MicroRNAs in normal and psoriatic skin

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Submitted 4 October 2013; accepted in final form 6 December 2013

Xia J, Zhang W. MicroRNAs in normal and psoriatic skin. Physiol Genomics 46: 113–122, 2014. First published December 10, 2013; doi:10.1152/physiolgenomics.00157.2013.—Psoriasis is a chronic and common human skin disorder currently with no cure. Psoriatic skin displays inflammatory, raised, and scaly lesions with widely aberrant gene expression. Recent studies have revealed critical roles that microRNAs play as a class of posttranscriptional gene regulator in skin development and skin diseases. A substantial number of novel microRNAs have been identified in skin, and much has been learned about the dysregulated expression and functional roles of microRNAs in psoriasis, as well as the robustness and plasticity of microRNA-mediated gene expression regulation. Here we review recent progresses in discovery, profiling, and characterization of microRNAs in human psoriatic skin, discuss insights to their biological functions, and share our view on remaining challenges to be addressed.

Psoriasis; skin development; microRNA; endogenous siRNA

HUMAN SKIN IS THE LARGEST organ of the body; it regenerates throughout the life of every individual, serves as the outermost barrier to prevent inner organs from dehydration, is able to repair when injured through a complex healing process, and responds to a variety of environmental stress and hazards (25, 54). Skin has developed a system of regulatory mechanisms to maintain these functions, orchestrated by various mediators that have local or systemic effects (79–81).

As a common skin disease, psoriasis is a chronic, autoimmune, and complex genetic disorder that affects 2–3% of the European population. Psoriatic skin has symptoms of inflammation and raised and scaly lesions (8). Three different types of cellular alteration occur in psoriatic involved skin: abnormal differentiation of keratinocyte, hyperproliferation of keratinocyte, and infiltration of immune cells (70). Much has been learned about the molecular components and cellular pathways of inflammation that contribute to the pathogenesis of psoriasis (30, 55, 107), as well as genetic and environmental factors that influence the onset and progression of psoriasis (35, 71).

Results from recent studies have indicated that microRNAs (miRNAs), as a novel regulator of gene expression, play important roles in psoriasis. miRNAs form a class of short, nonprotein-coding, regulatory RNAs that play critical roles in nearly all biological processes, including cell differentiation, development, and metabolism, as well as complex diseases (6, 12). The maturation of miRNAs involves multiple steps, during which two intermediate forms of miRNAs, primary (pri-) and precursor (pre-miRNAs), are produced sequentially. In this process, RNase III enzyme Drosha and partner double-stranded RNA (dsRNA) binding protein Dgcr8 cleave pri-miRNAs to produce hairpin-shaped pre-miRNAs that are recognized by Exportin5 and are subsequently transported from nucleus to cytoplasm. There, another RNase III enzyme Dicer cleaves the pre-miRNAs to release ~22 nucleotide (nt) dsRNA duplexes, namely miRNA/miRNA* duplexes, with ~2 nt 3’ overhangs. One strand of a RNA duplex, termed mature miRNA, is then loaded into an Argonaute protein in the RNA-induced silencing complex to exert its regulatory function by binding to target transcripts (6, 12).

Next-generation sequencing (NGS) is an enabling, high-throughput sequencing technology that is capable of profiling the expression of various RNA species in a genome-wide scale with single-nucleotide resolution (41, 53). Compared with hybridization-based techniques, such as quantitative PCR and microarray, which are more suitable for profiling known genes, NGS directly sequences RNA transcripts and thus is able to facilitate de novo discovery of novel miRNA genes and accurate quantification of miRNA expression (9, 32). Recent studies, particularly those based on NGS, have discovered a pool of miRNAs, miRNA-like RNAs, and their variants that are more diverse than we previously anticipated. These diverse miRNAs include canonical and noncanonical miRNAs (7, 13, 57, 65), miRNA-like RNAs (4, 22, 96, 97), and miRNA isoforms (58, 62) (Fig. 1). Canonical miRNAs are generated from a biogenesis pathway that requires Drosha and Dicer, as discussed earlier. Noncanonical miRNAs are produced from alternative biogenesis pathways where the essential player of the canonical miRNA biogenesis pathway, Drosha, is typically not involved. The first example of noncanonical miRNA is the class of mirtrons, which arise from short, ~60 to 100 nt long, debranched intron lariats that form stem-loop structures that serve as substrates for Dicer cleavage, thus bypassing the Drosha/dgcr8 processing (7, 16, 65, 73). Dicer-dependent but Dgcr8-independent miRNA-like RNAs can also arise from...
local hairpin formation within larger noncoding RNA (ncRNA) species, such as small nucleolar RNAs (snoRNAs) (4, 10, 22). miRNAs are typically defined as the most abundant small RNAs on pre-miRNA hairpins. Nevertheless, other less abundant but cognate small RNAs from the same pre-miRNAs, which differ by a few bases from miRNAs, have been reported (46, 72). These miRNA variants have been named miRNA isoforms, or isomiRs (59), and observed to exist in animals, plants, and viruses (21, 23, 68). isomiRs can function as regular miRNAs (31, 95, 98); they often share common mRNA targets with their companion miRNAs but may also have their own exclusive target genes (98). The emergence of such diverse miRNAs as additional gene expression regulators with potential functions complementary to that of canonical miRNAs reflects the robustness and plasticity of miRNA-mediated gene expression regulation.

It has been estimated that miRNAs regulate over one-third of protein-encoding mRNAs in humans (24, 50). In mammalian skin development, recent studies have revealed that the interaction between miRNAs and their target mRNAs is critical in regulating distinct signaling pathways during cell differentiation (48, 104). Noncanonical miRNAs, miRNA-like RNAs, and isomiRs in normal and psoriatic human skin are also of interest and have recently been reported (38, 96). Dysregula-
tion of miRNAs and their regulated targets has been implicated in the pathogenesis of psoriasis (75, 82, 108) as well as other forms of disorder of the skin, including malignant melanoma (11, 60, 76, 77). Such knowledge of the aberrantly expressed small ncRNAs (sncRNAs) in psoriatic skin has suggested functional roles of sncRNAs in psoriasis. An interesting and emerging theme from these studies is that miRNAs that take important parts in early skin development can also function in critical ways in psoriasis. This theme of “what goes wrong early in life can go wrong again later in life” echoes a similar scenario that dysregulation of some miRNAs critically important during early cardiovascular muscle development contributes to cardiovascular diseases (51). This emerging theme on miRNAs suggests that these miRNAs may potentially be excellent candidates for therapeutic treatment of psoriasis. We discuss in this short review the latest development in characterization of miRNAs and recent findings about their regulatory functions during skin development and in normal and psoriatic skin. We finish by sharing our view on challenges to be addressed for future development.

miRNAs DURING SKIN DEVELOPMENT

Significant progress has been made in identifying miRNAs and characterizing their specific functions in skin morphogenesis and homeostasis. Several miRNAs with such functions have been studied (Table 1). Initial efforts have been devoted to discovering novel miRNAs (45), investigating phenotypes of Dicer and Dgcr8 skin conditional knockouts (1, 102), and determining their spatiotemporal patterns of expression in mammalian skin by using cloning and sequencing methods (102).

By investigating phenotypes of mutants knocking out either Dicer or Dgcr8 in murine embryonic skin, the importance of miRNAs in skin development has been revealed (1, 102). Overall, mutants of Dicer and Dgcr8 conditional knockouts present similar phenotypes of severe defects in murine embryonic skin development (103). Specifically, both mutants have been characterized by rough skin, failure to gain weight, defects in hair follicle down-growth, abnormal apoptosis, and no more than 6-day survival after birth (103). Hyperproliferation has been observed in the Dicer conditional knockout epidermis, showing the importance of miRNAs in regulating epidermal proliferation (1).

Profiling miRNA expression at epidermal skin and hair follicle at embryonic day 17.5 (E17.5) has revealed a set of differentially expressed murine miRNAs between the two places. (102). For example, the highly expressed microRNA (miR)-199 family is only found in the hair follicle but not from the epidermis at E17.5, indicating a potential regulatory function that relates to hair morphogenesis (102). Further investigation by profiling miRNA expression in murine skin at embryonic day 13.5 (E13.5) and day 15.5 (E15.5) has revealed that miR-203 is barely detectable in E13.5 skin but emerges as one of the most abundant miRNAs after E15.5. Phenotypic difference between E13.5 and E15.5 skin is also prominent. When only single-layered multipotent embryonic skin stem cells exist at E13.5, the stratification of epidermis begins after E15.5, suggesting that miR-203 has a critical function in skin differentiation and stratification (104).

To appreciate the spatiotemporal pattern of miR-203 expression, in situ hybridization (ISH) has been carried out to localize the expression of miR-203 in great detail. It has been shown that the expression of miR-203 depends on the stages of skin development and is cell specific; it is highly expressed only in differentiated cells of mature skin such as suprabasal epidermis and inner root sheath of the hair follicle, but not in progenitor or stem cells such as basal epidermis and outer root sheath (48, 104). Furthermore, analysis of zebrafish skin (94) has demonstrated that the expression of miR-203 is restricted to the outermost layer of skin, indicating a conserved function of miR-203 contributing to skin differentiation across species.

To comprehed the physiological mechanism of miR-203 functions in differentiated skin cells, targets of miR-203 have been examined. Among these targets, the transcription factor p63, which plays an essential role in maintaining “stemness” in the skin, has been well examined (78). Highly expressed miR-203 in suprabasal layer has been shown to repress the expression of p63, thus reducing the proliferative potential of terminally differentiating keratinocytes (48, 104).

Besides miR-203, recent studies have also recognized several other miRNAs involved in skin development and homeostasis (Table 1 and Fig. 2). For example, repression of miR-34 transcription by p63 is important to maintain cell cycle progression in epidermal cells (2). miR-125b has been shown to be highly expressed in skin stem cells, in contrast to dramatically lower expression in their early progeny (106). In an inducible mouse system, miR-125b has been implicated as a repressor of stem cell differentiation (106). In addition, miR-205 has been found restricted to basal progenitor cells and had roles in maintaining the expansion of skin stem cells by antagonizing negative regulators of PI(3)K signaling (91). miR-200 family and miR-205 are both highly expressed in normal skin. These miRNAs have been shown to target ZEB1 and ZEB2, which are both transcriptional repressors of E-cadherin (17, 28, 42, 67). Downregulation of the miR-200 family and miR-205 induces an epithelial-to-mesenchymal transition in conjuction with upregulation of ZEB1 and ZEB2 (28).

Table 1. miRNAs that function in skin development

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Function</th>
<th>Ref. List No.</th>
</tr>
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<tbody>
<tr>
<td>miR-203</td>
<td>induced in suprabasal layer to inhibit cell proliferation by repressing p63; regulate the transition from basal to suprabasal layer in epidermis</td>
<td>48, 104</td>
</tr>
<tr>
<td>miR-34a/c</td>
<td>repressed by p63 in epidermal cells to maintain cell cycle progression and expression of cyclin D1 and Cdk4</td>
<td>2</td>
</tr>
<tr>
<td>mir-125b</td>
<td>expressed in skin stem cells to balance self-renewal and early lineage commitment</td>
<td>106</td>
</tr>
<tr>
<td>mir-200/miR-205</td>
<td>restricted to basal layer. 1) maintains proliferation of progenitor cells 2) maintains epithelial-mesenchymal transition</td>
<td>91, 28, 42, 67</td>
</tr>
</tbody>
</table>
miRNAs in normal and psoriatic-involved skin.

**Novel miRNAs and miRNA Variations**

A recent study has adopted NGS to comprehensively profile microRNAome in human psoriatic and normal skin (38). Compared with the results from profiling murine skin (102), the miRNAs that are most abundantly expressed in human and murine skin largely overlap. Furthermore, the depth of the collected data has not only allowed detection of low abundant miRNAs but also enabled discovery of novel human miRNAs at an unprecedented rate (38). Overall, >200 loci in the human genome have been identified to host novel miRNAs and miRNA candidates, and 38 novel miRNAs from this profiling study have been registered in miRBase (38). Many of these miRNAs and candidates have been reaffirmed as miRNAs in other studies. Over one-third of the novel miRNAs are intronic, and some are of particular interest in skin. The genomic loci of the intronic miRNAs suggest their coexpression and functional relationship with their host genes. For example, miR-4632, a newly identified mirtron, is located within TNFRSF1B, which has been implicated in psoriatic arthritis (69). Intronic miR-6510 is encoded in KRT15, which shows downregulation in psoriatic skin (30). A known human-specific miRNA, miR-944, is located in an intron of p63, a well-characterized transcription factor with essential function in maintaining stemness in skin (78).

Several novel miRNAs align uniquely to the antisense strands of known miRNAs (38). For example, miR-203-AS has been identified as a distinct miRNA on the DNA strand at the locus antisense to miR-203 (38). A few known miRNA loci, such as miR-103, encode similar but distinct miRNAs on both sense and antisense strands. As a note, distinct function of antisense miRNA has been described in detail at the miR-1ab-4 locus in *Drosophila melanogaster*, where sense and antisense miRNAs coordinately regulate Hox genes during larval development (84).

A variety of noncanonical miRNAs and miRNA-like RNAs have been discovered in human psoriatic and normal skin with NGS-based profiling (38, 96). The largest group of noncanonical miRNAs discovered is the class of mirtrons; >100 mirtron candidates have been identified in human skin (38, 96), which have important implications for biogenesis and function. Genomic loci of many ncRNAs, such as snoRNAs and tRNAs, may also host Dicer-dependent but Dgcr8-independent miRNA-like RNAs. For example, miRNA-like RNAs have been identified from small cajal body-associated RNAs, ACA45, in human (22) and mouse (4), as well as psoriatic and normal skin (38). Although there have been several snoRNA-derived miRNAs in eukaryotes, only one tRNA-derived miRNA has been reported so far (4). In this case, a murine tRNA, IleTAT, folds into an alternative secondary structure consisting of a stem-loop hairpin from which mmu-miR-1983 is derived (4). hsa-miR-1983, a homolog of mmu-miR-1983 located within human tRNA-IleTAT, has been detected to express in psoriatic and normal human skin at a lower level than mmu-miR-1983 in murine stem cells (96).

A systematic analysis has revealed that miRNA isoforms, i.e., isomiRs, can consistently originate from a majority of miRNA hairpins in diverse tissues and across species. Of particular interest is the class of so-called 5′-isomiRs, whose 5′-ends shift by a few bases from the 5′-ends of the cognate miRNAs (98). A number of miRNA loci that are essential for skin development and homeostasis produce substantial amounts of 5′-isomiRs. Particularly, the 5′-isomiR from hsa-miR-203 hairpin accumulates to the same level of abundance as the major miR-203 in human psoriatic and normal skin (Table 2) (98). Murine mmu-miR-203 hairpin also carries an abundant 5′-isomiR in murine skin (98), suggesting that the mechanism of generating 5′-isomiRs is conserved in mammals. In addition, miR-142 and miR-223, two hematopoietic-specific miRNAs, have high 5′-heterogeneities in human psoriatic lesions (98). miR-142 is highly expressed in dendritic cells (86) and miR-223 in neutrophils in humans and mice (5, 47). The abundant expression of miR-142 and miR-223 can be anticipated, given that immune cells including dendritic cells and neutrophils are known to infiltrate in affected skin lesions (38, 44).

With a large pool of novel miRNA candidates, experimental validation further supports endogenous expression of novel miRNAs in human skin. For example, stem-loop quantitative real-time PCR (qRT-PCR) has showed that novel miRNAs, including miR-203-AS, miR-3613, and miR-4490, have visible bands of the same length of miR-203 in normal skin (38). Stem-loop qRT-PCR uses primers specific to sequences in the loop regions of miRNAs so that they have higher RT efficiency than the conventional qRT-PCR and are able to discriminate among paralogous miRNAs (15, 43).

Ectopic expression in HEK293 cells has indicated that miR-203-AS undergoes miRNA biogenesis processing and incorporates into the Argonaute 2 (AGO2) protein (38). qRT-
PCR has also confirmed the expression of miRNA-like RNAs from noncanonical miRNAs, including miR-6499 (mirtron) and miRNAs from tRNA-pseudo-TTA and snoRNA-U60, in normal human skin (96). Ectopic expression of these miRNA-like RNAs has shown their enrichment in AGO2 immunoprecipitates, suggesting that they can associate with AGO2 protein (96).

Aberrant Expression of miRNAs in Psoriatic Skin

In addition to playing critical roles in skin development, miRNAs also regulate gene expression in skin disorders, including psoriasis. Earlier studies using microarray-based miRNA profiling have implicated the involvement of a number of miRNAs in pathogenesis of psoriasis (83, 108). Profiling using NGS, which is sensitive to low abundant transcripts, has identified 125 miRNAs from a diverse origins of canonical and noncanonical miRNAs as well as 5'–isomiRs that exhibit more than twofold differential expression in psoriatic skin (Supplemental Table S1) (38, 96, 98). The majority of the differentially expressed miRNAs are upregulated (90 upregulated vs. 35 downregulated) in psoriatic-involved (PP) skin vs. normal (NN) skin. This result is consistent with the overall mRNA expression changes in psoriatic skin, where there are more upregulated than downregulated mRNA transcripts and substantially fewer differentially expressed miRNAs between psoriatic-uninvolved (PN) skin vs. NN skin (30, 107).

A subset of differentially expressed (DE) miRNAs has been validated by stem-loop qRT-PCR, including 12 significantly DE miRNAs (8 upregulated and 4 downregulated) in PP skin compared with PN or NN skin (38). The most highly upregulated miRNAs in psoriatic skin include the abundantly expressed miR-21 and miR-31 (4- and 43-fold, respectively; Table 2), which are potential proangiogenic miRNAs (92). Upregulation of miR-21 and miR-31 has been observed in various cancers including skin cancer (74), miR-155, miR-142-3p, and miR-146a, which regulate various cell fate decisions in hematopoietic development (27), show ~2.5-fold upregulation in PP vs. NN skin (Table 2). miR-203-AS, discovered in human skin, has also been validated by qRT-PCR to have a 2.7-fold upregulation in psoriatic skin (38). Interestingly, miR-203, which plays a critical role in skin development, shows a moderate 1.6-fold upregulation in psoriatic vs. normal skin (38).

The most significantly downregulated miRNAs in psoriatic skin include miR-124 and miR-4490 (5.8- and 8.0-fold downregulated, respectively; Table 2). miR-124 also exhibits downregulation in the cutaneous squamous cell, one of the most common skin cancers (100). Several highly expressed miRNAs including miR-10a, miR-99a, and miR-100 (Table 2) in human skin also exhibit significant downregulation (2- to 2.3-fold) in psoriatic skin.

Although the overall abundance of noncanonical miRNAs is low in human skin, 12 of them, including hsa-miR-1983 (Supplemental Table S1), show differential expression in PP and/or PN skin (96). The differential expression of hsa-miR-937 (Table 2), which is recently reclassified as a mirtron, has also been validated by qRT-PCR (96). Furthermore, 18 5’–isomiRs also exhibit aberrant expression in psoriatic skin (98). One prominent example is the 5’–isomiR from miR-203; the 5’–isomiR is more than twofold upregulated in psoriasis. In addition, 5’–isomiRs from miR-142 and miR-223 are upregulated by 2.3- and 2.5-fold in psoriatic vs. normal skin. Given the high abundance and upregulation of many 5’–isomiRs in psoriasis, DE miRNAs and 5’–isomiRs may be important for pathogenesis of psoriasis.

Overall, the dramatic alteration in miRNA expression in psoriatic skin reflects the pathogenic features of psoriasis such as defects in skin cells, infiltration of immune cells, and impaired vasculature.

### Functions of DE miRNAs in Psoriasis

An early study exploring the function of miRNAs in psoriasis has revealed an upregulation of miR-203 in psoriatic skin; miR-203 has been implicated in targeting suppressor of cytokine signaling 3 (SOCS3) (83). However, subsequent studies have challenged the conclusion that SOCS3 is a genuine target

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**Table 2. miRNAs that express aberrantly in human psoriatic skin and relative amount of their companion 5’–isomiRs**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Description</th>
<th>Fold Change (PP/NN)</th>
<th>IsomiRs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-31</td>
<td>regulates cytokine/chemokine expression via targeting serine/threonine kinase 40 (STK40)</td>
<td>42.9</td>
<td>0.4</td>
</tr>
<tr>
<td>miR-977</td>
<td>mirtron; upregulated in psoriatic skin</td>
<td>6.4</td>
<td>1.3</td>
</tr>
<tr>
<td>miR-135b/miR-7</td>
<td>targets late cornified envelope-1B (LCE1B); impairs barrier development and response to environmental stimuli</td>
<td>5.6/3.1</td>
<td>0.4/1.0</td>
</tr>
<tr>
<td>miR-1983</td>
<td>tRNA-derived human homolog of murine miR-1983</td>
<td>4.9</td>
<td>0.2</td>
</tr>
<tr>
<td>miR-21</td>
<td>contributes to T cell-derived psoriatic skin inflammation</td>
<td>4.0</td>
<td>0.6</td>
</tr>
<tr>
<td>miR-203-AS</td>
<td>antisense to miR-203</td>
<td>2.7</td>
<td>7</td>
</tr>
<tr>
<td>miR-155/miR-142/miR-146a</td>
<td>cell fate decisions in hematopoietic development</td>
<td>2.7/2.5/2.3</td>
<td>1.1/46/0.8</td>
</tr>
<tr>
<td>miR-223</td>
<td>suppresses cell proliferation by targeting IGF-IR</td>
<td>2.5</td>
<td>9.8</td>
</tr>
<tr>
<td>miR-203</td>
<td>regulates the transition from basal to suprabasal layer in epidermis</td>
<td>1.6</td>
<td>43</td>
</tr>
<tr>
<td>miR-205</td>
<td>primarily in the basal layer expressed in normal skin and regulates the transcriptional repressor of E-cadherin to maintain epithelial-mesenchymal transition</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>miR-99a/miR-100</td>
<td>inhibits angiogenesis by repressing the mammalian target of rapamycin (mTOR) in endothelial cells</td>
<td>–2.1/–2.3</td>
<td>3.1/0.7</td>
</tr>
<tr>
<td>mir-10a</td>
<td>regulates the behavior of endothelial cells during angiogenesis</td>
<td>–2.3</td>
<td>22</td>
</tr>
<tr>
<td>miR-124</td>
<td>downregulation in skin cancer, cutaneous squamous cells</td>
<td>–5.9</td>
<td>23</td>
</tr>
<tr>
<td>miR-4490</td>
<td>novel miRNA from intergenic region</td>
<td>–8.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fold change is a value of miRNA expression in psoriatic skin (PP) divided by that in normal skin (NN). IsomiRs, the percentage of reads mapping to miRNA hairpins identified as 5’–isomiRs.

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1 The online version of this article contains supplemental material.
Review of miR-203 (48, 104). Furthermore, miR-203 has been characterized as an inhibitor of cell proliferation by targeting the transcription factor p63 in normal skin (Table 1 and Fig. 2) (48, 104). Thus, it is arguable for miR-203 to be downregulated in psoriatic skin, potentially accounting for the hyperproliferative skin involving a proinflammatory response. miR-203, however, has been observed to be slightly upregulated in PP skin in several studies (38, 83, 108), suggesting a minor effect of miR-203 to suppress the pathogenic hyperproliferation in psoriatic skin. Although further study is needed to reconcile the disparity over miR-203 targets and to determine the function of miR-203 in psoriatic skin, it has been speculated that the newly identified miR-203-AS as well as its cognate miRNA isoform (see below) may indicate their role in psoriasis (38, 98).

miR-21 and miR-31 are among the most abundant miRNAs with dramatic upregulation (4- and 40-fold, respectively; Table 2) in psoriatic lesions (38). The expression of miR-21 in epidermal cells and dermal T cells between psoriatic and healthy skin has also been examined, showing an elevated expression in both cell types (56). Inhibition of miR-21 increases the rate of apoptosis in activated T cells, suggesting that miR-21 suppresses apoptosis in activated T cells. Therefore, overexpression of miR-21 in psoriatic lesions may reflect the infiltration of activated T cells in psoriatic skin, contributing to T cell-derived psoriatic skin inflammation (56). Overexpression of miR-31 has been shown to contribute to skin inflammation in psoriasis lesions by regulating the production of inflammatory mediators and leukocyte chemotaxis to the skin (99). Specifically, miR-31 regulates cytokine/chemokine expression via targeting serine/threonine kinase 40 (STK40), a negative regulator of NF-κB signaling, in keratinocytes (99). Besides, transforming growth factor-β1, a cytokine highly expressed in psoriasis epidermis, has been shown to induce upregulation of miR-31 in keratinocytes in vitro and in vivo (99).

miR-135b and miR-7, which are upregulated in psoriatic skin (Table 2), have been reported to regulate late cornified envelope-1B (LCE1B) through direct interactions with the 3′-untranslated region of LCE1B (39). The LCE gene family is known to have dozens of members that are clustered within the epidermal differentiation complex region on human chromosome 1. LCE genes are transcriptionally regulated in response to environmental stimuli such as calcium levels and ultraviolet light (36). In skin, LCE1B is normally expressed in a skin barrier but shows a 2.1-fold downregulation in psoriatic skin (30). Therefore, downregulation of gene LCE1B targeted by upregulated miR-135b and miR-7 may result in defects in barrier development, contributing to the pathogenesis of psoriasis.

Downregulated miRNAs are of the same significance as upregulated miRNAs. miR-10a regulates the behavior of endothelial cells during angiogenesis by positively titrating proangiogenic signaling in zebrafish and human (33). Downregulation of miR-10a may contribute to abnormal behavior of endothelial cells in psoriasis. miR-100 is an antiangiomiR that has been shown to inhibit angiogenesis by repressing the mammalian target of rapamycin (mTOR) in endothelial cells (29). miR-99a has the same seed (ACCCGUA) as miR-100 and thus is anticipated to have a similar target specificity (49). Indeed, miR-99a and miR-100 have been shown to target mTOR in human skin (37). Downregulation of miR-99a and miR-100 has been observed in diverse cancers such as esophageal squamous cell carcinoma and endometrioid endometrial carcinoma (85, 90). Given the function of miR-99a and miR-100 as inhibitors of angiogenesis, downregulation of the two miRNAs in psoriatic skin may have an implication in pathogenic characteristics of psoriasis, particularly increased angiogenesis.

A system-wide target analysis has revealed that DE miRNAs show anticorrelated expression with their target mRNAs (38, 96). In particular, 287 DE miRNAs express anticorrelated with 55 DE canonical miRNAs (43 upregulated and 12 downregulated), and 59 DE miRNAs exhibit expression anticorrelated with nine noncanonical miRNAs (7 upregulated and 2 downregulated). A gene ontology analysis has shown that the anticorrelated targets of canonical DE miRNAs are significantly enriched in biological processes such as response to cytokine stimulus, organic substance, and estrogen stimulus. The genes related to cytokine stimulus are of particular interest because overexpression of proinflammatory cytokines, including IFN-alpha and TNF-alpha, has been implicated in the pathophysiology of psoriasis (44). The gene ontology analysis also shows that the enriched molecular functions involve transcription factor activity and cytoskeletal protein binding. These functional terms are consistent with the early results from mRNA transcriptome analyses in Refs. 30, 87, suggesting that miRNAs may respond to the dysregulated transcriptome of psoriasis.

Target analyses show that more than half of the targets between miRNAs and their 5′-isomiRs are different, implying their potentially distinct functions (98). For example, hsa-miR-203 and its most abundant 5′-isomiR share about half (53.7%) of their common targets. The 5′-isomiR of miR-203 may have additional and complementary functions with miR-203 by putatively regulating p63 to affect multiple pathways during skin development. Overall, DE 5′-isomiRs have a total of 148 anticorrelated pairs with 110 distinct target genes (21 upregulated and 89 downregulated) in psoriatic and normal skin (98).

While 5′-isomiRs are highly expressed in diverse tissues including skin, their impact on mRNA expression remains to be studied. Recent studies investigating a few examples of 5′-isomiRs (3, 16, 34) have shown that 5′-isomiRs of miR-223 have exclusive targets that are significantly de-repressed after the miR-223 gene was knocked out in neutrophils, indicating a direct impact of 5′-isomiRs on target gene expression (16, 98). Existing experimental evidence from in vitro target cleavage assays supports the notion that miR-142-5p and its 5′-isomiR have different target specificities (3). In light of the fact that 5′-isomiRs are functional, the dysregulated 5′-isomiRs, including those from the loci of abundantly expressed hsa-miR-203, hsa-miR-142, and hsa-miR-223, are of particular interest in psoriatic skin.

Cell Type-specific miRNA Expression in Psoriatic Skin

RNAISH has been adopted to localize miRNA expression to determine cell-specific expression patterns (40, 94). It has helped reveal the spatial pattern of miR-203 expression in the suprabasal layer of the epidermis. Adaption of ISH of miRNAs in psoriatic skin has also revealed that miR-135b, one of the upregulated miRNAs, is expressed in the suprabasal epidermis of psoriatic epidermis but is excluded from the basal layer (Fig.
and endo-siRNAs in psoriatic skin. These results indicate MDM4 is tightly regulated by miRNAs miRNA miR-19b that is upregulated by 2.1-fold in psoriasis. 

Thus, the downregulation of MDM4 in psoriasis, which may be mediated by the novel Alu-SINE-derived siRNA, might be one target, such as MDM4 (mouse double minute 4 homolog), are anticorrelated with the Alu-derived endo-siRNAs (96). This endo-siRNA and the host gene may be transcribed or processed within an intron of the gon-4-like (GON4L) gene on chromosome 1, where three Alu-SINEs appear in tandem. In sharp contrast to the huge upregulation of the endo-siRNA, the expression of the host gene GON4L shows little variation between PP and NN skin. This discrepancy implies that the endo-siRNA and the host gene may be transcribed or processed independently. The cluster of the siRNAs appears only in the first two Alu SINEs, suggesting that the repetitive elements may harbor the novel endo-siRNAs instead of the intron. A few targets, such as MDM4 (mouse double minute 4 homolog), are anticorrelated with the Alu-derived endo-siRNAs (96). This gene is an inhibitor of the 53 that is frequently upregulated in cancers (20, 89) but modestly downregulated in psoriasis (61). Thus, the downregulation of MDM4 in psoriasis, which may be mediated by the novel Alu-SINE-derived siRNA, might be one factor that distinguishes the benign hyperplasia in PP skin from cancerous tumors. MDM4 is also a putative target of canonical miRNA miR-19b that is upregulated by 2.1-fold in psoriasis. These results indicate MDM4 is tightly regulated by miRNAs and endo-siRNAs in psoriatic skin.

SUMMARY AND FUTURE DIRECTIONS

Although still in its infancy, the study of miRNAs and endo-siRNAs in mammalian skin and skin disorders has already provided deep insights into this novel layer of gene regulation. Knowledge about the aberrantly expressed sncRNAs in psoriatic skin has laid a foundation for understanding the roles of miRNAs and endo-siRNAs in psoriasis. We now have a good collection of miRNAs as well as their validated and putative targets, many of which are discussed here, that provide not only new knowledge of psoriasis mechanisms, but also novel threads for future research.

An interesting and converging theme has emerged from the results and findings obtained so far, i.e., two key miRNAs, miR-203 and miR-205 (Tables 1 and 2 and Fig. 2), which play essential roles in early skin development also function in critical ways in psoriasis. This theme of “what goes wrong early in life can go wrong again later in life” echoes a similar observation that miR-1 and miR-133 are critically important during early cardiovascular muscle development as well as in cardiovascular diseases (51). This emerging theme on miRNAs suggests that these miRNAs may potentially be excellent candidates for therapeutic treatment of psoriasis.

Despite significant progress that has been made so far, recent results and findings require more research to be done to unravel the complex genetic networks involved in the etiology of autoimmune skin disorders such as psoriasis. First, it is important, albeit challenging, to build a high-quality regulatory network underlying psoriasis. Such a network can be used to identify the major genetic components of the disease. Building such a network, nevertheless, requires a comprehensive profile of the total transcriptome, including mRNA genes, miRNAs, and other sncRNA species. A good collection of such transcriptome data can also be used to address the second challenge, i.e., identification and characterization of potential long noncoding RNAs (lncRNAs) in psoriasis. Although little results on IncRNA have been reported yet for psoriasis, it is possible to hypothesize the involvement of novel genetic elements, particularly lncRNAs, since extensive research has not brought us close to the genetic culprits of the disease. The third challenge is to integrate the information of genotypic variations, e.g., single nucleotide polymorphisms and copy number variations, with the data of transcriptome variations in hunting for causal genetic variations that ultimately lead to disease phenotypes. The results from biological assays analyzing individual genes and genome-wide profiling have shown that both techniques are effective and complementary to each other. It will be more effective, although challenging, to integrate the results from genome-wide profiling with experimental functional analyses of key elements. For example, the current results have pointed to a few miRNAs, e.g., miR-203, miR-205, and miR-125, to be critical players in skin development and psoriasis. Their regulatory functions need to be further scrutinized in animal models or cell lines with, e.g., loss- and/or gain-of-function mutants of these miRNAs. The recent progress on transgenic technologies, such as the latest TALEN (105) and CRISPR/Cas (18, 52, 101) techniques, can be adopted to meet the challenge of such functional analysis. They can be used to manipulate individual genes for their functions in psoriasis and also to develop animal models for psoriasis research.

Finally, we are optimistic with the prospects of this field. Because of the easy accessibility of the skin and the collective effort of the research community, we anticipate that skin-related diseases will be among the first to be treated with small RNA-based therapies.

ACKNOWLEDGMENTS

Thanks to Kevin Zhang for a critical reading of the manuscript.

Physiol Genomics • doi:10.1152/physiolgenomics.00157.2013 • www.physiolgenomics.org

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