MicroRNA regulation of epithelial-to-mesenchymal transition during re-epithelialisation: assessing an open wound

Wager LJ & Leavesley DI

Background: MicroRNAs regulate many biological processes, one being epithelial-to-mesenchymal transition (EMT) and another re-epithelialisation. EMT in cancer metastasis has been likened to the initiation of re-epithelialisation in wound healing.

Contribution: This review critically analyses cutaneous wound models microRNAs used to investigate microRNA effects on wound healing to discuss the potential of EMT as a mechanism that accounts for these effects. It also integrates microRNAs observed to affect EMT in cancer and EMT models, with roles for EMT in wound-healing re-epithelialisation, to identify microRNAs of interest to wound-healing research.

Keywords: *EMT, re-epithelialisation, microRNAs, healing, wound.*

ABSTRACT

It is becoming increasing clear that microRNAs contribute to the regulation of many biological processes, including wound healing. After injury, keratinocytes need to undergo what is known as an epithelial-to-mesenchymal transition (EMT) to initiate re-epithelialisation. During this process, keratinocytes reduce their attachment to the underlying matrix, extend membrane protrusions, become motile and migrate over the wound bed, affecting wound closure. MicroRNAs that regulate EMT are aberrantly upregulated in keratinocytes at the edge of non-healing wounds and potentially play a role in the chronicity of these wounds. *In vitro* and *in vivo*, downregulation of these microRNAs promotes EMT and migration, facilitating re-epithelialisation in wound models. This review will focus on the role of microRNAs that regulate or have potential to regulate EMT and re-epithelialisation during wound healing.

INTRODUCTION

As humans, our skin takes on more than a functional role; unlike internal organs it is a tissue with which we associate, recognise and interact. It is our interface with our surroundings and, most importantly, it forms a protective barrier that insulates us from our environment. Keratinocytes comprise and maintain our outermost layer of skin, the epidermis. Keratinocytes follow a route of stratifying differentiation, beginning in the stratum basale and ending in the stratum corneum to continuously replenish the epidermis. However, as our outermost layer of cells, the epidermis is exposed to a harsh environment. Consequently, the skin suffers daily insults that result in physical, chemical, microbial and thermal damage to its integrity that must be repaired. It is crucial for survival that keratinocytes repair damage and quickly restore barrier integrity.

Wager LJ * & Leavesley DI

Tissue Repair and Regeneration Program, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology
Brisbane, Qld 4059, Australia
Tel +61 7 3138 6440
Email l.wager@qut.edu.au

* *Corresponding author*

The epidermal keratinocyte phenotype is normally a sedentary, polarised cell, displaying attachment to a basement membrane and cohesion with nearby epithelium. Thus, in order to repair tissue damage, keratinocytes must undergo what is termed a partial epithelial-to-mesenchymal transition (EMT) (reviewed in [1–3]). It is a process that is essentially a cascade of molecular and cellular changes. Ultimately epithelial traits expressed during skin homeostasis, when keratinocytes are sedentary and tightly cohesive, are exchanged for mesenchymal traits as keratinocytes become migratory, spindle-shaped cells. These changes are required for keratinocytes to migrate across the wound bed during skin repair (reviewed in [3]). Briefly, during the process of EMT, epithelia reduce their attachment to neighbouring cells, they replace top-to-bottom polarity for front-to-rear polarity and also *de novo* express pro-migratory proteinases and extracellular matrix proteins. Together these alterations permit keratinocytes to invade the wound bed and begin re-epithelialisation. Upon wound closure, the EMT phenotype that facilitated re-epithelialisation is reversed and keratinocytes revert to their homeostatic, non-migratory state via the opposite process termed mesenchymal-to-epithelial transition (MET).

The changes for wound healing are very similar to changes that take place during tumour metastasis (reviewed in [4] and summarised in Figure 1), whereby cancer cells migrate to different regions of the body. Research into EMT has led researchers to characterise three phenotypes that classify an EMT based on the biological context:

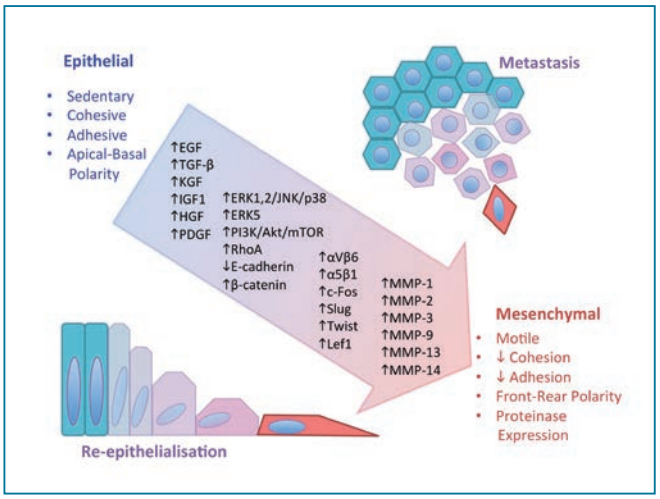

Figure 1: Molecular and morphological characteristics common to EMT and re-epithelialisation. The schematic shows the traits and genes altered during the process of EMT that allow epithelial cells to become motile during processes such as wound repair or metastasis.

types I, II and III. These feature common mechanisms, but result in functional differences[5]. Type I EMT is unique to embryogenesis, organogenesis and development. The EMT that occurs during wound healing/re-epithelialisation is classed as Type II EMT. Importantly, type II EMT occurs in the presence of inflammation[5]. Type III EMT encompasses cancer metastasis and is defined primarily by its initiation, at which point cells are transformed to a neoplastic state with the acquisition of mutations in oncogenes or tumour suppressor genes, generating a cell and tissue environment that promotes EMT[5].

The gene expression changes and signalling molecules that promote EMT in cancer metastasis have been extensively documented[6]. In contrast, the molecular mechanisms that regulate EMT during acute and delayed re-epithelialisation are poorly described. In this regard, new mechanisms have been recognised that play a substantial role in regulating gene expression and function.

MicroRNAs and re-epithelialisation

MicroRNAs are a family of short (usually 22 nucleotides in length) non-coding RNA transcripts able to regulate the transcription and translation of mRNAs, that is, microRNAs do not produce proteins, but can determine the amount of proteins expressed by cells (reviewed in [7]). By pairing to a complementary sequence of nucleotides in 'target' mRNA transcripts, before protein translation, microRNAs together with accessory proteins form what is known as an RNA-induced silencing complex (RISC). This is the protein/RNA complex that inhibits mRNA translation into proteins or induces mRNA degradation. This ultimately reduces the translation of protein from the target mRNA transcript (Figure 2). Importantly, their short length and even shorter seed sequence of 2–8 nucleotides (within the microRNA strand required to initiate pairing) permits a single microRNA to regulate hundreds of target mRNAs and downstream proteins. Furthermore, any mRNA transcript may contain target sites recognised by a single microRNA and/or multiple sites for many individual microRNA species. Thus, microRNAs are able to regulate vast numbers of cellular proteins and functions.

While only discovered in the last decade, it is now well established that microRNAs regulate almost all biological processes[8]. Importantly, microRNAs have been identified to drive and regulate the EMT process[9]. This is especially true for cancer development and metastasis, where microRNAs play clear roles as oncogenes and drivers of metastasis (reviewed in [10]). In turn, evidence for the role of microRNAs in skin development, maintenance, disease and wound healing is accumulating[11]. This review aims to integrate and highlight some of our knowledge of microRNAs in wound healing re-epithelialisation and EMT in tissue repair and discuss the potential key role for microRNAs in these processes.

MicroRNAs micromanage EMT modules in tissue repair

Like most processes in wound healing, EMT is a complex cascade of overlapping events and is subject to a hierarchy of microRNA regulators. In skin biology, microRNAs miR-203 and miR-21 (described later) are thought to occupy the role of 'master regulators'. Highly expressed in the epidermis, these microRNAs reduce the expression of proteins that regulate the rate of gene transcription, known as transcription factors. There are many distinct yet interrelated cellular changes that take place downstream of these master regulators, referred to as 'modules', that comprise an EMT. These modules include cytoskeletal reorganisation, cell detachment and extracellular matrix (ECM) regulation; all key processes in enabling a cell to migrate. Overall, modules of EMT are affected by the overarching effects of microRNAs such as miR-203, but downstream of such master regulators microRNAs also interact with targets that are specific to just one process or protein active within a module, providing complex layering of regulation. Considering the many similarities shared by cancer metastasis and wound healing (Figure 3), the microRNAs acting downstream of master regulators may act to 'fine tune' the overarching EMT signal and restrict an otherwise malignant cell phenotype to the confines of wound healing. In the following sections, these microRNAs that act on specific aspects that comprise an EMT are discussed in the context of re-epithelialisation and their EMT module.

Cell detachment and the roles of SNAIL, SLUG and ZEB proteins

Characteristic of all epithelia, keratinocytes form tight junctions, adherent junctions and gap junctions with adjacent cells under normal conditions. Desmosomes are the most abundant cell-cell junction in epithelia and must disassemble to allow keratinocytes to migrate during the wound healing processes[12,13]. E-cadherin is a major component of desmosomes and thus downregulation of E-cadherin is often used as a key indicator of EMT[5]. In this regard, another well-established 'signature' of EMT is the increased expression of ZEB transcription factor family members, ZEB1 and ZEB2, that repress E-cadherin expression to facilitate cell migration[5,14-17].

Transcription factors are powerful controllers of gene expression, driving or inhibiting the transcription of genes into mRNA that can be translated into proteins. SNAI1 (SNAIL) and SNAI2 (SLUG) are

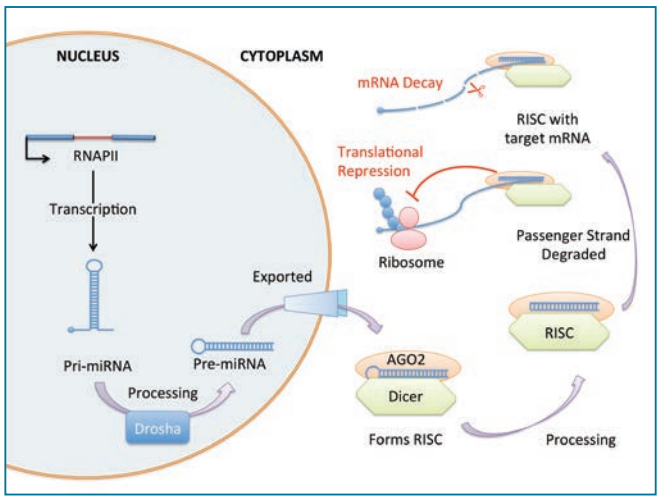

Figure 2: MicroRNA biogenesis and function. MicroRNAs are transcribed from DNA by RNA polymerase II (RNAPII) creating pri-microRNAs, which are cleaved by the enzyme Drosha generating hairpin structures termed pre-microRNAs. These are exported into the cytoplasm via Exportin-5, where they assemble with a number of proteins, including cofactor proteins Dicer and Argonaute 2 (AGO2), to form the RNA induced silencing complex (RISC). Further processing removes the RNA hairpin loop and one strand of the remaining RNA duplex is degraded to produce a mature microRNA. In this form RISC may associate with complementary mRNA to (in most cases) repress mRNA translation or induce mRNA degradation.

two transcription factors whose expression also reduces cell cohesion as an aspect of EMT induction[18]. *In vitro* and *in vivo* studies have revealed that efficient wound healing requires SLUG upregulation in keratinocytes at the wound edge[19,20]. Moreover, re-epithelialisation is impaired in mouse wound models that lack SLUG expression[21]. In contrast to SLUG, evidence for SNAIL in wound healing is sparse. So too, is evidence demonstrating that these transcription factors are regulated by microRNAs at the wound edge. However, in stark contrast microRNAs that regulate SLUG, SNAIL and ZEB proteins in studies of cancer metastasis and EMT are well documented.

Evidence from cancer research shows that members of the miR-200 family: miR-200a, miR-200b, miR-200c, miR-141 and miR-429 and miR-205 decrease SLUG expression during metastasis and EMT models, in this manner promoting an epithelial phenotype[15,17]. SLUG also acts in the opposite direction to inhibit the expression of miR-1 and miR-200b[22]. In this case, increased SLUG maintains a mesenchymal phenotype and correlates with advancing prostate cancer metastasis[22]. Likewise, upregulation of miR-124 in breast cancer attenuates the expression of SLUG and inhibits EMT and reduces their metastatic potential[23]. Downstream of SLUG, decreased miR-205 expression increases metastasis by permitting ZEB protein translation in prostate, bladder and breast cancers[24-26]. SNAIL has also been implicated in SLUG and miR-200 family EMT regulation. In breast cancer cell lines, SNAIL upregulation decreases miR-200b and miR-203 to promote EMT[27]. Conversely, miR-203 also targets and represses SNAIL, in this case to maintain an epithelial phenotype[27].

Transforming growth factor β (TGF-β) is an established inducer of EMT. Its network of EMT regulation includes, but is not limited to, many of the transcription factors outlined above (reviewed in [28,29]). For example, in MDCK cells (a common EMT model system), TGF-β exposure establishes and maintains EMT via a signalling loop that includes miR-200 and the ZEB transcription factors[30]. Furthermore, TGF-β expression is suppressed by miR-30a[31] and miR-199a[32] through the attenuation of SNAIL in lung carcinoma cells. Most importantly, TGF-β is a cytokine robustly produced at the site of injury to drive wound healing (reviewed in [33]). So far SNAIL, the ZEB transcription factors and many of the microRNAs outlined above have not been investigated in re-epithelialisation. However, the importance of TGF-β to their regulation and to healing together with the established role of SLUG in re-epithelialisation provides firm evidence that microRNAs may modulate EMT at the wound edge.

Cytoskeletal reorganisation via Rho-GTPases

Cells must change their shape in order to migrate, forming a leading edge and a trailing edge. Cell motility is generated by the protrusion of the leading edge, attachment to the substrate, then detachment and retraction of its trailing edge in a cycle of cytoskeletal dissolution and reorganisation[34]. Cytoskeletal reorganisation mediates these changes in epithelial cells and is thus characteristic of an EMT[14]. Cytoskeletal reorganisation is observed in wound edge keratinocytes during re-epithelialisation[34]. Accordingly, proteins that comprise the cytoskeleton occupy a pivotal role in re-epithelialisation. The Rho-GTPase family of proteins are key regulators of the cytoskeletal changes required to mediate cell migration[35]. Activation of RhoA signalling pathways increases the rate of wound closure in mouse wound models[36] and *in vitro* scratch wound closure assays[37,38]. Reports from cancer research show that the expression of the Rho family of proteins can be regulated by microRNAs[37,39,40].

TGF-β induced EMT in breast cancer can occur via upregulating miR-155 expression which in turn suppresses RhoA expression[41]. Deletion of miR-155's binding site from RhoA's mRNA transcript abolished this EMT induction, confirming miR-155's regulatory role[41]. Net1A is required for TGF-β-induced activation of the RhoA pathway and is involved in cytoskeletal regulation during cell migration[42]. MiR-24 represses NET1A and attenuates TGF-β-stimulated keratinocyte migration[42]. It also directly repressed other cytoskeletal modulators, including PAK4, Tks5, and ArhGAP19 in human and mouse keratinocytes, ultimately inducing differentiation and impairing cell mobility in mouse epidermis *in vivo* and human keratinocytes *in vitro*[43].

RhoA is also indirectly regulated in human keratinocytes and corneal keratinocytes by miR-205 and miR-184 via the lipid phosphatase SHIP2[44]. Downregulation of SHIP2 expression by miR-205 leads to increased healing *in vitro*[44]. In other reports, miR-205 expression is decreased in TGF-β-induced EMT[45]. A critical assessment of miR-205's effect on metastasis across a variety of carcinomas indicates that miR-205's effect on metastasis and EMT seems to be context-specific[46]. Thus, if we are to define the effect of miR-205 on EMT in wound healing, further investigations are necessary.

Two isoforms of miR-483 have been implicated in re-epithelialisation. During wound healing, the level of miR-483-3p ('-3p' denoting the

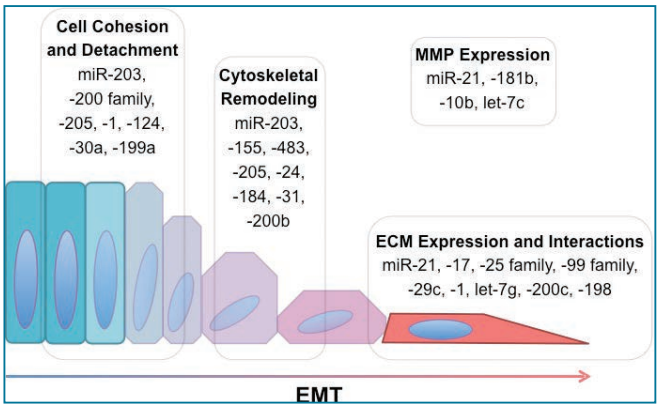

Figure 3: MicroRNAs can regulate many modules of EMT. An illustration that summarises the many microRNAs mentioned in this review and their niches within the overlapping processes that comprise an EMT. MiR-25 family members: miR-25/32/92abc/363/363-3p/367), miR-99 family members: miR-99ab/100.

strand of miR-483 originating from the 3' arm of the primary microRNA hairpin) expression in acutely wounded keratinocytes increases upon wound closure[47]. Further analysis shows that miR-483-3p represses several known promoters of cell proliferation[47]. Accordingly, overexpression of miR-483-3p in HaCaT keratinocytes delays *in vitro* wound closure[47]. Recently miR-483-5p, the complementary strand to miR-483-3p (comprising the 5' arm of the miR-483 precursor hairpin) was found to suppress the RhoA-family member, RhoGDI1 and induce metastasis via EMT in lung adenocarcinoma[48]. While the effect of miR-483-5p on EMT has not been confirmed in re-epithelialisation, it is possible that miR-483 plays a dual role in healing and warrants further investigation.

WAVE-3, a member of the Wiskott-Aldrich syndrome protein (WASP) family of proteins, modulates the actin cytoskeleton and promotes cell migration downstream of Rho-GTPase member, Rac[49]. Important findings by Jiang and associates have linked reduced WAVE-3 expression with non-healing skin lesions[50]. Whilst microRNA regulation of WAVE proteins have yet to be shown to directly regulate wound healing, miR-31 and miR-200b downregulate WAVE-3 leading to increased breast and prostate cancer invasion *in vitro*[51,52]. Furthermore, WAVE-3 expression is essential for TGF-β-induced EMT in triple-negative breast cancer cells[53]. These results suggest that miR-31 and miR-200b could play a role in regulating migration during wound healing, although this has yet to be tested.

The studies outlined above exemplify that microRNAs control changes in the cytoskeleton during EMT and demonstrate the potential for microRNAs to act in a similar way during re-epithelialisation. It is also evident that further research is necessary to confirm the roles of microRNA during wound-healing.

MicroRNAs, EMT and the ECM

The ECM provides a scaffold for cells consisting of molecules such as collagens, laminins and fibronectin[54]. Cells interact with these extracellular structures through receptors, namely the integrins, that bind to the ECM and use this scaffold to 'crawl' into the wounded area. Their adhesion to these molecules also provides cues for the initiation and cessation of keratinocyte migration[34]. Increased expression of ECM proteins is a hallmark of EMT in wound edge keratinocytes and cancer cells alike[55,56]. In many studies of cancer metastasis the expression of ECM proteins is regulated by microRNAs[57].

Collagen-1 is the most abundant protein in the dermis. Upon wounding, keratinocytes migrate into the wound bed and are exposed to collagen-1. They are stimulated to produce collagenase-1 among other proteinases to digest collagen matrices and aid in their migration[58]. Aside from digesting collagen-1, interaction with collagen-1 is also necessary for efficient keratinocyte migration[54]. Furthermore, collagen-1 is able to induce EMT in alveolar epithelial cells via TGF-β-dependent and independent means[59,60]. Research in cancer and wound healing document that microRNAs regulate and are regulated by collagens. MiR-21 regulates collagen fibril deposition in mouse skin largely via effects on dermal fibroblasts[61]. However, keratinocytes are also a major source of miR-21 in the wound environment[61]. In a feed-forward loop, collagen-1 was itself found to induce miR-21 expression and furthermore, in this manner drive EMT in lung and breast cancer cells[62]. This loop induces and maintains mesenchymal characteristics in both human lung and breast cancer cell lines when grown in a collagen-1-rich gel[62]. These results indicate that keratinocyte interaction with collagen at the wound edge may be a mechanism of EMT induction and mesenchymal phenotype maintenance instrumental in re-epithelialisation.

MiR-21 is not the only microRNA to regulate collagen expression, miR-29c has been shown to regulate the expression of multiple collagen isoforms (collagen 1A1, 1A2, 3A1, 4A1, 4A2, 15A1) and laminin-1 in nasopharyngeal carcinomas[63]. Increases in miR-29 expression induce EMT and invasiveness in many tumours via amplified ECM biosynthesis[64,65]. However, there also exists evidence to the contrary, in which miR-29 expression inhibits EMT[66]. Nevertheless, the majority of data indicate that microRNAs and deposited collagen may establish signalling loops that reinforce an invasive phenotype in epithelial cells. However, like many microRNAs described in this review, miR-29's function seems to be dictated by context and emphasises the need for investigation from a healing perspective.

Fibronectin comprises the majority of the haemostatic plug after injury and provides a preliminary matrix scaffold at the site of injury[67]. Evidence from cancer research indicates that several microRNAs can regulate fibronectin expression. In laryngeal squamous cell carcinoma, breast cancer and mammary endometrial cancer cells, expression of microRNAs miR-1[68], let-7g[69] and miR-200c[70] impedes cell migration by suppressing the production of fibronectin. Similarly, miR-17 overexpression can reduce fibronectin translation by endothelial cells and impede their migration in a scratch-wound[71]. Furthermore, an isoform of fibronectin (containing the extra domain A) can induce EMT in colorectal cancer cells[72]. This type of fibronectin is deposited by keratinocytes and fibroblasts in the wound environment[73], but so far it is unknown whether this process is regulated by microRNAs or whether fibronectin-induced EMT in colon epithelium takes place at the wound edge.

Laminins are heterotrimeric ECM glycoproteins that form the principle components of the basement membrane and act as major ligands for integrins[74]. Laminins bind to these integrin receptors and stimulate inside-out and outside-in signalling during healing that is highly complex[74]. Activated keratinocytes at the wound edge are known to express distinct forms of laminins opposed to keratinocytes distal to the wound[75]. Furthermore, laminin receptors such as integrin α6β4 are also upregulated by keratinocytes at the wound edge and are crucial to re-epithelialisation[73,76].

It is coming to light that microRNAs are able to regulate laminin expression and that doing so affects cell migration and invasion in cancer. Recent findings reveal that all three isoforms of miR-29 (a,b and c) are involved in cancer progression by suppressing laminin γ1, reducing prostate cell migration and invasion[77]. Laminin-332 is expressed by keratinocytes at the wound-edge to speed their migration into the wound bed[78]. Laminin-332 is also microRNA-regulated; miR-205 is able to control the deposition of laminin-332 on prostate basement membrane and upregulation of its receptor α6β4 in prostate cells[79]. Replacing of miR-205 in miRNA-deficient prostate cancers restores basement membrane laminin-332 and integrin α6β4 expression on prostate cancer cells, reducing prostate cancer invasion[79]. Integrin α6β4 comes under further microRNA regulation[73]. Two microRNA families, miR-25/32/92abc/363/363-3p/367 and miR-99ab/100, are consistently downregulated by the integrin α6β4 overexpression in breast cancer[80]. Of genes regulated by α6β4, fifty-four were identified as genes targeted by these microRNA families, suggesting an extensive microRNA contribution to α6β4 signalling[80]. Furthermore, many of the gene targets predicted in this study are associated with cell motility[80]. Together these data highlight a deficit in our understanding of microRNA regulation of ECM deposition and the potential for signalling during re-epithelialisation.

Meanwhile, an exciting discovery demonstrates that aberrant miR-198 upregulation by keratinocytes comprising the edge of chronic wounds, significantly decreases laminin γ2 (LAMC2) expression[81]. Conversely, during re-epithelialisation of acute wounds, increased TGF-β expression in wound-edge keratinocytes suppresses miR-198 expression and increases LAMC2 deposition[81]. These authors did not discuss the possibility of EMT as an underlying process, but it is worth mentioning that LAMC2 induces EMT in lung adenocarcinoma[82] and that, conversely, LAMC2 expression is increased by oral squamous carcinoma cells undergoing TGF-β-induced EMT[83]. This is important, as a positive-feedback loop involving integrins and TGF-β is a mechanism proposed to induce EMT in some cancers[84]. Collectively these findings indicate that integrins and laminins are intimately linked with EMT. The discovery of miR-198-reduced LAMC2 heralds firm evidence that microRNA expression is associated with a pathological cutaneous condition in humans. The complexity of the ECM and integrin network indicates that similar discoveries will come from further investigation of this field.

Matrix metalloproteinases

To add additional complexity to the ECM and intergrin networks described above, individual components are also altered by enzymatic cleavage by the many proteases secreted into the wound milieu, including matrix metalloproteinases (MMPs). MMPs are enzymes that perform an indispensable role in wound healing. MMPs digest collagen and other ECM proteins via hydrolysis of peptide bonds to deconstruct ECM barriers that would otherwise impede the migration of cells during epithelial cell migration[85,86]. MMPs can also cleave cytokines, chemokines, surface proteins, receptors, junctional proteins and others structures, affecting signalling changes in the wound environment[85,86]. Indeed, keratinocytes at the wound margin express specific MMPs that promote re-epithelialisation[86]. With these functions in mind, it comes as no surprise that MMPs also play a role in migration and invasion of cancer[87]. In this regard, MMP expression is considered a hallmark of cancer metastasis and EMT.

To date, miR-21 is the only microRNA reported to regulate MMPs during wound healing[88]. This occurs indirectly via modulation of tissue inhibitor of MMP3 (TIMP3), which is known to inhibit MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, MMP-14 and MMP-15. TIMP-3 expression is also reported to stabilise the basement membrane during cutaneous healing[89].

MicroRNA-10b, an unusual microRNA, in that unlike the majority of microRNAs it often increases mRNA target expression, is reported to increase MMP-9 expression in nasopharyngeal cancer metastasis[90]. Notably, aberrant MMP-9 expression is associated with non-healing wounds[91]. The shortage of evidence that microRNA effects on MMPs in wound healing, in light of known effects documented in cancer studies warrants further investigation of these molecules in a wound setting.

MicroRNA-203

Much of the knowledge discussed so far that implicates microRNAs in wound healing, is derived from the study of cancer metastasis with a smattering of wound-related data. However, miR-203 and miR-21 have been studied from a cutaneous wound-healing perspective, using epidermal wound models in rodents, and deserve particular attention in this review.

MicroRNA-203 is one of the most abundant microRNAs found in the epidermis and arguably one of the most influential. Its importance stems primarily from its ability to target and repress expression of the multi-isoform transcription factor, p63[92,93]. Expressed in basal keratinocytes, p63 is responsible for initiating epidermal development, maintaining epidermal stratification and determining epidermal cell fate[94,95]. In turn p63 expression was found to increase in hyperproliferative epidermis after wounding[96] and in the leading edge of migrating wound keratinocytes[97].

In the stratifying epidermis miR-203 is expressed by suprabasal keratinocytes[92,93]. Here, miR-203 represses the translation of the p63 gene, reducing the assembly of both primary isoforms of p63 and thus restricts p63 proteins to basal keratinocytes[92,96]. Currently, it is known that p63 protein isoform ΔNp63 is the primary isoform expressed in basal keratinocytes and is responsible for the expression of their characteristic cytokeratins –14 and –5[98,99]. The culmination of these works point to a role for miR-203 in wound healing and led to investigation of miR-203 in cutaneous wound models.

In a mouse wound model, *in situ* hybridisation studies revealed that miR-203 is downregulated at the wound edge during re-epithelialisation[100].

Furthering this work in human epidermal keratinocyte cultures, these authors found that down-regulating miR-203 removes inhibition of RAPH1 expression[100]. RAPH1 induces the formation of specialised membrane protrusions termed lamellipodia[101] that are necessary for cell migration and re-epithelialisation[102]. For epithelia to extend lamellipodia requires extensive cytoskeletal reorganisation facilitated by EMT. Indeed, assessing research literature provides evidence that miR-203 downregulation by wound edge keratinocytes may be a key facilitator of EMT.

A study of EMT in MDCK cells attributed EMT regulation and cell cycle arrest to the expression of two p63 isoforms, TAp63 and ΔNp63, respectively[103]. Keratinocytes *de novo* expressed TAp63 at the wound edge during re-epithelialisation, when miR-203 expression declines, further supporting the possibility that miR-203 downregulation may be required to promote EMT during re-epithelialisation.

As mentioned above, ΔNp63 is a key determinant of keratinocyte cell fate. Its expression is associated with stem cell properties, namely the clonogenic capacity of basal keratinocytes[93]. Both ΔNp63 and TAp63 are upregulated in the hyperproliferative epidermis after wounding[96]. Observations *in vitro* from metastasising primary breast tumour revealed that invasive strands of tumour cells extending from the primary tumour mass, consistent with tumour EMT, revert to a basal phenotype expressing p63 and cytokeratin-14[104]. This basal state necessary for cancer migration is consistent with the miR-203 mediated downregulation in keratinocytes during re-epithelialisation reported by Viticchie *et al.*[100] and an indication of microRNA-regulated EMT.

The extent of miR-203 regulation broadens further still when one considers its transcription factor targets. In breast cancer, miR-203 has been described as part of a "core network" of EMT regulating factors interacting with the chief EMT drivers SNAIL[27] and SLUG[105]. Important early work found that SLUG is expressed in the margins of the healing wound[19,106]. As mentioned, TGF-β is another potent EMT inducer[28,29] and is also upregulated at the wound site after injury[33]. Thus these factors share a common expression pattern. Analysis of TGF-β-induced EMT in MDCK cells, provides strong evidence that miR-203 is a crucial downstream effector of TGF-β-induced EMT[107]. The results from this model also revealed a concomitant up-regulation of SLUG and downregulation of miR-203 in response to TGF-β signalling[107]. Furthermore, TGF-β was found to induce SLUG expression, suppressing its own inhibitor; miR-203 and entering a double-negative feedback-loop resulting in strong miR-203 repression and initiation of EMT[107].

Taken together, these works evidence that miR-203 is downregulated at the wound edge, is suppressed by TGF-β, and that a key effector of re-epithelialisation and EMT, SLUG, is upregulated in miR-203's absence. All considered, there exists a strong case placing miR-203 as an important suppressor of wound healing and suggests that a chief mechanism of action may be miR-203's ability to suppress EMT by targeting SLUG and p63.

MicroRNA-21

Many groups have attempted to define the role of miR-21 in wound healing, yet current data supports neither enhanced nor delayed healing. Yang and colleagues report increased endogenous miR-21 expression during healing in a mouse epidermal wound model, concomitant with TGF-β induction at the wound edge[88]. The authors then delayed re-epithelialisation by knocking down endogenous miR-21 expression[88]. These results build on the previous finding that TGF-β upregulates miR-21 in keratinocytes *in vitro*[108]. In a link to pathology, TGF-β signalling is attenuated in non-healing venous ulcers[109]. Interestingly, in contrast to these results it has been reported that increasing miR-21 levels impaired re-epithelialisation in ex vivo human and *in vivo* rat skin wound models[110]. Experiments aiming to resolve miR-21's role in healing, revealed that miR-21 antagonists injected subdermally delay re-epithelialisation[61]. Thus the sum of evidence currently favours miR-21 as a promoter of re-epithelialisation.

To better understand the apparent discrepancies in miR-21's effect on healing, targets suspected of mediating miR-21's function have been interrogated. Yang *et al.* investigated T-cell lymphoma invasion, metastasis-inducing protein 1 (TIAM-1) and TIMP-3 (discussed earlier)[88]. Pastar *et al.,* on the other hand, explored the roles of the leptin receptor (LepR) and early growth response factor 3 (EGR3)[110]. Interestingly, in light of these miR-21 targets, neither author discussed a possible role for miR-21 in regulating wound-edge EMT. Yet, investigating these targets in the literature reveals evidence that EMT may be an overarching process encompassing miR-21's effect on re-epithelialisation. To this effect miR-21 target LepR, is suppressed during re-epithelialisation and is also targeted and repressed by miR-200c expression[15]. As a member of the miR-200 family, miR-200c has been thoroughly associated with EMT induction[17]. Furthermore, in a study of breast cancer cells, miR-200c's inhibitory effect on metastasis was linked to LepR suppression[70]. To continue, suppression of the miR-21 target, TIMP-3, permits migration and invasion in several cancer types[111-114] and its expression is suppressed by several microRNAs upregulated during metastasis[114-116], including miR-21 in melanoma[115]. Moreover, the EMT transcription factor SNAIL also suppresses TIMP-3 during induction of an EMT[117]. The miR-21 target, TIAM-1, was discovered as a factor dictating T lymphocyte invasion and is implicated in cancer metastasis under the regulation of miR-21[118,119]. In wound-healing, TIAM-1 is important for the redistribution of hemidesmosome components which are crucial components of lamellipodia at the wound edge[76]. All considered, these studies indicate that miR-21 plays a role in wound healing. However, for now, resolution of a definitive role for miR-21 in healing requires further experimentation, but clues may come from the wealth of miR-21 knowledge acquired in cancer research.

MiR-21 has a convincing association with cancer metastasis thus being ascribed "oncomiR" status, firmly placing miR-21 in the field of oncology[120]. Indeed, miR-21 induces EMT in many epithelial cell types including, but not limited to, human proximal tubular epithelial cells[121], lung epithelial cells[122] and prostate basal cells[123] producing a neoplastic state. However, like studies of miR-21 in wound healing, the role of miR-21 during EMT in cancer appears to be contextual and, to date, the mechanisms behind this phenomenon are unknown. It seems a major aspect of miR-21 function is intrinsic to its relationship with TGF-β. TGF-β's function in cancer is paradoxical: in the early stages of tumourigenesis TGF-β

provides tumour suppressive functions, and in later stages of tumour development, TGF-β induces migratory and invasive attributes[28,124]. As mentioned, exposing cells to TGF-β is a well-established EMT model and has provided data resolving the role of other microRNAs in EMT, especially the miR-200 family[30,125,126]. HaCaT cells exposed to TGF-β undergo EMT and increase their expression of miR-21[127]. However, subsequent miR-21 over-expression and knockdown experiments could not provide evidence to support miR-21 as an EMT mediator[127,128]. Interestingly, induction of miR-21 in the mouse neoplastic keratinocyte-papilloma-derived cell line resulted in *de novo* expression of several EMT markers[128]. Pertaining to this model, it was concluded that EMT can be driven by miR-21 expression in keratinocytes, but only in the context of premalignancy[128]. The mechanisms behind this switch in functional outcomes due to TGF-β exposure are unclear, but evidence indicates that microenvironment ECM components, integrins and microRNAs, contribute to the change[28,124]. Nonetheless it is a fortuitous link to wound healing that keratinocytes were used in these experiments and provides more clues to miR-21's role here, as indeed, a drastically altered microenvironment occurs in the healing wound. Keratinocytes at the wound edge are reported to enter a state of "activation" during re-epithelialisation[106,129], where they are hyper-responsive to growth factors and cytokines in the wound environment. Whether this state of activation is sufficient to permit miR-21-induced EMT akin to that observed by Bornachea and colleagues in neoplastic keratinocytes, is yet to be tested[128].

Thus, *in vivo* cutaneous wound-healing models have established that miR-21 does have a role in re-epithelialisation. However, the question as to whether miR-21 regulates EMT at the wound-edge remains equivocal. The wealth of data linking miR-21 to EMT, the strong connection between miR-21 and the injury-induced cytokine TGF-β, and the findings that miR-21 can inhibit or enhance re-epithelialisation, supports the possibility that miR-21 upregulation at the wound edge may initiate and maintain EMT during re-epithelialisation.

CONCLUSIONS

Work comparing facets of EMT in wound healing and cancer has presented compelling evidence of the similarity inherent in these seemingly disparate cellular processes[4]. Evidence of key functions and regulatory roles for microRNAs in EMT is accumulating. In this vein, a growing number of studies are focussed on the roles microRNA might occupy in the wound-healing cascade. However, while it is acknowledged that type II EMT occurs in epithelium at the wound edge, the mechanisms that regulate EMT during re-epithelialisation remain poorly understood. It is evident that microRNAs are key regulators of EMT and some regulate re-epithelialisation at the wound edge. However, it is also clear that a large deficit remains in our understanding of microRNA regulation of wound healing, particularly in the regulation of the individual modules, like cytoskeletal remodelling, that comprise an EMT. Thus, discerning the species of microRNAs that regulate wound-edge EMT warrants further research. As these data accumulate, we will gain a clearer understanding of the role that non-coding RNAs play in normal physiological processes and how dysregulation leads to disease and morbidity. Ultimately, better understanding microRNAs in wound healing may lead to novel therapeutics in wound healing. In this regard, microRNAs even display certain advantages over protein therapeutics. For example, synthetic microRNAs are many times less expensive to generate compared to proteins. As described here, they act upstream of protein synthesis and in this way affect complex networks of signalling. However, inherent in this benefit is the increased risk of off-target effects. Yet it is clear that microRNAs present exciting possibilities for improved wound care and that further research in this field is well founded.

ACKNOWLEDGEMENT

Funded by the Wound Management Innovation Cooperative Research Centre and the Australian Government.

REFERENCES

1. Nakamura M & Tokura Y. Epithelial–mesenchymal transition in the skin. J Dermatol Sci 2011; 61(1):7–13.
2. Yan C, Grimm WA, Garner WL, Qin L, Travis T & Tan N. Epithelial to mesenchymal transition in human skin wound healing is induced by tumor necrosis factor-α through bone morphogenic protein-2. Am J Pathol 2010; 176(5):2247–58.
3. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG & Sawaya A. Epithelialization in wound healing: a comprehensive review. Adv Wound Care 2014; 3(7):445–64.
4. Leopold PL, Vincent J & Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. Semin Cancer Biol 2012; 22(5–6):471–83.
5. Kalluri R& Weinberg RA. The basics of epithelial–mesenchymal transition. J Clin Investig 2009; 119(6):1420–8.
6. Lamouille S, Xu J & Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15(3):178–96.
7. Ul Hussain M. Micro-RNAs (miRNAs): genomic organisation, biogenesis and mode of action. Cell Tissue Res 2012; 349(2):405–13.
8. Sayed D & Abdellatif M. MicroRNAs in development and disease. Physiol Rev 2011; 91(3):827–87.
9. Lamouille S, Subramanyam D, Blelloch R & Derynck R. Regulation of epithelial–mesenchymal and mesenchymal–epithelial transitions by microRNAs. Curr Opin Cell Biol 2013; 25(2):200–7.
10. Lujambio A & Lowe SW. The microcosmos of cancer. Nature 2012; 482(7385):347–55.
11. Riemondy K, Hoefert JE & Yi R. Not miR-ly micromanagers: the functions and regulatory networks of microRNAs in mammalian skin. Wiley Interdiscip Rev RNA 2014; 5(6):849–65.
12. Simpson CL, Patel DM & Green KJ. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol 2011; 12(9):565–80.
13. Li L, Hartley R, Reiss B, Sun Y, Pu J & Wu D. E-cadherin plays an essential role in collective directional migration of large epithelial sheets. Cell Mol Life Sci 2012; 69(16):2779–89.
14. Moreno-Bueno G, Peinado H, Molina P, Olmeda D, Cubillo E & Santos V. The morphological and molecular features of the epithelial-to-mesenchymal transition. Nat Protoc 2009; 4(11):1591–613.
15. Park S-M, Gaur AB, Lengyel E & Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 2008; 22(7):894–907.
16. Moreno-Bueno G, Cubillo E, Sarrió D, Peinado H, Rodríguez-Pinilla SM & Villa S. Genetic profiling of epithelial cells expressing E-cadherin repressors reveals a distinct role for Snail, Slug, and E47 factors in epithelial–mesenchymal transition. Cancer Res 2006; 66(19):9543–56.

17. Díaz-Martín J, Díaz-López A, Moreno-Bueno G, Castilla M, Rosa-Rosa JM & Cano A. A core microRNA signature associated with inducers of the epithelial-to-mesenchymal transition. J Pathol 2014; 232(3):319–29.
18. Savagner P, Yamada KM & Thiery JP. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial–mesenchymal transition. J Cell Biol 1997; 137(6):1403–19.
19. Savagner P, Kusewitt DF, Carver EA, Magnino F, Choi C & Gridley T. Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes. J Cell Physiol 2005; 202(3):858–66.
20. Aomatsu K, Arao T, Abe K, Kodama A, Sugioka K & Matsumoto K. Slug is upregulated during wound healing and regulates cellular phenotypes in corneal epithelial cells. Invest Ophthalmol Vis Sci 2012; 53(2):751–6.
21. Hudson LG, Newkirk KM, Chandler HL, Choi C, Fossey SL & Parent AE. Cutaneous wound reepithelialization is compromised in mice lacking functional Slug (Snai2). J Dermatol Sci 2009; 56(1):19–26.
22. Liu YN, Yin JJ, Abou-Kheir W, Hynes PG, Casey OM & Fang L. MiR-1 and miR-200 inhibit EMT via Slug-dependent and tumorigenesis via Slug-independent mechanisms. Oncogene 2013; 32(3):296–306.
23. Liang Y-J, Wang Q-Y, Zhou C-X, Yin Q-Q, He M & Yu X-T. MiR-124 targets Slug to regulate epithelial–mesenchymal transition and metastasis of breast cancer. Carcinogenesis 2012: bgs383.
24. Lee JY, Park MK, Park JH, Lee HJ, Shin DH & Kang Y. Loss of the polycomb protein Mel-18 enhances the epithelial–mesenchymal transition by ZEB1 and ZEB2 expression through the downregulation of miR-205 in breast cancer. Oncogene 2014; 33(10):1325–35.
25. Tran MN, Choi W, Wszolek MF, Navai N, Lee IL & Nitti G. The p63 protein isoform DeltaNp63alpha inhibits epithelial–mesenchymal transition in human bladder cancer cells: role of MIR-205. J Biol Chem 2013; 288(5):3275–88.
26. Tucci P, Agostini M, Grespi F, Markert EK, Terrinoni A & Vousden KH. Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. Proc Natl Acad Sci U S A 2012; 109(38):15312–7.
27. Moes M, Le Bechec A, Crespo I, Laurini C, Halavatyi A & Vetter G. A novel network integrating a miRNA-203/SNAI1 feedback loop which regulates epithelial to mesenchymal transition. PloS One 2012; 7(4):e35440.
28. Morrison CD, Parvani JG & Schiemann WP. The relevance of the TGF-beta Paradox to EMT-MET programs. Cancer Lett 2013; 341(1):30–40.
29. Moustakas A & Heldin C-H (Eds). Induction of epithelial–mesenchymal transition by transforming growth factor β. Semin Cancer Biol 2012; Elsevier.
30. Gregory PA, Bracken CP, Smith E, Bert AG, Wright JA & Roslan S. An autocrine TGF-β/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial–mesenchymal transition. Mol Biol Cell 2011; 22(10):1686–98.
31. Kumarswamy R, Mudduluru G, Ceppi P, Muppala S, Kozlowski M & Niklinski J. MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snai1 and is downregulated in non-small cell lung cancer. Int J Cancer 2012; 130(9):2044–53.
32. Suzuki T, Mizutani K, Minami A, Nobutani K, Kurita S & Nagino M. Suppression of the TGF-β1-induced protein expression of SNAI1 and N-cadherin by miR-199a. Genes Cells 2014; 19(9):667–75.
33. Weber CE, Li NY, Wai PY & Kuo PC. Epithelial–mesenchymal transition, TGF-β, and osteopontin in wound healing and tissue remodeling after injury. J Burn Care Res 2012; 33(3):311–8.
34. Abreu-Blanco MT, Watts JJ, Verboon JM & Parkhurst SM. Cytoskeleton responses in wound repair. Cell Mol Life Sci 2012; 69(15):2469–83.

35. Spiering D & Hodgson L. Dynamics of the Rho-family small GTPases in actin regulation and motility. Cell Adh Migr 2011; 5(2):170–80.
36. Zhang M, Liu N-Y, Wang X-E, Chen Y-H, Li Q-L & Lu K-R. Activin B promotes epithelial wound healing *in vivo* through RhoA-JNK signaling pathway. PloS One 2011; 6(9):e25143.
37. Desai LP, Aryal AM, Ceacareanu B, Hassid A & Waters CM. RhoA and Rac1 are both required for efficient wound closure of airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2004; 287(6):L1134–L44.
38. Yin J, Lu J & Fu-Shin XY. Role of small GTPase Rho in regulating corneal epithelial wound healing. Invest Ophthalmol Vis Sci 2008; 49(3):900.
39. Omelchenko T, Vasiliev J, Gelfand I, Feder H & Bonder E. Rho-dependent formation of epithelial "leader" cells during wound healing. Proc Natl Acad Sci U S A 2003; 100(19):10788–93.
40. Yin J, Lu J & Fu-Shin XY. Role of small GTPase Rho in regulating corneal epithelial wound healing. Invest Ophthalmol Vis Sci 2008; 49(3):900–9.
41. Kong W, Yang H, He L, Zhao J-j, Coppola D & Dalton WS. MicroRNA-155 is regulated by the transforming growth factor β/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol 2008; 28(22):6773–84.
42. Papadimitriou E, Vasilaki E, Vorvis C, Iliopoulos D, Moustakas A & Kardassis D. Differential regulation of the two RhoA-specific GEF isoforms Net1/Net1A by TGF-beta and miR-24: role in epithelial-to-mesenchymal transition. Oncogene 2012; 31(23):2862–75.
43. Amelio I, Lena AM, Viticchie G, Shalom-Feuerstein R, Terrinoni A & Dinsdale D. miR-24 triggers epidermal differentiation by controlling actin adhesion and cell migration. J Cell Biol 2012; 199(2):347–63.
44. Yu J, Peng H, Ruan Q, Fatima A, Getsios S & Lavker RM. MicroRNA-205 promotes keratinocyte migration via the lipid phosphatase SHIP2. FASEB J 2010; 24(10):3950–9.
45. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A & Farshid G. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008; 10(5):593–601.
46. Orang AV, Safaralizadeh R & Hosseinpour Feizi M. Insights into the diverse roles of miR-205 in human cancers. Asian Pac J Cancer Prev 2014; 15(2):577–83.
47. Bertero T, Gastaldi C, Bourget-Ponzio I, Imbert V, Loubat A & Selva E. miR-483-3p controls proliferation in wounded epithelial cells. FASEB J 2011; 25(9):3092–105.
48. Song Q, Xu Y, Yang C, Chen Z, Jia C & Chen J. miR-483-5p promotes invasion and metastasis of lung adenocarcinoma by targeting RhoGDI1 and ALCAM. Cancer Res 2014; 74(11):3031–42.
49. Cory GO & Ridley AJ. Cell motility: braking WAVEs. Nature 2002; 418(6899):732–3.
50. Jiang WG, Ye L, Patel G & Harding KG. Expression of WAVEs, the WASP (Wiskott-Aldrich syndrome protein) family of verprolin homologous proteins in human wound tissues and the biological influence on human keratinocytes. Wound Repair Regen 2010; 18(6):594–604.
51. Sossey-Alaoui K, Bialkowska K & Plow EF. The miR200 family of microRNAs regulates WAVE3-dependent cancer cell invasion. J Biol Chem 2009; 284(48):33019–29.
52. Sossey-Alaoui K, Downs-Kelly E, Das M, Izem L, Tubbs R & Plow EF. WAVE3, an actin remodeling protein, is regulated by the metastasis suppressor microRNA, miR-31, during the invasion-metastasis cascade. Int J Cancer 2011; 129(6):1331–43.
53. Taylor MA, Davuluri G, Parvani JG, Schiemann BJ, Wendt MK & Plow EF. Upregulated WAVE3 expression is essential for TGF-beta-mediated EMT and metastasis of triple-negative breast cancer cells. Breast Cancer Res Treat 2013; 142(2):341–53.
54. O'Toole E. Extracellular matrix and keratinocyte migration. Clin Exp Dermatol 2001; 26(6):525–30.
55. Leopold PL, Vincent J & Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. Semin Cancer Biol 2012; 22(5–6):471–83.
56. Nakamura M & Tokura Y. Epithelial–mesenchymal transition in the skin. J Dermatol Sci 2011; 61(1):7–13.
57. Rutnam ZJ, Wight TN & Yang BB. miRNAs regulate expression and function of extracellular matrix molecules. Matrix Biol 2013; 32(2):74–85.
58. Pilcher B, Sudbeck B, Dumin J, Welgus H & Parks W. Collagenase-1 and collagen in epidermal repair. Arch Dermatol Res 1998; 290(1):S37–S46.
59. DeMaio L, Buckley ST, Krishnaveni MS, Flodby P, Dubourd M & Banfalvi A. Ligand-independent transforming growth factor-β type I receptor signalling mediates type I collagen-induced epithelial–mesenchymal transition. J Pathol 2012; 226(4):633–44.
60. Shintani Y, Maeda M, Chaika N, Johnson KR & Wheelock MJ. Collagen I promotes epithelial-to-mesenchymal transition in lung cancer cells via transforming growth factor-β signaling. Am J Respir Cell Mol Biol 2008; 38(1):95–104.
61. Wang T, Feng Y, Sun H, Zhang L, Hao L & Shi C. miR-21 regulates skin wound healing by targeting multiple aspects of the healing process. Am J Pathol 2012; 181(6):1911–20.
62. Li C, Nguyen HT, Zhuang Y, Lin Y, Flemington EK & Guo W. Post-transcriptional up-regulation of miR-21 by type I collagen. Mol Carcinog 2011; 50(7):563–70.
63. Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA & Cheng YJ. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci U S A 2008; 105(15):5874–8.
64. Jiang H, Zhang G, Wu J-H & Jiang C-P. Diverse roles of miR-29 in cancer (Review). Oncol Rep 2014; 31(4):1509–16.
65. Wang Y, Zhang X, Li H, Yu J & Ren X. The role of miRNA-29 family in cancer. Eur J Cell Biol 2013; 92(3):123–8.
66. Li H, Solomon E, Muggy SD, Sun D & Zolkiewska A. Metalloprotease-disintegrin ADAM12 expression is regulated by Notch signaling via microRNA-29. J Biol Chem 2011; 286(24):21500–10.
67. Midwood KS, Mao Y, Hsia HC, Valenick LV & Schwarzbauer JE. Modulation of cell-fibronectin matrix interactions during tissue repair. J Investig Dermatol Symp Proc 2006; 11(1):73–8.
68. Wang F, Song G, Liu M, Li X & Tang H. miRNA-1 targets fibronectin1 and suppresses the migration and invasion of the HEp2 laryngeal squamous carcinoma cell line. FEBS Lett 2011; 585(20):3263–9.
69. Qian P, Zuo Z, Wu Z, Meng X, Li G & Zhang W. Pivotal role of reduced let-7g expression in breast cancer invasion and metastasis. Cancer Res 2011; 71(20):6463–74.
70. Howe EN, Cochrane DR & Richer JK. Targets of miR-200c mediate suppression of cell motility and anoikis resistance. Breast Cancer Res 2011; 13(2):R45.
71. Shan SW, Lee DY, Deng Z, Shatseva T, Jeyapalan Z & Du WW. MicroRNA MiR-17 retards tissue growth and represses fibronectin expression. Nat Cell Biol 2009; 11(8):1031–8.
72. Ou J, Peng Y, Deng J, Miao H, Zhou J & Zha L. Endothelial cell-derived fibronectin extra domain A promotes colorectal cancer metastasis via inducing epithelial–mesenchymal transition. Carcinogenesis 2014: bgu090.
73. Koivisto L, Heino J, Hakkinen L & Larjava H. Integrins in wound healing. Adv Wound Care (New Rochelle) 2014; 3(12):762–83.
74. Domogatskaya A, Rodin S & Tryggvason K. Functional diversity of laminins. Annu Rev Cell Dev Biol 2012; 28:523–53.
75. Tzu J & Marinkovich MP. Bridging structure with function: structural, regulatory, and developmental role of laminins. Int J Biochem Cell Biol 2008; 40(2):199–214.
76. Litjens SH, de Pereda JM & Sonnenberg A. Current insights into the formation and breakdown of hemidesmosomes. Trends Cell Biol 2006; 16(7):376–83.

77. Nishikawa R, Goto Y, Kojima S, Enokida H, Chiyomaru T & Kinoshita T. Tumor-suppressive microRNA-29s inhibit cancer cell migration and invasion via targeting LAMC1 in prostate cancer. Int J Oncol 2014; 45(1):401–10.
78. Litjens SH, de Pereda JM & Sonnenberg A. Current insights into the formation and breakdown of hemidesmosomes. Trends Cell Biol 2006; 16(7):376–83.
79. Gandellini P, Profumo V, Casamichele A, Fenderico N, Borrelli S & Petrovich G. miR-205 regulates basement membrane deposition in human prostate: implications for cancer development. Cell Death Differ 2012; 19(11):1750–60.
80. Gerson KD, Maddula VS, Seligmann BE, Shearstone JR, Khan A & Mercurio AM. Effects of beta4 integrin expression on microRNA patterns in breast cancer. Biol Open 2012; 1(7):658–66.
81. Sundaram GM, Common JE, Gopal FE, Srikanta S, Lakshman K & Lunny DP. 'See-saw' expression of microRNA-198 and FSTL1 from a single transcript in wound healing. Nature 2013; 495(7439):103–6.
82. Moon Y, Rao G, Kim J, Shim H, Park K & An S. LAMC2 enhances the metastatic potential of lung adenocarcinoma. Cell Death Differ 2015.
83. Richter P, Umbreit C, Franz M, Berndt A, Grimm S & Uecker A. EGF/TGFβ1 co-stimulation of oral squamous cell carcinoma cells causes an epithelial–mesenchymal transition cell phenotype expressing laminin 332. J Oral Pathol Med 2011; 40(1):46–54.
84. Mamuya FA & Duncan MK. aV integrins and TGF-β-induced EMT: a circle of regulation. J Cell Mol Med 2012; 16(3):445–55.
85. Steffensen B, Hakkinen L & Larjava H. Proteolytic events of wound-healing — coordinated interactions among matrix metalloproteinases (MMPs), integrins, and extracellular matrix molecules. Crit Rev Oral Biol Med 2001; 12(5):373–98.
86. Martins VL, Caley M & O'Toole EA. Matrix metalloproteinases and epidermal wound repair. Cell Tissue Res 2013; 351(2):255–68.
87. Nistico P, Bissell MJ & Radisky DC. Epithelial–mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. Cold Spring Harb Perspect Biol 2012; 4(2).
88. Yang X, Wang J, Guo SL, Fan KJ, Li J & Wang YL. miR-21 promotes keratinocyte migration and re-epithelialization during wound healing. Int J Biol Sci 2011; 7(5):685–90.
89. Vaalamo M, Leivo T & Saarialho-Kere U. Differential expression of tissue inhibitors of metalloproteinases (TIMP-1,-2,-3, and-4) in normal and aberrant wound healing. Hum Pathol 1999; 30(7):795–802.
90. Sun X-J, Liu H, Zhang P, Zhang X-D, Jiang Z-W & Jiang C-C. miR-10b promotes migration and invasion in nasopharyngeal carcinoma cells. Asian Pac J Cancer Prev 2013; 14(9):5533–7.
91. Rohl J & Murray R. Matrix metalloproteinases during wound healing — a double edged sword. Wound Practice Research 2013; 21(4):174–82.
92. Yi R, Poy MN, Stoffel M & Fuchs E. A skin microRNA promotes differentiation by repressing 'stemness'. Nature 2008; 452(7184):225–9.
93. Lena A, Shalom-Feuerstein R, di Val Cervo PR, Aberdam D, Knight R & Melino G. miR-203 represses stemness by repressing Np63. Cell Death Differ 2008; 15(7):1187.
94. Mills AA, Zheng B, Wang X-J, Vogel H, Roop DR & Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398(6729):708–13.
95. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N & Bronson RT. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398(6729):714–8.

96. Bamberger C, Hafner A, Schmale H & Werner S. Expression of different p63 variants in healing skin wounds suggests a role of p63 in reepithelialization and muscle repair. Wound Repair Regen 2005; 13(1):41–50.

97. Kurokawa I, Mizutani H, Kusumoto K, Nishijima S, Tsujita-Kyutoku M & Shikata N. Cytokeratin, filaggrin, and p63 expression in reepithelialization during human cutaneous wound healing. Wound Repair Regen 2006; 14(1):38–45.

98. Romano RA, Ortt K, Birkaya B, Smalley K & Sinha S. An active role of the ΔN isoform of p63 in regulating basal keratin genes K5 and K14 and directing epidermal cell fate. PLoS One 2009; 4(5).

99. Romano R-A, Smalley K, Magraw C, Serna VA, Kurita T & Raghavan S. ΔNp63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation. Development 2012; 139(4):772–82.

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

101. Krause M, Leslie JD, Stewart M, Lafuente EM, Valderrama F & Jagannathan R. Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. Dev Cell 2004; 7(4):571–83.

102. Koivisto L, Alavian K, Häkkinen L, Pelech S, McCulloch CA & Larjava H. Glycogen synthase kinase-3 regulates formation of long lamellipodia in human keratinocytes. J Cell Sci 2003; 116(18):3749–60.

103. Zhang Y, Yan W & Chen X. P63 regulates tubular formation via epithelial-to-mesenchymal transition. Oncogene 2014; 33(12):1548–57.

104. Cheung KJ, Gabrielson E, Werb Z & Ewald AJ. Collective invasion in breast cancer requires a conserved basal epithelial program. Cell 2013; 155(7):1639–51.

105. Zhang Z, Zhang B, Li W, Fu L, Fu L & Zhu Z. Epigenetic silencing of miR-203 upregulates SNAI2 and contributes to the invasiveness of malignant breast cancer cells. Genes Cancer 2011; 2(8):782–91.

106. Arnoux V, Nassour M, L'Helgoualc'h A, Hipskind RA & Savagner P. Erk5 controls Slug expression and keratinocyte activation during wound healing. Mol Biol Cell 2008; 19(11):4738–49.

107. Ding X, Park SI, McCauley LK & Wang C-Y. Signaling between transforming growth factor β (TGF-β) and transcription factor SNAI2 represses expression of microRNA miR-203 to promote epithelial–mesenchymal transition and tumor metastasis. J Biol Chem 2013; 288(15):10241–53.

108. Zavadil J, Narasimhan M, Blumenberg M & Schneider RJ. Transforming growth factor-beta and microRNA: mRNA regulatory networks in epithelial plasticity. Cells, Tissues, Organs 2006; 185(1–3):157–61.

109. Pastar I, Stojadinovic O, Krzyzanowska A, Barrientos S, Stuelten C & Zimmerman K. Attenuation of the transforming growth factor β-signaling pathway in chronic venous ulcers. Mol Med 2010; 16(3–4):92.

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

111. Li J, Zhang Y, Zhang W, Jia S, Tian R & Kang Y. Genetic heterogeneity of breast cancer metastasis may be related to miR-21 regulation of TIMP-3 in translation. Int J Surg Oncol 2013; 2013:875078.

112. Martin del Campo SE, Latchana N, Levine KM, Grignol VP, Fairchild ET & Jaime-Ramirez AC. MiR-21 enhances melanoma invasiveness via inhibition of tissue inhibitor of metalloproteinases 3 expression: *in vivo* effects of MiR-21 inhibitor. PloS One 2015; 10(1):e0115919.

113. Wang N, Zhang CQ, He JH, Duan XF, Wang YY & Ji X. MiR-21 down-regulation suppresses cell growth, invasion and induces cell apoptosis by targeting FASL, TIMP3, and RECK genes in esophageal carcinoma. Dig Dis Sci 2013; 58(7):1863–70.

114. Xu C, Hou Z, Zhan P, Zhao W, Chang C & Zou J. EZH2 regulates cancer cell migration through repressing TIMP-3 in non-small cell lung cancer. Med Oncol 2013; 30(4):713.

115. Martin Del Campo SE, Latchana N, Levine KM, Grignol VP, Fairchild ET & Jaime-Ramirez AC. MiR-21 Enhances melanoma invasiveness via inhibition of tissue inhibitor of metalloproteinases 3 expression: *in vivo* effects of MiR-21 inhibitor. PLoS One 2015; 10(1):e0115919.

116. Yang X, Du WW, Li H, Liu F, Khorshidi A & Rutnam ZJ. Both mature miR-17-5p and passenger strand miR-17-3p target TIMP3 and induce prostate tumor growth and invasion. Nucleic Acids Res 2013; 41(21):9688–704.

117. Jin H, Yu Y, Zhang T, Zhou X, Zhou J & Jia L. Snail is critical for tumor growth and metastasis of ovarian carcinoma. Int J Cancer 2010; 126(9):2102–11.

118. Cottonham CL, Kaneko S & Xu L. miR-21 and miR-31 converge on TIAM1 to regulate migration and invasion of colon carcinoma cells. J Biol Chem 2010; 285(46):35293–302.

119. Huang YH, Lin YH, Chi HC, Liao CH, Liao CJ & Wu SM. Thyroid hormone regulation of miR-21 enhances migration and invasion of hepatoma. Cancer Res 2013; 73(8):2505–17.

120. Kumarswamy R, Volkmann I & Thum T. Regulation and function of miRNA-21 in health and disease. RNA Biol 2011; 8(5):706–13.

121. Wang J-Y, Gao Y-B, Zhang N, Zou D-W, Wang P & Zhu Z-Y. miR-21 overexpression enhances TGF-β1-induced epithelial-to-mesenchymal transition by target smad7 and aggravates renal damage in diabetic nephropathy. Mol Cell Endocrinol 2014; 392(1):163–72.

122. Yamada M, Kubo H, Ota C, Takahashi T, Tando Y & Suzuki T. The increase of microRNA-21 during lung fibrosis and its contribution to epithelial–mesenchymal transition in pulmonary epithelial cells. Respir Res 2013; 14(95).

123. Coppola V, Musumeci M, Patrizii M, Cannistraci A, Addario A & Maugeri-Sacca M. BTG2 loss and miR-21 upregulation contribute to prostate cell transformation by inducing luminal markers expression and epithelial–mesenchymal transition. Oncogene 2013; 32(14):1843–53.

124. Massagué J. TGFβ signalling in context. Nat Rev Mol Cell Biol 2012; 13(10):616–30.

125. Wendt MK, Tian M & Schiemann WP. Deconstructing the mechanisms and consequences of TGF-β-induced EMT during cancer progression. Cell Tissue Res 2012; 347(1):85–101.

126. Xiong M, Jiang L, Zhou Y, Qiu W, Fang L & Tan R. The miR-200 family regulates TGF-β1-induced renal tubular epithelial to mesenchymal transition through Smad pathway by targeting ZEB1 and ZEB2 expression. Am J Physiol Renal Physiol 2012; 302(3):F369–F79.

127. Wang T, Zhang L, Shi C, Sun H, Wang J & Li R. TGF-β-induced miR-21 negatively regulates the antiproliferative activity but has no effect on EMT of TGF-β in HaCaT cells. Int J Biochem Cell Biol 2012; 44(2):366–76.

128. Bornachea O, Santos M, Martínez-Cruz AB, García-Escudero R, Duenas M & Costa C. EMT and induction of miR-21 mediate metastasis development in Trp53-deficient tumours. Scientific reports 2012; 2.

129. Stojadinovic O, Pastar I, Vukelic S, Mahoney MG, Brennan D & Krzyzanowska A. Deregulation of keratinocyte differentiation and activation: a hallmark of venous ulcers. J Cell Mol Med. 2008; 12(6B):2675–90.