

EXPERT
REVIEWS

miRNAs in inflammatory skin diseases and their clinical implications

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miRNAs are a class of non-coding RNA molecules that modulate gene expression post-transcriptionally. They have a major impact on several physiological and pathological cellular processes including modulation of the innate and the adaptive immune system. The role of miRNAs in skin biology is still incomplete; however, it is known that miRNAs are implicated in various cellular processes of both normal and diseased skin. Some miRNAs appear to be consistently deregulated in several different inflammatory skin diseases, including psoriasis and atopic dermatitis, indicating a common role in fundamental biological processes. The clinical implications of miRNAs are intriguing, both from a diagnostic and a therapeutic perspective. Accordingly, there is emerging evidence for the clinical potential of miRNAs as both biomarkers and possible therapeutic targets in skin diseases. Future studies will hopefully establish the biological significance of miRNAs in skin biology, paving the way for new miRNA-based diagnostic and therapeutic applications in dermatology.

KEYWORDS: allergic contact dermatitis • atopic dermatitis • biomarkers • miRNAs • non-coding RNA • psoriasis

The human skin is a complex immunological organ, that forms an effective barrier between the internal and external environment, preventing invasion of pathogens and allowing regulation of water loss and protection against allergens and irritants. Structurally, the human skin has two compartments, the epidermis and the dermis, both of which are composed of a plethora of different cells that interact in a complex network to maintain the barrier function. Of these cells, there are both resident and infiltrating immune cells, which have a significant role in the interplay between the innate and the adaptive immune system. Disturbance of the skin barrier is a central event in various inflammatory skin diseases, including psoriasis and atopic dermatitis (AD).

Although much effort has been made to characterize the underlying disease mechanisms of various inflammatory skin diseases through extensive research, much still remains unclear. However, it has become evident that most inflammatory skin diseases are influenced by a variety of genetic and environmental factors. In addition, a number of different molecules have been proposed to contribute to the

complicated disease mechanisms. Among these, the miRNAs, which are small regulatory non-coding RNA molecules, have been shown to be involved in various cellular processes in both normal skin and diseased skin.

miRNAs

miRNAs are an abundant class of highly conserved small non-coding RNA molecules that modulate gene expression post-transcriptionally [1–3]. The first miRNAs to be discovered, *lin-4* and *let-7*, were found in *Caenorhabditis elegans* as genes important in coordination of the developmental timing [4,5]. The term miRNA was introduced in 2000, when the first human miRNA was discovered [6]. Since their discovery, they have received considerable attention in basic research and much progress has been made in elucidating miRNA expression patterns and functions. Currently, 2588 mature miRNAs in humans have been registered in the miRBase database (release 21.0, June 2014, [7]) and it is estimated that more than 60% of all human protein-coding genes are regulated by miRNAs, making them one of the most abundant classes of gene-regulatory molecules [8].

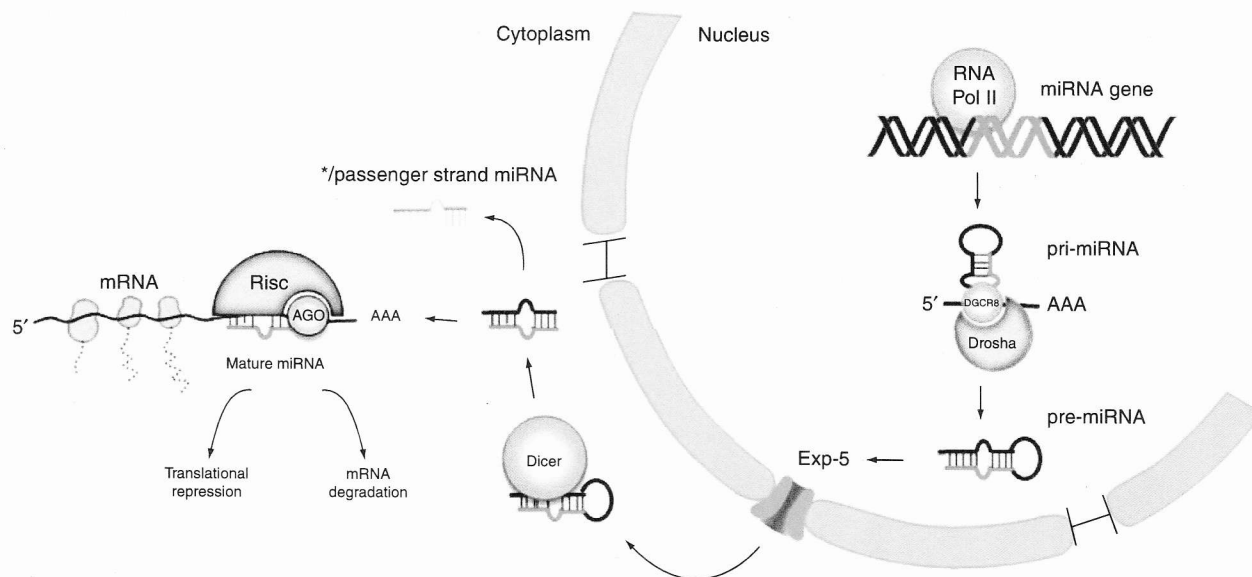


Figure 1. miRNA biogenesis. Most of the mammalian miRNAs are transcribed from different regions of the genome by RNA polymerase II. The primary miRNA transcript (pri-miRNA) is usually several kilobases long, poladenylated at its 3' end and 5'-capped, similarly to mRNAs. The pri-miRNA is processed by the microprocessor that includes Drosha, an RNase III enzyme and its cofactor DGCR8 (DiGeorge syndrome critical region gene 8), to produce the 60- to 70-nucleotide hairpin precursor miRNA (pre-miRNA). The pre-miRNA is actively exported from the nucleus to the cytoplasm by exportin 5 (Exp-5). In the cytoplasm, the pre-miRNA is further cleaved by a second RNase III enzyme, Dicer, which results in generation of a small 21- to 24-nucleotide RNA duplex. The two strands of the duplex are separated, and one of the strands (designated the mature strand) is incorporated into an RISC, the core components of which are members of the AGO. Strand selection is determined by inherent features of the miRNA duplex, including thermodynamic asymmetry/stability. The other strand (the passenger strand or miRNA*) is assumed to be excluded from RISC incorporation and is subsequently degraded. The mature miRNA guides RISC to its specific mRNA target and directs inhibition of translation or mRNA degradation [9]. AGO: Argonaute protein family; RISC: RNA-induced silencing complex.

miRNA biogenesis

miRNAs modulate gene expression by sequence-specific base pairing, usually within the 3' untranslated region (UTR) of the target mRNA. In mammalian cells, more than 70% of miRNAs are located within an intron, with the majority residing in a protein-coding gene. The remaining miRNAs are located in non-coding RNA transcripts [9]. The majority of mammalian miRNAs are transcribed by RNA polymerase II [10]. The transcribed long primary miRNA is processed by the microprocessor consisting – among other molecules – of Drosha (an RNase III enzyme) and its cofactor DGCR8 (DiGeorge syndrome critical region gene 8), to produce the 60- to 70-nucleotide hairpin precursor miRNA (pre-miRNA) [9]. After nuclear processing, the pre-miRNA is transferred to the cytosol by exportin 5 [11], where another RNase III enzyme, Dicer, cleaves the pre-miRNA into 21- to 24-nucleotide duplex miRNA. The strand destined to be the mature sequence is then incorporated into the RNA-induced silencing complex (RISC) [12], whose core components contain members of the Argonaute protein family [2]. The mature miRNA strand serves as a guide for recognition of target mRNAs, which requires stringent Watson–Crick base pairing to the 5'-end of the miRNA seed region centered on nucleotides 2–7 [13]. Depending on the level of miRNA–mRNA

complementarity [2,10], two different mechanisms of RISC-mediated gene regulation occur. At sites with extensive complementarity, the miRNA can mediate mRNA cleavage, while sites with a lower degree of complementarity may lead to translational repression or mRNA destabilization [14], the latter being more common in mammals [15]. An overview of miRNA biogenesis is given in **FIGURE 1**.

miRNA function

Given that miRNAs modulate more than half of mammalian mRNAs [8], it is apparent that miRNAs have a major impact on several physiological and pathological cellular processes including development, differentiation, proliferation and apoptosis [16]. miRNAs are described as fine-tuners of gene expression, as modest repression appears to be the most common regulatory outcome of an miRNA–mRNA target interaction [17]. Several factors influence the effect of an individual miRNA on target gene regulation. miRNA-mediated regulation is believed to have a widespread influence on protein levels. Thus, a single miRNA can target >100 mRNA targets [13,17] and at the same time, a single mRNA can be regulated by multiple miRNAs [3], making identification of miRNA targets a major challenge. There are currently a number of computational approaches to aid the

prediction of miRNA targets, for example, miRWalk, targetscan, PicTar and RNAhybrid [18]. Other factors that may influence the impact of an miRNA on target gene regulation include the temporal and tissue-specific expression of an miRNA and its targets, the ratio between target and miRNA concentration, competition with other miRNAs for RISC, co-expression of functionally redundant miRNAs [3] and expression of decoys [19]. In addition, a few studies have shown that miRNAs can activate translation [20] and target the 5' UTR of mRNA [21]. Finally, miRNAs have been proposed to be subject to as much complex regulation as they exert [9]. Indeed, epigenetic modifications, such as DNA methylation and histone acetylation, have been demonstrated to influence miRNA expression, making miRNA-mediated gene regulation particularly complex.

Considering the relatively high number of human miRNAs identified so far, their diverse expression patterns and their complex regulation along with the significant number of putative mRNA targets, it is not surprising that miRNAs have been implicated in modulation of both the innate immune response and the adaptive immune response, and also in a broad range of diseases including cancers [22], immunological diseases [23] and several diseases of the skin. Furthermore, since the discovery of miRNAs, intensive research has not only provided us with new insights into gene regulation, but it has also indicated new directions for future diagnostic and therapeutic applications. Elmén *et al.* conducted a pioneering study demonstrating the use of systemically administered locked nucleic acid (LNA) anti-miRs in non-human primates [24]. Later, Lanford *et al.* demonstrated that miravirsen (SPC3649; Santaris Pharma, Hørsholm, Denmark), a LNA-modified oligonucleotide that targets miR-122 (which is crucial for the functional infection of hepatitis C virus [HCV]), has a long-lasting suppressive effect on HCV viremia in chronically HCV-infected chimpanzees without any obvious side effects [25]. Since this non-human primate study was published, miravirsen has gone through two Phase I clinical trials that have successfully shown that the drug is also safe in humans [26,27], and a Phase IIa clinical trial [28] in patients with treatment-naïve chronic HCV infection to monitor tolerability, safety, pharmacokinetics and efficacy [29,30]. In addition to miravirsen, the first miRNA replacement therapy has recently advanced into the clinic [30]. In May 2013, Mirna Therapeutics (Austin, TX, USA) announced the commencement of a Phase 1 study of the liposome-formulated miR-34 mimic-based drug, designated as MRX34, in patients with primary liver cancer or metastatic cancer with liver involvement [30,31]. Altogether, the results have been encouraging and constitute a landmark breakthrough in miRNA-based therapeutics.

miRNAs in normal skin

The first evidence that miRNAs are essential for development of the skin came from the observation that mice carrying an epidermis-specific Dicer or DGCR8 deletion failed to produce mature miRNAs, and developing hair germs were found to evert rather than invaginate, thereby perturbing the organization of the epidermis [32,33]. In agreement with this, high

expression of several miRNAs in the epidermis and hair follicles has been found to be required for normal skin development [32,33]. Currently, the most studied miRNA in the skin is miR-203, which has been found to be preferentially expressed in the skin, in particular in the supra-basal layer of the epidermis [34]. By modifying the spatiotemporal expression of miR-203 *in vivo*, Yi *et al.* showed that miR-203 promotes epidermal differentiation by restricting its proliferative potential and inducing exit from the cell cycle [35]. The transcription factor $\Delta Np63\alpha$, which is critical for the maintenance of 'stemness' in the skin, was identified as a conserved target for miR-203 [35,36]. Together, these studies indicated that miR-203 defines a molecular boundary between proliferative basal progenitors and terminally differentiating supra-basal cells [35]. Since these pioneering studies, which have highlighted the importance of miRNAs in controlling mammalian skin development and function, miRNAs have been the subject of significant interest in dermatology. Currently, several investigations have indicated that miRNAs have a role in the pathogenesis of different cutaneous diseases, including psoriasis, AD, allergic contact dermatitis (ACD), cutaneous T-cell lymphoma (CTCL), malignant melanoma and basal and squamous cell carcinoma. In the following section, we consider some of the most important inflammatory cutaneous diseases where miRNAs have been studied.

miRNAs in inflammatory skin diseases

Psoriasis

Psoriasis is a systemic inflammatory skin disease affecting approximately 2–3% of the world's population [37]. The disease is considered to have a multifactorial etiology involving both environmental factors and genetic susceptibility. However, the exact cause of psoriasis still remains unknown. Histologically, psoriasis is characterized by epidermal hyperproliferation with parakeratosis, elongation of the rete ridges, exaggerated vascularity and epidermal and dermal infiltration of activated immune cells [37].

The first evidence that miRNAs are implicated in psoriasis came from a study by Sonkoly *et al.* demonstrating that psoriasis-affected skin has a specific miRNA expression profile compared with healthy human skin and to another chronic inflammatory skin disease, AD [34]. Zibert *et al.* identified several dysregulated miRNAs when they compared lesional and non-lesional psoriatic skin with healthy skin [38], partially confirming the findings of Sonkoly *et al.* [34]. Subsequently, we demonstrated that miRNA detection in psoriatic skin is robust regardless of the preservation method [39]. Two recent studies using next-generation sequencing (NGS) have expanded the list of deregulated miRNAs and non-canonical miRNAs in lesional psoriatic skin [40,41]. Together, the majority of the miRNAs currently described as being differentially expressed in psoriatic lesions have been shown to be involved in inflammation (e.g., miR-21, miR-142-3p and miR-146a) [34,40,42] and keratinocyte (KC) proliferation and differentiation (e.g., miR-99a, miR-100, miR-125b and miR-203) [34–36,43,44]. However, miR-31 has been demonstrated to be involved in the cross-talk between

epithelial cells and immune cells [45] emphasizing the fact that the exact role of the deregulated miRNAs in psoriasis may be much more complex.

As the previously established differences in global miRNA expression in lesional psoriatic skin relative to non-lesional psoriatic skin and healthy skin were based on whole tissue extracts (punch biopsies), they correspond to global miRNA changes from a mixture of cells including KCs, leucocytes and endothelial cells. To improve our understanding of the role of miRNAs in psoriasis, we recently investigated whether certain disease-related miRNAs could be specifically confined to the epidermis or to immune cells in the dermal inflammatory infiltrates [46]. By combining laser capture microdissection (LCM) and bar-coded small RNA cDNA libraries coupled with NGS, we identified several highly abundant miRNAs in the psoriatic epidermis. In agreement with previous studies using whole tissue extracts, miR-99a and miR-125b were found to be significantly downregulated in lesional epidermis [34,40]. Recently, miR-125b has been reported to target fibroblast growth factor receptor 2, thereby modulating KC proliferation and differentiation [44]. Moreover, using *in situ* hybridization, Xu *et al.* demonstrated that miR-125b expression was downregulated in all epidermal layers in psoriatic skin compared with healthy skin, and they suggested that the major cell type responsible for the reduced miR-125b expression in whole tissue samples was KCs [44]. However, from our study using LCM it appears that miR-125b is more abundant in the dermal compartment than in the epidermal compartment, based on the total read frequencies [46]. Since miR-125b expression has been shown to be downregulated during differentiation to effector T cells [47], we suggest that apart from having an important role in KC proliferation, miR-125b may also play an important role in immune cells present in the dermal inflammatory infiltrates in psoriasis, but further investigations are warranted to elucidate this in more detail. Other deregulated miRNAs in psoriatic epidermis are miR-21, miR-142-3p, miR-146a and miR-223, which have all been shown to be associated with inflammation [42]. miR-146a and miR-223 are preferentially expressed by immune cells [34,48]; thus, the observed increase in their expression in the psoriatic epidermis is probably caused by migration of inflammatory cells into the epidermis. Using *in situ* hybridization, Joyce *et al.* reported expression of miR-142-3p in lumen-like structures within the psoriatic epidermis and in immune cells present in the dermis [40].

Psoriatic skin is characterized by a significant increase in infiltrating immune cells such as T cells, dendritic cells (DCs), granulocytes and – to a lesser degree – B cells [49]. The importance of these inflammatory aggregates in dermis is supported by the observation that they are not present in non-lesional skin, and that effective treatment leads to their disappearance [50]. Several miRNAs have been found to be deregulated in the cells present in these dermal inflammatory infiltrates. Among them are miR-155, miR-21, miR-142-3p, miR-146a, miR-150, miR-223, miR-99a and miR-125b [46], which have all been reported to be expressed by various immune cell

subsets including T cells, B cells, Treg cells, granulocytes and DCs [42,47,48,51]. Many miRNAs appear to be active in several different kinds of immune cells, making it challenging to define the specific functions of single miRNAs. Another possible role of the deregulated miRNAs may be the involvement in the altered angiogenesis seen in psoriasis. miRNA-21, miR-100 and miR-378 are among the most abundant deregulated miRNAs in lesional skin [40]. Interestingly, they have all been reported to be involved in angiogenesis [52], so these miRNAs may contribute to the characteristic exaggerated vascularity and increased level of VEGF seen in psoriatic lesions.

Apart from the characteristic lesions of psoriatic skin, psoriasis is also characterized by systemic changes involving systemic inflammation with increased levels of among others IL-17, IL-1ra and TNF- α , and an increased risk of acquiring other systemic conditions such as diabetes mellitus and cardiovascular disease [37]. Recently, a small number of studies have emerged demonstrating that peripheral blood cells from patients with psoriasis are associated with miRNAs with deregulated expression [53,54]. Zhao *et al.* showed that increased miR-210 levels induce immune dysfunction by targeting FOXP3 in CD4⁺ T cells from patients with psoriasis [53]. Interestingly, we found that alterations in miRNA expression levels in psoriatic lesions actually appear to be reflected in the miRNome of peripheral blood mononuclear cells (PBMCs) from psoriasis patients [46]. It is conceivable that the miRNA expression profile of the PBMCs arises as part of a psoriasis-associated immune response, but this hypothesis requires further experimental confirmation.

Finally, in recent years several studies have been aimed at understanding the contribution of genetics to the risk of psoriasis using different approaches, such as twin studies [55] and genome-wide association studies [56]. However, many of these studies have focused on the protein-coding regions of the human genome, so the contribution of genetic variants involving non-coding regions – including the miRNAs – is largely unexplored in psoriasis. In 2011, a study demonstrated that a genetic variation in the 3' UTR miR-492 binding site of basigin, a member of the immunoglobulin superfamily, was associated with psoriasis [57]. Recently, Zhang *et al.* investigated the association between a functional SNP in miR-146a and the risk of psoriasis in the Chinese Han population. Their findings showed a significantly increased risk of psoriasis in subjects with the rs2910164 miR-146a CG and GG genotypes [58]. In addition, this specific allele in miR-146a appeared to weaken its suppression of the proliferation of KCs, probably through the target gene EGFR, thus highlighting the possible contribution of genetic polymorphism in miRNA genes to the pathogenesis of psoriasis [58].

In summary, several studies have provided evidence that psoriatic skin and cells from the blood are indeed characterized by specific miRNA expression patterns. An overview of a selected panel of deregulated miRNAs in psoriasis can be found in FIGURE 2. In all previous studies involving global miRNA expression in psoriatic skin, miR-21, miR-142-3p and miR-146a have been found to be significantly increased, emphasizing their importance in psoriasis. The fact that it also

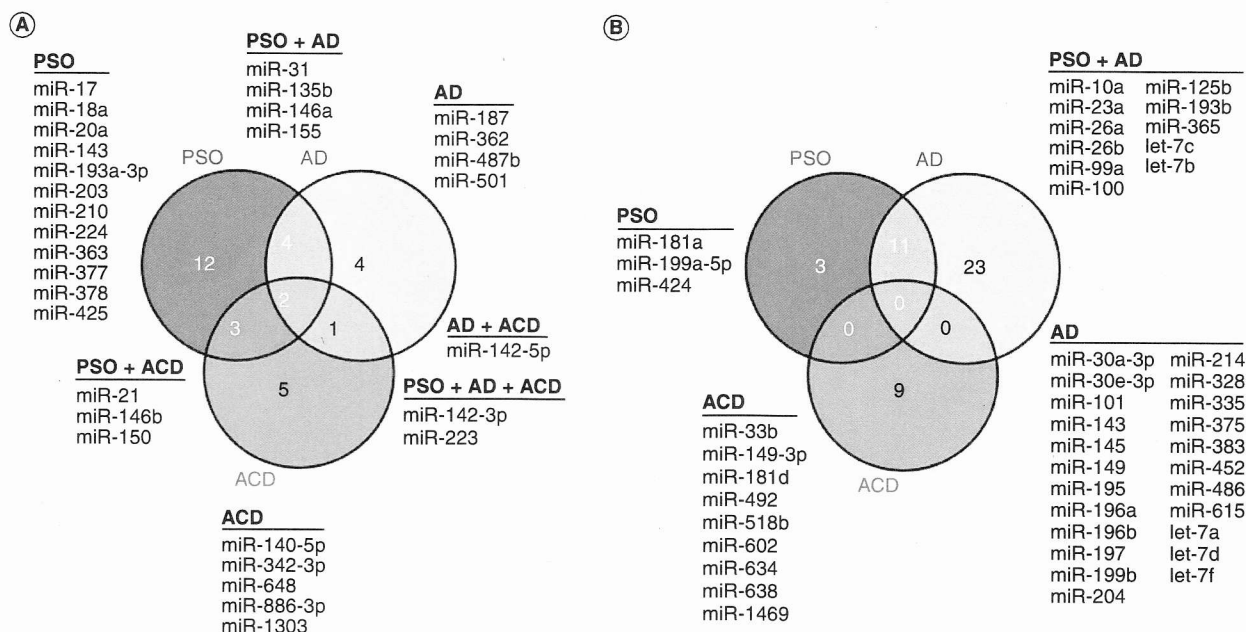


Figure 2. miRNAs whose expression is deregulated in inflammatory skin diseases. Venn diagrams showing a selected number of overlapping upregulated (A) and downregulated (B) miRNAs in the inflammatory skin diseases PSO, AD and ACD [34,38,40,46,58,60–62]. ACD: Allergic contact dermatitis; AD: Atopic dermatitis; PSO: Psoriasis.

became apparent recently that genetic polymorphism in miR-146a can affect miRNA activity and contribute to disease susceptibility in psoriasis is very interesting, but further studies are still required to uncover the specific functions of the miRNAs in the pathogenesis of psoriasis.

Atopic dermatitis

AD is a common chronic inflammatory skin disease characterized by relapsing pruritic skin lesions [59]. AD often presents in infancy and childhood, but it can persist throughout adulthood. AD is a complex disease with changes in the skin barrier function and also in the innate and adaptive immune system. The exact cause of AD is unknown, but the disease is probably the result of interactions between genetic and environmental factors [59].

Only a few studies have investigated the expression of miRNAs in AD. miRNAs were first associated with AD when a small study using an miRNA microarray revealed a number of deregulated miRNAs in skin lesions obtained from patients with AD, compared with psoriatic and healthy skin [34]. In active lesions from patients with AD, miR-155 was found to be one of the most upregulated [60]. It was found to be expressed mainly in infiltrating immune cells, including CD4⁺ T cells and DCs [60]. The authors showed that miR-155 was upregulated during T-cell differentiation and activation. Functionally, the cytotoxic T-lymphocyte-associated protein 4, which is an important negative regulator of T-cell responses, was found to be a direct target of miR-155, thus suggesting that miR-155 contributes to the chronic skin inflammation by increasing the proliferative

response of T cells through the inhibition of cytotoxic T-lymphocyte-associated protein 4 [60]. Recently, Rebane *et al.* demonstrated that miR-146a alleviates chronic skin inflammation in AD through suppression of innate immune responses in KCs [61]. The expression of miR-146a was significantly increased in KCs and chronic lesional skin of patients with AD, and the authors showed that miR-146a inhibited the expression of several proinflammatory factors including CCL5 and CCL8 [61]. Besides miR-155 and miR-146a, 43 other miRNAs have been found to be deregulated in AD lesions [60]. Notably, many of those have also been described as being altered in psoriatic skin, indicating that they may have a common role in the inflammation present in both pathologies (FIGURE 2).

Allergic contact dermatitis

ACD is a clinical term used to describe an inflammatory reaction that is caused by type IV delayed-type hypersensitivity responses to allergens that come in contact with the skin. The clinical manifestations of acute ACD are often erythema, papules and vesicles. There are two distinct phases in ACD, the sensitization (induction) phase and the challenge (effector) phase. Only miRNA expression in the challenge phase has been studied. Vennegaard *et al.* were the first to describe altered miRNA expression in ACD [62]. They analyzed the global miRNA expression in skin from subjects who had been sensitized with diphenylcyclopropenone (DPCP), a strong contact allergen. miRNA-21, miR-223, miR-142-3p and miR-142-5p were found to be the most upregulated miRNAs in the inflammatory

lesions [62]. Interestingly, these four miRNAs were also found to be upregulated in the skin of a contact hypersensitivity mouse model during the challenge reaction to 2,4-dinitrofluorobenzene, suggesting that this mouse model could be a valuable tool for further investigation of the involvement of miRNAs in ACD [62]. In addition, using barcoded small RNA sequencing, Gulati *et al.* recently confirmed the aberrant expression of miR-21, miR-223, miR-142-3p and miR-142-5p in skin lesions obtained 3 and 14 days after challenge with DPCP in sensitized subjects [63]. Whether the aberrant miRNAs identified after challenge with DPCP are specifically related to DPCP – or to other allergens also and quite separately from irritant reactions – must await further investigation.

Given that ACD is mainly a T-cell-mediated skin disease and that miR-21, miR-223, miR-142-3p and miR-142-5p have all previously been associated with the T-cell-driven skin diseases psoriasis and AD [34,38,40,46,60], the upregulation of these four miRNAs in ACD is most likely related to the increased infiltration and activation of T cells in the skin upon challenge with the allergen. Interestingly, Gulati *et al.* also identified six miRNAs that were significantly upregulated 120 days after challenge, when clinically the lesions were fully resolved [63]. This may indicate a long-lasting allergen-mediated immune reaction in the skin, which would explain the persistent upregulation of some miRNAs. Over the three time points studied (3, 14 and 120 days), many but not all miRNAs were uniquely expressed at certain times, and expression of one miRNA in particular (miR-140-5p) increased progressively over time [63]. This might suggest that miRNAs expressed exclusively at different time points may be related to changes in the adaptive immune response or may be due to regulatory mechanisms in the skin.

Clinical implications of miRNAs in skin diseases

The clinical implications of miRNAs being involved in skin diseases are attractive both from a diagnostic and from a therapeutic perspective, as the miRNAs have emerged as being a family of important regulatory molecules with far-reaching implications for the practice of medicine. In addition, the skin is easily accessible and detection of miRNA in the skin is robust, irrespective of the preservation method used [39,64,65], enabling retrospective studies on, for instance, archived formalin-fixed, paraffin-embedded samples. Today, there are several examples of miRNAs being valuable as biomarkers, and a number of studies have gradually emerged showing that the miRNAs also have therapeutic potential in skin diseases.

Recently, Ralfkiaer *et al.* provided the first evidence that an miRNA classifier based on only three miRNAs measured in the skin can distinguish benign skin disorders from CTCL [66]. Given that mycosis fungoides, the most common type of CTCL, initially presents as patches and plaques resembling benign inflammatory skin lesions such as psoriasis and dermatitis, early diagnosis of mycosis fungoides is a challenge. These authors assessed 148 patient samples – including 63 samples from patients with CTCL and 85 samples from patients with benign lesions – using microarray miRNA profiling [66]. The

most induced miRNAs in CTCL were miR-326, miR-663b and miR-711, whereas the most repressed ones were miR-203 and miR-205. A qRT-PCR-based classifier consisting of miR-155, miR-203 and miR-205 distinguished CTCL from benign skin disorders with high specificity and sensitivity, and with a classification accuracy of 95% – indicating that miRNAs have a high diagnostic potential in CTCL [66]. This was further supported by validation of the three miRNA classifier in a new, independent cohort of 78 patients using a standard TaqMan qRT-PCR assay [67]. Here, all but four patients in this large cohort were classified correctly and the minimal miRNA classifier could distinguish between patients with malignant disease and those with benign disease with a sensitivity of 90%, a specificity of 97.9% and an overall accuracy of 93.7% [67].

In 2008, the current concept of intracellular miRNA regulation was challenged when a number of studies reported the presence of circulating miRNAs in the cell-free environment in the blood [68,69]. Plasma and serum miRNAs were found to be remarkably stable compared with mRNAs, despite the presence of high levels of RNases [68,69]. Notably, specific serum miRNA signatures were identified for patients with prostate cancer and lung cancer, providing evidence that circulating serum miRNAs carry fingerprints for various diseases that can serve as novel diagnostic biomarkers [68,69]. Since the discovery of miRNAs in blood, they have been detected in a variety of other human body fluids including saliva, breast milk, cerebrospinal fluid and urine [70]. Many reports have confirmed the existence of plasma and serum miRNAs, and there has been intensive research focusing on the potential of circulating miRNAs as diagnostic and prognostic tools for various human diseases [71]. The substantial interest in circulating miRNAs is reflected in the exponential increase in the number of publications involving miRNAs in the blood (>4000 since 2008, [72]). Still, the origin and biological significance of circulating miRNAs remain unclear. Most circulating miRNAs may actually come from blood cells [73], exosomes [74], apoptotic bodies [75], high-/low-density lipoproteins [76] and/or RNA-binding proteins [77].

Regarding inflammatory skin diseases, assessment of the prospect of miRNAs being used as circulating biomarkers is still in its infancy. In psoriasis, a few studies have investigated the expression of miRNAs in the serum as potential biomarkers [78–81]. We recently identified several deregulated miRNAs in whole blood, plasma and PBMCs from patients with psoriasis [54]. Interestingly, of the miRNAs that were deregulated in PBMCs we observed a notable overlap with miRNAs that are deregulated in the dermal inflammatory infiltrates of psoriatic skin, suggesting that alterations in the expression levels of miRNA in psoriatic lesions are reflected in the blood of these patients [46]. To evaluate the potential of miRNAs as blood biomarkers for psoriasis, we measured the expression of a panel of miRNAs in PBMCs obtained from patients with psoriasis – at baseline, after 3–5 weeks, and after 7–10 weeks of treatment with methotrexate (MTX) [54]. MTX is the standard therapy for moderate-to-severe psoriasis, and it appears to exert its effects by acting as both an immune-modulatory agent and

an anti-metabolite. Although not statistically significant, a pairwise comparison of the miRNA expression levels at baseline and after MTX treatment identified that both miR-223 and miR-143 were downregulated in PBMCs already after 3–5 weeks of treatment [54]. Pivarsci *et al.* reported a similar decrease in the expression levels of a number of miRNAs including miR-223, albeit in serum, after 12 weeks of anti-TNF- α treatment, but not after MTX treatment [80]. This discrepancy may have arisen because different compartments were investigated (PBMCs or serum). Taken together, these findings suggest that downregulation of miR-223 in PBMCs is not specifically related to a specific treatment, but rather to the immunosuppressive effect of the treatment on cells in which miR-223 is abundantly expressed.

Circulating miRNAs were initially described in 2008 [68,69], but many questions and challenges still remain. As miRNAs are considered to be 'fine-tuners' of gene regulation, often resulting in relatively small fold changes, large-scale studies are required to increase the statistical power and to establish miRNAs as reliable blood biomarkers for skin diseases in future clinical settings. Nevertheless, blood from patients with psoriasis is characterized by a specific miRNA signature and these initial findings support that miRNAs indeed have the potential to serve as novel blood biomarkers for psoriasis activity [54,80].

As several studies have revealed roles of miRNAs in many aspects of dermatology and since the skin is easily accessible, the prospect of modulating miRNA activity in dermatological diseases is commencing. Interestingly, a recent study demonstrated that inhibition of miR-21 by LNA-modified anti-miR-21 compounds ameliorated disease pathology in patient-derived psoriatic skin xenotransplants in mice and in a psoriasis-like mouse model [82]. These findings suggested that miR-21 may have a causal role in the pathogenesis of epidermal hyperplasia involving inhibition of tissue inhibitor of matrix metalloproteinase-3 followed by an increase in TNF- α -converting enzyme/a disintegrin and metalloproteinase-17 and thereby TNF- α , which was previously proposed as a mechanism involved in psoriasis [38]. To establish the anti-miR-21 oligo as a new treatment for psoriasis, further studies are warranted to investigate the influence of miR-21 on other cells, pathways and disease mechanisms in psoriasis.

Other examples of therapeutic interventions with RNA interference are topical small interfering RNA-based treatments for the autosomal dominant skin disorder pachyonychia congenita [83–85], and also for reducing pigmentation in melasma (which presents as a discoloration of the skin) [86]. Additional future studies using technologies such as cell-penetrating peptides, creams and microneedle arrays will reveal the potential of miRNAs as novel therapeutic targets in skin diseases.

Conclusion

In recent years, intensive research has provided us with an improved understanding of how miRNAs modulate gene expression. Even though our knowledge of the role of miRNAs in skin biology is still incomplete, it is now evident that

miRNAs are indeed involved in various cellular processes in both normal and diseased skin. Still, only a few skin-specific miRNAs have been described. To date, the majority of studies have investigated specific patterns of miRNA expression in diseased skin relative to healthy skin, so both benign inflammatory skin diseases (such as psoriasis and AD) and malignant skin diseases (including basal cell carcinoma and malignant melanoma) have been associated with specific miRNA signatures [87,88]. Some miRNAs appear to be consistently deregulated in several skin diseases, indicating a common role in fundamental cellular processes in these pathologies. For example, aberrant expression of miRNAs involved in the innate and adaptive immune systems have been reported for several inflammatory skin diseases including miR-21, miR-142-3p, miR-146a, miR-150, miR-155, miR-181, miR-210 and miR-223. There is mounting evidence for the importance of miRNAs in normal immune cell functions, and the aberrant expression seen in many inflammatory skin diseases may be related to infiltration of immune cells and disruption of normal immune cell development, homeostasis and function.

Today, the vast majority of studies on miRNA expression in skin diseases have been confined to skin tissue samples. However, since the discovery of circulating miRNAs, many reports have proposed miRNAs as diagnostic biomarkers for various diseases. For a number of skin disorders, for example, psoriasis, several attempts have been made to identify soluble biomarkers; however, there are still no specific biomarkers that can accurately predict disease progression and therapeutic response. Now, initial studies on miRNAs have indicated that miRNAs do indeed have the potential to serve as soluble biomarkers for skin diseases including psoriasis [54,80]. In conclusion, the current descriptive studies of miRNAs in skin and blood samples from patients with various skin diseases provide a basis for further investigations addressing the exact role of miRNAs in skin physiology and pathology and their potential as soluble biomarkers. Moreover, despite the fact that investigation of the diagnostic and therapeutic potential of miRNAs is still in its infancy, with the significant progress that has recently been made, the future field of miRNA-based diagnostics and therapeutics in dermatology appears very promising.

Expert commentary

In the field of cutaneous biology, several studies have shown that lesions from different skin diseases are characterized by specific miRNA signatures. Unfortunately, the specific role and importance of most of these differentially expressed miRNAs still remain unclear. In addition, the reports of aberrantly expressed miRNAs in the same skin disease are often only partly consistent with each other. One explanation for this inconsistency could be the use of whole tissue extracts, which, for example, have various degrees of infiltration of immune cells in the dermis and epidermis and do not allow the study of miRNA expression in specific cells and regions. Indeed, most previous studies on global miRNA expression in different skin diseases have been based on whole tissue extracts including

both the epidermis and the dermis – and therefore represent global changes in miRNA from a mixture of cells. Using LCM to isolate epidermal and dermal cells from psoriatic skin, we found that the enrichment of specific cells – and thereby the additional information about aberrantly expressed miRNAs that can be obtained – is favorable, and we therefore suggest that LCM should be considered as a supplement to using whole tissue skin samples when trying to investigate miRNA expression and function in skin diseases in future studies [46].

Regarding circulating miRNAs, similar challenges are faced. The blood consists of a mixture of different cell types that rapidly respond to changes in their environment [89], resulting in alterations in miRNA expression levels immediately after sample collection. Thus, many issues need to be thoroughly investigated in order to establish a single miRNA or a panel of miRNAs as reliable systemic biomarkers in future clinical studies in dermatology. Concordantly with previous studies [68,69,90], it will be crucial to initially determine the influence of technical factors such as the sample collection procedure (e.g., duration of stasis) and processing of the samples (e.g., type of collection tube, storage conditions and centrifugation protocol) and subject-specific variables including gender, ethnicity, nutritional status and smoking habits, on miRNA expression levels before implementation in a clinical setting. In addition, standard procedures for extraction, detection, quantification and data analysis (including normalization strategy and empirical validation of stable endogenous control miRNA for each type of blood compartment) of circulating miRNAs are required. Furthermore, it will be important to determine the source of the circulating miRNAs and the mechanism by which miRNAs reach the bloodstream [91]. Many of the miRNAs detected in the blood may reflect differences in counts of blood cells from which the miRNAs may be derived. Because cellular miRNAs are much more abundant than extracellular miRNAs in the blood, even a small degree of contamination of the isolated serum or plasma samples with viable blood cells or lysed cells will significantly alter the miRNA expression profiles [90]. Thus, at this point the search for miRNA blood biomarkers for skin diseases should continue to focus on either circulating miRNAs that are not highly expressed in blood cells or miRNA expression and function in specific immune cells that have been isolated using flow cytometry.

Finally, as miRNAs are considered to be ‘fine-tuners’ of gene regulation, causing relatively small fold changes, larger prospective studies are certainly warranted in order to achieve statistical significance and to verify that miRNAs may be able to serve as blood-based biomarkers of skin diseases. Preferentially, combinations of deregulated miRNAs or known biomarkers might increase the specificity and sensitivity of the biomarkers in diagnosing a particular skin disease, identify patients with comorbidities or even predict resistance to specific treatments and thereby avoid unnecessary treatment and possible adverse events. Many questions still remain, but future studies will hopefully establish the biological significance and the specific roles of miRNAs in dermatology. In addition, with the technological advances in the field of small RNA therapeutics, a

window may have been opened regarding the prospect of using our understanding of the activities of miRNA as a means of treating a variety of skin diseases.

Five-year view

Even though our knowledge of miRNAs in dermatology has been rapidly expanding during the last 10 years, our exact understanding of the role of miRNAs in inflammatory skin diseases is still limited. There is no question that within the next 5 years, new discoveries will pave the way for an increased insight into the role of miRNA regulation in healthy and diseased skin, which will hopefully lead to new diagnostic and therapeutic applications in dermatology.

Future studies covering systematic miRNA profiling of the different cells present in the skin by high-throughput methods will be necessary to increase our understanding of the impact of miRNAs on skin physiology and pathology. In addition, prospective studies should investigate whether the aberrant miRNA expression observed in skin diseases is causative, or simply a result of altered gene expression in specific cells. This type of investigation may involve more functional and mechanistic studies including parallel mRNA expression profiling and subsequent protein analysis, which will provide us with an increased understanding of the significance of miRNA regulation in the cellular processes involved in various skin diseases. Given that miRNAs generally function through the coordinated activity of several gene targets to modulate signaling pathways, a key challenge for the future study of miRNA activities is to move beyond the paradigm of studying an individual miRNA, and instead to focus on delineating the interactions of several miRNAs and their targets in larger biological networks. Moreover, as the majority of miRNAs are transcribed by RNA polymerase II, they are themselves subject to regulation, including epigenetic modifications by chromatin remodeling and DNA methylation. As with the study by Boele *et al.* where they found disrupted miR-21 degradation pathways across a wide variety of cancers and other proliferative diseases including psoriasis [92], future studies should try to reveal the additional layers of regulation associated with miRNAs – including investigations of altered expression of components involved in miRNA biogenesis. Recently, a number of studies have also demonstrated that genetic polymorphism in miRNA genes and/or miRNA binding sites of target genes can affect miRNA activity and contribute to disease susceptibility [57,58]. The complexity of miRNAs is certainly very apparent, and our incomplete understanding of the exact role of miRNAs in skin physiology and pathology and of the clinical implications of miRNAs awaits further investigation.

Collectively, many questions remain to be answered, but within a 5-year period we will have gained important insights. This will hopefully highlight the miRNA pathways involved in specific skin diseases and point to new ways of treating skin disorders, with a view to improving patient management and outcome. Given that recent genome-wide surveys have led to the remarkable discovery that over 70% of the human genome is transcribed to tens of thousands of non-protein-coding RNA

(ncRNA) transcripts [93,94], other classes of ncRNAs (e.g., long ncRNAs) will probably also receive much attention in future studies, leading to new exciting discoveries that may improve our understanding of the complex gene-regulatory networks and of skin physiology in general.

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Key issues

- miRNAs are an abundant class of highly conserved small non-coding RNA molecules that modulate gene expression at the post-transcriptional level.
- miRNAs have been shown to be important for normal skin development.
- miRNA detection in skin is robust, irrespective of the preservation method.
- miRNAs have been implicated in the pathogenesis of various cutaneous diseases, including the inflammatory skin diseases psoriasis, atopic dermatitis and allergic contact dermatitis.
- The specific patterns of miRNA expression in skin can be used as biomarkers to differentiate benign skin diseases from malignant ones.
- miRNA may have the potential to serve as soluble biomarkers in blood for skin diseases such as psoriasis.
- The diagnostic and therapeutic potential of miRNAs remains in its infancy, but current developments in miRNA-based diagnostics and therapeutics in the field of dermatology are certainly promising.

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