Extracellular RNA Biomarkers for Chronic Non-Healing Wounds

Rebecca Rowlands[1,3], Matthew Wynn[2,3], Joe Harvey[4], Kehinde Ross[1,3].

[1]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom.

[2]School of Nursing, Liverpool John Moores University, Liverpool, United Kingdom.

[3]Institute for Health Research, Liverpool John Moores University, Liverpool, United Kingdom.

[4]Centre for Dermatology Research, The University of Manchester, Manchester, United Kingdom.

Correspondence
Kehinde Ross
School of Pharmacy and Biomolecular Sciences
Liverpool John Moores University
Liverpool
L3 3AF
Email: o.k.ross@ljmu.ac.uk

ORCIDs
Matthew Wynn: https://orcid.org/0000-0001-9021-4747
Joe Harvey: https://orcid.org/0000-0001-9047-5672
Kehinde Ross: https://orcid.org/0000-0003-0252-1152

Key words:
diabetic foot ulcers; cell-free RNA; exosomes; extracellular vesicles; circular RNA; long non-coding RNA; microRNA; skin; wound healing.

Abbreviations

A1BG-AS1	A1BG antisense RNA 1
CASC2	Cancer susceptibility candidate 2
ceRNA	competitive endogenous RNA
cfNA	cell-free nucleic acid
cfRNA	cell-free RNA
circRNA	circular RNA
DFU	diabetic foot ulcer
DLEU1	Deleted in Lymphocytic Leukaemia 1
ECM	Extracellular matrix
EV	Extracellular vesicle
HOTAIR	HOX transcript antisense intergenic RNA
lncRNA	Long non-coding RNA
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
mRNA	Messenger RNA
miRNA	MicroRNA
MMP	Matrix metalloproteinase
ncRNA	non-coding RNA
NICE	National Institute for Health and Care Excellence (NICE)
NEAT	Nuclear enriched abundant transcript
PTEN	Phosphatase and TENsin homolog deleted on chromosome 10
sEV	Small extracellular vesicle
T2DM	type 2 diabetes mellitus
TIMP	Tissue inhibitor of matrix metalloproteinase
tRF	transfer RNA fragments
VLU	Venous leg ulcer
VEGF	Vascular endothelial growth factor
WAKMAR	Wound and keratinocyte migration-Associated RNA

Abstract

Chronic non-healing wounds represent a major clinical challenge, often associated with diabetes, vascular insufficiencies, and aging. Despite the substantial burden that such wounds place on patients and healthcare systems, few biomarkers have been approved for prediction of wound healing trajectories and outcomes, limiting opportunities to inform clinical management decisions or quantify patient responses to interventions. Recent advances have identified cell-free nucleic acids as powerful tools for gaining molecular insights because they offer a non-invasive, dynamic snapshot of physiological and pathological processes occurring throughout the body. In particular, cell-free RNAs from non-coding RNA families including microRNA , long non-coding RNA , circular RNA and transfer RNA fragments can be profiled on a large scale to reveal novel disease signatures to support biomarker development. The presence of such non-coding RNAs in serum, plasma or other biofluids provides a rich resource for uncovering new parameters that can support biomarker development for wound repair. In this review article, we highlight some of the current challenges associated with biomarkers for wound healing in clinical practice. We then survey microRNAs, long non-coding RNA and circular RNAs landscape in relation to their utility as biomarkers in diabetic foot ulcers and other chronic wounds. Collectively, these extracellular RNAs offer a multifaceted view of wound biology and may serve as non-invasive biomarkers for stratifying wound severity, predicting healing outcomes, and guiding personalized interventions.

Introduction

Chronic wounds are open wounds that often take months to heal, if they heal at all. These wounds include diabetic foot ulcers (DFU), venous leg ulcers (VLU), arterial ulcers , pressure ulcers and surgical wounds healing by secondary intention that fail to achieve full closure [1, 2]. Collectively these wounds are common, with major implications for those affected and the health systems that deliver their care. For instance, the costs of managing chronic wounds was estimated at US$25 billion per annum in the USA and the prevalence 1–2% of the population in developed countries [3]. More recently, Guest and colleagues put the costs of wound management across the National Health Service of the United Kingdom at £8.3 billion, of which £5.6 billion was for managing unhealed wounds [4]. In a primary care setting in Barcelona, Spain, a 3-year evaluation put wound care costs at around US$40 million, which extrapolated to $2 billion across Spain [5]. Beyond these economic considerations, chronic wounds also have a substantial negative impact on health-related quality of life, which overall are comparable to those observed in congestive heart failure or chronic obstructive pulmonary disease [6]. Further, there is evidence that the 5-year mortality rate for patients after diabetes-related amputations is almost 50%, which is twice that reported for breast cancer [7]. It is also worth noting that there are limited effective treatments to promote wound healing across all wound types, and those that are evidence-based are mostly physical or mechanical in nature, such as negative pressure wound therapy, advanced dressings and compression therapy rather than biologically active innovations implemented in a stratified medicine framework [8, 9].

Wound healing progresses through well-established phases of inflammation, proliferation and remodelling, with co-ordinated interactions between diverse cell types orchestrating completion of the process [10, 11]. In chronic wounds, inflammation fails to resolve, neovascularisation is curtailed, extracellular matrix (ECM) formation and turnover is disrupted and keratinocytes adopt a hyperproliferative phenotype that prevents differentiation, migration and re-epithelialisation of the wound [12-14]. Against this backdrop, the ability to characterise the wound status and predict healing outcomes using biomarkers may offer a new framework for clinical management of chronic wounds by enabling personalised interventions that target the underlying molecular and cellular dysfunction associated with non-healing wounds. , However, although our understanding of

the cell and molecular biology of wound healing has grown tremendously in recent years, this has not been paralleled with a similar increase in understanding of the biomarker profiles of the wound microenvironment or indeed the blood-based biomarker profile associated with different types of chronic wounds, and issues with study quality often limit the conclusions that can be drawn [15-17]. Unsurprisingly then, no biomarkers are routinely recommended in national clinical guidelines for wound care in the UK. The National Institute for Health and Care Excellence (NICE) guidance on wound management does not recommend any biomarkers for routine clinical use in wound assessment or treatment, though several biomarkers have been explored in research settings (**Table 1).** The specificity of current wound care biomarkers such as proteases and cytokines is unclear, and spatiotemporal changes to their levels during wound healing makes their deployment as simple biomarkers more difficult. This highlights the gap between biological insight and practical diagnostic tools. In the UK, the only biomarker for wounds which has been evaluated by NICE is the WoundCheck Protease status test [18]. However, according to NICE (2016), this has so far only been tested in one small (n=35) prospective study, and its value therefore remains unclear.

Table 1. Illustrative biomarkers in wound healing identified in the literature, derived from references [19-23]

Biomarker	Type	Clinical Relevance	Strengths	Limitations
C-Reactive Protein	Acute-phase protein	Indicates systemic inflammation; elevated in infected or non-healing wounds	Easily measurable; widely used in clinical settings	Non-specific; elevated in various inflammatory conditions
Interleukins (e.g. IL-6, IL-8)	Cytokine	Pro-inflammatory; elevated levels may indicate impaired healing or infection	Sensitive to changes in inflammatory status	Levels can fluctuate; not specific to wound healing
Tumor Necrosis Factor-alpha	Cytokine	Promotes inflammation; high levels associated with chronic wounds	Key mediator in inflammation; potential therapeutic target	Systemic effects; elevated in various diseases
Matrix Metalloproteinases	Enzymes	Involved in extracellular matrix remodelling; elevated in chronic wounds	Reflects tissue remodelling activity; potential target for therapy	Overexpression can impair healing; requires balance with inhibitors
Tissue inhibitor of matrix metalloproteinases	Protease inhibitors	Reduced levels of TIMPS may worsen the impact of raised MMPs	May be measured from wound fluid.	Must be interpreted alongside MMPs to be clinically meaningful.
Vascular Endothelial Growth Factor	Growth factor	Stimulates angiogenesis; crucial for tissue regeneration	Promotes blood vessel formation; therapeutic potential	Overexpression may lead to abnormal angiogenesis
Heparin-binding EGF-like Growth Factor	Growth factor	Enhances keratinocyte migration and proliferation; aids re-epithelialization	Potent mitogen; involved in multiple healing phases	Potential role in tumorigenesis; requires controlled expression
Copper Peptide	Peptide complex	Stimulates collagen synthesis; promotes wound contraction and angiogenesis	Enhances tissue regeneration; antioxidant properties	Limited clinical data; requires further research
Angiopoietin-like 4	Glycoprotein	Modulates vascular permeability; promotes keratinocyte migration	Involved in angiogenesis; potential therapeutic target	Complex role in metabolism and cancer; requires careful modulation

Early proteomics studies on rodent wound fluid sought to identify peptides that may promote wound repair or have antimicrobial properties [24, 25]. In addition, proteomics-based analyses of wound fluid have gained traction for biomarker discovery to understand or predict the healing trajectory of a wound, particularly in relation to proteases and cytokines, as well as small molecules and microbes [21, 23, 26-28]. RNA-based biomarkers, particularly cell-free RNAs (cfRNA), may offer greater molecular specificity and reflect dynamic gene regulation processes central to healing and chronicity. Their presence in accessible fluids such as wound exudate and plasma makes them promising candidates for non-invasive, prognostic tools capable of personalising wound care. However, the cell-free nucleic acid (cfNA) landscape of wound fluid has received limited attention whether in relation to DNA, messenger RNAs (mRNA), non-coding RNAs (ncRNA). Hence the potential of wound fluid-derived cfNAs as an alternative biomarker for monitoring and predicting outcomes for patients with open wounds remains obscure.

Cell-free nucleic acids , particularly cell-free DNA, have emerged as tractable analytes for testing a range of conditions including non-invasive prenatal testing, tumour profiling and tracking, transplant surveillance and pathogen detection in infectious diseases [29]. The potential to gain deeper molecular and mechanistic insight into the underlying disease process has also led to a surge of interest in the exploitation of cfRNA as biomarkers for cancer. Broadly, the study of cfRNA bifurcates into those that focus on mRNA and those that focus on non-coding RNAs (**Figure 1**).

For ncRNA, the potential of cfRNA profiling is enormous because at least four categories of well-defined ncRNAs have been established: microRNAs (miRNA), long non-coding RNAs (lncRNA), circular RNAs (circRNA) and transfer RNA fragments (tRF) (**Figure 1**). In many cases, the assessment of these ncRNAs as biomarkers has been linked to studies on small extracellular vesicles (sEVs; exosomes).

Within this article, we evaluate the potential of ncRNAs, alone or associated with sEVs, as biomarkers that can be marshalled to monitor and predict the trajectories and outcomes of chronic non-healing wounds.

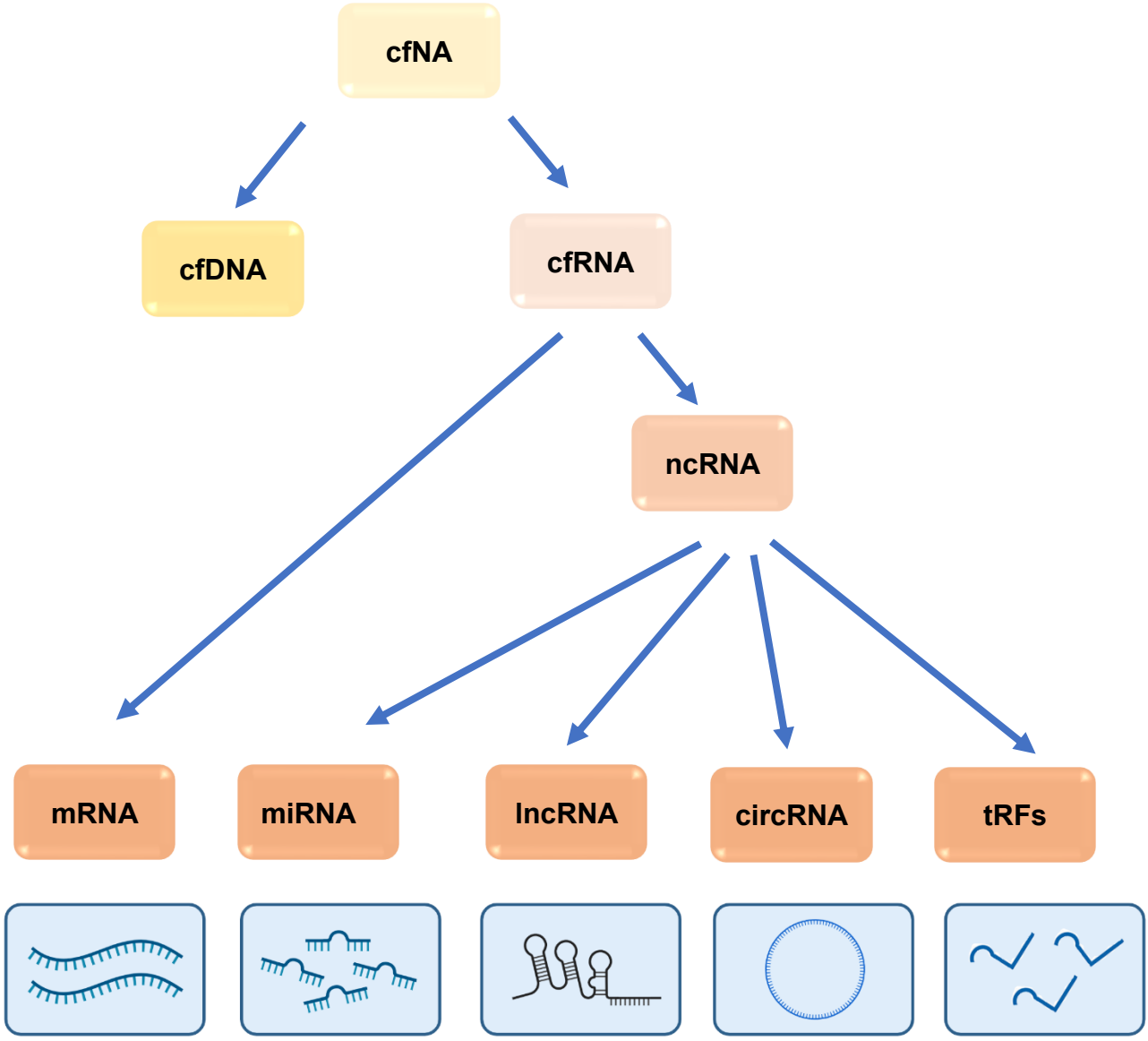

Figure 1: Cell-free nucleic acids (cfNA) amenable to analysis as biomarkers for wound repair. The cfNAs may be quantified in wound fluid, serum, saliva, urine and exosomes derived from these biofluids; ncRNA, non-coding RNA; mRNA, messenger RNA; miRNA, microRNA; lncRNA, long non-coding RNA, circRNA, circular RNA, tRF, tRNA fragments. Depictions of the RNAs were generated in BioRender.

Cell-free RNA

The presence of stable miRNAs in serum and plasma first highlighted the potential of miRNAs as a bloodborne biomarker that could be exploited for diagnostic or prognostic purposes in disease [30, 31]. These studies did not examine cfNAs in the context of bloodborne sEVs, which have since become a focus of much biomarker discovery work. Interestingly, more recent efforts have sought to uncover cell-free plasma mRNA biomarkers with diagnostic and prognostic potential for cancer [32-34], coronary disease [35], Alzheimer's disease [36, 37] and paediatric inflammatory syndromes such as bacterial/viral infection and Kawasaki disease [38]. Indeed, there is evidence that plasma cf-mRNAs may be enriched in cancer EVs [39]. However, the biomarker potential of cf-mRNA in plasma or plasma derived EVs from chronic wound patients remains under-explored, as the focus remains on miRNA, lncRNA and circRNA. These diverse RNA species may, together, offer exciting opportunities to uncover biomarker patterns that can support wound management, and we now appraise their potential in that regard.

MicroRNAs

MicroRNAs are endogenous small, non-coding RNA molecules, typically 22 nucleotides long, that regulate gene expression in a post-transcriptional manner by binding to the 3'-untranslated region (UTR) of target mRNAs [40]. This function of miRNA is intrinsically linked to their association with Argonaute proteins to form the RNA-induced silencing complex (RISC) which orchestrates the assembly of large multi-protein complexes that drive destabilisation, degradation or translation repression of target mRNAs [41].

Importantly, miRNAs play critical roles in numerous physiological and pathological processes, with a wide variety of roles in wound healing, modulating the expression of genes involved in proliferation, migration, differentiation, neovascularization and inflammation across multiple cell types as has been reviewed elsewhere by us and others [42-47]. These include miR-34a-5p and miR-34c-5p, which promote inflammation signalling in keratinocytes and impair wound closure in mouse wounds [48]; miR-132 which dampens keratinocyte inflammatory cytokine and chemokine production while elevating both keratinocyte proliferation and fibroblast migration [49, 50] and miR-129-5p and miR-335-5p which dampen the expression of matrix metalloproteinase 9 (MMP-9) [51]. In addition, miR-21-5p exerts pro-healing actions through an extensive network that includes anti-Inflammatory

regulation of nuclear factor kappa B (NF-κB) via programmed cell death 4 (PDCD4), promotion of keratinocyte migration by silencing of tissue inhibitor of metalloproteinases (TIMP-3) and regulation of fibroblast function by targeting sprouty homolog 2 (SPRY /2), as reviewed recently [52]. Notably, miR-21-5p is transferred from keratinocytes to myeloid cells via sEVs to shift the latter towards a pro-healing fibroblast-like phenotype that granulation tissue to support wound healing [53]. Further, multiple miRNAs regulate neovascularisation, with elevation of miR-221, miR-222, miR-92a, and miR-301a-3p inhibiting angiogenesis, while miR-296, miR-126, miR-378, and miR-210 promoted angiogenesis [47]. For more insight regarding the roles of miRNA in skin healing, the reader is referred to a recent review by Doghish and colleagues [54].

Cell-free MicroRNAs as Biomarkers for Chronic Wounds

Differential miRNAs expression in wound tissue from DFUs has received particular attention, as reviewed elsewhere [38], though studies defining the miRNA signatures of VLU has also been reported [55]. However, it remains unclear whether circulating miRNA can serve as prognostic biomarkers that would enable healthcare providers to predict the trajectory of wound healing and adjust treatment plans accordingly. Indeed, only a limited number of studies appear to have examined cf-miRNAs in wound fluid or blood from patients with DFU and other wounds, as summarised in **Table 2.**, These include seminal work by Ren and colleagues, which assessed miRNA in sEVs from wound fluid. Differential expression of 211 miRNAs, reporting 58 miRNAs that were elevated and 153 that were depleted in DFU-derived EVs compared to control EVs [56]. The study focused on miR-205-5p and miR-195-5p which they linked to the regulation of vascular endothelial growth factor A (VEGFA): transfer of the DFU-derived EVs into endothelial cells elevated the expression of these miRNAs and diminished VEGFA expression. Given the importance of VEGF, it appears feasible that these may have value as biomarkers. Levels of miR-205-5p might be carefully calibrated during wound repair, as both pro-migratory and anti-migratory effects have been reported in keratinocytes [57, 58] but whether the high levels of miR-205 in DFU EVs reflect elevated expression in structural cells of skin and blood vessels as opposed to EV released from infiltrating blood cells remains to be established. It might not matter in any case as putative transfer of miR-205-5p in a paracrine manner may contribute to impaired healing. The

important consideration from our perspective is whether monitoring miR-205-5p has prognostic value in predicting patient outcomes to standard of care. It should also be noted, however, that cf-miRNAs have not been formally validated in clinical contexts, whereas circulating miRNAs with biomarker potential have been established in clinical settings linked to cancer, ischemic stroke and myocardial infarction, as illustrated by several recent studies [59-61].

Table 2: Extracellular miRNAs and lncRNAs as potential biomarkers in DFU

Condition	Sample	RNA	Evaluated in EVs	Sample size	Ref.
DFU	Serum	miR-205-5p↑	Yes	21 patients 18 controls	[56]
DFU	Wound fluid	miR-195-5p↑ miR-205-5p↑	Yes	21 patients 14 DM controls 18 healthy controls	[56]
DFU	Plasma	let-7e-5p ↑ miR-17-5p ↑ miR-191-5p ↑ miR-33a-5p ↑	No	41 patients 50 controls	[62]
DFU	Plasma	miR-203a-3p↑	Plasma	64 patients 52 controls	[63]
DFU	Serum -derived exosomes	miR-15a-3p↑	Detected in both serum and serum-derived exosomes	10 DFU patients 10 patients with non-diabetic foot wounds	[64]
DFU	Serum	lncRNA A1BG-AS1↑	No	77 DFU patients 85 T2DM patients 75 healthy controls	[65]
DFU	Serum	lncRNA DLEU1↑	No	71 DFU patients 71 Healthy controls	[66]

EV, Extracellular vesicle
lncRNA, long non-coding RNA
DLEU1, Deleted in Lymphocytic Leukaemia 1
DFU, diabetic foot ulcer
DM, diabetes mellitus
T2DM, type 2 diabetes mellitus patients

Long non-coding RNAs

Long non-coding RNAs are RNA transcripts that have traditionally been defined as consisting of at least 200 nucleotides but having little or no protein-coding capacity. While the 200 nt lower limit for lncRNA size has served as a useful cut-off to date, a recent Consensus Statement has sought to re-define lncRNAs as >500 nt [67]. This is to distinguish lncRNA more clearly from certain ncRNAs that are over 200 nt long but are not classical lncRNAs. The 60-300 nt long small nucleolar RNAs involved in ribosomal RNA modification and the small nuclear RNAs that execute pre-mRNA splicing, which are typically ~150 nt long but can be 60-450 nt long, fall into this category of intermediate RNAs [68, 69].

Although a few notable lncRNAs such as X-inactive specific and H19 had been discovered early in relation to developmental processes [70, 71], it was the characterization of large-scale mouse, human and fly transcriptome datasets that established the pervasive nature of lncRNA transcripts [72-75]. The recent NONCODEV6 study estimates the human genome has just over 173,00 lncRNAs, but the number of functional human lncRNAs seems to be 20,000-60,000 [76, 77].

Combined with their genomic and structural diversity and low sequence conservation, the relatively large number of lncRNAs has made the validation and functional annotation of lncRNAs challenging but they have been implicated in mRNA decay, structural scaffolding, chromatin remodeling, epigenetic regulation, transcriptional and post-transcriptional regulation, RNA splicing and editing and in development [78, 79]. Competitive endogenous RNA (ceRNA) binding represents another framework for understanding lncRNA function, and involves sequestration of endogenous miRNA by lncRNA [36,37]. Thus, the lncRNA competes with mRNA targets to capture miRNAs, thereby limiting the effective concentration of target miRNA that is available to mediate repression [36]. It should be noted that a significant proportion of so-called lncRNAs are now known to encode peptides via short open reading frames [80, 81]. These micropeptides, usually less than 100 amino acids long, have been implicated in a range of functions associated with protein phosphorylation, mRNA modulation and interactions with proteins associated with subcellular organelle membranes [82]. The *TINCR* (Terminal differentiation-Induced Non-Coding RNA) gene is one such lncRNA that is now known to encode an 87-amino acid long peptide that has been implicated in promoting keratinocyte proliferation to support wound healing [83]. However, this appears to be the exception among several lncRNAs implicated in wound repair, to which we now turn.

LncRNA and wound healing

Multiple lncRNAs have been implicated in wound healing as well as other aspects of skin biology, including keratinocyte differentiation, melanocyte behaviour and hair growth [84, 85]. From the epidermal perspective, such lncRNAs include wound and keratinocyte migration-associated lncRNAs (WAKMAR1 and WAKMAR2) [86, 87]; TET2-interacting long noncoding RNA, which contributes to disrupted ECM homeostasis by promoting expression of MMP-9 promoter [88] and lncRNA SNHG26 which shifts keratinocyte progenitor cells from the inflammatory to the proliferative state during wound healing [89]. Interestingly, keratinocyte sEVs delivered the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) into macrophages, shifting them towards a pro-healing phenotype [90]. In fibroblasts, lncRNA cancer susceptibility candidate 2 (CASC2) levels were lower in wound tissues of DFU patients and CASC2 overexpression promoted fibroblast migration and proliferation and reduced their apoptosis [91]. On the other hand, lncRNA growth arrest-specific transcript 5 was elevated in diabetic wounds and drove macrophages towards a pro-inflammatory M1 phenotype [92]. The roles of these and other lncRNAs in relation to inflammation, angiogenesis and ECM turnover have been reviewed elsewhere recently [93]. Here, we first focus on lncRNAs associated with wound healing where there is also evidence of loading into EVs (H19, HOTAIR, NEAT1) then highlight two lncRNAs (A1BG-AS1 and DLEU1) identified as candidate biomarkers in DFU patient sera.

LncRNA-H19

Long noncoding RNA H19 has been established as a key regulator of programmed cell death and autophagy [94]. Recent evidence has emerged to suggest that H19 is downregulated in human mouse diabetic skin and elevated in exosomes from human hair follicle mesenchymal stem cells [95, 96]. Functionally, exosomal H19 appeared to promote wound repair promoting fibroblast proliferation through anti-inflammatory mechanisms that involved abrogation of pyroptosis, a form of programmed cell death mediated by the gasdermin family of pore-forming proteins [95-97]. This putative ability of H19 to dampen pyroptosis was associated with improved healing of diabetic mouse skin wounds through better re-epithelialisation and neovascularisation, and studies on HaCaT epidermal keratinocytes showcased suppression of the NLRP3, caspase-1, IL-β, and IL-18 axes of inflammation. However, it is not clear at present

if H19 levels are altered in exosomes from wound fluid, serum or plasma of patients with DFU or other chronic wounds. .

HOTAIR

HOTAIR (HOX Antisense Intergenic RNA) is a 2158 nucleotide lncRNA that was first identified in human fibroblasts following transcriptional profiling of the four HOX chromosomal loci present [98]. Mechanistically, HOTAIR was found to promote transcriptional silencing of chromosomal domains by Polycomb Repressive Complex 2-dependent H3K27 methylation [98]. Since then, multiple other roles have been established for HOTAIR, including serving as a ceRNA to sequester miRNAs as well as post-translational functions including ubiquitination and subsequent degradation of protein targets, as reviewed in [99]. In relation to wound healing, ethanol treatment was shown to boost the vascularization bioactivity of endothelial cell-derived EVs through mechanisms that included elevation of HOTAIR and MALAT1 within the EV cargo and downregulation of the anti-angiogenic miRNA miR-106b [100]. Additional evidence from the same group showed that HOTAIR overexpression in mesenchymal stem cells yielded EVs with raised HOTAIR levels to support wound healing in diabetic mice via increased angiogenesis [101]. HOTAIR expression increases after a burn injury in mouse skin and intradermal injections of HOTAIR-overexpressing epidermal stem cells promoted re-epithelialization and wound closure [102].

There is evidence in some contexts (laryngeal squamous cell cancer, acute myeloid leukaemia and liver fibrosis) that HOTAIR promotes methylation of phosphatase and TENsin homolog deleted on chromosome 10 (*PTEN)* by marshalling DNA Methyltransferase 3 beta expression [103-105]. The levels of such HOTAIR-dependent methylation of *PTEN* during wound healing have not been established to our knowledge but growing evidence links miRNA-mediated downregulation of PTEN to wound repair mechanisms [106-108].

Early studies considered the potential of serum-derived exosomal HOTAIR, in combination with exosomal miRNA-21, as candidate diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma [109]. Further investigations revealed HOTAIR elevation in urinary exosomes in bladder cancer [110], serum-derived EVs from lung cancer patients [111], colorectal cancer cell-derived exosomes [112] and in serum-derived exosomes from breast cancer patients, where HOTAIR levels appeared to have diagnostic and prognostic potential [113]. Together, these studies highlight the potential of HOTAIR as a biomarker

amenable to detection in liquid biopsies. However, the extent to which exosomal HOTAIR levels vary in serum or wound fluid from patients with different types of complex wounds compared to those whose wounds heal within a relatively short time frame has not been established.

Nuclear enriched abundant transcript

Nuclear enriched abundant transcript 1 (NEAT1) lncRNAs include, a short isoform of 3.7 kb (originally termed MENε, now known as NEAT1_1) and a large 23 kb which was initially known as MEN β but is now called NEAT1_2 [114, 115]. This longer NEAT1_1 isoform has been established as a central orchestrator of the assembly of paraspeckles, membraneless organelles implicated in cancer, stress responses and developmental processes, as reviewed in [116]. Elevation of NEAT1 has been implicated in multiple cancers, which mechanisms of pathogenesis often associated with ceRNA effects of NEAT that lead to sequestration of miRNA targets and subsequent elevation of various transcription factors and enzymes associated with oncogenesis [117, 118].

There is is evidence linking NEAT1 to angiogenesis as it downregulated in chronic DFU compared to acute DFU, liberating miR-146 which in turn represses matG, an important angiogenic transcription factor [119]. Depletion of NEAT1 was associated with impaired endothelial cell migration and network formation. Notably, exosomal NEAT1 has been reported in some contexts including serum-derived exosomes of rheumatoid arthritis patients [120], cancer-associated fibroblasts [121] and endothelial cells under oxidative stress [122]. However, NEAT1 has not been associated with exosomes from chronic wounds to our knowledge.

LncRNA A1BG-AS1

A recent survey of lncRNA-mRNA coexpression network analysis in peripheral blood monocytes identified 12 lncRNAs associated with inflammation in type 2 diabetes mellitus (T2DM) peripheral blood monocytes [123]. In a subsequent study comparing serum levels of lncRNA A1BG antisense RNA 1 (A1BG-AS1) in DFU, T2DM and control subjects (n = 77, 85 and 75, respectively), A1BG-AS1 was modestly (about 2-fold) upregulated in patients with DFUs [65]. Close analysis revealed a correlation with fasting blood glucose, glycated hemoglobin

and Wagner grade scores when segregated into high versus low A1BG-AS1 serum levels [65]. Both univariate and multivariate analysis suggested A1BG-AS1 levels may have potential as a biomarker for predicting the risk of DFU in T2DM patients and quantifying severity of DFU [65]. High glucose was shown to raise A1BG-AS1 expression in human dermal fibroblasts and reduce miR-214 levels, but it remains to be seen whether this translates into A1BG-AS1-mediated effects of autocrine exosomal miR-214-3p-depenent angiogenesis reported early on by Verhaar and colleagues [124]. However, longitudinal studies are required to validate the potential utility of monitoring serum A1BG-AS1 levels in DFU patients.

LncRNA DLEU1

Deleted in Lymphocytic Leukaemia 1 (DLEU1) has been established as a cancer-associated lncRNA that is upregulated in various tumours [125]. Analysis of sera from 71 DFU patients and matched controls revealed a modest 1.5-fold increase in lncRNA DLEU1 expression in the DFU cohort. Unlike the above-mentioned study of lncRNA A1BG-AS1, the study on serum DLEU1 did not present deep analysis based on low *versus* high levels of lncRNA DLEU1 [66]. Nonetheless, there was some suggestion that serum lncRNA DLEU1 had biomarker potential for monitoring DFU. Functionally, lncRNA DLEU1 appeared to have an anti-angiogenic function based on studies of cultured endothelial cells but the impact of DLEU1 on wound-related angiogenesis *in vivo* has yet to be determined.

Notably, none of the above studies examined lncRNA in plasma or serum-derived sEV or in wound exudates. Hence there is likely to be more scope to uncover lncRNA biomarkers with prognostic value in managing chronic wound patients. On the other hand, circular RNAs may also be promising as chronic wound biomarkers, and we now consider their potential in that regard.

Circular RNAs: A Brief Background

Circular RNAs are ncRNAs characterized by their covalently closed-loop structure and absence of 5' cap and poly(A) tail typically found in linear RNAs [126]. Interest in circular RNAs has grown tremendously over the last decade but their discovery goes back to the 1970s, with the first report of circular RNA viroids that infect plant hosts by Kleinschmidt and colleagues [127] followed by observation of circular RNAs in Hela cells by Hsu and Coca-Prados [128]. For

an elegant summary of the history of circRNAs, the reader is referred to a review by Kadener and colleagues [129]. The studies that moved circRNA from relative obscurity into the limelight emerged in 2013, when deep sequencing revealed their prevalence across human and other animal transcriptomes [130, 131] and their roles as competing endogenous RNAs that sequester miRNAs and thus reduce their availability to interact with target mRNA transcripts were defined [132, 133]. Interestingly, although primarily defined as non-coding RNA, evidence has emerged to show circRNA can be translated into protein [134-136].

Most circRNAs are derived from direct "back-splicing" of pre-mRNA exons though several other circRNAs have been defined based on the mechanism of biogenesis [137]. In any case, the circular structure of circRNAs renders them resistant to exonucleases, making them more stable than their linear counterparts. As a result, although their abundance tends to be low, they have emerged as promising biomarkers of disease, with high specificity and sensitivity [138, 139]. Further, a recent study of over 1000 human plasma samples, along with urine, bile and cerebrospinal fluid samples, revealed that circRNAs appeared to be preferentially sorted into EVs compared to linear RNAs, enabling functional enrichment [140]. Importantly, as with miRNA and lncRNA, circRNAs are also loaded into exosomes and have thus gained traction as novel biomarkers for cancer and other diseases [141-144].

Circular RNAs: Emerging Roles in Cutaneous Wound Healing

Recent studies have implicated circRNAs in both keratinocyte and fibroblast functions during wound repair [145-150]. Work from the Landén group on DFU [145] lay much of the foundation for understanding circRNA expression in chronic wounds. Wang, Landén and colleagues found that the expression of hsa_circ_0084443 (now known as circ_PRKDC) was reduced in normal wounds compared with intact skin, but expression of circ_PRKDC in DFUs was higher than in normal wounds [145]. Elevated circPRKDK may impair DFU by reducing keratinocyte migration via mechanisms in which circ_PRKDC sequesters miR-17-3p and miR-31, in turn modulating the activity of multiple pathways [145-147]. In an unrelated study that also used the Wang dataset as the starting point, Xiong and colleagues also found that a circRNA, circRNA-080968 was upregulated in DFU tissues compared to that of non-DFU wounds and its overexpression impaired keratinocyte migration [151]. Another circRNA implicated in wound repair is circCDK13, which was identified by analysis of the Wang dataset [145] followed by delineation of circRNAs depleted upon exposure of keratinocytes and fibroblasts to the advanced glycation end product-bovine serum albumin, to mimic the DFU environment [148]. CircCDK13 promoted the migration and proliferation of keratinocytes and

fibroblasts [148]. Notably, circCDK13 harboured N^6-methyladenosine modifications that facilitated interactions with insulin-like growth factor 2 mRNA-binding protein 3, an important RNA-binding protein [148]. In addition, Landen and colleagues define the circRNA network in VLUs and uncovered hsa-CHST15_0003 and hsa-TNFRSF21_0001 as upregulated circRNAs that appear to impair keratinocyte migration but boost proliferation, perhaps contributing to the pathologic hyperproliferation and impaired differentiation of keratinocytes at the wound edge in VLUs [152]. Interestingly, by segregating keratinocytes and fibroblasts prior to RNA sequencing, the Landén group also recently identified a bifunctional circRNA, CircGLIS3(2) that supports fibroblasts during wound repair [149]. The CircGLIS3(2) RNA stimulates ECM production while the 131-amino-acid protein encoded by CircGLIS3(2) enhances fibroblast proliferation via interactions with the transcription factor basic transcription Factor 3 [149]. Together, these studies showcase the importance of circRNAs in wound healing and raise the prospect of their being exploited as biomarkers in chronic wounds (Table 3).

Nevertheless, much remains to be done to establish the prognostic biomarker potential of circRNA in chronic wound exudates and in longitudinal contexts of healing versus non-healing wounds. For instance, there is some evidence that hsa_circ_0000907 and hsa_circ_0057362 in serum and serum-derived sEV may serve as potential biomarkers for early DFU diagnosis [153]. However, it is not clear if these circRNAs have prognostic value in predicting wound healing trajectories. Further, Bindereif and colleagues showed that circRNAs were associated with platelet-derived EVs [93]. It will therefore be interesting to uncover the circRNA landscape in platelet EVs at different stages of healing and non-healing wounds.

Conclusion

Are the most promising prospective cfRNA biomarkers to be found in wound fluid or in bloodborne EVs of serum and plasma? Can even less invasive liquid biopsies like saliva and urine provide cfRNA markers relevant to chronic wound management? In much the same way that wound fluid has been studied extensively to identify proteomic markers with prognostic value, attention should now be turned to defining the full RNA signatures of wound fluid and wound fluid-derived exosomes from a range of patients with diverse types of chronic non-healing wounds. Crucially, it will be important to compare healing and non-healing wounds to establish correlations between cfRNA expression and healing times in order to maximise the clinical relevance of such biomarker discovery programmes.

Critically, cfRNA alone may not suffice to achieve the high sensitivity and specificity crucial to ensure accurate identification of wound prognosis without false positives or false negatives. Hence, they will need to be integrated with other biomarkers, such as DFU

signatures from serum and plasma proteomics datasets [154-156]. Notably, a longitudinal study from Jozic and coworkers recently contrasted the proteomic profiles of chronic wounds using discarded wound dressings as a source of cells, EVs and soluble proteins and identified protein signatures that segregated healing from non-healing wounds [157]. Further, Veves and colleagues used a machine learning approach to identify serum proteins that delineated fast healing DFUs from slow healing ones [158]. Separately, metabolite profiling recently identified 402 small molecule metabolites in DFU exudates, though their predictive capabilities remain to be established [159]. Going forward, it will be interesting to exploit integrative approaches to connect cfRNA, protein and metabolite signatures to pathophysiological processes associated with chronic wounds datasets from the Veves and Landen laboratories [55, 152, 160, 161]. Such multi-omics integration promises to yield ever deeper insight into the molecular networks associated with chronic wounds. for sensitive biomolecular detection in clinical diagnostics.

Challenges remain in terms of standardising protocols for sample acquisition to support cfRNA and other biomarker profile studies, as has been outlined recently in relation to circulating miRNAs for cancer [162]. Nevertheless, there are exciting prospects for exploiting emerging biosensor technologies to real-world monitoring of cfRNAs in serum or exudates of chronic wound patients. These include a quantum-dot based triple sensor to detect lncRNA, miRNA and mRNA [163] and an upconversion nanoparticle-based lateral flow assay that was optimised for miR-21 detection [164]. Convergence of these and related technologies with machine learning algorithms [165] over the next few years will help shift cfRNA from the laboratory bench to clinical practice to improve patient outcomes.

Acknowledgments

RR was funded by a Liverpool John Moores University Institute for Health Research internship. JH is funded by the NIHR Manchester Biomedical Research Centre (NIHR203308). We thank Gemma Farrington for help with artwork. We would also like to thank the anonymous reviewers of the initial manuscript for comments that helped to significantly improve the work.

Table 3: Circular RNAs as potential biomarkers in wound healing

Condition	Sample	RNA	Sample size for validation	Ref.
DFU	Skin	hsa_circ_0084443* ↑ in DFU compared to NW (115 ↑ and 111 ↓ circRNAs in DFU *vs.* NWs)	19 DFU patients 8 healthy controls	[145]
DFU	Serum and Serum derived exosomes	hsa_ circ_0000907 ↑ hsa_circ_0057362 ↑ in DFU compared to both controls and non-DFU	65 DFU patients 65 non-DFU DM patients 70 healthy controls	[153]
DFU	Skin	hsa_circRNA_072697 ↑ (25 ↑ and 40 ↓ circRNAs in DFU *vs.* NWs)	9 DFU patients 8 healthy controls	[166]
DFU	Skin	circRNA-80968↑ circRNA-081069↑ circRNA-100980↑ (515 ↑ and 615 ↓ circRNAs in DFU *vs.* NW)	37 DFU patients 16 non-DFU DM patients 18 healthy controls	[151]
VLU	Skin	hsa-TNFRSF21_0001 hsa-CHST15_0003	5 VLU patients 5 healthy controls	[152]

*Now known as circ_PRKDC
DFU, diabetic foot ulcer
VLU, venous leg ulcer
NW, normal wound
circRNA, circular RNA

REFERENCES

1 Sen, C. K. (2023) Human Wound and Its Burden: Updated 2022 Compendium of Estimates. Adv Wound Care (New Rochelle). **12**, 657–670. https://doi.org/10.1089/wound.2023.0150

2 McCaughan, D., Sheard, L., Cullum, N., Dumville, J. and Chetter, I. (2018) Patients' perceptions and experiences of living with a surgical wound healing by secondary intention: A qualitative study. Int J Nurs Stud. **77**, 29–38. https://doi.org/10.1016/j.ijnurstu.2017.09.015

3 Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., et al. (2009) Human skin wounds: a major and snowballing threat to public health and the economy. Wound repair and regeneration. **17**, 763–771. https://doi.org/10.1111/j.1524-475X.2009.00543.x

4 Guest, J. F., Fuller, G. W. and Vowden, P. (2020) Cohort study evaluating the burden of wounds to the UK's National Health Service in 2017/2018: update from 2012/2013. BMJ Open. **10**, e045253. https://doi.org/10.1136/bmjopen-2020-045253

5 Diaz-Herrera, M. A., Gonzalez-Duran, M., Rodriguez-Martinez, F. J., Tujillo-Flores, G., Tuset-Mateu, N., Verdu-Soriano, J., et al. (2025) The financial burden of chronic wounds in primary care: A real-world data analysis on cost and prevalence. Int J Nurs Stud Adv. **8**, 100313. https://doi.org/10.1016/j.ijnsa.2025.100313

6 Olsson, M., Jarbrink, K., Divakar, U., Bajpai, R., Upton, Z., Schmidtchen, A. and Car, J. (2019) The humanistic and economic burden of chronic wounds: A systematic review. Wound repair and regeneration. **27**, 114–125. https://doi.org/10.1111/wrr.12683

7 Armstrong, D. G., Wrobel, J. and Robbins, J. M. (2007) Guest Editorial: are diabetes-related wounds and amputations worse than cancer? Int Wound J. **4**, 286–287. https://doi.org/10.1111/j.1742-481X.2007.00392.x

8 Qiu, R. and Johal, H. (2022) Cochrane in CORR(R): Negative Pressure Wound Therapy for Surgical Wounds Healing by Primary Closure. Clin Orthop Relat Res. **480**, 1646–1652. https://doi.org/10.1097/CORR.0000000000002313

9 de Moraes Silva, M. A., Nelson, A., Bell-Syer, S. E., Jesus-Silva, S. G. and Miranda, F., Jr. (2024) Compression for preventing recurrence of venous ulcers. Cochrane Database Syst Rev. **3**, CD002303. https://doi.org/10.1002/14651858.CD002303.pub4

10 Rodrigues, M., Kosaric, N., Bonham, C. A. and Gurtner, G. C. (2019) Wound Healing: A Cellular Perspective. Physiol Rev. **99**, 665–706. https://doi.org/10.1152/physrev.00067.2017

11 Pena, O. A. and Martin, P. (2024) Cellular and molecular mechanisms of skin wound healing. Nat Rev Mol Cell Biol. **25**, 599–616. https://doi.org/10.1038/s41580-024-00715-1

12 Frykberg, R. G. and Banks, J. (2015) Challenges in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle). **4**, 560–582. https://doi.org/10.1089/wound.2015.0635

13 Mayorca-Guiliani, A. E., Leeming, D. J., Henriksen, K., Mortensen, J. H., Nielsen, S. H., Anstee, Q. M., et al. (2025) ECM formation and degradation during fibrosis, repair, and regeneration. NPJ Metab Health Dis. **3**, 25. https://doi.org/10.1038/s44324-025-00063-4

14 Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., et al. (2014) Epithelialization in Wound Healing: A Comprehensive Review. Adv Wound Care (New Rochelle). **3**, 445–464. https://doi.org/10.1089/wound.2013.0473

15 Falanga, V., Isseroff, R. R., Soulika, A. M., Romanelli, M., Margolis, D., Kapp, S., et al. (2022) Chronic wounds. Nat Rev Dis Primers. **8**, 50. https://doi.org/10.1038/s41572-022-00377-3

16 Rembe, J. D., Garabet, W., Augustin, M., Dissemond, J., Ibing, W., Schelzig, H. and Stuermer, E. K. (2025) Immunomarker profiling in human chronic wound swabs reveals IL-1 beta/IL-1RA and CXCL8/CXCL10 ratios as potential biomarkers for wound healing, infection status and regenerative stage. J Transl Med. **23**, 407. https://doi.org/10.1186/s12967-025-06417-2

17 Westby, M. J., Dumville, J. C., Stubbs, N., Norman, G., Wong, J. K., Cullum, N. and Riley, R. D. (2018) Protease activity as a prognostic factor for wound healing in venous leg ulcers. Cochrane Database Syst Rev. **9**, CD012841. https://doi.org/10.1002/14651858.CD012841.pub2

18 National Institute for Health and Care Excellence. (2016) Woundchek Protease Status for assessing elevated protease status in chronic wounds. In Medtech innovation briefing. MIB83 ed.)^eds.)

19 Goh, Y. Y., Pal, M., Chong, H. C., Zhu, P., Tan, M. J., Punugu, L., et al. (2010) Angiopoietin-like 4 interacts with matrix proteins to modulate wound healing. J Biol Chem. **285**, 32999–33009. https://doi.org/10.1074/jbc.M110.108175

20 Goto, T. and Saligan, L. N. (2020) Wound Pain and Wound Healing Biomarkers From Wound Exudate: A Scoping Review. J Wound Ostomy Continence Nurs. **47**, 559–568. https://doi.org/10.1097/WON.0000000000000703

21 Patel, S., Maheshwari, A. and Chandra, A. (2016) Biomarkers for wound healing and their evaluation. J Wound Care. **25**, 46–55. https://doi.org/10.12968/jowc.2016.25.1.46

22 Pickart, L., Vasquez-Soltero, J. M. and Margolina, A. (2015) GHK Peptide as a Natural Modulator of Multiple Cellular Pathways in Skin Regeneration. Biomed Research International. **2015**. https://doi.org/Artn 648108
10.1155/2015/648108

23 Lindley, L. E., Stojadinovic, O., Pastar, I. and Tomic-Canic, M. (2016) Biology and Biomarkers for Wound Healing. Plast Reconstr Surg. **138**, 18S–28S. https://doi.org/10.1097/PRS.0000000000002682

24 Hartman, E., Wallblom, K., van der Plas, M. J. A., Petrlova, J., Cai, J., Saleh, K., et al. (2020) Bioinformatic Analysis of the Wound Peptidome Reveals Potential Biomarkers and Antimicrobial Peptides. Front Immunol. **11**, 620707. https://doi.org/10.3389/fimmu.2020.620707

25 Huang, C. M., Wang, C. C., Barnes, S. and Elmets, C. A. (2006) In vivo detection of secreted proteins from wounded skin using capillary ultrafiltration probes and mass spectrometric proteomics. Proteomics. **6**, 5805–5814. https://doi.org/10.1002/pmic.200600163

26 Mota, F. A. R., Pereira, S. A. P., Araújo, A. R. T. S., Passos, M. L. C. and Saraiva, M. L. M. F. S. (2021) Biomarkers in the diagnosis of wounds infection: An analytical perspective. Trac-Trend Anal Chem. **143**. https://doi.org/ARTN 116405
10.1016/j.trac.2021.116405

27 Harvey, J., Mellody, K. T., Cullum, N., Watson, R. E. B. and Dumville, J. (2022) Wound fluid sampling methods for proteomic studies: A scoping review. Wound repair and regeneration. **30**, 317–333. https://doi.org/10.1111/wrr.13009

28 Longfield, M. S., Lourdesamy, J., Min, D. and Twigg, S. M. (2023) Do diabetes-related foot ulcer wound fluid measures have clinical utility as biomarkers for healing? A systematic review. J Wound Care. **32**, xlvii–lxii. https://doi.org/10.12968/jowc.2023.32.Sup4a.xlvii

29 Loy, C., Ahmann, L., De Vlaminck, I. and Gu, W. (2024) Liquid Biopsy Based on Cell-Free DNA and RNA. Annual review of biomedical engineering. **26**, 169–195. https://doi.org/10.1146/annurev-bioeng-110222-111259
30 Chen, X., Ba, Y., Ma, L., Cai, X., Yin, Y., Wang, K., et al. (2008) Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. **18**, 997–1006. https://doi.org/cr2008282 [pii]
10.1038/cr.2008.282
31 Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. **105**, 10513–10518. https://doi.org/0804549105 [pii]
10.1073/pnas.0804549105
32 Larson, M. H., Pan, W., Kim, H. J., Mauntz, R. E., Stuart, S. M., Pimentel, M., et al. (2021) A comprehensive characterization of the cell-free transcriptome reveals tissue- and subtype-specific biomarkers for cancer detection. Nature communications. **12**, 2357. https://doi.org/10.1038/s41467-021-22444-1
33 Roskams-Hieter, B., Kim, H. J., Anur, P., Wagner, J. T., Callahan, R., Spiliotopoulos, E., et al. (2022) Plasma cell-free RNA profiling distinguishes cancers from pre-malignant conditions in solid and hematologic malignancies. NPJ Precis Oncol. **6**, 28. https://doi.org/10.1038/s41698-022-00270-y
34 Albrecht, L. J., Howner, A., Griewank, K., Lueong, S. S., von Neuhoff, N., Horn, P. A., et al. (2022) Circulating cell-free messenger RNA enables non-invasive pan-tumour monitoring of melanoma therapy independent of the mutational genotype. Clin Transl Med. **12**, e1090. https://doi.org/10.1002/ctm2.1090
35 Akat, K. M., Lee, Y. A., Hurley, A., Morozov, P., Max, K. E., Brown, M., et al. (2019) Detection of circulating extracellular mRNAs by modified small-RNA-sequencing analysis. JCI Insight. **5**. https://doi.org/10.1172/jci.insight.127317
36 Cisterna-Garcia, A., Beric, A., Ali, M., Pardo, J. A., Chen, H. H., Fernandez, M. V., et al. (2023) Cell-free RNA signatures predict Alzheimer's disease. iScience. **26**, 108534. https://doi.org/10.1016/j.isci.2023.108534
37 Toden, S., Zhuang, J., Acosta, A. D., Karns, A. P., Salathia, N. S., Brewer, J. B., et al. (2020) Noninvasive characterization of Alzheimer's disease by circulating, cell-free messenger RNA next-generation sequencing. Sci Adv. **6**. https://doi.org/10.1126/sciadv.abb1654
38 Wang, L., Wang, C., Huang, C., Zhou, Z., Yang, R., Huang, Y., et al. (2024) Role of microRNAs in diabetic foot ulcers: Mechanisms and possible interventions. Diabetes research and clinical practice. **217**, 111858. https://doi.org/10.1016/j.diabres.2024.111858
39 Kim, H. J., Rames, M. J., Goncalves, F., Kirschbaum, C. W., Roskams-Hieter, B., Spiliotopoulos, E., et al. (2023) Selective enrichment of plasma cell-free messenger RNA in cancer-associated extracellular vesicles. Commun Biol. **6**, 885. https://doi.org/10.1038/s42003-023-05232-z
40 Bartel, D. P. (2018) Metazoan MicroRNAs. Cell. **173**, 20–51. https://doi.org/10.1016/j.cell.2018.03.006
41 Iwakawa, H. O. and Tomari, Y. (2022) Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. Mol Cell. **82**, 30–43. https://doi.org/10.1016/j.molcel.2021.11.026

42 Fawcett, S., Al Kassas, R., I, M. D., Hughes, A. T., Ghali, F. and Ross, K. (2022) A time to heal: microRNA and circadian dynamics in cutaneous wound repair. Clin Sci (Lond). **136**, 579–597. https://doi.org/10.1042/CS20220011
43 Ross, K. (2021) MiR equal than others: MicroRNA enhancement for cutaneous wound healing. J Cell Physiol. **236**, 8050–8059. https://doi.org/10.1002/jcp.30485
44 Dykes, I. M. and Ross, K. (2021) Restoring the Final Frontier: Exosomal MicroRNA and Cutaneous Wound Repair. Biomolecular Research Reports. **1**, 1–15. https://doi.org/10.24377/BRR601
45 Bibby, G., Krasniqi, B., Reddy, I., Sekar, D. and Ross, K. (2021) Capturing the RNA Castle: Exploiting MicroRNA Inhibition for Wound Healing. FEBS J. https://doi.org/10.1111/febs.16160
46 Gowtham, A. and Kaundal, R. K. (2025) Exploring the ncRNA landscape in exosomes: Insights into wound healing mechanisms and therapeutic applications. International journal of biological macromolecules. **292**, 139206. https://doi.org/10.1016/j.ijbiomac.2024.139206
47 de Carvalho, M. R., Yang, H., Stechmiller, J. and Lyon, D. E. (2025) MicroRNA Expression in Chronic Venous Leg Ulcers and Implications for Wound Healing: A Scoping Review. Biol Res Nurs. **27**, 339–351. https://doi.org/10.1177/10998004241291062
48 Wu, J., Li, X., Li, D., Ren, X., Li, Y., Herter, E. K., et al. (2020) MicroRNA-34 Family Enhances Wound Inflammation by Targeting LGR4. J Invest Dermatol. **140**, 465–476 e411. https://doi.org/10.1016/j.jid.2019.07.694
49 Li, D., Wang, A., Liu, X., Meisgen, F., Grunler, J., Botusan, I. R., et al. (2015) MicroRNA-132 enhances transition from inflammation to proliferation during wound healing. J Clin Invest. **125**, 3008–3026. https://doi.org/10.1172/JCI79052
50 Li, X., Li, D., Wikstrom, J. D., Pivarcsi, A., Sonkoly, E., Stahle, M. and Landen, N. X. (2017) MicroRNA-132 promotes fibroblast migration via regulating RAS p21 protein activator 1 in skin wound healing. Scientific reports. **7**, 7797. https://doi.org/10.1038/s41598-017-07513-0
51 Wang, W., Yang, C., Wang, X. Y., Zhou, L. Y., Lao, G. J., Liu, D., et al. (2018) MicroRNA-129 and -335 Promote Diabetic Wound Healing by Inhibiting Sp1-Mediated MMP-9 Expression. Diabetes. **67**, 1627–1638. https://doi.org/10.2337/db17-1238
52 Xie, J., Wu, W., Zheng, L., Lin, X., Tai, Y., Wang, Y. and Wang, L. (2022) Roles of MicroRNA-21 in Skin Wound Healing: A Comprehensive Review. Front Pharmacol. **13**, 828627. https://doi.org/10.3389/fphar.2022.828627
53 Sinha, M., Sen, C. K., Singh, K., Das, A., Ghatak, S., Rhea, B., et al. (2018) Direct conversion of injury-site myeloid cells to fibroblast-like cells of granulation tissue. Nature communications. **9**, 936. https://doi.org/10.1038/s41467-018-03208-w
54 Mansour, R. M., Mageed, S. S. A., Awad, F. A., Sadek, M. M., Adel, S. A., Ashraf, A., et al. (2025) miRNAs and their multifaceted role in cutaneous wound healing. Funct Integr Genomics. **25**, 33. https://doi.org/10.1007/s10142-025-01535-y
55 Liu, Z., Zhang, L., Toma, M. A., Li, D., Bian, X., Pastar, I., et al. (2022) Integrative small and long RNA omics analysis of human healing and nonhealing wounds discovers cooperating microRNAs as therapeutic targets. Elife. **11**. https://doi.org/10.7554/eLife.80322
56 Liu, J., Wang, J., Fu, W., Wang, X., Chen, H., Wu, X., et al. (2021) MiR-195-5p and miR-205-5p in extracellular vesicles isolated from diabetic foot ulcer wound fluid decrease angiogenesis by inhibiting VEGFA expression. Aging (Albany NY). **13**, 19805–19821. https://doi.org/10.18632/aging.203393

57 Yu, J., Peng, H., Ruan, Q., Fatima, A., Getsios, S. and Lavker, R. M. (2010) MicroRNA-205 promotes keratinocyte migration via the lipid phosphatase SHIP2. FASEB J. **24**, 3950–3959. https://doi.org/10.1096/fj.10-157404
58 Wang, T., Zhao, N., Long, S., Ge, L., Wang, A., Sun, H., et al. (2016) Downregulation of miR-205 in migrating epithelial tongue facilitates skin wound re-epithelialization by derepressing ITGA5. Biochim Biophys Acta. **1862**, 1443–1452. https://doi.org/10.1016/j.bbadis.2016.05.004
59 Hedayat, S., Cascione, L., Cunningham, D., Schirripa, M., Lampis, A., Hahne, J. C., et al. (2024) Circulating microRNA Analysis in a Prospective Co-clinical Trial Identifies MIR652-3p as a Response Biomarker and Driver of Regorafenib Resistance Mechanisms in Colorectal Cancer. Clin Cancer Res. **30**, 2140–2159. https://doi.org/10.1158/1078-0432.CCR-23-2748
60 Toor, S. M., Aldous, E. K., Parray, A., Akhtar, N., Al-Sarraj, Y., Abdelalim, E. M., et al. (2022) Circulating MicroRNA Profiling Identifies Distinct MicroRNA Signatures in Acute Ischemic Stroke and Transient Ischemic Attack Patients. International journal of molecular sciences. **24**. https://doi.org/10.3390/ijms24010108
61 Danielson, K. M., Shah, R., Yeri, A., Liu, X., Camacho Garcia, F., Silverman, M., et al. (2018) Plasma Circulating Extracellular RNAs in Left Ventricular Remodeling Post-Myocardial Infarction. EBioMedicine. **32**, 172–181. https://doi.org/10.1016/j.ebiom.2018.05.013
62 Fuentevilla-Alvarez, G., Soto, M. E., Robles-Herrera, G. J., Vargas-Alarcon, G., Samano, R., Meza-Toledo, S. E., et al. (2024) Analysis of Circulating miRNA Expression Profiles in Type 2 Diabetes Patients with Diabetic Foot Complications. International journal of molecular sciences. **25**. https://doi.org/10.3390/ijms25137078
63 Liu, L., Chen, R., Jia, Z., Li, X., Tang, Y., Zhao, X., et al. (2022) Downregulation of hsa-miR-203 in peripheral blood and wound margin tissue by negative pressure wound therapy contributes to wound healing of diabetic foot ulcers. Microvasc Res. **139**, 104275. https://doi.org/10.1016/j.mvr.2021.104275
64 Xiong, Y., Chen, L., Yu, T., Yan, C., Zhou, W., Cao, F., et al. (2020) Inhibition of circulating exosomal microRNA-15a-3p accelerates diabetic wound repair. Aging (Albany NY). **12**, 8968–8986. https://doi.org/10.18632/aging.103143
65 Wu, F., Wang, L., Zuo, H. and Tian, H. (2025) LncRNA A1BG-AS1 regulates the progress of diabetic foot ulcers via sponging miR-214-3p. Endocr J. **72**, 295–306. https://doi.org/10.1507/endocrj.EJ24-0440
66 Yang, M. and Gu, Y. (2024) LncRNA DLEU1 promotes angiogenesis in diabetic foot ulcer wound healing by regulating miR-96-5p. Ir J Med Sci. **193**, 241–247. https://doi.org/10.1007/s11845-023-03471-x
67 Mattick, J. S., Amaral, P. P., Carninci, P., Carpenter, S., Chang, H. Y., Chen, L. L., et al. (2023) Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol. **24**, 430–447. https://doi.org/10.1038/s41580-022-00566-8
68 Jorjani, H., Kehr, S., Jedlinski, D. J., Gumienny, R., Hertel, J., Stadler, P. F., et al. (2016) An updated human snoRNAome. Nucleic Acids Res. **44**, 5068–5082. https://doi.org/10.1093/nar/gkw386
69 Kiss, T. (2004) Biogenesis of small nuclear RNPs. J Cell Sci. **117**, 5949–5951. https://doi.org/10.1242/jcs.01487
70 Morey, C., Rougeulle, C. and Ouimette, J. F. (2025) Unleashing XIST from X-chromosome inactivation. Curr Opin Cell Biol. **92**, 102446. https://doi.org/10.1016/j.ceb.2024.102446

71 Liao, J., Chen, B., Zhu, Z., Du, C., Gao, S., Zhao, G., et al. (2023) Long noncoding RNA (lncRNA) H19: An essential developmental regulator with expanding roles in cancer, stem cell differentiation, and metabolic diseases. Genes Dis. **10**, 1351–1366. https://doi.org/10.1016/j.gendis.2023.02.008
72 Okazaki, Y., Furuno, M., Kasukawa, T., Adachi, J., Bono, H., Kondo, S., et al. (2002) Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. Nature. **420**, 563–573. https://doi.org/10.1038/nature01266
73 Ota, T., Suzuki, Y., Nishikawa, T., Otsuki, T., Sugiyama, T., Irie, R., et al. (2004) Complete sequencing and characterization of 21,243 full-length human cDNAs. Nat Genet. **36**, 40–45. https://doi.org/10.1038/ng1285
74 Tupy, J. L., Bailey, A. M., Dailey, G., Evans-Holm, M., Siebel, C. W., Misra, S., et al. (2005) Identification of putative noncoding polyadenylated transcripts in Drosophila melanogaster. Proc Natl Acad Sci U S A. **102**, 5495–5500. https://doi.org/10.1073/pnas.0501422102
75 Liao, Q., Liu, C., Yuan, X., Kang, S., Miao, R., Xiao, H., et al. (2011) Large-scale prediction of long non-coding RNA functions in a coding-non-coding gene co-expression network. Nucleic Acids Res. **39**, 3864–3878. https://doi.org/10.1093/nar/gkq1348
76 Iyer, M. K., Niknafs, Y. S., Malik, R., Singhal, U., Sahu, A., Hosono, Y., et al. (2015) The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. **47**, 199–208. https://doi.org/10.1038/ng.3192
77 Hon, C. C., Ramilowski, J. A., Harshbarger, J., Bertin, N., Rackham, O. J., Gough, J., et al. (2017) An atlas of human long non-coding RNAs with accurate 5' ends. Nature. **543**, 199–204. https://doi.org/10.1038/nature21374
78 Jarroux, J., Morillon, A. and Pinskaya, M. (2017) History, Discovery, and Classification of lncRNAs. Adv Exp Med Biol. **1008**, 1–46. https://doi.org/10.1007/978-981-10-5203-3_1
79 Shields, E. J., Petracovici, A. F. and Bonasio, R. (2019) lncRedibly versatile: biochemical and biological functions of long noncoding RNAs. Biochem J. **476**, 1083–1104. https://doi.org/10.1042/BCJ20180440
80 Ji, Z., Song, R., Regev, A. and Struhl, K. (2015) Many lncRNAs, 5'UTRs, and pseudogenes are translated and some are likely to express functional proteins. Elife. **4**, e08890. https://doi.org/10.7554/eLife.08890
81 Mohapatra, S., Banerjee, A., Rausseo, P., Dragomir, M. P., Manyam, G. C., Broom, B. M. and Calin, G. A. (2024) FuncPEP v2.0: An Updated Database of Functional Short Peptides Translated from Non-Coding RNAs. Noncoding RNA. **10**. https://doi.org/10.3390/ncrna10020020
82 Wang, B. Y., Gao, Q., Sun, Y. and Qiu, X. B. (2024) Biochemical targets of the micropeptides encoded by lncRNAs. Noncoding RNA Res. **9**, 964–969. https://doi.org/10.1016/j.ncrna.2024.04.005
83 Nita, A., Matsumoto, A., Tang, R., Shiraishi, C., Ichihara, K., Saito, D., et al. (2021) A ubiquitin-like protein encoded by the "noncoding" RNA TINCR promotes keratinocyte proliferation and wound healing. PLoS Genet. **17**, e1009686. https://doi.org/10.1371/journal.pgen.1009686
84 Tang, L., Liang, Y., Xie, H., Yang, X. and Zheng, G. (2020) Long non-coding RNAs in cutaneous biology and proliferative skin diseases: Advances and perspectives. Cell Prolif. **53**, e12698. https://doi.org/10.1111/cpr.12698

85 Yu, H., Wang, Y., Wang, D., Yi, Y., Liu, Z., Wu, M., et al. (2022) Landscape of the epigenetic regulation in wound healing. Frontiers in physiology. **13**, 949498. https://doi.org/10.3389/fphys.2022.949498
86 Li, D., Kular, L., Vij, M., Herter, E. K., Li, X., Wang, A., et al. (2019) Human skin long noncoding RNA WAKMAR1 regulates wound healing by enhancing keratinocyte migration. Proc Natl Acad Sci U S A. **116**, 9443–9452. https://doi.org/10.1073/pnas.1814097116
87 Herter, E. K., Li, D., Toma, M. A., Vij, M., Li, X., Visscher, D., 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. https://doi.org/10.1016/j.jid.2018.11.033
88 Zhou, L., Ren, M., Zeng, T., Wang, W., Wang, X., Hu, M., et al. (2019) TET2-interacting long noncoding RNA promotes active DNA demethylation of the MMP-9 promoter in diabetic wound healing. Cell death & disease. **10**, 813. https://doi.org/10.1038/s41419-019-2047-6
89 Li, D., Liu, Z., Zhang, L., Bian, X., Wu, J., Li, L., et al. (2024) The lncRNA SNHG26 drives the inflammatory-to-proliferative state transition of keratinocyte progenitor cells during wound healing. Nature communications. **15**, 8637. https://doi.org/10.1038/s41467-024-52783-8
90 Kuang, L., Zhang, C., Li, B., Deng, H., Chen, R. and Li, G. (2023) Human Keratinocyte-Derived Exosomal MALAT1 Promotes Diabetic Wound Healing by Upregulating MFGE8 via microRNA-1914-3p. International journal of nanomedicine. **18**, 949–970. https://doi.org/10.2147/IJN.S399785
91 He, M., Tu, L., Shu, R., Meng, Q., Du, S., Xu, Z. and Wang, S. (2022) Long Noncoding RNA CASC2 Facilitated Wound Healing through miRNA-155/HIF-1alpha in Diabetic Foot Ulcers. Contrast Media Mol Imaging. **2022**, 6291497. https://doi.org/10.1155/2022/6291497
92 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. https://doi.org/10.1016/j.jid.2019.12.030
93 Preusser, C., Hung, L. H., Schneider, T., Schreiner, S., Hardt, M., Moebus, A., et al. (2018) Selective release of circRNAs in platelet-derived extracellular vesicles. J Extracell Vesicles. **7**, 1424473. https://doi.org/10.1080/20013078.2018.1424473
94 Xia, Y., Pei, T., Zhao, J., Wang, Z., Shen, Y., Yang, Y. and Liang, J. (2024) Long noncoding RNA H19: functions and mechanisms in regulating programmed cell death in cancer. Cell Death Discov. **10**, 76. https://doi.org/10.1038/s41420-024-01832-8
95 Yang, H., Zhang, Y., Du, Z., Wu, T. and Yang, C. (2023) Hair follicle mesenchymal stem cell exosomal lncRNA H19 inhibited NLRP3 pyroptosis to promote diabetic mouse skin wound healing. Aging (Albany NY). **15**, 791–809. https://doi.org/10.18632/aging.204513
96 Yu, P., Guo, J., Li, J., Shi, X., Xu, N., Jiang, Y., et al. (2022) lncRNA-H19 in Fibroblasts Promotes Wound Healing in Diabetes. Diabetes. **71**, 1562–1578. https://doi.org/10.2337/db21-0724
97 Dai, Z., Liu, W. C., Chen, X. Y., Wang, X., Li, J. L. and Zhang, X. (2023) Gasdermin D-mediated pyroptosis: mechanisms, diseases, and inhibitors. Front Immunol. **14**, 1178662. https://doi.org/10.3389/fimmu.2023.1178662
98 Rinn, J. L., Kertesz, M., Wang, J. K., Squazzo, S. L., Xu, X., Brugmann, S. A., et al. (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. **129**, 1311–1323. https://doi.org/10.1016/j.cell.2007.05.022

99 Amicone, L., Marchetti, A. and Cicchini, C. (2023) The lncRNA HOTAIR: a pleiotropic regulator of epithelial cell plasticity. J Exp Clin Cancer Res. **42**, 147. https://doi.org/10.1186/s13046-023-02725-x
100 Lamichhane, T. N., Leung, C. A., Douti, L. Y. and Jay, S. M. (2017) Ethanol Induces Enhanced Vascularization Bioactivity of Endothelial Cell-Derived Extracellular Vesicles via Regulation of MicroRNAs and Long Non-Coding RNAs. Scientific reports. **7**, 13794. https://doi.org/10.1038/s41598-017-14356-2
101 Born, L. J., Chang, K. H., Shoureshi, P., Lay, F., Bengali, S., Hsu, A. T. W., et al. (2022) HOTAIR-Loaded Mesenchymal Stem/Stromal Cell Extracellular Vesicles Enhance Angiogenesis and Wound Healing. Adv Healthc Mater. **11**, e2002070. https://doi.org/10.1002/adhm.202002070
102 Shi, Y., Yang, R., Tu, L. and Liu, D. (2020) Long non-coding RNA HOTAIR promotes burn wound healing by regulating epidermal stem cells. Molecular medicine reports. **22**, 1811–1820. https://doi.org/10.3892/mmr.2020.11268
103 Li, D., Feng, J., Wu, T., Wang, Y., Sun, Y., Ren, J. and Liu, M. (2013) Long intergenic noncoding RNA HOTAIR is overexpressed and regulates PTEN methylation in laryngeal squamous cell carcinoma. Am J Pathol. **182**, 64–70. https://doi.org/10.1016/j.ajpath.2012.08.042
104 Zhou, W., Xu, S., Chen, X. and Wang, C. (2021) HOTAIR suppresses PTEN via DNMT3b and confers drug resistance in acute myeloid leukemia. Hematology. **26**, 170–178. https://doi.org/10.1080/16078454.2021.1880733
105 Yu, F., Chen, B., Dong, P. and Zheng, J. (2017) HOTAIR Epigenetically Modulates PTEN Expression via MicroRNA-29b: A Novel Mechanism in Regulation of Liver Fibrosis. Mol Ther. **25**, 205–217. https://doi.org/10.1016/j.ymthe.2016.10.015
106 Li, Q., Zhao, H., Chen, W., Huang, P. and Bi, J. (2019) Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. The international journal of biochemistry & cell biology. **114**, 105570. https://doi.org/10.1016/j.biocel.2019.105570
107 Wei, Q., Wang, Y., Ma, K., Li, Q., Li, B., Hu, W., et al. (2022) Extracellular Vesicles from Human Umbilical Cord Mesenchymal Stem Cells Facilitate Diabetic Wound Healing Through MiR-17-5p-mediated Enhancement of Angiogenesis. Stem Cell Rev Rep. **18**, 1025–1040. https://doi.org/10.1007/s12015-021-10176-0
108 Wei, Q., Su, J., Meng, S., Wang, Y., Ma, K., Li, B., et al. (2024) MiR-17-5p-engineered sEVs Encapsulated in GelMA Hydrogel Facilitated Diabetic Wound Healing by Targeting PTEN and p21. Adv Sci (Weinh). **11**, e2307761. https://doi.org/10.1002/advs.202307761
109 Wang, J., Zhou, Y., Lu, J., Sun, Y., Xiao, H., Liu, M. and Tian, L. (2014) Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. Med Oncol. **31**, 148. https://doi.org/10.1007/s12032-014-0148-8
110 Berrondo, C., Flax, J., Kucherov, V., Siebert, A., Osinski, T., Rosenberg, A., et al. (2016) Expression of the Long Non-Coding RNA HOTAIR Correlates with Disease Progression in Bladder Cancer and Is Contained in Bladder Cancer Patient Urinary Exosomes. PLoS One. **11**, e0147236. https://doi.org/10.1371/journal.pone.0147236
111 Chen, L., Huang, S., Huang, J., Chen, Q. and Zhuang, Q. (2021) Role and Mechanism of Exosome-Derived Long Noncoding RNA HOTAIR in Lung Cancer. ACS Omega. **6**, 17217–17227. https://doi.org/10.1021/acsomega.1c00905

112 Xie, Z., Xia, J., Jiao, M., Zhao, P., Wang, Z., Lin, S., et al. (2023) Exosomal lncRNA HOTAIR induces PDL1(+) B cells to impede anti-tumor immunity in colorectal cancer. Biochem Biophys Res Commun. **644**, 112–121. https://doi.org/10.1016/j.bbrc.2023.01.005

113 Tang, S., Zheng, K., Tang, Y., Li, Z., Zou, T. and Liu, D. (2019) Overexpression of serum exosomal HOTAIR is correlated with poor survival and poor response to chemotherapy in breast cancer patients. Journal of biosciences. **44**. https://doi.org/10.1007/s12038-019-9861-y

114 Sasaki, Y. T., Ideue, T., Sano, M., Mituyama, T. and Hirose, T. (2009) MENepsilon/beta noncoding RNAs are essential for structural integrity of nuclear paraspeckles. Proc Natl Acad Sci U S A. **106**, 2525–2530. https://doi.org/10.1073/pnas.0807899106

115 Sunwoo, H., Dinger, M. E., Wilusz, J. E., Amaral, P. P., Mattick, J. S. and Spector, D. L. (2009) MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. Genome Res. **19**, 347–359. https://doi.org/10.1101/gr.087775.108

116 Hirose, T., Ninomiya, K., Nakagawa, S. and Yamazaki, T. (2023) A guide to membraneless organelles and their various roles in gene regulation. Nat Rev Mol Cell Biol. **24**, 288–304. https://doi.org/10.1038/s41580-022-00558-8

117 Li, K., Yao, T., Zhang, Y., Li, W. and Wang, Z. (2021) NEAT1 as a competing endogenous RNA in tumorigenesis of various cancers: Role, mechanism and therapeutic potential. Int J Biol Sci. **17**, 3428–3440. https://doi.org/10.7150/ijbs.62728

118 Farzaneh, M., Masoodi, T., Ghaedrahmati, F., Radoszkiewicz, K., Anbiyaiee, A., Sheykhi-Sabzehpoush, M., et al. (2023) An updated review of contribution of long noncoding RNA-NEAT1 to the progression of human cancers. Pathol Res Pract. **245**, 154380. https://doi.org/10.1016/j.prp.2023.154380

119 Architha, T., Juanitaa, G. R., Vijayalalitha, R., Jayasuriya, R., Athira, G., Balamurugan, R., et al. (2024) LncRNA NEAT1/miR-146a-5p Axis Restores Normal Angiogenesis in Diabetic Foot Ulcers by Targeting mafG. Cells. **13**. https://doi.org/10.3390/cells13050456

120 Liu, R., Jiang, C., Li, J., Li, X., Zhao, L., Yun, H., et al. (2021) Serum-derived exosomes containing NEAT1 promote the occurrence of rheumatoid arthritis through regulation of miR-144-3p/ROCK2 axis. Ther Adv Chronic Dis. **12**, 2040622321991705. https://doi.org/10.1177/2040622321991705

121 Fan, J. T., Zhou, Z. Y., Luo, Y. L., Luo, Q., Chen, S. B., Zhao, J. C. and Chen, Q. R. (2021) Exosomal lncRNA NEAT1 from cancer-associated fibroblasts facilitates endometrial cancer progression via miR-26a/b-5p-mediated STAT3/YKL-40 signaling pathway. Neoplasia. **23**, 692–703. https://doi.org/10.1016/j.neo.2021.05.004

122 Guo, L., Chen, Y., Feng, X., Sun, D., Sun, J., Mou, S., et al. (2022) Oxidative stress-induced endothelial cells-derived exosomes accelerate skin flap survival through Lnc NEAT1-mediated promotion of endothelial progenitor cell function. Stem Cell Res Ther. **13**, 325. https://doi.org/10.1186/s13287-022-03013-9

123 Huang, L., Xiong, S., Liu, H., Li, M., Zhang, R., Liu, Y. and Hu, X. (2023) Bioinformatics Analysis of the Inflammation-Associated lncRNA-mRNA Coexpression Network in Type 2 Diabetes. J Renin Angiotensin Aldosterone Syst. **2023**, 6072438. https://doi.org/10.1155/2023/6072438

124 van Balkom, B. W., de Jong, O. G., Smits, M., Brummelman, J., den Ouden, K., de Bree, P. M., et al. (2013) Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood. **121**, 3997–4006, S3991–3915. https://doi.org/10.1182/blood-2013-02-478925

125 Song, C., Zhang, J., Zhao, Z., Yang, Y., Meng, D., Wang, J., et al. (2020) DLEU1: A Functional Long Noncoding RNA in Tumorigenesis. Current pharmaceutical design. **26**, 1742–1748. https://doi.org/10.2174/1381612826666200122145305
126 Kristensen, L. S., Andersen, M. S., Stagsted, L. V. W., Ebbesen, K. K., Hansen, T. B. and Kjems, J. (2019) The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. **20**, 675–691. https://doi.org/10.1038/s41576-019-0158-7
127 Sanger, H. L., Klotz, G., Riesner, D., Gross, H. J. and Kleinschmidt, A. K. (1976) Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci U S A. **73**, 3852–3856. https://doi.org/10.1073/pnas.73.11.3852
128 Hsu, M. T. and Coca-Prados, M. (1979) Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature. **280**, 339–340. https://doi.org/10.1038/280339a0
129 Patop, I. L., Wust, S. and Kadener, S. (2019) Past, present, and future of circRNAs. EMBO J. **38**, e100836. https://doi.org/10.15252/embj.2018100836
130 Salzman, J., Gawad, C., Wang, P. L., Lacayo, N. and Brown, P. O. (2012) Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS One. **7**, e30733. https://doi.org/10.1371/journal.pone.0030733
131 Jeck, W. R., Sorrentino, J. A., Wang, K., Slevin, M. K., Burd, C. E., Liu, J., et al. (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. **19**, 141–157. https://doi.org/10.1261/rna.035667.112
132 Memczak, S., Jens, M., Elefsinioti, A., Torti, F., Krueger, J., Rybak, A., et al. (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. **495**, 333–338. https://doi.org/10.1038/nature11928
133 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. https://doi.org/10.1038/nature11993
134 Pamudurti, N. R., Bartok, O., Jens, M., Ashwal-Fluss, R., Stottmeister, C., Ruhe, L., et al. (2017) Translation of CircRNAs. Mol Cell. **66**, 9–21 e27. https://doi.org/10.1016/j.molcel.2017.02.021
135 Fan, X., Yang, Y., Chen, C. and Wang, Z. (2022) Pervasive translation of circular RNAs driven by short IRES-like elements. Nature communications. **13**, 3751. https://doi.org/10.1038/s41467-022-31327-y
136 Hwang, H. J. and Kim, Y. K. (2024) Molecular mechanisms of circular RNA translation. Exp Mol Med. **56**, 1272–1280. https://doi.org/10.1038/s12276-024-01220-3
137 Geng, X., Jia, Y., Zhang, Y., Shi, L., Li, Q., Zang, A. and Wang, H. (2020) Circular RNA: biogenesis, degradation, functions and potential roles in mediating resistance to anticarcinogens. Epigenomics. **12**, 267–283. https://doi.org/10.2217/epi-2019-0295
138 Zhang, Z., Yang, T. and Xiao, J. (2018) Circular RNAs: Promising Biomarkers for Human Diseases. EBioMedicine. **34**, 267–274. https://doi.org/10.1016/j.ebiom.2018.07.036
139 Verduci, L., Tarcitano, E., Strano, S., Yarden, Y. and Blandino, G. (2021) CircRNAs: role in human diseases and potential use as biomarkers. Cell death & disease. **12**, 468. https://doi.org/10.1038/s41419-021-03743-3
140 Zhao, J., Li, Q., Hu, J., Yu, H., Shen, Y., Lai, H., et al. (2024) Circular RNA landscape in extracellular vesicles from human biofluids. Genome medicine. **16**, 126. https://doi.org/10.1186/s13073-024-01400-w

141 Li, Y., Zheng, Q., Bao, C., Li, S., Guo, W., Zhao, J., et al. (2015) Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Res. **25**, 981–984. https://doi.org/10.1038/cr.2015.82
142 Yuan, X., Mao, Y. and Ou, S. (2023) Diagnostic accuracy of circulating exosomal circRNAs in malignances: A meta-analysis and systematic review. Medicine (Baltimore). **102**, e33872. https://doi.org/10.1097/MD.0000000000033872
143 Lou, Y., Yan, J., Liu, Q., Miao, M. and Shao, Y. (2024) Biological functions and molecular mechanisms of exosome-derived circular RNAs and their clinical implications in digestive malignancies: the vintage in the bottle. Annals of medicine. **56**, 2420861. https://doi.org/10.1080/07853890.2024.2420861
144 Zeng, Q., Liu, C. H., Ampuero, J., Wu, D., Jiang, W., Zhou, L., et al. (2024) Circular RNAs in non-alcoholic fatty liver disease: Functions and clinical significance. RNA Biol. **21**, 1–15. https://doi.org/10.1080/15476286.2023.2290769
145 Wang, A., Toma, M. A., Ma, J., Li, D., Vij, M., Chu, T., et al. (2020) Circular RNA hsa_circ_0084443 Is Upregulated in Diabetic Foot Ulcer and Modulates Keratinocyte Migration and Proliferation. Adv Wound Care (New Rochelle). **9**, 145–160. https://doi.org/10.1089/wound.2019.0956
146 Han, D., Liu, W., Li, G. and Liu, L. (2021) Circ_PRKDC knockdown promotes skin wound healing by enhancing keratinocyte migration via miR-31/FBN1 axis. J Mol Histol. **52**, 681–691. https://doi.org/10.1007/s10735-021-09996-8
147 He, Z. and Xu, X. (2022) Circ_0084443 Inhibits Wound Healing Via Repressing Keratinocyte Migration Through Targeting the miR-17-3p/FOXO4 Axis. Biochem Genet. **60**, 1236–1252. https://doi.org/10.1007/s10528-021-10157-5
148 Huang, Q., Chu, Z., Wang, Z., Li, Q., Meng, S., Lu, Y., et al. (2024) circCDK13-loaded small extracellular vesicles accelerate healing in preclinical diabetic wound models. Nature communications. **15**, 3904. https://doi.org/10.1038/s41467-024-48284-3
149 Niu, G., Toma, M. A., Geara, J., Bian, X., Chen, Y., Luo, L., et al. (2025) Collaborative Duality of CircGLIS3(2) RNA and Protein in human Wound Repair. Adv Sci (Weinh). **12**, e2416784. https://doi.org/10.1002/advs.202416784
150 Wang, Q., Niu, G., Liu, Z., Toma, M. A., Geara, J., Bian, X., et al. (2025) Circular RNA circASH1L(4,5) protects microRNA-129-5p from target-directed microRNA degradation in human skin wound healing. Br J Dermatol. **192**, 468–480. https://doi.org/10.1093/bjd/ljae405
151 Fu, Z., Jiang, Z., Liao, X., Liu, M., Guo, G., Wang, X., et al. (2023) Upregulation of circ_0080968 in diabetic foot ulcer inhibits wound healing via repressing the migration and promoting proliferation of keratinocytes. Gene. **883**, 147669. https://doi.org/10.1016/j.gene.2023.147669
152 Toma, M. A., Liu, Z., Wang, Q., Zhang, L., Li, D., Sommar, P. and Landen, N. X. (2022) Circular RNA Signatures of Human Healing and Nonhealing Wounds. J Invest Dermatol. **142**, 2793–2804 e2726. https://doi.org/10.1016/j.jid.2022.03.024
153 Chen, Z. J., Shi, X. J., Fu, L. J., Liu, J., Shi, K., Zhang, W. B. and Su, P. K. (2020) Serum and exosomal hsa_circ_0000907 and hsa_circ_0057362 as novel biomarkers in the early diagnosis of diabetic foot ulcer. Eur Rev Med Pharmacol Sci. **24**, 8117–8126. https://doi.org/10.26355/eurrev_202008_22498
154 Feng, Y., Jiang, B., Luo, Q., Liao, C., Liu, M. and Jiang, L. (2025) Plasma Proteomics-Based Identification of Novel Predictive and Diagnostic Inflammatory Biomarkers for Diabetic

Foot. Journal of proteome research. **24**, 3017–3031. https://doi.org/10.1021/acs.jproteome.5c00037
155 Theocharidis, G., Sumpio, B., Wang, E., Mezghani, I., Giurini, J. M., Kalavros, N., et al. (2024) Use of Serum Protein Measurements as Biomarkers that Can Predict the Outcome of Diabetic Foot Ulceration. Adv Wound Care (New Rochelle). **13**, 426–434. https://doi.org/10.1089/wound.2023.0126
156 Yu, X. T., Wang, F., Ding, J. T., Cai, B., Xing, J. J., Guo, G. H. and Guo, F. (2022) Tandem mass tag-based serum proteomic profiling revealed diabetic foot ulcer pathogenesis and potential therapeutic targets. Bioengineered. **13**, 3171–3182. https://doi.org/10.1080/21655979.2022.2027173
157 Soto, V., Jurczuk, V., Tose, L. V., Alcalde Pretel, C., Garcia, V., Farhadi, L., et al. (2025) Turning Waste Into Insight: A Novel Proteomic Approach to Non-Invasive Wound Biomarker Discovery. Mol Cell Proteomics. **24**, 101058. https://doi.org/10.1016/j.mcpro.2025.101058
158 Sumpio, B. J., Yeo, K., Theocharidis, G., Mezghani, I., Fokoue, A., Gray, A., et al. (2025) Integrated machine learning analysis of proteomic and transcriptomic data identifies healing associated targets in diabetic wound repair. Scientific reports. **15**, 34355. https://doi.org/10.1038/s41598-025-16914-5
159 Gould, L. and Mahmoudi, M. (2024) Analysis of Biogenic Amines and Small Molecule Metabolites in Human Diabetic Wound Ulcer Exudate. ACS Pharmacol Transl Sci. **7**, 2894–2899. https://doi.org/10.1021/acsptsci.4c00418
160 Theocharidis, G., Thomas, B. E., Sarkar, D., Mumme, H. L., Pilcher, W. J. R., Dwivedi, B., et al. (2022) Single cell transcriptomic landscape of diabetic foot ulcers. Nature communications. **13**, 181. https://doi.org/10.1038/s41467-021-27801-8
161 Liu, Z., Bian, X., Luo, L., Bjorklund, A. K., Li, L., Zhang, L., et al. (2025) Spatiotemporal single-cell roadmap of human skin wound healing. Cell Stem Cell. **32**, 479–498 e478. https://doi.org/10.1016/j.stem.2024.11.013
162 Metcalf, G. A. D. (2024) MicroRNAs: circulating biomarkers for the early detection of imperceptible cancers via biosensor and machine-learning advances. Oncogene. **43**, 2135–2142. https://doi.org/10.1038/s41388-024-03076-3
163 Ratre, P., Nazeer, N., Bhargava, A., Thareja, S., Tiwari, R., Raghuwanshi, V. S. and Mishra, P. K. (2023) Design and Fabrication of a Nanobiosensor for the Detection of Cell-Free Circulating miRNAS-LncRNAS-mRNAS Triad Grid. ACS Omega. **8**, 40677–40684. https://doi.org/10.1021/acsomega.3c05718
164 Chen, C., Hu, S., Tian, L., Qi, M., Chang, Z., Li, L., et al. (2024) A versatile upconversion-based multimode lateral flow platform for rapid and ultrasensitive detection of microRNA towards health monitoring. Biosensors & bioelectronics. **252**, 116135. https://doi.org/10.1016/j.bios.2024.116135
165 Bhaiyya, M., Panigrahi, D., Rewatkar, P. and Haick, H. (2024) Role of Machine Learning Assisted Biosensors in Point-of-Care-Testing For Clinical Decisions. ACS Sens. **9**, 4495–4519. https://doi.org/10.1021/acssensors.4c01582
166 Tian, M., Dong, J., Yuan, B. and Jia, H. (2021) Identification of potential circRNAs and circRNA-miRNA-mRNA regulatory network in the development of diabetic foot ulcers by integrated bioinformatics analysis. Int Wound J. **18**, 323–331. https://doi.org/10.1111/iwj.13535

Supplementary Information

Search Strategies for Tables 2 and 3

Searches were performed on PubMed between January and June 2025. Search terms included:

circulating microRNA wound healing: 49 hits
circulating microRNA diabetic foot ulcers: 10 hits
circulating microRNA venous leg ulcers: 0 hits
circulating microRNA arterial leg ulcers: 0 hits
circulating microRNA pressure ulcers: 1 hit
circulating microRNA surgical wounds healing by secondary intent: 0 hits
exosomal microrna biomarkers foot ulcers: 2 hits
lncRNA diabetic foot ulcers NOT reviews: 37 hits.
circulating lncRNA diabetic foot ulcers NOT reviews: 1 hit
lncRNA exosomes wound healing skin: 17 hits
circular RNA diabetic foot ulcers: 22 hits
circulating circular RNA wound healing: 4 hits.
circulating circular RNA diabetic foot ulcer: 0 hits
exosome circular RNA wound fluid: 1 hit
circular RNA wound fluid: 9

Review articles were excluded, and papers were hand searched by one author (KR) to select those most relevant.