VU-relevant miRNAs, targets of which were also enriched for the VU-related mRNA modules. We performed gene ontology analyses for the genes regulated by 8 VU-associated miRNAs. The upregulated miRNAs in VUs were predicted to promote inflammatory response but impair cell proliferation, but the downregulated miRNAs might be needed for cell proliferation and migration. We experimentally tested the combined effects of miR-34a-5p, miR-424-5p, and miR-516-5p upregulated in VUs. Simultaneous overexpression of miR-34a-5p and miR-424-5p had stronger inhibitory effects on keratinocyte proliferation and migration, whereas miR-34a-5p and miR-516b-5p cooperatively promoted expression of the pro-inflammatory chemokine CCL20.

In **Paper III**, we identified a skin-specific lncRNA HOXC13-AS, which was downregulated in human wound-edge keratinocytes. We performed single-cell RNA sequencing in the human skin and showed that HOXC13-AS was highly expressed in granular keratinocytes. Correlation analysis was performed for HOXC13-AS in the granular cells, and the top 50 correlated genes with HOXC13-AS were associated with keratinocyte differentiation and immune response. The expression of HOXC13-AS was inhibited by the EGFR signaling pathway but gradually increased in keratinocyte differentiation. The transcriptomic analysis showed that keratinocyte differentiation-related genes were enriched for the downregulated genes after HOXC13-AS silencing. Additionally, we confirmed the pro-differentiation effect of HOXC13-AS in the suspension-induced differentiation model and organotypic epidermis by examining the expression of differentiation markers. Using RNA pulldown and RIP, we revealed HOXC13-AS could physically bind to COPA, and the interaction interferes with the retrograde transport from the Golgi to the ER. Our data suggest that highly expressed HOXC13-AS impairs retrograde transport and promotes ER stress and epidermal differentiation.

In **Paper IV**, we focused on the evolutionary conserved lncRNA SNHG26, which was upregulated in the wound-edge basal keratinocytes in humans and mice. We further demonstrated that SNHG26 was a nuclear-located and polyadenylated non-coding RNA with a length of 3100 nts in human primary keratinocytes. Importantly, Snhg26-KO mice showed delayed re-epithelialization and exacerbated inflammatory response. By single-cell RNA sequencing analysis, we found decreased migratory but increased inflammatory progenitors in the wound edge of Snhg26 KO mice. Moreover, SNHG26 silencing enhanced TNFα-induced inflammatory response but impaired keratinocyte migration and re-epithelialization of human *ex vivo* wounds. Mechanistically, using RNA pulldown and RIP, we demonstrated the physical

interaction between SNHG26 and ILF2 protein. By ChIP and ChIRP analysis, we found SNHG26 directed ILF2 from the inflammatory genomic loci to the LAMB3 genomic locus during the phase transition, rewiring the gene expression program and promoting wound healing.

6 Points of perspective

Based on the results of this thesis, future research can focus on:

- Further investigate the role of WAKMAR1 and HOXC13-AS *in vivo*; although these two lncRNAs are not conserved in the mouse, we can transplant human skin in mouse models and generate transgenic mice expressing human WAKMAR1 and HOXC13-AS.
- Understanding the role of VU-relevant miRNAs *in vivo*; we can modulate miRNA expression using miRNA mimics and inhibitors, and generate miRNA knockout and transgenic mouse models.
- Exploring the role of HOXC13-AS in Darier's disease, a rare skin disorder associated with ER stress; this may contribute to our understanding of the pathogenesis of skin diseases.
- Investigating the function of SNHG26 in animal models mimicking chronic wounds; this may facilitate its clinical translation.
- Using single-cell and spatial technologies to study noncoding RNAs in human skin wound healing; this could identify more cell- and context-specific noncoding RNAs, which may reinforce their therapeutic potential.

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8 References

Adiewere, P., Gillis, R.B., Imran Jiwani, S., Meal, A., Shaw, I., and Adams, G.G. (2018). A systematic review and meta-analysis of patient education in preventing and reducing the incidence or recurrence of adult diabetes foot ulcers (DFU). Heliyon *4*, e00614.

Anderson, K.M., Anderson, D.M., McAnally, J.R., Shelton, J.M., Bassel-Duby, R., and Olson, E.N. (2016). Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. Nature *539*, 433-436.

Anthony, D., Alosoumi, D., and Safari, R. (2019). Prevalence of pressure ulcers in long-term care: a global review. J Wound Care *28*, 702-709.

Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., and Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. Wound Repair Regen *16*, 585-601.

Bartel, D.P. (2018). Metazoan MicroRNAs. Cell *173*, 20-51.

Beck, R., Rawet, M., Wieland, F.T., and Cassel, D. (2009). The COPI system: molecular mechanisms and function. FEBS Lett *583*, 2701-2709.

Bernard, D., Prasanth, K.V., Tripathi, V., Colasse, S., Nakamura, T., Xuan, Z., Zhang, M.Q., Sedel, F., Jourdren, L., Coulpier, F.*, et al.* (2010). A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. EMBO J *29*, 3082-3093.

Blumberg, S.N., Maggi, J., Melamed, J., Golinko, M., Ross, F., and Chen, W. (2012). A histopathologic basis for surgical debridement to promote healing of venous ulcers. J Am Coll Surg *215*, 751-757.

Bodnar, R.J., Yates, C.C., and Wells, A. (2006). IP-10 blocks vascular endothelial growth factor-induced endothelial cell motility and tube formation via inhibition of calpain. Circ Res *98*, 617-625.

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics *30*, 2114-2120.

Bragazzi, N.L., Sellami, M., Salem, I., Conic, R., Kimak, M., Pigatto, P.D.M., and Damiani, G. (2019). Fasting and Its Impact on Skin Anatomy, Physiology, and Physiopathology: A Comprehensive Review of the Literature. Nutrients *11*.

Bucalo, B., Eaglstein, W.H., and Falanga, V. (1993). Inhibition of cell proliferation by chronic wound fluid. Wound Repair Regen *1*, 181-186.

Buchstein, N., Hoffmann, D., Smola, H., Lang, S., Paulsson, M., Niemann, C., Krieg, T., and Eming, S.A. (2009). Alternative proteolytic processing of hepatocyte growth factor during wound repair. Am J Pathol *174*, 2116-2128.

Bushati, N., and Cohen, S.M. (2007). microRNA functions. Annu Rev Cell Dev Biol *23*, 175-205.

Cabili, M.N., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., Regev, A., and Rinn, J.L. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev *25*, 1915-1927.

Cai, N., Li, C., and Wang, F. (2019). Silencing of LncRNA-ANCR Promotes the Osteogenesis of Osteoblast Cells in Postmenopausal Osteoporosis via Targeting EZH2 and RUNX2. Yonsei Med J *60*, 751-759.

Candi, E., Schmidt, R., and Melino, G. (2005). The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol *6*, 328-340.

Cañedo-Dorantes, L., and Cañedo-Ayala, M. (2019). Skin Acute Wound Healing: A Comprehensive Review. International Journal of Inflammation *2019*, 3706315.

Cerase, A., Pintacuda, G., Tattermusch, A., and Avner, P. (2015). Xist localization and function: new insights from multiple levels. Genome Biol *16*, 166.

Chen, S.X., Zhang, L.J., and Gallo, R.L. (2019). Dermal White Adipose Tissue: A Newly Recognized Layer of Skin Innate Defense. J Invest Dermatol *139*, 1002-1009.

Cheng, C.H., Leferovich, J., Zhang, X.M., Bedelbaeva, K., Gourevitch, D., Hatcher, C.J., Basson, C.T., Heber-Katz, E., and Marx, K.A. (2013). Keratin gene expression profiles after digit amputation in C57BL/6 vs. regenerative MRL mice imply an early regenerative keratinocyte activated-like state. Physiol Genomics *45*, 409-421.

Chomiczewska, D., Trznadel-Budzko, E., Kaczorowska, A., and Rotsztejn, H. (2009). [The role of Langerhans cells in the skin immune system]. Pol Merkur Lekarski *26*, 173-177.

Chu, C., Qu, K., Zhong, F.L., Artandi, S.E., and Chang, H.Y. (2011). Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. Mol Cell *44*, 667-678.

Chu, C., Quinn, J., and Chang, H.Y. (2012). Chromatin isolation by RNA purification (ChIRP). J Vis Exp.

Chu, D.H. (2012). Chapter 7. Development and Structure of Skin. In Fitzpatrick's Dermatology in General Medicine, 8e, L.A. Goldsmith, S.I. Katz, B.A. Gilchrest, A.S. Paller, D.J. Leffell, and K. Wolff, eds. (New York, NY: The McGraw-Hill Companies).

Cichorek, M., Wachulska, M., Stasiewicz, A., and Tyminska, A. (2013). Skin melanocytes: biology and development. Postepy Dermatol Alergol *30*, 30-41.

Consortium, E.P. (2004). The ENCODE (ENCyclopedia Of DNA Elements) Project. Science *306*, 636-640.

Courvoisier, D.S., Righi, L., Bene, N., Rae, A.C., and Chopard, P. (2018). Variation in pressure ulcer prevalence and prevention in nursing homes: A multicenter study. Appl Nurs Res *42*, 45-50.

D'Souza, S.J., Vespa, A., Murkherjee, S., Maher, A., Pajak, A., and Dagnino, L. (2002). E2F-1 is essential for normal epidermal wound repair. J Biol Chem *277*, 10626-10632.

De Pessemier, B., Grine, L., Debaere, M., Maes, A., Paetzold, B., and Callewaert, C. (2021). Gut-Skin Axis: Current Knowledge of the Interrelationship between Microbial Dysbiosis and Skin Conditions. Microorganisms *9*.

De Rosa, L., Secone Seconetti, A., De Santis, G., Pellacani, G., Hirsch, T., Rothoeft, T., Teig, N., Pellegrini, G., Bauer, J.W., and De Luca, M. (2019). Laminin 332-Dependent YAP Dysregulation Depletes Epidermal Stem Cells in Junctional Epidermolysis Bullosa. Cell Rep *27*, 2036-2049 e2036.

Dealey, C., Brindle, C.T., Black, J., Alves, P., Santamaria, N., Call, E., and Clark, M. (2015). Challenges in pressure ulcer prevention. Int Wound J *12*, 309-312.

Del Rosso, J.Q., and Levin, J. (2011). The clinical relevance of maintaining the functional integrity of the stratum corneum in both healthy and disease-affected skin. J Clin Aesthet Dermatol *4*, 22-42.

Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D.G., *et al.* (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res *22*, 1775-1789.

Diaz-Herrera, M.A., Martinez-Riera, J.R., Verdu-Soriano, J., Capillas-Perez, R.M., Pont-Garcia, C., Tenllado-Perez, S., Cunillera-Puertolas, O., Berenguer-Perez, M., and Gea-Caballero, V. (2021). Multicentre Study of Chronic Wounds Point Prevalence in Primary Health Care in the Southern Metropolitan Area of Barcelona. J Clin Med *10*.

Dimitrova, N., Zamudio, J.R., Jong, R.M., Soukup, D., Resnick, R., Sarma, K., Ward, A.J., Raj, A., Lee, J.T., Sharp, P.A., *et al.* (2014). LincRNA-p21 activates p21 in cis to promote Polycomb target gene expression and to enforce the G1/S checkpoint. Mol Cell *54*, 777-790.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics *29*, 15-21.

Eaglstein, W.H., and Falanga, V. (1997). Chronic wounds. Surg Clin North Am *77*, 689-700.

Eckhart, L., Lippens, S., Tschachler, E., and Declercq, W. (2013). Cell death by cornification. Biochim Biophys Acta *1833*, 3471-3480.

Eming, S.A., Hammerschmidt, M., Krieg, T., and Roers, A. (2009). Interrelation of immunity and tissue repair or regeneration. Semin Cell Dev Biol *20*, 517-527.

Eming, S.A., Koch, M., Krieger, A., Brachvogel, B., Kreft, S., Bruckner-Tuderman, L., Krieg, T., Shannon, J.D., and Fox, J.W. (2010). Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. J Proteome Res *9*, 4758-4766.

Eming, S.A., Krieg, T., and Davidson, J.M. (2007). Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol *127*, 514-525.

Eming, S.A., Martin, P., and Tomic-Canic, M. (2014). Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med *6*, 265sr266.

Eming, S.A., Wynn, T.A., and Martin, P. (2017). Inflammation and metabolism in tissue repair and regeneration. Science *356*, 1026-1030.

Engreitz, J.M., Haines, J.E., Perez, E.M., Munson, G., Chen, J., Kane, M., McDonel, P.E., Guttman, M., and Lander, E.S. (2016). Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature *539*, 452-455.

Engreitz, J.M., Pandya-Jones, A., McDonel, P., Shishkin, A., Sirokman, K., Surka, C., Kadri, S., Xing, J., Goren, A., Lander, E.S., *et al.* (2013). The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. Science *341*, 1237973.

Engreitz, J.M., Sirokman, K., McDonel, P., Shishkin, A.A., Surka, C., Russell, P., Grossman, S.R., Chow, A.Y., Guttman, M., and Lander, E.S. (2014). RNA-RNA interactions enable specific targeting of noncoding RNAs to nascent Pre-mRNAs and chromatin sites. Cell *159*, 188-199.

Eriksson, A., Siegbahn, A., Westermark, B., Heldin, C.H., and Claesson-Welsh, L. (1992). PDGF alpha- and beta-receptors activate unique and common signal transduction pathways. EMBO J *11*, 543-550.

Ertosun, M.G., Hapil, F.Z., and Osman Nidai, O. (2016). E2F1 transcription factor and its impact on growth factor and cytokine signaling. Cytokine Growth Factor Rev *31*, 17-25.

Fagerberg, L., Hallstrom, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K.*, et al.* (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics *13*, 397-406.

Falanga, V. (1988). Occlusive wound dressings. Why, when, which? Arch Dermatol *124*, 872-877.

Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. Lancet *366*, 1736-1743.

Falanga, V., Brem, H., Ennis, W.J., Wolcott, R., Gould, L.J., and Ayello, E.A. (2008). Maintenance debridement in the treatment of difficult-to-heal chronic wounds. Recommendations of an expert panel. Ostomy Wound Manage *Suppl*, 2-13; quiz 14-15.

Falanga, V., Fujitani, R.M., Diaz, C., Hunter, G., Jorizzo, J., Lawrence, P.F., Lee, B.Y., Menzoian, J.O., Tretbar, L.L., Holloway, G.A.*, et al.* (1999). Systemic treatment of venous leg ulcers with high doses of pentoxifylline: efficacy in a randomized, placebo-controlled trial. Wound Repair Regen *7*, 208-213.

Falanga, V., Isseroff, R.R., Soulika, A.M., Romanelli, M., Margolis, D., Kapp, S., Granick, M., and Harding, K. (2022). Chronic wounds. Nat Rev Dis Primers *8*, 50.

Falanga, V., Margolis, D., Alvarez, O., Auletta, M., Maggiacomo, F., Altman, M., Jensen, J., Sabolinski, M., and Hardin-Young, J. (1998). Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. Arch Dermatol *134*, 293-300.

Falanga, V., and Sabolinski, M. (1999). A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. Wound Repair Regen *7*, 201-207.

Fatica, A., and Bozzoni, I. (2014). Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet *15*, 7-21.

Feisst, V., Meidinger, S., and Locke, M.B. (2015). From bench to bedside: use of human adipose-derived stem cells. Stem Cells Cloning *8*, 149-162.

Feldmeyer, L., Keller, M., Niklaus, G., Hohl, D., Werner, S., and Beer, H.D. (2007). The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. Curr Biol *17*, 1140-1145.

Fonder, M.A., Lazarus, G.S., Cowan, D.A., Aronson-Cook, B., Kohli, A.R., and Mamelak, A.J. (2008). Treating the chronic wound: A practical approach to the care of nonhealing wounds and wound care dressings. J Am Acad Dermatol *58*, 185-206.

Frank, D.N., Wysocki, A., Specht-Glick, D.D., Rooney, A., Feldman, R.A., St Amand, A.L., Pace, N.R., and Trent, J.D. (2009). Microbial diversity in chronic open wounds. Wound Repair Regen *17*, 163-172.

Freedberg, I.M., Tomic-Canic, M., Komine, M., and Blumenberg, M. (2001). Keratins and the keratinocyte activation cycle. J Invest Dermatol *116*, 633-640.

Friedlander, M.R., Mackowiak, S.D., Li, N., Chen, W., and Rajewsky, N. (2012). miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res *40*, 37-52.

Gallant-Behm, C.L., Piper, J., Dickinson, B.A., Dalby, C.M., Pestano, L.A., and Jackson, A.L. (2018). A synthetic microRNA-92a inhibitor (MRG-110) accelerates angiogenesis and wound healing in diabetic and nondiabetic wounds. Wound Repair Regen *26*, 311-323.

Gao, C., Lu, W., Lou, W., Wang, L., and Xu, Q. (2019). Long noncoding RNA HOXC13-AS positively affects cell proliferation and invasion in nasopharyngeal carcinoma via modulating miR-383-3p/HMGA2 axis. J Cell Physiol *234*, 12809-12820.

Gebert, L.F.R., and MacRae, I.J. (2019). Regulation of microRNA function in animals. Nat Rev Mol Cell Biol *20*, 21-37.

Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Alitalo, K., Shima, D.*, et al.* (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol *161*, 1163-1177.

Golebiewska, E.M., and Poole, A.W. (2015). Platelet secretion: From haemostasis to wound healing and beyond. Blood Rev *29*, 153-162.

Gonzalez, A.C., Costa, T.F., Andrade, Z.A., and Medrado, A.R. (2016). Wound healing - A literature review. An Bras Dermatol *91*, 614-620.

Gottrup, F., and Apelqvist, J. (2012). Present and new techniques and devices in the treatment of DFU: a critical review of evidence. Diabetes Metab Res Rev *28 Suppl 1*, 64-71.

Greenberg, M.V.C., and Bourc'his, D. (2019). The diverse roles of DNA methylation in mammalian development and disease. Nat Rev Mol Cell Biol *20*, 590-607.

Grey, J.E., Harding, K.G., and Enoch, S. (2006). Pressure ulcers. BMJ *332*, 472-475.

Groff, A.F., Sanchez-Gomez, D.B., Soruco, M.M.L., Gerhardinger, C., Barutcu, A.R., Li, E., Elcavage, L., Plana, O., Sanchez, L.V., Lee, J.C.*, et al.* (2016). In Vivo Characterization of Linc-p21 Reveals Functional cis-Regulatory DNA Elements. Cell Rep *16*, 2178-2186.

Grose, R., Hutter, C., Bloch, W., Thorey, I., Watt, F.M., Fassler, R., Brakebusch, C., and Werner, S. (2002). A crucial role of beta 1 integrins for keratinocyte migration in vitro and during cutaneous wound repair. Development *129*, 2303-2315.

Grose, R., and Werner, S. (2004). Wound-healing studies in transgenic and knockout mice. Mol Biotechnol *28*, 147-166.

Guan, D., Altan-Bonnet, N., Parrott, A.M., Arrigo, C.J., Li, Q., Khaleduzzaman, M., Li, H., Lee, C.G., Pe'ery, T., and Mathews, M.B. (2008). Nuclear factor 45 (NF45) is a regulatory subunit of complexes with NF90/110 involved in mitotic control. Mol Cell Biol *28*, 4629-4641.

Guinea-Viniegra, J., Zenz, R., Scheuch, H., Hnisz, D., Holcmann, M., Bakiri, L., Schonthaler, H.B., Sibilia, M., and Wagner, E.F. (2009). TNFalpha shedding and epidermal inflammation are controlled by Jun proteins. Genes Dev *23*, 2663-2674.

Gurtner, G.C., Werner, S., Barrandon, Y., and Longaker, M.T. (2008). Wound repair and regeneration. Nature *453*, 314-321.

Gushiken, L.F.S., Beserra, F.P., Bastos, J.K., Jackson, C.J., and Pellizzon, C.H. (2021). Cutaneous Wound Healing: An Update from Physiopathology to Current Therapies. Life (Basel) *11*.

Guttman, M., Amit, I., Garber, M., French, C., Lin, M.F., Feldser, D., Huarte, M., Zuk, O., Carey, B.W., Cassady, J.P.*, et al.* (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature *458*, 223-227.

Hacisuleyman, E., Goff, L.A., Trapnell, C., Williams, A., Henao-Mejia, J., Sun, L., McClanahan, P., Hendrickson, D.G., Sauvageau, M., Kelley, D.R.*, et al.* (2014). Topological organization of multichromosomal regions by the long intergenic noncoding RNA Firre. Nat Struct Mol Biol *21*, 198-206.

Hafemeister, C., and Satija, R. (2019). Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. Genome Biol *20*, 296.

Hanna, J., Hossain, G.S., and Kocerha, J. (2019). The Potential for microRNA Therapeutics and Clinical Research. Front Genet *10*, 478.

Hannemann, N., Cao, S., Eriksson, D., Schnelzer, A., Jordan, J., Eberhardt, M., Schleicher, U., Rech, J., Ramming, A., Uebe, S.*, et al.* (2019). Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis. J Clin Invest *129*, 2669-2684.

Hansen, T.B., Jensen, T.I., Clausen, B.H., Bramsen, J.B., Finsen, B., Damgaard, C.K., and Kjems, J. (2013). Natural RNA circles function as efficient microRNA sponges. Nature *495*, 384-388.

Hansen, T.B., Wiklund, E.D., Bramsen, J.B., Villadsen, S.B., Statham, A.L., Clark, S.J., and Kjems, J. (2011). miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. EMBO J *30*, 4414-4422.

Hao, J., Ho, J.N., Lewis, J.A., Karim, K.A., Daniels, R.N., Gentry, P.R., Hopkins, C.R., Lindsley, C.W., and Hong, C.C. (2010). In vivo structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. ACS Chem Biol *5*, 245-253.

Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W.M., 3rd, Zheng, S., Butler, A., Lee, M.J., Wilk, A.J., Darby, C., Zager, M.*, et al.* (2021). Integrated analysis of multimodal single-cell data. Cell *184*, 3573-3587 e3529.

He, Z.Y., Wei, T.H., Zhang, P.H., Zhou, J., and Huang, X.Y. (2019). Long noncoding RNA-antisense noncoding RNA in the INK4 locus accelerates wound healing in diabetes by promoting lymphangiogenesis via regulating miR-181a/Prox1 axis. J Cell Physiol *234*, 4627-4640.

Herter, E.K., Li, D., Toma, M.A., Vij, M., Li, X., Visscher, D., Wang, A., Chu, T., Sommar, P., Blomqvist, L.*, et al.* (2019). WAKMAR2, a Long Noncoding RNA Downregulated in Human Chronic Wounds, Modulates Keratinocyte Motility and Production of Inflammatory Chemokines. J Invest Dermatol *139*, 1373-1384.

Hilliard, B.A., Mason, N., Xu, L., Sun, J., Lamhamedi-Cherradi, S.E., Liou, H.C., Hunter, C., and Chen, Y.H. (2002). Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. J Clin Invest *110*, 843-850.

Hobbs, R.M., Silva-Vargas, V., Groves, R., and Watt, F.M. (2004). Expression of activated MEK1 in differentiating epidermal cells is sufficient to generate hyperproliferative and inflammatory skin lesions. J Invest Dermatol *123*, 503-515.

Homs-Romero, E., Romero-Collado, A., Verdu, J., Blanch, J., Rascon-Hernan, C., and Marti-Lluch, R. (2021). Validity of Chronic Venous Disease Diagnoses and Epidemiology Using Validated Electronic Health Records From Primary Care: A Real-World Data Analysis. J Nurs Scholarsh *53*, 296-305.

Hon, C.C., Ramilowski, J.A., Harshbarger, J., Bertin, N., Rackham, O.J., Gough, J., Denisenko, E., Schmeier, S., Poulsen, T.M., Severin, J.*, et al.* (2017). An atlas of human long non-coding RNAs with accurate 5' ends. Nature *543*, 199-204.

Hou, J., Wang, P., Lin, L., Liu, X., Ma, F., An, H., Wang, Z., and Cao, X. (2009). MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. J Immunol *183*, 2150-2158.

Hu, J., Zhang, L., Liechty, C., Zgheib, C., Hodges, M.M., Liechty, K.W., and Xu, J. (2020). Long Noncoding RNA GAS5 Regulates Macrophage Polarization and Diabetic Wound Healing. J Invest Dermatol *140*, 1629-1638.

Hu, W., Yuan, B., Flygare, J., and Lodish, H.F. (2011). Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. Genes Dev *25*, 2573-2578.

Huarte, M., Guttman, M., Feldser, D., Garber, M., Koziol, M.J., Kenzelmann-Broz, D., Khalil, A.M., Zuk, O., Amit, I., Rabani, M.*, et al.* (2010). A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell *142*, 409-419.

Hubner, G., Brauchle, M., Smola, H., Madlener, M., Fassler, R., and Werner, S. (1996). Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. Cytokine *8*, 548-556.

Hutchinson, J.N., Ensminger, A.W., Clemson, C.M., Lynch, C.R., Lawrence, J.B., and Chess, A. (2007). A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics *8*, 39.

Inman, G.J., Nicolas, F.J., Callahan, J.F., Harling, J.D., Gaster, L.M., Reith, A.D., Laping, N.J., and Hill, C.S. (2002). SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol *62*, 65-74.

Inui, M., Martello, G., and Piccolo, S. (2010). MicroRNA control of signal transduction. Nat Rev Mol Cell Biol *11*, 252-263.

Iyer, M.K., Niknafs, Y.S., Malik, R., Singhal, U., Sahu, A., Hosono, Y., Barrette, T.R., Prensner, J.R., Evans, J.R., Zhao, S.*, et al.* (2015). The landscape of long noncoding RNAs in the human transcriptome. Nat Genet *47*, 199-208.

Joung, J., Engreitz, J.M., Konermann, S., Abudayyeh, O.O., Verdine, V.K., Aguet, F., Gootenberg, J.S., Sanjana, N.E., Wright, J.B., Fulco, C.P.*, et al.* (2017). Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature *548*, 343-346.

Jull, A., Waters, J., and Arroll, B. (2002). Pentoxifylline for treatment of venous leg ulcers: a systematic review. Lancet *359*, 1550-1554.

Jun, J.I., and Lau, L.F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat Cell Biol *12*, 676-685.

Kanitakis, J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol *12*, 390-399; quiz 400-391.

Katz, M.H., Alvarez, A.F., Kirsner, R.S., Eaglstein, W.H., and Falanga, V. (1991). Human wound fluid from acute wounds stimulates fibroblast and endothelial cell growth. J Am Acad Dermatol *25*, 1054-1058.

Kiesler, P., Haynes, P.A., Shi, L., Kao, P.N., Wysocki, V.H., and Vercelli, D. (2010). NF45 and NF90 regulate HS4-dependent interleukin-13 transcription in T cells. J Biol Chem *285*, 8256-8267.

Kolarsick, P.A., Kolarsick, M.A., and Goodwin, C. (2011). Anatomy and physiology of the skin. Journal of the Dermatology Nurses' Association *3*, 203-213.

Kolev, V., Mandinova, A., Guinea-Viniegra, J., Hu, B., Lefort, K., Lambertini, C., Neel, V., Dummer, R., Wagner, E.F., and Dotto, G.P. (2008). EGFR signalling as a negative regulator

of Notch1 gene transcription and function in proliferating keratinocytes and cancer. Nat Cell Biol *10*, 902-911.

Konieczny, P., Xing, Y., Sidhu, I., Subudhi, I., Mansfield, K.P., Hsieh, B., Biancur, D.E., Larsen, S.B., Cammer, M., Li, D.*, et al.* (2022). Interleukin-17 governs hypoxic adaptation of injured epithelium. Science *377*, eabg9302.

Kopp, F., and Mendell, J.T. (2018). Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell *172*, 393-407.

Korsunsky, I., Millard, N., Fan, J., Slowikowski, K., Zhang, F., Wei, K., Baglaenko, Y., Brenner, M., Loh, P.R., and Raychaudhuri, S. (2019). Fast, sensitive and accurate integration of single-cell data with Harmony. Nat Methods *16*, 1289-1296.

Kozomara, A., Birgaoanu, M., and Griffiths-Jones, S. (2019). miRBase: from microRNA sequences to function. Nucleic Acids Res *47*, D155-D162.

Kretz, M., Siprashvili, Z., Chu, C., Webster, D.E., Zehnder, A., Qu, K., Lee, C.S., Flockhart, R.J., Groff, A.F., Chow, J.*, et al.* (2013). Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature *493*, 231-235.

Kretz, M., Webster, D.E., Flockhart, R.J., Lee, C.S., Zehnder, A., Lopez-Pajares, V., Qu, K., Zheng, G.X., Chow, J., Kim, G.E.*, et al.* (2012). Suppression of progenitor differentiation requires the long noncoding RNA ANCR. Genes Dev *26*, 338-343.

Lai, X., Eberhardt, M., Schmitz, U., and Vera, J. (2019). Systems biology-based investigation of cooperating microRNAs as monotherapy or adjuvant therapy in cancer. Nucleic Acids Res *47*, 7753-7766.

Landen, N.X., Li, D., and Stahle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci *73*, 3861-3885.

Langer, V., Bhandari, P.S., Rajagopalan, S., and Mukherjee, M.K. (2015). Negative pressure wound therapy as an adjunct in healing of chronic wounds. Int Wound J *12*, 436-442.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol *10*, R25.

Lau, K., Paus, R., Tiede, S., Day, P., and Bayat, A. (2009). Exploring the role of stem cells in cutaneous wound healing. Exp Dermatol *18*, 921-933.

Lauer, G., Sollberg, S., Cole, M., Flamme, I., Sturzebecher, J., Mann, K., Krieg, T., and Eming, S.A. (2000). Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. J Invest Dermatol *115*, 12-18.

Lee, S., Kopp, F., Chang, T.C., Sataluri, A., Chen, B., Sivakumar, S., Yu, H., Xie, Y., and Mendell, J.T. (2016). Noncoding RNA NORAD Regulates Genomic Stability by Sequestering PUMILIO Proteins. Cell *164*, 69-80.

Li, D., Li, X.I., Wang, A., Meisgen, F., Pivarcsi, A., Sonkoly, E., Stahle, M., and Landen, N.X. (2015a). MicroRNA-31 Promotes Skin Wound Healing by Enhancing Keratinocyte Proliferation and Migration. J Invest Dermatol *135*, 1676-1685.

Li, D., Niu, G., and Landen, N.X. (2022). Beyond the Code: Noncoding RNAs in Skin Wound Healing. Cold Spring Harb Perspect Biol *14*.

Li, D., Peng, H., Qu, L., Sommar, P., Wang, A., Chu, T., Li, X., Bi, X., Liu, Q., Serezal, I.G.*, et al.* (2020a). miR-19a/b and miR-20a promote wound healing by regulating the inflammatory response of keratinocytes. J Invest Dermatol.

Li, D., Wang, A., Liu, X., Meisgen, F., Grunler, J., Botusan, I.R., Narayanan, S., Erikci, E., Li, X., Blomqvist, L., *et al.* (2015b). MicroRNA-132 enhances transition from inflammation to proliferation during wound healing. J Clin Invest *125*, 3008-3026.

Li, E., and Zhang, Y. (2014). DNA methylation in mammals. Cold Spring Harb Perspect Biol *6*, a019133.

Li, G., Zhou, R., Zhang, Q., Jiang, B., Wu, Q., and Wang, C. (2016). Fibroproliferative effect of microRNA-21 in hypertrophic scar derived fibroblasts. Exp Cell Res *345*, 93-99.

Li, J., Chen, J., and Kirsner, R. (2007). Pathophysiology of acute wound healing. Clin Dermatol *25*, 9-18.

Li, W., Zhu, Q., Zhang, S., Liu, L., Zhang, H., and Zhu, D. (2020b). HOXC13-AS accelerates cell proliferation and migration in oral squamous cell carcinoma via miR-378g/HOXC13 axis. Oral Oncol *111*, 104946.

Li, X., Wang, Q., Rui, Y., Zhang, C., Wang, W., Gu, J., Tang, J., and Ding, Y. (2019). HOXC13-AS promotes breast cancer cell growth through regulating miR-497-5p/PTEN axis. J Cell Physiol *234*, 22343-22351.

Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics *30*, 923-930.

Lin, S., and Gregory, R.I. (2015). MicroRNA biogenesis pathways in cancer. Nat Rev Cancer *15*, 321-333.

Liu, N., Wang, Z., Liu, D., and Xie, P. (2019). HOXC13-AS-miR-122-5p-SATB1-C-Myc feedback loop promotes migration, invasion and EMT process in glioma. Onco Targets Ther *12*, 7165-7173.

Liu, T., Zhang, L., Joo, D., and Sun, S.C. (2017). NF-kappaB signaling in inflammation. Signal Transduct Target Ther *2*, 17023-.

Liu, Z., Zhang, L., Toma, M.A., Li, D., Bian, X., Pastar, I., Tomic-Canic, M., Sommar, P., and Xu Landén, N. (2022). Integrative small and long RNA omics analysis of human healing and nonhealing wounds discovers cooperating microRNAs as therapeutic targets. eLife *11*, e80322.

Loots, M.A., Lamme, E.N., Zeegelaar, J., Mekkes, J.R., Bos, J.D., and Middelkoop, E. (1998). Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. J Invest Dermatol *111*, 850-857.

Lopes, T.S., Videira, L., Saraiva, D., Agostinho, E.S., and Bandarra, A.J.F. (2020). Multicentre study of pressure ulcer point prevalence in a Portuguese region. J Tissue Viability *29*, 12-18.

Lopez-Pajares, V., Qu, K., Zhang, J., Webster, D.E., Barajas, B.C., Siprashvili, Z., Zarnegar, B.J., Boxer, L.D., Rios, E.J., Tao, S., *et al.* (2015). A LncRNA-MAF:MAFB transcription factor network regulates epidermal differentiation. Dev Cell *32*, 693-706.

Losquadro, W.D. (2017). Anatomy of the Skin and the Pathogenesis of Nonmelanoma Skin Cancer. Facial Plast Surg Clin North Am *25*, 283-289.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol *15*, 550.

Mackowiak, S.D. (2011). Identification of novel and known miRNAs in deep-sequencing data with miRDeep2. Curr Protoc Bioinformatics *Chapter 12*, Unit 12 10.

MacLeod, A.S., and Mansbridge, J.N. (2016). The Innate Immune System in Acute and Chronic Wounds. Adv Wound Care (New Rochelle) *5*, 65-78.

MacNeil, S. (2007). Progress and opportunities for tissue-engineered skin. Nature *445*, 874-880.

Maderal, A.D., Vivas, A.C., Eaglstein, W.H., and Kirsner, R.S. (2012). The FDA and designing clinical trials for chronic cutaneous ulcers. Semin Cell Dev Biol *23*, 993-999.

Madhyastha, R., Madhyastha, H., Nakajima, Y., Omura, S., and Maruyama, M. (2012). MicroRNA signature in diabetic wound healing: promotive role of miR-21 in fibroblast migration. Int Wound J *9*, 355-361.

Maksimovic, S., Nakatani, M., Baba, Y., Nelson, A.M., Marshall, K.L., Wellnitz, S.A., Firozi, P., Woo, S.H., Ranade, S., Patapoutian, A.*, et al.* (2014). Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. Nature *509*, 617-621.

Margolis, D.J., Bilker, W., Santanna, J., and Baumgarten, M. (2002). Venous leg ulcer: incidence and prevalence in the elderly. J Am Acad Dermatol *46*, 381-386.

Martin, P. (1997). Wound healing--aiming for perfect skin regeneration. Science *276*, 75-81.

Matsui, T., and Amagai, M. (2015). Dissecting the formation, structure and barrier function of the stratum corneum. Int Immunol *27*, 269-280.

Meigel, W.N., Gay, S., and Weber, L. (1977). Dermal architecture and collagen type distribution. Arch Dermatol Res (1975) *259*, 1-10.

Meisgen, F., Xu Landen, N., Wang, A., Rethi, B., Bouez, C., Zuccolo, M., Gueniche, A., Stahle, M., Sonkoly, E., Breton, L.*, et al.* (2014). MiR-146a negatively regulates TLR2-induced inflammatory responses in keratinocytes. J Invest Dermatol *134*, 1931-1940.

Memczak, S., Jens, M., Elefsinioti, A., Torti, F., Krueger, J., Rybak, A., Maier, L., Mackowiak, S.D., Gregersen, L.H., Munschauer, M.*, et al.* (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature *495*, 333-338.

Mercer, T.R., Dinger, M.E., and Mattick, J.S. (2009). Long non-coding RNAs: insights into functions. Nat Rev Genet *10*, 155-159.

Mervis, J.S., and Phillips, T.J. (2019). Pressure ulcers: Prevention and management. J Am Acad Dermatol *81*, 893-902.

Miscianinov, V., Martello, A., Rose, L., Parish, E., Cathcart, B., Mitic, T., Gray, G.A., Meloni, M., Al Haj Zen, A., and Caporali, A. (2018). MicroRNA-148b Targets the TGF-beta Pathway to Regulate Angiogenesis and Endothelial-to-Mesenchymal Transition during Skin Wound Healing. Mol Ther *26*, 1996-2007.

Mohseni, S., Bayani, M., Bahmani, F., Tajabadi-Ebrahimi, M., Bayani, M.A., Jafari, P., and Asemi, Z. (2018). The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. Diabetes Metab Res Rev *34*.

Moll, R., Divo, M., and Langbein, L. (2008). The human keratins: biology and pathology. Histochem Cell Biol *129*, 705-733.

Morlando, M., and Fatica, A. (2018). Alteration of Epigenetic Regulation by Long Noncoding RNAs in Cancer. Int J Mol Sci *19*.

Mostafa, W.Z., and Hegazy, R.A. (2015). Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res *6*, 793-804.

Nelson, E.A., and Adderley, U. (2016). Venous leg ulcers. BMJ Clin Evid *2016*.

Nickoloff, B.J., Lingen, M.W., Chang, B.D., Shen, M., Swift, M., Curry, J., Bacon, P., Bodner, B., and Roninson, I.B. (2004). Tumor suppressor maspin is up-regulated during keratinocyte senescence, exerting a paracrine antiangiogenic activity. Cancer Res *64*, 2956-2961.

Nithya, S., Radhika, T., and Jeddy, N. (2015). Loricrin - an overview. J Oral Maxillofac Pathol *19*, 64-68.

Noh, J.H., Kim, K.M., McClusky, W.G., Abdelmohsen, K., and Gorospe, M. (2018). Cytoplasmic functions of long noncoding RNAs. Wiley Interdiscip Rev RNA *9*, e1471.

Nojima, T., Tellier, M., Foxwell, J., Ribeiro de Almeida, C., Tan-Wong, S.M., Dhir, S., Dujardin, G., Dhir, A., Murphy, S., and Proudfoot, N.J. (2018). Deregulated Expression of Mammalian lncRNA through Loss of SPT6 Induces R-Loop Formation, Replication Stress, and Cellular Senescence. Mol Cell *72*, 970-984 e977.

Nolan, C.J., Damm, P., and Prentki, M. (2011). Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet *378*, 169-181.

Nourreddine, S., Lavoie, G., Paradis, J., Ben El Kadhi, K., Meant, A., Aubert, L., Grondin, B., Gendron, P., Chabot, B., Bouvier, M.*, et al.* (2020). NF45 and NF90 Regulate Mitotic Gene Expression by Competing with Staufen-Mediated mRNA Decay. Cell Rep *31*, 107660.

Obagi, Z., Damiani, G., Grada, A., and Falanga, V. (2019). Principles of Wound Dressings: A Review. Surg Technol Int *35*, 50-57.

Ozcan, G., Ozpolat, B., Coleman, R.L., Sood, A.K., and Lopez-Berestein, G. (2015). Preclinical and clinical development of siRNA-based therapeutics. Adv Drug Deliv Rev *87*, 108-119.

Panatta, E., Lena, A.M., Mancini, M., Smirnov, A., Marini, A., Delli Ponti, R., Botta-Orfila, T., Tartaglia, G.G., Mauriello, A., Zhang, X.*, et al.* (2020). Long non-coding RNA uc.291 controls epithelial differentiation by interfering with the ACTL6A/BAF complex. EMBO Rep *21*, e46734.

Pappas, P.J., You, R., Rameshwar, P., Gorti, R., DeFouw, D.O., Phillips, C.K., Padberg, F.T., Jr., Silva, M.B., Jr., Simonian, G.T., Hobson, R.W., 2nd*, et al.* (1999). Dermal tissue fibrosis in patients with chronic venous insufficiency is associated with increased transforming growth factor-beta1 gene expression and protein production. J Vasc Surg *30*, 1129-1145.

Pastar, I., Nusbaum, A.G., Gil, J., Patel, S.B., Chen, J., Valdes, J., Stojadinovic, O., Plano, L.R., Tomic-Canic, M., and Davis, S.C. (2013). Interactions of methicillin resistant Staphylococcus aureus USA300 and Pseudomonas aeruginosa in polymicrobial wound infection. PLoS One *8*, e56846.

Pastar, I., Stojadinovic, O., Yin, N.C., Ramirez, H., Nusbaum, A.G., Sawaya, A., Patel, S.B., Khalid, L., Isseroff, R.R., and Tomic-Canic, M. (2014). Epithelialization in Wound Healing: A Comprehensive Review. Adv Wound Care (New Rochelle) *3*, 445-464.

Philippeos, C., Telerman, S.B., Oules, B., Pisco, A.O., Shaw, T.J., Elgueta, R., Lombardi, G., Driskell, R.R., Soldin, M., Lynch, M.D.*, et al.* (2018). Spatial and Single-Cell Transcriptional Profiling Identifies Functionally Distinct Human Dermal Fibroblast Subpopulations. J Invest Dermatol *138*, 811-825.

Pizzino, G., Irrera, N., Galfo, F., Pallio, G., Mannino, F., D'Amore, A., Pellegrino, E., Ieni, A., Russo, G.T., Calapai, M.*, et al.* (2018). Effects of the antagomiRs 15b and 200b on the altered healing pattern of diabetic mice. Br J Pharmacol *175*, 644-655.

Ponting, C.P., and Haerty, W. (2022). Genome-Wide Analysis of Human Long Noncoding RNAs: A Provocative Review. Annu Rev Genomics Hum Genet *23*, 153-172.

Pool, J.G. (1977). Normal hemostatic mechanisms: a review. Am J Med Technol *43*, 776-780.

Pradhan, S., Khatlani, T., Nairn, A.C., and Vijayan, K.V. (2017). The heterotrimeric G protein Gbeta(1) interacts with the catalytic subunit of protein phosphatase 1 and modulates G protein-coupled receptor signaling in platelets. J Biol Chem *292*, 13133-13142.

Quinn, J.J., and Chang, H.Y. (2016). Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet *17*, 47-62.

Ramirez, H.A., Pastar, I., Jozic, I., Stojadinovic, O., Stone, R.C., Ojeh, N., Gil, J., Davis, S.C., Kirsner, R.S., and Tomic-Canic, M. (2018). Staphylococcus aureus Triggers Induction of miR-15B-5P to Diminish DNA Repair and Deregulate Inflammatory Response in Diabetic Foot Ulcers. J Invest Dermatol *138*, 1187-1196.

Rashid, F., Shah, A., and Shan, G. (2016). Long Non-coding RNAs in the Cytoplasm. Genomics Proteomics Bioinformatics *14*, 73-80.

Reddy, M.A., Chen, Z., Park, J.T., Wang, M., Lanting, L., Zhang, Q., Bhatt, K., Leung, A., Wu, X., Putta, S.*, et al.* (2014). Regulation of inflammatory phenotype in macrophages by a diabetes-induced long noncoding RNA. Diabetes *63*, 4249-4261.

Reinke, J.M., and Sorg, H. (2012). Wound repair and regeneration. Eur Surg Res *49*, 35-43.

Reynolds, L.E., Conti, F.J., Silva, R., Robinson, S.D., Iyer, V., Rudling, R., Cross, B., Nye, E., Hart, I.R., Dipersio, C.M.*, et al.* (2008). alpha3beta1 integrin-controlled Smad7 regulates reepithelialization during wound healing in mice. J Clin Invest *118*, 965-974.

Rinn, J.L., Kertesz, M., Wang, J.K., Squazzo, S.L., Xu, X., Brugmann, S.A., Goodnough, L.H., Helms, J.A., Farnham, P.J., Segal, E.*, et al.* (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell *129*, 1311-1323.

Rippa, A.L., Kalabusheva, E.P., and Vorotelyak, E.A. (2019). Regeneration of Dermis: Scarring and Cells Involved. Cells *8*.

Roche, E.D., Renick, P.J., Tetens, S.P., Ramsay, S.J., Daniels, E.Q., and Carson, D.L. (2012). Increasing the presence of biofilm and healing delay in a porcine model of MRSA-infected wounds. Wound Repair Regen *20*, 537-543.

Rodrigues, M., Kosaric, N., Bonham, C.A., and Gurtner, G.C. (2019). Wound Healing: A Cellular Perspective. Physiol Rev *99*, 665-706.

Schlackow, M., Nojima, T., Gomes, T., Dhir, A., Carmo-Fonseca, M., and Proudfoot, N.J. (2017). Distinctive Patterns of Transcription and RNA Processing for Human lincRNAs. Mol Cell *65*, 25-38.

Seidel, D., Storck, M., Lawall, H., Wozniak, G., Mauckner, P., Hochlenert, D., Wetzel-Roth, W., Sondern, K., Hahn, M., Rothenaicher, G.*, et al.* (2020). Negative pressure wound therapy compared with standard moist wound care on diabetic foot ulcers in real-life clinical practice: results of the German DiaFu-RCT. BMJ Open *10*, e026345.

Shan, K., Liu, C., Liu, B.H., Chen, X., Dong, R., Liu, X., Zhang, Y.Y., Liu, B., Zhang, S.J., Wang, J.J., *et al.* (2017). Circular Noncoding RNA HIPK3 Mediates Retinal Vascular Dysfunction in Diabetes Mellitus. Circulation *136*, 1629-1642.

Shi, J., Ma, X., Su, Y., Song, Y., Tian, Y., Yuan, S., Zhang, X., Yang, D., Zhang, H., Shuai, J., *et al.* (2018). MiR-31 Mediates Inflammatory Signaling to Promote Re-Epithelialization during Skin Wound Healing. J Invest Dermatol *138*, 2253-2263.

Sindrilaru, A., Peters, T., Wieschalka, S., Baican, C., Baican, A., Peter, H., Hainzl, A., Schatz, S., Qi, Y., Schlecht, A., *et al.* (2011). An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J Clin Invest *121*, 985-997.

Singh, A., Halder, S., Menon, G.R., Chumber, S., Misra, M.C., Sharma, L.K., and Srivastava, A. (2004). Meta-analysis of randomized controlled trials on hydrocolloid occlusive dressing versus conventional gauze dressing in the healing of chronic wounds. Asian J Surg *27*, 326-332.

Sorokin, L. (2010). The impact of the extracellular matrix on inflammation. Nat Rev Immunol *10*, 712-723.

Sorrell, J.M., and Caplan, A.I. (2004). Fibroblast heterogeneity: more than skin deep. J Cell Sci *117*, 667-675.

Sorrell, J.M., and Caplan, A.I. (2009). Fibroblasts—a diverse population at the center of it all. International review of cell and molecular biology *276*, 161-214.

Statello, L., Guo, C.J., Chen, L.L., and Huarte, M. (2021). Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol *22*, 96-118.

Steed, D.L. (1995). Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. J Vasc Surg *21*, 71-78; discussion 79-81.

Stojadinovic, O., Minkiewicz, J., Sawaya, A., Bourne, J.W., Torzilli, P., de Rivero Vaccari, J.P., Dietrich, W.D., Keane, R.W., and Tomic-Canic, M. (2013). Deep tissue injury in development of pressure ulcers: a decrease of inflammasome activation and changes in human skin morphology in response to aging and mechanical load. PLoS One *8*, e69223.

Stojadinovic, O., Pastar, I., Nusbaum, A.G., Vukelic, S., Krzyzanowska, A., and Tomic-Canic, M. (2014). Deregulation of epidermal stem cell niche contributes to pathogenesis of nonhealing venous ulcers. Wound Repair Regen *22*, 220-227.

Stojadinovic, O., Pastar, I., Vukelic, S., Mahoney, M.G., Brennan, D., Krzyzanowska, A., Golinko, M., Brem, H., and Tomic-Canic, M. (2008). Deregulation of keratinocyte differentiation and activation: a hallmark of venous ulcers. J Cell Mol Med *12*, 2675-2690.

Stojadinovic, O., and Tomic-Canic, M. (2013). Human ex vivo wound healing model. Methods Mol Biol *1037*, 255-264.

Stone, R.C., Stojadinovic, O., Sawaya, A.P., Glinos, G.D., Lindley, L.E., Pastar, I., Badiavas, E., and Tomic-Canic, M. (2020). A bioengineered living cell construct activates metallothionein/zinc/MMP8 and inhibits TGFbeta to stimulate remodeling of fibrotic venous leg ulcers. Wound Repair Regen *28*, 164-176.

Strecker-McGraw, M.K., Jones, T.R., and Baer, D.G. (2007). Soft tissue wounds and principles of healing. Emerg Med Clin North Am *25*, 1-22.

Stunova, A., and Vistejnova, L. (2018). Dermal fibroblasts-A heterogeneous population with regulatory function in wound healing. Cytokine Growth Factor Rev *39*, 137-150.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S.*, et al.* (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A *102*, 15545-15550.

Sugiura, K., Muro, Y., Futamura, K., Matsumoto, K., Hashimoto, N., Nishizawa, Y., Nagasaka, T., Saito, H., Tomita, Y., and Usukura, J. (2009). The unfolded protein response is activated in differentiating epidermal keratinocytes. J Invest Dermatol *129*, 2126-2135.

Taganov, K.D., Boldin, M.P., Chang, K.J., and Baltimore, D. (2006). 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 *103*, 12481-12486.

Tang, H., Wang, X., Zhang, M., Yan, Y., Huang, S., Ji, J., Xu, J., Zhang, Y., Cai, Y., Yang, B.*, et al.* (2020). MicroRNA-200b/c-3p regulate epithelial plasticity and inhibit cutaneous wound healing by modulating TGF-beta-mediated RAC1 signaling. Cell Death Dis *11*, 931.

Tichon, A., Gil, N., Lubelsky, Y., Havkin Solomon, T., Lemze, D., Itzkovitz, S., Stern-Ginossar, N., and Ulitsky, I. (2016). A conserved abundant cytoplasmic long noncoding RNA modulates repression by Pumilio proteins in human cells. Nat Commun *7*, 12209.

Todd, D.J., Lee, A.H., and Glimcher, L.H. (2008). The endoplasmic reticulum stress response in immunity and autoimmunity. Nat Rev Immunol *8*, 663-674.

Toulon, A., Breton, L., Taylor, K.R., Tenenhaus, M., Bhavsar, D., Lanigan, C., Rudolph, R., Jameson, J., and Havran, W.L. (2009). A role for human skin-resident T cells in wound healing. J Exp Med *206*, 743-750.

Trevor, L.V., Riches-Suman, K., Mahajan, A.L., and Thornton, M.J. (2020). Adipose Tissue: A Source of Stem Cells with Potential for Regenerative Therapies for Wound Healing. J Clin Med *9*.

Tsai, H.I., Zeng, X., Liu, L., Xin, S., Wu, Y., Xu, Z., Zhang, H., Liu, G., Bi, Z., Su, D.*, et al.* (2021). NF45/NF90-mediated rDNA transcription provides a novel target for immunosuppressant development. EMBO Mol Med *13*, e12834.

Turksen, K. (2019). Cell Biology and Translational Medicine, Volume 4: Stem Cells and Cell Based Strategies in Regeneration (Springer International Publishing).

Tziotzios, C., Profyris, C., and Sterling, J. (2012). Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics Part II. Strategies to reduce scar formation after dermatologic procedures. J Am Acad Dermatol *66*, 13-24; quiz 25-16.

Ulitsky, I., and Bartel, D.P. (2013). lincRNAs: genomics, evolution, and mechanisms. Cell *154*, 26-46.

Veves, A., Falanga, V., Armstrong, D.G., Sabolinski, M.L., and Apligraf Diabetic Foot Ulcer, S. (2001). Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care *24*, 290-295.

von Mering, C., Jensen, L.J., Kuhn, M., Chaffron, S., Doerks, T., Kruger, B., Snel, B., and Bork, P. (2007). STRING 7--recent developments in the integration and prediction of protein interactions. Nucleic Acids Res *35*, D358-362.

Wall, I.B., Moseley, R., Baird, D.M., Kipling, D., Giles, P., Laffafian, I., Price, P.E., Thomas, D.W., and Stephens, P. (2008). Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. J Invest Dermatol *128*, 2526-2540.

Wan, G., Hu, X., Liu, Y., Han, C., Sood, A.K., Calin, G.A., Zhang, X., and Lu, X. (2013). A novel non-coding RNA lncRNA-JADE connects DNA damage signalling to histone H4 acetylation. EMBO J *32*, 2833-2847.

Wang, T., Feng, Y., Sun, H., Zhang, L., Hao, L., Shi, C., Wang, J., Li, R., Ran, X., Su, Y.*, et al.* (2012). miR-21 regulates skin wound healing by targeting multiple aspects of the healing process. Am J Pathol *181*, 1911-1920.

Wang, T., Li, W., Ye, B., Zhang, S., Lei, X., and Zhang, D. (2021). FTO-stabilized lncRNA HOXC13-AS epigenetically upregulated FZD6 and activated Wnt/beta-catenin signaling to drive cervical cancer proliferation, invasion, and EMT. J BUON *26*, 1279-1291.

Watkin, L.B., Jessen, B., Wiszniewski, W., Vece, T.J., Jan, M., Sha, Y., Thamsen, M., Santos-Cortez, R.L., Lee, K., Gambin, T.*, et al.* (2015). COPA mutations impair ER-Golgi transport and cause hereditary autoimmune-mediated lung disease and arthritis. Nat Genet *47*, 654-660.

Watt, F.M. (1983). Involucrin and other markers of keratinocyte terminal differentiation. J Invest Dermatol *81*, 100s-103s.

Watt, F.M., and Fujiwara, H. (2011). Cell-extracellular matrix interactions in normal and diseased skin. Cold Spring Harb Perspect Biol *3*.

Wei, M., Yang, D., Chen, L., Wu, L., Jiang, Q., Niu, N., and Yang, T. (2021). The prevalence and prevention of pressure ulcers: A multicenter study of nine nursing homes in eastern China. J Tissue Viability *30*, 133-136.

Werner, S., Smola, H., Liao, X., Longaker, M.T., Krieg, T., Hofschneider, P.H., and Williams, L.T. (1994). The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science *266*, 819-822.

Wernig, G., Chen, S.Y., Cui, L., Van Neste, C., Tsai, J.M., Kambham, N., Vogel, H., Natkunam, Y., Gilliland, D.G., Nolan, G.*, et al.* (2017). Unifying mechanism for different fibrotic diseases. Proc Natl Acad Sci U S A *114*, 4757-4762.

West, J.A., Davis, C.P., Sunwoo, H., Simon, M.D., Sadreyev, R.I., Wang, P.I., Tolstorukov, M.Y., and Kingston, R.E. (2014). The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites. Mol Cell *55*, 791-802.

Wilkinson, H.N., and Hardman, M.J. (2020). Wound healing: cellular mechanisms and pathological outcomes. Open Biol *10*, 200223.

Wlaschek, M., Peus, D., Achterberg, V., Meyer-Ingold, W., and Scharffetter-Kochanek, K. (1997). Protease inhibitors protect growth factor activity in chronic wounds. Br J Dermatol *137*, 646.

Wu, J., Li, X., Li, D., Ren, X., Li, Y., Herter, E.K., Qian, M., Toma, M.A., Wintler, A.M., Serezal, I.G.*, et al.* (2020). MicroRNA-34 Family Enhances Wound Inflammation by Targeting LGR4. J Invest Dermatol *140*, 465-476 e411.

Wu, T.H., Shi, L., Lowe, A.W., Nicolls, M.R., and Kao, P.N. (2019). Inducible expression of immediate early genes is regulated through dynamic chromatin association by NF45/ILF2 and NF90/NF110/ILF3. PLoS One *14*, e0216042.

Wu, Y., Zhong, J.L., Hou, N., Sun, Y., Ma, B., Nisar, M.F., Teng, Y., Tan, Z., Chen, K., Wang, Y.*, et al.* (2017). MicroRNA Let-7b inhibits keratinocyte migration in cutaneous wound healing by targeting IGF2BP2. Exp Dermatol *26*, 116-123.

Xing, Y.H., Yao, R.W., Zhang, Y., Guo, C.J., Jiang, S., Xu, G., Dong, R., Yang, L., and Chen, L.L. (2017). SLERT Regulates DDX21 Rings Associated with Pol I Transcription. Cell *169*, 664-678 e616.

Xing, Z., Lin, A., Li, C., Liang, K., Wang, S., Liu, Y., Park, P.K., Qin, L., Wei, Y., Hawke, D.H.*, et al.* (2014). lncRNA directs cooperative epigenetic regulation downstream of chemokine signals. Cell *159*, 1110-1125.

Xiong, D., Wu, W., Kan, L., Chen, D., Dou, X., Ji, X., Wang, M., Zong, Z., Li, J., and Zhang, X. (2020). LINC00958 and HOXC13-AS as key candidate biomarkers in head and neck squamous cell carcinoma by integrated bioinformatics analysis. PeerJ *8*, e8557.

Xu, J., Wu, W., Zhang, L., Dorset-Martin, W., Morris, M.W., Mitchell, M.E., and Liechty, K.W. (2012). The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. Diabetes *61*, 2906-2912.

Xue, M., Zhao, R., March, L., and Jackson, C. (2022). Dermal Fibroblast Heterogeneity and Its Contribution to the Skin Repair and Regeneration. Adv Wound Care (New Rochelle) *11*, 87-107.

Yager, D.R., Zhang, L.Y., Liang, H.X., Diegelmann, R.F., and Cohen, I.K. (1996). Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. J Invest Dermatol *107*, 743-748.

Yang, X., Wang, J., Guo, S.L., Fan, K.J., Li, J., Wang, Y.L., Teng, Y., and Yang, X. (2011). miR-21 promotes keratinocyte migration and re-epithelialization during wound healing. Int J Biol Sci *7*, 685-690.

Yates, C.C., Krishna, P., Whaley, D., Bodnar, R., Turner, T., and Wells, A. (2010). Lack of CXC chemokine receptor 3 signaling leads to hypertrophic and hypercellular scarring. Am J Pathol *176*, 1743-1755.

Ye, J., Jin, H., Pankov, A., Song, J.S., and Blelloch, R. (2017). NF45 and NF90/NF110 coordinately regulate ESC pluripotency and differentiation. RNA *23*, 1270-1284.

Yin, Y., Lu, J.Y., Zhang, X., Shao, W., Xu, Y., Li, P., Hong, Y., Cui, L., Shan, G., Tian, B.*, et al.* (2020). U1 snRNP regulates chromatin retention of noncoding RNAs. Nature *580*, 147-150.

Zhang, G.Y., Wu, L.C., Liao, T., Chen, G.C., Chen, Y.H., Zhao, Y.X., Chen, S.Y., Wang, A.Y., Lin, K., Lin, D.M.*, et al.* (2016). A novel regulatory function for miR-29a in keloid fibrogenesis. Clin Exp Dermatol *41*, 341-345.

Zhang, X., Wang, W., Zhu, W., Dong, J., Cheng, Y., Yin, Z., and Shen, F. (2019). Mechanisms and Functions of Long Non-Coding RNAs at Multiple Regulatory Levels. Int J Mol Sci *20*.

Zhou, J.F., Shi, Y.T., Wang, H.G., Yang, X.Z., and Wu, S.N. (2019). Overexpression of long noncoding RNA HOXC13-AS and its prognostic significance in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci *23*, 7369-7374.

Zhou, L., Chen, J., Li, Z., Li, X., Hu, X., Huang, Y., Zhao, X., Liang, C., Wang, Y., Sun, L.*, et al.* (2010). Integrated profiling of microRNAs and mRNAs: microRNAs located on Xq27.3 associate with clear cell renal cell carcinoma. PLoS One *5*, e15224.

Zhou, R., Zhang, Q., Zhang, Y., Fu, S., and Wang, C. (2015). Aberrant miR-21 and miR-200b expression and its pro-fibrotic potential in hypertrophic scars. Exp Cell Res *339*, 360-366.

Ziegler, C., Graf, J., Faderl, S., Schedlbauer, J., Strieder, N., Forstl, B., Spang, R., Bruckmann, A., Merkl, R., Hombach, S.*, et al.* (2019). The long non-coding RNA LINC00941 and SPRR5 are novel regulators of human epidermal homeostasis. EMBO Rep *20*.

Zuckerman, B., Ron, M., Mikl, M., Segal, E., and Ulitsky, I. (2020). Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex. Mol Cell *79*, 251-267 e256.