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1 **Towards topical microRNA-directed therapy for epidermal**
2 **disorders**

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19 **Key words:** microRNA; psoriasis; keratinocyte; cell migration; cell penetrating
20 peptides; liposomes; liquid crystal nanoparticles; squamous cell carcinoma; spherical
21 nucleic acids; stem cells.

22

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2

3 **Abbreviations**

4	cSCC	Cutaneous squamous cell carcinoma
5	DOTAP	1,2-dioleoyloxy-3-trimethylammonium propane
6	DOPE	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine
7	hBD-2	Human beta defensin 2
8	IFN γ	Interferon gamma
9	IL	Interleukin
10	Keratin 17	KRT17
11	LCNPs	Liquid crystalline nanoparticles
12	miRNA	MicroRNA
13	MO	Monoolein
14	siRNA	Short/small interfering RNA
15	SNA-NC	Spherical nucleic acid-nanoparticle conjugates
16	STAT3	Signal transduction and activator of transcription 3
17	TACE	Tumour necrosis factor alpha (TNF α)-converting enzyme
18	TGF β	Transforming growth factor beta
19	TIMP-3	Tissue inhibitor of metalloproteinase 3
20	TNF α	Tumour necrosis factor alpha

21

22

1 **Abstract**

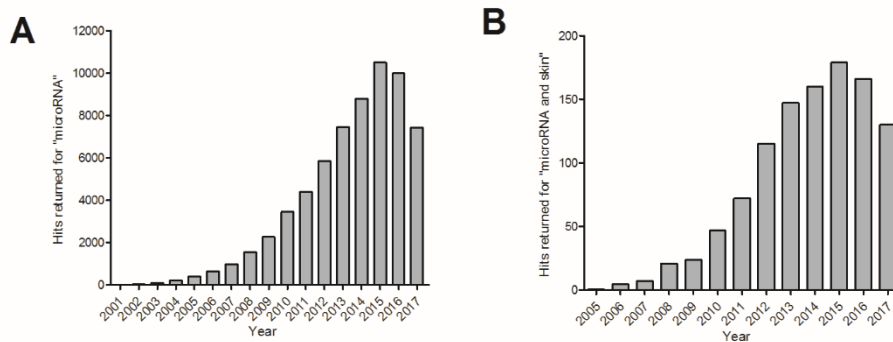
2 There remains an unmet dermatological need for innovative topical agents that
3 achieve better long term outcomes with fewer side effects. Modulation of the
4 expression and activity of microRNA (miRNAs) represents an emerging translational
5 framework for the development of such innovative therapies because changes in the
6 expression of one miRNA can have wide-ranging effects on diverse cellular
7 processes associated with disease. In this short review, the roles of miRNA in
8 epidermal development, psoriasis, cutaneous squamous cell carcinoma and re-
9 epithelisation are highlighted. Consideration is given to the delivery of
10 oligonucleotides that mimic or inhibit miRNA function using vehicles such as cell
11 penetrating peptides, spherical nucleic acids, deformable liposomes and liquid
12 crystalline nanodispersions. Formulation of miRNA-directed oligonucleotides with
13 such skin-penetrating epidermal agents will drive the development of RNA-based
14 cutaneous therapeutics for deployment as primary or adjuvant therapies for
15 epidermal disorders.

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1 **Introduction**

2 Few discoveries in recent bioscience history have had as wide-ranging an impact as
 3 the observation that small endogenous non-protein coding RNAs regulate the
 4 expression of multiple gene targets in diverse species. Following early reports by the
 5 Victor Ambros (1) and Gary Ruvkun (2) laboratories, the breakthrough came in 2001
 6 with 3 *Science* papers that showcased the widespread nature of microRNA (miRNA)
 7 expression in metazoan organisms (3-5). Since then, the number of microRNA-
 8 related entries in PubMed has grown exponentially to over 64,000 (Fig. 1A).

FIGURE 1



9

10 **Fig. 1:** Growth in the PubMed records returned using the search term (A)
 11 “microRNA” (A) or “microRNA and skin (B).

12 The miRNA revolution stems from the ability of these small non-coding RNA
 13 (ncRNA) molecules, typically ~22 nucleotides long, to regulate post-transcriptional
 14 expression across most of the genome, thereby fine-tuning numerous pathways that
 15 control cell behaviour (6, 7). Small ncRNAs are defined as being less than 200 nt
 16 long, and in addition to miRNAs, include small-interfering RNAs (siRNAs), piwi-
 17 interacting RNAs (piRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNA
 18 (snoRNAs) (8-10).

1 More recently, long ncRNAs (defined as >200 nt long) have garnered
2 attention as central regulators of physiological and pathological processes, despite
3 being expressed at 15-230 lower levels than protein coding transcripts (8). One such
4 lncRNA, PRINS (Psoriasis susceptibility-related RNA Gene Induced by Stress), has
5 long been associated with psoriasis, a debilitating skin disorder characterised by
6 complex interplay of cytokines from immune and skin cells (11, 12). Recent efforts
7 have defined a plethora of differentially expressed lncRNAs psoriatic skin (13-15)
8 and in cutaneous squamous cell carcinoma (cSCC) (16-18). The roles of lncRNA in
9 epidermal homeostatic and skin diseases have been very recently reviewed by
10 Botchkareva (19) so will not be considered further here.

11 As the largest and most accessible organ of the body, the skin represents a
12 major tissue for pharmacologic drugs targeting cutaneous disorders as well as
13 systemic delivery of active agents. The goal of this short review is to highlight the
14 emerging consensus on the global mechanism of miRNA action, the roles of miRNA
15 in development and disease with reference to the epidermis, the outer layer of the
16 skin. The translational prospects for topical delivery of RNA-directed oligonucleotides
17 for skin disorders will focus on cell penetrating peptides (CPPs), spherical nucleic
18 acid (SNA)-gold nanoparticles, deformable liposomes and liquid crystalline
19 nanoparticles (LCNPs). Nanoparticles based on natural or synthetic polymers such
20 as chitosan or poly(lactide-co-glycolic acid) will not be considered herein as they
21 have received little attention for cutaneous oligonucleotide delivery and have been
22 reviewed elsewhere for dermatological disorders (20). For broader consideration of
23 the issues associated with the design and delivery of oligonucleotide-based therapy
24 to diverse tissues, see the recent review by Juliano (21).

25

1 **MicroRNA Biogenesis**

2 Mature miRNAs arise from hairpin precursors (pre-miRNAs) which are themselves
3 largely derived from intergenic or intronic regions of coding and non-coding
4 transcripts as reviewed elsewhere (22-24). Gene repression by miRNAs is mediated
5 by the RNA-induced silencing complex (RISC), the assembly of which has been
6 reviewed recently (25). In its mature form, the RISC consists of a single-stranded
7 mature miRNA bound to an Argonaute (AGO) protein, of which there are four
8 paralogs in human cells (AGO1–AGO4). By guiding AGO proteins to the 3'
9 untranslated region (3' UTR) of target mRNA, the miRNA facilitates sequence-
10 specific repression of gene output (6, 22-26). Recent structural studies have yielded
11 exquisite insight into the mechanistic and conformational basis for guide miRNA
12 binding to AGO2 and target RNAs (27, 28).

13

14 **Mechanisms of microRNA action**

15 Mature miRNA duplexes consist of a guide strand which mediates RISC action and a
16 passenger or minor strand (miRNA*) that was considered to be degraded (29).
17 However, recent studies have shown miRNA* also regulate gene expression via
18 RISC-dependent binding to the 3'UTR of mRNA transcripts (30, 31). For this and
19 other reasons (32, 33) mature miRNAs are now designated miR-#-5p or miR-#-3p
20 according to the precursor hairpin arm from which they arise.

21 Early studies indicated that a given miRNA can downregulate the expression
22 of hundreds of target genes at the mRNA and protein levels (34-37). What has been
23 somewhat controversial is whether such miRNA-dependent attenuation of gene
24 output relies primarily on destabilisation and degradation of mRNA or on inhibition of
25 translational (38). Several studies suggested miRNAs function by blocking initiation

1 or elongation phases of translation (39-43) and that, at least in HeLa cells,
2 translational inhibition was the dominant effect of miRNAs (44, 45). However, the
3 Bartel group observed little change in the translational efficiency of ribosomes on
4 target mRNAs in HeLa cells, accounting for around 16% of the observed miRNA-
5 dependent repression. In contrast 84% of miRNA-mediated gene repression could
6 be attributed to mRNA destabilisation (46). Subsequent investigations on cell lines,
7 primary macrophages, mouse liver and primary B cells revealed that while
8 translational repression dominates miRNA action immediately following robust
9 miRNA induction, this is soon subsumed within a much greater mRNA destabilization
10 effect that maintains steady-state repression (47).

11

12 **MicroRNAs and epidermal development**

13 The epidermis forms the outer part of the skin and consists predominantly of
14 keratinocytes stratified from a basal layer of viable cells to an outermost layer of
15 terminally differentiated keratinocytes. Studies returned by PubMed in relation to
16 “microRNA and skin” have also grown exponentially over the last decade,
17 showcasing the efforts that have been made to define the contributions of
18 microRNAs to normal and pathological skin biology (Fig. 1B). From a developmental
19 perspective, studies with mouse models have revealed that miR-203, miR-205 and
20 miR-214 function as central controllers of epidermal morphogenesis. Several
21 independent laboratories revealed that by downregulating the stem-cell associated
22 transcription factor p63 along with other targets, miR-203 supports the initial
23 commitment of embryonic stem cells to the keratinocyte lineage and bolsters the
24 transition from proliferation to differentiation during stratification of the epidermis (48-
25 51). Loss of miR-205 led to derepression of phosphatases and other antagonists of

1 the pro-survival protein Akt, resulting in a dramatic reduction in Akt activation in
2 interfollicular progenitors and HFSCs (52). Hence the main function of miR-205
3 appears to be maintenance of the proliferative capacity of basal cells in the nascent
4 epidermis, hair follicle stem cells (HFSCs), outer root sheath, oesophagus and
5 tongue (52).

6 For miR-214, studies on transgenic mice have revealed multiple roles in the
7 regulation of both embryonic hair follicle development and postnatal hair cycling (53).
8 Keratinocyte-specific overexpression of miR-214 reduced proliferation of hair matrix
9 cells and interfollicular keratinocytes, leading to thinner hair shafts and thinner
10 epidermises, respectively (53). For further details on miRNA functions in skin
11 development, see the excellent review by Yi and colleagues (54).

12 Mouse studies have provided valuable insights concerning miRNA function in
13 the developing epidermis. However, little consideration has been given to concerted
14 efforts to use human cell and tissue models to uncover similar understanding of
15 miRNA function. Ablation or ectopic expression of miRNAs in human induced
16 pluripotent stem cells (hiPSCs) could provide a distinctive framework for
17 characterising miRNA function in epidermal stratification and folliculogenesis, given
18 that both mouse and human pluripotent SCs have been differentiated into
19 multipotent keratinocytes that generate interfollicular keratinocytes, stratified
20 epidermal equivalents and hair follicle cells (55-60). With over 700 hiPSC lines now
21 available from the Human Induced Pluripotent Stem Cells Initiative (61), the path is
22 clear for renewed efforts to decipher miRNA function using these cell lines as an
23 alternative to mouse-based studies. Importantly, miRNA expression can be silenced
24 in pluripotent SCs using transcription activator-like effector nucleases (TALENs) (62,
25 63) or with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

1 and CRISPR-associated protein 9 (Cas9) nuclease gene editing (64, 65). Therefore,
2 CRISPR-Cas9 reagents can be introduced into iPSCs using nucleofection or non-
3 lipid based chemical transfection with GeneJuice® (66, 67). The utility of CPPs and
4 SNAs in delivery of TALEN and CRISPR-Cas9 reagents to iPSCs for subsequent
5 differentiation into epidermal keratinocytes or hair follicle cells for 'omics studies,
6 morphometric assessment, drug screening and exposome analyses (68) warrants
7 detailed investigation.

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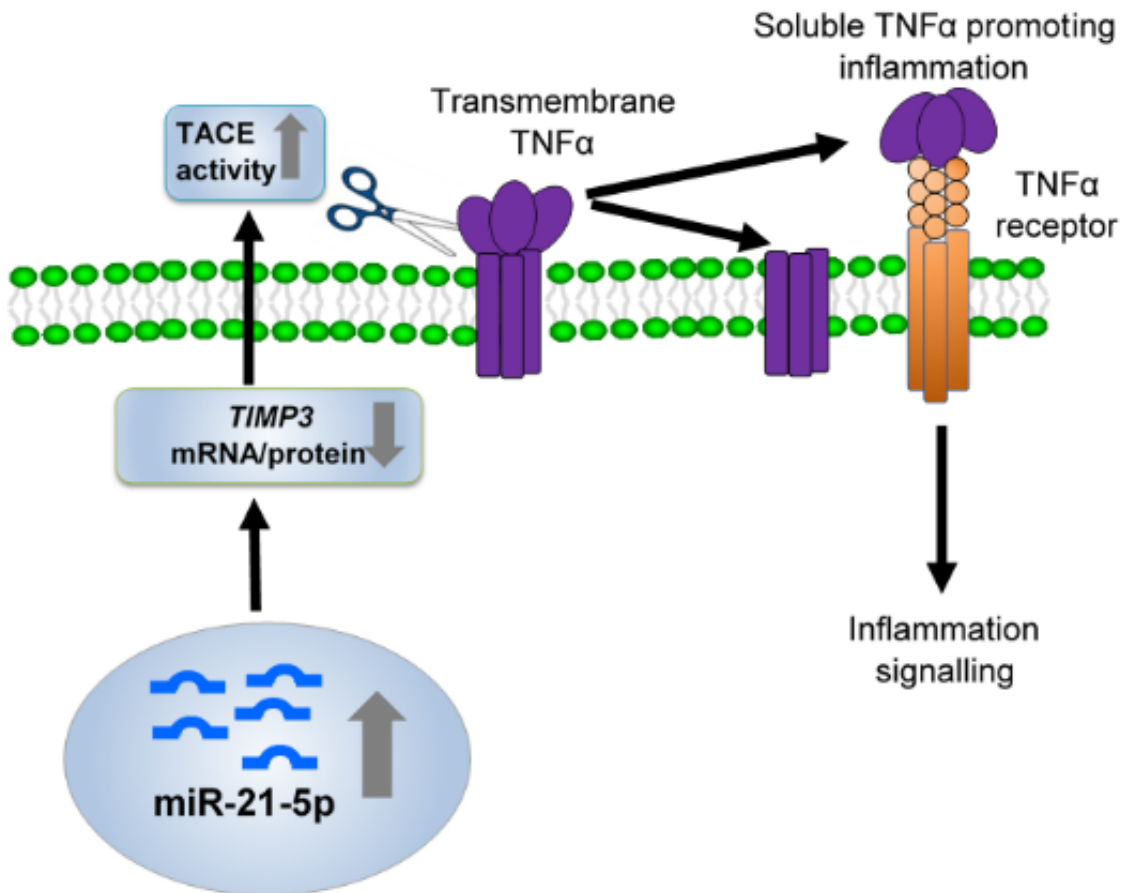
9 **MicroRNAs and psoriasis**

10 The major inflammatory molecules associated with epidermal inflammation and
11 hyperplasia in psoriasis include interferon gamma (IFN γ), tumour necrosis factor
12 alpha (TNF α), transforming growth factor beta (TGF β) and interleukins (ILs)
13 including IL-1, IL-17 and IL-22). Medium-scale and comprehensive screens revealed
14 multiple miRNAs were dysregulated in psoriasis (69, 70), among which miR-21-5p
15 functions as key mediator of epidermal inflammation (71). As illustrated in Fig. 2,
16 miR-21-5p downregulates tissue inhibitor of metalloproteinase 3 (TIMP-3) in
17 keratinocytes, as reported in several independent studies (71-73). The subsequent
18 elevation of tumour necrosis factor alpha (TNF α)-converting enzyme (TACE; also
19 known as ADAM17) activity results in enhanced release of soluble TNF α from
20 keratinocytes to promote epidermal inflammation (71, 74). Notably, the depletion of
21 TIMP-3 in a psoriasis-like mouse and in patient-derived xenografts on
22 immunodeficient mice was reversed using anti-miR-21 oligonucleotides and this was
23 associated with downregulation of inflammatory cytokines including IL-17, IL-23 and
24 TNF α (71). Importantly, epidermal thickness of the xenografts was reduced by
25 intradermal injection of anti-miR-21 every 48 h for 30 days. However, this decrease

1 in epidermal thickness was observed in 8 out of 11 psoriasis cases (73%), leaving
2 open the question of what factors precluded anti-miR-21 efficacy in the non-
3 responders.

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9 **Fig. 2:** Schematic depiction of a miRNA-21-dependent inflammation pathway.

10 Elevation of miR-21 through mechanisms that are yet to be fully defined in
11 keratinocytes leads to post-transcriptional repression of TIMP-3. Unfettered from
12 TIMP-3 inhibition, TACE activity is enhanced, boosting the processing of
13 transmembrane TNFα precursor into the soluble form that promotes inflammation
14 through the TNFα receptor.

1 Interestingly, elevated levels of miR-21-3p have also been linked to skin
2 inflammation (75). As the passenger or minor strand of the pre-miR-21 duplex, miR-
3 21-3p appeared to be around 1,000 times lower than miR-21-5p levels in the mouse
4 epidermis. Nevertheless, pharmacologic or UV-dependent activation of the
5 peroxisome proliferator-activated receptor (PPAR β/δ) nuclear hormone receptor led
6 to a significant increase of miR-21-3p levels in mouse skin and the HaCaT
7 keratinocyte cell line (75). In psoriatic skin, miR-21-3p was raised almost 4-fold
8 compared to healthy skin (75). Importantly, although these observations were made
9 using a rather small cohorts ($n \geq 4$ independent biopsies) earlier work by Bowcock
10 and colleagues had observed an almost 9-fold increase in miR-21-3p levels in
11 lesional psoriatic skin compared to healthy skin, using 24 and 20 independent
12 biopsies, respectively (70). Thus elevation of miR-21-3p appears to be strongly
13 associated with psoriasis. Whether inhibition of miR-21-3p ameliorates psoriasiform
14 inflammation remains to be seen. Nonetheless, topical anti-miR-21 oligonucleotides
15 may need to target both miR-21-5p and miR-21-3p in the psoriatic epidermis for
16 maximal therapeutic efficacy.

17 The relationships between cytokines and miRNA dysregulation in
18 keratinocytes have not been fully defined. That said, TGF β 1 has been shown to
19 promote miR-21-5p and miR-21-3p expression in HaCaT keratinocytes (72, 73, 75-
20 77). Furthermore, miR-31 was induced by TGF β 1 in primary human keratinocytes,
21 but not significantly changed by TNF α , IL-22, IL-6 or IFN γ (78). This contrasts with
22 recent work by Wang and collaborators, who showed that each of these four
23 cytokines could elevate miR-31 levels in primary keratinocytes by ≥ 2 fold, depending
24 on concentration (79). In any case, levels of miR-31 were significantly elevated in
25 psoriatic biopsies (78, 79). Importantly, inhibition of miR-31 reduced the basal and

1 TNF α -dependent expression of inflammatory cytokines and chemokines in human
2 keratinocytes and reduced epidermal thickening and keratinocyte hyperproliferation
3 in the imiquimod mouse model (78, 79). Taken together, these studies also suggest
4 miR-31 functions within a positive feedback loop in keratinocytes: IL-6 activates NF-
5 κ B to drive miR-31 expression and miR-31 represses serine/threonine kinase 40
6 (STK40), a suppressor of NF- κ B-dependent transcription to further enhance miR-31
7 levels. It will be interesting to evaluate this hypothesis and define the implications of
8 such a regulatory loop quantitatively.

9 The morphological alterations associated with the psoriatic epidermis are
10 strongly linked to IL-22 activity (80). Little is known about the impact of IL-22 on
11 keratinocyte miRNA expression. We have shown that IL-22 induces miR-184 in
12 reconstituted human epidermis and in HaCaT keratinocytes, as does oncostatin M
13 (81). Inhibition of the JAK/STAT pathway abrogated the cytokine-dependent
14 expression of miR-184. Interestingly, we demonstrated the RISC effector AGO2 was
15 downregulated by miR-184, suggesting a miR-184:AGO2 axis of dysregulation in
16 psoriasis AGO2 (81). However, the extent to which miR-184 modulates epidermal
17 homeostasis is unclear though recent studies on HaCaT keratinocytes suggest that
18 miR-184 stimulates keratinocyte proliferation and reduces apoptosis (82). Beyond
19 miR-184, IL-22 has also been shown to promote the expression of miR-197 in
20 keratinocytes, and this was associated with increased binding of STAT3 to the miR-
21 197 promoter (83).

22 Interestingly, miR-146a was among the first miRNAs found to be elevated in
23 psoriatic skin (69). Evidence from the Sonkoly and Pivarcsi group indicated that Toll-
24 like receptor (TLR) ligands induced a sustained increase in miR-146a in
25 keratinocytes, parsimoniously, to downregulate the expression of inflammatory

1 chemokines such as IL-8 and CCL-20 (84, 85). The picture that emerges is one in
2 which miR-146a dampens TLR-dependent epidermal inflammation by suppressing
3 TRAF6 (TNF receptor-associated factor 6) and IRAK1 (IL-1 receptor-associated
4 kinase 1) consistent with early work by Baltimore and colleagues (86). Attenuation of
5 TRAF6 and IRAK1, both of which mediate TLR signalling, in turn impaired activation
6 of the nuclear factor kappa B (NF- κ B) transcriptional programme and the recruitment
7 of inflammatory cells (84, 85). More recent work showed miR-146a induction in
8 keratinocytes exposed to IL-17, a central driver of psoriatic inflammation in the skin
9 (87). Activation of NF- κ B, expression of IL-8 and the ability of keratinocytes to attract
10 neutrophils was abrogated in cells loaded with a synthetic miR-146a mimic,
11 presumably due to downregulation of TRAF6, which is known to mediate IL-17A
12 signalling to NF- κ B (88, 89). Importantly, intradermal injection of the miR-146a mimic
13 into mouse blocked the development of psoriasis-like inflammation (87). Obviously,
14 intradermal injection is not appropriate for psoriasis patients, hence, like with anti-
15 miR-21, there is an urgent need for validated topical strategies to deliver miR-146a
16 mimics to psoriatic skin in clinical trials.

17 Interestingly, a functional polymorphism in the *miR-146a* gene appeared to
18 protect against early onset of psoriasis, apparently due to the anti-inflammatory
19 impact of elevated levels of miR-146a in keratinocytes (87). Patients with the CC
20 rs2910164 genotype were slightly protected against psoriasis when compared to
21 those with the GC or GG genotypes. This raises the intriguing prospect of a
22 prophylactic regimen based on miR-146a delivery to patients at higher risk of
23 developing the disease.

1 Several miRNAs have also been reported to be downregulated in psoriatic
2 skin, including miR-125b (90), miR-99a (91) and miR-424 (92). The roles of these
3 and other miRNAs in psoriasis have been reviewed in detail elsewhere (93-96).

4

5 **MicroRNAs and cutaneous squamous cell carcinoma**

6 Non-melanoma skin cancers are the most common malignancies in the world and
7 represent a growing public health challenge due to population ageing, UV exposure,
8 indoor tanning and other environmental factors (97, 98). Although low-risk localised
9 cSCC lesions can be treated by surgical methods, advanced metastatic disease has
10 an estimated mortality rate of >70% despite various chemotherapeutic options (99,
11 100).

12 Early work indicated that miR-21-5p and miR-184 were elevated in cSCC
13 compared to normal skin (101). Several independent studies have confirmed miR-
14 21-5p upregulation in cSCC (102-108). Furthermore, miR-21-3p, miR-31 and miR-
15 135b appear to be consistently elevated in cSCC (75, 106, 107, 109). With miR-21
16 and miR-31 also being strongly linked to psoriasis as discussed above, it would be
17 interesting to unravel the associated genomic or exposomal factors that determine
18 the propensity of keratinocytes with elevated miR-21 or elevated miR-31 to proceed
19 down psoriatic or cSCC paths.

20 In contrast to the raise levels of a few miRNAs, the majority of differentially
21 expressed miRNAs detected in cSCC were down-regulated (103, 104, 107, 109).
22 This evokes questions about the global mechanisms underpinning miRNA alterations
23 in cSCC and their relevance to cSCC therapy. Conceptually, reversing the overall
24 depletion of miRNAs may offer translational benefits that exceed those of replacing
25 individual miRNAs. However, the key factors leading to miRNA depletion in cSCC

1 cells have not been elucidated and the mechanistic explanations for downregulation
2 of specific miRNAs, such as miR-124, miR-125b and miR-204 cSCC remain obscure
3 (103, 107, 110, 111). Furthermore, although mutations in NOTCH1 and in TGF β
4 receptors have been defined as primary drivers of cSCC (112, 113), the relationships
5 between impaired NOTCH1 or TGF β receptor signalling and miRNA depletion have
6 not been investigated.

7 Conflicting observations have been made in relation to miR-205 in cSCC, with
8 some studies suggesting no differential expression between normal skin and cSCC
9 (101, 103) and others reporting elevation of miR-205 (105, 108). Notably, in an oral
10 SCC cell line, elevated miR-205 was associated with sustained Akt signalling and
11 cell survival via repression of SH2-containing phosphoinositide 5'-phosphatase
12 (SHIP2) (114). Hence, in cases where miR-205 is raised in cSCC, the mechanisms
13 of carcinogenesis may be related to the roles of miR-205 in epidermal development
14 (52). Other miRNAs implicated in cSCC include miR-34a, miR-199a and miR-361-
15 5p, as reviewed in (115).

16

17 **MicroRNAs in keratinocyte migration and wound healing**

18 Non-healing chronic wounds are also a burgeoning public health issue associated
19 with the rising incidence of diabetes and the ageing population (116). Co-ordinated
20 re-epithelisation of the wound surface by migrating keratinocytes is a crucial element
21 of wound closure (117). Some miRNAs associated with psoriasis, such as miR-21,
22 miR-203 and miR-31, have also been implicated in keratinocyte migration (72, 118,
23 119) Raised expression of TGF β 1 in wounded mouse skin correlates with the
24 induction of miR-21-5p in keratinocytes, and upregulation of miR-21 in the migrating
25 cells mediates the early phase of wound contraction (72, 120). However, although

1 inhibition of miR-21-5p with anti-sense oligonucleotides impaired keratinocyte
2 migration in these studies, the putative ability of a miR-21 mimic to promote wound
3 healing was not examined. Furthermore, as highlighted above, elevated miR-21-5p
4 has been associated with cSCC. Therefore, the translational potential of a miR-21-5p
5 mimic in relation to wound healing remains unclear.

6 Other miRNAs regulating keratinocyte migration include miR-483-3p, miR-24,
7 miR-205, and miR-132 (121-125). Upregulated expression of miR-483-3p also
8 occurs scratch-injured cultures of human keratinocytes and wounded skin in mice to
9 sustain keratinocyte proliferation, peaking at the final stage of the wound closure
10 process (121). Elevation of miR-24 during keratinocyte differentiation controls cell
11 mobility and promotes differentiation by regulating the expression of several proteins
12 associated with remodelling of the actin cytoskeleton (122). Likewise, miR-205
13 activity has been shown to stimulate keratinocyte migration, and least partly by
14 regulate filamentous actin polymerization and loosening cell attachment to the
15 extracellular matrix (123). However, more recent studies from Su and colleagues
16 suggest that raised miR-205 in the migratory front of chronic non-healing venous
17 ulcers impairs wound healing (124). Inhibition of miR-205 derepressed integrin
18 subunit alpha 5 (ITGA5), a component of the classical fibronectin receptor,
19 enhancing wound closure in monolayer scratch assays and wounded mouse skin
20 (124)., Interestingly, elevated expression of miR-210 has also been observed in
21 keratinocytes at the edge of ischemic wound tissue (126). Consequently, a clinical
22 trial has been launched to evaluate the relationship between miR-210 and clinical
23 outcomes in patients with chronic venous leg ulcers (NCT02024243). Thus both
24 miR-205 and miR-210 have potential as targets for wound therapy, and vehicles for

1 effective delivery of anti-miR-205 and anti-miR-210 oligonucleotides to keratinocytes
2 will likely be required for translational purposes.

3

4 **Topical targeting of microRNAs in cutaneous disease**

5 Validated agents for cutaneous delivery of miRNA-directed oligonucleotides (miRNA
6 mimics or miRNA inhibitors) for translational and clinical benefit have not yet been
7 realised. The epidermis presents a physical and immunological barrier against the
8 external environment and associated pathogens (127, 128). The stratum corneum
9 (SC) provides most of the epidermal barrier function, composed as it is of non-viable
10 keratinocyte squames embedded in a lipid-rich matrix, making it largely impermeable
11 to water and to hydrophilic and lipophilic substances greater than 500 Da (129-131).
12 Hence drug delivery to the viable epidermis and beyond requires optimisation of
13 multiple parameters to secure efficient delivery without evoking an irritation response
14 (132). We now consider emerging approaches for conveying RNA-directed
15 oligonucleotides into the epidermis, focusing on CPPs, SNA-gold nanoparticles,
16 deformable liposomes and LCNPs. The transmission of miRNA-directed
17 oligonucleotides to the epidermis is an emerging field that can take advantage of the
18 technologies already being explored for cutaneous delivery of siRNA and other
19 oligonucleotides. Physical approaches (microneedles, microporation) and active
20 methods (electroporation, iontophoresis, sonophoresis) for oligonucleotide delivery
21 into skin have been reviewed previously by Mitragotri and colleagues (133).

22

23 **Cell penetrating peptides**

24 Extensive studies on the potential of cell penetrating peptides CPPs for drug delivery
25 have been performed since the HIV TAT peptide and the *Drosophila* peptide

1 penetratin were first defined as CPPs (134, 135). Sequences presented in Table 1
 2 illustrate the diversity of selected CPPs known to traverse the SC into the viable
 3 epidermis. Broadly, CPPs are thought to enter cells through endocytosis-driven
 4 pathways or via direct translocation across the lipid bilayer, and the reader is
 5 referred to the excellent review by Bechara and Sagan for mechanistic details (136).

6 Early work by Khavari and collaborators indicated that conjugation of the
 7 immunosuppressant cyclosporine A (CsA) to the CPP poly-arginine (R7) enabled
 8 CsA to cross the SC of mouse and human skin (137). More recently, a SPACE (skin
 9 penetrating and cell entering) peptide was isolated by iterative selection from an in
 10 vitro phage display library and shown to facilitate delivery of CsA into porcine
 11 epidermis (138, 139). In a comparison of CPP-mediated entry of CsA into porcine
 12 skin, Mitragotri and colleagues observed little difference in performance of the
 13 SPACE peptide and R7 (140). A third peptide, TD-1, also delivered CsA into the
 14 skin, albeit with slightly lower efficiency than SPACE peptide and R7. Although all
 15 three skin-penetrating peptides (SPPs) showed minimal irritation, the toxicity profile
 16 of the SPACE peptide was lower than that of R7 and TD-1.

17

18 **Table 1: Sequences of selected skin penetrating peptides**

CPP	Primary Sequence	Refs.
IMT-P8	RRWRRWNRFNRRRCR	(141)
PepFect6	Stearyl-AGYLLGK(K(K2(tfq4)))INLKALAALAKKIL-NH2L*	(142)
Poly-Arginine (R7)	RRRRRRRR	(140)
SPACE peptide	AC-TGSTQHQC-G	(138)
TD-1	ACSSSPSKHCG	(143)

19 *PepFect6 is stearylated analogue of transportan-10, with four trifluoroquinoline moieties attached via
 20 a lysine triplex

1 The SPACE peptide has been shown to deliver covalently conjugated siRNA
2 through the SC to silence target gene expression in mouse skin (138, 144, 145). In
3 the rat footpad model, TD-1 transmitted siRNA throughout the epidermis and
4 reduced expression of a target gene (143). Hence, although the use of SPACE
5 peptide, R7 or TD-1 to deliver miRNA-directed oligonucleotides has not been
6 evaluated to my knowledge use of these CPPs should be generalizable to miRNA
7 mimics or inhibitors.

8 An *in silico* screening approach recently yielded a 15-amino acid arginine-rich
9 CPP, IMT-P8, penetrated cells 10 times more efficiently than TAT or a related IMT-
10 P4 peptide (141). The efficiency of IMT-P8 appears to stem not just from the helical
11 structure it adopts but also the specific clustering of arginine residues on one phase
12 of the helix and two critical tryptophan residues on the opposite phase (Fig. 3).

13 The main mechanism of cell entry by IMT-P8 appears to be macropinocytosis
14 mediated by cell-surface proteoglycans (141). Interestingly, the N-terminal portion of
15 the IMT-P8 primary sequence (RRWRRWNRFRRCR) is similar to the R₆W₃
16 peptide (RRWWRRWRR) reported by Sagan and colleagues (146). A comparison of
17 the skin-penetrating capacities of these two peptides should be revealing in terms of
18 the extent to which residues 10-15 of IMT-P8 are required for activity.

19 In tests on shaved mouse skin, FITC-labelled IMT-P8, with or without a
20 peptide cargo, appeared to partition predominantly to the SC (147). Relatively little
21 distribution was evident in the bulk of the epidermis. Similarly, a large (green
22 fluorescent protein) cargo attached to IMT-P8 appeared to be targeted mainly to hair
23 follicles (147). Hence, utility of IMT-P8 for therapeutic miRNA transport into the
24 epidermis will depend on the emergence of further evidence that IMT-P8 cargoes
25 localise and function within interfollicular keratinocytes. Alternatively, IMT-P8 may

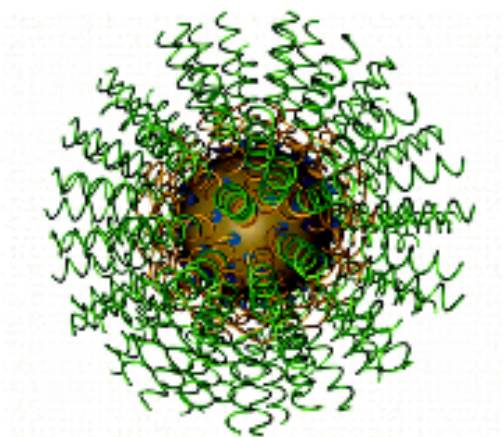
1 One limitation of the CPPs covered above is the requirement for covalent
2 conjugation to their cargoes. In contrast, PepFect6 (PF6), developed by the Langel
3 group, formed stable complexes with siRNA simply upon mixing the two molecules
4 (142, 151). PepFect6 is an analogue of transportan 10 designed with an N-terminal
5 stearyl moiety that enhances membrane insertion and four trifluoromethylquinoline
6 derivatives to promote endosomal escape (142, 152). Recently, subcutaneous
7 injection of PF6 CPP complexed with a miR-146a mimic was shown to suppress
8 inflammatory gene expression in a mouse model of irritant contact dermatitis (153).
9 There is arguably a case, therefore, for merging PF6 with SPACE peptide, R7 or TD-
10 1 in order to combine the oligonucleotide-binding capacity of PF6 with the ability of
11 the SPPs to cross the SC into the viable epidermis.

12

13 **Spherical nucleic acids and gold nanoparticles**

14 Various gold nanoparticles (AuNP) are also under investigation for oligonucleotide
15 delivery, reviewed in (154). Spherical nucleic acid nanoparticle conjugates (SNA-NC)
16 are particularly intriguing AuNPs that have been shown to rapidly enter over 50 cell
17 types (155). The original SNA-NC (depicted in Figure 4) consisted of a 3-dimensional
18 (3D) gold core decorated with a densely packed shell of covalently immobilized,
19 highly oriented oligonucleotides (156). Uptake of SNA-NC appears to occur via lipid-
20 raft–dependent, caveolae-mediated endocytosis upon binding to class A scavenger
21 receptors (157).

22 Work Paller and colleagues showed that such SNA-NC constructed with
23 siRNA duplexes against the epidermal growth factor receptor (EGFR) distributed
24 rapidly and extensively throughout the epidermis of hairless mouse skin and human
25 skin equivalents upon topical application (158). More importantly, the expression of



1

2 **Fig 4:** Depiction of a spherical nucleic acid illustrating a gold core surrounded by a
3 densely packed layer of covalently attached oligonucleotides. The core can be based
4 on other metals or on polymers, and appears dispensable for SNA function.
5 Reprinted with permission from Cutler et al., ref. (155). Copyright 2012, American
6 Chemical Society.

7

8

9

10 EGFR at the mRNA and protein levels was strongly suppressed in both skin models,
11 with no apparent toxicity (158). Similar siRNA-based SNA-NCs were found to
12 promote wound healing by downregulating ganglioside-monosialic acid 3 (GM3)
13 synthase (159). Notably, the gold core of SNA-NC can be replaced with a
14 biocompatible porous silica (SiO₂) shell without loss of SNA functionality (160). This
15 enhances the translational potential of SNAs given that silica can be degraded under
16 physiological conditions (161). Furthermore, exchanging the gold core for a hollow
17 silica shell showed that the emergent ability of SNAs to function as delivery agents is
18 due to the dense layer of oriented oligonucleotides as opposed to the inorganic core
19 (160).

1 The SNA-NC approach has been explored for targeting miRNA. Mirkin and
2 co-workers produced SNA-NC with miR-205 mimics to facilitate miRNA replacement
3 in prostate cancer cells (162). Although SNA-NC:miR-205 lowered the expression of
4 a target gene by 52%, compared to a non-targeting control SNA-NC, the effects of
5 SNA-NC:miR-205 on the transcriptome as a whole have not been established. Such
6 insights would reveal the putative differential impact that SNA-NC:miR-205 has on
7 miR-205-regulated transcripts. In any case, as miR-205 has been reported to
8 promote epidermal and corneal keratinocyte migration (123) the wound healing
9 potential of SNA-NC:miR-205 deserves exploration.

10 Whereas the SNA-NC:miR-205 study aimed to raise effective miR-205 levels,
11 a recent study constructed SNA-NC with antisense oligonucleotides to inhibit miRNA
12 function. Using SNA-NC:anti-miR-99b, inflammation-related depletion of a miR-99b
13 target gene was reversed in a mouse model of sepsis (163).

14 These miRNA-directed SNA-NC proof-of-concept studies combined with the
15 established abilities of topically applied SNA-NC to permeate the epidermis support
16 the development of SNA-NC as modulators of miRNA activity in the skin. Initial
17 efforts focussed on SNA-NC:miR-146a and SNA-NC:anti-miR-21 will be highly
18 relevant for psoriasis.

19 Non-covalent AuNP conjugates have also been evaluated for oligonucleotide
20 delivery (154). Recently, a layer-by-layer approach was used to generate chitosan-
21 coated AuNPs/siRNA/chitosan formulations that penetrated porcine ear skin under
22 iontophoresis (164). However, although AuNPs coated with chitosan or other
23 polymer is of broad biomedical interest, there is a paucity of data regarding their
24 utility for oligonucleotide delivery to the skin. The reader is referred to an excellent
25 review on polymer-coated AuNPs for further insight (165).

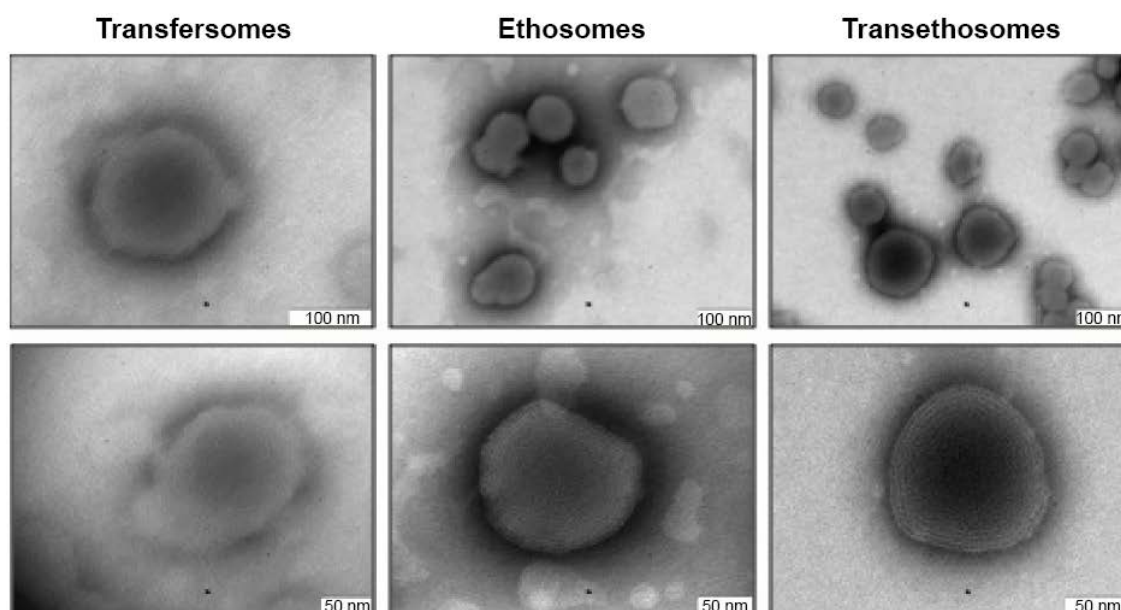
1 **Deformable liposomes**

2 Various “elastic” “flexible” or “ultradeformable” liposomes have recently emerged for
3 topical delivery of therapeutic agents (166-169). Elasticity in this context relates to
4 the presence of both stabilizing and destabilizing molecules within a given lipid
5 bilayer and the ability to redistribute within that bilayer (166). The exploitation of
6 liposomes for siRNA delivery has been broadly reviewed recently by Chourasia and
7 colleagues (170). Here, Transfersomes®, ethosomes®, transethosomes and
8 SECosomes have been selected to illustrate the potential of deformable liposomes
9 for topical cutaneous drug delivery.

10

11 **Transfersomes:** The first generation of elastic vesicles developed by Cevc and
12 colleagues were Transfersomes® consisting of phosphatidylcholine and a single
13 chain surfactant such as sodium cholate as an edge activator (171-174). Other edge
14 activators include Span 60/65/80, and Tween 20/60/80 (175, 176). Transfersomes®
15 have been shown to mediate transcutaneous delivery of large macromolecules
16 including protein immunogens (171), DNA vaccines (177, 178), insulin (173),
17 interleukin-2 (179), hydrophobic macromolecules such as lycopene (180) and
18 tretinoin (181). In addition, both glucocorticosteroids (182) and the nonsteroidal anti-
19 inflammatory drugs diclofenac (183, 184) and meloxicam (184-186) have been
20 formulated in Transfersomes®. Efforts to deploy Transfersomes® specifically for
21 inflammatory skin disease include formulation of tacrolimus for atopic dermatitis
22 (187) but use of Transfersomes® in miRNA-directed applications has not been
23 reported to our knowledge. The microscopic structures of Transfersomes®,
24 ethosomes® and transethosomes are depicted in Fig. 5.

25



1

2 **Fig 5:** Transmission electron micrographs of ultradeformable liposomes that been
3 compared directly for topical epidermal drug delivery. Reprinted from Ascenso et al.,
4 ref. (176) under the Creative Commons Attribution Non-Commercial (unported, v3.0)
5 License. Dove Medical Press Limited.

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7

8 **Ethosomes:** Work by Touitou and collaborators led to the development of
9 transfersome-like liposomes in which high dosing with ethanol was deployed to
10 enhance skin permeation (188). Ethosomal lipids assembled into phospholipid
11 bilayers in dispersions of up to 45% ethanol, and any solubilisation of the
12 phospholipid appeared to be limited (188). A diverse range of drugs have been
13 loaded into ethosomes® (168, 189). For psoriasis, these include methotrexate (190),
14 tretinoin (191) and 5-aminolevulinic acid, a second-generation photosensitizer for a
15 photodynamic therapy (192). Ethosomal entrapment has also been reported to
16 enhance the permeation of paclitaxel across the SC and improve anticancer activity
17 of the drug in a cSCC cell line (193). As with Transfersomes® though, the potential

1 of ethosomes® for oligonucleotide delivery into the epidermis remains an
2 underexplored area of research.

3

4 **Transethosomes:** By combining the edge activator approach of Transfersomes®
5 with the high ethanol dose of ethosomes®, Song and colleagues designed
6 transethosomes (175). Initial studies suggested enhance penetration of a lipophilic
7 drug compared to Transfersomes® or ethosomes® (175). In more recent tests using
8 porcine ear skin, Simões and colleagues found that transethosomes enabled deeper
9 penetration of vitamin E into the viable epidermis compared to both Transfersomes®
10 and ethosomes® (176). A similar comparison of Transfersomes®, ethosomes® and
11 transethosomes for delivery of fluorescently-labelled miRNA inhibitors or mimics
12 would provide a strong framework for pushing the use of these deformable vesicles
13 for miRNA-directed therapy.

14 Specific comparison of Transfersomes®, ethosomes® or transethosomes for
15 oligonucleotide transport into the epidermis does not appear to have been reported.
16 However, cationic elastic liposomes based on 1,2-dioleoyloxy-3-trimethylammonium
17 propane (DOTAP) and sodium cholate have been shown to convey oligonucleotides
18 into mouse epidermis (194, 195) and cadaveric human skin (196). Functional
19 efficacy was demonstrated using antisense oligonucleotides targeting IL-13 for
20 atopic dermatitis (194) and siRNA targeting BRAF in melanoma cells (196). Similar
21 liposomes comprising 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and
22 ceramide in addition to DOTAP and sodium cholate have also been tested for siRNA
23 delivery in A431 cSCC cells. Liposomes loaded with siRNA against signal transducer
24 and activator of transcription 3 (STAT3) attenuated target gene expression in A431

1 cells (197). However, no permeation of porcine ear skin was observed beyond the
2 SC unless the specimens were also subjected to iontophoresis (197).

3 Silencing of keratin 17 (KRT17) has also been explored for anti-psoriatic
4 potential through topical application of siRNA in a liposome-emulsion formulation
5 though the details of the liposome were not reported (198). Importantly, however,
6 Wang and colleagues showed siKRT17 silenced expression of KRT17 human
7 psoriatic skin xenografts on mouse models, and this was associated with histological
8 and clinical improvement including a reduction in epidermal thickness and
9 substantial clearance of erythema and scales (198).

10

11 **SECosomes:** Building on initial work showing siRNA-delivery to melanocytes using
12 DOTAP/sodium cholate elastic liposomes (199), Lambert and co-workers combined
13 DOTAP and sodium cholate with cholesterol as a stabilizer and 30% ethanol to
14 enhance penetration, creating surfactant-ethanol-cholesterol-osomes (SECosomes).
15 These SECosomes were shown to transmit siRNA to a skin-humanized mouse
16 model of psoriasis to silence expression of human beta-defensin 2 (hBD-2, encoded
17 by *DEF4*), an anti-microbial peptide that is highly over-expressed in psoriatic skin
18 (200, 201). More recently, by altering the cholesterol composition and replacing
19 sodium cholate with DOPE, the group produced a modified SECosome (DDC642)
20 with increased ability to deliver siRNA into the viable epidermis of normal and
21 psoriatic skin explants (202). The penetration of DDC642:siRNA lipoplexes into *ex*
22 *vivo* psoriatic was associated with strong suppression of the target hBD-2 within 48
23 h. In addition, DDC642 mediated pre-miR-145 and anti-miR-203 oligonucleotide in
24 melanocytes and keratinocytes, respectively, to modulate target mRNA levels (202).
25 However, anti-miR-203 had little effect on target protein levels raising questions

1 about the optimisation required to achieve biologically relevant miRNA-dependent
2 outcomes with DDC642. Very recently, the group also demonstrated the ability of
3 DDC642 complexed with siRNAs to repress targets in the reconstructed 3D psoriasis
4 skin model available from MatTek Corporation (203). Combining siRNAs against
5 hBD-2, thymic stromal lymphopoietin (TSLP) and KRT17 into a single DDC642
6 formulation silenced the first two of these genes by 38% and 45%, respectively
7 (203). However, individual siRNA formulations, including siKRT17 were more
8 effective at reducing the levels of distinct psoriasis markers indicating the synergistic
9 potential of a multi-targeted approach requires further evaluation.

10

11 **LeciPlex**

12 Liposomal vesicles in the form of self-assembled nanocarriers composed of lecithin
13 phospholipids and cationic lipids such as didodecyldimethylammonium bromide
14 (DDAB) or cetyltrimethylammonium bromide (CTAB) have also been developed
15 recently for drug delivery (204, 205). These LeciPlex nanocarriers transported
16 hydrophobic drug molecules to the SC, viable epidermis and dermis. Some evidence
17 of *in vivo* drug efficacy on a rat model of acne was observed when LeciPlex vesicles
18 were loaded with the antibacterial agent azelaic acid (204). However, the utility of
19 LeciPlex nanocarriers for transcutaneous delivery of oligonucleotides remains to be
20 determined. Although the abilities of DDAB and CTMA to trigger irritation raises
21 concerns over their suitability for skin therapy (204), the low cost of DDAB is an
22 important consideration for development of cost-effective liposomal nanocarriers,
23 given that DDAB h
24 as been estimated to cost 1/800 the price of DOTAP (206). Hence, the abilities of
25 recently reported DDAB-poly(ethylene glycol) nanoassemblies to deliver miRNA-

1 directed oligonucleotides across the SC into the viable epidermis warrants
2 investigation (206).

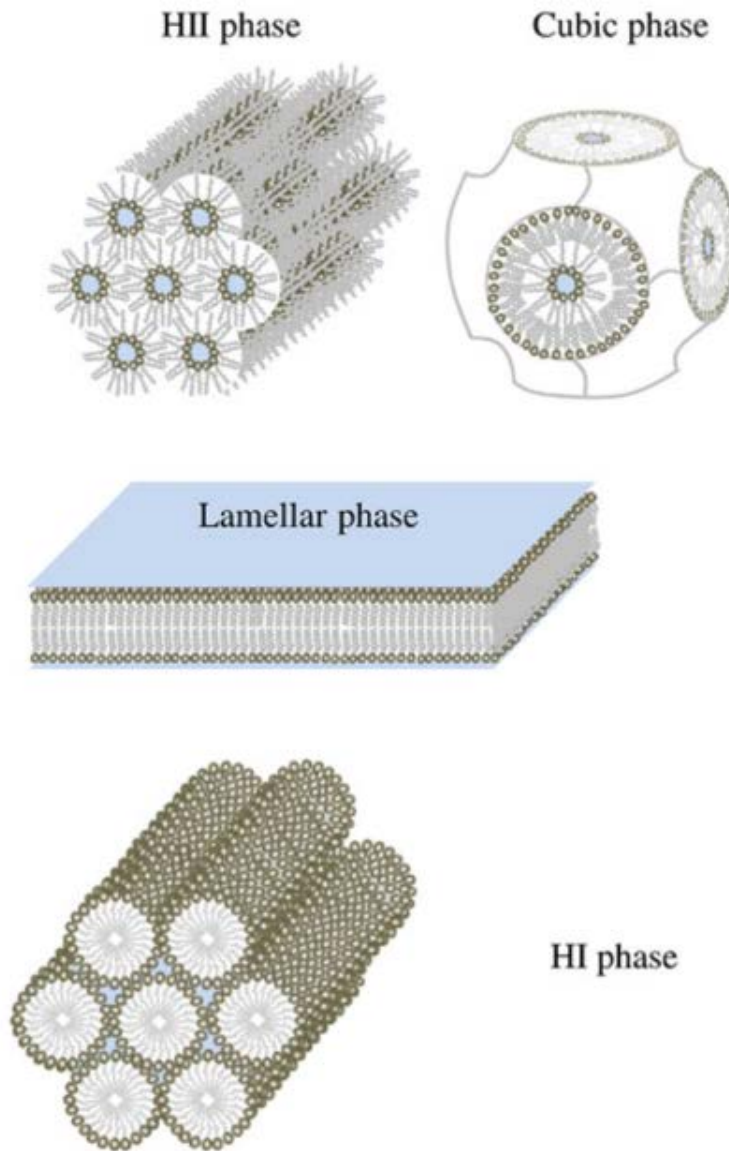
3

4 **Liquid crystalline nanoparticles**

5 Liquid crystalline phase aggregates are lipid-based alternatives to liposomes that
6 have received attention for topical siRNA delivery. When amphiphilic lipids are
7 placed in an aqueous environment, they can self-organise into diverse liquid
8 crystalline structures including the lamellar phase, cubic phase and reverse
9 hexagonal phase (207-209), depicted in Fig. 6.

10 Monoolein (MO; glycerol monooleate) is widely used for the generation of
11 such liquid crystalline nanoparticles (LCNPs) and the geometries of cubic phase
12 (cubosomes) and reverse hexagonal phase LCNPs make them particularly attractive
13 vehicles for drug delivery and controlled release (210-213).

14 Early work by the Bentley group found that both cubic and reverse hexagonal
15 phases of aqueous MO LCNPs enhanced the accumulation of CsA in the epidermis
16 and dermis of porcine skin and hairless mice (214). Addition of oleic acid (OA)
17 enabled formation of the reverse hexagonal phase at room temperature (214). More
18 recently, MO:OA nanodispersions incorporating cationic polymer polyethylenimine
19 (PEI) or cationic lipid oleylamine (OAM) were shown to transmit siRNA across the
20 SC into the viable epidermis of hairless mice and silence expression of the GAPDH
21 target (215). Importantly, using optimised MO:OA:PEI:aqueous phase dispersions,
22 siRNA was targeted to IL-6 in a reconstituted human psoriasis skin model, leading to
23 a 3-fold reduction in secreted IL-6 levels (216). Interestingly, functionalisation of



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Fig. 6: Liquid crystalline phase structures. Representation of the reverse hexagonal (HII), cubic, lamellar and tubular (HI) phases that can emerge depending on the relative cross-sectional dimensions of polar head groups and hydrophobic regions. Adapted from Jouhet, ref. (209) under the Creative Commons Attribution License. Frontiers Media.

1 MO:OA:PEI:aqueous phase nanodispersions enhanced siRNA-mediated
2 suppression of TNF α in a phorbol ester-induced model of inflammation on hairless
3 mouse skin (217). Together, these developments suggest Bentley's LCNPs should
4 prove useful in studies aimed at delivering miRNA-directed oligonucleotides,
5 especially anti-miR-21 and miR-146a mimics, to reconstituted in vitro and mouse
6 models of psoriatic skin. In addition, functionalisation with SPACE peptide,
7 polyarginine or IMT-P8 may enhance the penetration of Bentley's LCNPs into the
8 epidermis even more than with the TAT peptide.

9

10 **Targeting the epidermal keratinocyte**

11 Diverse receptors including scavenger receptors, receptor tyrosine kinases, G
12 protein-coupled receptors, integrins and TLRs can be selectively targeted for
13 oligonucleotide delivery (21). In addition, glycosaminoglycan (GAGs) have also been
14 targeted to promote uptake of a range of cargoes in hard-to-transduce cells (218).
15 For specific targeting of keratinocytes, a particularly novel approach has been
16 developed using an anti-desmoglein (Dsg) monoclonal antibodies (219). Desmoglein
17 is a non-classical cadherin found in the desmosomes of the epidermis (220). Stanley
18 and collaborators cloned a single-chain variable fragment (scFv) of a patient-derived
19 anti-Dsg antibody to yield Px44. As Px44 does not include the antibody effector
20 region, antibody-induced inflammation is avoided (219). In their studies, specific
21 localization of a Px44-conjugated protein cargo to epidermal keratinocytes was
22 observed after intradermal injection of the complexes into human skin xenografts on
23 mice (219). The obvious corollary would be to determine whether Px44 can enhance
24 the overall efficacy of epidermal oligonucleotide delivery with the vehicles surveyed
25 above.

1 **Conclusions**

2 In summary, we are beginning to witness the deployment of the diverse delivery
 3 vehicles surveyed above for topical delivery of siRNA, miRNA mimics and miRNA
 4 inhibitors to the epidermis (Table 2). Translating the promising initial findings to
 5 successful epidermal miRNA-dependent therapy will need to be supported with
 6 system-wide proteogenomic analyses of reconstituted human psoriatic epidermis
 7 models (221-223) exposed to the various topical vectors surveyed herein. In line with
 8 the need for affordable psoriasis treatments highlighted by the World Health
 9 Organisation (224), it will also be important to maintain cost-effectiveness for any
 10 therapies that emerge. In the meantime, clinical trials of nanoscale approaches for
 11 topical miRNA-directed therapy are likely to emerge in the near future for psoriasis,
 12 cSCC and wound healing.

13

14 **Table 2: Topical RNA interference for epidermal disease**

Target	Vehicle	Model	Refs.
<i>miR-146a</i>	PepFect6/miR-146a mimic ¹	Mouse ear irritant contact dermatitis	(153)
<i>EGFR</i>	SNA-NC/siRNA	Hairless mouse skin; reconstituted skin equivalents	(158)
<i>GM3S</i>	SNA-NC/siRNA	Diabetic mouse wounds	(159)
<i>STAT3</i>	chitosan-coated AuNPs/siRNA/ chitosan ²	Porcine ear skin	(164)
<i>STAT3</i>	Cationic liposomes/siRNA ²	Porcine ear skin	(197)
<i>DEFB4</i>	DDC642 SECosome/siRNA	Reconstituted psoriatic skin	(202)
<i>SOCS3</i>	DDC642 SECosome/anti-miR-203	Keratinocytes	(202)
<i>FSCN1</i>	DDC642 SECosome/pre-miR-145	Melanocytes	(202)
<i>IL-6</i>	Liquid crystalline nanodispersions/siRNA	Reconstituted psoriatic skin	(216)
<i>TNFα</i>	Liquid crystalline nanodispersions with TAT peptide	Hairless mouse skin with chemically-induced inflammation	(217)

15 ¹ PepFect6/miR-146a mimic delivered locally by subcutaneous injection

16 ² Iontophoresis was required for penetration beyond the stratum corneum

17

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5

REFERENCES

- 6 1. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs
7 with antisense complementarity to *lin-14*. *Cell*. 1993;75(5):843-54.
- 8 2. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-
9 nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature*.
10 2000;403(6772):901-6.
- 11 3. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for
12 small expressed RNAs. *Science*. 2001;294(5543):853-8.
- 13 4. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable
14 regulatory roles in *Caenorhabditis elegans*. *Science*. 2001;294(5543):858-62.
- 15 5. Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science*.
16 2001;294(5543):862-4.
- 17 6. Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. *Cell Death Differ*.
18 2015;22(1):22-33.
- 19 7. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets
20 of microRNAs. *Genome Res*. 2009;19(1):92-105.
- 21 8. Dykes IM, Emanuelli C. Transcriptional and Post-transcriptional Gene Regulation by Long
22 Non-coding RNA. *Genomics Proteomics Bioinformatics*. 2017;15(3):177-86.
- 23 9. Liu Q, Paroo Z. Biochemical principles of small RNA pathways. *Annu Rev Biochem*.
24 2010;79:295-319.
- 25 10. Dupuis-Sandoval F, Poirier M, Scott MS. The emerging landscape of small nucleolar RNAs in
26 cell biology. *Wiley interdisciplinary reviews RNA*. 2015;6(4):381-97.
- 27 11. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annual review of*
28 *immunology*. 2014;32:227-55.
- 29 12. Sonkoly E, Bata-Csorgo Z, Pivarcsi A, Polyanka H, Kenderessy-Szabo A, Molnar G, et al.
30 Identification and characterization of a novel, psoriasis susceptibility-related noncoding RNA gene,
31 PRINS. *J Biol Chem*. 2005;280(25):24159-67.
- 32 13. Tsoi LC, Iyer MK, Stuart PE, Swindell WR, Gudjonsson JE, Tejasvi T, et al. Analysis of long non-
33 coding RNAs highlights tissue-specific expression patterns and epigenetic profiles in normal and
34 psoriatic skin. *Genome Biol*. 2015;16:24.
- 35 14. Gupta R, Ahn R, Lai K, Mullins E, Debbaneh M, Dimon M, et al. Landscape of Long Noncoding
36 RNAs in Psoriatic and Healthy Skin. *J Invest Dermatol*. 2016;136(3):603-9.
- 37 15. Ahn R, Gupta R, Lai K, Chopra N, Arron ST, Liao W. Network analysis of psoriasis reveals
38 biological pathways and roles for coding and long non-coding RNAs. *BMC Genomics*. 2016;17(1):841.
- 39 16. Sand M, Bechara FG, Sand D, Gambichler T, Hahn SA, Bromba M, et al. Expression profiles of
40 long noncoding RNAs in cutaneous squamous cell carcinoma. *Epigenomics*. 2016;8(4):501-18.
- 41 17. Piipponen M, Nissinen L, Farshchian M, Riihila P, Kivisaari A, Kallajoki M, et al. Long
42 Noncoding RNA PICSAR Promotes Growth of Cutaneous Squamous Cell Carcinoma by Regulating
43 ERK1/2 Activity. *J Invest Dermatol*. 2016;136(8):1701-10.

- 1 18. Ponzio G, Rezzonico R, Bourget I, Allan R, Nottet N, Popa A, et al. A new long noncoding RNA
2 (lncRNA) is induced in cutaneous squamous cell carcinoma and down-regulates several anticancer
3 and cell differentiation genes in mouse. *J Biol Chem.* 2017;292(30):12483-95.
- 4 19. Botchkareva NV. The Molecular Revolution in Cutaneous Biology: Noncoding RNAs: New
5 Molecular Players in Dermatology and Cutaneous Biology. *J Invest Dermatol.* 2017;137(5):e105-e11.
- 6 20. Zhang Z, Tsai PC, Ramezanli T, Michniak-Kohn BB. Polymeric nanoparticles-based topical
7 delivery systems for the treatment of dermatological diseases. *Wiley Interdiscip Rev Nanomed*
8 *Nanobiotechnol.* 2013;5(3):205-18.
- 9 21. Juliano RL. The delivery of therapeutic oligonucleotides. *Nucleic Acids Res.*
10 2016;44(14):6518-48.
- 11 22. Finnegan EF, Pasquinelli AE. MicroRNA biogenesis: regulating the regulators. *Crit Rev*
12 *Biochem Mol Biol.* 2013;48(1):51-68.
- 13 23. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol.* 2014;15(8):509-24.
- 14 24. Stroynowska-Czerwinska A, Fiszer A, Krzyzosiak WJ. The panorama of miRNA-mediated
15 mechanisms in mammalian cells. *Cell Mol Life Sci.* 2014;71(12):2253-70.
- 16 25. Kobayashi H, Tomari Y. RISC assembly: Coordination between small RNAs and Argonaute
17 proteins. *Biochim Biophys Acta.* 2016;1859(1):71-81.
- 18 26. Meister G. Argonaute proteins: functional insights and emerging roles. *Nat Rev Genet.*
19 2013;14(7):447-59.
- 20 27. Elkayam E, Kuhn CD, Tocilj A, Haase AD, Greene EM, Hannon GJ, et al. The structure of
21 human argonaute-2 in complex with miR-20a. *Cell.* 2012;150(1):100-10.
- 22 28. Schirle NT, Sheu-Gruttadauria J, MacRae IJ. Structural basis for microRNA targeting. *Science.*
23 2014;346(6209):608-13.
- 24 29. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol.*
25 2009;10(2):126-39.
- 26 30. Yang JS, Phillips MD, Betel D, Mu P, Ventura A, Siepel AC, et al. Widespread regulatory
27 activity of vertebrate microRNA* species. *RNA.* 2011;17(2):312-26.
- 28 31. Okamura K, Phillips MD, Tyler DM, Duan H, Chou YT, Lai EC. The regulatory activity of
29 microRNA* species has substantial influence on microRNA and 3' UTR evolution. *Nat Struct Mol Biol.*
30 2008;15(4):354-63.
- 31 32. Marco A, Macpherson JI, Ronshaugen M, Griffiths-Jones S. MicroRNAs from the same
32 precursor have different targeting properties. *Silence.* 2012;3(1):8.
- 33 33. Guo L, Lu Z. The fate of miRNA* strand through evolutionary analysis: implication for
34 degradation as merely carrier strand or potential regulatory molecule? *PLoS One.* 2010;5(6):e11387.
- 35 34. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis
36 shows that some microRNAs downregulate large numbers of target mRNAs. *Nature.*
37 2005;433(7027):769-73.
- 38 35. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein
39 output. *Nature.* 2008;455(7209):64-71.
- 40 36. Selbach M, Schwanhaussner B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread
41 changes in protein synthesis induced by microRNAs. *Nature.* 2008;455(7209):58-63.
- 42 37. Hendrickson DG, Hogan DJ, McCullough HL, Myers JW, Herschlag D, Ferrell JE, et al.
43 Concordant regulation of translation and mRNA abundance for hundreds of targets of a human
44 microRNA. *PLoS Biol.* 2009;7(11):e1000238.
- 45 38. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational
46 repression and mRNA decay. *Nat Rev Genet.* 2011;12(2):99-110.
- 47 39. Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, et al. Inhibition of
48 translational initiation by Let-7 MicroRNA in human cells. *Science.* 2005;309(5740):1573-6.
- 49 40. Humphreys DT, Westman BJ, Martin DI, Preiss T. MicroRNAs control translation initiation by
50 inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci U S A.*
51 2005;102(47):16961-6.

- 1 41. Petersen CP, Bordeleau ME, Pelletier J, Sharp PA. Short RNAs repress translation after
2 initiation in mammalian cells. *Mol Cell*. 2006;21(4):533-42.
- 3 42. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, et al. MicroRNA silencing
4 through RISC recruitment of eIF6. *Nature*. 2007;447(7146):823-8.
- 5 43. Djuranovic S, Nahvi A, Green R. miRNA-mediated gene silencing by translational repression
6 followed by mRNA deadenylation and decay. *Science*. 2012;336(6078):237-40.
- 7 44. Bethune J, Artus-Revel CG, Filipowicz W. Kinetic analysis reveals successive steps leading to
8 miRNA-mediated silencing in mammalian cells. *EMBO Rep*. 2012;13(8):716-23.
- 9 45. Janas MM, Wang E, Love T, Harris AS, Stevenson K, Semmelmann K, et al. Reduced
10 expression of ribosomal proteins relieves microRNA-mediated repression. *Mol Cell*. 2012;46(2):171-
11 86.
- 12 46. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to
13 decrease target mRNA levels. *Nature*. 2010;466(7308):835-40.
- 14 47. Eichhorn SW, Guo H, McGeary SE, Rodriguez-Mias RA, Shin C, Baek D, et al. mRNA
15 Destabilization Is the Dominant Effect of Mammalian MicroRNAs by the Time Substantial Repression
16 Ensues. *Mol Cell*. 2014;56(1):104-15.
- 17 48. Nissan X, Denis JA, Saidani M, Lemaitre G, Peschanski M, Baldeschi C. miR-203 modulates
18 epithelial differentiation of human embryonic stem cells towards epidermal stratification.
19 *Developmental biology*. 2011;356(2):506-15.
- 20 49. Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, Aberdam D, Knight RA, Melino G, et al.
21 miR-203 represses 'stemness' by repressing DeltaNp63. *Cell Death Differ*. 2008;15(7):1187-95.
- 22 50. Yi R, Poy MN, Stoffel M, Fuchs E. A skin microRNA promotes differentiation by repressing
23 'stemness'. *Nature*. 2008;452(7184):225-9.
- 24 51. Jackson SJ, Zhang Z, Feng D, Flagg M, O'Loughlin E, Wang D, et al. Rapid and widespread
25 suppression of self-renewal by microRNA-203 during epidermal differentiation. *Development*
26 (Cambridge, England). 2013;140(9):1882-91.
- 27 52. Wang D, Zhang Z, O'Loughlin E, Wang L, Fan X, Lai EC, et al. MicroRNA-205 controls neonatal
28 expansion of skin stem cells by modulating the PI(3)K pathway. *Nat Cell Biol*. 2013;15(10):1153-63.
- 29 53. Ahmed MI, Alam M, Emelianov VU, Poterlowicz K, Patel A, Sharov AA, et al. MicroRNA-214
30 controls skin and hair follicle development by modulating the activity of the Wnt pathway. *J Cell Biol*.
31 2014;207(4):549-67.
- 32 54. Riemondy K, Hoefert JE, Yi R. Not miR-ly micromanagers: the functions and regulatory
33 networks of microRNAs in mammalian skin. *Wiley interdisciplinary reviews RNA*. 2014;5(6):849-65.
- 34 55. Bilousova G, Chen J, Roop DR. Differentiation of mouse induced pluripotent stem cells into a
35 multipotent keratinocyte lineage. *J Invest Dermatol*. 2011;131(4):857-64.
- 36 56. Yang R, Zheng Y, Burrows M, Liu S, Wei Z, Nace A, et al. Generation of folliculogenic human
37 epithelial stem cells from induced pluripotent stem cells. *Nature communications*. 2014;5:3071.
- 38 57. Petrova A, Celli A, Jacquet L, Dafou D, Crumrine D, Hupe M, et al. 3D In vitro model of a
39 functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent
40 stem cells. *Stem cell reports*. 2014;2(5):675-89.
- 41 58. Gnedeva K, Vorotelyak E, Cimadamore F, Cattarossi G, Giusto E, Terskikh VV, et al. Derivation
42 of hair-inducing cell from human pluripotent stem cells. *PLoS One*. 2015;10(1):e0116892.
- 43 59. Veraitch O, Mabuchi Y, Matsuzaki Y, Sasaki T, Okuno H, Tsukashima A, et al. Induction of hair
44 follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent
45 LNGFR(+)/THY-1(+) mesenchymal cells. *Scientific reports*. 2017;7:42777.
- 46 60. Kidwai FK, Liu H, Toh WS, Fu X, Jokhun DS, Movahednia MM, et al. Differentiation of human
47 embryonic stem cells into clinically amenable keratinocytes in an autogenic environment. *J Invest*
48 *Dermatol*. 2013;133(3):618-28.
- 49 61. Kilpinen H, Goncalves A, Leha A, Afzal V, Alasoo K, Ashford S, et al. Common genetic
50 variation drives molecular heterogeneity in human iPSCs. *Nature*. 2017;546(7658):370-5.

- 1 62. Zhang Z, Xiang D, Heriyanto F, Gao Y, Qian Z, Wu WS. Dissecting the roles of miR-302/367
2 cluster in cellular reprogramming using TALE-based repressor and TALEN. *Stem cell reports*.
3 2013;1(3):218-25.
- 4 63. Wang X, Wang Y, Huang H, Chen B, Chen X, Hu J, et al. Precise gene modification mediated
5 by TALEN and single-stranded oligodeoxynucleotides in human cells. *PLoS One*. 2014;9(4):e93575.
- 6 64. Ma Y, Yao N, Liu G, Dong L, Liu Y, Zhang M, et al. Functional screen reveals essential roles of
7 miR-27a/24 in differentiation of embryonic stem cells. *EMBO J*. 2015;34(3):361-78.
- 8 65. Luo Y, Xu X, An X, Sun X, Wang S, Zhu D. Targeted Inhibition of the miR-199a/214 Cluster by
9 CRISPR Interference Augments the Tumor Tropism of Human Induced Pluripotent Stem Cell-Derived
10 Neural Stem Cells under Hypoxic Condition. *Stem Cells Int*. 2016;2016:3598542.
- 11 66. Chatterjee P, Cheung Y, Liew C. Transfecting and nucleofecting human induced pluripotent
12 stem cells. *J Vis Exp*. 2011(56).
- 13 67. Niu X, He W, Song B, Ou Z, Fan D, Chen Y, et al. Combining Single Strand
14 Oligodeoxynucleotides and CRISPR/Cas9 to Correct Gene Mutations in beta-Thalassemia-induced
15 Pluripotent Stem Cells. *J Biol Chem*. 2016;291(32):16576-85.
- 16 68. Krutmann J, Bouloc A, Sore G, Bernard BA, Passeron T. The skin aging exposome. *J Dermatol*
17 *Sci*. 2017;85(3):152-61.
- 18 69. Sonkoly E, Wei T, Janson PC, Saaf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel
19 regulators involved in the pathogenesis of Psoriasis? *PLoS ONE*. 2007;2(7):e610.
- 20 70. Joyce CE, Zhou X, Xia J, Ryan C, Thrash B, Menter A, et al. Deep sequencing of small RNAs
21 from human skin reveals major alterations in the psoriasis miRNAome. *Human molecular genetics*.
22 2011;20(20):4025-40.
- 23 71. Guinea-Viniegra J, Jimenez M, Schonhaler HB, Navarro R, Delgado Y, Concha-Garzon MJ, et
24 al. Targeting miR-21 to treat psoriasis. *Science translational medicine*. 2014;6(225):225re1.
- 25 72. Yang X, Wang J, Guo SL, Fan KJ, Li J, Wang YL, et al. miR-21 promotes keratinocyte migration
26 and re-epithelialization during wound healing. *Int J Biol Sci*. 2011;7(5):685-90.
- 27 73. Wang T, Zhang L, Shi C, Sun H, Wang J, Li R, et al. TGF-beta-induced miR-21 negatively
28 regulates the antiproliferative activity but has no effect on EMT of TGF-beta in HaCaT cells. *The*
29 *international journal of biochemistry & cell biology*. 2012;44(2):366-76.
- 30 74. Sato K, Takaishi M, Tokuoka S, Sano S. Involvement of TNF-alpha converting enzyme in the
31 development of psoriasis-like lesions in a mouse model. *PLoS One*. 2014;9(11):e112408.
- 32 75. Degueurce G, D'Errico I, Pich C, Ibberson M, Schutz F, Montagner A, et al. Identification of a
33 novel PPARbeta/delta/miR-21-3p axis in UV-induced skin inflammation. *EMBO molecular medicine*.
34 2016;8(8):919-36.
- 35 76. Zavadil J, Narasimhan M, Blumenberg M, Schneider RJ. Transforming growth factor-beta and
36 microRNA:mRNA regulatory networks in epithelial plasticity. *Cells, tissues, organs*. 2007;185(1-
37 3):157-61.
- 38 77. Wang J, Qiu Y, Shi NW, Zhao JN, Wang YC, Jiang H, et al. microRNA-21 mediates the TGF-
39 beta1-induced migration of keratinocytes via targeting PTEN. *Eur Rev Med Pharmacol Sci*.
40 2016;20(18):3748-59.
- 41 78. Xu N, Meisgen F, Butler LM, Han G, Wang XJ, Soderberg-Naucler C, et al. MicroRNA-31 is
42 overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in
43 keratinocytes via targeting serine/threonine kinase 40. *J Immunol*. 2013;190(2):678-88.
- 44 79. Yan S, Xu Z, Lou F, Zhang L, Ke F, Bai J, et al. NF-kappaB-induced microRNA-31 promotes
45 epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. *Nature communications*.
46 2015;6:7652.
- 47 80. Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, et al. IL-22 and IL-20 are key
48 mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. *J Mol Med*.
49 2009;87(5):523-36.
- 50 81. Roberts JC, Warren RB, Griffiths CE, Ross K. Expression of microRNA-184 in keratinocytes
51 represses argonaute 2. *J Cell Physiol*. 2013;228(12):2314-23.

- 1 82. Bi X, Cao Y, Chen R, Liu C, Chen J, Min D. MicroRNA-184 Promotes Proliferation and Inhibits
2 Apoptosis in HaCaT Cells: An In Vitro Study. *Med Sci Monit.* 2016;22:3056-61.
- 3 83. Lerman G, Sharon M, Leibowitz-Amit R, Sidi Y, Avni D. The crosstalk between IL-22 signaling
4 and miR-197 in human keratinocytes. *PLoS One.* 2014;9(9):e107467.
- 5 84. Meisgen F, Xu Landen N, Wang A, Rethi B, Bouez C, Zuccolo M, et al. MiR-146a negatively
6 regulates TLR2-induced inflammatory responses in keratinocytes. *J Invest Dermatol.*
7 2014;134(7):1931-40.
- 8 85. Meisgen F, Xu Landen N, Bouez C, Zuccolo M, Gueniche A, Stahle M, et al. Activation of toll-
9 like receptors alters the microRNA expression profile of keratinocytes. *Exp Dermatol.*
10 2014;23(4):281-3.
- 11 86. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of
12 microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc*
13 *Natl Acad Sci U S A.* 2006;103(33):12481-6.
- 14 87. Srivastava A, Nikamo P, Lohcharoenkal W, Li D, Meisgen F, Xu Landen N, et al. MicroRNA-
15 146a suppresses IL-17-mediated skin inflammation and is genetically associated with psoriasis. *J*
16 *Allergy Clin Immunol.* 2016.
- 17 88. Bulek K, Liu C, Swaidani S, Wang L, Page RC, Gulen MF, et al. The inducible kinase IKKi is
18 required for IL-17-dependent signaling associated with neutrophilia and pulmonary inflammation.
19 *Nature immunology.* 2011;12(9):844-52.
- 20 89. Zhong B, Liu X, Wang X, Chang SH, Liu X, Wang A, et al. Negative regulation of IL-17-
21 mediated signaling and inflammation by the ubiquitin-specific protease USP25. *Nature immunology.*
22 2012;13(11):1110-7.
- 23 90. Xu N, Brodin P, Wei T, Meisgen F, Eidsmo L, Nagy N, et al. MiR-125b, a microRNA
24 downregulated in psoriasis, modulates keratinocyte proliferation by targeting FGFR2. *J Invest*
25 *Dermatol.* 2011;131(7):1521-9.
- 26 91. Lerman G, Avivi C, Mardoukh C, Barzilai A, Tessone A, Gradus B, et al. MiRNA expression in
27 psoriatic skin: reciprocal regulation of hsa-miR-99a and IGF-1R. *PLoS One.* 2011;6(6):e20916.
- 28 92. Ichihara A, Jinnin M, Yamane K, Fujisawa A, Sakai K, Masuguchi S, et al. microRNA-mediated
29 keratinocyte hyperproliferation in psoriasis vulgaris. *Br J Dermatol.* 2011;165(5):1003-10.
- 30 93. Hawkes JE, Nguyen GH, Fujita M, Florell SR, Callis Duffin K, Krueger GG, et al. microRNAs in
31 Psoriasis. *J Invest Dermatol.* 2016;136(2):365-71.
- 32 94. Wang MJ, Xu YY, Huang RY, Chen XM, Chen HM, Han L, et al. Role of an imbalanced miRNAs
33 axis in pathogenesis of psoriasis: novel perspectives based on review of the literature. *Oncotarget.*
34 2016.
- 35 95. Huang RY, Li L, Wang MJ, Chen XM, Huang QC, Lu CJ. An Exploration of the Role of
36 MicroRNAs in Psoriasis: A Systematic Review of the Literature. *Medicine (Baltimore).*
37 2015;94(45):e2030.
- 38 96. Liu Q, Wu DH, Han L, Deng JW, Zhou L, He R, et al. Roles of microRNAs in psoriasis:
39 Immunological functions and potential biomarkers. *Exp Dermatol.* 2017;26(4):359-67.
- 40 97. U.S. Department of Health and Human Services. The Surgeon General's Call to Action to
41 Prevent Skin Cancer. Washington, DC: Office of the Surgeon General.; 2014.
- 42 98. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of
43 disease, nodal metastasis, and deaths from disease in the United States, 2012. *J Am Acad Dermatol.*
44 2013;68(6):957-66.
- 45 99. Cranmer LD, Engelhardt C, Morgan SS. Treatment of unresectable and metastatic cutaneous
46 squamous cell carcinoma. *Oncologist.* 2010;15(12):1320-8.
- 47 100. Burton KA, Ashack KA, Khachemoune A. Cutaneous Squamous Cell Carcinoma: A Review of
48 High-Risk and Metastatic Disease. *Am J Clin Dermatol.* 2016;17(5):491-508.
- 49 101. Dziunycz P, Iotzova-Weiss G, Eloranta JJ, Lauchli S, Hafner J, French LE, et al. Squamous cell
50 carcinoma of the skin shows a distinct microRNA profile modulated by UV radiation. *J Invest*
51 *Dermatol.* 2010;130(11):2686-9.

- 1 102. Darido C, Georgy SR, Wilanowski T, Dworkin S, Auden A, Zhao Q, et al. Targeting of the
2 tumor suppressor GRHL3 by a miR-21-dependent proto-oncogenic network results in PTEN loss and
3 tumorigenesis. *Cancer Cell*. 2011;20(5):635-48.
- 4 103. Xu N, Zhang L, Meisgen F, Harada M, Heilborn J, Homey B, et al. MicroRNA-125b down-
5 regulates matrix metalloproteinase 13 and inhibits cutaneous squamous cell carcinoma cell
6 proliferation, migration, and invasion. *J Biol Chem*. 2012;287(35):29899-908.
- 7 104. Sand M, Skrygan M, Georgas D, Sand D, Hahn SA, Gambichler T, et al. Microarray analysis of
8 microRNA expression in cutaneous squamous cell carcinoma. *J Dermatol Sci*. 2012;68(3):119-26.
- 9 105. Bruegger C, Kempf W, Spoerri I, Arnold AW, Itin PH, Burger B. MicroRNA expression differs in
10 cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. *Exp*
11 *Dermatol*. 2013;22(6):426-8.
- 12 106. Ge Y, Zhang L, Nikolova M, Reva B, Fuchs E. Strand-specific in vivo screen of cancer-
13 associated miRNAs unveils a role for miR-21(*) in SCC progression. *Nat Cell Biol*. 2016;18(1):111-21.
- 14 107. Toll A, Salgado R, Espinet B, Diaz-Lagares A, Hernandez-Ruiz E, Andrades E, et al. MiR-204
15 silencing in intraepithelial to invasive cutaneous squamous cell carcinoma progression. *Mol Cancer*.
16 2016;15(1):53.
- 17 108. Stojadinovic O, Ramirez H, Pastar I, Gordon KA, Stone R, Choudhary S, et al. MiR-21 and miR-
18 205 are induced in invasive cutaneous squamous cell carcinomas. *Arch Dermatol Res*.
19 2017;309(2):133-9.
- 20 109. Olsz EB, Seline LN, Schock AM, Duncan NE, Lopez A, Lazar J, et al. MicroRNA-135b Regulates
21 Leucine Zipper Tumor Suppressor 1 in Cutaneous Squamous Cell Carcinoma. *PLoS One*.
22 2015;10(5):e0125412.
- 23 110. Yamane K, Jinnin M, Etoh T, Kobayashi Y, Shimozono N, Fukushima S, et al. Down-regulation
24 of miR-124/-214 in cutaneous squamous cell carcinoma mediates abnormal cell proliferation via the
25 induction of ERK. *Journal of molecular medicine*. 2013;91(1):69-81.
- 26 111. Harada M, Jinnin M, Wang Z, Hirano A, Tomizawa Y, Kira T, et al. The expression of miR-124
27 increases in aged skin to cause cell senescence and it decreases in squamous cell carcinoma.
28 *Bioscience trends*. 2017;10(6):454-9.
- 29 112. South AP, Purdie KJ, Watt SA, Haldenby S, den Breems N, Dimon M, et al. NOTCH1 mutations
30 occur early during cutaneous squamous cell carcinogenesis. *J Invest Dermatol*. 2014;134(10):2630-8.
- 31 113. Cammareri P, Rose AM, Vincent DF, Wang J, Nagano A, Libertini S, et al. Inactivation of
32 TGFbeta receptors in stem cells drives cutaneous squamous cell carcinoma. *Nature communications*.
33 2016;7:12493.
- 34 114. Yu J, Ryan DG, Getsios S, Oliveira-Fernandes M, Fatima A, Lavker RM. MicroRNA-184
35 antagonizes microRNA-205 to maintain SHIP2 levels in epithelia. *Proc Natl Acad Sci U S A*.
36 2008;105(49):19300-5.
- 37 115. Yu X, Li Z. The role of miRNAs in cutaneous squamous cell carcinoma. *J Cell Mol Med*.
38 2016;20(1):3-9.
- 39 116. Whittam AJ, Maan ZN, Duscher D, Wong VW, Barrera JA, Januszyk M, et al. Challenges and
40 Opportunities in Drug Delivery for Wound Healing. *Adv Wound Care (New Rochelle)*. 2016;5(2):79-
41 88.
- 42 117. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling,
43 and translation. *Science translational medicine*. 2014;6(265):265sr6.
- 44 118. Viticchie G, Lena AM, Cianfarani F, Odorisio T, Annicchiarico-Petruzzelli M, Melino G, et al.
45 MicroRNA-203 contributes to skin re-epithelialization. *Cell death & disease*. 2012;3:e435.
- 46 119. Li D, Li X, Wang A, Meisgen F, Pivarcsi A, Sonkoly E, et al. MicroRNA-31 Promotes Skin
47 Wound Healing by Enhancing Keratinocyte Proliferation and Migration. *J Invest Dermatol*.
48 2015;135(6):1676-85.
- 49 120. Wang T, Feng Y, Sun H, Zhang L, Hao L, Shi C, et al. miR-21 regulates skin wound healing by
50 targeting multiple aspects of the healing process. *Am J Pathol*. 2012;181(6):1911-20.

- 1 121. Bertero T, Gastaldi C, Bourget-Ponzio I, Imbert V, Loubat A, Selva E, et al. miR-483-3p
2 controls proliferation in wounded epithelial cells. *FASEB J.* 2011;25(9):3092-105.
- 3 122. Amelio I, Lena AM, Viticchie G, Shalom-Feuerstein R, Terrinoni A, Dinsdale D, et al. miR-24
4 triggers epidermal differentiation by controlling actin adhesion and cell migration. *J Cell Biol.*
5 2012;199(2):347-63.
- 6 123. Yu J, Peng H, Ruan Q, Fatima A, Getsios S, Lavker RM. MicroRNA-205 promotes keratinocyte
7 migration via the lipid phosphatase SHIP2. *FASEB J.* 2010;24(10):3950-9.
- 8 124. Wang T, Zhao N, Long S, Ge L, Wang A, Sun H, et al. Downregulation of miR-205 in migrating
9 epithelial tongue facilitates skin wound re-epithelialization by derepressing ITGA5. *Biochim Biophys*
10 *Acta.* 2016;1862(8):1443-52.
- 11 125. Li D, Wang A, Liu X, Meisgen F, Grunler J, Botusan IR, et al. MicroRNA-132 enhances
12 transition from inflammation to proliferation during wound healing. *J Clin Invest.* 2015;125(8):3008-
13 26.
- 14 126. Biswas S, Roy S, Banerjee J, Hussain SR, Khanna S, Meenakshisundaram G, et al. Hypoxia
15 inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model
16 of ischemic wounds. *Proc Natl Acad Sci U S A.* 2010;107(15):6976-81.
- 17 127. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of
18 chemicals. *Biochim Biophys Acta.* 2009;1788(11):2362-73.
- 19 128. Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. *Exp Dermatol.*
20 2008;17(12):1063-72.
- 21 129. Segre JA. Epidermal barrier formation and recovery in skin disorders. *J Clin Invest.*
22 2006;116(5):1150-8.
- 23 130. Iwai I, Han H, den Hollander L, Svensson S, Ofverstedt LG, Anwar J, et al. The human skin
24 barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules
25 associated with the ceramide sphingoid moiety. *J Invest Dermatol.* 2012;132(9):2215-25.
- 26 131. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds
27 and drugs. *Exp Dermatol.* 2000;9(3):165-9.
- 28 132. Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S. Design principles of chemical
29 penetration enhancers for transdermal drug delivery. *Proc Natl Acad Sci U S A.* 2005;102(13):4688-
30 93.
- 31 133. Zakrewsky M, Kumar S, Mitragotri S. Nucleic acid delivery into skin for the treatment of skin
32 disease: Proofs-of-concept, potential impact, and remaining challenges. *J Control Release.*
33 2015;219:445-56.
- 34 134. Copolovici DM, Langel K, Eriste E, Langel U. Cell-penetrating peptides: design, synthesis, and
35 applications. *ACS Nano.* 2014;8(3):1972-94.
- 36 135. Dinca A, Chien WM, Chin MT. Intracellular Delivery of Proteins with Cell-Penetrating
37 Peptides for Therapeutic Uses in Human Disease. *International journal of molecular sciences.*
38 2016;17(2):263.
- 39 136. Bechara C, Sagan S. Cell-penetrating peptides: 20 years later, where do we stand? *FEBS Lett.*
40 2013;587(12):1693-702.
- 41 137. Rothbard JB, Garlington S, Lin Q, Kirschberg T, Kreider E, McGrane PL, et al. Conjugation of
42 arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. *Nat*
43 *Med.* 2000;6(11):1253-7.
- 44 138. Hsu T, Mitragotri S. Delivery of siRNA and other macromolecules into skin and cells using a
45 peptide enhancer. *Proc Natl Acad Sci U S A.* 2011;108(38):15816-21.
- 46 139. Chen M, Kumar S, Anselmo AC, Gupta V, Slee DH, Muraski JA, et al. Topical delivery of
47 Cyclosporine A into the skin using SPACE-peptide. *J Control Release.* 2015;199:190-7.
- 48 140. Kumar S, Zakrewsky M, Chen M, Menegatti S, Muraski JA, Mitragotri S. Peptides as skin
49 penetration enhancers: mechanisms of action. *J Control Release.* 2015;199:168-78.
- 50 141. Gautam A, Sharma M, Vir P, Chaudhary K, Kapoor P, Kumar R, et al. Identification and
51 characterization of novel protein-derived arginine-rich cell-penetrating peptides. *European journal*

- 1 of pharmaceuticals and biopharmaceuticals : official journal of Arbeitsgemeinschaft für
2 Pharmazeutische Verfahrenstechnik eV. 2015;89:93-106.
- 3 142. Andaloussi SE, Lehto T, Mager I, Rosenthal-Aizman K, Oprea II, Simonson OE, et al. Design of
4 a peptide-based vector, PepFect6, for efficient delivery of siRNA in cell culture and systemically in
5 vivo. *Nucleic Acids Res.* 2011;39(9):3972-87.
- 6 143. Lin CM, Huang K, Zeng Y, Chen XC, Wang S, Li Y. A simple, noninvasive and efficient method
7 for transdermal delivery of siRNA. *Arch Dermatol Res.* 2012;304(2):139-44.
- 8 144. Chen M, Zakrewsky M, Gupta V, Anselmo AC, Slee DH, Muraski JA, et al. Topical delivery of
9 siRNA into skin using SPACE-peptide carriers. *J Control Release.* 2014;179:33-41.
- 10 145. Ruan R, Chen M, Sun S, Wei P, Zou L, Liu J, et al. Topical and Targeted Delivery of siRNAs to
11 Melanoma Cells Using a Fusion Peptide Carrier. *Scientific reports.* 2016;6:29159.
- 12 146. Delaroché D, Aussédât B, Aubry S, Chassaing G, Burlina F, Clodic G, et al. Tracking a new cell-
13 penetrating (W/R) nonapeptide, through an enzyme-stable mass spectrometry reporter tag.
14 *Analytical chemistry.* 2007;79(5):1932-8.
- 15 147. Gautam A, Nanda JS, Samuel JS, Kumari M, Priyanka P, Bedi G, et al. Topical Delivery of
16 Protein and Peptide Using Novel Cell Penetrating Peptide IMT-P8. *Scientific reports.* 2016;6:26278.
- 17 148. Goodarzi HR, Abbasi A, Saffari M, Fazelzadeh Haghighi M, Tabei MB, Noori Dalooi MR.
18 Differential expression analysis of balding and nonbalding dermal papilla microRNAs in male pattern
19 baldness with a microRNA amplification profiling method. *Br J Dermatol.* 2012;166(5):1010-6.
- 20 149. Wang EHC, DeStefano GM, Patel AV, Drill E, Harel S, Cela C, et al. Identification of
21 differentially expressed miRNAs in alopecia areata that target immune-regulatory pathways. *Genes
22 and immunity.* 2017;18(2):100-4.
- 23 150. Sellheyer K. Spiradenoma and cylindroma originate from the hair follicle bulge and not from
24 the eccrine sweat gland: an immunohistochemical study with CD200 and other stem cell markers.
25 *Journal of cutaneous pathology.* 2015;42(2):90-101.
- 26 151. van Asbeck AH, Beyerle A, McNeill H, Bovee-Geurts PH, Lindberg S, Verdurmen WP, et al.
27 Molecular parameters of siRNA-cell penetrating peptide nanocomplexes for efficient cellular
28 delivery. *ACS Nano.* 2013;7(5):3797-807.
- 29 152. Anko M, Majhenc J, Kogej K, Sillard R, Langel U, Anderlüh G, et al. Influence of stearyl and
30 trifluoromethylquinoline modifications of the cell penetrating peptide TP10 on its interaction with a
31 lipid membrane. *Biochim Biophys Acta.* 2012;1818(3):915-24.
- 32 153. Urgard E, Lorents A, Klaas M, Padari K, Viil J, Runnel T, et al. Pre-administration of PepFect6-
33 microRNA-146a nanocomplexes inhibits inflammatory responses in keratinocytes and in a mouse
34 model of irritant contact dermatitis. *J Control Release.* 2016;235:195-204.
- 35 154. Ding Y, Jiang Z, Saha K, Kim CS, Kim ST, Landis RF, et al. Gold nanoparticles for nucleic acid
36 delivery. *Mol Ther.* 2014;22(6):1075-83.
- 37 155. Cutler JI, Auyeung E, Mirkin CA. Spherical nucleic acids. *J Am Chem Soc.* 2012;134(3):1376-
38 91.
- 39 156. Rosi NL, Giljohann DA, Thaxton CS, Lytton-Jean AK, Han MS, Mirkin CA. Oligonucleotide-
40 modified gold nanoparticles for intracellular gene regulation. *Science.* 2006;312(5776):1027-30.
- 41 157. Choi CH, Hao L, Narayan SP, Auyeung E, Mirkin CA. Mechanism for the endocytosis of
42 spherical nucleic acid nanoparticle conjugates. *Proc Natl Acad Sci U S A.* 2013;110(19):7625-30.
- 43 158. Zheng D, Giljohann DA, Chen DL, Massich MD, Wang XQ, Iordanov H, et al. Topical delivery
44 of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation. *Proc Natl Acad Sci
45 U S A.* 2012;109(30):11975-80.
- 46 159. Randeria PS, Seeger MA, Wang XQ, Wilson H, Shipp D, Mirkin CA, et al. siRNA-based
47 spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase
48 knockdown. *Proc Natl Acad Sci U S A.* 2015;112(18):5573-8.
- 49 160. Young KL, Scott AW, Hao L, Mirkin SE, Liu G, Mirkin CA. Hollow spherical nucleic acids for
50 intracellular gene regulation based upon biocompatible silica shells. *Nano Lett.* 2012;12(7):3867-71.

- 1 161. Park JH, Gu L, von Maltzahn G, Ruoslahti E, Bhatia SN, Sailor MJ. Biodegradable luminescent
2 porous silicon nanoparticles for in vivo applications. *Nat Mater*. 2009;8(4):331-6.
- 3 162. Hao L, Patel PC, Alhasan AH, Giljohann DA, Mirkin CA. Nucleic acid-gold nanoparticle
4 conjugates as mimics of microRNA. *Small*. 2011;7(22):3158-62.
- 5 163. Wang X, Hao L, Bu HF, Scott AW, Tian K, Liu F, et al. Spherical nucleic acid targeting
6 microRNA-99b enhances intestinal MFG-E8 gene expression and restores enterocyte migration in
7 lipopolysaccharide-induced septic mice. *Scientific reports*. 2016;6:31687.
- 8 164. Labala S, Jose A, Venuganti VV. Transcutaneous iontophoretic delivery of STAT3 siRNA using
9 layer-by-layer chitosan coated gold nanoparticles to treat melanoma. *Colloids and surfaces B,
10 Biointerfaces*. 2016;146:188-97.
- 11 165. Muddineti OS, Ghosh B, Biswas S. Current trends in using polymer coated gold nanoparticles
12 for cancer therapy. *International journal of pharmaceutics*. 2015;484(1-2):252-67.
- 13 166. Loan Honeywell-Nguyen P, Wouter Groenink HW, Bouwstra JA. Elastic vesicles as a tool for
14 dermal and transdermal delivery. *J Liposome Res*. 2006;16(3):273-80.
- 15 167. Hua S. Lipid-based nano-delivery systems for skin delivery of drugs and bioactives. *Front
16 Pharmacol*. 2015;6:219.
- 17 168. Akhtar N, Khan RA. Liposomal systems as viable drug delivery technology for skin cancer
18 sites with an outlook on lipid-based delivery vehicles and diagnostic imaging inputs for skin
19 conditions'. *Prog Lipid Res*. 2016;64:192-230.
- 20 169. Benson HA. Elastic Liposomes for Topical and Transdermal Drug Delivery. *Methods Mol Biol*.
21 2017;1522:107-17.
- 22 170. Singh Y, Tomar S, Khan S, Meher JG, Pawar VK, Raval K, et al. Bridging small interfering RNA
23 with giant therapeutic outcomes using nanometric liposomes. *J Control Release*. 2015;220(Pt A):368-
24 87.
- 25 171. Paul A, Cevc G, Bachhawat BK. Transdermal immunization with large proteins by means of
26 ultradeformable drug carriers. *European journal of immunology*. 1995;25(12):3521-4.
- 27 172. Paul A, Cevc G, Bachhawat BK. Transdermal immunisation with an integral membrane
28 component, gap junction protein, by means of ultradeformable drug carriers, transfersomes.
29 *Vaccine*. 1998;16(2-3):188-95.
- 30 173. Cevc G, Gebauer D, Stieber J, Schatzlein A, Blume G. Ultraflexible vesicles, Transfersomes,
31 have an extremely low pore penetration resistance and transport therapeutic amounts of insulin
32 across the intact mammalian skin. *Biochim Biophys Acta*. 1998;1368(2):201-15.
- 33 174. Cevc G, Schatzlein A, Richardsen H. Ultradeformable lipid vesicles can penetrate the skin and
34 other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and
35 direct size measurements. *Biochim Biophys Acta*. 2002;1564(1):21-30.
- 36 175. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier,
37 transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo
38 evaluation. *Colloids and surfaces B, Biointerfaces*. 2012;92:299-304.
- 39 176. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praca FG, et al. Development,
40 characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes,
41 ethosomes, and transethosomes. *International journal of nanomedicine*. 2015;10:5837-51.
- 42 177. Gupta PN, Mishra V, Rawat A, Dubey P, Mahor S, Jain S, et al. Non-invasive vaccine delivery
43 in transfersomes, niosomes and liposomes: a comparative study. *International journal of
44 pharmaceutics*. 2005;293(1-2):73-82.
- 45 178. Mahor S, Rawat A, Dubey PK, Gupta PN, Khatri K, Goyal AK, et al. Cationic transfersomes
46 based topical genetic vaccine against hepatitis B. *International journal of pharmaceutics*.
47 2007;340(1-2):13-9.
- 48 179. Hofer C, van Randenborgh H, Lehmer A, Hartung R, Breul J. Transcutaneous IL-2 uptake
49 mediated by Transfersomes depends on concentration and fractionated application. *Cytokine*.
50 2004;25(4):141-6.

- 1 180. Ascenso A, Pinho S, Eleuterio C, Praca FG, Bentley MV, Oliveira H, et al. Lycopene from
2 tomatoes: vesicular nanocarrier formulations for dermal delivery. *Journal of agricultural and food*
3 *chemistry*. 2013;61(30):7284-93.
- 4 181. Ascenso A, Salgado A, Euleterio C, Praca FG, Bentley MV, Marques HC, et al. In vitro and in
5 vivo topical delivery studies of tretinoin-loaded ultradeformable vesicles. *European journal of*
6 *pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische*
7 *Verfahrenstechnik eV*. 2014;88(1):48-55.
- 8 182. Cevc G, Blume G. Hydrocortisone and dexamethasone in very deformable drug carriers have
9 increased biological potency, prolonged effect, and reduced therapeutic dosage. *Biochim Biophys*
10 *Acta*. 2004;1663(1-2):61-73.
- 11 183. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal
12 administration in ultradeformable drug carriers, Transfersomes. *Biochim Biophys Acta*.
13 2001;1514(2):191-205.
- 14 184. Ghanbarzadeh S, Arami S. Enhanced transdermal delivery of diclofenac sodium via
15 conventional liposomes, ethosomes, and transfersomes. *Biomed Res Int*. 2013;2013:616810.
- 16 185. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Characterization and In Vitro Skin
17 Permeation of Meloxicam-Loaded Liposomes versus Transfersomes. *J Drug Deliv*. 2011;2011:418316.
- 18 186. Duangjit S, Obata Y, Sano H, Onuki Y, Opanasopit P, Ngawhirunpat T, et al. Comparative
19 study of novel ultradeformable liposomes: menthosomes, transfersomes and liposomes for
20 enhancing skin permeation of meloxicam. *Biological & pharmaceutical bulletin*. 2014;37(2):239-47.
- 21 187. Lei W, Yu CX, Lin H, Zhou X. Development of tacrolimus-loaded transfersomes for deeper
22 skin penetration enhancement and therapeutic effect improvement in vivo. *Asian Journal of*
23 *Pharmaceutical Sciences*. 2013;8(6):336-45.
- 24 188. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes - novel vesicular carriers for
25 enhanced delivery: characterization and skin penetration properties. *J Control Release*.
26 2000;65(3):403-18.
- 27 189. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. *Crit Rev Ther Drug*
28 *Carrier Syst*. 2003;20(1):63-102.
- 29 190. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of
30 an anti-psoriatic agent via ethanolic liposomes. *J Control Release*. 2007;123(2):148-54.
- 31 191. Raza K, Singh B, Lohan S, Sharma G, Negi P, Yachha Y, et al. Nano-lipoidal carriers of tretinoin
32 with enhanced percutaneous absorption, photostability, biocompatibility and anti-psoriatic activity.
33 *International journal of pharmaceutics*. 2013;456(1):65-72.
- 34 192. Fang YP, Huang YB, Wu PC, Tsai YH. Topical delivery of 5-aminolevulinic acid-encapsulated
35 ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the
36 penetration behavior. *European journal of pharmaceutics and biopharmaceutics : official journal of*
37 *Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV*. 2009;73(3):391-8.
- 38 193. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of
39 ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-
40 inflammatory activity on human volunteers. *J Control Release*. 2005;106(1-2):99-110.
- 41 194. Kim ST, Lee KM, Park HJ, Jin SE, Ahn WS, Kim CK. Topical delivery of interleukin-13 antisense
42 oligonucleotides with cationic elastic liposome for the treatment of atopic dermatitis. *J Gene Med*.
43 2009;11(1):26-37.
- 44 195. Hattori Y, Date M, Arai S, Kawano K, Yonemochi E, Maitani Y. Transdermal Delivery of Small
45 Interfering RNA with Elastic Cationic Liposomes in Mice. *J Pharm (Cairo)*. 2013;2013:149695.
- 46 196. Dorrani M, Garbuzenko OB, Minko T, Michniak-Kohn B. Development of edge-activated
47 liposomes for siRNA delivery to human basal epidermis for melanoma therapy. *J Control Release*.
48 2016;228:150-8.
- 49 197. Jose A, Labala S, Venuganti VV. Co-delivery of curcumin and STAT3 siRNA using deformable
50 cationic liposomes to treat skin cancer. *Journal of drug targeting*. 2016:1-15.

- 1 198. Chang T, Sun L, Wang Y, Wang D, Li W, Li C, et al. Inhibition of keratin 17 expression with
2 antisense and RNAi strategies: exploring novel therapy for psoriasis. *Exp Dermatol.* 2011;20(7):555-
3 60.
- 4 199. Geusens B, Lambert J, De Smedt SC, Buyens K, Sanders NN, Van Gele M. Ultradefortable
5 cationic liposomes for delivery of small interfering RNA (siRNA) into human primary melanocytes. *J*
6 *Control Release.* 2009;133(3):214-20.
- 7 200. Geusens B, Van Gele M, Braat S, De Smedt SC, Stuart MC, Prow TW, et al. Flexible
8 nanosomes (SECosomes) enable efficient siRNA delivery in cultured primary skin cells and in the
9 viable epidermis of ex vivo human skin. *Advanced Functional Materials.* 2010;20(23):4077-90.
- 10 201. Bracke S, Carretero M, Guerrero-Aspizua S, Desmet E, Illera N, Navarro M, et al. Targeted
11 silencing of DEFB4 in a bioengineered skin-humanized mouse model for psoriasis: development of
12 siRNA SECosome-based novel therapies. *Exp Dermatol.* 2014;23(3):199-201.
- 13 202. Desmet E, Bracke S, Forier K, Taevernier L, Stuart MC, De Spiegeleer B, et al. An elastic
14 liposomal formulation for RNAi-based topical treatment of skin disorders: Proof-of-concept in the
15 treatment of psoriasis. *International journal of pharmaceutics.* 2016;500(1-2):268-74.
- 16 203. Eline D, Van Gele M, Grine L, Remaut K, Lambert J. Towards the development of a RNAi-
17 based topical treatment for psoriasis: Proof-of-concept in a 3D psoriasis skin model. *Exp Dermatol.*
18 2017.
- 19 204. Shah SM, Ashtikar M, Jain AS, Makhija DT, Nikam Y, Gude RP, et al. LeciPlex, invasomes, and
20 liposomes: A skin penetration study. *International journal of pharmaceutics.* 2015;490(1-2):391-403.
- 21 205. Date AA, Srivastava D, Nagarsenker MS, Mulherkar R, Panicker L, Aswal V, et al. Lecithin-
22 based novel cationic nanocarriers (LeciPlex) I: fabrication, characterization and evaluation.
23 *Nanomedicine (Lond).* 2011;6(8):1309-25.
- 24 206. Jin Y, Wang S, Tong L, Du L. Rational design of didodecyldimethylammonium bromide-based
25 nanoassemblies for gene delivery. *Colloids and surfaces B, Biointerfaces.* 2015;126:257-64.
- 26 207. Lindblom G, Rilfors L. Nonlamellar phases formed by membrane lipids. *Adv Colloid Interface*
27 *Sci.* 1992;41:101-25.
- 28 208. Shah JC, Sadhale Y, Chilukuri DM. Cubic phase gels as drug delivery systems. *Advanced drug*
29 *delivery reviews.* 2001;47(2-3):229-50.
- 30 209. Jouhet J. Importance of the hexagonal lipid phase in biological membrane organization.
31 *Front Plant Sci.* 2013;4:494.
- 32 210. Libster D, Aserin A, Garti N. Interactions of biomacromolecules with reverse hexagonal liquid
33 crystals: drug delivery and crystallization applications. *J Colloid Interface Sci.* 2011;356(2):375-86.
- 34 211. Chen Y, Ma P, Gui S. Cubic and hexagonal liquid crystals as drug delivery systems. *Biomed*
35 *Res Int.* 2014;2014:815981.
- 36 212. Milak S, Zimmer A. Glycerol monooleate liquid crystalline phases used in drug delivery
37 systems. *International journal of pharmaceutics.* 2015;478(2):569-87.
- 38 213. Duttagupta AS, Chaudhary HM, Jadhav KR, Kadam VJ. Cubosomes: Innovative
39 Nanostructures for Drug Delivery. *Current drug delivery.* 2016;13(4):482-93.
- 40 214. Lopes LB, Lopes JL, Oliveira DC, Thomazini JA, Garcia MT, Fantini MC, et al. Liquid crystalline
41 phases of monoolein and water for topical delivery of cyclosporin A: characterization and study of
42 in vitro and in vivo delivery. *European journal of pharmaceutics and biopharmaceutics : official journal*
43 *of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV.* 2006;63(2):146-55.
- 44 215. Vicentini FT, Depieri LV, Polizello AC, Del Ciampo JO, Spadaro AC, Fantini MC, et al. Liquid
45 crystalline phase nanodispersions enable skin delivery of siRNA. *European journal of pharmaceutics*
46 *and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische*
47 *Verfahrenstechnik eV.* 2013;83(1):16-24.
- 48 216. Depieri LV, Borgheti-Cardoso LN, Campos PM, Otaguiri KK, Vicentini FT, Lopes LB, et al. RNAi
49 mediated IL-6 in vitro knockdown in psoriasis skin model with topical siRNA delivery system based
50 on liquid crystalline phase. *European journal of pharmaceutics and biopharmaceutics : official*
51 *journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV.* 2016;105:50-8.

- 1 217. Petrilli R, Eloy JO, Praca FS, Del Ciampo JO, Fantini MA, Fonseca MJ, et al. Liquid Crystalline
2 Nanodispersions Functionalized with Cell-Penetrating Peptides for Topical Delivery of Short-
3 Interfering RNAs: A Proposal for Silencing a Pro-Inflammatory Cytokine in Cutaneous Diseases. *J*
4 *Biomed Nanotechnol.* 2016;12(5):1063-75.
- 5 218. Dixon JE, Osman G, Morris GE, Markides H, Rotherham M, Bayoussef Z, et al. Highly efficient
6 delivery of functional cargoes by the synergistic effect of GAG binding motifs and cell-penetrating
7 peptides. *Proc Natl Acad Sci U S A.* 2016;113(3):E291-9.
- 8 219. Kouno M, Lin C, Schechter NM, Siegel D, Yang X, Seykora JT, et al. Targeted delivery of tumor
9 necrosis factor-related apoptosis-inducing ligand to keratinocytes with a pemphigus mAb. *J Invest*
10 *Dermatol.* 2013;133(9):2212-20.
- 11 220. Simpson CL, Patel DM, Green KJ. Deconstructing the skin: cytoarchitectural determinants of
12 epidermal morphogenesis. *Nat Rev Mol Cell Biol.* 2011;12(9):565-80.
- 13 221. Guilloteau K, Paris I, Pedretti N, Boniface K, Juchaux F, Huguier V, et al. Skin Inflammation
14 Induced by the Synergistic Action of IL-17A, IL-22, Oncostatin M, IL-1{alpha}, and TNF-{alpha}
15 Recapitulates Some Features of Psoriasis. *J Immunol.* 2010.
- 16 222. Bracke S, Desmet E, Guerrero-Aspizua S, Tjabringa SG, Schalkwijk J, Van Gele M, et al.
17 Identifying targets for topical RNAi therapeutics in psoriasis: assessment of a new in vitro psoriasis
18 model. *Arch Dermatol Res.* 2013;305(6):501-12.
- 19 223. Swindell WR, Remmer HA, Sarkar MK, Xing X, Barnes DH, Wolterink L, et al. Proteogenomic
20 analysis of psoriasis reveals discordant and concordant changes in mRNA and protein abundance.
21 *Genome medicine.* 2015;7(1):86.
- 22 224. World Health Organization. Global Report on Psoriasis. Switzerland. 2016.