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Contributions of Helen Vaher to original publications:

Study I: performed the experiments (analyzed the patient data, performed expression analysis of CARD10, IRAK1, CCL5, IL-8, FERMT1, and NUMB in human keratinocytes, mice keratinocytes, and mice fibroblasts and performed the corresponding cell culture experiments, analyzed FERMT1 function by immunofluorescence analysis and western blot, performed correlation analysis), analyzed the data, contributed with the design of the study and writing the manuscript.

Study II: responsible for the design of the study, performed the experiments (analyzed the patient data, performed expression analysis of miR-146a, miR-146b, IRAK1, CARD10, SERPINB2, CCL5, CXCL5, and IL-8 in human keratinocytes and performed the corresponding cell culture experiments, analyzed SERPINB2 function by immunofluorescence analysis, human neutrophils chemotaxis assay, and annexin V-FITC 7-AAD staining, performed correlation analysis), analyzed the data, prepared the figures, and wrote the manuscript.

Study III: performed the experiments (analyzed the patient data, performed expression analysis of miR-10a, HAS3, and Ki67 in human keratinocytes, and performed the corresponding cell culture experiments,

analyzed miR-10a function by cell cycle analysis, analyzed HAS function by immunofluorescence analysis), analyzed the data, prepared the figures, contributed with the design of the study, and writing the manuscript.

* These authors contributed equally to this work

ABBREVIATIONS

AD	atopic dermatitis
ADAMTSL5	a disintegrin-like and metalloprotease domain containing thrombospondin type 1 motif-like 5
AKT	protein kinase B
AMP	antimicrobial peptide
C/EBPβ	CCAAT-enhancer-binding-protein-β
CARD10	caspase recruitment domain-containing protein 10
CCL	c-c motif chemokine ligand
CD	cluster of differentiation
c-Fos	proto-oncogene C-Fos
C-MYC	MYC proto-oncogene
CTLA-4	cytotoxic T lymphocyte–associated antigen
CXCL	chemokine (C-X-C motif) ligand
DC	dendritic cells
DCGR8	DiGeorge syndrome critical region gene 8
FLG	filaggrin
Foxp3	forkhead box P3
GM-CSF	granulocyte-macrophage colony-stimulating factor (GM-CSF)
GPCR	G-protein-coupled receptor
GW182	glycine-tryptophan protein of 182 kDa
hBD2	human β-defensin-2
HEK	human embryonic kidney
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HOXB4	homeobox B4
Hsc70	heat shock cognate protein 70
Hsp90	heat shock protein 90
IFN	interferon
IFNGR	interferon-gamma receptor
IgE	immunoglobulin E
IKK	IκB kinase
IL	interleukin
ILC	innate lymphoid cells
IRAK	interleukin-1 receptor-associated kinase
IRF	interferon regulatory factor
IκBα	NF-kappa-B inhibitor alpha
JAK	Janus kinase
KRT5	keratin 5
LPS	lipopolysaccharides
MALT1	mucosa-associated lymphoid tissue lymphoma translocation protein 1
MAP3K7	mitogen-activated protein kinase kinase knase 7
miR-10a	hsa-miR-10a-5p

miR-146a/b	hsa-miR-146a-5p and hsa-miR-146b-5p
miRNA	microRNA
mRNA	messenger RNA
Msi2	Musashi RNA binding protein 2
NF-κB	nuclear factor kappa B
NK	natural killer
nt	nucleotides
PASI	psoriasis area and severity index
PBMC	peripheral blood mononuclear cell
PI3K	phosphoinositide-3-kinase–protein kinase
PKIα	protein kinase inhibitor α
PLA2G4D	phospholipase A2 group IVD
PPP6C	protein phosphatase 6
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA
PSORS1	psoriasis susceptibility 1
PV	*psoriasis vulgaris*
RanGTP	RAs-related nuclear protein GTP
RISC	RNA induced silencing complex
RORα	RAR-related orphan receptor alpha
SERPINB2	serpin peptidase inhibitor B2
Skp2	S-phase kinase associated protein 2
SNP	single-nucleotide polymorphism
STAT	signal transducer and activator of transcription
STK40	serine/threonine kinase 40
TGF	transforming growth factor
TGIF1	TGF-β induced factor homeobox 1
Th	T helper
TIAM1	T-lymphoma invasion and metastasis-inducing protein 1
TLR	Toll-like receptors
TNF	tumor necrosis factor
TRAF6	TNF receptor-associated factor 6
Treg	regulatory T cell
TSLP	thymic stromal lymphopoietin
TWIST1	Twist-related protein 1
TYK2	tyrosine kinase 2
UTR	untranslated region
XPO5	exportin 5
βTRC	β-transducing repeat-containing gene

1. INTRODUCTION

The skin is the outermost layer of our body serving as the first-line defense against environmental factors and pathogens. To maintain this function, various cell types in the skin, including keratinocytes, fibroblasts, and innate and adaptive immune cells, like dendritic cells, macrophages, mast cells, and T cells, need to work and communicate together. Disruption of this balance can lead to inflammatory skin diseases, such as atopic dermatitis (AD), *psoriasis vulgaris* (PV), and vitiligo (Gould, 2018; Richmond & Harris, 2014), which are characterized by skin rashes accompanied by itching, redness, and the presence of inflammation. These diseases are relatively common among the general population and affected people suffer from loss of quality of life, and the accompanying socio-economic costs are high (Lim et al., 2017).

Inflammatory skin diseases like PV and AD are usually caused by genetic predisposition and environmental factors leading to impaired epithelial function and altered immunity (Eyerich & Eyerich, 2018). The involvement of keratinocytes as major regulators in both the acute and the chronic phase of skin inflammation through cytokine production and surface molecule expression has been established (Albanesi et al., 2005). This will lead to an increased immune cell infiltration in the skin. The epithelial and immune cells start to produce specific cytokines characteristic to a discrete disease. For example, AD is characterized by the production of interleukin (IL)-4 that is typical to T helper 2 (Th) or group 2 innate lymphoid cells (ILC) response in the acute phase, whereas cytokines like interferon (IFN)-γ and IL-22 are involved in the chronic phase (Weidinger & Novak, 2016).

MicroRNAs (miRNAs) are short ~22 nucleotides (nt) single-stranded non-coding RNA molecules that together with protein partners regulate gene expression at the post-transcriptional level (Bartel, 2004; Lau et al., 2001; Lee et al., 1993). Each miRNA binds through partial complementarity to messenger RNA (mRNA) 3' untranslated region (3'UTR) and thereby functions as a guide bringing proteins that initiate the degradation to target mRNA or inhibition of Translation. As a single miRNA can target numerous or even hundreds of mRNAs (Baek et al., 2008; Selbach et al., 2008), it is thought that more than 60% of our protein-coding genes are affected by miRNAs (Friedman et al., 2009).

Since their discovery, miRNAs have been implicated in many human pathologies, including skin diseases. However, there is still relatively little known about their functions in association with skin diseases. In this thesis, we investigated the role of hsa-miR-146a-5p, hsa-miR-146b-5p (miR-146a/b) and hsa-miR-10a-5p (miR-10a). miR-146a and miR-146b belong to the miR-146 family, and as they differ only by two nucleotides in the area not binding to mRNA, they are thought to target the same set of genes and are mostly known about their anti-inflammatory function in various cell types (Paterson & Kriegel, 2017; Taganov et al., 2006). miR-10a belongs to the miR-10 family and the function of miR-10a is often associated with various cancers (Lund, 2010). In

our studies, we set out to determine the function and regulation of miR-146a/b and miR-10a in association with the two most common chronic inflammatory skin diseases PV and AD, respectively. We aimed to identify novel direct targets of miR-146a/b and miR-10a and how these particular targets and miRNAs themselves may influence the development of PV or AD as well as inflammation and proliferation of keratinocytes, the processes tightly associated with both these diseases.

2. LITERATURE REVIEW

2.1. Skin immunity

One of the most important functions of the skin is to act as a physical barrier and to protect the organism from infections. The outermost layer of the skin, the epidermis, is considered to be the primary physical barrier between the organism and the environment. The epidermis is composed of different cells, of which keratinocytes, melanocytes, and Langerhans cells are considered the main cell type of the epidermis (Nordlund, 2007). The structure and cellular composition of the skin are more precisely described in figure 1. Tight regulation of keratinocyte proliferation is vital to the structure and function of the epidermis. Usually, 96% of human basal keratinocytes are in the G0/G1 cell cycle phase and are not proliferating due to limited space in the basal layer and leading to contact inhibition (Bata-Csorgo et al., 1993; Bauer et al., 1980). During differentiation, the basal layer keratinocytes divide and migrate upwards forming first the spinous layer, then the granular layer, and in the end the cornified layer and eventually shed from the surface (Losquadro, 2017).

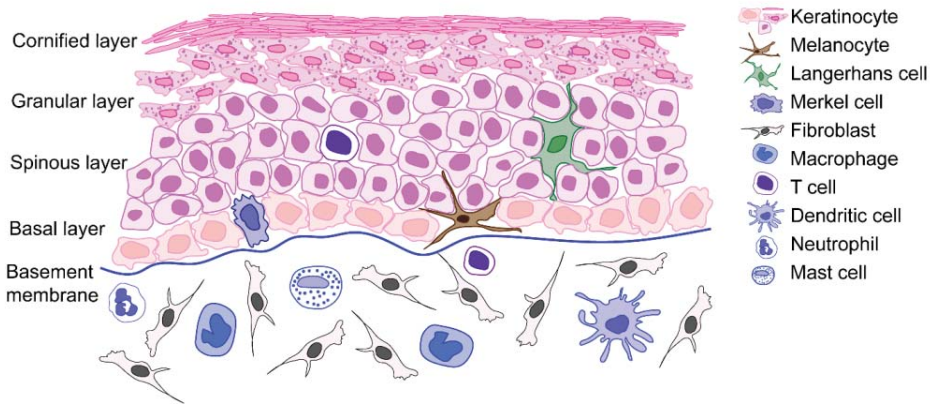

Figure 1. Cellular composition of the skin. The epidermis is mostly comprised of keratinocytes and has four layers: basal layer, spinous layer, granular layer, and cornified layer. The basal layer consists of keratinocytes, melanocytes, and Merkel cells and is usually one cell layer thick. Merkel cells are responsible for the light touch sensation and melanocytes produce melanin for skin pigmentation. Langerhans cells and T cells mediate and control immune responses in the epidermis (Chambers & Vukmanovic-Stejic, 2020). The second layer of the skin is the dermis, which is separated from the epidermis by the basement membrane. The most abundant cells in the dermis are the fibroblasts that produce collagen, elastin, and other proteins (Rippa et al., 2019). The dermis also protects from pathogens and other toxic substances by immune cells, like mast cells, T cells, dendritic cells (DC), and macrophages (Gould, 2018).

In the normal healthy epidermis, keratinocytes from the basal layer move to the spinous layer in 13 days (Weinstein & Van Scott, 1965). The layers are characterized by specific morphological and biochemical features indicating the state of differentiation. For example, keratin 5 (KRT5) and KRT14 are mostly expressed by basal layer keratinocytes, and the markers of early differentiation KRT1 and KRT10 are expressed in the spinous layer (Goleva et al., 2019).

2.1.1. The role of keratinocytes in inflammation

Interestingly, keratinocytes do not only fulfill the role of a structural element, but they also participate in the regulation of innate and adaptive immune responses (Nestle et al., 2009). First, keratinocytes can have direct protective functions as they produce antimicrobial peptides (AMPs) (Clausen & Agner, 2016). AMPs have antimicrobial activity against bacteria, fungi, and viruses and they can modify cell migration, proliferation, differentiation, and cytokine production (Clausen & Agner, 2016; Takahashi & Gallo, 2017). Keratinocytes can produce different AMPs, like human β-defensin-2 (hBD2), LL-37, psoriasin, RNase7, and adrenomedullin, in response to stimuli (Clausen & Agner, 2016). For example, IL-12, IL-23, and IL-27 enhance hBD2 secretion and production in keratinocytes (Kanda & Watanabe, 2008).

Secondly, another role of keratinocytes is to transmit signals from the environment to the immune cells in the skin. As keratinocytes can sense various signals through Toll-like receptors (TLR) (Lebre et al., 2007), they can be activated to produce chemokines and cytokines, which leads to the recruitment and activation of various types of immune cells (Miller, 2008; Miller & Modlin, 2007). In addition, keratinocytes also constitutively or when induced produce cytokines like tumor necrosis factor (TNF), members of the IL-1 cytokine family, and thymic stromal lymphopoietin (TSLP), (Albanesi et al., 2005; Soumelis et al., 2002). Several factors are known to induce the production of TSLP from keratinocytes including other cytokines, viruses, bacteria, fungi, and allergens (Varricchi et al., 2018). This has a prominent role in promoting Th2 cytokine responses in the skin as TSLP-activated DCs promote T cells to differentiate into Th2 cells (Soumelis et al., 2002). Additionally, the activation of the inflammasome signaling pathway will lead to the production of pro-inflammatory cytokines IL-1β and IL-18, members of the IL-1 cytokine family (Martinon et al., 2009). Furthermore, keratinocytes express receptors for a broad array of cytokines, which makes them sensitive to the inflammatory environment. One of the receptors that keratinocytes constitutive express is the IFN-γ receptor complex making them sensible to IFN-γ (Albanesi et al., 2005; Barker et al., 1990).

Another mode of how keratinocytes help to regulate immune cell trafficking is the expression of different chemokines (Figure 2). Resting keratinocytes express low levels of IL-8 and c-c motif chemokine ligand 27 (CCL27) mRNA (Anttila et al., 1992; Morales et al., 1999). When stimulated, the expression of

these and other chemokines can be upregulated remarkably. For example, when keratinocytes are stimulated with TNF-α or IL-1β, the expression of CCL27 is induced (Homey et al., 2000), which attracts a high number of T-cells into the skin (Nedoszytko et al., 2014). Some of the other known chemokines produced by keratinocytes in response to various stimuli are chemokine (C-X-C motif) ligand 1 (CXCL1), CCL20, and CCL5 (Albanesi et al., 2001). IL-8 and CXCL1 are known for attracting neutrophils, CCL20 mediates the movement of dendritic and Langerhans cells, and CCL5 attracts eosinophils and T-cells (Nedoszytko et al., 2014).

In addition to their capacity to produce proinflammatory cytokines and chemokines, keratinocytes are also capable of producing anti-inflammatory cytokines, like transforming growth factor (TGF)-β and IL-10, and receptor inhibitors (Stadnyk, 1994). The main functions of IL-10 are to limit inflammation and downregulate overwhelming immune response by regulating Th1-Th2 balance, inhibiting macrophage and monocyte activation, cytokine production, antigen presentation, and T-cell activation (Weiss et al., 2004). Interestingly, keratinocytes can produce receptor inhibitors for the IL-1 family IL-1Ra and IL-36Ra (Bigler et al., 1992; Johnston et al., 2011). They bind, respectively, to the same receptors as IL-1α and IL-1β, and IL-36α, IL-36β, and IL-36γ, however, preventing the activation of further signaling, including in keratinocytes. Therefore, keratinocytes by producing IL-1Ra and IL-36Ra can modulate the biological responses to stimuli that induce respective IL-1 family member expression in the cells (Lowes et al., 2013; Sims & Smith, 2010). In summary, keratinocytes are an important part of the immunoregulatory network of the skin as they help to regulate both immune homeostasis and inflammatory responses.

2.1.2. Immune cell functions in the skin

The skin is rich in different types of immune cells of which some are resident cells, and some migrate there in case of infection or inflammation (Figure 2). The main immune cell population residing in the epidermis in non-inflamed conditions is a specialized subset of tissue-resident macrophages called Langerhans cells (Doebel et al., 2017; Otsuka et al., 2018), which account for 3–5% of epidermal cells (Merad et al., 2008). It has been shown that Langerhans cells in mice can promote the Th17 cells by secreting IL-6 in response to pathogens such as *Candida albicans* (Igyarto et al., 2011). However, antigen presentation by Langerhans cells can also result in T cell anergy or deletion indicating that they have a possible immunoregulatory role and could be involved in peripheral tolerance induction (Igyarto & Kaplan, 2013).

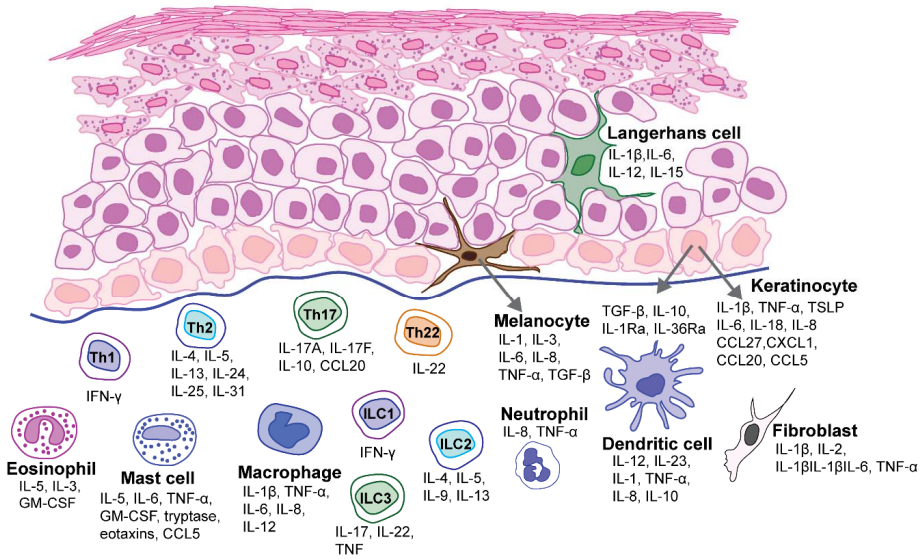

Figure 2. Immune cell functions in the skin. Among different immune cells some are resident cells and some migrate to the skin in the case of infection or inflammation. Each cell subset is characterized by a specific cytokine profile. While Langerhans cells reside in the epidermis (Doebel et al., 2017; Otsuka et al., 2018) another subset of antigen presenting cells in the skin is dermal DCs (Schraml & Reis e Sousa, 2015; Waisman et al., 2017). Even in uninflamed conditions, a diverse types of T helper cells, including Th1, Th2, Th17, and Th22 are present in the skin (Raphael et al., 2015). In addition, different type ILCs (ILC1, ILC2, and ILC3) that all have specific cytokine profiles play a role in barrier immunity (Kobayashi et al., 2020; Vivier et al., 2018). Additionally, different types of granulocytes can be recruited, including neutrophils, eosinophils, basophils, and mast cells (Bochner & Gleich, 2010; Kita et al., 1991; Nauseef & Borregaard, 2014).

Another cell type playing an important role in skin immunity is DCs. DCs are known as antigen-presenting cells and therefore link innate and adaptive immune responses. DC can phagocytose pathogens, and in response, they start to produce pro-inflammatory mediators that attract innate immune cells to the infection site (Schraml & Reis e Sousa, 2015; Waisman et al., 2017). There are different subsets of DC in the dermis, including conventional DC1s and DC2s, and, also during inflammation, plasmacytoid DCs, and monocyte-derived DCs (Collin & Bigley, 2018; Worbs et al., 2017). Different subsets of DCs have specialized functions and can produce specific cytokines and may initiate distinct inflammatory responses following activation. For example, conventional DC2s express a wide range of receptors, such as lectins, TLRs, nucleotide-binding oligomerization domain-like receptors and retinoic acid-inducible gene-I-like receptors (Collin & Bigley, 2018; Kashem et al., 2017) and can activate Th1, Th2, Th17, and cluster of differentiation (CD8+) T cells (Di Blasio et al.,

2016; Nizzoli et al., 2016; Sittig et al., 2016). Conventional DC2s, when stimulated, can secrete different cytokines including IL-12, IL-23, IL-1, TNF-α, IL-8 and, IL-10 (Nizzoli et al., 2013; Nizzoli et al., 2016; Sittig et al., 2016). In most cases, the DCs participate in the clearance of infectious agents. However, in other circumstances, the activation of DCs can lead to pathological tissue responses and persistent inflammation. For instance, plasmacytoid DCs have been implicated in the pathogenesis of several autoimmune diseases, for example, systemic lupus erythematosus, due to their ability to sense self-nucleic acids and to produce type I interferon (Berggren et al., 2015; Lande et al., 2011).

T lymphocytes play a central role in the immune system as potent effectors and regulators of immunity. In the case of noninflamed skin, both $CD4^+$ T cells and $CD8^+$ T cells are present in the skin (Klicznik et al., 2018). The T cells are divided between different layers of the skin. The majority of T cells are in the dermis, most of which are $CD4^+$ T cells in the perivascular area. The epidermis contains few T cells, of which the majority are $CD8^+$ tissue-resident memory T cells that help to protect the body from infections (Clark et al., 2012). $CD8^+$ T cells are also known as killer or cytotoxic T lymphocytes and they also can summon circulating memory T cells to the site of virus reactivation or reentry of pathogens (Heath & Carbone, 2013). The $CD4^+$ T cell population consists of a diverse set of T helper cells, including Th1, Th2, Th17, Th22, regulatory T (Treg) cells (Raphael et al., 2015), which all produce characteristic cytokines, and have specific roles also in the skin. For instance, Th1 cells secrete IFN-γ and protect against intracellular pathogens, such as Mycobacteria and viruses (Sallusto, 2016). Th2 cells mediate isotype class-switching to immunoglobulin E (IgE) and that contributes to host defense against parasitic worms (Geha et al., 2003). Th2 cells and the cytokines they secrete (IL-4, IL-5, IL-13, IL-24, IL-25 and, IL-31) have a central role in various diseases, including AD, asthma, chronic rhinosinusitis, and food allergy (Morita et al., 2016). Th17 cells can be divided into non-pathogenic and pathogenic Th17 cells. The physiological role of the non-pathogenic Th17 cells is to protect against extracellular bacteria and fungi by controlling neutrophil migration to the skin. They secrete IL-17A, IL-17F, IL-10, and, CCL20 (Stadhouders et al., 2018). However, when the Th17 cells are activated by IL-23 in combination with IL-1β and IL-6, they turn into pathogenic Th17 cells that express IL-17A, IL-17F, IL-22, CCL9, and CXCR3 and are associated with PV, rheumatoid arthritis, multiple sclerosis, and AD (Kurebayashi et al., 2013; Yasuda et al., 2019). Additionally, there are distinct IL-22–producing CD4+ (Th22) and CD8+ (Tc22) T cells (Nograles et al., 2009). Th22 cells are defined by the production of IL-22, which together with other members of the IL-20 family mediate epidermal hyperplasia and inhibit terminal differentiation (Nograles et al., 2009; Sa et al., 2007) as well as function in tissue remodeling as they can produce fibroblast growth factor isoforms, CCL15 and CCL23 (Eyerich et al., 2009). Treg cells are an immunosuppressive subset of T cells, which secrete cytokine IL-10 and thereby relieve inflammatory of skin diseases (Honda et al., 2011).

ILCs are considered as the innate counterpart of adaptive T cells and they can be activated by alarmins, cytokines, and other inflammatory mediators expressed by tissue-resident cells enabling rapid responses to environmental signals (Kobayashi et al., 2020; Vivier et al., 2018). The ILCs have three groups based on the expression of key transcription factors. Group 1 ILCs comprise natural killer (NK) cells and ILC1s and express the transcription factor T-bet and they are able to produce IFN-γ, granzymes, and perforins. They mediate type 1 immunity and can react to intracellular microorganisms such as viruses and bacteria (Kobayashi et al., 2020). Group 2 ILCs are ILC2s and they express the transcription factors GATA Binding Protein 3, RAR-related orphan receptor (ROR) alpha, and MYC proto-oncogene (C-MYC). They produce IL-4, IL-5, IL-9, and IL-13 in response to parasites and allergens and participate in type 2 immunity (Akdis et al., 2020). Additionally, ILC2s have been shown to be upregulated in AD skin and contribute to the increase in type 2 cytokine production in the skin (Salimi et al., 2013). Group 3 ILCs comprise lymphoid tissue-inducer cells and ILC3s and their development are dependent on the expression of RORγt and they produce IL-17, IL-22, and TNF which they produce in response to extracellular bacteria and fungi (Polese et al., 2020). In the case of PV, it has been shown that there is an increase of ILC3s in the lesional skin of PV patients contributing to the development and maintenance of PV (Dyring-Andersen et al., 2014; Teunissen et al., 2014).

In response to the activation of tissue-resident immune cells and keratinocytes and their subsequent release of chemokines, different types of granulocytes, including neutrophils, eosinophils, basophils, and mast cells are recruited to the skin. Neutrophils are the first cell type to be recruited to the skin after the activation of antigen-presenting cells in response to infection (Nauseef & Borregaard, 2014). Neutrophils can efficiently phagocytose pathogens, degrade them by synthesizing reactive oxygen species inside phagolysosomes, kill pathogens by degranulation of AMPs, or create extracellular traps to trap pathogens (Bardoel et al., 2014). Eosinophils participate in antiparasitic and allergic responses. The activation of eosinophils leads to the secretion of IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and degranulation of cationic toxins that help to kill microbes and parasites (Bochner & Gleich, 2010; Kita et al., 1991). Eosinophils also participate in inflammatory responses, which may lead to tissue damage, fibrosis, and the development of hypercoagulability (Rosenberg et al., 2013). As a result, eosinophils are important in different eosinophilic dermatosis and many allergy-related diseases including allergic drug eruption, urticaria, allergic contact dermatitis, and AD (Heymann, 2006). Mast cells can secrete mediators that regulate eosinophil activation and recruitment including IL-5, IL-6, TNF-α, GM-CSF, tryptase, eotaxins, CCL5 (Shakoory et al., 2004). Both eosinophils and mast cells are associated with Th2 responses, and therefore, they also play a role in allergic diseases, including allergic contact dermatitis, AD, allergic rhinitis, and bronchial asthma (Ito et al., 2011; Kawakami et al., 2009).

2.1.3. Pro-inflammatory signaling pathways

In the case of inflammation, pro-inflammatory signaling pathways are activated in the different types of cells in the skin, including keratinocytes. Among the key signaling pathways activated during inflammation is the nuclear factor kappa B (NF-κB) and the Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathways (Shi et al., 2017; Villarino et al., 2017). The activation of both pathways is described in figure 3. The NF-κB signaling pathway can be activated by many stimulatory molecules, like TNF-α, IL-17, or infections by various pathogens (Osborn et al., 1989; Pahl, 1999; Shalom-Barak et al., 1998). The NF-κB transcription factor can induce the expression of hundreds of target genes (Zhang et al., 2017), many of them are related to the host immune response, including cytokines or chemokines like CCL5, IL-1α, and IL-8, or regulation of apoptosis or cell growth (Kunsch & Rosen, 1993; Mori & Prager, 1996; Pahl, 1999).

Another signaling pathway commonly activated in inflammatory skin diseases is the JAK/STAT pathway. Over 60 cytokines and growth factors use the JAK/STAT pathway for their signaling (Howell et al., 2019). For example cytokines like IL-19, IL-20, IL-22 and, IL-23, which are also highly expressed in psoriatic lesions, signal through the JAK/STAT pathway (Dumoutier et al., 2001; Witte et al., 2014). There are four Janus kinases (JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)) and seven STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6). In mammals JAK1, JAK2, and TYK2 are expressed everywhere. However, JAK3 expression is specific to hematopoietic and lymphoid tissues (Ghoreschi et al., 2009). The activation of STAT induced gene expression will lead to different effector responses. For example, STAT1 and STAT4 activate the antiviral Th1 type response and STAT3 regulates the anti-bacterial/fungal Th17 type response (Welsch et al., 2017). Cytokines can activate different STAT proteins at the same level or one of the STATs more strongly. For instance, INF-γ activates both STAT1 and STAT3, however, the STAT3 response is much weaker (Qing & Stark, 2004). Also, one STAT protein can be activated by different cytokines. For example, IL-6, IL-10, IL-21, IL-22, and IL-23 all signal through STAT3 (Hirahara et al., 2015; Yang et al., 2011).

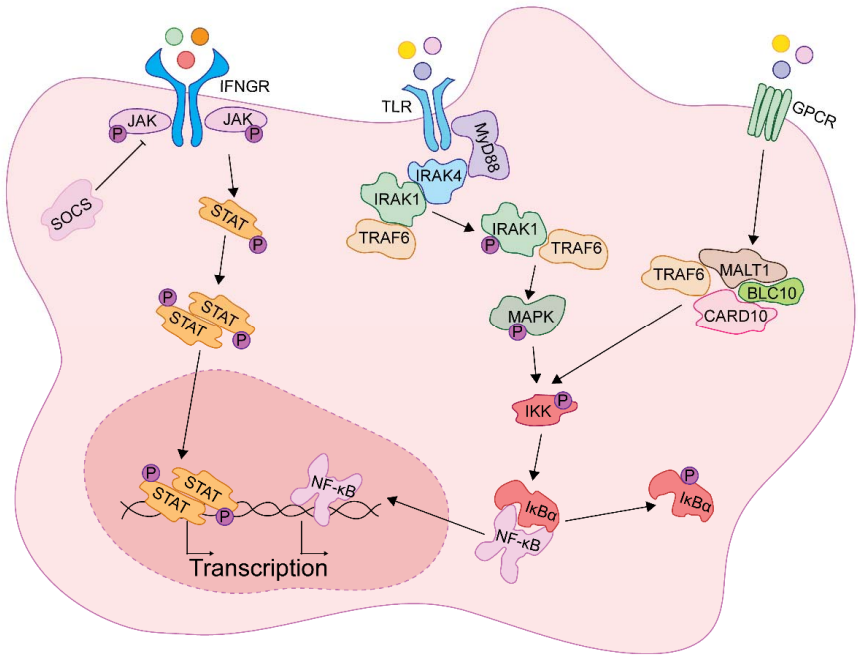

Figure 3. NF-κB and JAK/STAT signaling pathways. Activation of the NF-κB and JAK/STAT signaling pathways occurs through different types of receptors, including the interferon-gamma receptor (IFNGR), TLR, and G protein-coupled receptors (GPCR) by various stimuli. In case of signaling through TLRs, a cascade of protein phosphorylation-associated events leads to the association of MyD88 with TLR intracellular part, recruitment of interleukin-1 receptor-associated kinase 4 (IRAK4), IRAK1, and TNF receptor-associated factor 6 (TRAF6) (Akira & Takeda, 2004; Cao et al., 1996). The phosphorylated IRAK1 and TRAF6 detach from the receptor, this eventually leads to the phosphorylation of mitogen-activated protein (MAP) kinases and the IκB kinase (IKK) complex, which will result in phosphorylation of the NF-kappa-B inhibitor alpha (IκBα) that will be ubiquitylated and therefore degraded enabling the NF-κB dimer to be imported to the nucleus where it activates the expression of its target genes (Akira & Takeda, 2004; Gilmore & Herscovitch, 2006). If the stimulatory signal comes from GPCR, the caspase recruitment domain-containing protein 10 (CARD10) forms a complex with mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), which leads to the phosphorylation of IKK, degradation of the complex and activation of NF-κB (Grabiner et al., 2007; Mahanivong et al., 2008). The activation of the JAK/STAT signaling pathways is initiated by binding of cytokine or growth factor to the corresponding transmembrane receptor, leading to the phosphorylation and activation of the JAKs. The activated JAKs then phosphorylate STAT monomers, which dimerize and translocate to the nucleus where they directly engage DNA regulatory elements to activate the transcription (Howell et al., 2019; Villarino et al., 2015). Different steps of the JAK/STAT pathway are tightly regulated, for example by suppressor of cytokine signaling (SOCS) family of proteins that act as negative regulators (Villarino et al., 2015).

The interplay between epidermal keratinocytes, stromal, and immune cells results in an effective host defense against various pathogens and maintains or restores skin homeostasis. Some immune cells, like skin-resident memory T cells, have the capacity to migrate into the skin, terminally differentiate and reside there and thereby maintain memory to protect against future reinvasion (Mueller et al., 2014). However, in the case of PV, it has been shown that $CD49a^{-}$ skin-resident memory T cells are responsible for the augmented IL-17 production (Cheuk et al., 2017). Other cells are rapidly recruited to the skin in case of infection. For example, neutrophils are considered as one of the first cell types to be recruited to the inflammation site after injury (Kovtun et al., 2018). Altered communication or dysregulation of these cell types can lead to chronic inflammatory skin diseases or other conditions like, inflammatory bowel disease and hematologic malignancies (Baumgart & Carding, 2007; Bachireddy et al., 2015). For example, in the case of psoriasis, the increased number of neutrophils contributes to the development and progression of the disease (Chiang et al., 2019). Chronic infections in case of inadequate skin immunity is also often associated with various pathogens, such as *Candida albicans* or *Staphylococcus aureus* (Giardino et al., 2016).

2.2. Chronic inflammatory skin diseases

2.2.1. Psoriasis vulgaris

Psoriasis is a common inflammatory skin disease with a prevalence of approximately 2–3 % in Europe and the United States (Parisi et al., 2013). It has two main forms PV and pustular psoriasis. The most common form of psoriasis, PV, also known as plaque psoriasis, representing almost 90% of psoriatic patients (Boehncke & Schon, 2015). There are two peak ages of onset, early-onset between 15 and 30 years, and late-onset 50 and 60 years (Boehncke & Schon, 2015). PV is characterized by often symmetrical monomorphic and demarcated erythematous plaques with white scaling that appear mostly on the scalp, trunk, and extensor surfaces. However, in some cases, it can lead to erythroderma affecting the entire body surface (Boehncke & Schon, 2015). Histologically, psoriatic skin lesions are characterized by acanthosis, the thickening of the epidermis, leukocyte infiltrates, and an increased number of tortuous and leaky vessels in the dermis (Griffiths & Barker, 2007) (Figure 4). PV is not only a skin disease: it is associated with comorbidities, such as psoriatic arthritis, cardiovascular disease, metabolic syndrome (obesity, hypertension, dyslipidemia, and diabetes), chronic kidney disease, gastrointestinal disease, mood disorder, malignancy, and systemic inflammation that increases the risk for death (Gelfand et al., 2007; Takeshita et al., 2017a, 2017b).

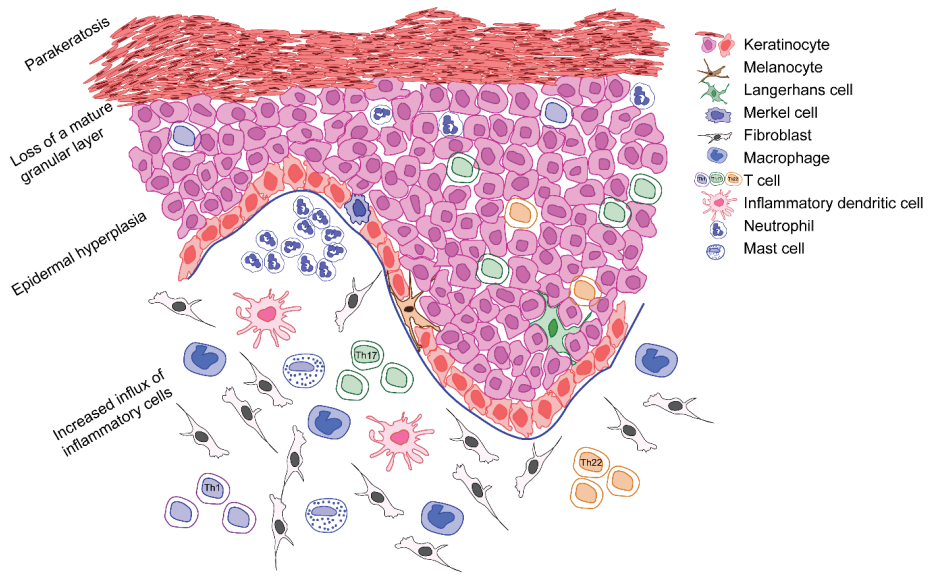

Figure 4. Cellular composition of psoriatic skin. The thickening of the epidermis or epidermal hyperplasia in psoriatic lesions is caused by increased keratinocyte proliferation, which may lead up to an eightfold increase in epidermal cell turnover (Bata-Csorgo et al., 1993). The increased transition of keratinocyte to the upper layers leads to altered protein expression, loss of a mature granular layer, and retention of keratinocyte nuclei (parakeratosis). There is an increased influx of inflammatory cells into the skin, including inflammatory DCs, neutrophils, effector Th1, Th17, and Th22 (Boehncke & Schon, 2015) that further drive the inflammation.

PV has a strong genetic background that is illustrated by the evidence that up to 70% of monozygotic twins and 20% of dizygotic twins share the disease (Farber et al., 1974; Griffiths & Barker, 2007). Also, epidemiologic studies have shown a higher prevalence of PV among the relatives of patients as compared to the general population (Boehncke & Schon, 2015). To date, genome-wide linkage studies have identified at least 60 chromosomal loci linked to psoriatic susceptibility (Elder, 2018). The PSORS1 locus, which is a 220-kb region found on chromosome 6p21.3, has been shown to contribute between 35% and 50% of the heritability of psoriasis and has the largest effect size (Nair et al., 1997; Trembath et al., 1997; Veal et al., 2002). One of the alleles in psoriasis susceptibility 1 (PSORS1) region is the human leukocyte antigen C (*HLA-Cw6 (C*06:02)*), which encodes a class I major histocompatibility complex molecule that is expressed on antigen-presenting cells and involved in CD8+ T-cell activation and it is present in more than 60% of patients and increases the risk for PV over 9–23 times (Gudjonsson et al., 2003). The presence of *HLA-Cw6* is more likely to be associated with early onset PV (Nair et al., 2006). In addition, many linked loci include candidate genes that are

related to immune pathways like antigen presentation, innate antiviral signaling, and Th17 cell activation (Tsoi et al., 2015). For example, many of the genes linked with PV are related to cytokine and cytokine receptors and their signaling, including *IL23A*, *IL12B*, *IL23R*, *TYK2* and *STAT3* (Tsoi et al., 2012). Another gene associated with PV and involved in NF-κB signaling is *CARD14,* which is highly expressed in keratinocytes (Scudiero et al., 2011; Tsoi et al., 2012). Mutations in *CARD14* have been shown to cause constitutive NF-κB activation, leading to enhanced production of pro-inflammatory cytokines and increased risk of PV (Berki et al., 2015; Sugiura et al., 2014).

2.2.1.1. Pathophysiology of PV

Psoriatic lesions usually develop in the same skin areas susceptible to frequent friction or minor trauma, known as Koebner's phenomenon (Weiss et al., 2002). The development of psoriatic lesion usually starts with immune activation following environmental stimuli, including exogenous trauma, infections or drugs (Di Meglio et al., 2014) and the loss of immune tolerance to psoriasis autoantigens, like LL37, a disintegrin-like and metalloprotease domain-containing thrombospondin type 1 motif-like 5 (ADAMTSL5), and phospholipase A2 group IVD (PLA2G4D)-generated neolipids (Arakawa et al., 2015; Cheung et al., 2016; Lande et al., 2014). Activated Th17 cells in the skin produce several cytokines, including IL-17A, IL-17F, TNF-α, and IL-22 (Blauvelt & Chiricozzi, 2018). IL-17A is considered as one of the major effector cytokines in PV, which can act synergistically with TNF-α to induce the expression and release of many psoriasis-related proteins from keratinocytes, including hBD2, lipocalin 2, S100 proteins, and LL37 (Wang et al., 2013). Th1 cells contribute to the increase of IFN-γ, TNF-α, and IL-12 in psoriatic lesions (Austin et al., 1999). TNF-α activates the NF-κB pathway that affects lymphocyte and keratinocyte survival, proliferation, and apoptosis (Ogawa et al., 2018). On the other hand, IL-22 produced by Th22 cells has been shown to cause epidermal hyperplasia, hypogranulosis, and parakeratosis. These processes are all associated with disruption of normal terminal differentiation of keratinocytes in *in vitro* reconstituted human epidermis, indicating that IL-22 is one of the cytokines contributing to acanthosis and parakeratosis in PV (Boniface et al., 2005; Sa et al., 2007; Wolk et al., 2009). Figure 5 describes the proposed mechanisms leading to loss of immune tolerance to psoriasis autoantigen LL37.

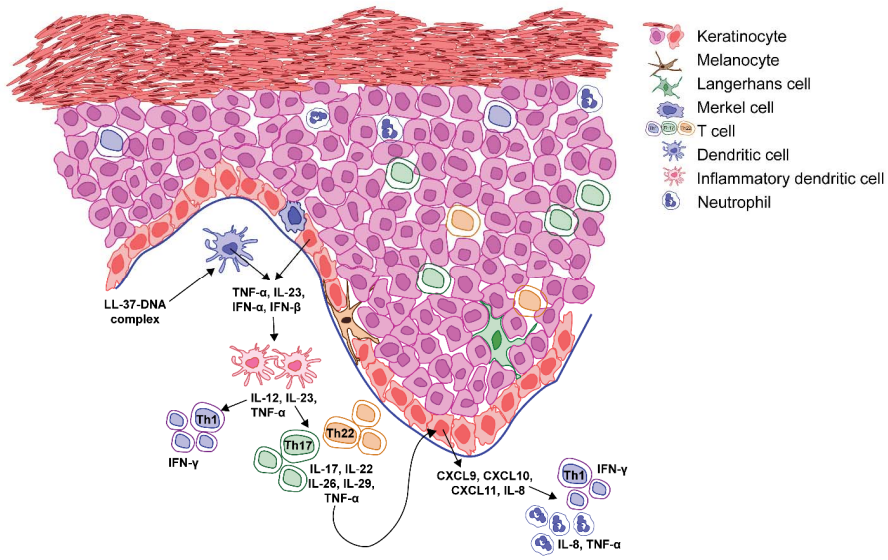

Figure 5. Pathophysiology of PV. The loss of immune tolerance to psoriasis auto-antigen LL37 in complex with DNA will lead to the activation of plasmacytoid DCs (Morizane et al., 2012). The activated plasmacytoid DCs and damaged keratinocytes start to produce type I IFN (IFN-α and IFN-β) and TNF-α, which results in further production of TNF-α, IL-12 and IL-23 by plasmacytoid and recruited inflammatory DCs (Lande et al., 2007; Lowes et al., 2014). From the produced cytokines, IL-12 promotes the differentiation of naïve CD4+ T cells into IFN-γ-producing Th1 cells. The cytokine IL-23 drives the polarization, clonal expansion, and activation of Th17 or Th22 cells, which leads to the production of IL-17 and IL-22 (Hawkes et al., 2018; Langrish et al., 2005; Lowes et al., 2014). Additionally, Th17 cells produce TNF-α, IL-26, and IL-29 that activate keratinocytes, which start to produce chemokines CXCL9, CXCL10, and CXCL11, which in turn recruit more Th1 cells (Stephen-Victor et al., 2016; Wolk et al., 2013). IL-17A is also known to upregulate the expression of TNF-α (Blauvelt & Chiricozzi, 2018) CXCL1, CXCL2, and IL-8 in keratinocytes, which attract neutrophils (Reich et al., 2015).

In the case of psoriasis, neutrophils secrete a variety of proinflammatory signals, including reactive oxygen species, IL-17, and neutrophil extracellular traps (Lin et al., 2011). Neutrophils also have a role in amplifying the IL-36 driven inflammatory processes in PV. The released extracellular traps contain several proteases including elastase (SERPINA1) and cathepsin G (SERPINA3), that are required for the cleavage of IL-36α, IL-36β and IL-36γ to their truncated and more biologically active forms (Clancy et al., 2017; Henry et al., 2016; Towne et al., 2011). IL-36α, IL-36β, and IL-36γ are highly expressed in psoriatic skin (Johnston et al., 2011; Keermann et al., 2015) and they, in turn, activate keratinocytes (Carrier et al., 2011). Also, IL-36 can stimulate the maturation and function of antigen-presenting cells. For example, monocytes and myeloid DCs both secrete more IL-1β and IL-6 when the cells were stimulated with IL-36 family members (Foster et al., 2014).

2.2.1.2. Therapy

PV is a chronic disease that usually requires long-term therapy. Most often, Psoriasis Area and Severity Index (PASI) is used to help quantify disease severity as PASI takes into consideration the affected body surface area along with redness, scaling, and cellular infiltration. PV is considered mild when the PASI values are <10, moderate at 10–20 and severe >20 (Spuls et al., 2010). The disease severity and other comorbidities will determine the most suited choice of therapy (Mrowietz et al., 2011). For the case of mild to moderate PV, it is often treated topically using a variation of vitamin D analogs, narrow-band phototherapy, and glucocorticoids. Traditionally moderate to severe PV have been treated with systemic treatment like methotrexate, cyclosporin A, and retinoids (Rendon & Schakel, 2019). In recent years, the treatment has shifted more towards the use of systemic therapies that target specific inflammatory pathways by using complex engineered molecules including monoclonal antibodies and receptor fusion proteins (Rendon & Schakel, 2019). The longest-used biologicals are those that inhibit TNF-α by binding to its soluble and membrane-bound form. Currently, three TNF-α inhibitors are used in the treatment of psoriatic disease. These include infliximab and adalimumab which are monoclonal antibodies and etanercept that is a fusion protein of the TNF receptor to the constant end of the IgG1 antibody (Campanati et al., 2019). Understanding the role of IL-23 in shaping the Th17 response in PV has led to the development of antibodies against IL-17A (secukinumab, ixekizumab, and brodalumab) (D'Adamio et al., 2019; Langley et al., 2014; Mease et al., 2017) and IL-23 (ustekinumab, guselkumab, tildrakizumab, risankizumab) (Famenini & Wu, 2013; Papp et al., 2017a; Reich et al., 2017), which are now all used in the treatment of PV.

2.2.2. Atopic dermatitis

AD is characterized by acute flare-ups and exacerbations, generalized skin dryness, recurrent eczematous lesions on dry skin accompanied by intensive pruritus. There are also a set of stigmata that are associated with AD, including Dennie–Morgan lines, hyperlinearity of the palms and soles, and Herthoge's sign (Weidinger & Novak, 2016). The cellular composition and histology of AD are shown in figure 6. The disease prevalence is up to 20% in children and about 2–5 % of the adult population in developed countries (Akdis et al., 2006; Barbarot et al., 2018). AD onset can be during early infancy and childhood but can persist or start in adulthood (Weidinger & Novak, 2016). The skin lesions mostly occur on flexural sites and in rare cases can generalize to secondary erythroderma (Weidinger & Novak, 2016). The skin of AD patients has an overall decrease in microbial diversity (Tauber et al., 2016) and is highly susceptible to complicating infections with *Staphylococci* and *Herpesviridae* (Weidinger & Novak, 2016). The loss of this diversity leading to *S. aureus*

dominance may occur shortly before AD flares (Kong et al., 2012). The increased levels of *S. aureus* in the epidermis correlates with increased IL-4, IL-13, IL-22, and TSLP and decreased expression of AMPs (Nakatsuji et al., 2016). The quality of life of AD patients is impaired especially in case of extensive lesions and itch (Holm et al., 2016). Several systemic comorbidities are associated with AD, including obesity, mental problems, and autoimmune diseases (Brunner et al., 2017). Also, AD-like food allergy in infants may progress to other atopic disorders, including asthma and allergic rhinitis in later childhood or adulthood (Schneider et al., 2016; Thomsen, 2015).

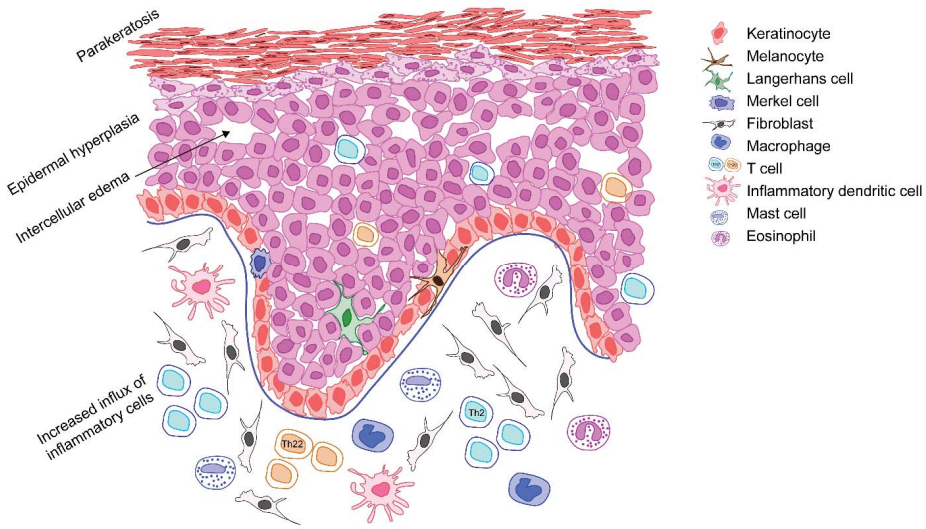

Figure 6. Cellular composition of AD skin. The acute lesions histologically are characterized by epidermal intercellular edema, thickening of the *stratum corneum*, and parakeratosis. Additionally, chronic lesions are characterized by diffuse epidermal hyperplasia (Kawakami et al., 2009). There are more inflammatory cells recruited to the skin, including inflammatory DCs, eosinophils, mast cells, and effector Th2 and Th22 (Weidinger & Novak, 2016).

AD is considered as a multifactorial and heterogeneous disease characterized by different clinical phenotypes based on interactions of susceptibility genes, impaired skin barrier integrity, and immune dysregulation (Weidinger & Novak, 2016). One of the defining factors in AD is a defective barrier function that is considered fundamental in its pathogenesis. It has been shown that epidermal expression of barrier function-related proteins, such as filaggrin (FLG), loricrin, and involucrin are decreased. The impaired epidermal barrier allows the interaction of skin DCs with different irritants, pathogens, and allergens (Czarnowicki et al., 2017). Consequently, the onset of AD is also affected by different environmental factors. For example, over 85% of AD patients are IgE sensitized to house dust mite and this may play a role in acute flare-ups (D'Auria et al., 2016).

Pruritus is one of the main symptoms of AD, the itch-scratch cycle induces further inflammation through the release of TSLP, IL-13, and IL-31 that stimulate nerve fibers and upregulate cellular pruritogens. The interplay between keratinocytes and immune cells is important in itch regulation because these cells generate mediators that contribute to the sprouting of nerve fibers and stimulate sensory nerve endings (Weidinger & Novak, 2016). IL-31 produced by Th2 cells has been shown to induce severe itching and dermatitis in transgenic mice and also in patients with AD (Cheung et al., 2010; Dillon et al., 2004; Takaoka et al., 2006). IL-31 induces a specific transcriptional profile in sensory neurons that causes nerve elongation and branching leading to increased sensitivity and sustained itch (Feld et al., 2016).

Genetically, AD is associated with at least 31 significant susceptibility loci, which have been linked to genes involved in skin barrier function, keratinocyte differentiation, innate and adaptive immune responses and cytokines, and chemokines (Paternoster et al., 2015). The strongest link with AD has the *FLG* gene, encoding the epidermal structural protein FLG that has a role in skin barrier function, keratinocyte differentiation, and epidermal homeostasis (Palmer et al., 2006). FLG deficiency is associated with early-onset, severe AD, greater allergen sensitization, and increased susceptibility to infections (Szegedi, 2015). However, even if *FLG* gene is associated with AD, the mutations in the *FLG* gene are found only in 15% to 50% of the patients, and 40% of *FLG* mutation carriers never develop AD (Palmer et al., 2006). Therefore, factors other than *FLG* gene play a role in the development of AD. For example, genes encoding other structural and tight junction proteins may have influence. Concordantly, reduced expression of Claudin-1 in the epidermis of AD patients inversely correlates with both total IgE levels and eosinophil numbers (De Benedetto et al., 2011). In addition, variations in genes encoding serine proteases and inhibitors have also been associated with AD as they play a role in epidermal barrier homeostasis, including regulation of stratum corneum desquamation, lipid barrier construction and cornified cell envelope (Werfel et al., 2016a). For example, the serine protease inhibitor Kazal-type 5 that is known to regulate proteolysis, keratinocyte differentiation, and maintenance of the normal skin barrier is associated with the incidence of AD in the Japanese population (Kato et al., 2003). Many other genes associated with AD are related to innate and adaptive immune responses, of which some are related to the Th2 type cytokines (*IL-4/IL13*) or IL-1 family receptors (*IL1RL1/IL18R1/IL18RAP*) (Tamari & Hirota, 2014). It has been also shown that JAK/STAT pathway-related loci have been associated with risk for AD, including polymorphisms within the *IL6R*, *IL2/IL21*, *IL7R*, *IL15RA/IL2RA* and *STAT3* genes (Paternoster et al., 2015).

2.2.2.1. Pathophysiology of AD

AD is a complex disease that involves both innate and adaptive immune counterparts of the immune system in its pathogenesis. The major cell types involved are keratinocytes, Th2 cells, ILC2s, Th22 cells, DCs, eosinophils, and mast cells, however, in particular patient groups also Th17, Th1 and cytotoxic T cells have influence (Czarnowicki et al., 2019; Malik et al., 2017). DCs are considered as one of the most important cell types, which have been shown after allergen challenge to increase the release of pro-inflammatory cytokines and chemokines to initiate Th2 immune responses in the skin of AD patients (Hammad et al., 2010; Novak, 2012). The Th2 cell type reactions involve ILC2s, eosinophils, mast cells, and itch-promoting cytokines like IL-31 and IL-4 (Werfel et al., 2016a). The Th2 type cytokines promote mast cell and eosinophil differentiation and antibody responses, including IgE isotype-switching (Gittler et al., 2012). The first events during initiation of AD may have different mechanistic routes. One scenario is depicted in figure 7.

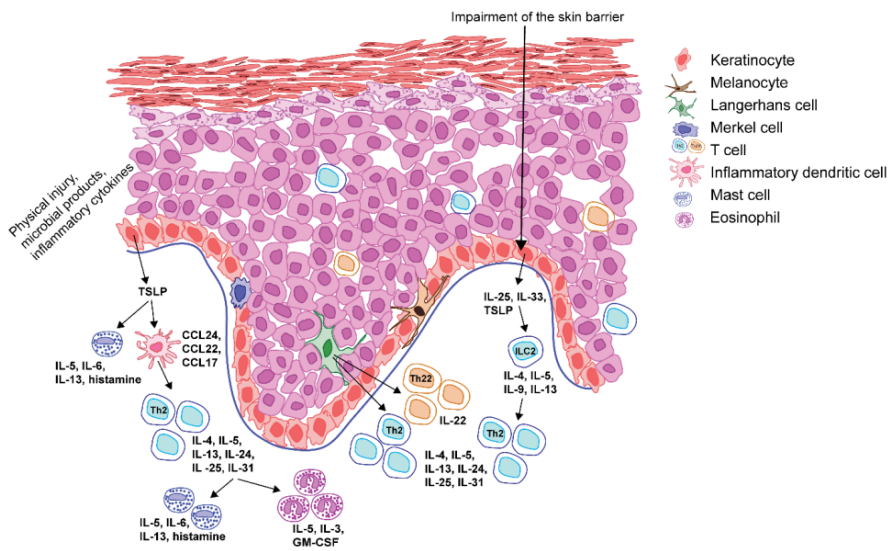

Figure 7. Pathophysiology of AD. As a result of physical injury, microbial products, or inflammatory cytokines, keratinocytes start to produce TSLP that activates inflammatory DCs that in turn induce the development of Th2 cells that express IL-4, IL-5, and IL-13. The Th2 type cytokines promote mast cell and eosinophil differentiation (Guttman-Yassky et al., 2011). Additionally, TSLP stimulates DCs to produce various inflammatory mediators including CCL24, CCL22, and CCL17 (Liu, 2007) and mast cells to produce IL-5, IL-6, IL-13 (Allakhverdi et al., 2007). Alternatively, the impairment of the skin barrier causes keratinocytes to produce IL-25, IL-33, and TSLP that in turn affect dermal group ILC2 cells to produce IL-5, IL-9, and IL-13 (Wang & Beck, 2016). This will activate the Th2 cells and the production of Th2 cytokines like IL-4, IL-13, and IL-33, which damage the skin barrier even further. Langerhans cells have been shown to induce both Th2 and Th22 cells (Fujita et al., 2009; Klechevsky et al., 2008).

It has been shown that IL-4 and IL-13 reduce FLG expression of human keratinocytes *in vitro* (Howell et al., 2009). Th2-associated cytokines also inhibit other barrier-related genes, such as loricrin and involucrin (Kim et al., 2008), the production of AMPs, like LL-37 and hBD2 (Clausen & Agner, 2016; D'Auria et al., 2016) leading to the increased infection of the skin of AD patients (Williams & Gallo, 2015). The effect of IL-9 is seen on T-cells, where it drives T-cell survival, proliferation, and secretion of inflammatory mediators (Clark & Schlapbach, 2017). Additionally, IL-9 affects also keratinocytes, where it induces the expression of vascular endothelial growth factor, which can be associated with epidermal changes (Ma et al., 2014).

Both Th22 and Tc22 cells have been shown to play an important role in the pathophysiology of AD, they produce IL-22 that is upregulated in lesional skin of chronic AD and the number of $CD8^+$ T cells producing IL-22 correlated with the disease activity score of AD (Nograles et al., 2009). Also, it has been shown that serum IL-22 levels positively correlated with serum levels of CCL17, a marker of AD severity (Hayashida et al., 2011). The chemokine CCL17 is considered to have a causative role in the accumulation of Th2 cells into lesional skin as its receptor CCR4 is mainly expressed on Th2 cells (Kakinuma et al., 2001). Studies with *in vitro* cultured human keratinocytes have shown that IL-22 is capable of downregulating the expression of FLG, loricrin, and involucrin in keratinocytes thereby contributing to the decreased barrier function as well (Boniface et al., 2005; Gutowska-Owsiak et al., 2011; Nograles et al., 2008). During the onset of the lesion Langerhans cells have been shown to also induce both Th2 and Th22/Tc22 cells (Fujita et al., 2009; Klechevsky et al., 2008).

Mast cells and eosinophils are both implicated in the development of AD and their numbers are increased in patients with AD (Kawakami et al., 2009; Simon et al., 2004). Mast cells contribute to the development of AD as they can express IL-22 (Mashiko et al., 2015) and might contribute to the IgE-mediated inflammation and eosinophil influx to tissues (Williams & Galli, 2000). Mast cells produce histamine and other inflammatory mediators that contribute to itching and inflammation in patients with AD (Kawakami et al., 2009). The role of histamine is mainly associated with erythema and edema in patients with AD (Kawakami et al., 2009). In the tissue, eosinophils can produce IL-4 and promote inflammation by the production of cationic granules, such as eosinophil peroxidase (Chu et al., 2014). The survival and mobilization of eosinophils are affected by the levels of Th2 cytokine IL-5, which is increased in the serum of patients with AD and also correlates with disease activity (Kondo et al., 2001).

2.2.2.2. Therapy

AD is a complex disease with many different features in its pathophysiology, therefore, the treatment of AD is relatively more complicated. In addition to conventional treatment of the disease, the patients are instructed to avoid factors that might irritate the skin, including environmental irritants and different

allergens as they may induce or exacerbate skin lesions (Plotz & Ring, 2010). The basic AD therapy includes treatment with topical agents that restore barrier function, like the use of different emollients, lipid-based barrier repair formulations. The endpoints are to hydrate the skin, prevent transepidermal water loss, and suppress pruritus (Fleischer & Boguniewicz, 2010). Also, agents that have anti-inflammatory effects and suppress the immune responses are used in the treatment. The use of topical corticosteroids and calcineurin inhibitors pimecrolimus and tacrolimus are the first-line therapy to treat areas of visible disease (Fleischer & Boguniewicz, 2010; Ruzicka et al., 1997). In addition, systemic immunosuppressive therapies, including systemic corticosteroids, cyclosporin A, and methotrexate are used to stop an acute exacerbation or in patients with aggressive AD (BuBmann et al., 2009; Darsow et al., 2010). To relieve the pruritus, antihistamines are used as an add-on therapy to other topical or systemic approaches (Sidbury & Hanifin, 2000). In line with the importance of Th2 responses in AD, the clinical efficacy of dupilumab has been demonstrated in adult and adolescent patients suffering moderate-to-severe AD (Beck et al., 2014; Renert-Yuval & Guttman-Yassky, 2020). Dupilumab is a human monoclonal antibody targeting the IL-4 receptor α (IL-4Rα) and thereby inhibits both IL-4 and IL-13 signaling. Dupilumab improves the symptoms of AD, pruritus, anxiety, and depression, and quality of life (Simpson et al., 2016).

Currently, several other Th2 type cytokine inhibitors are in the development for the treatment of AD. For instance, nemolizumab targeting IL-31 is in phase II clinical trials and has shown improvements in clinical scores, pruritus, and sleep loss (Kabashima et al., 2018). Also, etokimab anti–IL-33 agent is being tested for the treatment of AD in a phase IIa clinical study (Renert-Yuval & Guttman-Yassky, 2020). As also Th22 cells are implicated in the development of AD, the use of antibodies against IL-22 are being tested. For example, fezakinumab, the IL-22–blocking monoclonal antibody has shown significant clinical improvements in patients with severe AD in a phase IIa clinical study (Guttman-Yassky et al., 2018).

2.2.3. Comparison of PV and AD

When comparing molecular mechanisms of disease pathologies in the skin of AD and PV patients, the two diseases are somewhat similar. Both, AD and PV lesions have epidermal hyperplasia, alterations in the barrier function and keratinocyte differentiation, increased immune cell infiltration, and increased expression of proinflammatory cytokines (Guttman-Yassky & Krueger, 2017). As epidermal keratinocytes respond to T-cell derived cytokines and change their growth and differentiation, the activation of keratinocytes plays an important role in lesion development and phenotype in both diseases (Guttman-Yassky & Krueger, 2017). AD and PV both have also a strong immune cell component, especially in different types of T cells that play a role in the development of the diseases (Dainichi et al., 2018). When comparing the types of T cells involved, then Th17 cells appear to be dominant in PV (Kim & Krueger, 2017; Papp et

al., 2017b) while in the case of AD, Th2 component is always represented and plays the most important role in triggering the disease (Guttman-Yassky & Krueger, 2017). However, in the chronic phase of AD, the disease is also characterized by other types of cytokines (IFN-γ, IL-17A, IL-22, IL-23, and IL-31) that are not related to the Th2 lineage, and also increase in other Th1 related chemokines, such as CXCL9, CXCL10 and CXCL11 occur (Gittler et al., 2012; Rebane et al., 2012). Interestingly, in different populations, the types of T cells involved in the pathogenesis of AD may vary. For instance, in Asian AD in addition to Th2 activation, substantial activation of Th17 cells has been identified in blood and acute AD skin lesions (Koga et al., 2008) indicating that the Asian AD phenotype shares features of both European American AD and PV (Noda et al., 2015). Additionally, types of T cells involved in AD change with age. It has been shown that in infants the frequency of Th1 is significantly lower compared with older patients (Czarnowicki et al., 2020). There are also other shared immune cell subsets between the diseases. For example, Th9 cells produce IL-9 which is increased both in PV and AD and is known to induce the production of inflammatory cytokines from other T cell subsets (Kaplan et al., 2015; Schlapbach et al., 2014). Additionally, both diseases share a common Th22 component (Gittler et al., 2012; Res et al., 2010). Also, myeloid DC that secrete IFN-α play a role in lesion formation and also are the largest population of dermal DCs in both diseases (Guttman-Yassky et al., 2007; Novak et al., 2010). The understanding of molecular pathways in both PV and AD has led to the development of different treatment options for the diseases (Campanati et al., 2019; Simpson et al., 2016). Therefore, understanding of both PV and AD disease mechanisms might lead to future treatment options.

2.3. miRNA biogenesis and function

Most miRNAs have independent genes (Lagos-Quintana et al., 2001; Lee & Ambros, 2001), however, some of the miRNAs originate from introns (Aravin et al., 2003; Lagos-Quintana et al., 2003; Lai et al., 2003) or exons of protein-coding genes (Hsu et al., 2006). miRNA biogenesis is comprised of several steps and leads to about ~22 nt long single stranded miRNA loaded to the RISC complex, which has capacity to bind mRNA 3'UTR and to initiate the degradation to target mRNA or suppress the translation (Baek et al., 2008; Selbach et al., 2008) (Figure 8). miRNAs are considered as fine-tuners of gene expression, as the effect of one miRNA on a single gene can be quite modest (Baek et al., 2008). However, as one mRNA can be regulated by many different miRNAs (Bartel, 2009) and when various miRNAs act in a coordinated manner, a strong mRNA suppression can take place (Nam et al., 2014). As mentioned in the introduction, about 60% of the protein-coding genes are thought to be regulated by miRNAs (Friedman et al., 2009). Therefore, most biological processes are influenced by miRNAs, including cell proliferation, differentiation, and apoptosis (Shenoy & Blelloch, 2014). For example, in the hair follicle bulge

cells and basal keratinocytes, miR-125 overexpression increases the proliferation (Zhang et al., 2011). miRNAs also help to regulate T helper cell differentiation, for instance, miR-19a contributes to Th2 cell development and type 2 cytokine production (Simpson et al., 2014). As an interesting finding, in some cases, miRNAs can increase the expression of their target genes. For example, when the RNA induced silencing complex (RISC) complex binds to the target mRNA, it can replace repressive RNA binding proteins and therefore increase the expression. For example, miR-466l increases the expression of IL-10 in macrophages by outcompeting tristetraprolin (Brooks & Blackshear, 2013; Ma et al., 2010). As various processes are regulated by miRNAs, their dysregulation is associated with many human diseases, including inflammatory skin diseases.

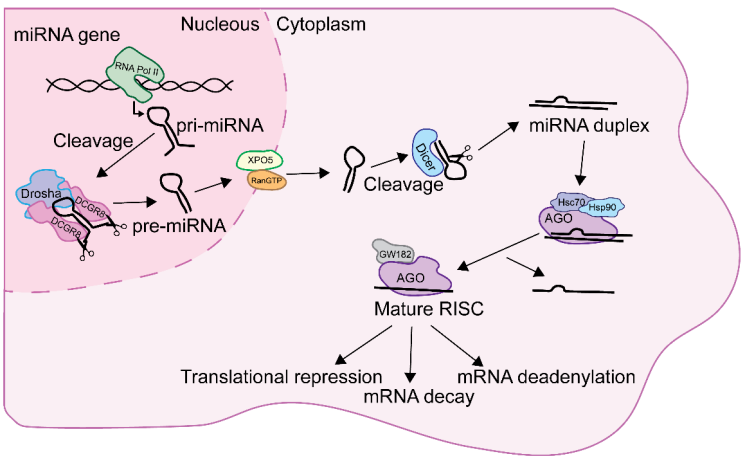

Figure 8: miRNA biogenesis and function. miRNA encoding genes are transcribed by RNA polymerase II as primary miRNA (pri-miRNA) transcript with the 5'end cap structure and poly(A) tail at 3' end (Lee et al., 2004). Next, the pri-miRNA is cleaved by RNase III enzyme Drosha in complex with two DiGeorge syndrome critical region gene 8 (DCGR8) (Denli et al., 2004; Han et al., 2004). As a result, approximately a 60–65 nt hairpin precursor miRNA (pre-miRNA) is formed (Lee et al., 2003), which then is transported to the cytoplasm by Exportin 5 (XPO5) in complex with a GTP-binding nuclear protein, RAS-related Nuclear protein GTP (RanGTP) (Bohnsack et al., 2004; Lund et al., 2004). In the cytoplasm, the hairpin is recognized by another RNase III enzyme, Dicer that cleaves off the loop structure resulting in approximately 22 nt long miRNA duplex (Bernstein et al., 2001; MacRae et al., 2007). For the miRNA to be functional, it needs to be loaded onto RISC containing the Argonaute (AGO) and glycine-tryptophan protein of 182 kDa (GW182) proteins. This is done by heat shock cognate protein 70 (Hsc70)/ heat shock protein 90 (Hsp90) chaperones (Iwasaki et al., 2010). Only one strand of the duplex is used in the RISC complex, while the other strand is separated by the N-domain of Ago protein (Diederichs & Haber, 2007; Hammond et al., 2000; Kwak & Tomari, 2012). Based on the 6–8 nt long seed sequence, which contains minimally nt 2–7 from 5' end of the miRNA (Bartel, 2009; Krol et al., 2010), the RISC complex binds to target mRNA 3′-UTR and causes translational repression, mRNA deadenylation and decay (Eichhorn et al., 2014; Huntzinger & Izaurralde, 2011). The figure is based on information from (Daugaard & Hansen, 2017).

Due to their altered expression pattern and capacity to modulate disease-related processes, the potential of miRNAs as biomarkers and therapeutic targets are intensively investigated. Accordingly, many studies show the dysregulation of miRNAs in various diseases, indicating their relevance as biomarkers (Neagu et al., 2020). Moreover, miRNAs can be found in body fluids, including urine and serum, where their levels reflect the expression of corresponding miRNA in blood cells or tissues. For example, in the case of PV, miR-223 and miR-143 levels in the peripheral blood corresponded to PASI, potentially helping to monitor treatment or disease progression (Lovendorf et al., 2014). However, although several biomarker platforms are in clinical trials, only a few commercial biomarker platform based miRNAs are available (Bonneau et al., 2019). Considering that up or downregulation of miRNAs is associated with disease pathogenesis, the modulation of expression miRNAs has been suggested to have therapeutic potential. Accordingly, miRNA mimics and inhibitors have reached clinical trials. As an example of some of the skin-related therapeutics that are in clinical trials is the miR-29b mimic (Remlarsen, MRG-201, miRagen Therapeutics) that is in phase I clinical trials for the treatment of scleroderma (Gallant-Behm et al., 2019) and the miR-155 inhibitor is in phase I in the treatment of cutaneous T cell lymphoma and mycosis fungoides (Cobomarsen, MRG-106, miRagen Therapeutics) (Seto et al., 2018). So far, none of the miRNA drug candidates have reached phase 3 trials, however, miR-122 repressor (Miravirsen, SPC3649, Roche) against Hepatitis C virus has completed phase 2 studies (Hanna et al., 2019).

2.4. miRNA functions in keratinocytes and chronic inflammatory skin diseases

The capacity of miRNAs to regulate different processes in the skin and their altered expression in various skin diseases have been demonstrated in numerous studies (Lovendorf et al., 2015; Singhvi et al., 2018). Accordingly, miRNAs have been shown to participate in the regulation of many physiological processes in the skin in health and diseases, including barrier function, pigmentation, aging, wound healing, and inflammation (Botchkareva, 2017; Hawkes et al., 2016). As maintaining the balance between keratinocyte proliferation, migration and differentiation are essential to maintain the structure and function of the epidermis, the large number of studies exploring miRNA functions in the skin are focused on keratinocytes (Landen et al., 2016) (Figure 9).

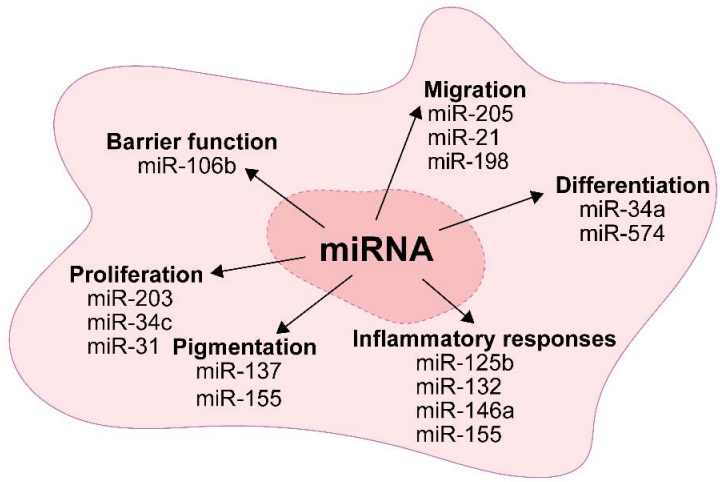

Figure 9. miRNA functions in keratinocytes. miRNAs can suppress or promote keratinocyte proliferation, migration, differentiation. Additionally, they regulate inflammatory responses and the barrier function of keratinocytes. Based on information from (Botchkareva, 2017; Hawkes et al., 2016; Landen et al., 2016).

2.4.1. miRNAs role in keratinocyte proliferation in association with PV and AD

An increasing number of miRNAs have been shown to regulate keratinocyte proliferation and may affect the development of skin diseases. For example, the expression of miR-203, miR-205, miR-31, miR-483-3p, and miR-210 is dysregulated in various skin diseases and they all have been shown to modulate keratinocyte proliferation through various targets important for the cell cycle progression (Singhvi et al., 2018).

One of the best-characterized miRNAs controlling keratinocyte proliferation is miR-203 that is also regarded as one of the central miRNAs in the skin. miR-203 expression is considered skin-specific, as its expression is more than 100-fold higher in the skin compared with other organs (Sonkoly et al., 2007). miR-203 is expressed in the suprabasal layer of the epidermis and is transcriptionally activated in the epithelial stem cell progeny upon their asymmetric cell division to basal and suprabasal cells (Jackson et al., 2013). There, miR-203 functions in the maintenance of basal layer keratinocyte proliferation as it inhibits the expression of the transcription factor p63, which is needed for the initiation of epithelial stratification (Koster et al., 2004; Lena et al., 2008). miR-203 also inhibits other genes important for the control of cell cycle, including S-phase kinase-associated protein 2 (Skp2), a cell cycle regulator, and Musashi RNA binding protein 2 (Msi2), an RNA-binding protein (Jackson et al., 2013). The expression of miR-203 is upregulated in hyper-proliferative keratinocyte

pathologies, including PV and chronic venous ulcers (Pastar et al., 2012; Sonkoly et al., 2007).

miRNAs regulate keratinocyte proliferation also under inflammatory conditions. For example, miR-31 transcription is NF-κB dependent and its expression in the cells is upregulated in response to IL-1α, IL-6, IL-17A, TNF-α, IFN-γ, or IL-22 (Yan et al., 2015). The target of miR-31 in keratinocytes is protein phosphatase 6 (PPP6C), an inhibitor of the G1–S phase transition in the cell cycle. When the expression of miR-31 is enhanced, this will lead to miR-31 mediated increase in basal keratinocyte proliferation leading to epidermal hyperplasia. It has been shown that in PV lesional skin, miR-31 expression is elevated and ppp6c expression is downregulated (Yan et al., 2015). In contrast to miR-31, miR-20a-3p has been shown to suppress the proliferation and induced apoptosis of HaCaT cells. Accordingly, in the psoriatic lesions, miR-20a-3p is downregulated and thereby it can contribute to hyperproliferation and aberrant apoptosis of keratinocytes. It has been also shown that IL-22, a cytokine known to play a role in both AD and PV pathogenesis can downregulate miR-20a-3p in HaCaT cells that are immortalized human keratinocyte cell line from adult human skin (Li et al., 2018). These results allow to conclude that the inflammatory environment can affect the miRNA levels and this may lead to the regulation of the proliferation of keratinocytes and contribute to the pathogenesis of skin diseases like AD and PV.

Besides proliferation, different miRNAs are indicated as positive or negative regulators for keratinocyte differentiation. For example, let-7b has been shown to promote differentiation of keratinocytes by targeting IL-6 in *in vitro* and *in vivo* studies (Wu et al., 2018). Interestingly, a recent study identified decreased let-7b-5p levels in circulating extracellular vesicles as a potential biomarker of psoriatic arthritis in patients with PV (Pasquali et al., 2020). Another miRNA, miR-23b-3p, which is expressed during the late step of human keratinocyte differentiation, was demonstrated to inhibit keratinocyte differentiation by targeting the SMAD transcriptional corepressor TGF-β induced factor homeobox 1 (TGIF1) and trough this to activate TGF-β signaling (Barbollat-Boutrand et al., 2017). One more miRNA, miR-143 has been associated with regulating the barrier function of the skin. miR-143 is downregulated in the lesional skin of AD patients compared to healthy controls (Sonkoly et al., 2010). In human epidermal keratinocytes, miR-143 targets IL-13Rα1, thereby regulating the effect of IL-13 on epidermal barrier function. As overexpression of miR-143 blocked the IL-13-induced downregulation of FLG, loricrin, and involucrin in keratinocytes (Zeng et al., 2016), indicating that this miRNA may have potential in the development of a therapeutic approach for AD.

2.4.2. miRNAs in the regulation of inflammatory responses of keratinocytes and association with PV and AD

As keratinocytes play a role in regulating immune responses and transmitting signals from the environment to the immune cells, the signals from keratinocytes must be tightly regulated. The first data linking miRNAs and inflammation came from a study in the human monocytic cell line THP-1. When these cells were stimulated with lipopolysaccharides (LPS), the upregulation of miR-146a/b, miR-132, and miR-155 was detected indicating that they may be involved in the regulation of inflammatory responses (Taganov et al., 2006). The first study concerning miRNAs in the skin inflammation was published by Sonkoly *et al.* in 2007, and it showed that miRNAs are dysregulated in the skin of PV and AD patients (Figure 10).

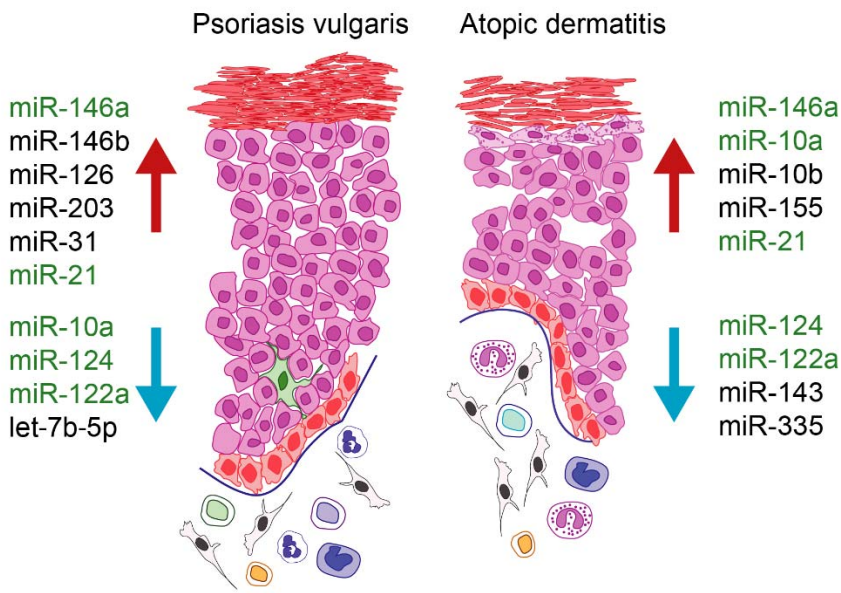

Figure 10. Examples of miRNAs dysregulated in the skin of PV and AD patients. Both diseases have specific miRNA expression profiles in the skin where various miRNAs are either upregulated or downregulated. Upregulation is indicated by a red and downregulation by a blue arrow. miRNAs changed in both AD and PV are marked in green. The figure is based on information from previously published results (Joyce et al., 2011; Rebane et al., 2014; Sonkoly et al., 2007).

Several dysregulated miRNAs have been shown to contribute to the pathogenesis of AD. One of the miRNAs downregulated in the chronic lesional skin of AD patients is miR-124. miR-124 itself downregulates cytokines IL-8, CCL5, and CCL8 in keratinocytes by inhibition of the expression of p65

through the direct binding to the 3′UTR of RELA proto-oncogene. Therefore, the reduced expression of miR-124 in the skin of AD patients could be responsible for the increased expression of the mentioned chemokines in the skin of AD patients (Yang et al., 2017). One of the miRNAs shown to be upregulated in AD is miR-155. In the skin, it was shown that miR-155 is mostly expressed in the infiltrating immune cells and cytotoxic T lymphocyte-associated antigen (CTLA-4) was identified as a direct target of miR-155. CTLA-4 is known as a negative regulator of T-cell activation therefore miR-155 decreases CTLA-4 expression and regulates the proliferation of Th cells (Sonkoly et al., 2010). Another direct target of miR-155 is protein kinase inhibitor α (PKIα): inhibition of miR-155 resulted in the increased expression of PKIα, tight junction proteins occludin and claudin-16, and decreased expression of TSLP HaCaT cells as well as in a mouse model of AD (Wang et al., 2019). Consequently, miR-155 overexpression could contribute to the pathogenesis of AD.

The cytokines involved in the pathogenesis of AD also regulate the miRNA expression in the skin. For example, IL-4 can regulate the expression of miRNAs involved in inflammation, angiogenesis, and apoptosis. When keratinocytes were stimulated with IL-4, the upregulation of 26 (for example miR-122-5p, miR-204-5p, miR-146b-5p, miR-335-3p, miR-34b-3p) and downregulation of one miRNA miR-147a (Bao et al., 2018) was found. The downregulated miR-147a function in keratinocytes has not been studied. However, its expression is also downregulated in non-small-cell lung cancer where it inhibited the growth and metastasis of cells from the non-small-cell lung cancer cell lines H1650 or H1299 by targeting CCL5 (D'Adamio et al., 2019). Therefore, the decreased levels of miR-147a in AD could be contributing to the increased levels of CCL5 in the skin of AD patients. Recently, IL-32 isotype IL-32γ was shown to have an inhibitory effect on the development of AD in AD mouse models by inhibition of miR-205. The inhibition of miR-205 decreased the expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and TSLP in TNF-α/lFN-γ-treated HaCaT cells through the inactivation of the NF-κB pathway (Lee et al., 2020).

miRNAs with altered expression also contribute to the pathogenesis of PV. Among them, miR-31 as one of the most highly overexpressed miRNAs in PV skin was shown to target serine/threonine kinase 40 (STK40), a negative regulator of NF-κB signaling. Accordingly, the inhibition of miR-31 in human primary keratinocytes caused the decreased expression of IL-1β, CXCL1, CXCL5, and IL-8 and suppressed the ability of keratinocytes to activate endothelial cells and attract leukocytes. Therefore, increased levels of miR-31 in the psoriatic skin could increase the inflammation by regulating the production of cytokines and leukocyte chemotaxis to the skin (Xu et al., 2013). Another highly overexpressed miRNA in psoriatic skin is miR-203. Cytokines TNF-α and IL-24 were identified as direct targets of miR-203, indicating that besides regulation of keratinocyte proliferation, miR-203 also controls cytokine signaling by repressing key pro-inflammatory cytokines (Primo et al., 2012). The expression of miR-203 was also shown to be upregulated by IL-17 in the ears of mice and

HaCaT cells (Xu et al., 2017). miR-126 expression is also upregulated in the lesional skin of PV patients and its expression positively correlated with PASI. The overexpression of miR-126 increased TNF-α, IFN-γ, IL-17A, and IL-22 expression, while decreased IL-10 expression in HaCaT cells. Also, miR-126 increased proliferation and decreased the apoptosis rate, hence, contributing to the pathogenesis of PV (Feng et al., 2018). Together the studies on miRNA functions in cell cultures and skin indicate that restoring the skin's normal miRNA profile could be one of the therapeutic options in AD and PV.

2.5. miR-146 family

The miR-146 family has two members, miR-146a-5p and miR-146b-5p (miR-146a/b) that are transcribed by two different genes, *MIR146A* and *MIR146B*. They are similar in the sequence differing only by two nucleotides in the 3′ part of the mature strand outside the seed region. Therefore, miR-146a/b are predicted to target the same set of genes. In the human genome, the *MIR146A* gene is located within a larger long noncoding RNA host gene, *MIR3142HG* (chromosome 5q33.3), while the *MIR146B* gene is located in an intergenic region of human chromosome 10 (10q24.32) (Taganov et al., 2006). Accordingly, their expression is regulated differently in cells. Both *MIR146A* and *MIR146B* are reported to be controlled by NF-κB and CCAAT-enhancer-binding-protein-β (C/EBPβ). However, it should be noted that while miR-146a expression is repeatedly shown to be strongly upregulated in response to NF-κB activation (Meisgen et al., 2014; Taganov et al., 2006), this seems to be not the case for miR-146b, despite its distant promoter region contains a binding site for NF-κB2 (Taganov et al., 2006). In addition, *MIR146A* is regulated by transcription factors C-MYC and interferon regulatory factor 3/7 (IRF3/7) while *MIR146B* is regulated by transcription factors Proto-Oncogene C-Fos (c-Fos) and STAT3/6 (Paterson & Kriegel, 2017). Based on miRBase data, the mature -5p strand of both miR-146a and miR-146b is regarded as the bioactive "guide strand" and the mature -3p strand as the "passenger strand" (Paterson & Kriegel, 2017).

As mentioned previously, the role of miR-146a/b in the regulation of immune responses was first shown in human monocytes, where miR-146a expression increased in response to a variety of microbial components and pro-inflammatory cytokines, like TNF-α and IL-1β. In this study, it was also shown that miR-146a/b directly represses the NF-κB signaling cascade members IRAK1 and TRAF6 and thereby participate in a negative feedback regulation of TLR and cytokine receptor signaling (Taganov et al., 2006). In Treg cells, the expression of miR-146a is needed for their suppressor function as it directly targets Stat1. As a result, miR-146a prevents the activation of Treg cells and their turning into IFNγ-producing Th1-like cells (Lu et al., 2010). In CD4 and CD8 T cells, the lack of miR-146a led to hyperresponsiveness after TCR stimulation, evident by higher proliferation, prolonged survival, exaggerated

activation phenotype, and increased effector cytokine production. These changes are due to altered NF-κB activity and changed expression of NF-κB responsive genes (Yang et al., 2012). Another study has demonstrated that mice lacking miR-146a develop several immune-related phenotypes around the age of 6–8 months characterized by splenomegaly, lymphadenopathy, and multi-organ inflammation and this correlates with the loss of peripheral T cell tolerance (Boldin et al., 2011). Additionally, miR-146a can act as a tumor suppressor in the context of the immune system, as mice lacking miR-146a have excessive production of myeloid cells and develop tumors in their secondary lymphoid organs (Boldin et al., 2011). Therefore, altering miR-146a expression might be a viable treatment option for myeloproliferative and inflammatory disorders. Recently it was shown that a miR-146a conjugated to a scavenger receptor/Toll-like receptor 9 agonist is a myeloid cell-selective NF-κB inhibitor able to target myeloid cells and leukemic cells leading to reduced expression of miR-146a targets IRAK1 and TRAF6 (Su et al., 2020).

The function of miR-146b has been mostly studied in different types of cancer. Interestingly, miR-146b can act as both an oncogene and a tumor suppressor depending on the type of cancer. The downregulation of miR-146b is associated with tumorigenesis and poor outcome in several types of cancer, including gliomas, B-cell lymphomas, breast cancer and hepatocellular carcinoma (Li et al., 2017; Liu et al., 2015; Wu et al., 2014; Xiang et al., 2014). The downregulation of miR-146b in breast cancer was associated with NF-κB-dependent production of IL-6 and subsequent STAT3 activation leading to increased migration and invasion of breast cancer cells (Xiang et al., 2014). In gliomas, there is an inverse correlation between miR-146b expression and the elevation of glioma grades and Ki-67 index and a positive correlation with patients' survival. In glioblastoma cell lines, the overexpression of miR-146b significantly reduced proliferation and increased apoptosis (Liu et al., 2015). However, in the case of thyroid cancer, miR-146b was shown to be one of the most upregulated miRNAs, which plays a role in cancer progression and development (Chou et al., 2017; Ramirez-Moya et al., 2018). The overexpression of miR-146b in thyroid epithelial cell lines led to the hyperactivation of phosphoinositide-3-kinase–protein kinase (PI3K)/protein kinase B (AKT) signaling, increased proliferation, migration, and invasion, as well as decreased apoptosis leading to a more aggressive tumoral phenotype (Ramirez-Moya et al., 2018).

Additionally, miR-146b does participate in the regulation of the immune system and immune responses. In human naïve thymic-derived Tregs, miR-146b is highly expressed compared with naive CD4 T cells. Inhibition of miR-146b led to enhanced TRAF6 expression and increased NF-κB activation causing a reduction in forkhead box P3 (Foxp3) and increased expression of anti-apoptotic genes associated with enhanced cell survival, proliferation, and suppressive function of Treg cells (Lu et al., 2016). It has been also shown that human immunodeficiency virus-1 (HIV-1) infection leads to the upregulation of miR-146b in CD4 T cells and CD4 T cell-derived extracellular vesicles,

whereas T cell-derived extracellular vesicles overexpressing miR-146b were able to transfer miR-146b mimics into endothelial cells and reduce endothelial inflammatory responses *in vitro* and *in vivo* (Balducci et al., 2019).

Despite differences in the expression regulation, some of the functions miR-146a/b have been shown to be overlapping. For example, both miR-146a/b play a role in humoral immunity by regulating germinal center reaction. More precisely, miR-146a controls germinal center responses in B cells by targeting multiple CD40 signaling pathway components. Interestingly, loss of only miR-146a from T cells does not alter humoral responses, however, the deletion of both miR-146a/b in T cells was required for the increase in follicular Th cell numbers and enhanced germinal center reactions (Utaijaratrasmi et al., 2018). Additionally, miR-146a/b both regulate DC apoptosis and cytokine production. Overexpression of miR-146a/b increased DC apoptosis and reduced cytokine production of IL-12p70, IL-6, and TNF-α. In the same study, it was also shown that the decreased levels of miR-146a/b can enhance the ability of DCs to activate NK cells producing IFN-γ (Park et al., 2015).

2.5.1. miR-146 family function in the skin

The increased expression of miR-146a/b in both PV and AD lesional skin was first shown by microarray analysis already several years ago (Sonkoly et al., 2007), however, the functions of miR-146a/b in keratinocytes and skin were started to be uncovered more recently. An initial study in human primary keratinocytes showed that miR-146a expression was upregulated in response to TLR2 stimulation in NF-κB and mitogen-activated protein kinase-dependent manner. The overexpression of miR-146a suppressed the production of IL-8, CCL20, and TNF-α functionally leading to the decreased chemotactic attraction of neutrophils by keratinocytes (Meisgen et al., 2014). Simultaneously to the study of Meisgen et al. (2014), it was shown that the expression of miR-146a can be induced in keratinocytes in response to heat-killed *S. aureus*, IL-13, IL-1β, TNF-α, and IL-17A (Rebane et al., 2014). Additionally, overexpression of miR-146a in keratinocytes revealed that miR-146a downregulated genes from NF-κB signaling, chemokine signaling, and cytokine-cytokine receptor interaction pathways. Moreover, in the MC903-dependent mouse model of AD, mice lacking miR-146a developed a stronger inflammation compared with WT mice, characterized by increased accumulation of infiltrating cells in the dermis, elevated expression of IFN-γ, CCL5, CCL8, and Ubiquitin D in the skin of miR-146a$^{-/-}$ mice (Rebane et al., 2014). Although mice lacking miR-146a produced much less IgE, in the serum of patients with AD, there was detected a negative relationship of miR-146a levels with serum IgE in allergic AD patients (Carreras-Badosa et al., 2018). This indicates, that miR-146a is needed for the production of IgE, however, when miR-146a is increased, it may act in the suppression of inflammation.

Similarly to AD, the function of miR-146a has also been studied in PV. It has been shown that miR-146a is upregulated in both lesional skin and peripheral blood mononuclear cells (PBMCs) of PV patients and the expression of miR-146a positively correlated with IL-17 levels in the lesions and peripheral blood (Xia et al., 2012). Another study demonstrated that a G-to-C polymorphism (rs2910164) in the miR-146a precursor leading to changes in the levels of miR-146a is associated with psoriasis in the Chinese Han population. More particularly, it was shown that miR-146a CG and GG genotypes significantly increased the risk of PV (Zhang et al., 2014). The same single-nucleotide polymorphism (SNP) was also studied in the Swedish PV cohort, in which PV patients with the CC genotype among HLA-C*06–negative patients were protected from PV compared with those with GC or GG genotypes (Srivastava et al., 2017). In the imiquimod-induced mouse model of PV, miR-146a$^{-/-}$ mice had an earlier onset and more severe skin inflammation with increased expression of S100a7, S100a8 and S100a9, Krt16, Il1b, and Cxcr2, epidermal hyperproliferation, and increased neutrophil infiltration (Srivastava et al., 2017). Recent clinical data support the significant role of miR-146a in PV, where after 12 weeks of treatment with adalimumab, a significant downregulation of miR-146a was observed in PBMCs. Additionally, miR-146a levels in PBMCs were inversely correlated with the PASI reduction induced by adalimumab treatment (Mensa et al., 2018). Concordant its anti-inflammatory function in AD and PV, it was also shown that subcutaneous pre-administration of miR-146a alleviated the ear-swelling and reduced the expression of pro-inflammatory cytokines in a mouse model of irritant contact dermatitis, showing that miR-146a could have a therapeutic effect in inflammatory skin conditions (Urgard et al., 2016).

The function of miR-146b has not been studied as in detail in the skin as the function of miR-146a. One of the few studies showing the role of miR-146b in skin demonstrated that miR-146b directly targets the atypical chemokine receptor (ACKR2) that functions as a high-capacity scavenger of proinflammatory CC-chemokines in the skin. As miR-146a/b have the same seed sequence, also miR-146a was able to reduce the expression of ACKR2 (Shams et al., 2018). It has been shown that miR-146b is one of the miRNAs that is upregulated in systemic sclerosis skin tissues and fibroblasts and additionally in normal skin fibroblasts and endothelial cells that were stimulated with serum from systemic sclerosis patients (Zhou et al., 2017). Considering the importance of miR-146a in the suppression inflammation, further studies are still needed to delineate novel miR-146a/b targets and to clarify the role of the miR-146 family in association with particular skin diseases.

2.6. miR-10 family

The miR-10 family contains two members miR-10a (miR-10-5p) and miR-10b. miR-10a is encoded upstream of *Homeobox B4* (*HOXB4)* within the homeobox clusters of developmental regulators on chromosome 17 (Lagos-Quintana et al., 2003; Tehler et al., 2011). miR-10b is encoded by a region, which is located near the HOXD cluster on chromosome 2 (Ma et al., 2007). In human embryonic kidney (HEK) 293 cells, the NF-κB transcription complex subunit p65 is the direct regulator of miR-10a expression as it can directly bind the miR-10a promoter in response to retinoic acid (Huang et al., 2010). The expression of miR-10b is induced by Twist-related protein 1 (TWIST1), which binds directly to the putative promoter of *MIRN10B* (Ma et al., 2007). miR-10a and miR-10b differ by only a single nucleotide in the mature sequence that is outside the seed sequence and are predicted to share common targets (Tehler et al., 2011).

It has been shown that miR-10a can regulate the differentiation of cells and developmental processes. miR-10a can regulate *HOX* genes by targeting various HOX transcripts including HOXA1 and HOXA3 (Garzon et al., 2006; Han et al., 2007). Retinoic acid induced miR-10a has been shown to regulate smooth muscle cell differentiation from embryonic stem cells (Huang et al., 2010). Accordingly, the function of miR-10a has been studied most in different kinds of cancers. For example, miR-10a can regulate the migration and invasion of hepatoma (Yan et al., 2013). In esophageal squamous cell carcinoma, miR-10a expression was reduced compared with control tissues while the overexpression of miR-10a in esophageal squamous cell carcinoma cell lines, EC109 and TE-3, decreased cell proliferation, and enhanced apoptosis. The effects of miR-10a on cell proliferation were mediated by targeting T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1) and indicate miR-10a as a tumor suppressor (Liu et al., 2018).

Additionally, there are studies showing the role of miR-10a in the regulation of immune responses and inflammation. For example, in arterial endothelial cells, miR-10a was shown to target the IκB/NF-κB regulators mitogen-activated protein kinase kinase knase 7 (MAP3K7) and β-transducing repeat-containing gene (βTRC) thereby regulating the activation of the NF-κB pathway (Fang et al., 2010). Another study demonstrates that miR-10a can influence the differentiation of CD4+ T cell subsets. Naturally occurring Treg cells that arise in the thymus have high miR-10a expression, which was shown to be induced by retinoic acid and TGF-β. The targets of miR-10a in T-cells were transcriptional repressor Bcl-6 and the corepressor Ncor2, and by that, miR-10a inhibited the conversion of inducible Treg cells into follicular helper T cells. In the same study, miR-10a was shown to repress the differentiation of the Th17 subset indicating that miR-10a has a role in the regulation of the plasticity of T helper cells (Takahashi et al., 2012). In inflammatory bowel disease patients, miR-10a expression was decreased in the inflamed mucosa compared with healthy controls. *In vitro* DC cell cultures, TNF and IFN-γ decreased and TGF-β increased miR-10a and IL-12/IL-23p40 and nucleotide-binding oligomerization domain-

containing protein 2 were identified as direct targets, indicating that miR-10a regulates inflammatory responses in DC (Wu et al., 2015).

2.6.1. miR-10 family function in the skin

The function of miR-10b has been studied in the context of various skin cancers and inflammatory diseases. It has been shown that the increased expression of miR-10b is associated with poor prognosis in melanoma (Bai et al., 2017). Additionally, miR-10b was found to be upregulated in aggressive squamous cell carcinomas. More particularly, miR-10b was associated with pro-metastatic function as it supported cancer stem cell-like properties, like increased adhesion, spheroid formation capacities, and associated cellular outgrowth of immortalized keratinocytes (Wimmer et al., 2020). In the case of ankylosing spondylitis, a common inflammatory rheumatic disease, Th17 cells upregulated miR-10b in response to IL-6 and TNF-α. On the other hand, miR-10b regulated the cytokine production of Th17 cells and suppressed IL-17A production, at least in part through direct inhibition of MAP3K7 expression (Chen et al., 2017).

The role of miR-10a in the skin is not well known. There are a few sequencing and array datasets that show the downregulation of miR-10a in PV compared with healthy skin (Joyce et al., 2011; Sonkoly et al., 2007) or upregulation in AD (Rebane et al., 2014). It should be noted, miR-10b expression in the keratinocytes is significantly lower than that of miR-10a (Rebane et al., 2014). From functional studies, it was shown that miR-10a expression is downregulated during wound healing and that this downregulation is inhibited in diabetic wounds as compared to normal skin wounds. Therefore, the downregulation of miR-10a was suggested to support the healing of skin wounds (Madhyastha et al., 2012). In line with this, another recent study identified miR-10a as one of the highest expressed miRNAs in the mouse skin, which was downregulated during wound healing (Simoes et al., 2019). Additionally, the miR-10 family was the most abundant among those downregulated in psoriatic keratinocytes as compared to keratinocytes separated from control skin biopsies (Srivastava et al., 2019). However, it should be noted that none of these studies showed the function of miR-10a during wound healing nor how miR-10a expression is regulated during this process.

2.7. Summary of the literature

Keratinocytes in the epidermis are an essential part of the skin immune regulatory network. They participate in the regulation of both, the innate and adaptive immune responses by transmitting signals from the environment to the immune cells by producing pro-inflammatory chemokines and cytokines as well as anti-inflammatory ligands (Nestle et al., 2009). In the case of chronic inflammatory

skin disease, pro-inflammatory signaling pathways are constitutively activated in the keratinocytes. These include the NF-κB signaling pathway, which activation is needed for many genes involved in cell adhesion, differentiation, growth, apoptosis, and acute-phase inflammatory responses (Kunsch & Rosen, 1993; Mori & Prager, 1996; Pahl, 1999). Another commonly activated pathway in inflammation is the JAK/STAT pathway, which is used by over 60 cytokines and growth factors (Howell et al., 2019). As a result of activation of these and other signaling pathways, miRNAs like miR-146a/b and miR-10a are expressed in keratinocytes. The functional role of miRNAs is to regulate gene expression at the post-transcriptional level (Bartel, 2004; Lau et al., 2001; Lee et al., 1993). Interestingly, miR-146a/b and miR-10a had been both shown to suppress the NF-κB signaling pathway activation. miR-10a was previously shown to target IκB/NF-κB regulators MAP3K7 or βTRC in endothelial cells (Fang et al., 2010). miR-146a/b was demonstrated to target multiple members of the NF-κB signaling pathway including IRAK1, TRAF6, and CARD10 (Rebane et al., 2014; Taganov et al., 2006). Both these miRNAs were detected to be upregulated in keratinocytes from AD patients as compared to controls as well as the anti-inflammatory function of miR-146a in AD had been demonstrated (Rebane et al., 2014).

Despite these former publications, several questions have remained to be elucidated. First, the previous study describing the anti-inflammatory function of miR-146a in AD by targeting the NF-κB suggested that miR-146a effect is more general, and therefore it may also have a function in PV (Rebane et al., 2014). Moreover, a large number of downregulated genes detected from array analysis suggested that besides the capacity to suppress the inflammation, miR-146a may have additional functions in keratinocytes (Rebane et al., 2014). Second, at least two previous studies had shown the increased expression of miR-146b besides miR-146a in the skin of PV patients (Lovendorf et al., 2015; Sonkoly et al., 2007), however, the functional impact of miR-146b in keratinocytes was not studied before. As miR-146a/b differ only by two nucleotides on the 3′-end of the mature strand and they were predicted to target the same set of genes, we could hypothesize that more robust repression of the NF-κB signaling pathway may occur when both these miRNAs are present. Thus, we could not exclude that miR-146 family members can have individual and/or overlapping functions, thereby adding another layer of complexity needed to be explored. As besides miR-146a/b, other miRNAs were detected to be differentially expressed in keratinocytes from AD patients in our previous array analysis (Rebane et al., 2014), we proposed that other miRNAs also may influence the disease development. As among them, miR-10a was previously shown to target MAP3K7 in endothelial cells (Fang et al., 2010) and regulate cell proliferation and differentiation (Garzon et al., 2006; Liu et al., 2018), we choose it for functional studies in keratinocytes to explore the relationship with AD.

3. AIMS OF THE STUDY

The overall aim of this study was to characterize the role of miR-146a/b and miR-10a in keratinocytes and how they contribute to the development of PV and AD, respectively.

The specific aims were:

– To characterize miR-146a/b expression and relationship with the target genes in the skin of PV patients and role in the regulation of keratinocyte proliferation and activation.
– To investigate the expression, regulation, and function of putative miR-146a/b target serpin peptidase inhibitor B2 (SERPINB2) in keratinocytes in association with PV.
– To describe the localization, regulation, and function of miR-10a in keratinocytes and AD.

4. MATERIALS AND METHODS

4.1. Patients (Study I, II and III)

Skin samples from patients with PV (Diagnosis Code L40. 0) were collected from the Dermatology Clinic of Tartu University Hospital and University Hospital Kiel (Germany). Skin samples from patients with AD (Diagnosis Codes L20.0, L20.8, L20.9) or healthy controls were collected only from the Dermatology Clinic of Tartu University Hospital. All participants provided written informed consent and the study was approved by the respective institutions' ethics committees (permit numbers 219/T-28 and 267/T-8) and performed according to the Declaration of Helsinki Principles. All the participants were unrelated Caucasians living either in Estonia or Germany. Control subjects were recruited from among health care personnel, medical students, and patients who turned to the dermatological outpatient clinic for surgical excision of nevi. None of the control subjects had any history of chronic inflammatory skin disease or a family history of psoriasis or AD. From each patient with PV or AD, two punch biopsy skin samples (3–4 mm in diameter) were taken, one from the marginal zone of lesional skin and another from non-sun-exposed non-lesional skin. From each control subject, one punch biopsy skin sample (3–4 mm in diameter) from non-sun-exposed skin was taken. The skin samples were immediately frozen and stored at -80˚C until RNA extraction. For *in situ* hybridization and immunofluorescence, skin biopsy specimens were embedded into the Tissue-Tek (Thermo Fisher Scientific, Waltham, MA, USA) before freezing.

In study I and II, the same 22 PV patients and 22 controls were used for RT-qPCR and correlation analyses. In study I, an additional four PV patient samples and three control samples were used for *in situ* hybridization and immunofluorescence. In study II, an additional 1 PV patients and 1 control subjects were used for immunofluorescence analysis. The PASI score of the PV patients ranged from 7 to 33. The patients were 9 women and 18 men aged between 21 to 65 years. The control individuals were 11 women and 15 men aged between 20 to 76 years. For study III, 10 AD patients were included in the study (5 women and 5 men, age between 22 to 58 years), and 15 control samples were used that were gender- and age-matched (7 men, 8 women, age 20–59 years). Elevated IgE or eosinophilia was detected for 9 out of 10 patients. One patient with AD was diagnosed with bronchial asthma.

4.2. Cell culture

4.2.1. Primary Human Epidermal Keratinocytes (Study I, II and III)

Pooled primary human keratinocytes from healthy adults (PromoCell, Heidelberg, Germany) were used in the experiments. To passage the keratinocytes, the cells were detached with trypsin (Thermo Fisher Scientific) at >80% confluency and re-seeded at 20–30% confluency. Trypsin neutralizing solution (Lonza, Basel, Switzerland) was used to neutralize the trypsin solution. The keratinocytes were used at passages between 5 to 8. Monolayer keratinocyte cultures were grown in Keratinocyte-SFM media with supplements, human recombinant Epidermal Growth Factor (5 ng/ml) and Bovine Pituitary Extract (50 μg/ml) (Thermo Fisher Scientific) and with Penicillin and Streptomycin mixture (0.5 U/ml) (Lonza, Basel, Switzerland). To grow the 3D keratinocyte culture in air-liquid interface 5×10^5 cells were seeded on ThinCert Cell Culture Inserts (Greiner Bio-One, Kremsmünster, Austria) (0,4 μm pore, 0.33 cm^2) on 24-well plates using Keratinocyte-SFM medium with supplements and Dulbecco's Modified Eagle Medium (both from Thermo Fisher Scientific) containing High Glucose, GlutaMAX™, and Pyruvate in 1:1 ratio. To observe the growth and monitor the quality of the 3D culture transepithelial resistance was measured every 24 hours using Millicell ERS-2 Voltohmmeter (Merk Millipore, Darmstadt, Germany). All the cells were grown at 37°C in a humidified incubator with 5% CO2 in the air. For monolayer keratinocyte differentiation experiment, the cells were grown in the presence of 1.8 mM calcium chloride.

4.2.2. Human primary fibroblasts (Study I)

To culture human primary fibroblasts, a piece of dermis was used, and the fibroblasts were isolated by the migration method. The piece of dermis was cut into 4×4 mm pieces and attached onto a culture dish, covered with Dulbecco's Modified of Eagle's Medium (DMEM) (Merck, Darmstadt, Germany) and supplemented with 10% fetal bovine serum (Merck) and with Penicillin and Streptomycin mixture (0.5 U/ml) (Lonza, Basel, Switzerland). To passage the fibroblasts, the cells were taken up with trypsin (Thermo Fisher Scientific) at >80% confluency and re-seeded at 20–30% confluency. The fibroblasts were grown at low passage numbers (2–3). The cells were grown at 37°C in a humidified incubator with 5% CO2 in the air.

4.2.3. Human peripheral blood mononuclear cells (Study I)

The PBMCs were isolated from freshly collected blood obtained from Blood Centre of Tartu University Hospital. PBMCs were prepared by density gradient centrifugation on Ficoll-PaqueTM Plus (GE Healthcare, Chicago, IL, USA).

4.3. Transfection and stimulation of cells (Study I, II and III)

Transfection with PepFect14 was used in all three studies. For the transfection, 2×10^4 cells were seeded per each well of 12-well plate or 5×10^3 cells per well of 96-well plate and after 24 h, the cells were transfected with PepFect14 as described in (Urgard et al., 2016). Here briefly, PepFect14 and miRNA mimics, siRNA or LNA-inhibitors at 30 or 60 nM concentration were mixed at molar ratio 17:1 in a final volume of 100 µl, incubated at room temperature for 1 h, mixed with 900 µl of the growth media and applied to the cells in the case of 12-well plates. For 96-well plates, the same molar ratio was used but the miRNA mimics, siRNA or LNA-inhibitors, and PepFect14 were incubated in a final volume of 60 µl at room temperature for 1 h, then mixed with 0,54 ml of the growth media and shared to the cells in six wells of 96-well plate as six replicates were usually done. All the used siRNAs, miRNAs, and LNA-inhibitors are shown in tables 1, 2, and 3 respectively. After 24 h, cells were either harvested or stimulated with cytokines for an additional 48 h shown in table 4.

In study I and III, in addition to PepFect14 transfection siPORT NeoFX (Thermo Fisher Scientific) was used. In that case, 2×10^4 keratinocytes per well of 12-well or 5×10^3 cells per well of 96-well plate were seeded 24 hours before the transfection, which was performed according to the manufacturer's protocol, 3 µl or 0.3 µl of siPORT NeoFX per well of 12-well or 96-well plate was used, respectively.

If only stimulations were performed, 4×10^4 cells were seeded per well of 12-well plate and stimulated after 24 h for an additional 48 h with cytokines shown in table 4 or left nonstimulated. In time point analysis, the cells were stimulated with indicated cytokines for 6, 12, 24, 48, or 72 h or left nonstimulated. For 3D ALI cultures the cells were seeded as described earlier and after 5 days stimulated for 48 h with cytokines or left nonstimulated.

Table. 1 siRNAs used in the studies.

	Assay ID/ Catalog number	Concen-tration	Manufacturer	Study
Silencer Select Validated CARD10	s26577	50 nM	Thermo Fisher Scientific	Study I
		30 nM		Study II
Silencer Select Validated FERMT1	s31076	50 nM	Thermo Fisher Scientific	Study I
Silencer Select Validated IRAK1	s323	50 nM	Thermo Fisher Scientific	Study I
		30 nM		Study II
Silencer Select Validated SERPINB2	s10018	30 nM	Thermo Fisher Scientific	Study II
Silencer™ Select Negative Control No. 1 siRNA	4390843	50 nM	Thermo Fisher Scientific	Study I
		30 nM		Study II

Table 2. miRNA mimics used in the studies.

	Assay ID/Catalog number	Concen-tration	Manufacturer	Study
Pre-miR™ miRNA Precursor Molecules Negative Control #1	AM17110	60 nM	Thermo Fisher Scientific	Study I
		30 nM		Study III
Pre-miR™ miRNA Precursor hsa-miR-146a-5p	PM10722/ AM17100	60 nM	Thermo Fisher Scientific	Study I
Pre-miR™ miRNA Precursor hsa-miR-10a-5p	PM10787/ AM17100	30 nM	Thermo Fisher Scientific	Study III
mirVana miRNA Mimic, Negative Control #1	4464058	30 nM	Thermo Fisher Scientific	Study I, Study III
*mir*Vana® miRNA mimic has-miR-146b-5p	MC10105/ 4464066	30 nM	Thermo Fisher Scientific	Study I
*mir*Vana® miRNA mimic hsa-miR-10a-5p	MC10787 /4464066	30 nM	Thermo Fisher Scientific	Study III
miRIDIAN miRNA hsa-miR-146a-5p mimic	C-300630-03-0020	30 nM	Dharmacon (Lafayette, CO, USA)	Study II
miRIDIAN miRNA hsa-miR-146b-5p mimic	C-300754-03-0005	30 nM	Dharmacon	Study II
miRIDIAN miRNA Mimic Negative Control #1	CN-001000-01-20	30 nM	Dharmacon	Study II

Table 3. LNA-inhibitors used in the studies.

	Catalog number	Concentration	Manu-facturer	Study
hsa-miR-146 miRCURY LNA™ miRNA Power Family Inhibitor	YFI0450021	30 nM	Qiagen (Hilden, Germany)	Study I, Study II
hsa-miR-146a-5p miRCURY LNA miRNA Inhibitor	YI04100676	30 nM	Qiagen	Study I
hsa-miR-10a-5p miRCURY LNA miRNA Inhibitor	YI04101337	10 nM or 30 nM	Qiagen	Study III
Negative control A miRCURY LNA miRNA Inhibitor Control	YI00199006	30 nM	Qiagen	Study I, Study II
		10 nM or 30 nM		Study III

Table 4. Cytokines used in the studies.

Cytokine	Concentration	Manufacturer	Study
IFN-γ	10 ng/ml	PeproTech (London, UK)	Study I, Study II, Study III
HB-EGF	25 ng/ml	PeproTech	Study III
IL-1β	10 ng/ml	PeproTech	Study I, Study III
IL-4	40 ng/ml	PeproTech	Study III
IL-6	20 ng/ml	PeproTech	Study II
IL-13	50 ng/ml	PeproTech	Study III
IL-17A	10 ng/ml	PeproTech	Study I, Study II, Study III
IL-20	20 ng/ml	PeproTech	Study II
IL-22	10 ng/ml	PeproTech	Study I
	20 ng/ml		Study III
IL-23	20 ng/ml	Thermo Fisher Scientific	Study II
Retinoic acid (ATRA)	300 ng/ml	Merck	Study III
TGF-β	20 ng/ml	R&D Systems (Minneapolis, MN, USA)	Study III
TNF-α	25 ng/ml	PeproTech	Study I, Study II, Study III

4.4. Isolation of RNA, cDNA synthesis and RT-qPCR (Study I, II and III)

All skin biopsies were submerged Qiazol (Qiagen, Hilden, Germany) cell lysis reagent and homogenized with Precellys 24 homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) at 4°C. Total RNA from frozen skin samples was isolated using the miRNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer´s protocols. Total RNA was extracted from *in vitro* cell culture samples with Direct-zol™ RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) (Study I and III), miRNeasy Mini Kit (Qiagen) (Study I, III) or total RNA mini kit (AA Biotechnology, Gdynia, Poland) (Study II) according to the manufacturer´s protocols. In-column DNase digestion was performed with DNase (Zymo Research) or with RNase-Free DNase Set (Qiagen). RNA concentration and quality were assessed with NanoDrop 2000c (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer, using Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA).

miRNA expression levels were quantified with TaqMan® miRNA Reverse Transcription Kit and TaqMan® miRNA Assays (TaqMan® miRNA Assays hsa-let-7a, TaqMan® miRNA Assays hsa-miR-146a, TaqMan® miRNA Assays hsa-miR-146b or TaqMan® miRNA Assays hsa-miR-10a) (all from Thermo Fisher Scientific) and 5× HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne, Tartu, Estonia). That were all used according to the manufacturer's

protocol. let-7a was used for normalization and relative expression was calculated using the ΔΔCt method. For quantification of mRNA expression levels, cDNA was synthesized from 0.1–1 µg of total RNA using oligo-dT (TAG Copenhagen, Frederiksberg, Denmark), RevertAid Reverse Transcriptase and RiboLock RNase Inhibitor (Thermo Fisher Scientific). For qPCR, 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne) was used. PCR primers were designed with the assistance of Primer 3 (Untergasser et al., 2012) and were ordered from TAG Copenhagen. A list of used primers can be seen in table 5. Target gene expression was normalized to EEF1A1 using ΔΔCt calculation. Machines used were ABI Prism 7900 (Thermo Fisher Scientific) or Viia7 (Thermo Fisher Scientific).

Table 5. Used primers.

Gene	Forward	Reverse
CARD10	5′-AGTGTGCCCAGCGGAAAGCC-3’	5’-GATGGCCCGGATCCTGCTGC-3’
CCL5	5′-AGTCGTCTTTGTCACCCGAAA-3’	5’-TCTCCATCCTAGCTCATCTC CAA-3’
EEF1A1	5′-CCACCTTTGGGTCGCTTTGCTGT-3’	5’-TGCCAGCTCCAGCAGCCTT CTT-3’
FERMT1	5’-TGATGCAGCCACCGGGATTCCA-3’	5’-CGA TGACCACCTGCCGGGT TTC-3’
IL-8	5’-GCAGCTCTGTGTGAAGGTG CAGTT-3’	5’-TTCTGTGTTGGCGCAGTGTG GTC-3’
IRAK1	5’-CACCTTCAGCTTTGGGGTGGT AGTG-3’	5’-CCAGCCTCCTCAGCCTCCTCT-3’
Ki67	5’-AAAGAGTGGCAACCTGCCTT-3’	5’-TTTGCAACAATCAGATTTGCT TCCG-3’
KRT10	5’-TCCCAACTGGCCTTGAAACAAT CCC-3’	5’-AGAGCTGCACACAGTAGCG ACCT-3’
MAP3K7	5’-AGCCTGCTTGAATCCAGTGT-3’	5’-TTGCGTGGGCAGCAGTATAA-3’
NUMB	5’-AATGCCTTCAGCACACCTGA-3’	5’-AGTC AGTGCCATTAGCTTG GAA-3’
SERPINB2	5’-GGATGGTCCTGGTGAATGCT-3’	5’-TGCGCTGAGCCGAGTTTAC-3’

4.5. Immunofluorescence (Study I and II)

Immunofluorescence was performed on either cryosections of skin biopsy samples or on primary human keratinocytes. For skin sample collection for immunofluorescence analysis, the bunch biopsy samples of healthy controls, nonlesional, and lesional skin of PV patients were snap-frozen in TissueTek (Thermo Fisher Scientific). Immunofluorescence staining of FERMT1 or SERPINB2 was performed on frozen skin sections of 10 or 5 µm, respectively. For the staining of SERPINB2 in keratinocytes, the cells were seeded on coverslips on a 24-well plate. The sections or cells were fixed with 4% formaldehyde in PBS for 10 min, permeabilized with 0.1% Triton-X-100 in PBS for 10 min,

and blocked with 10% goat serum (Thermo Fisher Scientific) and 0.5% BSA (Merck, Darmstadt, Germany) in PBS for 1 h. The skin sections or cells were further incubated for 1 hour either with rabbit polyclonal anti-FERMT1 antibody (HPA041966) (Atlas Antibodies, Stockholm, Sweden) or mouse monoclonal anti-SERPINB2 antibody (MAB8550) (R&D Systems, Minneapolis, MN, USA). The omission of the primary antibody was used as a negative control. Alexa-488 labeled anti-rabbit-IgG (Thermo Fisher Scientific) with a dilution of 1:2000 or Alexa-488 labeled anti-mouse-IgG (Thermo Fisher Scientific) at dilution of 1:1000 were used respectively as secondary antibodies. Before and after antibody incubations, samples were washed 3×5 min with PBS. Further, the slides were stained with 4′,6-diamidino-2-phenylindol (DAPI) (Roche, Basel, Switzerland) (1μg/mL) for 10 min. After that, the slides were washed twice with PBS and were mounted either with StayBrite Hardset Mounting Medium (Biotium, Fremont, CA, USA) or fluorescent mounting medium (Agilent, Santa Clara, CA, United States) and analyzed with Leica DM5500 B microscope (Leica Microsystems, Wetzlar, Germany).

4.6. *In situ* hybridization (ISH) (Study I and III)

In study I and III, miRNA ISH Buffer, Controls Kit, and miRCURY LNA™ Detection probes (all from Qiagen) were used according to the manufacturer's protocol. In study I, ISH was performed on 10 μm frozen sections using 5`-DIG and 3`-DIG-labeled hsa-miR-146a-5p (619856-360), hsa-miR-146b (615935-360) miRCURY LNA™ Detection probes. In study III, ISH was performed on 6 μm frozen skin sections using 5`-DIG and 3`-dig-labeled miRCURY LNA™ Detection probes: hsa-miR-10a-5p (612528-360) and Custom Detection Probe as a scrambled control (339115). Prehybridization, hybridization, and washing were performed at 50 °C for miR-146a and 48 °C for miR-146b and miR-10a. For detection, slides were incubated with alkaline phosphatase-conjugated sheep anti-DIG-AP (1:1500, Roche) for 1h at room temperature. The staining was visualized by adding BM purple alkaline phosphatase substrate (Roche).

4.7. Western blot (Study I)

The human primary keratinocytes were lysed using 1× Laemmli buffer and then boiled at 95°C for 5 minutes before the loading to the gel. A total of 10 μl of protein solution was loaded in each line of 10% SDS PAGE gel. Antibodies were diluted in Tris-buffered saline containing 0.05% Tween 20 and 5% milk. Primary antibodies were used at the following dilutions: mouse monoclonal anti-GAPDH 1:1000 (Santa Cruz Biotechnology, Dallas, TX, USA) and rabbit polyclonal anti-FERMT1 antibody 1:250 (HPA041966, Atlas Antibodies). For secondary antibodies, anti-rabbit and -mouse IgG horseradish peroxidase-conjugated antibodies were used 1:2000 (Cell Signaling Technology, Danvers,

MA, USA). For signal detection, Amersham ECL Select Western blotting kit (GE Healthcare) was used according to the manufacturer's protocol. The results were visualized using ImageQuant™ RT ECL™ (GE Healthcare) and were quantified using ImageJ software. The mean intensity of the background value was subtracted from the mean intensity of each protein band and then normalized against the mean values of GAPDH.

4.8. The enzyme-linked immunosorbent assay (ELISA) (Study II)

For study II, the quantity of secreted SERPINB2, CCL5, and IL-8 were measured from the keratinocyte culture supernatants using the Serpin B2 DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA) or the CCL5 or IL-8 ELISA MAX™ Deluxe sets (BioLegend). ELISAs were performed according to the manufacturer's protocol. The data were acquired with Ledetect 96 Microplate reader (Labexim Products, Lengau, Austria) and the concentrations were calculated by using the Four Parameter Logistic Regression model in an online analysis software Myassays.com (MyAssays Limited, Brighton, United Kingdom).

4.9. Luminescent Cell Viability Assay (Study I and III)

Keratinocytes were transfected on 96-well plates as described previously. For study III, the cell proliferation was analyzed after 72h of the transfection of miR-10a and for study I after 24h of the transfection of miR-146a/b. CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA) was used according to the manufacture's protocol. Luminescent Cell Viability Assay measures luciferase activity that is proportional to the amount of cellular ATP.

4.10. Thymidine Incorporation Assay (Study I)

Keratinocytes were transfected with miR-146a or negative control mimics on 96-well plates as described previously. Cytokines were added after 24 hours and a radioactive nucleoside ^{3}H thymidine (Hartmann Analytic, Braunschweig, Germany) at an end-concentration of 1 μCi/ml after 48 hours. During cell-division, ^{3}H thymidine was incorporated into the replicating DNA. After 14 hours, cells were harvested onto a glass-fiber filter, mounted with a cocktail containing scintillation liquid, and the counts were monitored using a scintillation beta-counter to measure the radioactivity in DNA recovered from the keratinocytes to determine the extent of cell division that has occurred. β-counts of the control cells were in the range of 2.7×10^{4} and 1.2×10^{6} counts per minute.

4.11. Cell cycle analysis (Study III)

For cell cycle analysis, the cells were first trypsinized and collected 48 h after transfection with miR-10a or negative control mimics. The cells were fixed for 30 minutes with ice-cold 70% ethanol, permeabilized in 0,1 % Triton X-100 in PBS for 10 minutes at room temperature, and stained with DAPI (1 µg/ml). Cell cycle phase distribution was determined with BD LSRFortessa™ cell analyzer (BD Biosciences Franklin Lakes, NJ, USA) by quantifying DAPI intensity and further analyzed using ModFit LT™ Software 2.5.1 (Verity Software House, Topsham, ME, USA).

4.12. Proliferation assay with Click-iT™ EdU Cell Proliferation Kit (Study III)

Click-iT® EdU Cell Proliferation Alexa Fluor® 488 Flow Cytometry assay (Thermo Fisher Scientific) was performed 48 h after the transfection of human primary keratinocytes with miR-10a or negative control mimics according to the manufacturer's instructions. EdU was added to the cells 2 hours before harvesting the cells. For analysis, BD LSRFortessa™ cell analyzer (BD Biosciences) was used.

4.13. Cloning and Luciferase assay (Study I)

Two FERMT1 3'UTR fragments, containing binding sites for miR-146a or the corresponding mutant binding sites of FERMT1 were cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) using the PCR primers containing NheI and SalI sites. The used cloning primers are shown in table 6. For Luciferase assay, human embryonic kidney epithelial cells HEK293 were transfected for 24 hours with 30 nM miR-146a and control (Pre-miR™ Precursors Molecules hsa-miR-146a or Negative Control #1, Thermo Fisher Scientific) and 50 ng of the reporter plasmid using siPORT NeoFX (Thermo Fisher Scientific) according to the manufacture's manual. Firefly and renilla luciferase activities were measured using the Dual-Luciferase® Reporter Assay System (Promega). Firefly luciferase activities were normalized to the values of the renilla luciferase.

Table 6. Used cloning primers.

Site	Forward	Reverse
FERMT1-1 3'UTR	5'-ATTGCTAGCACCCACTGC TGGCACATCCCT-3'	5'-ATTGTCGACGCAGTTCCAT GAAGG ACAGGCG-3'
FERMT1-2 3'UTR	5'-ATAGCTAGCATTAACAGCTG CCTGAATT-3'	5'-ATAGTCGACAAGTCTGTCT CTAT GTGGCT-3'
FERMT1-1 3'UTR for mut	5'-TGGTTACAGTAGTC TGACTTGC-3'	5'-GCAAGTCAGACTACTGT AAC-3'
FERMT1-2 3'UTR for mut	5'-ATTTTTCAGTAGTCCTAG GATG-3'	5'-CATCCTAGGACTACT GAAAAAT-3'

4.14. Array, pathway and target analysis (Study I and Study III)

For study I, a previously published dataset E-MTAB-1739, in which miR-146a or negative control mimics were transfected to keratinocytes and the cells were stimulated with IFN-γ or TNF-α or left nonstimulated (Rebane et al., 2014) and where mRNA profiling was done with Illumina HumanHT-12 Expression BeadChip (Illumina, San Diego, CA, USA) was used. The dataset was reanalyzed to determine the effect of miR-146a in nonstimulated keratinocytes. For study III, mRNA profiling of cells where miR-10a or negative control mimics were transfected to keratinocytes and the cells were stimulated with IL-1β or left nonstimulated, was done with Illumina HumanHT-12 Expression BeadChips version 4 (Illumina) and data analyzed with Illumina GenomeStudio V2009.1 Gene Expression Module using Quantile normalization and background subtraction with Illumina custom errors model. For both studies, additional analyses and visualizations were performed using Microsoft Excel and Multi Experiment Viewer 4.8.1. Pathway analysis was performed with g:Profiler (http://biit.cs.ut.ee/gprofiler) (Reimand et al., 2007), which estimates the significance of the overlap between the functional groups and the list of studied genes by calculating enrichment P-value using Fisher's one-tailed test. For study III, additionally, genes with average expression values below 50 or average detection P values above 0.05 in all conditions, were discarded. The remaining genes were sorted based on gene expression fold change between control transfected and miR-10a transfected cells and only the 200 most upregulated genes were used in g:Profiler analysis. For both studies, putative direct targets for miR-146a or miR-10a were selected using Targetscan (http://www. targetscan.org/vert_71/) search, choosing only highly conserved and those with better context score (<-0.15) (Agarwal et al., 2015; Lewis et al., 2005).

4.15. Statistical analyses (Study I, II and III)

Statistical analyses were performed with GraphPad Prism 5 or 6 (GraphPad Software, San Diego, CA, USA) by ANOVA when more than 2 conditions were compared or Student's t-test, otherwise. For the linear regression analysis, Pearson's chi-squared test was used. Results were considered significant at $P<0.05$. Data are presented as mean with the standard error of the mean (SEM).

5. RESULTS

5.1. Both miR-146a and miR-146b are upregulated in the skin of PV patients and in response to pro-inflammatory cytokines in keratinocytes (Study I)

The upregulation of miR-146a/b in PV has been shown previously by miRNA expression profiling (Sonkoly et al., 2007). To confirm the previous array result, we used RT-qPCR revealing the increased expression of miR-146a/b in the lesional skin of PV patients compared with healthy controls (Figure 11A). To determine the localization of miR-146a/b in the tissue, we performed *in situ* hybridization analysis of skin sections from healthy controls, non-lesional and lesional skin of PV patients. While only modest miR-146a expression, which was still stronger than that of miR-146b, was found in the epidermis of the control skin, a robust miR-146a signal was detected in the epidermis of PV patients lesional skin sample (Figure 11B). The expression of miR-146a in PV patients' lesional skin was the highest in the granular layer of the epidermis. Comparatively, miR-146b expression in the lesional skin of PV patients was lower than that of miR-146a, and therefore, its localization was not so clearly defined (Figure 11B). Interestingly, in one of the PV patients, the expression of miR-146b was higher in the dermis compared with the epidermis of the lesional skin.

Next, we compared the levels of miR-146a/b in the healthy skin with the levels of miR-146a/b in human primary keratinocytes, fibroblasts, and peripheral blood mononuclear cells. Compared with the skin, the levels of miR-146a/b were lower in the keratinocytes and fibroblasts, however, highest in the peripheral blood mononuclear cells (Figure 11C). To delineate which cytokines might be responsible for the increased miR-146a/b levels in the skin, human primary keratinocytes in proliferating or differentiating conditions and fibroblasts were stimulated with different pro-inflammatory cytokines. In monolayer keratinocyte and fibroblast cultures, miR-146a was upregulated in response to IL-17A, TNF-α and, IL-1β (Figure 12A, 12B), while miR-146b was upregulated in response to IFN-γ in keratinocytes and response to IL-1β in fibroblasts (Figure 12A, 12B). In the keratinocyte 3D differentiation model, miR-146a was upregulated in response to IL-17A, TNF-α, and, IL-1β and miR-146b with IFN-γ and IL-22 (Figure 12C). To evaluate the reliability of the 3D model, the trans-epithelial electrical resistance (TER) was measured every 24 hours (Figure 12D). These data together demonstrate that miR-146a/b is elevated in the lesional skin of PV patients and upregulated by pro-inflammatory cytokines in keratinocytes and fibroblasts.

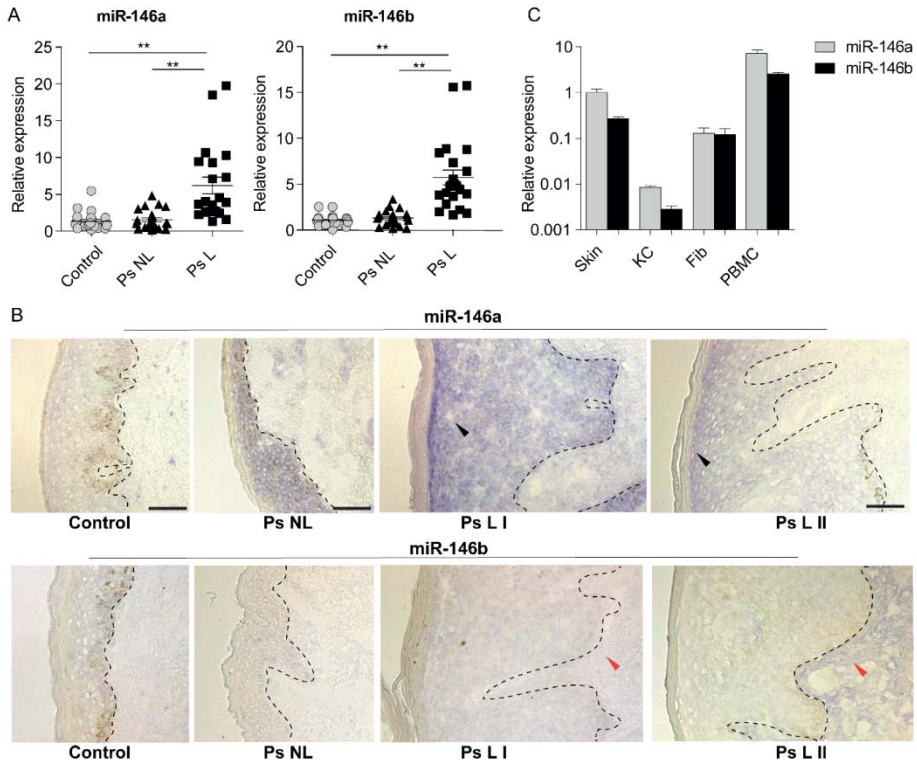

Figure 11. The expression of miR-146a/b is increased in the skin of PV patients. A) Relative expression of miR-146a/b in the nonlesional (Ps NL) and lesional (Ps L) skin of PV patients and healthy controls (Control). The expression of miR-146a/b was measured with RT-qPCR. **B)** *In situ* hybridization images from nonlesional (Ps NL) and lesional (Ps L) skin of PV patients and healthy controls. The expression of miR-146a/b is marked in blue. Bar = 75 μm. The line between the epidermis and dermis is indicated with a dashed line. **C)** Relative expression of miR-146a/b in the skin, human primary keratinocytes (KC), fibroblasts (Fib), and peripheral blood mononuclear cells (PBMCs), (n = 5). The expression of miR-146a/b was measured with RT-qPCR. Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

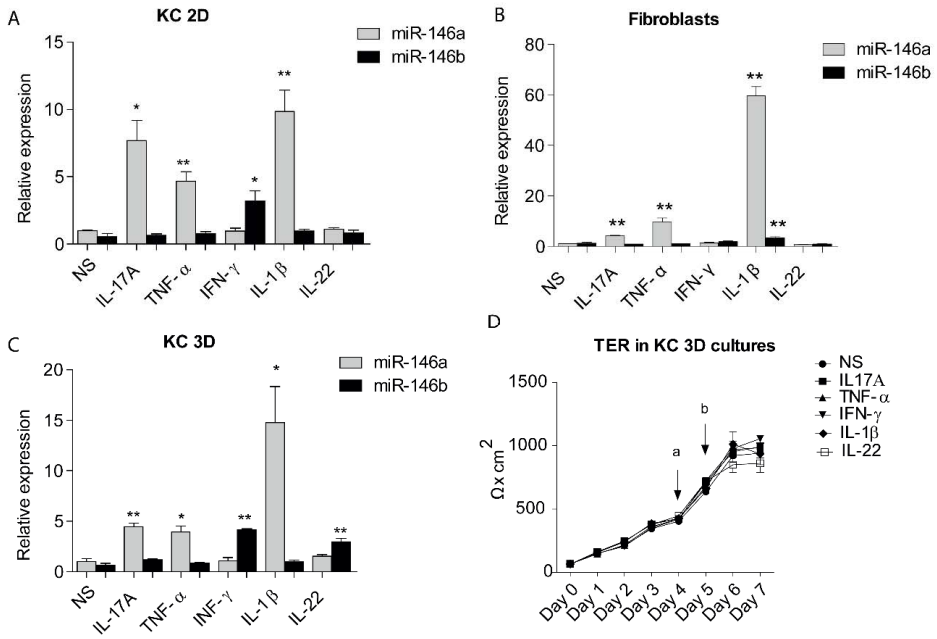

Figure 12. The expression of miR-146a/b in human primary keratinocytes and transepithelial resistance measurements of keratinocyte 3D cultures. **A)** Human monolayer keratinocytes (KC 2D), **B)** human primary fibroblasts, **C)** reconstituted epidermis (KC 3D) were stimulated with shown cytokines for 48 hours or left nonstimulated (NS). The expression of miR-146a/b was measured with RT-qPCR. Data are shown compared with the mean expression of miR-146a in NS cells (=1) (n = 4). **D)** Transepithelial resistance measurements (TER) of keratinocyte 3D cultures. At day 0, 50 000 KCs were seeded per each well. The apical media was removed on day 4 (a) and after 24 hours (b) the KC 3D cultures were stimulated with indicated cytokines for 48h or left nonstimulated (NS). Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

5.2. miR-146a/b inhibit keratinocyte proliferation (Study I)

Increased keratinocyte proliferation is a known feature of both AD and PV (Dainichi et al., 2018). Interestingly, a pathway analysis of a previously published array data (Rebane et al., 2014) revealed that proliferation-related genes (Figure 13A) were enriched among the set of 102 genes suppressed by miR-146a in nonstimulated keratinocytes (Study I, Supplementary Table S2). Next, we did a ^{3}H thymidine assay to assess the effect of miR-146a on keratinocyte proliferation. We transfected keratinocytes with either miR-146a mimics or control or miR-146a inhibitor (LNA-146a) or control LNA to suppress the endogenous levels of miR-146a and stimulated keratinocytes with TNF-α or IFN-γ or left nonstimulated. Figure 13B demonstrates that miR-146a over-

expression led to reduced keratinocyte proliferation in nonstimulated cells while in the case of inhibition of miR-146a, cell proliferation increased in all conditions (Figure 13C). To further confirm our results and to estimate the effect of miR-146b, we next carried out ATP dependent assay where Luciferase activity is measured as the outcome. Figure 13D demonstrates that miR-146b can suppress cell proliferation to a similar extent as miR-146a and if endogenous levels of both miRNAs are reduced by LNA targeting both miR-146a/b, there is an increase in keratinocyte proliferation. These data together demonstrate that miR-146a/b inhibit the proliferation of primary keratinocytes.

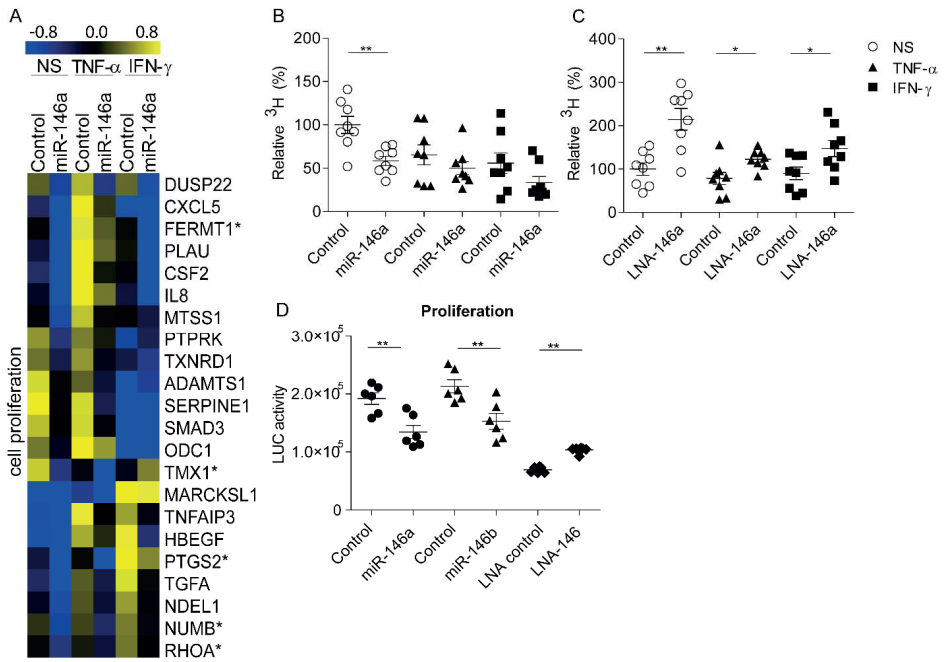

Figure 13. miR-146a/b inhibit proliferation of human primary keratinocytes. A) Keratinocytes were transfected with control or miR-146a for 24 hours and then stimulated with IFN-γ or TNF-α for 48 hours or left nonstimulated (NS). Log2 values of expression signals are mean-centered for each gene separately. Asterisks mark the proposed miR-146a direct targets. **B, C)** Keratinocytes were transfected either **(B)** with control or miR-146a or **(C)** with control LNA (Control) or miR-146a inhibitor (LNA-146a) for 24 hours and then stimulated with IFN-γ or TNF-α for 24 hours or left nonstimulated (NS). ^{3}H thymidine was added for another 14 hours. **D)** Keratinocytes were transfected either with control or miR-146a or miR-146b or with control LNA (Control) or LNA inhibitor for miR-146a/b (LNA-146) for 24 hours and then the proliferation assay was performed. Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

5.3. miR-146a/b putative target genes are upregulated in PV (Study I and III)

It is previously shown that miR-146a directly targets members of the NF-κB pathway like IRAK1 and CARD10 and CCL5 (Crone et al., 2012; Rebane et al., 2014; Taganov et al., 2006) and it also inhibits IL-8 (Meisgen et al., 2014; Rebane et al., 2014). In addition, we choose for further studies FERMT1 and SERPINB2 as they both were downregulated by miR-146a in array analysis and predicted to be putative direct miR-146a targets by Targetscan (Agarwal et al., 2015; Lewis et al., 2005). Additionally, they could potentially influence disease-related processes in PV as FERMT1 had been previously associated with keratinocyte adhesion, proliferation, and migration (Herz et al., 2006; Lai-Cheong et al., 2009) and SERPINB2 had been demonstrated to have protease inhibitor function (Kruithof et al., 1995). From the tested genes, IRAK1, CCL5, IL-8, FERMT1, and SERPINB2 were detected to be up-regulated in the lesional skin of PV patients compared with healthy controls and nonlesional skin of PV patients (Figure 14). FERMT1 and SERPINB2 were also upregulated in the non-lesional skin. From the tested genes, only CARD10 was downregulated in the lesional skin of PV patients compared with healthy controls. These data indicate that both miR-146a/b and putative target genes are upregulated in the skin of PV patients because of inflammation-associated signaling and that increased level of miR-146a/b is not sufficient to suppress the targets to the level characteristic to non-inflamed condition.

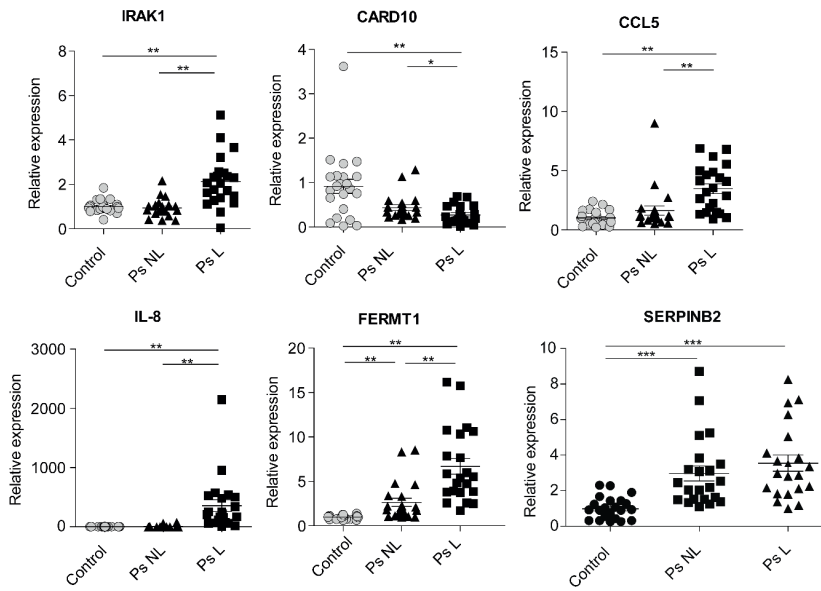

Figure 14. The expression of miR-146a/b target genes is changed in PV patients' skin. Relative mRNA expression in the nonlesional (Ps NL) and lesional (Ps L) skin of PV patients compared with the mean of healthy controls (Control). Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

5.4. miR-146a may regulate the proliferation of keratinocytes through FERMT1 (Study I)

Among putative miR-146a target genes, *FERMT1* (also known as Kindlin-1) was previously shown to act as a positive regulator of keratinocyte proliferation (Herz et al., 2006). In addition, it was known that mutations in the *FERMT1* gene cause Kindler syndrome characterized by multiple skin problems, including blistering, premature aging, and increased cancer risk (Duperret & Ridky, 2014). Thus, we next performed immunofluorescence analysis showing FERMT1 protein expression was the highest in the basal layer of keratinocytes and with increased expression in the lesional skin of PV patients (Figure 15A). To confirm the effect of miR-146a on FERMT1 expression, keratinocytes were transfected with miR-146a and stimulated with TNF-α, IFN-γ, IL-17A, or left nonstimulated followed by RT-qPCR and western blot. In most of the conditions, FERMT1 expression was suppressed by miR-146a both at mRNA (Figure 15B) and protein level (Figure 15C). Based on Targetscan (Agarwal et al., 2015; Lewis et al., 2005) prediction, two putative miR-146a binding sites in the 3' untranslated region (3' UTR) of FERMT1 mRNA was found. Both these binding sites together with flanking areas or the same areas with the mutated binding sites were inserted into the luciferase reporter vector. Following luciferase assays demonstrated that the overexpression of miR-146a led to the suppression of luciferase activity of the reporters containing FERMT1 3' UTR regions with miR-146a binding sites but not with the mutant versions (Figure 15D). The vector containing CARD10 3'UTR was used as a positive control (Crone et al., 2012; Rebane et al., 2014). These results demonstrate that FERMT1 is one of the targets through which miR-146a may mediate the effect on keratinocyte proliferation.

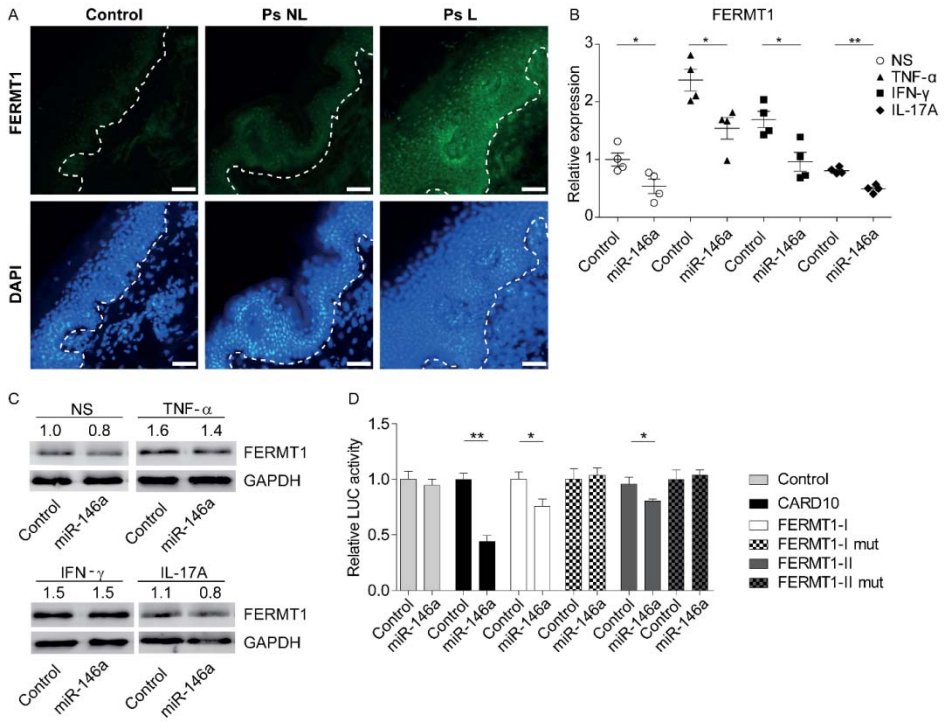

Figure 15. Positive regulator of proliferation FERMT1 is upregulated in PV and is a direct target of miR-146a. A) Immunofluorescence analysis of FERMT1 expression (Green) in the nonlesional (Ps NL) and lesional (Ps L) skin of PV patients and healthy controls (Control). DAPI labeling is blue. The white line indicates the border between epidermis and dermis. White bar=50 µm. **B, C)** Keratinocytes were transfected with control or miR-146a for 24 hours and then stimulated with shown cytokines for 48 hours or left nonstimulated (NS). **B)** Relative FERMT1 expression was measured by RT-qPCR. **C)** FERMT1 protein expression was measured by western blot. **D)** HEK293 cells were transfected with control or miR-146a for 24 hours. The relative firefly luciferase (LUC) activity is normalized to the value of control miRNA and empty vector (Control; =1) (n = 8). Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

5.5. SERPINB2 is indirectly suppressed by miR-146a and has an anti-inflammatory effect in keratinocytes (Study III)

The function of SERPINB2 in the skin was not well understood. To delineate its role in the skin, we first visualized SERPINB2 expression by immunofluorescence analysis from skin biopsies of healthy controls, non-lesional, and lesional skin of PV patients. In healthy controls and non-lesional skin of PV patients, SERPINB2 expression was localized in the granular layer (Figure 16A). In the case of lesional skin, SERPINB2 expression was detectable all over the epidermis with the strongest expression in the cornified layer (Figure 16A). Additionally, we were able to confirm that there was a positive relationship between PASI and SERPINB2 mRNA expression in lesional skin samples from PV patients (Figure 16B). Interestingly, we detected a negative relationship between SERPINB2 mRNA and miR-146a even though both miR-146a and SERPINB2 were upregulated in the lesional skin of PV patients (Figure 16C). Next, we stimulated human primary keratinocytes with psoriasis-related cytokines. From the tested cytokines SERPINB2 expression was strongly upregulated in response to IFN-γ and TNF-α and slightly by IL-6 (Figure 16D). The upregulation of SEPRINB2 in response to IFN-γ and TNF-α was confirmed on protein level by immunofluorescence analysis (Figure 16E). Also, there was an increase in the levels of secreted SERPINB2 in response to IFN-γ and TNF-α measured by ELISA (Figure 16F). Inhibition of the expression of SERPINB2 itself by siRNAs led to the increased mRNA levels of IL-8 when stimulated with TNF-α and CCL5 when stimulated with IFN-γ (Figure 16G). Using the supernatants from the same experiment, we detected increased levels of IL-8 in nonstimulated conditions and when stimulated with TNF-α and increased CCL5 when stimulated with IFN-γ when SERPINB2 expression was inhibited (Figure 16H). Together, these results show that SERPINB2 is upregulated in the lesional skin of PV patients and response to pro-inflammatory cytokines. Additionally, these results imply that SERPINB2 has an anti-inflammatory function in keratinocytes as it can suppress the levels of pro-inflammatory chemokines IL-8 and CCL5.

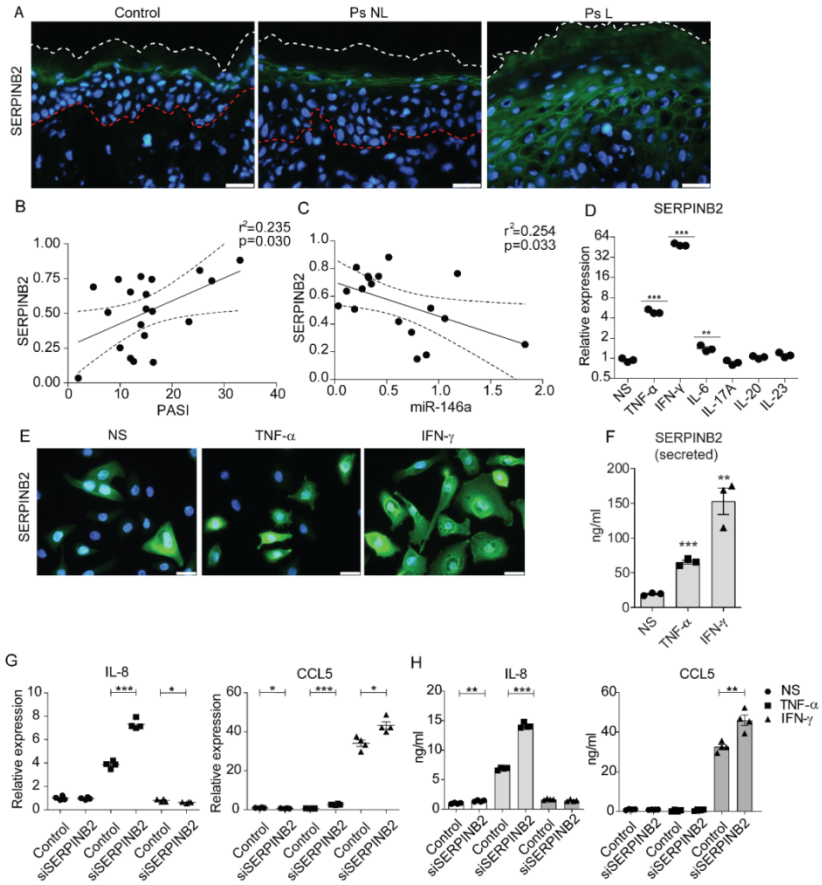

Figure 16. SERPINB2 is increased in the skin of PV patients and has an anti-inflammatory effect in keratinocytes. A) Immunofluorescence analysis of SERPINB2 expression (green) in the nonlesional (Ps NL) and lesional (Ps L) skin of PV patients and healthy controls (Control). DAPI labeling is blue. The white line indicates the outer border of the stratified layer. The red line indicates the border between epidermis and dermis. White bar=25 µm. **B)** Linear regression analysis between SERPINB2 and PASI in the lesional skin of PV patients. 95% confidence intervals (CI) are shown as a dotted line. Data is presented on the log10 scale. **C)** Linear regression analysis between SERPINB2 and miR-146a in the lesional skin of PV patients. 95% confidence intervals (CI) are shown as a dotted line. Data is presented on the log10 scale. **D-F)** Human keratinocytes were stimulated with shown cytokines for 48 hours or left nonstimulated (NS). **D)** The expression of SERPINB2 was measured with RT-qPCR. **E)** SERPINB2 level in keratinocyte supernatants was measured by ELISA. **F)** Immunofluorescence analysis of SERPINB2 expression (green) in the stimulated keratinocytes. DAPI labeling is blue. White bar=25 µm. **G,H)** Keratinocytes were transfected either with control siRNA (Control) or siRNA targeting SERPINB2 and after 24 h stimulated with IFN-γ or TNF-α for 48 h or left non-stimulated (NS). The expression of indicated chemokines was measured with RT-qPCR **(G)** or by ELISA **(H)**. Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

5.6. miR-146a and miR-146b have a similar capacity to suppress psoriasis-related target genes (Study I and III)

To explore whether the miR-146b effect on the targets is indeed similar to miR-146a, we transfected keratinocytes with miR-146a, miR-146b, or control in three conditions, using cells left nonstimulated or stimulated with TNF-α or IFN-γ and analyzed mRNA levels of CARD10, IRAK1, CCL5, IL-8, FERMT1, and SERPINB2. In most cases, miR-146a and miR-146b suppressed the mRNA expression of studied genes and had a similar effect in tested cytokine conditions (Figure 17). These results indicate that miR-146a/b target similar genes to the same extent in keratinocytes.

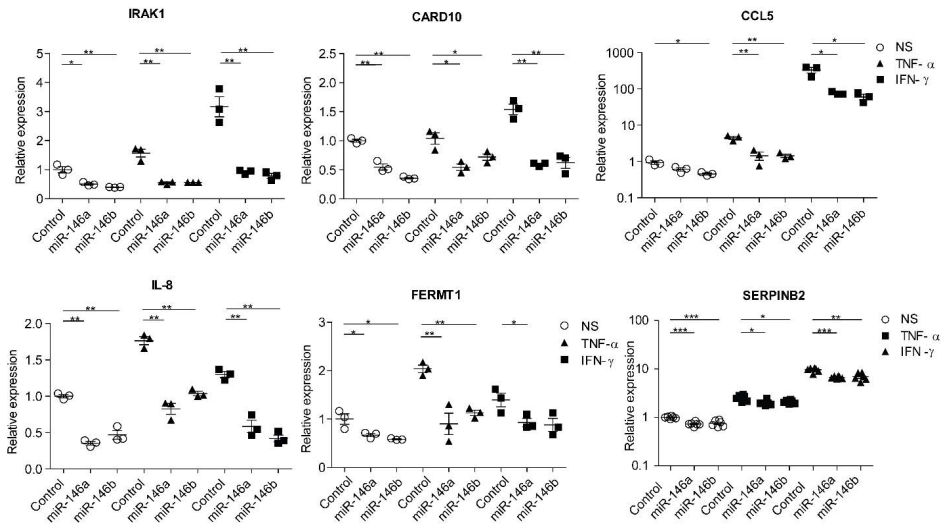

Figure 17. miR-146a/b inhibit psoriasis-related genes in keratinocytes. Keratinocytes were transfected with control or miR-146a or miR-146b for 24 hours and then stimulated with IFN-γ or TNF-α for 48 hours or left nonstimulated (NS). Relative mRNA expression was measured by RT-qPCR. Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

5.7. The endogenous level of miR-146a/b is sufficient to suppress the target genes in the keratinocytes (Study I and III)

To clarify whether the endogenous level of miR-146a/b is sufficient to suppress the target genes in the keratinocytes, we used LNA-miR-146 family inhibitor suppressing both, miR-146a/b. Once again, we did this in three conditions, in cells left nonstimulated or stimulated with TNF-α or IL-17A. Except in the case of SERPINB2 where the cells were left nonstimulated or stimulated with TNF-α or IFN-γ instead of IL-17A as IL-17A did not affect SERPINB2 expression and IFN-γ had the strongest effect on SERPINB2 levels. In all tested conditions the suppression of miR-146a/b led to the increased expression of the target genes IRAK1, CARD10, CCL5, and IL-8 (Figure 18). However, in the case of SERPINB2, the effect of miR-146a/b suppression was only visible when the cells were stimulated with IFN-γ (Figure 18). These data demonstrate that endogenous levels of miR-146a/b are sufficient to have an effect on target genes in keratinocytes as well as further suggests that miR-146a/b have the capacity to suppress the studied target genes also in the skin of PV patients.

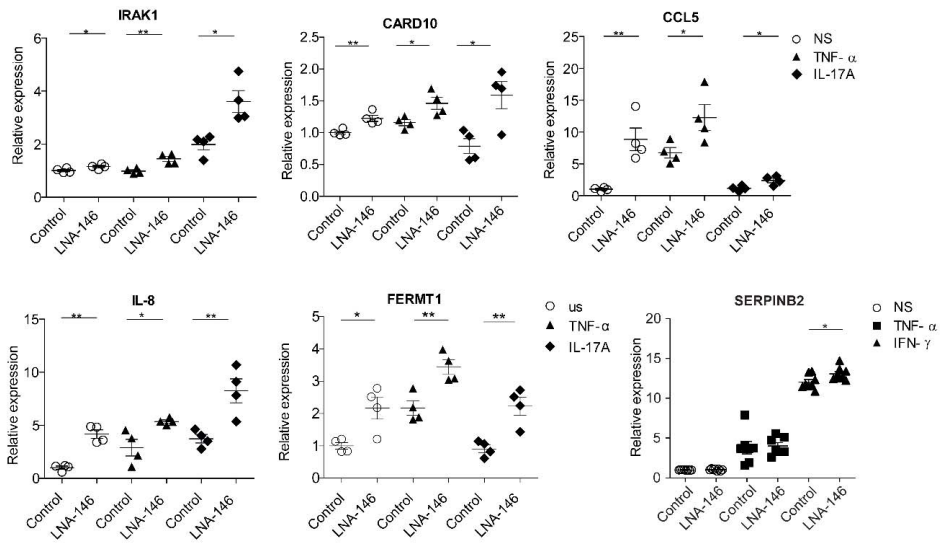

Figure 18. Inhibition of miR-146a/b endogenous levels leads to increased expression of psoriasis-related genes in keratinocytes. Keratinocytes were transfected with control LNA (Control) or LNA inhibitor for miR-146a/b (LNA-146) for 24 hours and then stimulated with shown cytokines for 48 hours or left nonstimulated (NS). Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

5.8. miR-10a is upregulated in the skin of AD patients and is downregulated in differentiated keratinocytes (Study II)

As previous miRNA profiling demonstrated miR-10a as one of the most highly upregulated miRNAs in keratinocytes from AD patients (Rebane et al., 2014), we next focused on the studies on the function of miR-10a. We first confirmed that miR-10a was upregulated both in the non-lesional and lesional skin of AD patients compared with healthy controls (Figure 19A). Further, *in situ* hybridization analysis demonstrated that miR-10a was more highly expressed in the epidermis and its expression was stronger in AD patients compared with healthy controls (Figure 19B). To delineate the factors that might induce miR-10a expression in the skin, we stimulated the keratinocytes with various pro-inflammatory cytokines as well as TGF-β and retinoic acid, which have been shown to regulate miR-10a expression in T cells and dendritic cells (Takahashi et al., 2012; Wu et al., 2015). As we could not detect cytokines to alter miR-10a levels in human primary keratinocytes (Study III, Figure S3. miR-10a-5p and miR-146a expression in stimulated KCs.) and *in situ* hybridization results suggested miR-10a to be increased in the basal layer, we next tested whether miR-10a expression was associated with different stages of keratinocyte differentiation. To investigate that, we differentiated keratinocytes in the presence of high levels of Ca^{2+} or grew the cells in a 3D model and used KRT10 as a marker of keratinocyte differentiation. Figure 19C, 19D demonstrates that in later time points when KRT10 levels were increased, miR-10a levels were decreased in both models. To investigate whether growth factors supporting the keratinocyte proliferation might play a role in regulating miR-10a levels, we stimulated proliferating and 3D keratinocyte cultures with heparin-binding EGF-like growth factor (HB-EGF). Indeed, miR-10a levels were increased in keratinocytes stimulated with HB-EGF compared with nonstimulated cells (Figure 19E, 19F). Together, these results demonstrate that miR-10a expression is increased in AD, in proliferating keratinocytes and in response to HB-EGF and is downregulated during differentiation of keratinocytes.

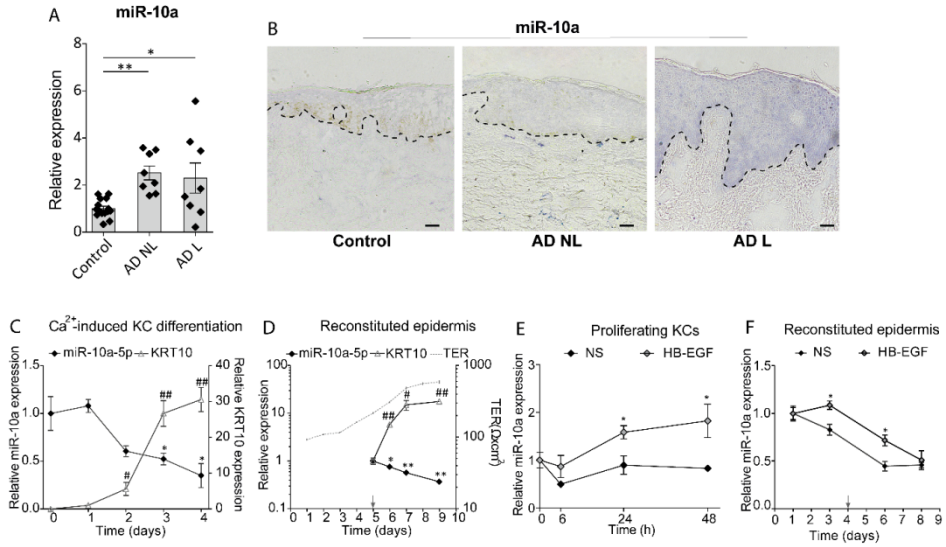

Figure 19. The expression of miR-10a in the skin and human primary keratinocytes. A,B) Relative expression of miR-10a by RT-qPCR **(A)** and *in situ* hybridization images **(B)** from nonlesional (AD NL) and lesional (AD L) skin of AD patients and healthy controls. **(B)** The expression of miR-10a appears in blue. Bar = 75 µm. The border between the epidermis and dermis is indicated with a dashed line. **C-F)** The expression analysis of miR-10a in monolayer and differentiation models. KRT10 was used as a differentiation marker. Data are shown as the mean ± SEM, n=4, student's t-test, *P < 0.05, **P < 0.01, #P < 0.05, ##P < 0.01. **C)** Keratinocytes were differentiated with high Ca^{2+} concentration. **D, F)** Reconstructed epidermis was used as a differentiation model. Cells were seeded on the membrane at day 0, and arrow indicates the time when the apical media was removed. Trans-epithelial electrical resistance (TER) was measured every 24 hours. In **F)**, the *in vitro* reconstructed epidermis was stimulated with HB-EGF or left nonstimulated (NS). **E)** The monolayer keratinocytes were stimulated with HB-EGF or left nonstimulated (NS).

5.9. miR-10a modulates cell cycle and inhibits proliferation (Study II)

To delineate the role of miR-10a in keratinocytes, we next analyzed a data set from the performed Illumina array analysis from the keratinocytes transfected miR-10 and control mimics and stimulated with IL-1β or left nonstimulated. 317 upregulated and 522 downregulated genes were detected in cells transfected with control and stimulated with IL-1β (differential P-value < 0.05) compared to cells transfected with control and left nonstimulated confirming the effect of IL-1β (Study III, Table S1. Top 200 genes induced by IL-1β). In the next step, we selected the top 200 genes downregulated by miR-10a-5p based on fold change in keratinocytes stimulated with IL-1β, of which only 14 genes were differentially expressed according to the selected P-value cut-off of 0.05. Considering that miRNA effects at a single gene level may be moderate, we included the full top 200 genes in the pathway analysis (Study III, Table S2. Top 200 genes downregulated by miR-10a-5p). The most prominent gene ontology functional group that was affected by miR-10a was cell cycle regulation with 63 downregulated genes (P-value of 7.0×10^{-18}) (Study III, Table S3. miRNA-10a-5p downregulates genes associated with cell cycle regulation in KCs). Out of these 63 genes, 13 were putative direct targets of miR-10a (Figure 20A) based on Targetscan (Agarwal et al., 2015; Lewis et al., 2005) prediction. As the array results revealed a strong potential miR-10a effect on cell proliferation, we next did a set of experiments with keratinocytes transfected with miR-10a or control mimic to assess the effect on proliferation. We first analyzed the cell cycle distribution based on DAPI, which revealed that in the cells transfected with miR-10, there were more cells in the G_1/G_0- and less in the S-phase compared with the control (Figure 20B). Concordantly, in 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay, fewer cells incorporated EdU and were in the S-phase when the cells were transfected with miR-10a (Figure 20C). Similarly, ATP-based proliferation assay revealed that increased levels of miR-10a inhibited cell proliferation while LNA-inhibition of miR-10a led to increased cell proliferation (Figure 20D). In line with this, the proliferation marker Ki-67 was downregulated in cells transfected with miR-10a in cells stimulated with IL-4, IL-17A, IL-1β, or left nonstimulated (Figure 20E). On the other hand, when the endogenous levels of miR-10a were inhibited, an increase in proliferation-associated marker Ki-67 levels was detected only in the case of IL-17A stimulations (Figure 20F). The levels of Ki-67 were also increased in the lesional skin of AD patients compared with healthy controls (Figure 20G). These results together show that miR-10a has the capacity to inhibit keratinocyte proliferation.

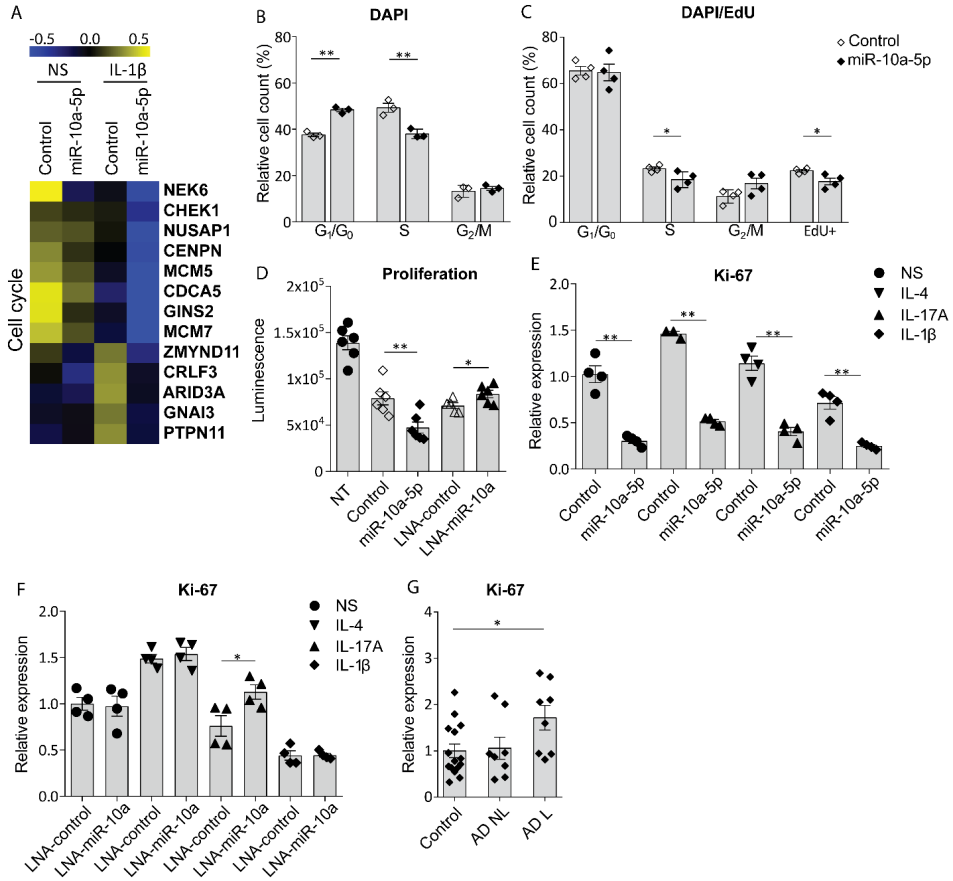

Figure 20. miR-10a inhibits keratinocyte proliferation. A-F) Keratinocytes were transfected either with miR-10a (miR-10a-5p) or control or miR-10a inhibitor (LNA-miR-10a) or control (LNA-control) for (A) 24 hours, (B, C, D) 48 hours or (E) 72 hours (A, C, E) and then stimulated with shown cytokines for additional 24 hours. **A)** Heat-map of miR-10a-influenced cell cycle associated predicted target genes. Log2 values of expression signals are mean-centered for each gene separately. The proposed direct targets of miR-10a are shown in bold. **B)** The cell cycle distribution based on the DAPI signal measured by flow cytometry. **C)** Cells were labeled with EdU for 2 hours before harvesting followed by measurement of DAPI and EdU signals by flow cytometry. **D)** CellTiter-Glo® Luminescent Cell Viability Assay. Nontrasnfected (NT) cells were included as an additional control. **E, F)** Relative Ki67 expression in transfected keratinocytes measured by RT-qPCR. **G)** Relative Ki67 expression in the nonlesional (AD NL) and lesional (AD L) skin of AD patients compared with the mean of healthy controls (Control) measured by RT-qPCR. **B-G)** Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01.

5.10. miR-10a has an anti-inflammatory effect in keratinocytes (Study II)

miR-10a has been suggested to regulate the NF-κB-mediated inflammatory pathway by targeting the IκB/NF-κB regulators MAP3K7 or βTRC in arterial endothelial cells (Fang et al., 2010). To confirm the effect of miR-10a on genes associated NF-κB pathway, we used the same experimental settings as for array analysis and analyzed mRNA levels of MAP3K7, CCL5, and IL-8 also by RT-qPCR. We were able to confirm the downregulation of MAP3K7, a previously known direct target of miR-10a, in keratinocytes transfected with miR-10a (Figure 21A) as well as two NF-κB-dependent chemokines IL-8 and CCL5 were downregulated in keratinocytes transfected with miR-10a and stimulated with IL-1β (Figure 21B). Based on our array data, cytokine signaling in the immune system (Figure 21C) appeared among other functional groups that were affected by miR-10a were in addition to cell cycle regulation cell adhesion, epithelium development, and abnormal skin morphology (Study III, Table S3. miRNA-10a-5p downregulates genes associated with cell cycle regulation in KCs). These results demonstrate that in addition to proliferation miR-10a is capable of suppressing the inflammatory responses of keratinocytes by inhibiting the NF-κB pathway leading to the reduced expression of chemokines IL-8 and CCL5.

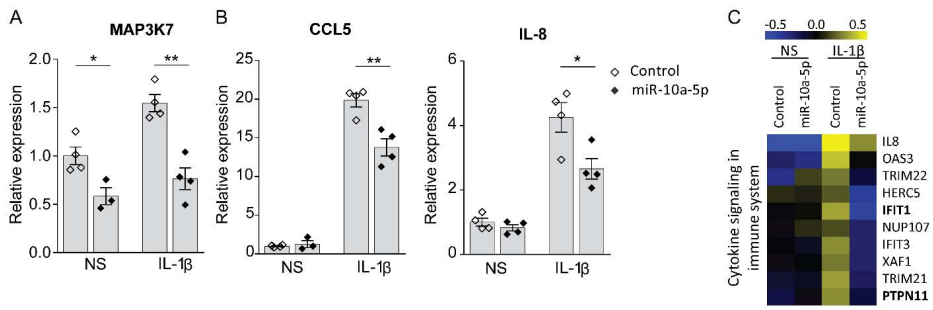

Figure 21. miR-10a has an anti-inflammatory effect in keratinocytes. Keratinocytes were transfected either with miR-10a (miR-10a-5p) or control for 24 hours and then stimulated with IL-1β or left nonstimulated for additional 24 hours. **A,B)** Relative MAP3K7, CCL5, and IL-8 expression in transfected keratinocytes measured by RT-qPCR. Data are shown as the mean ± SEM, student's t-test, *P < 0.05, **P < 0.01. **C)** Heatmap of miR-10a influenced genes from the cytokine signaling of the immune system pathway. Log2 values of expression signals are mean-centered for each gene separately. The proposed direct targets of miR-10a are shown in bold.

6. DISCUSSION

6.1. The levels of miR-146a/b are increased in PV and regulated differently in keratinocytes and fibroblasts

PV and AD are common chronic inflammatory skin diseases that have a high negative impact on patients' quality of life (Werfel et al., 2016b). AD is associated with other atopic diseases like allergic rhinoconjunctivitis, allergic bronchial asthma, and food allergy and as the patients have a systemic Th2 type inflammation. In addition, AD is associated with ulcerative colitis (Werfel et al., 2016a). PV on the other hand is associated with for example, metabolic syndrome and arthritis (Dainichi et al., 2018). However, in both diseases' patients suffer from chronic cutaneous inflammation manifesting in the form of skin lesions. PV and AD are considered diseases that are mediated by specific types of T cells and the cytokines they produce, while the activation of other immune cells and their substantial migration to the skin also contributes to the pathogenesis of both diseases (Guttman-Yassky & Krueger, 2017). The cytokines produced by immune cells activate various signaling pathways in keratinocytes leading to the changes seen in the epidermis, including epidermal hyperplasia, changes in keratinocyte differentiation, and parakeratosis, accounting for most of the overall PV and AD phenotypes (Guttman-Yassky et al., 2011). Therefore, the role of keratinocytes in the development of PV and AD cannot be overlooked.

In our study, we show the increased expression of miR-146a/b in the lesional skin of PV patients compared with healthy controls indicating that both miR-146a and miR-146b play a role in the pathophysiology of PV. This is in line with previously published results where the increased expression of miR-146a/b in PV skin has been shown (Lovendorf et al., 2015; Sonkoly et al., 2007). In line with these results is the notion that miR-146a deficiency leads to earlier PV onset and more severe skin inflammation in the imiquimod-induced model of PV compared with wild type mice (Srivastava et al., 2017). The increased miR-146a expression is not only characteristic of PV, it has been shown that miR-146a is also upregulated in the non-lesional and lesional skin of AD patients (Rebane et al., 2014). Additionally, the same study showed that miR-146a-deficient mice compared with wild type mice develop a stronger inflammation in a mouse model of AD (Rebane et al., 2014). This data demonstrates that miR-146a has an anti-inflammatory function in different chronic inflammatory skin diseases and not only PV.

When comparing the expression of miR-146a and miR-146b, we could identify that miR-146a expression is higher in healthy skin, keratinocytes, and PBMCs. However, in fibroblasts, the expression level of these two miRNAs is relatively similar. This indicated that there is a need to study these miRNAs in detail as they might have different functions in keratinocytes and fibroblasts.

When studying the localization of miR-146a/b in the skin of PV patients, we could detect the increased expression of miR-146a in the epidermis of PV patients indicating that even though the expression of miR-146a was higher in cultured fibroblasts, in disease conditions, miR-146a is more strongly upregulated in the keratinocytes. The expression of miR-146b was lower than miR-146a expression in healthy skin. Interestingly, in PV patients, the expression of miR-146b was higher in some cases in the dermis compared with epidermis. The levels of miR-146b might be dependent on the type of immune cells infiltrating the dermis and stage of the disease. In line with this result, in the previous finding, miR-146b was only upregulated in the reticular dermis of PV lesional skin compared with PV non-lesional skin but not in the epidermis (Lovendorf et al., 2015). This indicates that in the case of PV, miR-146b might have a more important role in in the dermis. However, future studies are needed to clarify the role of miR-146b in the dermis and in association with PV.

When fibroblasts and keratinocytes were stimulated with proinflammatory cytokines, the expression changes of miR-146a and miR-146b were observed to be different. miR-146a was upregulated in response to IL-17A, TNF-α, and IL-1β in fibroblasts and keratinocytes. miR-146b was differently regulated in keratinocytes and fibroblasts and in case of keratinocyte differentiation. In monolayer keratinocytes, the expression of miR-146b was upregulated in response to IFN-γ and in the 3D model in response to IFN-γ and IL-22. In addition, in fibroblasts, miR-146b was upregulated in response to IL-1β. The lack of effect of IL-22 on miR-146b in monolayer keratinocytes can be explained by the fact that the expression of IL-22 receptor components IL-10RB and IL22RA1 is low in proliferating keratinocytes (Study I, Figure S2. Relative expression of marker genes and transepithelial resistance measurements of keratinocyte 3D cultures). The effect of IL-17A, TNF-α, and IL-1β on miR-146a expression in keratinocytes was previously shown (Rebane et al., 2014). The mentioned cytokines will lead to the activation of NF-κB and MAPK pathways (Aggarwal, 2003; Li et al., 2019). Thus, our results confirm the previous studies demonstrating that miR-146a expression is induced trough the NF-kB pathway (Taganov et al., 2006). The effect of IFN-γ on miR-146b expression in keratinocytes is in line with the results that IFN-γ primarily activates the JAK/STAT pathway (Boehm et al., 1997). Previously, miR-146b was shown to be induced by STAT3/6 (Paterson & Kriegel, 2017). The lack of effect of IFN-γ on miR-146b expression in fibroblasts, however, is interesting, as fibroblasts are known also to express the IFN-γ receptor components even in nonstimulated conditions (Takaoka et al., 2000). Together these data show the need to study these miRNAs in parallel both keratinocytes and fibroblasts as they are regulated differently and might have specific functions in the dermis or epidermis and this could contribute to the pathogenesis of PV.

6.2. miR-146a/b suppress keratinocyte proliferation and may be the associated with PV

To further clarify the role of miR-146a/b in keratinocytes, we performed a pathway analysis of a previously published array data (Rebane et al., 2014) revealing that in nonstimulated keratinocytes, cell proliferation-related genes were enriched among the set of 102 genes suppressed by miR-146a. Concordantly, we observed that the overexpression of both miR-146a and miR-146b inhibited the proliferation of keratinocytes. It has been also shown that miR-146a–deficient mice have increased epidermal hyperproliferation and that miR-146-deficient mice have thicker epidermis compared with WT mice (Srivastava et al., 2017). These findings are in line with the effect of SNP rs2910164 in the coding region of the *MIR146A.* In the case of CG and GG alleles, there was an increased risk of PV and decreased levels of miR-146a that also leads to an increase of keratinocyte proliferation. The effect on keratinocyte proliferation was suggested to be mediated through the epidermal growth factor receptor (Zhang et al., 2014). We also performed genetic analysis using previous GWAS dataset. Interestingly, our *in silico* analysis showed evidence for the presence of two independent PV susceptibility loci in the *MIR146A* gene region rs2961920 (odds ratio = 1.12, P = 0.0015) 853 bp upstream from miR-146a encoding sequence and rs184776122 (odds ratio = 0.80, P = 0.005) 37 kb downstream of the *MIR146A* gene. The marker rs2961920 was in perfect linkage disequilibrium (r^2 = 1) with a functional polymorphism rs2910164 (Study I, Supplementary Table S4. In silico association analysis of genetic markers of *MIR146A* and *MIR146B*) that was shown to be associated with PV in the Han Chinese population (Zhang et al., 2014) and Caucasians (Srivastava et al., 2017). miR-146b has not been shown to be genetically associated with any skin diseases, however, there appears to be a link between miR-146b and gastric cancer: an SNP (rs1536309) 1066 bp upstream of the TTS of the miR-146b-5p precursor was found to be a prognostic factor for gastric cancer while individuals carrying the C allele were protected (Wang et al., 2018). Our results together suggest that both miR-146a/b have a role in regulating keratinocyte proliferation.

To better understand the effect of miR-146a in PV, we also analyzed the expression of previously characterized inflammatory-related targets (IRKA1, CARD10, CCL5, IL-8) as well as less known targets SERPINB2 and FERMT1, in the skin of PV patients. Interestingly, we found that only the expression of CARD10 was downregulated in the lesional skin of PV patients while the other targets, IRAK1, CCL5, IL-8, SERPINB2, and FERMT1 were upregulated in the lesional skin of PV patients. FERMT1 was chosen from the array data, and because it was one of the genes implicated in the regulation of cell proliferation (Duperret & Ridky, 2014; Herz et al., 2006) and it was also a previously uncharacterized target for miR-146a. Similarly, SERPINB2 contained a miR-146a/b putative binding site, as well as we expected it to have a functional influence on disease development because of its protease inhibitor activity (Kruithof et al., 1995). Also, the increased levels of SERPINB2 in PV (Lyons-

Giordano et al., 1994) and other inflammatory or autoimmune diseases, such as asthma (Woodruff et al., 2007) and lupus erythematosus (Bechtel et al., 1996) had been shown earlier. Additionally, CCL5 was shown to be also upregulated in AD (Rebane et al., 2012), IRAK1 is upregulated in PV (Xia et al., 2012) and IL-8 is known to be upregulated in both AD and PV (Nedoszytko et al., 2014). Thus, these proteins regulate inflammatory responses in different conditions and are not only specific for PV. Apart from CARD10, miR-146a and its putative target genes were significantly upregulated in diseased conditions indicting to a strong activation of inflammatory signaling pathways, including the NF-κB and JAK/STAT pathway. These results demonstrate that the increased expression of miR-146a in the skin of PV patients is not sufficient to fully suppress the inflammation during the disease.

Among miR-146a putative target genes, we considered FERMT1 important and performed several experiments to demonstrate miR-146a influence and to describe its function in association with PV. We show that the expression of FERMT1 in healthy skin was predominant in the basal layer. Interestingly, in the case of PV lesional skin, the expression of FERMT1 was detected also in other areas of the epidermis. This suggests that increased expression of FERMT1 in the skin is mostly due the changes in keratinocytes. We also showed that the expression of FERMT1 was suppressed by miR-146a on both mRNA and protein levels and confirmed that FERMT1 is a direct target of miR-146a. Previously, it was described that FERMT1 localizes only to the dermal-epidermal junction, especially in the basal keratinocyte layer (Herz et al., 2006; Siegel et al., 2003). Our result that FERMT1 is also expressed in the outer layers further indicates that it contributes to PV pathogenesis as it is known that in FERMT1 deficient skin, keratinocytes have reduced proliferation and increased apoptosis (Herz et al., 2006; Siegel et al., 2003). Additionally, mutations in the *FERMT1* gene were shown to cause Kindler syndrome, a rare autosomal recessive disorder that is characterized by blistering, photosensitivity, and cutaneous atrophy (Jobard et al., 2003; Siegel et al., 2003). Our data together suggest that one of the functions of miR-146a in PV could be the suppression of keratinocyte hyperproliferation by downregulating FERMT1, a known positive regulator of keratinocyte proliferation. However, as miR-146a similar to other miRNAs has many target genes, the anti-proliferative effect of miR-146a is most probably mediated through multiple targets. The identification of additional miR-146a/b targets could further deepen our understanding of the role these miRNAs play in the development of inflammatory skin diseases.

6.3. The function of SERPINB2 in keratinocytes and association with PV

When we carried out the analyses and experiments with miR-146a/b targets in association with PV, we also performed linear regression analysis to search for an association of miR-146a/b and the target gene expression (Study I, Figure

S3. Correlation analysis miR-146a/b and target genes in the skin of PV patients). Interestingly, none of the target genes showed a significant positive or negative relationship with miR-146a levels in the skin of PV patient, except for SERPINB2 which relative expression was in a negative relationship with miR-146a in the skin of PV patients. Going through the literature, we found that SERPINB2 a clade B or OVA-like serine protease inhibitor subgroup member of the serpin superfamily, which is also known as PAI2 and functions in its extracellular form as an inhibitor of extracellular protease urokinase plasminogen activator and tissue plasminogen activator (Kruithof et al., 1995). That function to convert plasminogen to plasmin which is a protease that is involved in the regulation of extracellular matrix degradation and cell migration (Schroder et al., 2014), and mediation of inflammation and tissue remodeling (Schuliga, 2015). Although shown to be overexpressed in PV and other diseased conditions like in the lesional epidermis of lupus erythematosus patients, (Bechtel et al., 1996; Gissler et al., 1993), the role of SERPINB2 in the skin is not so clear. Both extracellular and intracellular functions for SERPINB2 have been proposed in keratinocytes. In its extracellular form, SERPINB2 can inhibit keratinocyte proliferation by inhibition of urokinase plasminogen activator, which has a growth stimulatory effect (Hibino et al., 1999) and is proposed to protect the Stratum corneum in inflammatory conditions (Schroder et al., 2016). Its intracellular functions are less know and as it has been previously suggested that SERPINB2 is considered poorly secreted by keratinocytes (Jensen et al., 1995) and therefore might have intracellular functions in the skin (Risse et al., 2000)

To detect the expression of SERPINB2 in the skin, we performed immunofluorescence analysis and showed that SERPINB2 protein was detectable in the granular layer of the epidermis in healthy controls and PV patients' non-lesional skin. However, in the lesional skin of PV patients, it was visible that SERPINB2 expression was increased in the cornified layer and to a lesser extent all over the epidermis. As mentioned at the beginning of this chapter, in the case of SERPINB2, we detected a negative relationship between SERPINB2 mRNA and miR-146a expression levels in the lesional skin of PV patients. As further evidence, ISH of miR-146a and immunofluorescence analysis of SERPINB2 demonstrate that they both localize in the epidermis. However, although the 3´UTR of SERPINB2 does contains a binding site for miR-146a, we were not able to confirm SERPINB2 as a direct target of miR-146a in keratinocytes. As siRNA silencing of CARD10 and IRAK1 led to the downregulation of SERPINB2 (Study II, Figure 3. The expression of SERPINB2 is suppressed by miR-146a/b in human primary keratinocytes.), the effect of miR-146a on SERPINB2 expression may be mediated through NF-κB members IRAK1 and CARD10. Additionally, regression analysis between PASI and SERPINB2 mRNA expression in lesional skin samples from PV patients revealed a positive relationship between SERPINB2 mRNA levels and disease severity. This indicates that SERPINB2 might be potentially used as a biomarker of disease severity. Previously, it has been shown that SERPINB2 could be used as a

biomarker to help distinguish between different types of asthma and its severity (Baos et al., 2019; Baos et al., 2018).

During analysis, which cytokines are capable of modulating SERPINB2 expression in keratinocytes, we found TNF-α and IFN-γ as most prominent factors that enhanced both the cellular expression and amount of secreted SERPINB2. It should be noted, that the SERPINB2 levels in the supernatants were relatively high indicating that keratinocytes do secret SERPINB2 from the cells efficiently. Contradictory to our results, previous studies concerning skin had stated that SERPINB2 is poorly secreted by keratinocytes (Jensen et al., 1995). Interestingly, more recent results have shown that SERPINB2 is secreted from the cells in response to inflammatory stimuli and is predominantly in its nonglycosylated 43kDa form as it released from endothelial cells (Boncela et al., 2013). Among cellular pathways important to the expression activation of SERPINB2, most probably the JAK/STAT signaling pathway contributes as IFN-γ had a very strong positive effect on SERPINB2 levels in the keratinocytes. Additionally, it is known that the expression activation of SERPINB2 is also mediated through the activation of the NF-κB signaling pathway (Mahony et al., 1999).

To study SERPINB2 function in keratinocytes, we performed siRNA inhibition of SERPINB in keratinocytes, which interestingly led to the increased expression and secretion of IL-8 and CCL5. Thus, our results show that one function of SERPINB2 in the skin could be the regulation of cytokine levels of keratinocytes and through that influence the immune cell influx into the skin, thereby also participating in the regulation of PV related processes in the skin.

6.4. Overexpression of single miR-146 family member suppresses and downregulation increases PV associated target genes in keratinocytes

To assess whether miR-146b in addition to miR-146a has an influence on IRAK1, CARD10, CCL5, IL-8, FERMT1, and SERPINB2 expression in keratinocytes, we overexpressed both miRNAs in the human keratinocytes and observed similar effects, showing that miR-146a/b complement each other´s function in keratinocytes. Additionally, the inhibition of both miR-146a/b led to the increase in IRAK1, CARD10, CCL5, IL-8, FERMT1, and SERPINB2 confirming that miR-146 family members are crucial to avoid excessive inflammation in keratinocytes. Interestingly, keratinocytes from mice deficient in miR-146a or miR-146b responded differently to pro-inflammatory cytokines. In case of nonstimulated, TNF-α, or IL-17A stimulated cells, keratinocytes from miR-146a deficient mice had a stronger inflammatory response as seen by the increased expression of Card10 and Cxcl1 as well as a higher expression of Fermt1 compared with wild type mice. However, miR-146b deficient mice keratinocytes did not respond in a similar manner (Study I, Figure S10.

Deficiency of miR-146a or miR-146b results in the increased expression of the target genes). It is known, miR-146a expression in cells is induced by NF-κB pathway and miR-146b trough the STAT3/6 (Paterson & Kriegel, 2017). Altogether, this allows more robust repression of the NF-κB pathway as both miRNAs can suppress the targets to a similar extent. Interestingly, as miR-146a itself is upregulated by the NF-κB, it forms a negative feedback loop as after upregulation, it can suppress several members from the NF-κB pathway, which in keratinocytes include IRAK1, TRAF6, and CARD10 (Meisgen et al., 2014; Rebane et al., 2014). This leads to the decreased expression of several cytokines that are responsible for the increased expression of miR-146a itself, including TNF-α and IL-1β (Meisgen et al., 2014; Rebane et al., 2014). Additionally, miR-146b also inhibits the NF-κB pathway, therefore miR-146b supposedly influences miR-146a expression while there is apparently no direct effect of miR-146a on miR-146b expression. The different inflammatory responses in mice keratinocytes from miR-146a or miR-146b knock out mice show the need to distinguish miR-146a/b effects from each other as they could have independent roles in the disease progression or development. More studies are needed to distinguish miR-146a/b roles in the skin and in association with PV.

6.5. The levels of miR-10a are increased in AD patients and the regulation in keratinocytes

AD is a chronic inflammatory skin disease associated with barrier defects, changes in keratinocyte proliferation, and immune dysregulation (Weidinger & Novak, 2016). Keratinocytes play a role in the development and maintaining inflammation in the skin as keratinocytes from AD patients produce higher levels of growth factors (GM-CSF), chemokines (CCL5), and cytokines (TNF-α, TSLP) as compared to normal keratinocytes (Soumelis et al., 2002). Before our work, a few studies had shown by microarray analysis or sequencing the expression changes of miR-10a in skin diseases. It has been shown that miR-10a is downregulated in PV (Joyce et al., 2011; Sonkoly et al., 2007), in systemic scleroderma (Zhou et al., 2017) and upregulated in keratinocytes from AD patients (Rebane et al., 2014). We confirmed the upregulation of miR-10a in the lesional and nonlesional skin of AD patients. Also, *in situ* hybridization showed that miR-10a is expressed in the epidermis of the skin and is increased in the lesional skin of AD patients.

Our attempt to delineate the factors influencing the expression of miR-10a identified the growth factor HB-EGF capable of upregulating miR-10a expression in keratinocytes and additionally show that miR-10a expression is downregulated during keratinocyte differentiation. It has been previously shown that miR-10a expression is upregulated by growth-inducing factors TGF-β or retinoic acid (Takahashi et al., 2012) and downregulated by TNF and IFN-γ in human DCs (Wu et al., 2015). However, these growth-inducing factors and proinflammatory cytokines did not alter miR-10a expression in keratinocytes in our

case. Interestingly, HB-EGF is also known to be upregulated in the lesional skin of AD patients (Malaisse et al., 2014) and the excess release of HB-EGF as a result of inflammation and tissue stress may result in the pathological hyperplasia of cells (Takenobu et al., 2003). On the other hand, pro-HB-EGF has been shown to suppress cell proliferation (Iwamoto et al., 1999) and the expression of pro-HB-EGF is also increased in response to pro-inflammatory cytokines IL-1β and TNF-α (Yoshizumi et al., 1992).

6.6. The function of miR-10a in keratinocytes

As there was very little known about the functions of miR-10a in keratinocytes, we first carried out array analysis from the keratinocytes transfected with miR-10 mimics. Interestingly, the gene ontology functional group that was affected by miR-10a the most was cell cycle regulation. To delineate the role of miR-10a in regulating keratinocyte proliferation, we next performed a set of experiments where we overexpressed miR-10a in keratinocytes and measured the effect on cell cycle progression. A higher number of cells in the G_1/G_0-phase and less in the S-phase were detected indicating a slower cell cycle progression. Similarly, miR-10a inhibited the cell cycle progression of human cardiomyocyte progenitor cells (Liang et al., 2014). Additionally, our results demonstrated that miR-10a downregulated the cell proliferation marker Ki-67 in keratinocytes even when the cells were stimulated with pro-inflammatory cytokines, while Ki-67 was upregulated in the lesional skin of AD patients. However, miR-10a's effect on cell proliferation appears to depend on the cell type or disease. For example, miR-10a has been shown to be upregulated and enhances cell migration, invasion, and growth in solid tumors like lung cancer (Yu et al., 2015) and thyroid carcinoma (Hudson et al., 2013). Concordantly, miR-10a has been shown to act as a tumor suppressor in the case of many cancers, including esophageal squamous cell carcinoma (Liu et al., 2018) and human cervical cancer (Zhai et al., 2017).

In addition to cell cycle regulation, we confirmed the effect of miR-10a on cytokine signaling in the immune system in keratinocytes. We show that miR-10a downregulates MAP3K7, IL-8, and CCL5 in keratinocytes. It is known that miR-10a also targets MAP3K7 in human aortic endothelial cells and thereby affects the NF-κB signaling pathway (Fang et al., 2010). In human mesangial cells, it was shown that IL-8 is a direct target of miR-10a (Tangtanatakul et al., 2017). In conclusion, we show for the first time that miR-10a inhibits cell cycle progression in keratinocytes and additionally affects cytokine signaling by regulating IL-8 and CCL5 in keratinocytes. Thus, the role of miR-10a in AD might be to control of proliferation of keratinocytes and to dampen the excessive inflammation.

6.7. General remarks and further studies

miR-10a and miR-146a/b are not considered skin-specific miRNAs, however, they are still relatively highly expressed in the skin. Based on previous report, miR-10a has the highest expression in healthy skin followed by miR-146a and then miR-146b (Sahmatova et al., 2016). We identified miR-146a/b and miR-10a to be expressed in the epidermis, and therefore, these three miRNAs can affect the development of PV and AD through their functions in keratinocytes. Interestingly, miR-146a/b and miR-10a seem to have similar functions in keratinocytes as they suppress inflammation and proliferation of the cells. The roles of miR-146a/b and miR-10a and targets are outlined in figure 22.

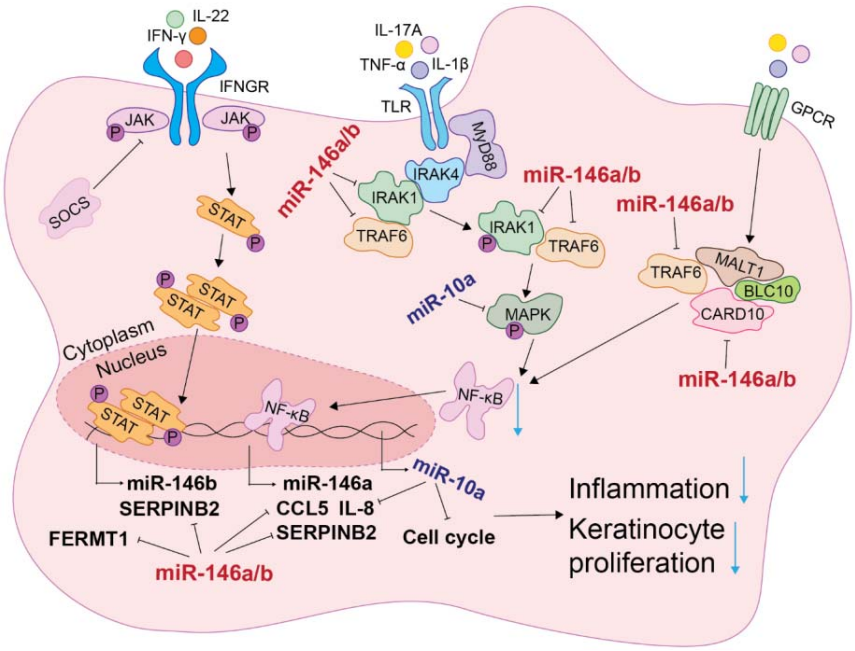

Figure 22. The function of miR-146a/b and miR-10a in keratinocytes. miR-146a/b and miR-10a are transcribed by independent genes with different genomic locations. miR-146a expression is upregulated in response to NF-κB activation and miR-146b is regulated by STAT3/6 in response to specific cytokines (Taganov et al., 2006) (Paterson & Kriegel, 2017). miR-10a expression is regulated by keratinocyte differentiation and the growth factor HB-EGF. miR-146a/b and miR-10a suppress multiple targets in keratinocytes including members of the NF-κB pathways leading to the reduced activity of NF-κB, indicated by the blue arrow (Meisgen et al., 2014; Rebane et al., 2014). miRNA target genes are prone to be enriched in particular biological pathways in order to stabilize the gene regulatory networks (Bracken et al., 2016). miR-146a/b both target SERPINB2, IRAK1, CARD10, CCL5, IL-8 and FERMT1. miR-10a targets MAP3K7, CCL5, and IL-8 in keratinocytes. Together miR-146a/b and miR-10a inhibit inflammation and proliferation of keratinocytes. Indicated by the blue arrow.

However, based on microarray analyses of the influenced genes, miR-146a/b have a significant effect on inflammation, and miR-10a influences more cell proliferation. It should be noted, that as our study was one of the first studies to show miR-10a function in the skin, further analysis of miR-10a function in AD mouse models would help to give deeper insight into the role of miR-10a in the development of AD. Still, based current knowledge that increased cell proliferation and skin inflammation are known features of both PV and AD (Boehncke & Schon, 2015; Weidinger & Novak, 2016), we conclude that miR-146a/b and miR-10a affect essential disease-associated processes in the skin.

As a result of the activation of the NF-κB pathway during inflammatory skin diseases, such as PV and AD, the expression of miRNAs themselves is changed. For example, miR-146a/b are highly upregulated in PV, however, miR-10a is downregulated (Sonkoly et al., 2007; Srivastava et al., 2019). In the case of AD, miR-10a and miR-146a are upregulated (Rebane et al., 2014; Sonkoly et al., 2007). This indicates that the changes in miRNA expression levels can be disease-specific and could be used as biomarkers to distinguish between different diseases, as it has been also shown for PV and different types of skin cancer (Lovendorf et al., 2014; Neagu et al., 2020). Moreover, considering the results from the functional studies demonstrating that miR-10a and miR-146a inhibit inflammatory responses and keratinocyte proliferation, we propose that overexpression of these miRNA may have therapeutic effect in case of inflammatory skin diseases. Indeed, it was shown that the subcutaneous pre-administration of cell penetrating peptide Pepfect6-miR-146a nanocomplexes alleviated the inflammation in a mouse model of irritant contact dermatitis indicated by the reduced expression of pro-inflammatory cytokines and attenuated ear-swelling (Urgard et al., 2016). In association with PV, it has been shown that the intradermal administration of miR-146a mimics alleviated imiquimod-induced psoriasiform skin inflammation indicated by the reduced epidermal thickening, decreased numbers of proliferating keratinocytes, and infiltrating neutrophils (Srivastava et al., 2017). Future studies will bring additional knowledge on the field of miRNA studies and eventually may lead to the development of miRNA-based therapeutics for various conditions, including chronic inflammatory diseases.

7. CONCLUSIONS

1. The expression of miR-146a/b and their target genes, IRAK1, CCL5, IL-8, and FERMT1 was shown to be increased while CARD10 was decreased in the lesional skin of PV patients compared with healthy controls. miR-146a/b suppress the proliferation of keratinocytes most probably through multiple targets, among which we characterize novel direct target FERMT1. Similarly to miR-146a, miR-146b suppresses the inflammation in keratinocytes by downregulating inflammation-related targets IRAK1, CARD10, CCL5, and IL-8.
2. The increased expression of SERPINB2 in the lesional and non-lesional skin of PV patients was shown at mRNA and protein levels. A positive relationship between PV severity and relative SERPINB2 mRNA expression and a negative relationship between the relative level of miR-146a and SERPINB2 mRNA were detected. In keratinocytes stimulated with IFN-γ and TNF-α, SERPINB2 mRNA, as well as intracellular and secreted protein levels, were significantly increased. miR-146a was shown to suppress SERPINB2 mRNA and protein levels indirectly. The inhibition of SERPINB2 increased the expression and protein levels of CCL5 and IL-8, thus indicating that although miR-146a has the capacity to suppress SERPINB2, they both possess anti-inflammatory influence and may act synergistically.
3. The increased expression of miR-10a in the lesional skin of AD patients was demonstrated. By ISH, miR-10a was shown to localize to the epidermis and there was increased expression of miR-10a in AD lesional skin. The expression of miR-10a was shown to be dependent on keratinocyte differentiation and was upregulated by HB-EGF. The array analysis, cell cycle measurements, and proliferation assays using miR-10a-transfected keratinocytes demonstrated that miR-10a hinders the progression of the cell cycle and thereby inhibits keratinocyte proliferation. Additionally, miR-10a moderately inhibits inflammation-associated genes in keratinocytes.

8. SUMMARY IN ESTONIAN

MikroRNA-d keratinotsüütide rakulise vastuse reguleerimisel naastulise psoriaasi ja atoopilise dermatiidi korral

Nahk on meie organismi suurim organ, mille põhifunktsiooniks on kaitsta meid väliskeskkonna mõjude, sealhulgas erinevate patogeenide eest. Oma funktsioonide täideviimiseks on nahal kolm kihti: epidermis, dermis ja hüpodermis. Epidermis jaguneb omakorda neljaks: basaalkiht, ogakiht, sõmerkoht ja sarvkiht. Keratinotsüüdid on epidermise peamine raku tüüp ja moodustavad 90% kõigist epidermise rakkudest. Jagunemisvõimelised keratinotsüüdid paiknevad epidermise basaalkihis ning edasisel diferentseerumisel migreeruvad nad välimistesse kihtidesse moodustades epidermise pindmised kihid. Keratinotsüütide proliferatsiooni kontroll on oluline epidermise struktuuri ja funktsiooni säilitamiseks. On teada, et suurem osa basaalkihi keratinotsüüte on tegelikult G0/G1 faasis ja ei jagune. Keratinotsüütide hüperproliferatsioon on paljude krooniliste põletikuliste nahahaiguste üheks põhiliseks tunnuseks. Lisaks oma põhifunktsioonile olla füüsiliseks barjääriks organismi ja keskkonna vahel, vahendavad keratinotsüüdid signaale keskkonnast imuunrakkudele ning seetõttu reguleerivad nii kaasasündinud kui ka omandatud immuunvastuseid. Häired keratinotsüütide signaliseerimises võivad olla seotud või olla üheks põhjuseks erinevate nahahaiguste, näiteks atoopiline dermatiidi ja psoriaasi puhul.

Psoriaas ja atoopiline dermatiit on kroonilised põletikulised nahahaigused ning on suhteliselt sagedased üldpopulatsioonis. Psoriaasi sagedus Euroopas ja Ameerika Ühendriikides on keskmiselt 2–3%. Kõige levinum psoriaasi vorm on naastuline psoriaas (*psoriaasis vulgaris*), mis moodustab ligi 90% kõigist juhtudest. Naastulist psoriaasi iseloomustavad sümmeetriliselt paiknevad punetavad infiltreeritud ja ketendavad naastud juustega kaetud peanahal ja jäsemete sirutuspindadel. Samas võib löövet olla ulatuslikult üle kogu keha ja haigel võib kujuneda erütrodermia, kus lööbest on haaratud rohkem kui 80% kehapinnast. Lisaks kvõib kaasneda nahalööbele psoriaatiline küünte- ja liigeskahjustus. Atoopilise dermatiidi sagedus on täiskasvanutel 2–5% ja lastel isegi kuni 20%. Atoopilist dermatiiti iseloomustab piinavalt sügelevate, erütematoossete, lihheniseeritud kollete esinemine näol ja jäsemete painutuspindadel, samas analoogselt psoriaasiga patsientidele võib lööve esineda generaliseeritult üle kogu keha. Tüüpiliselt on ka kahjustatud patsientide nahabarjäär. Mõlemal haigusel on geneetiline eelsoodumus, kuid ka keskkonna faktorid mängivad rolli haiguste avaldumises. Samuti mängivad mõlema haiguse patogeneesis olulist rolli keratinotsüüdid, mis mõjutavad erinevaid haiguse faase läbi tsütokiinide tootmise ja oma pinnaretseptorite ekspressiooni. Selle tulemusena suureneb immuunrakkude liikumine nahka ning kujuneb psoriaasile või atoopilisele dermatiidile vastav iseloomulik immuunkeskkond nahas.

miRNA-d on lühikesed üheahelalised mittekodeerivad RNA molekulid, mis reguleerivad geenieksprессiooni transkriptsiooni järgselt. miR-146a ja miR-

146b (miR-146a/b) kuuluvad miR-146 perekonda ja kuna nad erinevad 3' alas ainult kahe nukleotiidi poolest, siis arvatakse, et nad inhibeerivad samu geene. miR-146a/b funktsiooni on eelnevalt uuritud nahas seoses põletikuliste protsessidega ja on näidatud, et nad on võimelised inhibeerima mitmeid NF-κB signaaliraja liikmed. miR-10a kuulub miR-10 perekonda ning selle funktsioon on nahas suhteliselt tundmatu. miR-10a funktsioone on enim uuritud seoses organismide arenguprotsesside ja rakkude diferentseerumisega, eelkõige seoses erinevate vähkkasvajatega. Antud töös uurisime miR-146a/b ja miR-10a ekspressiooni nahas ja nende funktsioone keratinotsüütides seoses vastavalt psoriaasiga ja atoopilise dermatiidiga.

Uurimistöö eesmärgid:
Käesoleva uuringu eesmärkideks oli iseloomustada miR-146a/b ja miR-10a rolle keratinotsüütides ning seda, kuidas need aitavad kaasa psoriaasi ja atoopilise dermatiidi arengule.

Uuringu täpsemateks eesmärkideks olid:

– Teha kindlaks, kas miR-146a/b ja sihtgeenide ekspressioon on muutunud naastulise psoriaasi patsientide nahas võrreldes kontrollproovidega ning analüüsida kas ja kuidas miR-146a/b mõjutavad psoriaasiga seotud protsesse, sealhulgas proliferatsiooni ja rakulisi vastuseid, keratinotsüütides.
– Selgitada välja oletatava miR-146a/b sihtgeeni, SERPINB2, ekspressiooni regulatsiooni mõjutavad faktorid ja tema funktsioonid keratinotsüütides seoses psoriaasiga.
– Kirjeldada miR-10a ekspressiooni atoopilise dermatiidi haigete nahas ning uurida miR-10a ekspressiooni regulatsiooni ja funktsioone keratinotsüütides ja seoses atoopilises dermatiidiga.

Materjal ja meetodid:
Antud töös kasutati naha proove, mis olid kogutud Tartu Ülikooli Nahahaiguste kliinikus ja Kieli Ülikooli haiglas Saksamaal. Kõiki uuringus teostatud katsed, kus kasutati patsientidelt ja tervetelt vabatahtlikelt pärinevaid materjale, on heaks kiidetud vastavate asutuste eetikakomiteede poolt. Samuti informeeriti kõiki doonoreid eelnevalt katse eesmärkidest ja kõigilt koguti allkirjastatud informeeritud kirjalikud nõusolekuvormid. Kogutud nahaproovid külmutati ja hoiustati RNA eraldamiseni või koelõikude valmistamiseni -80º C juures. Uuringutes I ja II värvati kokku 27 naastulise psoriaasi patsienti ja 27 kontrolli RT-qPCR, korrelatsiooni analüüside, *in situ* hübridisatsiooni ja immuunfluorestsents analüüside jaoks. Patsientide vanus varieerus 21st 65 aastani (keskmine 48); 27st patsendist 9 olid naised ja 18 mehed. PASI väärtus varieerus 7–33ni. Uuringus III kasutati 10 atoopilise dermatiidi patsiendi naha biopsiat. Atooplise dermatiidi patsientidest 5 olid naised ja 5 mehed vanuses 22 kuni 58. Lisaks kasutati 15 sobiva soo ja vanusega kontrollindiviidi (7 meest ja 8 naist vanuses 20 kuni 59). Antud proovides analüüsiti miR-146a/b ja miR-10a ning CARD10, IRAK1, CCL5, IL-8, FERMT1, SERPINB2, Ki67 ja MAP3K7 mRNA ja/või valgu ekspressiooni.

Selgitamaks välja kas miR-146a/b võib olla seotud psoriaasi patogeneesiga, kasutati rakukultuuride mudeleid. Kõigepealt stimuleeriti naha primaarseid keratinotsüüte ning fibroblaste psoriaasiseoseliste tsütokiinidega (IFN-γ, TNF-α, IL-1β, IL-17A ja IL-22) ning analüüsiti miR-146a/b ekspressiooni. miR-146a/b mõju proliferatsioonile kindlaks tegemiseks transfekteeriti miRNA-de prekursorid või inhibiitorid keratinotsüütidesse ning hinnati mõju proliferatsioonile ja eluvõimele. Kasutades proliferatsiooni hindamiseks radioaktiivselt märgistatud tümidiini ning eluvõime hindamiseks CellTiter-Glo® Luminescent Cell Viability analüüsikomplekti, mis kvantifitseerib rakkudes olevat ATP-d. Samuti analüüsiti geenide CARD10, IRAK1, CCL5, IL-8, FERMT1, SERPINB2 ekspressiooni RT-qPCR-ga.

miR-146a mõju uurimiseks FERMT1 mRNA ja valgu ekspressioonile, transfekteeriti keratinotsüüdid miR-146a prekursoriga ja analüüsiti FERMT1 taset nii mRNA tasemel kasutade RT-qPCRi, kui ka valgu tasemel kasutades western blotti. Tuvastamaks, kas FERMT1 on miR-146a otsene sihtmärk, kloneeriti FERMT1 3'UTR fragmendid, mis sisaldasid kas miR-146a või muteeritud miR-146a seondumissaiti, pmirGLO Dual-Luciferase ekspressiooni vektorisse ning mõõdeti kasutades Dual-Luciferase® Reporter Assay süsteemi *firefly* ja *renilla* lutsiferaasi aktiivsust.

SERPINB2 funktsiooni uurimiseks stimuleeriti keratinotsüüte põletikku soodustavate tsütokiinidega ja analüüsiti SERPINB2 taset rakkudes mRNA tasemel kasutades RT-qPCR-i ja valgu tasemel kasutades immuunfluorestsentsanalüüsi ja ELISA-t. Lisaks pärsiti endogeense SERPINB2 avaldumist siRNA-de abil, misjärel analüüsiti IL-8 ja CCL5 mRNA ja valgu tasemeid kasutades esimesel juhul RT-qPCR-i ja teisel juhul ELISA meetodit.

miR-10a ekspressiooni regulatsiooni uurimiseks kasvatati inimese primaarseid keratinotsüüte nii 2D kui ka 3D kultuuris ning stimuleeriti HB-EGF-iga, et hinnata keratinotsüütide diferentseerumise mõju miR-10a ekspressioonile. miR-10a taset analüüsiti RT-qPCR-ga. miR-10a funktsioonide kindlaks tegemiseks transfekteeriti keratinotsüütidesse miR-10a prekursorid ja hinnati mõju transkriptoomile mRNA kiibil. Järgmisena transfekteeriti keratinotsüütidesse miR-10a prekursorid ja inhibiitorid ning analüüsiti mõju rakkude proliferatsioonile kasutades läbivoolutsütomeetriat ning hinnati proliferatsiooni markergeeni Ki67 ekspressiooni RT-qPCR-i abil. Lisaks analüüsiti geenide CCL5, IL-8 ja MAP3K7 ekspressiooni RT-qPCR-iga.

Uurimistöö peamised tulemused ja järeldused:

Antud töös tegime kindlaks, et miR-146a/b ja nende sihtmärkgeenide IRAK1, CCL5, IL-8 ja FERMT1 ekspressioon oli kõrgem, samal ajal kui CARD10 ekspressioon oli madalam psoriaasi patsientide lööbelises nahas. Samuti näitasime, et miR-146a/b ekspressioon suureneb keratinotsüütides ja fibroblastides vastusena põletikulistele tsütokiinidele. Leidisime, et miR-146a/b surub alla keratinotsüütide proliferatsiooni tõenäoliselt läbi mitme sihtgeeni, mille hulgas iseloomustasime uue miR-146a otsese sihtgeeni FERMT1. Sarnaselt miR-146a-le pärsib miR-146b ka keratinotsüütides põletikuliste protsesside ning prolife-

ratsiooniga seotud sihtgeenide IRAK1, CARD10, CCL5, IL-8, SERPINB2 ja FERMT1 avaldumist, kusjuures miR-146a/b endogeense taseme allasurumisel oli samade sihtgeenide tase kõrgem.

Teiseks uurisime antud töös SERPINB2 funktsioone keratinotsüütides seoses psoriaasiga. Tuvastasime SERPINB2 suurenenud ekspressiooni psoriaasi patsientide nahas mRNA ja valgu tasemel, avastasime positiivne seose psoriaasi raskusastme ja SERPINB2 mRNA suhtelise ekspressiooni vahel ning negatiivse seose miR-146a ja SERPINB2 mRNA suhteliste tasemte vahel. Leidsime, et IFN-γ ja TNF-α-ga stimuleeritud keratinotsüütides tõusis märkimisväärselt SERPINB2 mRNA ning ka rakusisese ja sekreteeritud valgu tase ning et miR-146a vähendab nendes tingimustes SERPINB2 avaldumist. Lisaks näitasime, et SERPINB2 endogeense taseme vähendamine suurendas CCL5 ja IL-8 mRNA ekspressiooni ja valgu taset.

Töö viimases osas tuvastasime miR-10a suurenenud ekspressiooni atoopilise dermatiidi patsientide nahas ning näitasime, et miR-10a lokaliseerub eelkõige epidermises. Leidsime, et miR-10a ekspressioon väheneb keratinotsüütide diferentseerumisel ja HB-EGF stimuleerib miR-10a ekspressiooni nii proliferee-ruvates kui ka diferentseeruvates keratinotsüütides. Lisaks näitasime, et miR-10a on võimeline keratinotsüütides takistama rakutsükli kulgu ja pärsib seeläbi keratinotsüütide proliferatsiooni. miR-10a inhibeeris ka mõõdukalt põletikuliste protsessidega seotud geene MAP3K7, IL-8 ja CCL5 keratinotsüütide.

Kokkuvõttes näitavad meie tulemused, et miR-146a/b on võimelised suruma alla proliferatsiooni ja põletikulisi protsesse keratinotsüütides, kuid miR-146a/b kõrgem ekspressioon psoriaasihaigete nahas ei ole suuteline ära hoidma sihtmärkgeenide ekspressiooni tõusu ja haigusenähte. Lisaks näitasime, et SERPINB2 omab võimet pärssida põletikulisi protsess keratinotsüütides. Samuti viitavad meie tulemused, et kuigi miR-146a inhibeerib SERPINB2 ekspressiooni on neil mõlemal põletikuvastane mõju ja nad võivad toimida keratinotsüütides ja psoriaasi haigete nahas sünergiliselt. Oma töö kolmandas artiklis leidsime, et miR-10a tase on atoopilises dermatiidis tõusnud ja mõjutab haigusseoselisi protsesse, nagu seda on proliferatsioon ja põletik, kuid tema taseme tõus atoopilise dermatiidi patsientide nahas ei ole piisav, et ära hoida atoopilise dermatiidi teket. Meie tulemused viitavad, et miR-146a/b ja miR-10a lokaalne manustamine võiks olla üks potentsiaalseid terapeutilisi võimalusi põletikuliste nahahaiguste korral.

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***

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Publications

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Tunnustused

2018	Valda ja Bernard Õuna mälestusfondi stipendium
2017	Ida-Euroopa dermatoloogia-alase teadustöö noore teadlase preemia uurimustöö "*SERPINB2 is regulated by the NF-kappa B pathway and miR-146a in human primary keratinocytes and psoriasis*" eest

Publikatsioonid

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