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Summary and perspectives
Chapter 8

Summary

Atopic dermatitis (AD) is a common inflammatory skin disease that is clinically characterized by a broad spectrum of manifestations. Clinically, AD skin is generally characterized by dryness (xerosis), itch (pruritis) and redness (erythema) and frequently present eczematous lesions. It is in particular the highly pruritic lesions that have a severe impact on the quality of life of the patients. In addition to the skin problems, a large group of the AD patients progresses into a secondary disease, such as allergic asthma and hay fever. AD is the result of interaction between susceptibility genes and the host environment. Important characteristics are an impaired skin barrier function and both local as well as systemic immunological abnormalities. An impaired barrier function is present in both lesional (affected) and non-lesional (less affected and normal appearing) skin of AD patients, and is illustrated by increased transepidermal water loss (TEWL), reduced stratum corneum (SC) hydration and increased SC permeability. The immunological abnormalities comprises changes in the innate immune response, e.g. increased expression of pro-inflammatory cytokines such as TSLP, and in the adaptive response, e.g. increased presence of T-helper 2 (Th2) cells. Initially, barrier dysfunction and the immunological abnormalities were proposed to be separate mechanisms of which either one was the initiating cause for AD. However, recent studies imply that there is interaction between these two mechanisms, e.g. cytokines have been shown to affect the SC lipid composition.

Increased prevalence of AD in industrialized countries and the likelihood of progression of AD into allergic rhinitis and asthma are becoming an emerging problem. The current treatments for AD are mainly aimed at skin inflammation and barrier restoration and/or consist of anti-microbial approaches.

Anti-inflammatory therapy targets the cutaneous inflammation in AD, which is crucial since inflammation is one of the major contributors to the symptoms and complications of AD. Barrier restoration includes application of moisturizers, to reduce the transepidermal water loss, or lipid enriched creams to restore the lipid deficiencies in AD and thereby improve the skin barrier function. Anti-microbial treatments are in particular aimed at Staphylococcus aureus, since almost all AD patients have an increased susceptibility to S. aureus colonization and recurrent infections by this bacterium. Whereas most of the available anti-inflammatory treatments are aimed at reduction of the inflammation in a systemic manner, less therapies are present that aim directly at restoration of the epidermis. Due to the limited efficacy as well as the adverse effects of the current treatments, there is much room for improvement. In particular novel treatments targeting S. aureus are desired, since increased bacterial colonization is a major problem in AD, as well as the emerging increase in resistance of these bacteria against antibacterial treatments.
Whereas much of our understanding about AD and its pathogenesis is obtained using various mouse models, their applicability for screening of newly developed compounds is limited due to the large differences in both skin morphology as well as skin barrier function between mouse and human skin. These differences have often resulted in difficulties in the translation of the obtained results towards humans and patients. As an alternative, in vitro three-dimensional human skin equivalents (HSEs) for AD (AD-HSEs) can be useful tools for increasing our understanding of AD pathogenesis, as well as for screening of new therapeutic compounds and pharmacological studies. Therefore, as an initial step, the primary aim of the studies described in this thesis was to develop reproducible novel HSEs that mimic important characteristics of AD. In the future, these HSEs might serve as a potential tool to screen newly developed compounds or formulations for the treatment of patients.

**Filaggrin mutations and their role in AD**

The discovery of the strong association between filaggrin (FLG) mutations and AD was a breakthrough in AD research, and resulted in an increased focus on the epidermal skin barrier and the role of FLG therein. Moreover, currently attempts are made in which FLG is used as a target for protein replacement therapy. However, the precise role of FLG in AD and why FLG mutations are a predisposing factor for AD are currently still not fully understood. The frequently used flaky tail (ft) mouse model contains a homozygous mutation in Flg and mimics AD in this regard. However its translation towards humans remains rather difficult, due to aforementioned differences between mice and man as well as the presence of other mutations, which were recently shown to be the cause for the barrier defects in these mice. To study the role of FLG in various aspects of AD in vitro, several HSEs have been developed in the research that is described in this thesis, which were used to address the following questions:

- Does reduced FLG expression result in a defective skin barrier?
- What is the effect of reduced FLG expression on epidermal *S. aureus* colonization?
- Does an explant HSE recapitulate all characteristics of the original AD biopsy in the presence or absence of FLG mutations?
- Does cytokine supplementation mimic lesional AD in a human skin equivalent?

The different HSEs and their application to address these questions are described in this thesis and are summarized below.
Establishing a reproducible HSE using a keratinocyte cell line

HSEs are usually established using primary keratinocytes that are obtained from surplus skin. These HSEs mimic human skin in many aspects including morphology, expression of various differentiation markers and several important aspects of the skin barrier\textsuperscript{31}. However, primary keratinocytes have several drawbacks, including a limited in vitro lifespan, large donor-to-donor variation and their limited availability. To overcome these limitations, immortalized cell lines can be an attractive alternative. Such cell lines provide a homogeneous and unlimited source of cells. However, their suitability to establish a functional HSE is not always evaluated. Many studies use the spontaneously immortalized HaCaT cell line to study epidermal biology, but HSEs established with these cells display a disturbed differentiation which probably resulted in poorer stratum corneum barrier properties\textsuperscript{32}. Although improved culture conditions have resulted in an improved epidermal organization of HaCaT-based HSEs, the SC barrier properties of these improved HaCaT-based HSEs have not been evaluated\textsuperscript{33}. In addition to HaCaT cells, another spontaneous keratinocyte cell line, NIKS\textsuperscript{®}, is currently available. These cells have been shown to be applicable for establishing reproducible HSEs\textsuperscript{34}. Nonetheless, these HSEs have not been evaluated for their SC properties.

Besides spontaneous immortalization, genetic engineering of keratinocytes can be used to generate cell lines. An example of such a genetically engineered cell line is the N/TERT cell line, which expresses hTERT to prevent telomere shortening and lack the p16\textsuperscript{INK4a} cell cycle control mechanism\textsuperscript{35}. In the studies described in this thesis, N/TERT cells were used to establish N/TERT based skin equivalents (NSEs), in order to establish a novel and reproducible skin equivalent. These NSEs were studied in great detail for their SC barrier properties.

The results described in chapter 2 indicate that these NSEs display many similarities to full-thickness (FT)-HSEs that are established with primary keratinocytes. NSEs have a comparable epidermal morphology and the expression of collagen IV, keratin 10, loricrin and filaggrin in NSEs was comparable to that of FT-HSE established with primary keratinocytes. In addition, NSEs display a comparable SC lipid organization and contain all 12 CER subclasses and a comparable distribution of ceramides, free fatty acids and cholesterol. Furthermore, the SC permeability for the lipophilic compound butyl para-aminobenzoic acid (butyl-PABA) was similar between both skin equivalents. However, some differences in the composition of one of the three main SC lipid classes, the ceramides, were observed, which may affect the outcome of topical application studies.

Besides N/TERT cells, genetic engineering of keratinocytes might provide novel and unlimited sources of keratinocytes, which can be used to establish improved HSEs that mimic features of native human skin even more closely. Additionally, the uniformity of keratinocyte cell lines allows a specific investigation of the role of different fibroblasts on epidermal morphogenesis through elimination of the keratinocyte donor differences.
Establishing a reproducible AD-HSE through FLG-knockdown

In addition to its reproducibility when used for generation of HSEs, cell lines are also convenient for transfection experiments. Transfection of immortalized cells results in novel genetically engineered cell lines that can be used for the generation of reproducible HSEs that mimic genetic features of skin diseases. To establish a FT-HSE that mimics the FLG mutations as seen in a large group of AD patients, N/TERT cells were transfected with shRNA to induce filaggrin knockdown (FLG-KD). This approach resulted in reduced FLG expression in these cells, which were subsequently used to establish the FT-HSEs that are described in chapter 3. This study revealed that this approach resulted in reduced FLG mRNA and protein expression in FT-HSEs after 14 days of air-exposed culturing. The reduction in FLG expression due to FLG-KD did not affect epidermal morphology, i.e. all four epidermal strata were present after FLG-KD. Furthermore, FLG-KD did not affect the expression of the early differentiation marker keratin 10, and the expression of the late differentiation marker loricrin. Evaluation of the SC barrier properties indicated that FLG-KD did not affect various SC lipid properties, i.e. the SC lipid composition, lipid organization and the SC permeability for a lipophilic compound were not affected. Based on these observations, the conclusion was drawn that FLG alone cannot be the sole contributing factor for the impaired barrier function as seen in AD.

An AD-HSE established by outgrowth of biopsies from AD patients

Besides using isolated primary keratinocytes or a keratinocyte cell line to establish HSEs, biopsies can be used as an alternative source. By placing small biopsies onto a fibroblast populated collagen matrix, keratinocytes from the biopsy are able to grow out and form an epidermis and thereby establish an explant HSE (Ex-HSE). In chapter 4, a study is described in biopsies from healthy skin are used to establish such a HSE. After establishing the Ex-HSE, a small piece of the outgrowth was taken and placed onto a secondary collagen matrix in order to establish a secondary Ex-HSE (2nd generation). This procedure was repeated again to establish a tertiary Ex-HSE (3rd generation). The results described in this chapter indicate that the expression of the differentiation markers, e.g. keratin 10, FLG and loricrin, was present in the Ex-HSE and in the 2nd and 3rd generation. Furthermore, the 2nd and 3rd generation Ex-HSEs displayed the presence of the three main SC lipid classes. Whereas the density of the lipid packing the SC of the 2nd and 3rd generation gradually reduced, the lamellar organization in the SC of these HSEs remained similar. However in comparison to human skin, all Ex-HSEs displayed alterations in the SC lipid properties.

Following the usage of healthy skin biopsies, in the following study we wanted to know whether biopsies of diseased skin could also be used to establish an Ex-HSE that maintain their in vivo characteristics. Chapter 5 describes this study in which biopsies from AD patients were used to establish an AD Ex-HSE. In addition, in this study the effect of FLG mutations on epidermal morphogenesis was evaluated, by using biopsies from AD patients with a homozygous FLG
mutation that were compared to wildtype AD patients. To examine whether the AD Ex-HSE recapitulate features of AD skin as well as the effects of FLG mutations on the outgrowth of the AD Ex-HSEs, we evaluated the expression of differentiation markers in vivo and in vitro, i.e. within the biopsy and the corresponding Ex-HSE from the same patient. The results from this study showed that FLG mutations resulted in reduced FLG protein expression in the biopsies, which was also present in their corresponding Ex-HSEs. FLG mutations did not affect the expression of keratin 10, involucrin, kallikrein 5 and Lekti. However, in the presence of FLG mutations reduced loricrin expression was observed in AD biopsies, which was also maintained in their corresponding Ex-HSEs. The observations from this study show that AD biopsies, with and without FLG mutations, maintain many characteristics in vitro when used for establishing an Ex-HSE. This study also revealed that loss of FLG expression due to mutations is not rescued by the increase in the expression of other proteins from the epidermal differentiation complex, i.e. involucrin and loricrin. These findings show the possibility of establishing an AD-HSE with primary cells from AD patients. Such an AD Ex-HSE might be a suitable tool in the later stages of development of FLG restoring therapies.

Reduced FLG expression as a risk factor for S. aureus colonization

In addition to intrinsic factors, such as the presence of FLG mutations, external factors play an important role in the development of AD. These external factors include bacterial colonization with Staphylococcus aureus. Almost all AD patients show increased and persistent S. aureus colonization, which secrets toxins that negatively affect disease severity17;36-38. Furthermore, these bacteria form biofilms, which makes their treatment more difficult39.

Since FLG breakdown products have been shown to affect bacterial growth in vitro40, an N/TERT based epidermal HSE (NEM) was developed to address the possible involvement of FLG in epidermal colonization with S. aureus. Therefore, FLG expression in this epidermal HSE was reduced through FLG-KD or through supplementation the culture medium with IL-31. The results from this study, which are described in chapter 6, show that both FLG-KD and IL-31 supplementation resulted in decreased FLG expression accompanied by an increased epidermal S. aureus colonization. The presence of S. aureus resulted in increased IL-8 secretion and increased expression of various anti-microbial peptides (AMPs), including human β defensin (hBD-) 2, hBD-3 and RNase 7. However, the presence of IL-31 shifted the epidermal response towards S. aureus from protective - towards inflammatory. This was illustrated by increased expression of IL-8 and prevention in the increased expression of AMPs. Furthermore, both S. aureus and IL-31 were able to affect the expression of various enzymes that are involved in stratum corneum lipid synthesis.

Mimicking lesional AD skin by supplementation of Th2 cytokines

Besides FLG mutations and skin barrier dysfunction, AD is characterized by various immunological abnormalities. In chapter 7, a study is described in which FT-HSEs were
established in the presence of a Th2 cytokine mixture, to mimic a lesional AD environment as closely as possible. The effects of this cytokine mixture on the epidermis were compared to lesional AD skin to evaluate whether supplementation of this cytokine mixture resulted in a HSE that mimics lesional AD skin. The results from this study indicate that a mixture of Th2 cytokines induced simultaneously alterations on various levels in the epidermis of HSEs; differentiation, expression of enzymes involved in the desquamation process and stratum corneum lipid synthesis as well as in the stratum corneum lipid organization. Supplementation of the Th2 cytokine mixture resulted in increased epidermal thickness that is also present in lesional AD skin. The marked changes in expression of keratin 10, loricrin and filaggrin indicate a delay in early differentiation as well as incomplete late differentiation. The expression of Lekti was increased by Th2 cytokines similar to what is seen in lesional AD skin. In the FT-HSE, the expression of various enzymes that are involved in SC lipid synthesis was reduced by supplementation of the Th2 cytokine mixture. Furthermore, presence of the Th2 cytokine mixture resulted in alterations in the SC lipid organization.

**In conclusion**

As a multifactorial inflammatory skin disease, AD is difficult to recapitulate into one HSE. The AD-HSEs that are described in this thesis all mimic various aspects of AD. These include reduced FLG expression, increased epidermal colonization with *S. aureus* and various features that can be observed in lesional AD skin. Depending on the research question these HSEs can be applied for various purposes. However several aspects of *in vitro* AD-HSEs can be further improved. The application of the currently available AD-HSEs and the opportunities for improvement of AD HSEs will be discussed below.
Future perspectives on in vitro AD human skin equivalents

AD-HSE 2.0: application and improvements of AD-HSEs
The currently developed AD-HSEs, including those described in this thesis, mimic some of the features of AD, e.g. reduced FLG expression. However, as described above, these AD-HSEs have several limitations. In particular, the generation of a lesional AD-HSE remains a challenge. Despite their limitations, the current AD-HSEs can be applied for various purposes, but to mimic AD more closely in vitro, several aspects could be improved. The applications of the current AD HSEs and the possible improvements are described below.

Evaluation of the role of FLG in AD
The discovery of FLG mutations as an important predisposing factor for AD allows the study of the role of FLG in epidermal homeostasis and in the skin barrier function in vitro. Such approaches include application of RNAi to knockdown FLG (FLG-KD) expression and thereby mimic FLG mutations.

Reduced FLG expression due to FLG-KD or due to FLG mutations did not affect the epidermal morphogenesis, expression of keratin 10, nor the SC thickness and SC lipid properties of HSEs, as shown in chapter 3 and 5 and in other FLG deficient HSEs. These in vitro studies are in line with in vivo studies with AD patients in which no correlation was found between presence of FLG mutations and SC barrier properties. However, some studies have shown that various SC lipid properties of HSEs are different from those observed in SC from native skin. Therefore, optimization of the SC lipid properties of the current HSEs allows the generation of FLG-KD HSEs that can be used to examine the role of FLG in the skin barrier even more accurately.

The consequences of reduced FLG, reduced levels of the FLG breakdown products PCA and UCA, were shown to affect epidermal S. aureus colonization (chapter 6), and to provide protection against UV. In addition, FLG mutations were found to be a cause for reduced NMF levels in the SC in vivo. Taken these observations together, it appears that in particular the FLG breakdown products are an important factor in the skin barrier, rather than the FLG protein itself. Therefore, FLG deficient HSEs might be useful for the development and screening of compounds for rehydration therapies, e.g. FLG protein replacement based therapies as has been described recently, rather than for the evaluation of the role of the FLG protein in the skin barrier function. However, usage of HSEs to evaluate FLG degradation products might require several improvements. A previous study has shown that the levels of PCA are depending on the environmental humidity. This study implies that environmental factors are important for the regulation of FLG degradation is different and that it is possibly altered in HSEs. Furthermore, the exact role of the NMF in regulation of skin hydration is currently under debate.
The finding of increased colonization of *S. aureus* on FLG deficient HSEs provides an important answer to the question concerning the contribution of FLG as an initiating factor in AD development. Increased presence of *S. aureus*, as well as the presence of an itch, caused by *S. aureus* toxins or other factors, induces a mechanical barrier disruption through scratching that results in an infection. This might trigger the cycle of barrier disruption – inflammation – worsening of barrier disruption.

**Mimicking the inflammatory environment in vitro**

Currently, only supplementation of Th2 cytokines has been used successfully to mimic epidermal features of lesional AD in FT-HSEs, as described in chapter 7 and in epidermal HSEs12. Using this approach, the effects of other AD related cytokines on the epidermis could be investigated, e.g. IL-17 and/or IL-22. Recently, these two cytokines were suggested to be involved in tissue repair and remodelling in AD46. Supplementation of these cytokines to the medium during generation of FT-HSEs might provide additional insight in their contribution to the epidermal changes as seen in AD. Although this approach can be used to study the biological mechanisms underlying these features of lesional AD *in vitro*, it surely has several disadvantages. In particular when such HSEs are used for screening anti-inflammatory compounds. In general, the concentration of cytokine(s) added to the medium is arbitrarily selected, since little is known about the concentration of various cytokines in lesional AD skin. Furthermore, the cytokine concentration in lesional AD skin is also subject to many variables, including disease severity but also treatment history. Although HSEs generated in the presence of cytokines can provide significant insight in the effects of a compound on reducing the inflammatory effects on the epidermis, translation to the *in vivo* situation should be done with precaution. Furthermore, currently, cytokines are relatively expensive which makes it unsuitable for high throughput screenings.

As an alternative, integration of immune cells, obtained from healthy individuals or from AD patients, is an attractive approach, since these immune cells can easily be collected from blood and can be cultured and modified *in vitro*. Whereas a previous attempt to incorporate Th2 cells in HSEs did not result in an AD phenotype47, optimization of the protocol for polarization of the T-cells might result in Th2 cells that induce an AD phenotype after introducing them into HSEs. In addition, T cells from AD patients might be an interesting alternative to *in vitro* polarized T cells. Next to Th2 cells, various other immune cells have been shown to be present in lesional AD skin, including eosinophils, mast cells and dendritic cells (DCs).

Eosinophils have immunoregulatory role by secretion of a large number of cytokines and chemokines. Whereas eosinophils are absent in normal skin, they can be found in lesional AD skin48. However, not much is known about the contribution of these cells to the AD phenotype, but they are thought to be involved in the switch from acute to chronic AD49. Introducing these immune cells in the HSE might provide insight in the contribution of these
cells to the epidermal abnormalities as seen in AD. Furthermore, investigation of these cells, in a 3D environment and their interaction with fibroblasts and keratinocytes might provide new targets for interfering in AD progression.

Mast cells are present in chronic AD lesions \(^5^0\) and have been shown to affect keratinocytes through the release of histamine. Furthermore, a recent publication has shown the applicability of HSEs to evaluate histamine-induced effects \(^5^1\). Introduction of (activated) mast cells in the HSE might therefore result in a relevant HSE to screen antihistamine therapies for AD.

Dendritic cells (DCs) are professional antigen-presenting cells that are present in an immature stage in various epithelia, including the skin, where they are called Langerhans cells (LCs). Upon activation, LCs migrate from the epidermis to the draining lymph nodes, which is essential for the initiation of an adequate immune response. A recent study showed that LCs can successfully be introduced in a HSE \(^5^2\). Introducing these cells might aid in discovering initiating factors for AD development, as well as its contribution in e.g. \textit{S. aureus} colonization and host defences \textit{in vitro}. Furthermore, DCs/LCs from AD patients or healthy patients might behave differently in HSEs. Whereas previous studies have shown that LCs and T cells can be successfully introduced in HSEs, the introduction of multiple cells into a single HSE might be difficult and requires optimization of various culturing protocols. Furthermore, incorporation of various cell types into a single HSE increases the variability, which might cause difficulties in the interpretation of the results.

\textbf{Studies on the AD bacterial environment \textit{in vitro}}

The increased colonization of \textit{S. aureus} on FLG-KD HSEs provides new opportunities to study the role of \textit{S. aureus} in AD \textit{in vitro}, using HSEs. Moreover, the role of FLG in colonization of other bacteria/pathogens, e.g. the commensal bacteria \textit{S. epidermidis}, and possibly in the alteration in the skin microbiome as seen in AD can be investigated \(^5^3;^5^4\). Using a FLG deficient HSE, the role of FLG in establishing a host environment favourable for pathogenic or less favourable for commensal bacteria, can be investigated. Besides increased colonization, \textit{S. aureus} was found to form biofilms in lesional AD \(^3^9\), which renders this bacterium harder to treat. \textit{S. epidermidis} has been shown to secret proteases, including Esp \(^5^5\), which inhibits \textit{S. aureus} biofilm formation and therefore this protease might provide new peptides that can be used to combat the \textit{S. aureus} biofilm formation in AD. The epidermal FLG-KD HSEs as described in chapter 6 might provide a valuable tool in screening such commensal-bacterial-derived peptides.

Furthermore, the presence of the SC on the HSEs allows the investigation of the interactions between bacteria and the epidermis. Currently, this epidermal FLG-KD HSE is being used for screening of AMP-derived peptides, for their ability to effectively kill \textit{S. aureus \textit{in vitro}}. If successful, such peptides might be promising for continuous, long-term treatment for mild...
and moderate-to-severe AD. Besides screening of peptides that are potential new treatments for AD, *S. aureus* colonization was found to induce alterations in the expression of enzymes that are involved in SC lipid synthesis. These findings imply that *S. aureus* might be an additional cause for the disrupted barrier in AD. Evaluation of the SC lipid composition of a (FLG deficient) HSE after inoculation with *S. aureus* might reveal an additional role of this bacterium in AD, and therefore create new possibilities for intervention in AD.

**The role of fibroblasts in AD**

Whereas much research for AD is focused on the role of the keratinocytes, the epidermis and the SC, little research has been performed to investigate the role of the fibroblasts. However, several studies suggest that these cells might also play an important role in the AD microenvironment. Cytokine stimulated dermal fibroblasts were shown to promote the migration of dendritic cells and to attract various immune cells by secretion of soluble compounds such as eotaxin and metalloproteinase-1356-58. In addition fibroblasts were shown to be involved in regulation of the pro-inflammatory response of the keratinocytes 59. The fibroblasts might play an additional role in regulating other epidermal alterations in AD, including in the modulation of the SC properties. In chapter 7 it was shown that addition of fibroblasts to a skin equivalent resulted in some changes in the epidermal response to Th2 cytokines that were absent in epidermal HSEs12.

Participation of fibroblasts has been shown in other skin diseases. In recessive epidermolysis bullosa simplex (REBS), the fibroblasts have been shown to play an important regulatory role in establishing the REBS phenotype *in vitro*60. In squamous cell carcinoma (SCC), the cancer-associated-fibroblasts (CAFs) have been shown to play an important role in SCC development61. An initial study using fibroblasts from AD patients showed that such fibroblasts in HSEs affect epidermal homeostasis62. Therefore, incorporation of dermal fibroblasts from AD patients (with or without FLG mutations) can be used to examine their role in keratinocyte-fibroblast interaction, in epidermal morphogenesis and in epidermal barrier properties.

Furthermore, whereas some studies have shown that monolayer fibroblasts respond to IL-4 and/or IL-13 by secretion of various molecules/proteins56;57, it would be interesting to evaluate how fibroblasts respond to inflammatory mediators in a 3D environment, and whether fibroblasts from AD patients display increased or decreased sensitivity to cytokines.

**Concluding remarks**

In this thesis, research is presented that describes several new HSEs for AD. These HSEs mimic various aspects of AD and have been applied to address the role of FLG in this skin disease. Whereas the findings in this research suggests a more indirect role of FLG in AD, through the FLG degradation products that appears to prevent increased bacterial colonization, the exact role of FLG as a predisposing factor for AD remains a subject for future research. As most
models, HSEs have some limitations, such as the lack of systemic aspects e.g. a closed system for vascularization and absence of the nerve system for the sensitization of itch. Studies that address these aspects, e.g. screenings of anti-pruritic compounds, will therefore, for the time being, require mouse models.

The HSEs that are presented in this thesis have shown their applicability and they belong to the first generation of AD-HSEs. Thereby they provide a solid starting point for the development of improved AD-HSEs. Such improved AD-HSEs can be used to screen new compounds designed for the treatment of AD, as well as to discover new points for intervention in this skin disease. Thereby, these AD-HSEs may contribute to the development of novel treatments for AD, which prevent the progression of AD into secondary diseases and have less adverse effects. In doing so, these AD-HSEs might contribute to (partly) relieve the heavy burden of the patients and their close relatives.
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