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AN EX*PADI*TION FOR CITRULLINATION IN THE DEVELOPING HAIR FOLLICLE

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An exPADItion for Citrullination in the Developing Hair Follicle

Thesis for Doctoral Degree (Ph.D.)

By

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“I'd take the awe of understanding over the awe of ignorance any day.”

- Douglas Adams

Popular science summary of the thesis

The skin is the body's largest organ, designed to offer protection from the sun, the wind, and angry cats. The protection is achieved by a strong barrier within the skin that makes sure that nothing gets in, like nasty bacteria, and that nothing leaks out. The skin is also the home of one of the most striking features of mammals, namely our hair.

The skin is made up of cells which are layered on top each other, like the layers of a cake if you will. In the bottom layer are stem cells, a type of immature cell that do not directly contribute to the protective barrier function of the skin, but instead all they do is divide. They divide because the upper level of the skin gets shed off by wear-and-tear and needs to be replaced. This is a continuous process, so occasionally a stem cell leaves the bottom layer and move upwards. In doing so they change their characteristics and lose their ability to divide, to become specialized skin cells that actively contribute to the protective skin barrier. The process of specialization is called differentiation. The genetic features that drive differentiation are tightly controlled, yet the full details of this control are not entirely understood.

In the production of a hair fibre, a highly complex and orchestrated relationship exists between stem cells and the cells they differentiate into. In summary, at the bottom of the hair follicle are a pool of stem cells. These divide tremendously, and as they move upwards, they start to differentiate. They can then differentiate into either becoming cells that will make the actual hair fibre, or they can differentiate into supportive structures within the hair follicle that will ensure that the hair is anchored, fed, and happy to be able to continue to grow. The processes that govern differentiation and the "decisions" that shape the hair follicle are, despite being well studied, still undergoing intense scrutiny, and are not fully comprehended.

Moreover, in an embryo which is growing and still acquiring all the features of a newborn, as in having functional organs, the processes of stem cell division and differentiation are essential to ensure proper and accurate growth. This is true in both embryonic skin and hair follicles.

For both maintenance of adult tissue as for embryonic growth, it is the genes that drives and controls the processes of cell division and differentiation. The product of an active gene is a protein, and proteins are the main operative units within a cell, be it a stem cell or a differentiated cell, it is just that the subset of proteins in the two different cell types differ. It is vital to understand how the different genes and proteins of different type of cells are regulated and controlled in order to understand the function of an organ.

As stated, the genes and proteins involved in skin and hair growth are highly controlled. One level of control is to modify the structure of the proteins themselves, with what is referred to as posttranslational modifications (PTMs). This thesis focuses on one such

PTM called citrullination. Citrullination is carried out by a family of proteins called peptidylarginine deiminases (PADIs), of which there are five family members named PADI1-4, and 6 (number 5 was lost due to nomenclature admin). When a protein is citrullinated by a PADI, that protein is changed in either how it is folded, how it binds to other proteins, or how stable it is. The contribution of PADIs and citrullination to hair follicle stem cell biology is overwhelmingly understudied. While there are many indications for the importance of both citrullination and PADIs in other types of stem cells in other tissues, the protagonist of this thesis, PADI4, had not even been described in hair follicles prior to the work presented herein.

Accordingly, the current thesis presents evidence for the existence of PADI4 in hair follicles as these form during embryonic and early neonatal development. From there, a functional validation of PADI4 (by removing it from the hair follicles and investigating the effects of its removal) shows that it plays a role in the processes of stem cell division and the "decisions" into which differentiation path the cells should take – PADI4 seems to influence the differentiation of the hair producing cells (rather than the supportive structures). Furthermore, we characterize which proteins are citrullinated by PADI4 and show that they are mostly associated with the process of synthesising proteins, a process called Translation. Going deeper, we show that some of the fundamental molecular pathways that regulate translation are altered when we remove PADI4, proposing that PADI4 has a far more important role in core cellular machinery than previously appreciated. This means that another important puzzle piece has been fitted into the grander scheme for how stem cell division and differentiation are controlled, and that citrullination has a role to play in that control.

In other terms of citrullination and PADI biology, another finding of work presented in this thesis is that two of the PADI family members, namely PADI2 and PADI3, can actually be produced in such a way that each gene that blueprints either PADI2 or PADI3 gives rise to an additional protein. Like little siblings to the main form of the proteins. These little siblings are called alternative isoforms. To clarify, PADI2 exists as a canonical protein, the one that is described and well known, yet when the PADI2 protein is being produced, another similar but smaller (shorter, in terms of the number of amino acids that are linked together to make a protein) protein can be produced from the same gene. These two proteins are called PAD2α for the well-known full-length protein, and PAD2β for the alternative isoform. The same was discovered for PADI3. The alternative PADI2β isoform was detected in stem cell-like brain cells (oligodendrocyte precursors) in the process of differentiating, and PADI3β in the cells in the process of differentiating in the hair follicle. Functionally, the alternative isoforms were shown to impede the enzymatic activity of their respective canonical counterparts and hamper citrullination, and were suggested to exist as a regulatory measure to impact tissue formation.

Lastly, a study characterizing the ID1 protein in skin stem cells during development, unearths its role in differentiation, and makes the conclusion that ID1 interacts with known modulators of skin stem cell behaviour (these factors are BMP/pSMAD1/5, TCF3, and CEBPα) in a novel manner.

Collectively, the studies presented in this thesis help to better understand the genetic and protein features of skin and hair follicle cell biology. Specifically, some conventions regarding PADI biology are challenged, whereas others are confirmed and corroborated with new evidence. The studies provide new insights for how citrullination is integrated into core physiological processes in stem cell behaviour, as well as identifying new avenues for further studies to explore by calling out the need for more accurate tools to identify and measure citrullination. All in all, citrullination was not believed to be part the hair follicle stem cell repertoire, but with the findings and postulations of this thesis, it now ought to be.

Abstract

During epidermal development, to assure proper tissue structure, highly complex transcriptional networks interact within the stem cell compartments of the epidermis and hair follicles (HFs) to balance the choice between self-renewal or differentiation. The full characterisation of the protein profiles resulting from those transcriptional networks, within the compartments of the HF, remains, however, incomplete. Moreover, the proteins themselves can be regulated via posttranslational modification (PTMs). One such PTM is citrullination, carried out by the peptidylarginine deiminase (PADI) family of enzymes. Although, PADIs have been described in other stem and progenitor cells, their role in hair follicle stem cell (HFSC) and progenitor lineages have remained elusive.

The main objectives of this thesis are to address the functional consequences of PADI expression in HFSCs during development.

Paper I identifies *Padi4* expression in the developing HF, where it is found to participate in restricting proliferation and lineage commitment of HF progenitors, as well as playing a role in the central mechanism for translational control, and by doing so altering the distinct sequential events that mark HF differentiation progression. As a result, we identify citrullination as a means to assert regulation of protein function in HFSCs and progenitors.

Paper II identifies alternative isoforms of PADI2 and PADI3, in oligodendrocytes and HF differentiated cells, respectively, and show that the alternative isoforms have an incumbering effect on the enzymatic activity and stability of their conventional counterparts.

Paper III is a review paper in which meta-analysis of published human citrullinomes in health and inflammatory disease reveals that citrullination is a commonplace yet highly dynamic molecular regulator of protein function. A strong case is made for the involvement of PADIs and citrullination in hair follicle stem cell biology and inflammatory alopecia.

Paper IV addresses the involvement of transcription factor ID1 in self-renewal and differentiation of epidermal progenitor cells during development. This study describes how ID1 facilitates synchronisation of progenitor proliferation and differentiation via TCF3-binding, and establishes a novel axis of coordination for how BMP-induction of *Id1* expression via pSMAD1/5 is supressed by CEBPα.

The combined efforts within this thesis demonstrate the clear and overarching importance of PADIs and citrullination in skin developmental physiology.

List of scientific papers

I. **Vikhe Patil, K.**, Genander, M. *PADI4 restricts proliferation of hair follicle progenitor cells by rewiring translation*. (Manuscript)

II. **Vikhe Patil, K.**, Meijer, M., Mak, K. H. M., Yang, W., Falcão A. M., Castelo-Branco, G., Genander, M. *Co-expression of PADI isoforms during progenitor differentiation enables functional diversity*. Accepted, Philosophical Transactions B (2023) (DOI: 10.1098/rstb.2022.0451)

III. **Vikhe Patil, K.**, Mak, K. H. M., Genander, M. *A Hairy Cituation – PADIs in Regeneration and Alopecia*. Front. Cell Dev. Biol. (2021) 9, 789676. DOI: 10.3389/fcell.2021.789676

IV. Kantzer, C. G*., Yang, W*., Grommisch, D., **Vikhe Patil, K.**, Mak, K. H. M., Shirokova, V., & Genander, M. *ID1 and CEBPA coordinate epidermal progenitor cell differentiation*. Development (2022) 149(22), dev201262. DOI: 10.1242/dev.201262
* These authors contributed equally to this work.

List of scientific papers not included in this thesis

I. Hong Yu, **Kim Vikhe Patil**, Chul Han, Brian Fabella, Barbara Canlon, Shinichi Someya, and Christopher R. Cederroth. *GLAST deficiency in mice exacerbates gap detection deficits in a model of salicylate-induced tinnitus*. Frontiers in Behavioral Neuroscience 10 (2016): 158.

II. **Kim Vikhe Patil**, Barbara Canlon, and Christopher R. Cederroth. *High quality RNA extraction of the mammalian cochlea for qRT-PCR and Transcriptome analyses*. Hearing research 325 (2015): 42-48.

III. Adam Trewin, Leonidas Lundell, Ben Perry, **Kim Vikhe Patil**, Alexander Chibalin, Itamar Levinger, Leon McQuade, and Nigel Stepto. *Effect of N-acetylcysteine infusion on exercise induced modulation of insulin sensitivity and signaling pathways in human skeletal muscle.* American Journal of Physiology-Endocrinology and Metabolism 309.4 (2015): E388-E397.

Contents

List of abbreviations

ACPA	Anti-citrulline protein antibodies
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
bHLH	Basic Helix-Loop-Helix
BMP	Bone-morphogenic protein
CCCA	Central centrifugal cicatricial alopecia
CEBP	CCAAT-enhancer-binding protein
DNMT3A	DNA Methyltransferase 3 Alpha
DP	Dermal papilla
E	Embryonic day
ECM	Extracellular matrix
EDA	Ectodysplasin
EDU	Epidermal differentiation unit
EGFR	Epidermal growth factor receptor
eIF	Eukaryotic translation initiation factor
EPU	Epidermal proliferative unit
FGF	Fibroblast growth factor
GSH	Glutathione
HF	Hair follicle
HFSC	Hair follicle stem cells
hnRNP	Heterogeneous nuclear ribonucleoprotein
HRNR	Hornerin
HS	Hair shaft
ID	Inhibitors of differentiation
IFE	Interfollicular epidermis
IFN	Interferon
IGF	Insulin-like growth factor
IL	Interleukin
iPSC	Induced pluripotent stem cell
IRF	Interferon response factors
IRS	Inner root sheath
IVL	Involucrin
JAG1/2	Jagged 1/2
K, *Krt*	Keratins

LEF1	Lymphoid enhancer factor-1
mESC	Mouse embryonic stem cells
MS	Mass spectrometry
mTOR	Mechanistic target of Rapamycin
NET	Neutrophil extracellular trap
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NICD	Notch intracellular domain
OPC	Oligodendrocyte precursor cells
ORS	Outer root sheath
P	Postnatal day
p63	Tumour protein 63
PADI	Peptidylarginine deiminase
PRMT	Protein arginine methyltransferases
PTM	Posttranslational modification
RA	Rheumatoid Arthritis
RBP-J	Recombination signal binding protein for immunoglobulin kappa
RNAPII	RNA polymerase II
Sca1	Stem cell antigen-1
SHH	Sonic hedge hog
TACs	Transit amplifying cells
TCF	T-cell factor
TCHH	Trichohyalin
TGF	Transforming growth factor
TMT	Tandem mass tag
TNF	Tumour necrosis factor
UHS	Uncombable hair syndrome
WNT	Wingless-related integration site

1 Introduction

1.1 The mammalian skin

1.1.1 Skin as a protective barrier

The skin, largest organ (D'Orazio et al., 2013) and arguably most definitive external feature of a mammalian individual as it marks the border between the physical self and the outside world. Encapsulating the internal world of bodily fluids, tissues, and cells, whilst simultaneously vigilantly protecting the precious and fragile internal environment from outside harm as presented by health-detrimental pathogens, injurious chemical and mechanical trauma, DNA-damaging UV radiation, and perhaps more than anything, providing waterproofing from the outside in and at the same time preventing leakage from the inside out. The skin also constitutes a major contributor to thermoregulation as it allows the passages of venules and arterioles to the surface of the body to increase or decrease loss of body heat, making use of the interface between the internal and external worlds that meet at the border. More than that in terms of thermoregulation, the skin contains the organ for sweating and a degree of insulation in form of subcutaneous and dermal fat deposits. In short, your skin keeps you safe within yourself, at just the right temperature.

Anatomically, the skin can be subdivided into two major compartments: the overlying epidermis and the underlying dermis. The epidermis composes the outermost protective barrier and consists of a multi-layered squamous epithelium which at regular intervals will stretch downwards and form hair follicles (HFs). The underlying dermis, on the other hand, is a connective tissue with a supportive role and contains a variety of different cell types such as dermal fibroblasts, immune cells, and adipocytes. The epidermis and dermis have in turn a high level of suborganization and are separated by the basement membrane, a sheet of extracellular matrix rich in collagen IV and laminin (Watt, 2014)

1.1.2 Anatomy of the adult interfollicular epidermis

The epidermis is subdivided into the interfollicular epidermis (IFE) and associated structures, namely (and perhaps most notably) the HFs, as well as the sebaceous glands and, in humans, sweat glands. The main cell type of the epidermis are the epithelial cells called keratinocytes, so named for their characteristic keratin filament expression. Lacking the outer protective shell of insects and crustaceans, most vertebrate species have evolved to utilize the keratin filaments to form a dense cytoskeletal structure to withstand the physical stresses the outside world continually subjects the skin to. Most keratinocyte subpopulations have further been classified based on their divergent and stereotypical expression of different keratin proteins both in the IFE and the HFs.

As mentioned, the epidermis is structurally highly organised – the stratified IFE is divided into four layered segments based on cell morphology and levels of differentiation, as determined by their dynamically shifting expression of different keratins and other structural proteins.

The basal layer, or *Stratum Basale,* consists of a single layer of proliferating, undifferentiated progenitors which maintains the entire epidermis by perpetuated cell division. Basal IFE cells are classically distinguished by the protein expression of keratins K5 and K14 as well as Stem cell antigen-1 (Sca1) (U. B. Jensen et al., 2008). Additionally, the basal layer is anchored to the basement membrane. The anchorage promotes the proliferative identity and restricts differentiation – only by delamination from the basement membrane can the keratinocytes acquire a fully differentiated characteristic (a process that is discussed at length below).

Basal cells that do delaminate generate the spinous layer, or *Stratum Spinosum*. The spinous layer consists of differentiated, postmitotic cells. Spinous cells have lost their integrin and K5/K14 expression and are instead marked by the differentiation markers K1/K10 and Involucrin (IVL) (Humbert et al., 2017; Watt, 1983). *Stratum spinosum* consists of several layers of cells, which progressively differentiate as they gradually rise towards the surface of the skin, concomitant with increased lipid metabolism. Spinous cells are characterized by their spiny appearance (hence the name) owning to the highly specialized intercellular junctions called desmosomes (Kowalczyk & Green, 2013) which provide strength and flexibility to the epidermis (Garrod & Chidgey, 2008).

The granular layer, *Stratum Granulosum*, is also marked by K1 and K10, but has massively increased the production of IVL as compared to the spinous layer. IVL, together with Loricrin (LOR), makes up the major compartment of the cornified envelope, an insoluble structure of crosslinked proteins and lipids functioning to mediate the epidermal barrier (Candi et al., 2005). The granular layer also prevents transcutaneous water loss through the formation of tight junctions (Yokouchi et al., 2016).

Finally, the uppermost layer of the skin, the *Stratum Corneum*, consists of denucleated, dead cells whose plasma membrane has been replaced by the cornified envelope (Candi et al., 2005). The cells of the cornified layer are shed off in what is called desquamation, and is the main reason for need of the continuous proliferation of the basal cells – to replenish the cells lost due to the constant wear-and-tear of the outermost layer of the skin (Matsui & Amagai, 2015).

Although the vast majority of the cells in the epidermis are keratinocytes, there are other cell types as well, including but not limited to, blood vessels, sensory cells of the peripheral nervous system (Zimmerman et al., 2014), melanocytes (Lo & Fisher, 2014), and cells of the adaptive and innate immune system (A. V Nguyen & Soulika, 2019) (predominantly T-

cells and Langerhans cells (K. Clayton et al., 2017)), all contributing to the function of the epidermis.

1.1.3 Molecular features of epidermal homeostasis

Instrumental to the proliferative and undifferentiated characteristics of the basal keratinocytes is the basement membrane, the epithelial extracellular matrix (ECM) that separates the epidermis from the dermis. The basal keratinocytes express several different integrins, predominantly $\alpha3\beta1$ and $\alpha6\beta4$, which provide anchorage to the basement membrane through linkage to secreted laminins therein, (Hsu et al., 2014) and in this way restrict delamination and in turn differentiation (Watt, 2002). To sustain balanced levels of proliferation the basal layer receives several signals from the underlying dermal fibroblasts, forming a dermal niche, in form of secreted growth factors such as insulin-like growth factors (IGFs), fibroblast growth factors (FGFs) 7 and 10, and epidermal growth factor receptor (EGFR) ligands (Hsu et al., 2014). Basal cell autonomous expression of positive and negative regulators of these incoming factors are employed in an auto- or paracrine fashion to fine-tune the cells' proliferative response. Such regulators include TGFα, TGFβ, and Mig6, which work in concert with the incoming dermal niche factors, but have the potential to induce hyper- or hypoproliferation of basal keratinocytes, depending on their respective positive or negative effect on growth factor signalling (Ferby et al., 2006; Kahata et al., 2018; Vassar & Fuchs, 1991). Another important regulator of basal proliferation is p63, which has been shown to be essential for the proliferative potential and stem cell state of basal keratinocytes. Although mostly described in embryonic epidermal development, p63 is also implicated in adult basal layer progenitors (De Rosa et al., 2019; Pellegrini et al., 2001). By binding up to 2000 genes (Senoo et al., 2007; A. Yang et al., 2006), p63 regulates proliferation through acting both as a transcriptional activator of basal layer genes (including integrins $\alpha3/5/6$ and $\beta4$, keratins 5 and 14, and several progenitor-associated transcription factors) and as a repressor of antiproliferation genes (including *p21*, *14-3-3σ*, and *Pten*) (Botchkarev & Flores, 2014; Senoo et al., 2007; Truong et al., 2006).

Although the role of basal keratinocytes is to continually provide new cells for the upper epithelium as it is shed through wear-and-tear, the question for how the proliferation-to-differentiation dynamics are maintained is left somewhat open. Addressing this question, two models for basal cell proliferation and differentiation have been put forward – the hierarchical and stochastic models.

The hierarchical model proposes that each stem cell of the IFE is slow cycling and divide only rarely but gives rise to progeny which are highly proliferative transit-amplifying cells (TACs) (Hsu et al., 2014), whom after several rounds of divisions all delaminate and differentiate upwards in the epidermal architecture. The hypothesis for the hierarchical model was formed early in the field of epidermal stem cell research where observations

of morphology and proliferation proposed the concept of epidermal proliferative units (EPUs) (Potten, 1974), in which a slow-cycling stem cell gives rise to about 10 TCAs which all undergo differentiation after a (seemingly) predetermined number of divisions. The idea of the EPU was later supported as studies utilizing mutagenic or retroviral cell-labelling (Ghazizadeh & Taichman, 2001; Mackenzie, 1997; Ro & Rannala, 2004) were able to demonstrate clonal expansion in a columnar fashion where clone size remained unchanged over time. Moreover, the concept of epidermal progenitor hierarchy gained further traction when a more recent study (Mascré et al., 2012) was able to demonstrate that the basal layer is maintained by two interdependent stem/progenitor cell population where a small number of *Krt14*-expressing slow-cycling label-retaining stem cells, with the capacity to self-renew, gave rise to an intermediate *Ivl*-expressing fast-cycling progeny of committed progenitors with the capacity to undergo a fixed number of cell division before exiting cell cycle and univariably differentiate, just as TACs where described to do in the EPU. Additionally, other studies (Gomez et al., 2013; Sada et al., 2016) described the existence of two distinctly different stem cell populations when studying behaviour kinetics of basal layer progenitors in scale and interscale regions of the mouse tail. Lineage tracing and mathematical modelling suggested that the basal layer consists of two spatially independent stem/progenitor populations with different proliferation frequencies (cell cycling-rates) and preferences for lineage differentiation in terms of the specific markers the differentiated progeny expressed depending on scale/interscale locality (Gomez et al., 2013). The basal layer cells were found to be controlled by epidermal *Eda*, WNT/β-cantenin, and *Lrig1*, and marked distinctly by either *Dxl1* and *Lgr6* (interscale) or *Slc1a3* (scale) (Sada et al., 2016). Collectively, these studies suggest that the different basal cell populations are not equally regulated transcriptionally or by signalling, nor do they display a random proliferation behaviour or differentiation program, indicating that they are not competing neutrally, but specifically.

In contrast to the hierarchical model, the stochastic model proposes that the basal layer consists of an equipotent progenitor population which compete neutrally with equal chance of each cell division giving rise to either 1) one cell that exit the cell cycle and differentiate, and one basal progenitor cell that will continue to divide; 2) two basal progenitor cells; or 3) two differentiated cells.

Several lineage tracing studies together with mathematical modelling have supported the stochastic model in several skin regions, suggesting that in randomly labelled basal cells, although individual clone sizes differ, fate choice is balanced over time and contributes to the population level homeostatically (E. Clayton et al., 2007; Doupé et al., 2010; Füllgrabe et al., 2015; Lim & Nusse, 2013; Piedrafita et al., 2020). Moreover, a study using live imaging in combination with lineage tracing to track cell fate choices was able to demonstrate that although the cell fate decisions of the basal layer was random, the delamination direction of differentiating progeny adhered to the pre-organized architecture of the

suprabasal epidermis into what the authors termed epidermal differentiation units (EDU, in a wink to the EPU) (Rompolas et al., 2016). In the EDU each basal cell divides stochastically but the differentiating progeny is funnelled into existing suprabasal spaces left by differentiated cells as they migrate upwards due to the demand of the ever-shedding corneal layer, giving rise to columnar shaped cell clones. In this way there some reconciliation between the two models as the EDU may help to explain the columnar observations that originally defined the EPU. Moreover, it is noteworthy that in the Mascré *et al.* study that demonstrated the existence of a slow-cycling pool of stem cells that gave rise to a fast-cycling population of committed progenitors, essentially supporting the hierarchical model, the committed progenitors in question adhered to the stochastic model during their lifetime as proliferative basal layer cells and was able to contribute to tissue homeostasis for up to a year prior to the "decision" of dividing into two post-mitotic cells.

In light of these models, single cell analyses have identified the existence of several transcriptionally divergent IFE basal layer progenitor populations (Dekoninck et al., 2020; Ghuwalewala et al., 2022; Haensel et al., 2020; Joost et al., 2016, 2020; Tan et al., 2013), confirming stem cell/progenitor heterogeneity. Although the basal cells may not then be entirely equal in performance, they appear to contribute to homeostasis in a manner akin to the stochastic model. The heterogeneity may reflect a difference in cell state rather than different cell type, since, although transcriptionally divergent, they appear follow a single differentiation program (Joost et al., 2016; Lin et al., 2020; Rognoni & Watt, 2018). Even so, there is still the observation that tail scale and interscale regions can be distinguished by distinctive markers in the respective suprabasal progeny. It appears, however, to be a singular event in the mouse IFE not observed elsewhere, possibly as a result from the transcriptional and marker disparities in the stem cell populations that give rise to each region, or possibly as a result of the particular spatially organised features of the scale/interscale structures (which are also tightly linked to the organised localisation of hair follicles in the tail) (Ghuwalewala et al., 2022; Gomez et al., 2013; E. Roy et al., 2016; Sánchez-Danés et al., 2016).

Along these lines, the difficulties to resolve the hierarchical/stochastic debate may largely come from that the interpretations of the previous studies have relied on assumptions for the parameters that define a stem cell, namely its basal localisation and selective marker expression, its cycling rate, and its ability to divide (at least once) prior to delamination (Gonzales & Fuchs, 2017). The more unbiased single-cell sequencing studies have subsequently been able to demonstrate that basal cells exist in a heterogeneity of cell states across the basal layer, in that basal cells can be both fast-cycling long-term inhabitants of the basal layer, as well as being slow-cycling without necessarily giving rise to TAC progeny as an intermediate (Ghuwalewala et al., 2022; Haensel et al., 2020; Joost et al., 2020).

To exemplify, Ghuwalewala *et al.* (Ghuwalewala et al., 2022) made a distinction between label retaining cells and non-label retaining cells to mark which cells do and do not remain in the basal layer long-term and how the two cell populations behave in terms of proliferation and differentiation. Interestingly, the two cell populations performed very similarly in proliferation kinetics and differentiation dynamics, highlighting that both fast and slow cycling basal layer cells contribute to the maintenance of the basal layer as well as giving rise to differentiating progeny at similar levels.

Additionally, another recent study (Cockburn et al., 2022) demonstrated that classical differentiation markers (most predominantly *Krt10,* followed by *Krt1* and *Krtdap*) were upregulated long before the delamination event, representing a commitment to differentiation, yet retaining basal cell marker expression, locality, and morphology. Moreover, some of the cells expressing the differentiation markers preserved their mitotic capacity. Although all such cell divisions were symmetrical (in that they would both terminally differentiate), both daughters of the basal *Krt10*+ cell were not obliged to differentiate immediately, as one of them could remain in in the basal layer and undergo another round of division.

Still, another study (Miroshnikova et al., 2018) demonstrated that in embryonic epidermis, local crowding of basal cells induced delamination in stochastically selected cells, which due to density-dependent shifts in the mechanical forces affecting cell shape experienced low cortical tension and increased cell-cell adhesion, which resulted in initiation of differentiation. Only after these events did the cells delaminate. However, a subsequent study of adult epidermal homeostasis (Mesa et al., 2018), found that differentiation and delamination is the delimiting factor for proliferation, that only after the delamination of a neighbouring cell will mitoses be triggered to fill the gap left by the differentiating cells. In either case, cell state was determined by the local environment created by the nearest neighbouring epidermal cells, and not by innate, cell-intrinsic, properties for being slow or fast cycling.

At any rate, delamination from the basement membrane is demonstrably tightly linked to differentiation and transformation from a dividing cell into a post-mitotic keratinocyte that will contribute directly to the barrier function of the skin. And as shown (Cockburn et al., 2022), the transcriptional events that lead to differentiation appear to be instigated prior to delamination. Delamination is in turn achieved by downregulation of integrins to allow release from the basement membrane. Ostensibly, there is a spectrum of different types of integrins expressed by basal cells, as well as the levels at which they are expressed, at different sites along the skin (Watt & Fujiwara, 2011). These differences are associated with readiness to detach from the basement membrane and have thus been correlated with stem cell identity of basal cells, where cells with high integrin-expression creates a more adhesive environment and are deemed as more stem-like (U. B. Jensen et

al., 1999; Jones et al., 1995), further highlighting the heterogeneity of progenitor cell states in the basal layer.

Moreover, Notch have been shown to be highly important in both late and early stages of keratinocyte differentiation. Functioning right at the transition from undifferentiated basal cell to differentiated suprabasal cell, Notch is active at the junction between basal and spinous layer cells (Nowell & Radtke, 2013). Accordingly, Notch 1-3 is expressed in the suprabasal cells together with Notch ligand Jagged-1 (JAG1), whereas basal cells express JAG2. When the Notch receptors (1-3) binds either of their ligands (JAG 1 or 2) on neighbouring cells, Notch is cleaved sequentially; first extracellularly by a disintegrin and metalloprotease (ADAM), and then intracellularly by γ-secretase to release its intracellular domain (NICD), which translocates to the nucleus where it binds transcription factor RBP-J (Watt et al., 2008). Upon binding, the role of RBP-J is two-footed: Notch signalling both induces expression of differentiation genes and suppresses expression of basal layer genes, two effects that are independently regulated. A canonical target of RBP-J is *Hes1*, a basic helix-loop-helix (bHLH) transcription factor, which when induced by Notch/RBP-J signalling enhances the expression of differentiation proteins *Krt1* and *Krt10* (Blanpain et al., 2006).Moreover, Notch signalling represses cell-cycle progression and restricts proliferation via induction of *p21/WAF1/Cip1* expression, a tumour-suppressor gene facilitating growth-arrest. Activation of *p21* is both directly induced by RBP-J binding to its promoter, as well as indirectly by increased NFAT signalling, mediated via HES1 (which represses NFAT inhibitor calcineurin) (Jaiswal & Singh, 2022; Mammucari et al., 2005). Furthermore, in a mechanism that is controlled separately from cell-cycle withdrawal, Notch-signalling suppresses several basal cell markers, including p63 and several integrins (Blanpain et al., 2006; Lefort et al., 2007; B.-C. Nguyen et al., 2006), as integrins themselves confer signalling for suppression of differentiation, rather than just providing physical attachment to the basement membrane (Evans et al., 2003; Watt, 2002; Watt et al., 2008). Moreover, Notch-dependent suppression of p63 acts via upregulation of CCAAT-enhancer-binding protein-α (CEBPα) and β (Lopez et al., 2009; X. Wang et al., 2008), together with concomitant suppression of interferon response factors (IRFs) (Restivo et al., 2011). Additionally, there is a reciprocal signalling crosstalk between Notch and p63, where in basal cells, p63 negatively regulates the expression of *Hes1* (Botchkarev & Flores, 2014; Nowell & Radtke, 2013), thus counteracting the pro-differentiation activity of Notch. In this way there is a mutual antagonism between p63 and Notch that may help to maintain a balanced proliferation-to-differentiation transition in the epidermis.

1.1.4 Anatomy and activity of the adult hair follicle

The defining feature of the IFE is that it is 'interfollicular', meaning that it semantically can only be defined by the structures between which it is located – namely the hair follicles (HF). The HF is arguably one of the most prominent features of mammalian skin. It is formed as an epithelial appendage of the IFE that invaginates downwards through the

dermis to form a funnel or tube-like structure, out of which the characteristic hair strand grows. Functionally, more than just providing the hair strand, the hair follicle forms the pilosebaceous unit (PSU) together with the sebaceous gland, which lubricates the skin and serves as an anchorage point for *arrector pili* muscle (Schepeler et al., 2014), sensory neurons (Zimmerman et al., 2014) and to some extent blood vessels (Hsu et al., 2014).

The HF displays a particular microanatomy divided into several distinct segments (Figure 1; (Schneider et al., 2009)). The uppermost part of the HF is called the infundibulum and connects the IFE to the HF (Figure 1A). Transcriptionally, the infundibulum is more similar

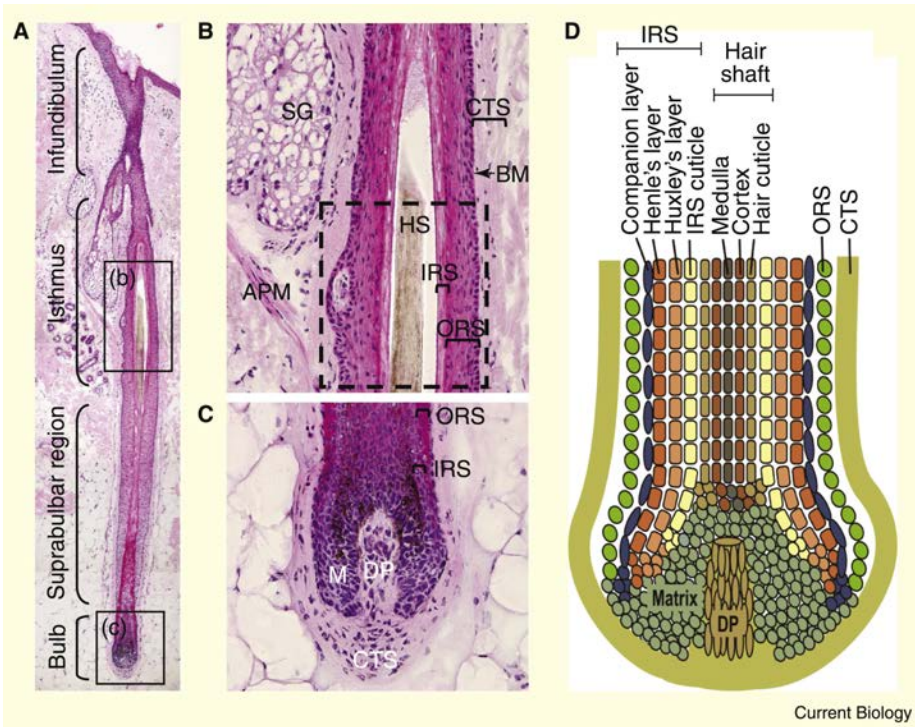

Figure 1. (A) Cross section of a human hair follicle during anagen, showing the permanent infundibulum and isthmus, and the anagen-associated suprabulbar and bulbar area of the hair follicle. (B) High magnification image of the isthmus. The dashed square indicates the approximate location of the bulge, where hair follicle stem cells reside. (C) High magnification image of the bulb. Matrix (M) is made up of proliferating TACs. (D) Schematic drawing illustrating the concentric layers of the outer root sheath (ORS), inner root sheath (IRS) and Hair shaft. The inner root sheath is composed of four layers: Companion layer (CL), Henle's layer, Huxley's layer, and the inner root sheath cuticle (BM: basal membrane; APM: arrector pili muscle; CTS: connective tissue sheath; DP: dermal papilla; M: matrix; HS: hair shaft, IRS: inner root sheath; ORS: outer root sheath; SG: sebaceous gland). (Schneider MR, Schmidt-Ullrich R, Paus R. *The hair follicle as a dynamic miniorgan*. Curr Biol. 2009 Feb 10;19(3):R132-42. doi: 10.1016/j.cub.2008.12.005. PMID: 19211055.) © *Elsevier, 2009. Reprinted with permission.*

to the IFE than to the lower parts of the HF (Joost et al., 2016, 2020). Below the infundibulum follows the junctional zone, which is the entry point for the lipid-filled, antimicrobial sebum secreted from the sebaceous gland. The sebum passes upwards through the infundibulum to lubricate the hair strand and the surface of the skin. Exploring the HF further downwards, following the junctional zone is the isthmus (Figure 1B), a short transitional segment between the opening of the sebaceous gland above and the attachment of the arrector pili muscle below, connecting the upper parts of the HF to the lower, stem cell rich, regions. The isthmus is also the site of a small group of stem cells defined by their expression of LRIG1 (Jensen et al., 2009) and LGR6 (Füllgrabe et al., 2015; Snippert et al., 2010), and contribute to maintaining the upper HF during telogen, as well as readily responding to wounding in the instance this occurs in the overlying IFE (Joost et al., 2018). Continuing below the isthmus, the lower adult HF consists of the bulge and the hair germ (Figure 2B and 2C), which both comprises of undifferentiated hair follicle stem cells (HFSCs). The hair germ, located just below the bulge cells, is also in direct contact with the underlying stromal niche, the dermal papilla (DP) (Figure 2C). The DP is made up of mesenchymal fibroblasts and functions as a specialised dermal signalling centre to provide the stem cells of the hair germ and bulge with activating or deactivating signals (Clavel et al., 2012; Rompolas & Greco, 2014).

The hair follicle undergoes cycles of growth (anagen), degeneration (catagen), and dormancy (telogen) (Müller-Röver et al., 2001). The HFSCs are instrumental for hair cycle progression in coordination with dermal signalling. The bulge and hair germ stem cell populations share some molecular signatures but differ in their respective mode of quiescence maintenance and responsiveness to activation. Bulge stem cells are characterized by the simultaneous expression of CD34, SOX9, K15, and to some extent Lgr5 (Joost et al., 2018; Mardaryev et al., 2011; Schepeler et al., 2014), whereas hair germ cells can be distinguished as ID1/ID3-positive CD34-negative cells expressing high levels of P-Cadherin (Genander et al., 2014; Greco et al., 2009; Joost et al., 2020; Müller-Röver et al., 1999).

1.1.5 **Mechanisms of hair follicle stem cell activation – telogen to anagen**

Telogen, the resting state, has been shown to be the default state of hair follicles, as an energy-efficient mode which is highly responsive to various stimuli and insults to initiate hair regeneration (Geyfman et al., 2015). Telogen can be subdivided into refractory telogen, a period when anagen cannot be re-initiated by stimulation, and competent telogen, when the hair follicle is highly sensitive to activation (Plikus & Chuong, 2014). The balance and ratio between activating (WNTs, FGF-7, FGF-10) and inhibiting (Bone-morphogenic proteins (BMPs) and FGF-18) cues, determines when HFSCs become activated (Geyfman et al., 2015).

During telogen, high BMP and low WNT signalling maintains HFSC quiescence (Hsu et al., 2014). Differentiated K6+ inner bulge cells secrete BMP6, whereas BMP2 and BMP4 is produced from subcutaneous adipocytes and dermal fibroblasts, respectively (Hsu et al., 2011; Plikus et al., 2008). Additionally, the potent quiescence factor FGF-18 is produced mainly by K6+ inner bulge cells, and to some extent by CD34+ bulge cells (Kimura-Ueki et al., 2012) and DPs (Greco et al., 2009). FGF-18 reaches peak upregulated expression in early (refractory) telogen, but actively downregulated (presumably via Foxp1 in the bulge (K6+ and CD34+ cells both) (Leishman et al., 2013)), in late (competent) telogen, in a process thought to prime the HF to anagenic signals – yet not inducing anagen itself. During late telogen, dermal niche signalling contributes to lowering the threshold for HFSC activation when BMP2 and BMP4 are downregulated in their respective mesenchymal constituent, but also as adipocyte precursor cells begin to express and secrete platelet-derived growth factor-α (PDGFα) to activate PDGFR-signalling in dermal papillae (Festa et al., 2011). Moreover, expression of activating factors FGF-7, FGF-10, TGFβ2, and BMP-inhibitor Noggin is elevated in the dermal papilla (Greco et al., 2009; Oshimori & Fuchs, 2012). Prior to actual telogen-to-anagen transition, activated WNT signalling in the hair germ is signified by stabilisation and nuclear localisation of the well-established WNT effector β-catenin (Lo Celso et al., 2004; Lowry et al., 2005), together with expression of β-catenin cofactors lymphoid enhancer factor-1 (LEF1) and T-cell factor (TCF) proteins (Greco et al., 2009). Additionally, the activated WNT signalling in the late telogen hair germ occurs concomitantly with increased expression of Ki67 and MAPK activation, indicative of activated proliferation signalling. Collectively, in the hair germ during late telogen, accumulation of DP-derived BMP-inhibition together with a gradual decrease of FGF-18 expression, acts in concert with FGF-7/10 signalling to achieve an elevated activation of WNT, as shown by both β-catenin stabilisation as well as a gradual transcriptional upregulation of WNT ligands (Greco et al., 2009; Kandyba et al., 2013; Y.-H. Li et al., 2013; Lim & Nusse, 2013; H. Yang et al., 2017), to cumulatively direct the hair germ stem cells towards a state of substantial proliferation – and once the tipping point is reached, anagen ensues (Lim & Nusse, 2013).

Subsequently, as anagen proceeds irrevocably, activated hair germ stem cells give rise to what is called the matrix – a pool of highly proliferative, undifferentiated transit amplifying cells (TACs). The matrix will start to secrete Sonic hedgehog (SHH) which in turn activates the bulge hair follicle stem cells (Hsu et al., 2014; Hsu et al., 2014). The bulge cells have up to this point remained largely quiescent in a WNT/β-catenin inhibitory state in which they partly supress WNT target gene transcription via TFC3/4-binding (Lien & Fuchs, 2014), and partly where they themselves express WNT-repressors such as *Dkk3*, *Wif1*, and *Sfrp*. In addition, the bulge cells are also being subject to active WNT-inhibition via BMP signalling (Lim & Nusse, 2013) (as exemplified by a BMPR1a KO model where *Lef1* was upregulated, and β-catenin stabilized (Plikus et al., 2008), as was *Wnt7a* in another BMPR1a cKO (Kandyba et al., 2013)) where BMP-effector pSMAD1/5 binds to enhancers and

promoters of key stemness genes that also mediate quiescence (Adam et al., 2018; Genander et al., 2014). Activated bulge stem cells will divide, migrate out from the bulge and eventually form the Outer root sheath (ORS) (Figure 2D). The ORS is a basal population of proliferative, undifferentiated K14+/K5+ cells expressing stem cell marker LHX2 (Folgueras et al., 2013; Rhee et al., 2006; H. Yang et al., 2017) that will expand and eventually envelope the entire HF all throughout the hair cycle (Rompolas et al., 2013; Sequeira & Nicolas, 2012). The lowermost portion of the ORS, which covers the bottom of the anagenic HF, is called the lower proximal cup and forms an interface between the ORS and the matrix (Xin et al., 2018). Furthermore, SHH from the matrix also signals to the dermal papilla to intensify the secretion of FGF-7 and Noggin to sustain proliferation of the lower ORS and the matrix itself (Hsu et al., 2014).

1.1.6 **Specification and differentiation of hair lineages**

To form a proper hair, the growing hair follicle requires several specialized cell populations, each originating from specific lineages following spatially and temporally controlled trajectories of differentiation (Yang et al., 2017). The differentiated lineages of the HF are ordered in seven concentric rings within the HF (Figure 2D), and are from outermost to innermost: 1) the companion layer; 2) the inner root sheath ([IRS] – in turn made up by three sublayers: Henle, Huxley and IRS cuticle); and 3) the hair shaft ([HS] also containing three sublayers: cuticle, cortex and medulla) (Joost et al., 2020; Schneider et al., 2009). All these layers can be distinguished from one another by their distinct keratin and structural protein expression patterns (Langbein et al., 2010).

During hair growth, the matrix cells adjacent to the DP become specified into distinct subtypes of lineage committed TACs according to a spatial organisation, in turn determined by a pre-ordered arrangement of signals emanating from the DP in an epithelial-mesenchymal crosstalk, manifesting a micro-niche (Yang et al., 2017). Initially, TAC progenitors thus far unspecified in their commitment to either IRS and HS lineages, display nuclear localisation of LEF1, which has translocated to replace the occupancy of TCF3/4 on key gene enhancer elements in order to facilitate proliferation (in a move initiated just prior to onset of anagen in hair germ cells where TCF3/4 induce the expression of WNT inhibitors (e.g. *Dkk3* and *Znrf3*)) (Adam et al., 2018). Next, specification occurs while the cells are still in a highly proliferative state, and they subsequently expand along their respective lineage. Moreover, lineage specification transpires via remodelling of cell identity-specific chromatin accessibility and epigenetic marks (such as H3K27ac), which modulate the binding sites for lineage specific transcription factors(Adam et al., 2015), perpetrating dynamic shifts in gene expression (Adam et al., 2018). The subsets of TACs can consequently be identified by their distinct intrinsic transcription factor expression profile (Joost et al., 2020), which is the result of divergent exposure to BMPs and WNTs from the DP together with the effectors and repressors of these pathways, and

the crosstalk between them (Ghahramani et al., 2018; Joost et al., 2020; Schneider et al., 2009).

Thusly, the TACs committed to the HS lineages follow a complicated route of initially being reliant on high levels of BMP signalling, acting via pSMAD1/5 binding of HS signature genes. However, in the later stage, terminally differentiating HS cells will express enhanced levels of LEF1 together with nuclear translocation of β-catenin (WNT) and follow a migratory path centrally in the HF (DasGupta & Fuchs, 1999). Fully formed HS will ultimately express K6, K40 (AE13), and K75 (although K75 will also be expressed to some extent by the companion layer).

On the other hand, TACs specified towards the IRS lineage will in the initial stages of specification rely on low levels of BMP signalling, inducing the expression of *Gata3*. In turn, *Gata3* is essential for commitment to, and expansion of, the IRS lineage (Kaufman et al., 2003). The levels of BMP signalling are gradually elevated in the IRS lineage as the TACs progressively become more differentiated, to sustain expression of IRS genes such as *Id1-3* and *Cux1* (Genander et al., 2014; Joost et al., 2020). IRS specification is also reliant on Notch signalling, as exemplified by Notch/RBPJ target gene *Hes1* expression (Adam et al., 2018; Joost et al., 2020). Terminally differentiated IRS will ultimately be distinguishable by K71 and Trichohyalin (TCHH or AE15) expression (Joost et al., 2016, 2020; Langbein et al., 2010).

Interestingly, LEF1 remains nuclear as the differentiation trajectories of each cell fate lineage progress, even though the differentiation of the IRS lineage is defined by a BMP-high, WNT-inhibitory, state. Even more intriguing is the fact that enhancer elements of the *Lef1* gene itself is bound by BMP effector pSMAD1/5 in HS-TACs. This dichotomy is achieved by LEF1 occupancy on genes that in turn are modulated by BMP and Notch/RBPJ effectors (Adam et al., 2018). In this way, the crosstalk between these contradictory signalling pathways achieves a thin but highly precise balancing act of perfectly executed transcriptional programs that generate the distinct differentiated cell populations that makes up a mature hair.

1.1.7 Translational coordination in epidermal progenitor specification

Additionally, the distinct and highly coordinated transcriptomic changes the progenitor and differentiating cells undergo to end up as terminally differentiated, also involves important dynamic shifts in protein translation. Crudely speaking, quiescent stem cells (such as bulge HFSC and pre-activated hair germ HFSCs) display low global translation, whereas lineage committed progenitors positioned right at the cusp of becoming differentiated display high levels of translation (Saba et al., 2021; Signer et al., 2014). Conversely, stem cells retain a high level of ribosomal biogenesis through higher rates of rRNA synthesis (through RNA polymerase I and III) and upregulated ribosomal (r)-protein expression (through RNA polymerase II and conventional translation of mRNA for protein

synthesis), than their immediate progenies. Located at the heart of the translational machinery is the mechanistic target of Rapamycin (mTOR). Low levels of translation are directly linked to low levels of mTOR abundance and activity. mTOR has a dual role in translation (G. Y. Liu & Sabatini, 2020): one is activation of the cap-dependent mRNA translational machinery involving eIF4E-binding protein (4E-BP) and eukaryotic initiation factor 4F (eIF4F) complex formation, the other is ribosomal biogenesis induction via a phosphorylation cascade of r-protein S6 by S6 Kinase (S6K). It may seem contradictory that stem cells retain high levels of ribosomal biogenesis while simultaneously display low levels of translation and mTOR, when mTOR also induces ribosomal biogenesis. This contrast is thought to be achieved by the coordinated activity of other signalling pathways and regulatory mechanisms which can engage RNA polymerases I and III for rRNA synthesis and the production of r-proteins (as well as the eukaryotic ribosomal biogenesis factors and small nucleolar RNAs required for rRNA processing) (Saba et al., 2021).

Specifically in the HF, the stem cells (bulge HFSC and pre-activation hair germ cells) exhibit markedly lower protein synthesis than the proliferative progenitors of the matrix, and the highest levels of translation can be observed during anagen in the IRS lineage, in both committed progenitors and terminally differentiated compartments (Blanco et al., 2016). This is correlated with increased mTOR activity (as evidenced by increased phosphorylated S6) in activated hair germ at the onset of anagen (Deng et al., 2015; H. Yang et al., 2017), through a process where mTOR effectively signals back to supress BMP to assist in the continuing proliferative activation in the matrix (Deng et al., 2015). Moreover, epidermal morphogenesis and barrier formation is regulated by a tight relationship between ribosomal homeostasis (via ribosomal rescue factor Pelota, encoded by the *Pelo* gene) and mTOR activity, where dysregulation of *Pelo* results in the aberrant upregulation of mTOR and protein synthesis causing severe skin barrier defects (Ding et al., 2016; Elkenani et al., 2016; Liakath-Ali et al., 2018). To take the matter further in highlighting the involvement of translation in epidermal progenitor cell commitment; in the event of oncogenic stress (Cai et al., 2020), as manifest by HRAS mutation-driven hyperproliferation, the progenitor cells of the epidermal basal layer were able to contain the tumour outgrowth by inhibiting self-renewal and driving differentiation through a process of upregulated translation, in this way linking cell fate with translation rates.

1.1.8 **Termination of hair growth – Catagen**

As the hair follicle grows downwards during anagen, BMP-inhibitory signals and SHH produced by the dermal papilla and the matrix respectively, are physically pushed away from the bulge HFSC, which duly reassumes quiescence (Hsu et al., 2014). Hence the enveloping ORS will not expand to accommodate lengthening of the HF, and the HF will not grow any further. Moreover, TACs have a limited number of divisions before they terminally differentiate, meaning that the matrix will eventually be diminished (Alonso &

Fuchs, 2006). Differentiation will thus slow and the HF will enter the regressive phase of catagen, the transitional state of degeneration between anagen and telogen (Alonso & Fuchs, 2006). The hallmark of catagen is apoptosis, where the IRS and almost all proliferating cells are eliminated, with the exception of some ORS (former bulge) cells (Y.-C. Hsu et al., 2011). Cells found in the upper and middle ORS will escape apoptosis and contribute to the new forming bulge and hair germ respectively, and subject to activation and hair growth in the next hair cycle. Accordingly, after a completed hair cycle, the bulge cells are the origin of the hair germ although these different stem cell population respond differently to the anagen-initiating stimuli (Greco et al., 2009).

The receding bottom of the HF will seal off into what is called the club and move upwards as the surrounding cells apoptose, until it reaches the upper permanent isthmus of the HF. The HS will hereafter be called the club-hair. The DP will trail the club upwards to reassume its place adjacent to the newly re-established hair germ and once again exert its suppressive behaviour on the HFSCs, entering (refractory) telogen (Alonso & Fuchs, 2006). Molecular cues for anagen-to-catagen transitions remain obscured, however there is some evidence that FGF-5, BMP2/4, and TGFβ1 signalling can induce catagen, together with crosstalk between K17 and TNFα downstream effector NF-κB, which can regulate the rate of apoptosis (Alonso & Fuchs, 2006; Schneider et al., 2009; Tong & Coulombe, 2006).

After the massive apoptotic event of catagen, the hair follicle re-enters telogen and quiescence. As the individual gets older, the telogenic phases get longer. This has been attributed to age-associated stem cell intrinsic alterations, rendering hair follicle stem cells less likely to respond to activating cues from the environment (Keyes et al., 2013).

1.1.9 Development of the murine epidermis and hair follicle

Skin is of dual germ layer origin where epidermis develops from the ectoderm and dermis from the mesoderm. WNT activation in the ectoderm counteracts FGF responsiveness and induces BMP expression, which promote an epidermal fate (Fuchs, 2007). Epidermal specification occurs when the surface ectoderm initiates expression of *p63* (at embryonic day E8.5) and the newly formed epidermal progenitors express *Krt8* (Koster et al., 2004; Zhao et al., 2015)). In a process involving massive chromatin landscape remodelling and the directed action of p63 with coordination of WNT/β-catenin to express K14/K5 (at E9.5), this leads to the formation of the epidermal basal layer (Fan et al., 2018; Koster & Roop, 2007)). Shortly after, the basal layer epithelium gives rise to a second layer of cells called the periderm (M'Boneko & Merker, 1988), a protective barrier of sorts that prevents pathological adhesion of immature epithelia during development (Richardson et al., 2014). However, as periderm gets shed as soon as the barrier function of stratified and differentiated epidermis has been established, it is not a permanent constituent of the epidermis, and the formation of periderm is not considered the earliest

event of epidermal stratification. Instead, onset of stratification occurs at approximately E12.5 (Lechler & Fuchs, 2005) when the basal layer gives rise to another transient suprabasal proliferative cell layer betwixt the basal cells and the periderm. In this intermediate cell layer, p63 cooperates with Notch to induce K1 expression, rendering the cells both K1+, K14+, and proliferative (Hu et al., 2018). Differentiation of the intermediate layer into spinous keratinocytes occur at E14-15 as the cells downregulate K14/K5, emphasizing K1 expression while inducing K10, as well as become post-mitotic (Damen et al., 2021; Koster et al., 2004, 2007; Koster & Roop, 2007). Henceforth, proliferation only occurs in the basal layer (see section: 1.1.3 "Molecular features of epidermal homeostasis"). The maturation into K1+, post-mitotic spinous cells from K1+, mitotic intermediate cells is also dependent on p63 via the control of IKKα, IRF6, and 14-3-3σ, who work in concert to withdraw the cell from the cell cycle (Koster & Roop, 2007). The subsequent formation of the granular layer by differentiation of the spinous cells is strongly influenced by an increasing extracellular calcium (Ca^{2+}) concentration gradient. Ca^{2+} signalling converges on protein kinase C (PKC) to induce the terminal differentiation markers Loricrin, Filaggrin, and transglutaminases. Furthermore, Notch signalling in interaction with its ligand JAG1 has also been implicated in inducing Loricrin and Filaggrin expression, and in turn proper granular layer differentiation (Nickoloff et al., 2002). It is within the granular layer the barrier function of the epidermis is established with the expression of desmosomes (Garrod & Chidgey, 2008; Kowalczyk & Green, 2013) and tight junctions (Yokouchi et al., 2016), a prosses heavily influenced by the expression and activity of transcription factor KLF4 (Segre, 2003).

At the point when the epidermis is consisting of a single layer of epithelial cells, interaction with the underlying mesoderm leads to the specification of the epidermal appendages, such as HFs. HF formation is subdivided into distinct morphologically developmental stages. Firstly, WNT/β-catenin signalling is indispensable for epidermal keratinocytes to condense locally and form the hair placodes, evenly spaced invaginations into the dermis. After epidermal specification (which was the result of WNT-mediated attenuation of sensitivity to FGF with induced expression of BMP), the basal layer expresses WNT (Park, 2022), and by some elusive molecular mechanism some of the basal layer cells become further responsive to WNT and some do not (Fuchs, 2007). The unresponsive cells form the IFE, whereas the WNT responsive cells receive FGF7 and -10 signals (via the expression of FGF-receptor FGFRIIIb) (Petiot et al., 2003) and BMP inhibitor Noggin (Botchkarev et al., 1999) from the underlying dermal mesenchyme. Moreover, the initiation of the hair placode involves the upregulation of LEF1 with concomitant reduction of TCF3 (H. Nguyen et al., 2006), where LEF1 associates with β-catenin and displaces TCF3 form its target genes, and LEF1 deficient mice display a marked reduction in the number of hair follicles formed embryogenically (van Genderen et al., 1994).

After specification of epidermal cells into the hair follicle lineage, the placode starts expressing morphogens such as WNT and SHH, which coordinate the spatial distribution and proliferative behaviours of the growing HF to specify cell fate (Matos et al., 2020; Ouspenskaia et al., 2016; Z. Xu et al., 2015). SHH expression also leads to the recruitment and condensation of mesenchymal cells to form the dermal condensate, located just below the placode. The dermal condensate matures into the dermal papilla. Signalling emanating from the dermal papilla induces proliferation and directed growth of the developing hair follicle through additional mesenchymal-epithelial crosstalk (Clavel et al., 2012; Fuchs, 2007).

The formation of placodes in mice occurs in waves, where the first wave at E13.5-E14.5 results in primary guard hairs, a process dependent on Ectodysplasin isoform A1 (EDA-A1)/EDAR/NF-κB signalling. The EDA-A1/EDAR pathway signals via NF-κB to induce *Shh* and cyclin D1 upregulation leading to placode growth, and it may also induce *Wnt10b* expression and activates β-catenin signalling by stabilisation and activation of LEF1 (Schmidt-Ullrich & Paus, 2005). Moreover, β-catenin/LEF1 working in conjuncture with BMP inhibition, achieved by EDA-mediated expression of connective tissue growth factor (CTGF) and follistatin (Pummila et al., 2007), results in the inhibition of E-cadherin, an important cell adhesion protein, which allows downward growth of the expanding hair follicle (Jamora et al., 2003).

The second and third waves of hair placode formation occurs between E16 and P0, respectively, and results in secondary awl, auchene, and zigzag hairs, a process independent of EDA-A1/EDAR/NF-κB, but which requires Noggin and LEF1 (Fuchs, 2007; Schmidt-Ullrich & Paus, 2005; Schneider et al., 2009). Although the hair types differ in their initiation process, they subsequently follow the same developmental pathways and require the same molecular cues for proliferation and differentiation. The activated growth brought about by the different signalling events occur in a cell population which is, just as in anagen, called the hair germ, and will form the basis for the expanding HF and the differentiated lineages with in it that gives rise to the hair itself.

During growth of the hair follicle, stem cell markers that initially were co-expressed in cells of the placode (eg. *Sox9*, *Lgr6*, and *Lrig1*) segregate to set the foundation for the cellular heterogeneity observed in the mature HF (Schepeler et al., 2014; Snippert et al., 2010). The ORS will be formed by the K14+ cells that maintain contact with the basement membrane and are dependent on SOX9 for their continued maintenance of their cell identity (Mikkola, 2007; Vidal et al., 2005). At E16, as the HF grows downwards and forms an elongated structure referred to as the peg, the dermal papilla starts to become enwrapped by the most proximal cells, i.e. the hair germ, analogous to anagen. The cells around the dermal papilla will form the matrix, which are, just as described in anagen, a highly proliferative population of cells that through sustained reciprocal epithelial-mesenchymal signalling will give rise to the differentiated structures of the hair follicle.

The initial differentiation lineage to appear in the peg is the IRS. Essential for specification of matrix cells to differentiate into IRS precursors are GATA3 and CCAAT displacement protein (CDP, encoded by the *Cutl1* gene) (Fuchs, 2007; Mikkola, 2007; Schmidt-Ullrich & Paus, 2005) in a process seemingly involving both the CDP and GATA3-mediated suppression of *c-myc* and *p21*, and the recruitment of histone deacetylases (HDACs) to repress target genes through epigenetic control (Ellis et al., 2001; Kaufman et al., 2003). Moreover, GATA3 being a pSMAD target indicates active BMP signalling, where ablation of *Bmpr1a* abrogates IRS differentiation altogether (Andl et al., 2004; Kaufman et al., 2003). Additionally, Notch pathway signalling is crucial for maintaining the IRS differentiation trajectory and cell fate (Estrach et al., 2006; Pan et al., 2004; Watt et al., 2008).

At around E18-19, the dermal papilla will be fully enveloped in what is called the bulb, and the first signs of the HS emerges within the hair follicle. HS specification is, as described for in anagen, fully dependent on WNT/β-catenin signalling (Merrill et al., 2001), as exemplified by several cortex and HS-cuticle keratins being direct WNT target genes (Fuchs, 2007; Merrill et al., 2001). Interestingly, BMP signalling (via BMPR1a) is required for cell cycle exit and fate commitment of matrix cells to both the IRS and HS lineages (Genander et al., 2014; Kobielak et al., 2003; Mikkola, 2007; Ming Kwan et al., 2004).

Maturation and growth of the HF will continue for another 7-8 days and reach full maturity when the bulb reaches the bottom of the dermis, usually at around P6. Matrix cells will continue to proliferate, and the HS will protrude visibly through the skin. Hair growth will be sustained until the onset of the first catagen, sometime between postnatal day P14 (neck) to P18 (lower back/tail) to initiate the first hair cycle. The embryonic formation of the hair follicle until the onset of catagen is referred to as morphogenesis, and although it shares a lot of features with anagen, is considered separate from the hair cycle (Alonso & Fuchs, 2006; Fuchs, 2007; Müller-Röver et al., 2001; Schmidt-Ullrich & Paus, 2005; Schneider et al., 2009).

Morphogenesis and the first postnatal hair cycle are highly synchronized in mice, occurring in waves across the skin. However, synchronization is lost over time and becomes less and less apparent as the mouse ages (Alonso & Fuchs, 2006; Müller-Röver et al., 2001; Schneider et al., 2009). The molecular reasons for this are not entirely clear (Plikus et al., 2008).

To conclude, skin and hair follicle biology is a powerful model system in which to study stem cell behaviour. Both during development and adult homeostasis are the highly organised signalling cues and transcriptional networks at such a complex level that new insights about cell biology is being revealed with each new study, even though the epidermal cell research field has been going for close to a century (Ferreira et al., 2021). As more research is being poured into this area, more and broader insights into fundamental cell biological aspects will be uncovered.

1.2 PADIs and citrullination

Citrullination, or deimination, of proteins is a post-translational modification where the positively charged amino acid arginine is converted to the neutral amino acid citrulline (Figure 2). Peptidylcitrulline, the presence of the non-essential amino acid citrulline within a protein, is thus not a product of translation but must be generated via enzymatic catalysation within an existing peptide chain. Peptidylcitrulline is also distinct from unbound citrulline which is an intermediate metabolite of the urea cycle.

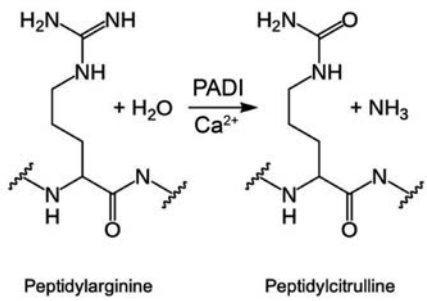

Figure 2. Citrullination of peptidylarginine by PADI enzymes results in peptidylcitrulline, in the presence of Ca^{2+}

Protein bound citrulline was first described in 1958 (Rogers, 1958), and was shortly after found to be an integral constituent within peptide sequences (Rogers, 1962). In 1977, it was shown that citrullination was the result of enzymatic catalytic activity (Rogers et al., 1977), and in 1981 the first enzymes carrying out citrullination were described (Fujisaki & Sugawara, 1981).

Catalysing citrullination are the highly conserved, calcium dependent, Peptidylarginine Deiminase (PADI) enzymes. PADIs exist across the animal kingdom, expressed in bacteria, fungi, and eukaryotic vertebrates (Crisp et al., 2015). A recent study demonstrated that PADIs was acquired by vertebrates by horizontal gene transfer from cyanobacteria (Cummings et al., 2022), whereas fungi and actinobacteria have developed enzymatically similar but structurally different PADI proteins – the cyanobacteria/vertebrate PADIs consists of a three-domain structure whereas the fungi/actinobacteria consists of two. Although the physiological purpose of fungal PADIs is unknown, they are catalytically active and are able to citrullinate peptides in a manner akin to mammalian PADIs (El-Sayed et al., 2019). Interestingly, other eukaryotes such as yeast, flies, and worms lack PADIs altogether (Crisp et al., 2015), suggesting that the evolutionary pressure to evolve PADIs did not take place in the Last Universal Common Ancestor, and no horizontal gene transfer occurred into the invertebrate eukaryotes (although the possibility of gene loss may still exist (Salzberg, 2017)). However in terms of evolution, the catalytic domain of PADIs contains a most highly conserved structural moiety, termed a pentein fold, which comprises a trio of Cys-His-Asn that carries out the core catalytic hydrolysis event of the citrullination itself (Linsky & Fast, 2010), suggesting that the PADI proteins have gained their chemical catalytic ability from a far more ancient source than cyanobacteria. Additionally, human pathogens *Porphyromonas gingivalis* and *Giardia Lamblia* both have citrullinating enzymes, called pPAD (Goulas et al., 2015) and gADI (Touz et al., 2008), respectively, which are evolutionary independent from PADIs. While genetically unrelated

to eukaryotic PADIs, the pPAD and gADI catalytic domain also contain the pentein fold, further highlighting the positive selection for this type of structure, and are also able to citrullinate substrates of eukaryotic PADIs (Wegner, Wait, et al., 2010). In an interesting turn of events, even plants may have the ability for protein citrullination. A recent study (Marondedze et al., 2021) discovered citrullinated proteins in the *Arabidopsis thaliana* proteome and were subsequently able to identify a catalytic motif in the genome, based on sequences for bacterial PADI catalytic domain. Although the identified enzyme (with the catchy name At5g08170) may not be homologous to PADIs, it functions in a calcium dependent manner (similar to PADIs) and generates citrullinated proteins. Collectively, these findings suggest that protein citrullination is an evolutionary advantageous PTM so highly specialized it is utilized to carry out functions other PTMs cannot, further expanding the levels of regulation of protein biology, and has been incorporated into complex biological processes across most kingdoms of life.

The evolutionary pressure on PADIs in vertebrates has been such that several gene duplications have occurred over time. Thus, there are currently five different mammalian PADIs described, namely PADI1, 2, 3, 4, and 6. There is no longer a PADI5, since when PADI5 was first identified in humans it was believed to be a novel PADI protein. It was later found that PADI5 was the human homolog of rodent PADI4, and was thus renamed (Vossenaar et al., 2003). However, before the approval to fuse the PADI4 and PADI5 nomenclature, PADI6 had already been discovered (Chavanas et al., 2004; Wright et al., 2003) and the fact of the matter was settled. The enzymatic activity of these removes the imine group of the arginine sidechain and replaces it with a ureido group (Figure 2), changing the overall charge of the protein and affecting intra-ionic interactions within the protein (Vossenaar et al., 2003). The exact effects of citrullination of the target protein is highly contextual and depend largely on the location within the peptide in which it occurs and may impact on protein biology by altering conformation, stability (as in degradation or turn-over), subcellular localisation, binding capabilities (to RNA, DNA, or other proteins), or interaction with other PTMs, either facilitating or antagonising covalent adduct such as acetylation or methylation (Christophorou, 2022).

In mammals, the PADI enzymes have classically been described to be differentially expressed according to both tissue type and subcellular localization (Table 1) (Christensen et al., 2022; Vossenaar et al., 2003). PADI1 expression was first observed in epidermis and uterus (Guerrin et al., 2003; Ishida-Yamamoto et al., 2002; Nachat, Mechin, Charveron, et al., 2005; Nachat, Méchin, et al., 2005; Rus'd et al., 1999; Terakawa et al., 1991), but has later also been implicated in preimplantation embryos (Zhang et al., 2016). PADI2 has the most widely distributed tissue expression, being described in the pituitary gland, brain, spinal cord, uterus, spleen, breast, pancreas, skin, immune cells, and skeletal muscle (Beato & Sharma, 2020; Falcão et al., 2019; Van Beers, Zendman, et al., 2013; Vossenaar et al., 2003; S. Wang & Wang, 2013). On the other hand, PADI3 expression has been suggested to be limited to epidermis and hair follicles (Kanno et al., 2000; M. C. Méchin et

al., 2020; Nachat, Mechin, Charveron, et al., 2005; Nachat, Méchin, et al., 2005; Vossenaar et al., 2003). Moreover, arguably the most studied PADI is PADI4, which has been described extensively in cells of myeloid origin such as leukocytes, bone marrow, spleen, blood, and lymphocytes, but also in embryos and various stem cell systems (Brahmajosyula et al., 2010; Brahmajosyula & Miyake, 2013; Christophorou et al., 2014; Kan et al., 2012; Nakashima et al., 2013; Young et al., 2022). PADI6 expression is detected in the oocytes in human ovaries, testes, and in the early murine embryo (Chavanas et al., 2004; Kan et al., 2011; Wright et al., 2003)).

PADI enzyme	*Tissue*	*Subcellular localization*
PADI1	Epidermis, uterus	Cytoplasm
PADI2	Pituitary gland, brain, spinal cord, uterus, spleen, breast, pancreas, skin, immune cells, skeletal muscle	Cytoplasm/Nucleus
PADI3	Epidermis, hair follicles, trophoblast	Cytoplasm
PADI4	Immune cells, mammary gland epithelium, mESC, iPSCs, pre-implantation embryos, brain, haematopoietic stem cells	Nucleus
PADI6	Ovaries, egg cells, testis, early embryo	Cytoplasm

Table 1. *Conventional view on PADI distribution*

From an evolutionary viewpoint, PADI2 is considered the ancestral PADI (Christophorou, 2022; Cummings et al., 2022; Villanueva-Cañas et al., 2022), whose gene duplication and modification has given rise to the other PADIs, to possibly generate a tissue appropriate substrate specificity or as a result of divergent demands in protein biology regulation due to specific tissue functions.

In addition to their suggested tissue specific expression profiles, PADI enzymes have been detected in both the cell nucleus and cytoplasm. PADI4 is the only family member reported to have an exclusive nuclear localization, containing an N-terminal nuclear localisation signal (NLS) (Asaga et al., 2001; Bicker & Thompson, 2013; Christophorou et al., 2014; Nakashima et al., 2002). PADI2 has been shown to be able to shuttle between the nucleus and cytoplasm (Cherrington et al., 2010, 2012; Falcão et al., 2019; Sharma et al., 2019), whereas PADI1, PADI3, and PADI6 are limited to the cytoplasm.

Digging deeper, the tissue distribution of the different PADI members is not a discrete manifestation. Indeed, in the example of trophoblast stem cell expression, PADI2 works in conjuncture with PADI3 to regulate histone citrullination and concomitant DNA methylation in maintaining the trophoblast stem cell state and facilitating proper differentiation (Ballasy et al., 2022). Moreover, scrutinizing the Human Protein Atlas (Uhlén et al., 2015) for PADI protein and mRNA (*PADI*) expression (Christophorou, 2022), a more

complex picture arises in terms of tissue distribution, apart from that already described: PADI1 localisation can be extended to oesophagus, testis, kidney and cervix; PADI2 is observable in digestive and gastrointestinal tract, kidney, bladder, testis and bone marrow, whereas *PADI2* mRNA is detectable in virtually all tissues listed in the database; *PADI3* can be detected in oesophagus and urinary bladder; and *PADI4* is, similar to *PADI2*, detectable to various degrees in most tissues, most markedly in bone marrow and lung.

Collectively, the evolutionary pressure to acquire, keep, and expand the PADI family appears to have been relatively high. Keeping this in mind, it is perhaps not unreasonable to argue that the diversity in tissue distribution that is being nuanced with each new finding is hinting at a wide functional applicability of PADIs and the citrullination they bring.

1.2.1 Structure of PADIs

The PADIs are structurally also highly conserved enzymes with a high level of sequence homology, both genetically and in their amino-acid sequences (M.-C. Méchin et al., 2007). In terms of the biochemical structure of the PADI enzymes, the most detailed studied family member is PADI4 (Bicker & Thompson, 2013). As shown by x-ray crystallography, the tertiary structure of PADI4 is subdivided into three structural domains, the N-terminal (PAD_N), middle (PAD_M), and C-terminal domain (PAD_C), and contains a total of five calcium binding sites (Arita et al., 2004). Three of these are located in the N-terminal and two help bridge the N- and C-terminals. Calcium binding brings about conformational changes, generating the active cleft in the C-terminal. Calcium must bind all five sites in order for PADI4 to become catalytically active. Additionally, PADI4 forms a head-to-tail homodimer to attain full enzymatic activity (Arita et al., 2004; C.-Y. Lee et al., 2017). Similarly, calcium-binding and homodimerization have also been shown for PADI2 (Slade et al., 2015) and PADI3 (Saijo et al., 2016). However PADI1 functions as a monomer and exhibits a broader substrate specificity (Saijo et al., 2016). Additionally, PADI6 seems to be catalytically inactive in that it is unable to citrullinate substrates which are readily citrullinated by the other PADIs *in vitro* (Knuckley et al., 2010). Based on sequence homology, several calcium binding sites as well as several amino acids in the stipulated active cleft are missing in PADI6, including Cys of the catalytic triad within the aforementioned pentein fold, suggesting a structural basis for its enzymatic inactivity (Chavanas et al., 2004; Raijmakers et al., 2007). However, the high-resolution structure of PADI6 remains to be reported. Nevertheless, it is still possible that PADI6 may have completely different substrate preferences than its family members and may require other cofactors than calcium to function (Raijmakers et al., 2007). Other possibilities suggests that PADI6 functions by simply binding to other proteins and in this way facilitates or obstructs its targets' participation in distinct protein complexes, thusly conveying a secondary function apart from citrullination. This is perhaps exemplified by the fact that despite PADI6 not having any (as of yet detectable) enzymatic activity, genetic mutations causing structural defects in the protein leads to diminished protein

abundance in oocytes and early embryos, and through a mechanism involving aberrant formation of cytoskeletal structures, causes early embryonic arrest, and consequently female infertility (Esposito et al., 2007; Qian et al., 2018; X. Wang et al., 2022; Y. Xu et al., 2016, 2022; Yurttas et al., 2008).

1.2.2 Calcium requirement and other cofactors for PADI activity

What is more, the involvement of calcium for PADI activity is well documented (Arita et al., 2004; Slade et al., 2015), and as the calcium binding sites are highly conserved structures in PADI1-4 and essential for PADI activity it is clear that it makes out an important regulating factor for when and how PADIs are recruited. Although many disease settings (such as Rheumatoid Arthritis (Tilvawala et al., 2018) (discussed in detail below)) or physiological settings (as in skin differentiation (PADI3 (Nachat, Méchin, et al., 2005; Senshu et al., 1996a)) are associated with high calcium concentrations, the calcium concentrations used *in vitro* to analyse PADI activity (0.5-20mM Ca^{2+}, most commonly used at 5-10mM) greatly exceed physiological intracellular calcium levels (reaching up to 10μM but usually lower than that (Atchison & Beierwaltes, 2013; Christophorou, 2022; Tour et al., 2007)). Due to this, it has been speculated (Christophorou, 2022) that signalling pathways regulating the local intracellular calcium environment such as opening of calcium channels on the plasma membrane or from intracellular calcium stores (like the endoplasmic reticulum) have been involved in controlling PADI activity. Indeed, neutrophils stimulated by ionophores or LPS (Neeli et al., 2008) will readily increase their intracellular calcium concentrations with consequently high PADI4 activity and hypercitrullination to follow. However, there are observations of PADI activity in cells that do not undergo these activation steps in terms of calcium influx. In light of this, a search for co-factors able to sensitise PADIs to lower levels of calcium or being able to active PADIs even in the absence of calcium, has been initiated. Thus far, allosteric interactors have been found in Rheumatoid Arthritis (Darrah et al., 2013; Demoruelle et al., 2021), where a subset of autoantibodies against PADI4 (with cross-reactivity with PADI3 [as of yet not implicated in Rheumatoid Arthritis]) bind to PADI4 in a way that poises the active site for activation and effectively lowering the requirements for calcium concentrations. Additionally, biochemical effectors of PADI activity have been found to include reducing agent Glutathione (GSH) (Damgaard et al., 2016) and bicarbonate (Zhou et al., 2018). Bicarbonate was interestingly able to induce PADI4 to carry out citrullination even without the presence of calcium. GSH on the other hand (Damgaard et al., 2016), was shown to be a physiological co-factor to the effects of calcium binding, as *in vitro* assays with supraphysiological levels of Ca^{2+} in the absence of GSH (or non-physiological reducing agent DTT) did not elicit measurable PADI activity. Conversely, in the presence of high levels of reactive oxygen species (ROS) PADI2- and PADI4-mediated hypercitrullination was inhibited (Damgaard et al., 2017). However, the biochemical mechanisms for this remains to be determined.

Jointly, these findings suggest that there are many aspects of PADI activity regulation to uncover with regards to the physiological biochemical mechanisms that may affect citrullination in both health and disease.

1.2.3 Citrullination in epidermis and hair follicles

In the epidermis, citrullination is an integral part of tissue homeostasis and barrier function where key components of the cornified envelop such as Filaggrin (FLG), FLG-2, Hornerin (HRNR), K1, and K10 are citrullinated (C.-Y. Hsu et al., 2011, 2017; Senshu et al., 1996b). Citrullination of these proteins enhances their crosslinking by transglutaminases, important for the mechanical resilience of the epidermis and formation of the cornified envelope. Furthermore, citrullination of FLG and FLG-2 facilitates their proteolysis to generate free amino acids, whose derivatives are components of the epidermal natural moisturizing factor (NMF), a collection of hygroscopic molecules that mediate water-retention in the upper *stratum corneum* (Cau et al., 2018). Additional citrullinated keratins in the epidermis include K2, K14, K6, K16, and K17 (M.-C. Méchin et al., 2020) – keratins associated with proliferation in undifferentiated epidermal progenitor cells.

The literature has traditionally stated that PADI3 is the only PADI in the HF, whereas PADI3 and PADI1 (and maybe PADI2 (M. C. Méchin et al., 2020) but with some ambiguity) are co-expressed in the epidermis (Nachat, Mechin, Charveron, et al., 2005; Nachat, Mechin, Takahara, et al., 2005). Stressing the importance of citrullination in proper skin barrier function, a recent study (Alioli et al., 2023) found that in reconstructed human epidermis, PADI1 appears to be the main citrullinating enzyme as its induced downregulation brought about a significant global decrease of citrullination, with a concurrent downregulation of LOR, FLG, and transglutaminases, resulting in a diminished cornified envelope and increased epidermal permeability allowing transcutaneous water-loss.

Turning to the HF, PADI3 is associated with terminal differentiation of HF lineages during anagen, and citrullinated proteins has so far only been detected in the IRS, medulla, and cuticle of the HS. The best verified target of PADI3 is Trichohyalin (TCHH), a structural protein abundant in IRS and medulla, and has an essential part in hair morphology and HF architecture (Méchin et al., 2007). In fact, TCHH was the first citrullinated protein ever discovered (Rogers et al., 1977). Citrullination of TCHH facilitates its solubility and subsequently its aggregation (Tarcsa et al., 1996a, 1997), which is required to provide mechanical strength in the IRS and enable air entrapment for thermal insulation in the medulla (Steinert et al., 2003). Additionally, K2, K25, K28 and K71 expressed in the IRS are citrullinated (Langbein et al., 2010), as well as S100A3, which functions to support the cuticle. Interestingly, all citrullinated proteins in the hair follicle that are not keratins, belong to the S100-fused protein family (FLGs, HRNR, TCHH, and S100A3), hinting at a preference based on function and/or structure for substrate specificity (Knuckley et al., 2010; Tarcsa et al., 1996b). Of note is that S100A3 can be citrullinated by all three of PADI1, 2, and 3

(albeit *in vitro*) but the enzymes exhibit divergent preferences for which Arg within the peptide they citrullinate (Kizawa et al., 2017), proposing that substrate specificity is non-overlapping and non-random between the PADIs.

However, despite the clear contribution of citrullination to the structural integrity and function of HF proteins, there remains some inconsistencies in the reported expression pattern of the PADIs (see "1.2. PADIs and citrullination") within the HF. Making use of transcriptomic and chromatin remodelling data sets in studies exploring the dynamic shifts in HFSC during activation and rest, it is possible to discern *Padi3* and *Padi4* expression in the activated hair germ (Lien et al., 2011), as well as increased chromatin accessibility for *Padi3* and *Padi4* (Adam et al., 2018). Moreover, turning to a single cell sequencing atlas for mouse skin and HF in anagen (Joost et al., 2020) to probe the cell type expression of *Padi* mRNA (as *Padi1/2/3* have been readily described in these tissues), *Padi1* and *Padi2* are essentially undetectable, whereas *Padi3* is expressed in the IRS and HS medulla of growing HFs (in line with previous reports) but with little to no expression in the epidermis. Curiously, this analysis also detects *Padi4* in a gradual expression pattern from lowest in the proliferating progenitors committed to HS differentiation, to highest in the terminally differentiated HS medulla, and to some extent in cortex and the IRS. Considering the signalling pathways (predominantly WNT and BMP) that govern the activation of HFSC and lineage specification of matrix cells, it is noteworthy that the increased chromatin accessibility of *Padi3* and *Padi4* correlates with pSMAD1/5 binding sites on the respective gene promoters (Genander et al., 2014), and that both *Padi3* and *Padi4* are WNT targets, albeit LEF1-independent (Pálmer et al., 2008) and LEF1-dependent (Adam et al., 2018), respectively. Given the role of PADI4 in hematopoietic stem cells, where PADI4 physically associates with LEF1 (Nakashima et al., 2013; Young et al., 2022), it is tempting to speculate that PADI4 may act to regulate the proliferative potential in activated HFSCs, although functional studies for PADI4 in the HF are needed to clarify the role for PADI4 during these cell states.

1.2.3.1 Human skin diseases associated with PADIs

Contemplating its involvement in skin and HF homeostasis, PADI-dysregulation has unsurprisingly been implicated in several human skin disorders. In psoriasis (Ishida-Yamamoto et al., 2000) as well as bullous congenital ichthyosiform erythroderma (Ishida-Yamamoto et al., 2002), K1 citrullination is diminished in lesioned epidermis, resulting in aberrant crosslinking with FLG. In both these diseases, blistering and scaling is observed in the lesioned areas, and it is believed that decreased K1 citrullination affects the overall structural organization of the epidermis. In the case of psoriasis, PADI1 was recently shown (Padhi et al., 2022) to be downregulated in lesional psoriatic skin with concomitant decreased levels of citrullinated FLG and K1, in a process mediated by interleukin (IL)-22. Furthermore, another skin disease displaying decreased levels of citrullinated FLG is atopic dermatitis (AD) (Winget et al., 2016), which also manifest high levels of IL-22 in AD

lesions (Hänel et al., 2013). Subsequentially, PADI1 was found to be decreased in AD lesions (He et al., 2021; Padhi et al., 2023), and this correlated with the decreased levels of citrullinated FLG (Padhi et al., 2023). Similar to psoriasis, PADI1 downregulation was mediated via IL-22, but also by IL-4 and IL-13. Besides, treatment with Janus kinase (JAK) 1/2 inhibitor baricitinib (a targeted therapy of AD (Chovatiya & Paller, 2021)) re-established the expression of PADI1 in IL-4, -13, and -22 treated keratinocytes (as the JAK pathway elicit inflammation in epidermis in response to these cytokines), suggesting that an inflammatory environment can act on keratinocytes to adopt a transcriptomic response that affect epidermal barrier function and in extension leading to disease.

Moreover, mutations in human *PADI3* were discovered to give rise to uncombable hair syndrome (UHS), a rare hair shaft dysplasia that appear in young children and is characterized by hair follicles that grow in multiple directions with dry, frizzy, and tousled hair that is impossible to flatten (Ü. Basmanav et al., 2016). Out of eleven children with UHS, nine exhibited *PADI3* mutations, whereas one had mutations in transglutaminase and another in *TCHH*, highlighting the interdependency of these proteins in generating the hair.

Peculiarly, in a recent study (Díez-Del-Molino et al., 2023) sequencing a cohort of 23 woolly mammoths to probe the positive selection of adaptive traits along the species' evolution over 700,000 years, several mutations in *PADI3* and *TCHH* was found, which correlated with those observed in UHS. The authors speculate that perhaps a frizzy hair phenotype was better adapted to a cold environment. However, if the children afflicted by UHS are better suited to withstand cold climate, is best left unsaid.

Additionally, central centrifugal cicatricial alopecia (CCCA), the most common form of scarring alopecia in women of African ancestry, was associated with missense mutations in *PADI3* (Malki et al., 2019). These mutations were found to result in decreased enzyme expression, activity, and abnormal intracellular localization. The expression of PADI3 substrates TCHH and S100A3 was concomitantly also decreased in CCCA. Manifestations of CCCA normally occurs after intensive grooming over a period of time, however family members without hair trauma also exhibited signs of CCCA, indicating a genetic component of the condition. Subsequently, genetic analysis revealed that the prevalence of *PADI3* mutation was higher in CCCA patients than in the control group, demonstrating the underlying pathology to be genetic but with external stressors significantly exacerbating the condition.

Together, these data further link PADI1 and PADI3 to epidermal barrier function and proper hair differentiation.

1.2.4 Citrullination of histones – a stable epigenetic modification

Because of its nuclear localization, the main reported activity of PADI4 is its ability to citrullinate arginine residues in histone tails. Histone modifications entail intricate epigenetic transcriptional regulatory networks, directing adaptive transitions between

transcriptionally permissive or repressive chromatin states in what is sometimes called the 'Histone code' (Jenuwein & Allis, 2001). Histone modifications include established ones such as histone methylation, acetylation, and phosphorylation (Fischer et al., 2008; Oki et al., 2007), but also more uncommon ones such as serotonylation (Farrelly et al., 2019), ubiquitylation and sumoylation (Bannister & Kouzarides, 2011). Citrullination is now also an acknowledged histone modification that partakes in chromatin dynamics and gene regulation and has been credited with both activating and repressive functions in a context dependent manner (Christophorou, 2022).

Over the years since PADI4 was discovered to citrullinate histone tails (Cuthbert et al., 2004; Fuhrmann & Thompson, 2016; Hagiwara et al., 2002; Volker et al., 2004; Y. Wang et al., 2004), the identification of histone citrullination have relied heavily on antibodies. Subsequent proteomic approaches employing high-throughput mass spectrometry were largely able to corroborate the histone citrullination profile put forward by the immunoreactivity assays (Chaerkady et al., 2021; Christophorou et al., 2014; Falcão et al., 2019; Fert-Bober et al., 2019; Tanikawa et al., 2018). Accordingly, PADI4 was found to citrullinate core histones H2A at position H2AR3, H3 at positions H3R2, H3R8, H3R17, and H3R26, and H4 at position H4R3 (Kolodziej et al., 2014), as well as linker histone H1 at position R54 (Christophorou et al., 2014). However, a recent study (Rebak et al., 2022) put great effort in improving the sensitivity and specificity of quantitative mass spectrometry-based proteomic detection of citrullination and was able to show significantly more citrullination sites within the histone tails, including those previously reported, but also identifying a large number of unreported citrullination sites on all core histones and linker histone H1, vastly expanding the possibilities and interpretation for how regularly and commonly citrullination is employed for physiological performances of the nucleosome.

Nevertheless, Histone citrullination operates in close coordination with other histone modifications as it can occur at unmodified or monomethylated arginine, and largely acts to counteract the activity of protein arginine methyltransferases (PRMTs) (Y. Wang et al., 2004). In general, histone methylation of H3R17 and H4R3 by PRMTs is considered a mark for activation of transcription. Because PADI4-mediated citrullination antagonises arginine methylation by PRMTs it was hence considered a repressive mark of transcription (S. Wang & Wang, 2013), sometimes by direct citrullination of the arginine target site (H3R17) to outcompete methylation (P. Li, Wang, et al., 2010), or in coordination with other histone modifying enzymes (S. Wang & Wang, 2013). In fact, the first enzyme identified to antagonise histone methylation was PADI4 (Cuthbert et al., 2004).

However, the effects of histone citrullination can be quite dynamic given the context in which it occurs. And despite the extensive histone citrullination landscape now identified (Rebak et al., 2022), no functional data for the newly discovered citrullination sites exist as of yet (given the recentness of reporting). Therefore, our current knowledge of the

biological impact of histone citrullinated is based on individual studies addressing the distinct histone modification within each given context.

As such, to give some examples of how direct histone citrullination may activate gene expression, citrullination of H3R8 was shown to result in gene activation by disabling the binding site on the histone tail for the repressive HP1α protein (Sharma et al., 2012). HP1α normally binds to trimethylated H3 lysine 9 (H3K9me3) at target promoters to silence their expression. However, when H3R8 was citrullinated, HP1α was unable to bind due to the loss of the adjacent docking site as previously made up by the polar sidechain of arginine. In activated T-cells, this resulted in upregulation of the cytokines TNFα and IL-8, as well as several human endogenous retrovirus (HERV) genes, in a manner reminiscent of that seen in multiple sclerosis.

In addition, methylation of H3K27 by polycomb repressive complex-2 (PRC2) represses gene transcription, however citrullination of adjacent R26 reduces methylation of K27 30,000-fold *in vitro* (Clancy et al., 2017), thus facilitating activated transcription. Interestingly, increased methylation states (mono- to trimethylation) of H3K27 did in turn slow citrullination rate of H3R26, but only up to 30-fold. Indeed, in ER(+) breast cancer cell lines, H3R26-citrullination facilitated the upregulation of several oestrogen receptor-α responsive genes, showcasing the delicate crosstalk of histone modifications and their contribution to transcriptional regulation.

Moreover, in contrast to the repressive function of histone citrullination ascribed to the antagonising activity towards PRMTs, another study (Zhang et al., 2011) probed the chromatin binding of PADI4 and found that it predominantly localised to the transcription start sites of actively transcribed genes, while interacting with other histone modifying enzymes and activating histone marks. Interestingly, while histone citrullination was not probed for in this study, PADI4 chromatin binding showed strong anti-correlated repressive histone marks H3K9me3 and H3K27me3 trimethylations. While PADI4 cannot citrullinate trimethylated arginine, it is conceivable that if histone methylation occurs sequentially, the initial methylation deposit is antagonised by citrullination, and if arginine is converted into citrulline it will not be reconverted (to be determined if it can) and no trimethylation can pursue.

To add complexity to the matter, it is not only PADI4 with the capacity to citrullinate histones, as PADI2 was found to citrullinate H3R26 in human breast cancer cell lines, leading to chromatin decondensation and transcriptional activation (Guertin et al., 2014; Zhang et al., 2012). PADI2 also specifically citrullinates H3R2+8+17 in canine mammary epithelium during the dioestrus stage to regulate the expression of lactation related genes (Cherrington et al., 2010).

It is evident that citrullination works in collaboration with other histone modifications and PADI4 exist in a gene-regulatory space together with other posttranslational modifying

enzymes. However, histone modifications such as methylation, acetylation and phosphorylation all have antagonising enzymes that removes the modification: Jumonji domain containing (JmjC) demethylases for arginine methylation and lysine-specific demethylases (LSD1), HDACS, and phosphatases, respectively (Bannister & Kouzarides, 2011). No such enzyme has been found for citrullination. De-citrullinating enzymes may even not exist, owning to the fact that citrullination is not a covalently bound adduct like the other modifications, but rather the stable transition from one amino acid to another. Instead, if citrullination is a permanent modification, its removal will likely be dependent on histone turnover and/or nucleosome exchange (Venkatesh & Workman, 2015). This is evident by the fact that after a stimulus for citrullination, the citrullination on the target disappears after a time (Cuthbert et al., 2004). The inability to remove histone citrullination through enzymatic catalysis may be a way to maintain certain transcriptional networks that are needed to maintain a specific cell state, e.g. pluripotency, even when the expression profile of PADIs fluctuate. Indeed, Rebak and Nielsen *et al.* (Rebak et al., 2022) showed in their proteomics study that even with the treatment of PADI4-specific inhibitor GSK484 that several histone citrullination sites remained unchanged. The unchanged histone citrullination sites were found predominantly on linker histone H1, implicating that in light of its importance in general chromatin decondensation for pluripotency maintenance (Christophorou et al., 2014), the ability to maintain an open chromatin state is favoured when the overall citrullinating ability is challenged. For stem cells, this may be a way to protect against aberrant signalling for upregulated proliferation or inaccurate cues for differentiation.

Nonetheless, these reports indicate that there are still many questions to answer regarding the intricacies of histone citrullination-mediated gene regulation. Collectively, citrullination of histones may function to regulate transcription directly, but more often it is with coordination, or by modulation, of other histone modifications and their associated enzymes. The search continues for the elusive de-citrullinase, or in absence of this, an adequate explanation for why citrullination is prioritized as a stable modification whose removal only involves protein turn-over.

1.2.5 Citrullination in stem and progenitor cells

PADI4 expression has been described in several stem cell systems and has been functionally linked to the maintenance of stemness. PADI4 acts to maintain pluripotency in both human induced pluripotent stem cells (iPSCs) and in mouse embryonic stem cells (mESCs) by mediating citrullination of histone H1. Citrullination of H1R54 at the DNA-binding site leads to H1 displacement and global chromatin decondensation. Moreover, PADI4 H3R2+R8+R17 citrullination at *Tcl1* and *Nanog* promoters correlate positively to their expression (Christophorou et al., 2014). In another study not addressing the contribution of any particular PADI to pluripotency maintenance, the authors instead identified histone H3R26 citrullination as a mark for chromatin occupancy of SMARCAD1 in naïve-state

pluripotent human ESCs (Xiao et al., 2017). In this study, SMARCAD1 was identified as a reader of H3R26Cit and co-localized on the genome not by DNA-binding but by preferential binding to the histone citrullination. Conversely, knocking down H3R26Cit increased H3K9me3 at SMARCAD1 binding regions as well as increased the chromatin condensation. In this process, the ESCs transitioned form a naïve to a primed pluripotency state, suggesting that histone citrullination is an integral constituent in pluripotency maintenance.

In another setting of mECS, PADI4 was also found to citrullinate HP1γ. HP1γ is a protein whose association with H3K9me3 is considered a corepressor of transcription. Citrullination of HP1γ inhibits its chromatin affinity, contributing to an open chromatin landscape. HP1γ citrullination, and PADI4 expression, decrease with differentiation. This leads to increased chromatin association of HP1γ, and subsequent repression of genes associated with stemness, as exemplified by *Nanog* (Wiese et al., 2019). This further implicates PADI4 in pluripotency maintenance.

Moreover, PADI4 has been found to regulate proliferation of haematopoietic multipotent progenitors by facilitating histone deacetylation by HDAC1 (Nakashima et al., 2013), leading to a LEF1-mediated suppression of *c-myc* expression, and in this way moderating the rate of proliferation. Furthermore, PADI4 binds the transcriptional regulator TAL1 (Kolodziej et al., 2014) and acts to influence lineage differentiation of haematopoietic progenitor cells by preventing, through direct H3 citrullination, PRMT6-mediated H3R2me2 methylation at TAL1 target genes.

What is more, histone citrullination of H4R3, H3R2+8+17, and H3R26 was found to be required for early embryo development as treatment with PADI inhibitor Cl-amidine (Knuckley et al., 2010; Luo et al., 2006) reduced citrullination, as well as histone acetylation, and arrested development at the 2-4 cell stage (Kan et al., 2012). PADI4 was later discovered in pre-implantation embryos, with concordant H3 citrullination (Brahmajosyula & Miyake, 2013). Surprisingly, in these early-stage embryos PADI4 is reported to be localized to the cytoplasm with a gradual translocation to the nucleus as the embryo develops. In contrast, PADI1 is believed to be the prevailing nuclear deiminase responsible for histone citrullination (Zhang et al., 2016). More research will have to go into the potential overlapping functions of the PADIs in early embryogenesis in general, and the function and localization of PADI1 in particular, as this has been largely overlooked. Regardless, histone citrullination is required for the development of the zygote from 2-cell stage to blastocyst.

In cells where PADI4 is not preferentially expressed, PADI2 assumes the role of histone citrullinating enzyme. In oligodendrocyte precursor cells (OPCs) (Falcão et al., 2019), PADI2 is not implicated in stem cell maintenance, but is instrumental for the correct differentiation trajectory of precursor cells, via committed oligodendrocyte intermediates, to terminally differentiated oligodendrocytes. Through citrullination of core

histones H2B and H3, and linker histone H1 (as well as a host of other proteins) was PADI2 integral to the differentiation process, as shown in mice with oligodendrocyte-specific ablation of PADI2 that displayed motor dysfunction and diminished myelination of the corpus callosum.

1.2.6 Non-histone citrullination convey transcriptional regulation and translational control

PADI4 has been shown to citrullinate a range of different proteins besides histones (Zhai et al., 2017). Citrullination of non-histone substrates commonly result in effects on transcription, translation, or RNA processing, highlighting that even though histones are not directly citrullinated, PADI4-mediated citrullination is a core modulation of key cellular processes.

For example, PADI4 citrullination of histone acetyltransferase P300 was shown to counteract PRMT4-mediated methylation of P300, altering the transcriptional coactivator complex assembly and function *in vitro*. P300 citrullination affects oestrogen receptor-mediated transcription (Y.-H. Lee et al., 2005), thus displaying the importance of demethylimination by PADI4 in hormone-dependent physiological responses in other ways than by histone citrullination.

PADI4 has also been found to citrullinate the NLS region of the tumour suppressor inhibitor of growth 4 (ING4), thus disrupting its affiliation with p53 and subsequently repressing p53-dependent transcription, resulting in suppressed p21 expression (Guo & Fast, 2011). Suppression of p21 expression in a context where p53 is activated, for example due to DNA-damage, may help the cell to avoid senescence or apoptosis, and can in this way bring about tumorigenicity.

Additionally, DNA Methyltransferase 3 Alpha (DNMT3A), an epigenetic modifier that methylates DNA *de novo*, was found to be citrullinated by PADI4. Expression of PADI4 and DNMT3A was positively correlated, as was citrullination with DNMT3A enzymatic activity. Also, citrullination of DNMT3A increased protein stability and consequently CpG methylation at the *p21* promoter (Deplus et al., 2014), generally considered a mark of repression. These data, of the close relationship of two epigenetic modifiers who are seemingly unrelated in their modes of operation, provides insight into how modifications to both protein and DNA may cooperate and assist in regulating the epigenetic landscape, a landscape that in turn is essential for genetic regulation. Hence, PADI4 is able to affect transcription through several mechanisms.

Lastly, PADI4 has also been found to autocitrullinate. Citrullination of several arginine residues in proximity to the active PADI4 cleft may render the enzyme inactive (Andrade et al., 2010), or modulate its ability to interact with other histone modifiers by interfering with protein-protein binding (Slack et al., 2011) adding complexity to the activity and function of PADI4 in gene regulation.

1.2.7 Citrullination in translation and RNA processing

There is growing evidence that citrullination is essential in the intermediate steps of post-transcriptional processing of mRNA to the initiation of translation, including translation factors and ribosomal proteins. Several studies (Chaerkady et al., 2021; Fert-Bober et al., 2019; C. Y. Lee et al., 2018; Lewallen et al., 2015; Rebak et al., 2022; Snijders et al., 2015; Tanikawa et al., 2018) have identified citrullination on RNA-processing proteins, such as splicing factors, RNA helicases, and factors stabilizing, transporting, and aiding RNA, to enable translation at one end, and transcription in another. Collectively, the evidence points towards an integral part for citrullination in RNA biology and remarking upon the surprising conformity of overlap in citrullinated targets associated with RNA-processing across studies, this seems to be a conserved and fundamental mechanism for accurate RNA physiology and biochemistry. In fact, citrullination is implicated in all steps involved in transcription initiation until the end of translation. As discussed extensively above, citrullination occurs on histones for the epigenetic control of gene activity, as well as in the regulation of several transcription factors. However, looking more closely on transcription itself, RNA polymerase II (RNAPII) is found to be citrullinated (Sharma et al., 2019) in a process involving transcription initiation via transcriptional paucity and elongation. Moreover, many members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family are continuously being identified as citrullinated. The hnRNPs are a multifunctional group of RNA- and protein-binding proteins which are highly associated with RNA-processing, and citrullination of these have been implicated in every aspect of their function; from interacting with RNAPII for initiation and release of nascent transcripts, to splicing, stabilisation, and transport of both nascent and mature mRNA (Xie et al., 2021). Complementary, several RNA helicases are among the RNA-processing proteins found citrullinated. RNA helicases are just like hnRNPs intricately linked to the whole life cycle of RNA, from transcription to translation (Naineni et al., 2023). Specifically, RNA helicases are important for the unwinding of mRNA for accurate binding to translation initiation factors and translation progression. In addition, many spliceosomal proteins are also citrullinated. Furthermore, these studies (Chaerkady et al., 2021; Fert-Bober et al., 2019; C. Y. Lee et al., 2018; Lewallen et al., 2015; Rebak et al., 2022; Snijders et al., 2015; Tanikawa et al., 2018) also identify many proteins involved in ribosomal biogenesis and of mature ribosomal proteins. Even further implications for the involvement of citrullination in translation is the finding that several (if not all) key eukaryotic translation initiation factors (eIFs) are citrullinated, which are required for cap-dependent translation initiation. A process where hnRNPs also are implicated.

The importance for citrullination of ribosome, translation initiation, and RNA-processing components is stressed by the fact that in the most sensitive and accurate identification of citrullination to date (Rebak et al., 2022), the citrullination of these proteins were stably detected both in baseline and calcium ionopohore-stimulated cells, an observation the authors termed the “core citrullinome”, so well established that hypercitrullination stimuli

does not affect it. However, whether citrullination is required for accurate ribosome assembly remains to be seen.

To give some examples of specific studies probing the contribution of citrullination in terms of translation, citrullination of the histone chaperone protein nucleophosmin (NPM1) leads to NPM1 translocation from the nucleoli to the nucleoplasm, negatively affecting ribosomal biogenesis (Tanikawa et al., 2009). Prior to the proteomic approaches in detecting citrullination in ribosomes, several ribosomal proteins had been found citrullinated, including the 40S subunit ribosomal protein S2 (RPS2). Citrullination of RPS2 antagonises PRMT3-mediated methylation of RPS2 (and *vice versa*) to regulate ribosome assembly (Guo et al., 2011). From this study it was speculated that it is possible that PADI4 and PRMTs have co-evolved to fine-tune the regulation of translation, as this constitutes a high energy expenditure of the cell.

Moreover, PADI2 was shown to citrullinate ribosomal proteins specifically, but also proteins associated with ribosomal assembly and translation initiation, both in proliferating OPC and differentiated oligodendrocytes (Falcão et al., 2019). Further evidence for the importance for citrullination in translation-associated processes and its implications in myelinated tissue comes from the study of amyotrophic lateral sclerosis (ALS) (Tanikawa et al., 2018). PADI4 genetic polymorphisms leads to low PADI4 expression in ALS, suggesting that PADI4 may have a protective function in neurodegenerative diseases. Furthermore, there are several examples where protein aggregation during ALS is inhibited through citrullination. For example, citrullination of RNA-processing proteins FUS, EWSR1, and TAF15 (collectively referred to as FET) and ribonucleoprotein family hnRNP, all important for accurate continuation of mRNA translation, was found to inhibit FET aggregation. Interestingly, both FET and hnRNP proteins are also subject to PRMT1 methylation (Jobert et al., 2009; Passos et al., 2006), further suggesting that citrullination by PADI4 may be more linked to transcriptional and translational regulation than previously thought.

Taken together, citrullination is thus as a PTM highly involved in regulating and facilitating translation and RNA-processing, a process so fundamental that citrullination can no longer be considered an anomalous event reserved for special occasions. However, despite the clear consensus regarding the citrullination of the RNA-processing factors, primarily, and the translation factors, secondly, very little functional analysis has gone into investigating the biological outcome of these citrullinations. As more evidence for the existence and abundance of citrullination in these processes accumulate, follow-up studies addressing their functionality are bound to follow.

1.2.8 Citrullination in disease – Implications for PADI2, PADI3, and PADI4

1.2.8.1 Anti-microbial defence

As PADI4 was first identified in human myeloid leukaemia HL60 cells (Nakashima et al., 1999), it is perhaps not surprising that a large share of the information uncovered about PADI4 has since then been collected from the study of lymphocytes and leukocytes, such as neutrophils (Neeli et al., 2008), monocytes and macrophages (Vossenaar, 2004), as well as in dendritic cells (DCs) and in DC- and macrophage-derived osteoclasts (Krishnamurthy et al., 2019). In neutrophils, PADI4 was shown to be essential for the formation of neutrophil extracellular traps (NETs), an innate antimicrobial response to pathogens (Hemmers et al., 2011; Lewis et al., 2015; P. Li, Li, et al., 2010; Thiam et al., 2020; Y. Wang et al., 2009). In order to neutralize pathogens, neutrophils express PADI4 thereby inducing hypercitrullination of chromatin, massive chromatin de-condensation and subsequent expulsion of chromatin from the neutrophil. The NETs entangle and immobilises the pathogen in question limiting its spread. Neutrophil cell death as a result of NET release is referred to as NETosis.

As NETs also contain antimicrobial agents produced by the neutrophil it can degrade virulence factors and also directly kill the pathogen (Agraz-Cibrian et al., 2019; Brinkmann et al., 2004; P. Li, Li, et al., 2010; Neeli et al., 2008; Y. Wang et al., 2009). One such agent is the Cathelicidin antimicrobial peptide LL-37, whose implication in NET-associated inflammation was shown when citrullinated by PADI2 and PADI4 (Casanova et al., 2020; Chapman et al., 2019) LL-37 displayed weakened microbicidal capability (Al-Adwani et al., 2020; Kilsgård et al., 2012), as well being worse at lowering levels of pro-inflammatory cytokines from virus-infected epithelial cells compared to non-citrullinated LL-37 (Casanova et al., 2020).

1.2.8.2 Rheumatoid arthritis

In the inflammatory disease Rheumatoid Arthritis (RA), autoantibodies against citrullinated proteins are strong diagnostic markers (Hermansson et al., 2010). The anti-citrulline protein antibodies (ACPAs) are produced by B-cells in the arthritic tissues starting from early, presymptomatic stages throughout disease-progression. ACPAs are generally considered one of the causative factors of the autoimmune inflammation seen in RA (Corsiero et al., 2016; Masson-Bessière et al., 2000; Van Venrooij et al., 2008).

Several studies (Tutturen et al., 2014; Van Beers, Schwarte, et al., 2013; Violeta et al., 2013; F. Wang et al., 2016) have in an accumulative effort been able to identify over 100 citrullinated proteins in RA joints, and in this way provided the potential origin of the targets for ACPAs. Examples of identified citrullinated proteins which ACPAs react towards include structural proteins such as Fibrinogen, Enolase, Vimentin and Filaggrin (Hermansson et al., 2010; Van Venrooij et al., 2008; Vossenaar, 2004; S. Wang & Wang, 2013; Wegner, Lundberg, et al., 2010). Furthermore, many studies have associated RA with

NET (Fert-Bober et al., 2019; Khandpur et al., 2013; K. H. Lee et al., 2017), as increased NET formation has been observed in neutrophils isolated from RA patient synovial fluid. NETosis was enhanced in neutrophils subjected to ACPAs and the protein content of the NETs exhibited autoantigens distinct to the RA profile (e.g. citrullinated Vimentin and Enolase), in a self-perpetuating circle further exacerbating inflammation in the RA afflicted joints (Khandpur et al., 2013). However, not all NETs contain citrullinated histones (Holmes et al., 2019; Tsourouktsoglou et al., 2020) suggesting that the NETs *per se* may not be the direct cause of the ACPAs. Instead, there is evidence that PADI2 and PADI4 are expulsed together with the NETs, citrullinated or not, in the RA synovial fluid, and that these subsequently extracellular PADIs retain enzymatic activity, further activated by the high calcium concentration in the extracellular space, and can aberrantly citrullinate proteins in the synovium (Spengler et al., 2015). To complicate matters further, one study has shown that viable neutrophils can express extracellular membrane-bound PADI2 and PADI4, which are enzymatically active, and are in this way suggested to contribute to the extracellular citrullinome associated with RA (Zhou et al., 2017).

Other cells lining the arthritic synovium expressing PADI4 include B-cells (which are also the producers of ACPAs), T-cells, macrophages, as well as fibroblast-like and endothelial cells. However, these cells do not seem to contribute to the synovial fluid RA citrullinome, but rather express PADI2 and PADI4 as part of a physiological inflammation response, and display an inflammation progression-dependent PADI4 expression, i.e. the more severe the inflammation, the higher the expression of PADI4 (X. Chang et al., 2004). Also, apart from being found in RA synovial fluid, extracellular PADI4 can be found in the fibrin deposits surrounding the synovial tissue, where PADI4 is closely correlated with apoptotic cells.

Moreover, in some populations, PADI4 gene polymorphism is strongly correlated with RA (Y. H. Lee et al., 2012; Suzuki et al., 2003; Too et al., 2012), but whether PADI4 activity is a cause or effect of inflammation in RA remains to be elucidated. Nevertheless, angiogenesis is a principal component of RA and endothelial cells of capillaries of the newly established vascularity express PADI4 (X. Chang et al., 2004), suggesting that PADI4 can contribute to the pathogenesis of RA, as the disease will not progress without the formation of vasculature. However, the extent of PADI4's contribution to this process in RA is not known.

Also, polymorphisms in PTPN22, a suppressor of leukocyte activation signals, is directly linked with the inability of PTPN22 to suppress PADI4 activity and thus enhancing inflammation-stimulated NETosis in RA (H.-H. Chang et al., 2015). PTPN22 is so far the only identified physiological allosteric regulator of PADI4 activity.

PADI4 could also drive the initial inflammatory response in a feedback-loop (Klopf et al., 2021; T. Liu et al., 2017; Neeli et al., 2008; Papayannopoulos, 2018; Schön & Erpenbeck, 2018). As neutrophils got activated by macrophage-derived cytokines IL-1β, IL-6, IL-8,

TNFα and IFNγ, the neutrophils themselves were able to express and release auto- and paracrine IL-8 and TNFα. This was in part attributed to a process wherein PADI4 citrullinates four sites of NF-κB within the p65 Rel homology domain, and in this way enhancing the interaction of p65 with Importin α3 and subsequently facilitating nuclear translocalisation of p65 to induce expression of its target genes IL-1β and TNFα (Sun et al., 2017). Interestingly, Importin α3 was later also found to be the main transporter of PADI4 into the nucleus by interaction with the PADI4 NLS region (Neira et al., 2022). Similarly, PADI4 could also directly augment inflammation signalling by driving inflammatory gene expression in granulocytes by citrullination of the transcription factor E2F-1 (Ghari et al., 2016), leading to the expression of cytokines IL-1β and TNFα which in turn stimulate NETosis.

Collectively, PADI4 is thus implicated at all levels of RA inflammation and disease progression.

1.2.8.3 Citrullination in other inflammatory and degenerative diseases

PADI4 is also implicated in the pathogenesis of systemic lupus erythematosus (SLE) (Garcia-Romo et al., 2011; Knight et al., 2015) and lupus nephritis (LN) (Massarenti et al., 2019), where again NETosis and autoimmunity are driving disease progression (K. H. Lee et al., 2017). In the case of SLE, citrullinated LL-37 was implicated in generating a stronger T-cell response (than non-citrullinated LL-37) and in this way potentially induce an expansion of autoreactive T-cells (Lande et al., 2020). Multiple sclerosis is, on the other hand, characterized by PADI2-mediated hypercitrullination of myelin basic protein, which together with inflammatory stimuli induce PADI4 expression and leads to increased H3 citrullination and exacerbated inflammation progression (Carrillo-Vico et al., 2010; Mastronardi et al., 2006; Sharma et al., 2012; Wood et al., 2008). Additionally, in Alzheimer's Disease (AD), PADI4-mediated citrullination in neurons and PADI2-mediated citrullination in astrocytes is linked to neurodegeneration, release of citrullinated antigens and consequently, an autoimmune response (Acharya et al., 2012). It is interesting that while PADI4 is suggested to have a protective function in ALS (Tanikawa et al., 2018), it appears to facilitate neurodegeneration in AD.

1.2.8.4 PADI4 in tumour biology

Much investigation has gone into PADI4 in the context of p53 and tumour biology. Normally, PADI4 and HDAC2 interact with one another and act as a corepressors of p53 target genes by maintaining high levels of histone H3 citrullination, with simultaneous hypoacetylation at the promoters of *p21, GADD45* and *PUMA* (P. Li et al., 2008; P. Li, Wang, et al., 2010). Upon DNA damage, PADI4 and HDAC2 dissociate from the promoter regions of *p21, GADD45* and *PUMA*, leading to a reduction in citrullination and a concomitant increase in arginine methylation and lysine acetylation which activates transcription. Both PADI4 and HDAC2 can also bind directly to p53, a binding which is abrogated upon DNA

damage in order to, in a p53-dependent fashion, recruit PRMT1, PRMT4 and P300 to induce transcription of p53-target genes (An et al., 2004; P. Li et al., 2008).

Similarly, PADI4 occupancy and citrullination of histones at the *OKL38* promoter is decreased in an p53-dependent manner upon DNA-damage, resulting in OKL38-mediated apoptosis through mitochondrial release of cytochrome *c* (Yao et al., 2008). To complicate matters further, DNA-damage has been shown to increase citrullination levels at histone H4R3 in a p53-dependent manner (Tanikawa et al., 2012). In this study, PADI4 was shown to facilitate chromatin fragmentation as well as citrullination of Lamin C, a nuclear membrane protein that congregates at fragmented nuclei, both hallmarks of apoptosis. Furthermore, mice lacking *Padi4* exhibited resistance to radiation-induced apoptosis, pointing towards an apoptotic pathway dependent on citrullination. Collectively, these studies highlight the complex nature of histone citrullination, where decreased histone citrullination facilitates p53-dependent apoptosis, while at the same time, increased citrullination at other histone sites is able to initiate p53-independet cell death.

The evident link between p53 and cancer provides a connection of PADI4 to tumorigenesis via the p53-PADI4 axis, in which apoptosis seem to play a major role. To intertwine these proteins further it has been shown that PADI4 contains an intronic p53-binding site, through which p53 can transactivate PADI4 expression upon cellular stress (Tanikawa et al., 2009). Indeed, PADI4 upregulation has been shown in a variety of cancer cell lines and human tumour tissues, including osteosarcoma, carcinoma of the colon, oesophagus, ovaries, breast, lung, and stomach (Cui et al., 2016; C. Liu et al., 2019; Mohanan et al., 2012; Tanikawa et al., 2012; S. Wang & Wang, 2013; Zhai et al., 2017; Zhang et al., 2011; Zheng et al., 2016). In many of these cancers, PADI4 was associated with the upregulation of a plethora of genes of various functions, and not solely classically p53-target genes. The PADI4-p53 pathway may well be affected in these situations, but PADI4 upregulation is nevertheless linked to regulation of transcription. Studies (Zhang et al., 2011) using the MCF-7 breast cancer cell line have shown that PADI4 associates with ELK-1 at the *c-Fos* promoter, a proto-oncogene that dimerize with c-Jun to form transcription factor complex AP-1. Following Epidermal Growth Factor (EGF) stimulation, ELK-1 is citrullinated. This in turn enables the subsequent phosphorylation of ELK-1 by ERK, allowing histone acetylation by P300, with the transcriptional activation of *c-Fos* (Zhang et al., 2011). Other genes that are upregulated in concert with upregulation of PADI4 (in a seemingly p53-independent manner) include *CXCR2, KRT14, TNFα* (Zheng et al., 2016) and *CA9* (C. Liu et al., 2019), to mention a few.

Moreover, increased expression of PADI4 in liver metastasis and citrullinated collagen type I in the surrounding ECM was found when compared to unaffected liver or the primary colorectal tumour and its surrounding ECM (Yuzhalin et al., 2018). This work also suggests that citrullination in the ECM may help in mesenchymal-to-epithelial transition

(MET), aiding in metastasis establishment and malignancy. Inversely, knockdown of PADI4 in breast cancer cells induced TGFβ signalling and increased epithelial-to-mesenchymal transition (EMT) (Stadler et al., 2013). PADI4 was show to citrullinate Glycogen synthase kinase-3β (GSK3β) and thus enable its nuclear localization, which in turn is necessary for the maintenance of an epithelial phenotype of tumour cells. Both these studies suggest that PADI4 is involved in directing the tumour cells towards an epithelial phenotype, but differ in their conclusions whether PADI4 in cancer facilitates or impedes malignancy.

What is interesting with the work that has been performed with PADI4 in association with p53 and cancer, are the type of cells and tissues in which PADI4 expression is shown and functional. There may of course be vastly aberrant transcriptional and proteomic profiles in many of the cancer tissues and cell lines being used, nevertheless PADI4 is commonly upregulated in tumours when compared to the healthy tissue. That there are detectable levels of PADI4 in the healthy tissue to begin with is by itself interesting, highlighting a more dynamic and varied expression pattern of PADI4 than previously anticipated (Vossenaar et al., 2003).

1.2.8.5 PADI3 in tumour biology

PADI3 was recently shown to be expressed in the human colon (X. Chang et al., 2019). Downregulation of PADI3 in colon cancer, as compared to adjacent colon tissue, was correlated with suppression of the tumour suppressive Sirt2/AKT/p21 pathway, thus facilitating tumour growth. Overexpression of PADI3 also resulted in diminished tumorgenicity in cell lines and *in vivo*. However, citrullination was not probed in this study, and the mechanisms by which PADI3 effect tumour growth remains unclear.

Also, PADI3 expression negatively correlated with the expression of Cyclin-dependent kinase regulatory subunit 1 (CKS1) in colorectal cancer (Chai et al., 2019). CKS1 is essential for cell proliferation, and its expression is facilitated by Heat shock protein 90 (HSP90). PADI3 was shown to correlate with the downregulation of HSP90 and thus inhibiting CKS1 expression, a process that was reversed upon PADI3 downregulation in colorectal cancer. Whether HSP90 is a direct substrate of PADI3 remains to be investigated.

These are the first studies of non-epidermal PADI3 expression, and recent work report expression of PADI3 in breast, oesophageal, gastric, hepatocellular as well as lung cancer. With the reported divergency of PADI-expression in mind, this may very well be true, however, needs to be thoroughly investigated and corroborated before any claims can be made about what contribution PADI3 has to these tissues, and whether PADI3 functions as a tumour suppressor or not.

1.2.8.6 PADI2 in tumour biology

In epithelial cells, PADI2 has been described in breast cancer, being transcriptomically profiled both in primary breast tumours as well as in luminal breast cancer cell lines (Beato

& Sharma, 2020; Blick et al., 2010; Mackay et al., 2009). Building of these data together with the finding of physiological histone citrullination by PADI2 in canine mammary epithelium (Cherrington et al., 2010), PADI2 was found in human mammary gland epithelia and subsequently in MCF-7 breast cancer cell line (Cherrington et al., 2012). In MCF-7 cells, PADI2 associated with chromatin and was found to regulate a unique subset of cancer-associated genes.

Moreover, PADI2 appears to promote epithelial-to-mesenchymal transition (EMT), and analyses of large cohorts of breast cancer patients revealed that upregulation of *PADI2* correlated with poor prognosis (Beato & Sharma, 2020; Curtis et al., 2012; Györffy et al., 2010; Koboldt et al., 2012; Sharma et al., 2019). Moreover, seeking mechanistic clarity to the correlation or causative implication of PADI2 in tumour biology, human PADI2 was overexpressed in skin of mice and was identified as an oncogenic driver of tumour formation (McElwee et al., 2014), where PADI2 indeed promoted EMT, with higher migration capability and invasiveness (Mohanan et al., 2017).

PADI2 has also been shown to play an important role in bone marrow mesenchymal stem cell (BMMSC) in multiple myeloma (MM) (McNee et al., 2017). Here, PADI2 was found to overexpressed in BMMSCs in MM patients. It was found that increased PADI2 resulted in induced upregulation of *IL-6* expression in a process wherein PADI2 aberrantly citrullinated histone H3R26, leading to chemotherapeutic resistance to bortezomib.

Collectively, PADI2 upregulation in various cancers, breast cancer in particular, correlates with adverse consequences for tumour progression, indicating that PADI2 may actually be part of the oncogenic mechanism. Research into the relevance for PADI2-mediated citrullination in cancer may open up new avenues for therapeutic interventions.

2 Research aims

PADI expression and citrullination goes hand in hand. The expression profile of the PADI enzymes grows ever more dynamic with each new study, and the once rigid dogma for the distribution of each PADI member is well underway to be expanded and nuanced. Accordingly, the functional outcome of PADI expression pattern is the deposition of citrullination. As the skin is an inexhaustible source for understanding developmental biology, the involvement of transcriptional regulatory networks to execute accurate developmental processes are of vital importance to understand. As such, the collective effort contributing to the current thesis has aimed at understanding when and how PADIs are expressed and contribute to epidermal and hair follicle development, and how specific regulatory elements carry out appropriate developmental programmes.

2.1 Paper I: PADI4 restricts proliferation of hair follicle progenitor cells by rewiring translation

Serendipitous identification of *Padi3* and *Padi4* containing BMP-response elements (pSMAD1/5-binding to *Padi3* and *Padi4* chromatin) in regenerating and lineage committed HF progenitors (Adam et al., 2018; Genander et al., 2014; Lien et al., 2011), and given the implications for PADI4 in stem cells, prompted the investigation into any possible function of PADI4 in HF stem and progenitor cells. Thusly, analysing the expression and function of PADI4 in hair follicles during development, this paper aimed at answering the following questions:

1. How does the expression profile of *Padi4* during hair morphogenesis look like?
2. In what way is PADI4 implicated in progenitor proliferation and differentiation in the different HF lineages?
3. What citrullinated targets are there in hair follicle development?
4. What is the functional outcome of those citrullinations?

2.2 Paper II: Co-expression of PADI isoforms during progenitor differentiation enables functional diversity

Database annotation and RNA sequencing suggested the existence of effectively more than one isoform of both PADI2 and PADI3. Testing the hypothesis that there might be at least two isoforms of PADI2 and PADI3 in mouse oligodendrocyte precursors and hair follicle progenitors, respectively, this study aimed at answering the questions:

1. Are the transcriptomic indications for multiple isoforms of *Padi2* and *Padi3* resulting in physical proteins?
2. What would the structure of the resulting proteins be?
3. Are the stipulated alternative isoforms enzymatically functional, and if so: do they differ in activity from their canonical counterparts?

4. Would any alternative isoforms differ in tissue distribution from their canonical counterparts?
5. What is the reasoning for retaining several protein isoforms?

2.3 Paper III: A Hairy Cituation – PADIs in Regeneration and Alopecia

A review paper aimed at addressing the influence of PADI expression and citrullination on hair follicle stem cells and inflammation in alopecia, trying to answer:

1. How does the citullinome differ from non-inflamed to inflamed tissue, are the citrullination targets similar (hinting at a set biological function for citrullination) or do they differ (suggesting a dynamic, context dependent, utilisation)?
2. Can we use published data sets from studies of hair follicle stem cells, unrelated to citrullination, to probe a possible expression pattern and participation in signalling pathways of PADIs?
3. Is it possible to draw any conclusion from other stem cell systems to infer understanding for PADIs in hair follicle stem cells during regeneration and differentiation?
4. Can we combine the knowledge from how PADIs interact with other posttranslational modification enzymes with how these modifiers are expressed in hair follicle stem cells to infer function of PADIs?
5. Are the implications for PADI expression and citrullination in inflammation, in both epithelial and immune cells, applicable to alopecia?

2.4 Paper IV: ID1 and CEBPA coordinate epidermal progenitor cell differentiation

Functionally, ID1 lacks a DNA-binding domain, and instead exerts its function by binding basic helix-loop-helix (bHLH) proteins and can in this way regulate transcriptional control of stem cell proliferation and differentiation dynamics. Although observed in basal layer epidermis, the role for ID1 in the developing epidermis has remained poorly understood. Given ID1's role in lineage specification during hair follicle lineage growth, this study set out to investigate the functional consequence of ID1 expression in embryonic epidermis.

Investigating the expression and functional consequence of ID1 activity in embryonic epidermis, this study aimed at answering:

1. Is there a functional relevance for ID1 during epidermal development?
2. What are the binding partners of ID1 in the developing epidermis and how are they related to either stem cell characteristics or epidermal stratification?
3. How can the expression dynamics of ID1 and its stipulated binding partners be correlated to stratification of epidermis?

3 Methods

3.1 Methods to detect citrullination

Methods to detect citrullinated proteins has mainly entailed antibody-based assays for immunohistochemistry and western blot. One of the more popular methods utilizes an anti-modified citrulline antibody (AMCA) assay where a peptidylcitrulline is chemically modified to receive an additional ureido group adduct, which is then recognizable by an anti-modified citrulline antibody (Senshu et al., 1992). This has proven useful when highlighting hypercitrullination in RA. Similarly, the preferred method to detect histone citrullination is by usage of anti-citrullinated histone antibodies (anti-HisCit) which specifically will recognize either H3R2, H3R8, H3R17 (individually or collectively (i.e., R2+R8+R17), or in any combination), H3R26, or H4R3, respectively. Quite understandably, these approaches are rather limited in terms of identifying novel substrates for citrullination, not to mention being able to robustly confirm citrullination sites with high confidence. Not to mention the relatively large amount of starting material needed to accurately perform immunoreactive assays, as is can be difficult to come by when considering patient material or animal tissue. To overcome this, recent studies applied Mass Spectrometry (MS) for the definitive determination of citrullination sites of candidate proteins (Christophorou et al., 2014; Falcão et al., 2019; Goëb et al., 2009; Hermansson et al., 2010; Snijders et al., 2015; F. Wang et al., 2016). In recent publications, a high throughput quantitative proteomic approach has been used by employing MS to yield what is referred to as the "citrullinome" (Chaerkady et al., 2021; Fert-Bober et al., 2019b, 2020; C. Y. Lee et al., 2018; Lewallen et al., 2015; Tanikawa et al., 2018; Tilvawala et al., 2018; Tutturen et al., 2014; Van Beers, Schwarte, et al., 2013). These studies revealed a pervasive occurrence of citrullinated proteins in most tissues examined, concomitant with the expression of PADI protein. PADI2 was the most prevalent family member detected (highest expression, widest tissue distribution) followed by PADI4, again exemplifying that citrullination is more frequent than previously estimated. Under physiological conditions, functional classification of citrullinated proteins revealed that citrullination was primarily associated with categories of cytoskeletal organisation and RNA-binding, as well as metabolic pathways, processes that are shared between most cell types. However, in a hyper-citrullinated library, where tissue samples were incubated with all PADI enzymes so as to citrullinate as many sites as possible, the type of proteins found citrullinated where specific to the physiological function associated with the tissue in question.

Drawbacks of the MS approach to identify citrullination is that it is biased towards highly abundant proteins, or inversely – low abundant peptides will be difficult to distinguish from the background of the full proteome, in concurrence with the unmodified peptide being in a much higher ratio, further masking the modified peptide. However, this may help

to explain why broad cellular processes are enriched among the citrullinated proteins during physiological conditions, and may also explain why a more cell function-specific citrullinome profile emerges when PADIs are enriched to yield a substantially higher number of citrullinated proteins.

Additional downsides when performing MS data-analysis is that the mass-shift on a citrullinated peptide compared to its unmodified counterpart is no more than 0.9840 Da, which incidentally is the same as another, and more frequent, PTM called deamidation, which occurs at asparagine and glutamine residues. Deamidation can hence be mistaken for citrullination if care is not taken (Fert-Bober et al., 2019). Using trypsin for enzymatic digestion in sample preparation for MS is common, however since trypsin cleaves after arginine, citrullination will mask this cleavage site (Bennike et al., 2013). In automated database searches for peptide spectrum matches is it therefore likely that a C-terminal citrulline constitutes a false positive, and will have to be validated further with, for example, the delta retention time shift (the difference in retention time two matched peptides exhibit depending on their respective modification state, i.e., the 0.9840 Da mass shift a citrullination entail). An additional drawback is that the databases may disregard a citrullination-induced missed cleavage as a technical 'missed cleavage' owning to the less-than-100% digestion efficiency of trypsin, resulting in a false negative. Another way to validate citrullinated peptides and to determine the citrullination site is to use the collision-induced dissociation (CID) spectra of fragmented molecules to detect the neutral loss of isocyanic acid (-NHCO), which results in a -43 Da mass shift as isocyanic acid is dissociated from the citrulline residue (Hao et al., 2009). Despite these limitations, MS has proven an essential tool to discover, identify, and validate citrullinated peptides in a variety of tissues, both in health and disease (Raijmakers et al., 2012; Verheul et al., 2018). Yet, it was not until recently that a robust enough protocol was developed (Bekker-Jensen et al., 2017) for in-depth and accurate high resolution proteomic detection that could be deployed to extraordinarily improve on the amount of citrullinated proteins that could be detected and the citrullination sites within those proteins. To put things into perspective, previous studies (Christophorou et al., 2014; Falcão et al., 2019; Fert-Bober et al., 2019) probing the physiological citrullinome in mouse, in three different cell lines, could when combined into a single data set amount to roughly 1200 citrullinated proteins, whereas the improved MS approach used by Rebak *et al*. (Rebak et al., 2022) in human HL60 cells (and stimulated to differentiate into granulocytes) were able to detect 4,008 proteins representing 14,056 citrullination sites, all ascribed to PADI4 alone. This proves that mass spectrometry is a most powerful tool in analysing citrullination, and that there is still much of protein citrullination biology left to discover.

In **Paper I**, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect citrullination in lysates from mouse primary keratinocyte progenitors. The sensitivity for citrullination was on par with previous studies (263 proteins to be exact).

The validation of the indicated citrullinations were based on the database search for a +1 Da shift not corresponding to deamidation, and the peptides were manually verified not to contain a positive citrullination on a C-terminal arginine, as a true citrullination would obstruct cleavage by trypsin. Moreover, we used tandem mass tagging (TMT) in the global proteomic analysis of each sample. TMT is an isobaric stable isotope labelling technique (Zecha et al., 2019), which enables the relative quantitative measurement of each identified protein as each sample is uniquely labelled. Subsequently, all samples can then be combined and processed together in the same mass spectrometry run. In addition, the identification of proteins allows its quantitative measurement due to that each protein within a given sample have the same mass tag. Quantification is thusly based on the TMT-reporter ion intensities, and in this way the different levels of a protein can be compared between samples.

4 Results

4.1 Paper I: PADI4 restricts proliferation of hair follicle progenitor cells by rewiring translation

Using embryonic and early neonatal timepoints relevant for hair morphogenesis (i.e., E18.5-P1 for the growth phase of differentiation initiation (Schmidt-Ullrich & Paus, 2005)) this study set out to characterize the expression pattern of PADI4 in hair follicles during development. Using FACS isolated TACs and differentiated cells at P2 and P4, we were able to detect expression of *Padi4* at both timepoints. Building on those data, using *in situ* hybridization for *Padi4* mRNA, *Padi4* was detectable at developmental timepoints reaching from E18.5 to P1. Specifically, *Padi4* localised mainly to structures associated with the HS; in what can be described as the precortex, (i.e. HS-lineage committed precursor cells) (DasGupta & Fuchs, 1999) at P0, and later in the medulla at P1. Also, at anagen, *Padi4* was expressed in all matrix TACs, in the compartments of proliferative and lineage committed cells, yet with highest signal in the fully formed HS.

Based on these observations, a *Krt14*-Cre:*Padi4*$^{fl/fl}$ (PADI4 KO) mouse was generated to achieve a skin-specific genetic ablation of *Padi4* from the onset of *Krt14*-driven Cre expression (at E12.5-13.5 (Beronja et al., 2010; Vasioukhin et al., 1999)). Phenotypically, mice lacking PADI4 displayed an increased proliferative capacity in the matrix at E18.5, specifically in the HS committed LEF1+ TAC population, which later conferred an expansion of the total number of LEF1+ cells within the HF as well as a more progressed differentiation of the HS. Notably, the expansion did not occur at the expense of the IRS lineage, which demonstrated no shifts in lineage commitment or differentiation.

Moreover, in primary mouse keratinocyte progenitors an overexpression of PADI4 (PADI4 OE) displayed a decrease in proliferation (opposite to the effects of KO), suggesting that PADI4 acts to restrict the proliferation frequency of epidermal progenitors.

Performing high-throughput liquid chromatography-tandem mass spectrometry (LC-MS), we were able to identify the PADI4-dependet citrullinome where citrullinated proteins enriched for functional processes associated with RNA-processing, the cytoskeleton, and translation. Citrullinated proteins included translation initiation factors, RNA-helicases, and hnRNPs, to mention a few. Further employing quantitative (TMT-labelling) proteomic analyses we turned to the proteome of the different conditions (WT, KO, and OE) for an explanation for the upregulated proliferative phenotype observed in KO – the KO proteome was highly enriched for processes associated with ribosomal biogenesis and translation.

Indeed, rRNA synthesis was correspondingly increased in KO. Although translational output was correspondingly increased in KO and decreased in PADI4 OE *in vitro*, rRNA synthesis was unaltered in OE.

Thus, analyses of protein synthesis in developing hair follicles revealed that lack of PADI4 enhanced global translation, even to some extent in proliferating TACs, contrary to WT where proliferation is separated form upregulation of translation (Blanco et al., 2016).

For mechanistic understanding, characterisation of key translational effectors revealed that in PADI4 KO the engaged activation of the AKT/mTOR pathway ultimately resulted in enhanced cap-dependent translation initiation, notably by circumventing the need for 4E-BP1, which demonstrated both downregulation and abolished phosphorylation. Additionally, mTOR-dependent signalling driving ribosomal biogenesis was also accelerated in PADI4 KO.

Taken together, the results of **Paper I** represent the first functional study of PADI4 in developing hair follicles, where its expression and biological relevance is described to contribute to the proliferative capacity and lineage commitment of hair follicle progenitors. Mechanistically, we map the PADI4-dependent citrullinome and unearth a citrullination-based regulation of translation and ribosomal biogenesis in epidermal progenitors.

4.2 Paper II: Co-expression of PADI isoforms during progenitor differentiation enables functional diversity

Revisiting previously published (Falcão et al., 2019) RNA-sequencing data from primary oligodendrocyte precursors (OPCs) suggested a second, albeit potentially truncated, isoform of *Padi2*. Later, PCR walking confirmed its existence (a method to probe an unknown genomic sequence adjacent a known sequence by "walking" through PCR products generated by primers originating from the known genomic sequence (Shapter & Waters, 2014), and its transcription start site was localised to a downstream accessible intronic region as a functional alternative promoter, bound by CCCTC-binding factor (CTCF) and enriched for H3K27ac and H3K4me3, indicating chromatin accessibility and active transcription. The alternative isoform was named *Padi2β*, whose mRNA was inferred to be translated into a 449 amino acids long protein truncated at its N-terminus (as compared to the 673 amino acids long canonical PADI2α). The resulting protein was 3D reconstructed and displayed a complete absence of the PAD_N domain and partial lack of PAD_M, including at least one Ca^{2+}-binding site.

Physiological incidence of *Padi2β* was later investigated in FACS isolated OPCs, as well as in juvenile (P21) and mature (P60) oligodendrocytes, and was found to be incrementally expressed with progression of differentiation, just as *Padi2α*, suggesting that *Padi2β* is dynamically regulated in oligodendrocyte lineage maturation *in vivo*. Functionally, *Padi2β* appeared to inhibit oligodendrocyte differentiation in an opposing effect to *Padi2α*, which is known to have a differentiation-promoting effect. Combined siRNA silencing of both *Padi2α* and *Padi2β* resulted in enhanced differentiation, akin to *Padi2β* silencing alone. In this way was *Padi2β* proposed to restrict oligodendrocyte lineage progression.

Reasoning that Padi2β could inhibit oligodendrocyte differentiation by modulating the activity of Padi2α, we next set out to probe the interacting effects of PADI2β on PADI2α on protein level by overexpression PADI2β in OPC cell lines. Even though PADI2β was overexpressed it could initially not be detected by immunoblotting, and only after the simultaneous treatment with a proteasome inhibitor was PADI2β observed. This suggested that PADI2β is unstable, expression is transient and possibly relatively low, yet is enough to moderate the effect of PADI2α in oligodendrocyte lineage differentiation.

Moreover, the existence of an alternative PADI2 isoform lent credence to the physical manifestation of an annotated, yet unconfirmed, PADI3 isoform. Both transcriptional and protein database predictions for a second PADI3 isoform suggested the existence of a N-terminally slightly shorter (corresponding to 10 amino acids) protein, duly named PADI3β. 3D generated models displayed a vestigial initiation of the N-terminal thought to affect dimerization or substrate binding.

Utilising FACS and *in situ* hybridisation *Padi3β* was detected and localised in the developing hair follicle, with slight expression in the proliferative progenitors of the hair follicle only to increase with lineage commitment and differentiation. Moreover, *Padi3α* and *Padi3β* appeared to co-localise in the hair follicle, suggesting a common feature of activity as their respective expression was not spatially restricted to different compartments.

Although N-terminus truncation of PADI3β did not affect its subcellular localisation (it remained cytoplasmic just as PADI3α), PADI3β was found to be enzymatically inactive. Additionally, co-expression of PADI3α and PADI3β resulted in the diminished catalytic activity of PADI3α. Furthermore, the two PADI3 isoforms co-precipitated with each other, hinting at the ability for heterodimerisation. Yet, when co-expressing the isoforms, both PADI3α and PADI3β levels decreased. Taken together, these data suggest an antagonistic effect of PADI3β on PADI3α. Whether the observed decrease in enzymatic activity upon co-expression is due to an inhibitory effect on catalytic ability, or a result of decreased protein stability remains to be seen.

Collectively, the results of **Paper II** were able to demonstrate the existence and physiological applicability of alternative PADI isoforms. PADI2 and PADI3 are seemingly unrelated in tissue distribution and substrate preferences, which results in vastly different biological functions, yet both PADI2 and PADI3 display transcriptional divergence and generate two isoforms with which the canonical, enzymatically relevant, isoform can interact with to modulate its own function or protein stability.

4.3 Paper III: A Hairy Cituation – PADIs in Regeneration and Alopecia

As this is a review paper the results are not those of an original research paper. Nevertheless, performing a meta-analysis by reanalysing published proteomic data from

healthy and inflamed human tissue reporting on the citrullination content within their datasets, we were able to revaluate the citrullinome in health and inflammation to portray the dynamic differences between these conditions. Commenting on those dynamics it was possible to conclude that citrullination is not an acute effect of altered cell state, but an integrated constituent of protein biology in all conditions and most cell types.

Moreover, as the review focused on PADIs and citrullination in hair follicle stem cells (HFSC), by revisiting published data looking at dynamic chromatin accessibility and transcriptomic changes in HFSCs during activation, regeneration, and lineage commitment, arguments for active transcription of *Padi3* and *Padi4* could be made. Also, by inferring observations about PADIs from other stem cell systems (e.g. the hematopoietic), and the known interaction of PADI4 with other enzymes catalysing PTMs (HDACs, PRMTs) together with the established functions of those PTM enzymes in the hair follicle, functions for, primarily, PADI4 could be reasoned.

Lastly, the collective research into the influence of citrullination on autoimmune inflammatory diseases was compiled, and used to propose a model for how citrullination at all levels of inflammation could contribute to the onset and progression of alopecia.

Jointly, the results of **Paper III** make a strong case for the ubiquity of citrullination in most cellular programmes, and the presence and probable function of PADI3 and PADI4 in hair follicle progenitors. As well as establishing the role for PADIs and citrullination in autoimmune inflammatory alopecia.

4.4 Paper IV: ID1 and CEBPA coordinate epidermal progenitor cell differentiation

Reanalysing an available single-cell RNA-sequencing dataset of early epidermal development (Fan et al., 2018; Genander et al., 2014), specifically looking at E13, revealed two main clusters: one associated with progenitor cell markers (*Krt15*, cluster 1) and the other with differentiation cell markers (*Krtdap*, cluster 2), with corresponding Gene Ontology profiling for both clusters. *Id1* was expressed in both clusters, but with significant enrichment in cluster 2. In line with this, ID1 protein localised to epidermal progenitors at E13.5, but as stratification became more progressed, ID1 became gradually more prominent in the basal layer with diminished expression with delamination and differentiation. *In vitro* data corroborated the downregulation of both *Id1* mRNA and protein during differentiation but noticed a lingering ID1 protein abundance at the onset of differentiation, suggesting a role for ID1 at the transitioning stage of epidermal progenitors committing to differentiation.

To study the functional consequence of *Id1* expression in epidermal development, *in utero* lentiviral injection of embryos with small hairpin (sh) RNA targeting *Id1* resulted in a severely thinner epidermis as the thickness of both the basal K14+ layer as well as the

spinous K10+ layer was reduced. There was also an increased frequency of cells doubly labelled with basal and suprabasal markers, as well as the anomalous display of proliferation in the suprabasal layer. The overall proliferation was however reduced.

Additionally, silencing of *Id1* resulted in decreased proliferation in progenitors *in vitro*, yet when asked to differentiate, *Id1* silencing resulted in preservation of proliferation in differentiated cells, which normally should become post-mitotic in the differentiation process. Likewise, differentiation genes were dysregulated in that they were upregulated in the progenitor state but failed to be properly induced upon receiving cues for differentiation, suggesting that in the absence of ID1, epidermal progenitor cells cannot properly commit to differentiation and shut down the proliferative machinery.

Moreover, RNA-sequencing of *shId1* transduced epidermal progenitors *in vitro* also exhibited an increased spinous cell genes expression profile as well as a downregulation of adhesion genes *Itga6*, *Itgb1*, *Itgb4*, and *Lama3*, all associated with basal layer progenitor cell identity. Further manipulation of *Id1* by overexpression (OE) reversed the phenotype and resulted in enhanced proliferation and RNA-sequencing revealed downregulation of spinous differentiation genes, hampering but not inhibiting differentiation.

By isolation of ID1-binding complexes, the class I bHLH transcription factors TCF3, TCF4, and TCF12 could be identified as ID1 binding partners.

Combination of the most differently expressed genes that overlap in *shId1* and *Id1* OE transcriptomic profiles, provided a gene list of ID1 candidate targets. Then, using transcription factor binding motif analysis for bHLH binding motifs on the target promoters, the TCF target genes could be identified as being directly influenced by ID1. Knockdown of *Tcf3* revealed a downregulation of markers of differentiation consistently upregulated upon *Id1* silencing, suggesting that ID1 maintains the progenitor transcriptomic profile by sequestering bHLH proteins such as TCF3.

Given that CEBPα was activated upon *Id1* silencing, analysis of CEBPα expression revealed a decrease when silencing *Tcf3*, and subsequent ChIP-qPCR confirmed TCF3 binding to *Cebpa* promoter and enhancer. However, TFC3 binding was not sufficient to drive *Cebpa* expression. Instead, it was found that CEBPα was able to bind the pSMAD1/5 binding motif of the *Id1* promoter, and this was interpreted as the way by which CEBPα suppresses BMP-induction of *Id1*. Another interpretation conceives that CEBPα binds and sequesters the pSMAD1/5 proteins themselves and in this way supress *Id1* expression. Either way, as CEBPα gets upregulated in differentiation it acts to suppress *Id1*, which in turn gradually releases its sequestration of bHLH proteins such as TCF3, and these can then go on to induce expression of differentiation genes. However, as TCF3-biding was not sufficient to drive *Cebpa* expression, what the inductive cue for its expression, as its exact mode of *Id1* suppression, are remains to be elucidated.

Taken together, the results of **Paper IV** establish ID1 in a central position for proper execution of proliferation and differentiation in the epidermis in the process of stratification. They also establish a hitherto obscured signalling axis between ID1, CEBPα, and BMP via bHLH transcription factors such as TCF3, essential in balancing proliferation and differentiation in developing epithelium.

5 Discussion

The skin, defining feature of individuality and sharp border between the self and the rest of the world, is a marvel of coordination between stem cell self-renewal, differentiation, and specific organotypic performance. The orchestrated transcriptional networks that is the result of extensive molecular signalling, give rise to complex structures of different cell identity to ultimately form the epidermal barrier and the hair, for protection and warmth. This thesis discusses the various molecular mechanisms at play in the epidermal and HF stem cells needed for their self-renewal and timely organised differentiation pathways.

In **Paper I** we showed the dynamic expression of *Padi4* in HF TACs as they commit to, and proceed with, differentiation. Investigating PADI4 in hair morphogenesis, genetic ablation of *Padi4* in developing HFs resulted in increased proliferation and an expansion of the WNT responsive LEF1+ HS-TACs as well as increased HS differentiation. Physiologically, WNT signalling is important for HS maturation, whereas BMP is important in the IRS lineage. Given that removal of *Padi4* results in enhanced HS maturation, points towards an impeding role for PADI4 on WNT signalling in the HS. As the IRS lineage is not affected by *Padi4* ablation, this in turn suggests that the BMP signalling in the IRS cells do not employ PADI4 for their maturation. The results are such that the accelerated effect of WNT in the HS lineage does not spill over and impinges on IRS, further suggesting that BMP can keep WNT at bay without the use of PADI4. Instead, these data, together with the finding that *Padi4* is a pSMAD1/5 target gene (Genander et al., 2014), suggest that BMP induces *Padi4* in the HS as part of the reciprocal crosstalk between WNT and BMP in the HF during lineage specification for correct spatiotemporal execution of hair formation.

By identifying the PADI4-dependent citrullinome we were, in line with previous studies, able to implicate RNA-processing, ribosomal biogenesis, and translation as key processes where citrullination is involved. Building on this, the main finding was that lack of PADI4 brings about upregulated translation and rRNA synthesis (for ribosomal biogenesis). Interestingly, in contrast to KO, rRNA synthesis was unaltered upon PADI4 OE, suggesting that as heightened citrullination does not correspond to reduced rRNA transcription the citrullination in this system does not act as an accelerator or obstructor *per se*, but a prerequisite for accurate and appropriate performance of this process.

Additionally, the finding that upregulation of translation also occurred in proliferating TACs, in contrast to WT where elevated translation is a sign of differentiation and thereby separated from proliferation (Blanco et al., 2016), proposes that the spatiotemporal aspect of proliferation restriction and enhancement of translational machinery is disjointed in PADI4 KO, i.e. differentiation-associated upregulation of translation can be initiated before cell cycle exit.

Moreover, translation was found to be upregulated via a process involving AKT/mTOR signalling, hence the clear assumption would be that this involves 4EBP1 phosphorylation by mTOR, to release eIF4E to freely participate in translation initiation. However, in PADI4 KO both p-4EBP1 and total levels of 4EBP1 were decreased. Conceivably, citrullination of 4EBP1 may then be required for its function, either by facilitating its phosphorylation, general stability, or binding to eIF4E. If citrullination is required for phosphorylation, one would expect less translation in its absence, as phosphorylation is needed for the release of eIF4E for cap-dependent translation. Yet, in the absence of PADI4, translation proceeds without phosphorylated 4EBP1. However, the reduced levels of the protein in total might offer an explanation for these seemingly contradictory observations: it has been shown that unbound, unphosphorylated 4EBP1 is degraded via ubiquitinylation, whereas unbound, phosphorylated 4EBP1 is stabilised and refractory to degradation (Elia et al., 2008; Hinnebusch, 2012; Yanagiya et al., 2012). In the absence of citrullination, this suggests two things: on the one hand, phosphorylation cannot happen; and on the other hand, 4EBP1 does not (cannot) bind eIF4E. Hence, lacking PADI4, 4EBP1 ends up unbound and unphosphorylated, and is degraded.

Another possibility is that citrullination of 4EBP1 has nothing to do with either binding ability or phosphorylation, but is instead required for the correct conformation of 4EBP1, where lack of citrullination results in a misfolded protein that is degraded based on its erroneous tertiary structure.

Nevertheless, these speculations propose the idea that without citrullination, 4EBP1 protein is not available, thus eIF4E is unbound and goes freely to initiate cap-dependent translation. In turn the demand for ribosomal biogenesis increases, mTOR is this activated in a feedback loop and activates its pathways associated with ribosomal biogenesis (via P70S6K and S6). Although we were not able to detect citrullinated 4EBP1 in our dataset, it has been found citrullinated in the recently massively improved mass spectrometry endeavour by Rebak *et al.* (Rebak et al., 2022), warranting further investigation.

In **Paper II** we describe the previously unverified alternative isoforms of PADI2 and PADI3 in physiological settings of progenitor cell differentiation progression. Both novel isoforms (named PADI2β and PAD3β, respectively) are the result of alternative promoter usage for transcription initiation and both result in truncated proteins. They also appear to modulate the behaviour or function of their respective canonical counterparts (the α-isoforms). Where PADI2β appears to work in an opposing manner to PADI2α on oligodendrocyte differentiation, PAD3β appears to lower the catalytic activity and stability of PAD3α.

As shown by siRNA silencing, *Padi2β* was proposed to restrict oligodendrocyte lineage progression, in a process where it possibly is needed to fine-tune various differentiation cues to execute properly timed oligodendrocyte maturation. Dysregulation of

oligodendrocyte differentiation and axon myelination have been described in various neurodegenerative diseases, such as multiple sclerosis, and in fact PADI2 was found to citrullinate myelin basic protein (MBP) (Wood et al., 2008) which was found to be detrimental for its function (Caprariello et al., 2018; Moscarello et al., 2013; Musse et al., 2008). Therefore, the fine-tuning and timely execution of oligodendrocyte differentiation is non-trivial, where the involvement of PADI2α, and now also PADI2β, is becoming clearer.

Similarly, PAD3β was co-expressed with PADI3α in the HF differentiating lineages, suggesting that its effect on protein stability is a means to fine-tune the catalytic activity of PADI3α in these cells. Whether the observed decrease in enzymatic activity upon co-expression is due to an inhibitory effect on catalytic ability, or a result of decreased protein stability remains to be seen. It will however be interesting to see what effect aberrant citrullination may have on the structural proteins that are the physiological substrates of PADI3, if PADI3α and PAD3β can be individually targeted for selective ablation.

Given the observation that PADI2β is unstable, expression is transient and possibly relatively low, however still sufficient to modulate the effect of PADI2α in oligodendrocyte lineage differentiation, together with the observation that PADI3β alters stability of PADI3α, it is feasible that co-expression halters citrullination. This proposes the idea that the utilization of alternative isoforms to modulate the activity of the main isoform is a circuitous way of coping with not having a de-citrullinase, to impede the effects of citrullination in the contexts where the alternative isoforms are expressed. However, our data further suggests that the two β-isoforms seems to be obligatorily following the expression of the α-isoforms, proposing that they would always subdue activity. Follow up studies would have to address whether the distinct transcription start sites identified in the current study could be regulated separately to confer the function of the β-isoforms. In any case, the possibility remains that they may also carry out other functions in addition to impeding their respective canonical counterparts.

The finding of two operative alternative isoforms of two separate PADI genes may additionally shine some light on the evolutionary origin of possessing and expressing several distinct PADI genes in the mammalian (and other) genomes. It is thought that the different PADIs have consecutively arisen via gene duplication of an ancestral PADI gene (argued to be PADI2 (Christophorou, 2022; Golenberg et al., 2020)). Perhaps this happened via a process where they first arose as alternative isoforms making use of alternative promoter sites and then gradually transitioned into *bona fide* genes, during millions of years of evolution. It is altogether perceivable that the observation of PADI2β and PADI3β is a continuation of that evolutionary process and that given enough positive selection over time, these will develop in genes in their own right someday. Only time will tell.

In **Paper III** we discuss the current understanding of PADIs and citrullination in HF stem cell and inflammatory biology. The main conclusion from this review is that citrullination is highly dynamic and general mechanism of protein biology in both health and disease. We were able to draw parallels from various stem cell contexts to infer function of PADIs in tissues and cells where they have been observed but not functionally characterized.

Moreover, scrutinizing the literature for PADI expression and function during bouts of inflammation, and gathering how the settings of inflammation (where citrullination is implicated) in various autoimmune disorders highly resemble those observed in alopecia, it was possible to generate a model in which citrullination takes a central place in inflammatory alopecia, and even pinpointing certain types of alopecia as autoimmune diseases. Lastly, what also emerged from the literature, was that essentially all cells involved in the inflammatory cascade express PADIs in one way or another, including epithelial cells, innate immune cells, and adaptive immune cells.

The proposed model is based on the current understanding of citrullination in inflammation as it has been investigated in a myriad of different contexts and might thusly not be complete. Also, as discussed above, the tools used to identify citrullination thus far may not provide the full picture. However, with the continued development of tools to accurately assess citrullination, it will be interesting to follow how the continual research into its role in inflammatory alopecia, and other diseases, as it unfolds.

Paper IV highlights the involvement of ID1 in the processes of progenitor cell proliferation and differentiation during epidermal development. The study uncovers an interdependence between ID1, BMP, TCF3, and CEBPα to correctly execute the appropriate transcriptional programmes that govern epidermal homeostatic stratification. First, ID1 is duly established as a major contributor to the epidermal progenitor state, where upregulation of *Id1* results in increased proliferation and downregulation correspondingly increases markers for differentiation. Targeting the embryonic epidermis as early as E9.5 by ultrasound-guided injection of lentiviral shRNA to silence *Id1* expression, the phenotype is striking in that the overall thickness of the epidermis is decreased concurrent with diminished proliferation. Yet as development progresses, even though overall stratification is perturbed, all markers for the different epidermal layers can be found, albeit in a massively compressed compartment, at least as far as the spinous layer is concerned. Our study did not include data of any potential barrier defects, yet to investigate the long-term effect of ID1 ablation on barrier formation and function would be of interest to address whether tight junctions within the granular layers would still be intact.

Moreover, bHLH transcription factor TCF3 was established as an ID1 binding partner during within epidermal progenitors, and subsequently the binding of TCF3 to *Cebpa* promoter and enhancer regions was demonstrated.

Additionally, CEBPα was shown to bind the pSMAD1/5 binding motif of the *Id1* promoter, and in this way was able to show that CEBPα suppresses BMP-induction of *Id1*.

During epidermal stratification in homeostasis *Id1* gets downregulated with differentiation and *Cebpa* gets upregulated. Our study showed that CEBP drives the decrease in ID1 by suppressing its induction by BMP by competing with pSMAD1/5 biding.

TCF3 is interesting with regards to its relationship with LEF1 in hair follicle progenitor activation and hair placode formation. When WNT signalling is activated, LEF1 associates with β-catenin and translocate to the nucleus where it outcompetes and displaces TFC3 from their shared binding sites on the chromatin (Adam et al., 2018; H. Nguyen et al., 2006). In the epidermal progenitors within the study, ID1 sequesters TCF3, and its targets remain inactivated, in terms of differentiation genes. It would therefore be of interest to investigate whether LEF1 is occupying any of the TCF binding sites in the presence of ID1. If that would be the case, as WNT is the activator of nuclear translocation of LEF1 and BMP the inducer of ID1, there would be a synergistic effect of WNT and BMP signalling. Moreover, the effect of Notch signalling on epidermal differentiation is well established (Blanpain et al., 2006). Since the binding alone of TCF3 to the *Cebpa* promoter was insufficient for the induction of gene expression, perhaps an investigation into whether Notch effectors are part of inducing *Cebpa* expression to enable downregulation of ID1 is warranted.

6 Conclusions and outlook

As far as model systems go for the study of stem cell behaviour in terms of proliferation and differentiation, and the mechanisms that drive these processes, few are as powerful as the epidermis and hair follicles. Given the implications for the broad contribution of citrullination to principal cellular processes, it was only a matter of time before the potential of PADIs was investigated in hair follicle stem cells.

As discussed, many reports that go looking for PADIs find them. The drawback from this is that the approach is often limited to one tissue or system of cells (such as various stem cells or immune cells). The result is thus that the standing belief is that tissue distribution of PADIs is limited and discrete. However, when turning to more unbiased databases such as single cell datasets or the Human Protein Atlas, PADI expression materialises as highly diverse. One reason for the discrepancies could be varied expression pattern owning to species differences, primarily between human and mouse. If so, this could be seen as yet another example of the dynamic utility of citrullination. Other, more mundane, reasons for the inconsistencies between the database annotations and the more directed searches for each individual PADI may come from the tools used to detect both citrullination and the proteins themselves. As these tools are being developed to become more sensitive, in combination with more unbiased approaches such as single cell sequencing (and/or spatial transcriptomics) the existence of PADIs within complex tissue will only become clearer. What this exercise in deduction really stresses, is that when looking for PADIs, one must keep an open mind and follow wherever the citrullination may lead.

In terms of PADI activity activation, the finding that certain factors can sensitise PADIs to lower concentrations of calcium or activate PADIs even in the absence of calcium (shown by bicarbonate) encourages continued search for other cofactors to help explain the activity of PADIs in low calcium environments. Perhaps as the field turns to embryonic development, there are undisclosed cofactors that underly the effect seen on PADI1, PADI2, PADI4, and even PADI6 in oocytes and preimplantation embryos. Moreover, are there cofactors which preferentially activate alternative isoforms of PADIs, or with diverging preferences for different (currently described) PADI members present in the same cell, such as PADI2 and PADI4 in certain stem cells and immune cells? It will be interesting to follow the therapeutic implications for any newly discovered cofactors.

Within the concept of the "core citrullinome", citrullination emerges not as something special and unusual only to rear its ugly head in suffering in disease, but something deeply integrated into the fabric of life. Then, however, the question arises about why the effects of PADI4 KO are not greater? To offer a humble opinion, there might be much more redundancy between PADI proteins than currently thought. Also, there might be the existence of further alternative PADI isoforms (as shown with PADI2β and PADI3β). Indeed, there are database annotations for additional *PADI2* and *PADI4* isoforms in human that

awaits verification and functional assessment. Arguments for this opinion are based on that no study has KO'd all five PADIs simultaneously, neither have the studies with full body KO of, for example, *Padi4* addressed the efficiency or magnitude of translational output. Moreover, it is possible that full KO of *Padi4*, which are achieved by deletions in exons (Hemmers et al., 2011), leaves any unidentified alternative isoforms unaffected, especially if these are under separate transcriptional control (although the available *Padi4*$^{-/-}$ KO available (Hemmers et al., 2011) has the active site of PAD4 removed, there may still be implications for isoforms lacking these as shown by the effects of PADI6 and our β-isoform study). As our own studies have shown, although the effects on translation are significant, they are not such that the overall phenotype on skin specific ablation of PADI4 are adverse, detrimental, or even result in gross anatomical manifestations, under the normal conditions we have studied them in. The implications for citrullination in ribosomal biology are then that it may be dispensable for overall function, but required for the accurate performance of those functions, as its removal perturbs protein synthesis. Given these results the next step is to investigate citrullination in translation in tumour biology, as many cancer types exhibit upregulated translation to uphold the high demand for growth that cancer entails (Robichaud et al., 2019; Ruggero, 2013; Song et al., 2021). Hence, in cancerous conditions, it is possible that the hitherto mild phenotype of PADI4 KO in hair follicles would be drastically different. Moreover, if KO of a seemingly important regulating feature does not result in a severe effect, it is possible that there is some hidden mechanism in place to protect against the loss of that feature in the face of changing conditions.

Perhaps also the coining of the term "core citrullinome" can give hints to why no de-citrullinating enzyme is identified yet. Conceivably, the maintenance of citrullination within these core biological processes may be innate in correct protein function to a degree where a cell cannot afford spending effort on removing the citrullination, but instead rely on protein turnover for its removal, i.e. that citrullination only disappears together with the protein it has become part of. Yet, citrullination appears to not be a mark for degradation of proteins, thus the cells would have to rely on normal rates for protein turnover for the modification to disappear. As most ribosomal proteins are synthesised *de novo* upon the requirements of the cell this synthesis would then also, invariably, include citrullination. This is not to say that there are no de-citrullinating enzymes, the discovery of one would explain so much – why citrullination is not found on every copy of a given protein, especially on particularly stable proteins, or how it disappears over time in histones if it is not by protein turnover, and provide yet another level of regulation of epigenetic transcriptional control.

These speculations go to show that the field is, despite its chronological age (dating back to 1958), still in its infancy. Only with more accurate methods of detection in combination

with asking the right questions, can the true functions and contributions of citrullination be unearthed.

Thus, as MS methodology is being further developed to increase sensitivity and resolution for the detection of citrullination, the contribution of citrullination to autoimmune diseases such as RA, lupus, and skin disorders, as well as neurodegenerative diseases such as multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease, where citrullination only just has been hinted at, can be thoroughly investigated. Moreover, a more detailed classification of PADIs and citrullination in cancer may open up novel areas of treatment or offer explanation to why some treatments are ineffective in some cancers. It will also guide the functional studies of specific citrullinated targets within the categories of RNA-processing and translation, as shifts in these fundamental biological processes are bound to entail significant proteomic alterations. The continued development of specific and sensitive methods to implement the great potential of MS will only help in unearthing the hidden secrets still locked away within the depths of citrullination.

Given that our study (Paper I) was conducted prior to that of Rebak *et al.* (Rebak et al., 2022), we could not rely on their high resolution citrullination detection. Future work will have to delve deeper into the citrullinome in keratinocyte progenitor, and may help to discern the PADI3- and PADI4-dependent citrullinomes. Additionally, the combination of high-resolution MS with single cell RNA-sequencing will yield an unprecedented powerful insight to the contribution of PADI4 in hair follicle development and into where, when, and how citrullination is deployed. If it turns out that histone citrullination is as integral in homeostatic hair follicle development as anticipated, the functional studies that can come of this brings with them great potential.

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8 References

Acharya, N. K., Nagele, E. P., Han, M., Coretti, N. J., DeMarshall, C., Kosciuk, M. C., Boulos, P. A., & Nagele, R. G. (2012). Neuronal PAD4 expression and protein citrullination: Possible role in production of autoantibodies associated with neurodegenerative disease. *Journal of Autoimmunity*, *38*(4), 369–380. https://doi.org/https://doi.org/10.1016/j.jaut.2012.03.004

Adam, R. C., Yang, H., Ge, Y., Lien, W.-H., Wang, P., Zhao, Y., Polak, L., Levorse, J., Baksh, S. C., Zheng, D., & Fuchs, E. (2018). Temporal Layering of Signaling Effectors Drives Chromatin Remodeling during Hair Follicle Stem Cell Lineage Progression. *Cell Stem Cell*, *22*(3), 398-413.e7. https://doi.org/10.1016/j.stem.2017.12.004

Adam, R. C., Yang, H., Rockowitz, S., Larsen, S. B., Nikolova, M., Oristian, D. S., Polak, L., Kadaja, M., Asare, A., Zheng, D., & Fuchs, E. (2015). Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice. *Nature*, *521*(7552), 366–370. https://doi.org/10.1038/nature14289

Agraz-Cibrian, J. M., Giraldo, D. M., & Urcuqui-Inchima, S. (2019). 1,25-Dihydroxyvitamin D3 induces formation of neutrophil extracellular trap-like structures and modulates the transcription of genes whose products are neutrophil extracellular trap-associated proteins: A pilot study. *Steroids*, *141*, 14–22. https://doi.org/https://doi.org/10.1016/j.steroids.2018.11.001

Al-Adwani, S., Wallin, C., Balhuizen, M. D., Veldhuizen, E. J. A., Coorens, M., Landreh, M., Végvári, Á., Smith, M. E., Qvarfordt, I., Lindén, A., Gräslund, A., Agerberth, B., & Bergman, P. (2020). Studies on citrullinated LL-37: detection in human airways, antibacterial effects and biophysical properties. *Scientific Reports*, *10*(1), 2376. https://doi.org/10.1038/s41598-020-59071-7

Alioli, A. C., Briot, J., Pons, C., Yang, H., Gairin, M., Goudounèche, D., Cau, L., Simon, M., & Méchin, M.-C. (2023). Down-regulation of peptidylarginine deiminase type 1 in reconstructed human epidermis disturbs nucleophagy in the granular layer and affects barrier function. *Cell Death Discovery*, *9*(1), 198. https://doi.org/10.1038/s41420-023-01509-8

Alonso, L., & Fuchs, E. (2006). The hair cycle. *Journal of Cell Science*, *119*(Pt 3), 391–393. https://doi.org/10.1242/jcs02793

An, W., Kim, J., & Roeder, R. G. (2004). Ordered Cooperative Functions of PRMT1, p300, and CARM1 in Transcriptional Activation by p53. *Cell*, *117*(6), 735–748. https://doi.org/https://doi.org/10.1016/j.cell.2004.05.009

Andl, T., Ahn, K., Kairo, A., Chu, E. Y., Wine-Lee, L., Reddy, S. T., Croft, N. J., Cebra-Thomas, J. A., Metzger, D., Chambon, P., Lyons, K. M., Mishina, Y., Seykora, J. T., Crenshaw III, E. B., & Millar, S. E. (2004). Epithelial Bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development. *Development*, *131*(10), 2257–2268. https://doi.org/10.1242/dev.01125

Andrade, F., Darrah, E., Gucek, M., Cole, R. N., Rosen, A., & Zhu, X. (2010). Autocitrullination of human peptidyl arginine deiminase type 4 regulates protein citrullination during cell activation. *Arthritis and Rheumatism*, *62*(6), 1630–1640. https://doi.org/10.1002/art.27439

Arita, K., Hashimoto, H., Shimizu, T., Nakashima, K., Yamada, M., & Sato, M. (2004a). Structural basis for Ca2+-induced activation of human PAD4. *Nature Structural & Molecular Biology*, *11*(8), 777–783. https://doi.org/10.1038/nsmb799

Arita, K., Hashimoto, H., Shimizu, T., Nakashima, K., Yamada, M., & Sato, M. (2004b). Structural basis for Ca2+-induced activation of human PAD4. *Nature Structural & Molecular Biology*, *11*(8), 777–783. https://doi.org/10.1038/nsmb799

Asaga, H., Nakashima, K., Senshu, T., Ishigami, A., & Yamada, M. (2001). Immunocytochemical localization of peptidylarginine deiminase in human eosinophils and neutrophils. *Journal of Leukocyte Biology*, *70*(1), 46–51.

Atchison, D. K., & Beierwaltes, W. H. (2013). The influence of extracellular and intracellular calcium on the secretion of renin. *Pflugers Archiv : European Journal of Physiology*, *465*(1), 59–69. https://doi.org/10.1007/s00424-012-1107-x

Ballasy, N. N., Bering, E. A., Kokorudz, C., Radford, B. N., Zhao, X., Dean, W., & Hemberger, M. (2022). Padi2/3 Deficiency Alters the Epigenomic Landscape and Causes Premature Differentiation of Mouse Trophoblast Stem Cells. In *Cells* (Vol. 11, Issue 16). https://doi.org/10.3390/cells11162466

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395. https://doi.org/10.1038/cr.2011.22

Beato, M., & Sharma, P. (2020). Peptidyl Arginine Deiminase 2 (PADI2)-Mediated Arginine Citrullination Modulates Transcription in Cancer. *International Journal of Molecular Sciences*, *21*(4). https://doi.org/10.3390/ijms21041351

Bekker-Jensen, D. B., Kelstrup, C. D., Batth, T. S., Larsen, S. C., Haldrup, C., Bramsen, J. B., Sørensen, K. D., Høyer, S., Ørntoft, T. F., Andersen, C. L., Nielsen, M. L., & Olsen, J. V. (2017). An Optimized Shotgun Strategy for the Rapid Generation of Comprehensive Human Proteomes. *Cell Systems*, *4*(6), 587-599.e4. https://doi.org/10.1016/j.cels.2017.05.009

Bennike, T., Lauridsen, K. B., Olesen, M. K., Andersen, V., Birkelund, S., & Stensballe, A. (2013). *Optimizing the Identification of Citrullinated Peptides by Mass Spectrometry : Utilizing the Inability of Trypsin to Cleave after Citrullinated Amino Acids Proteomics & Bioinformatics*. *6*(12), 288–295. https://doi.org/10.4172/jpb.1000293

Beronja, S., Livshits, G., Williams, S., & Fuchs, E. (2010). Rapid functional dissection of genetic networks via tissue-specific transduction and RNAi in mouse embryos. *Nat Med*, *16*(7), 821–827. http://dx.doi.org/10.1038/nm.2167

Bicker, K. L., & Thompson, P. R. (2013). The protein arginine deiminases: Structure, function, inhibition, and disease. *Biopolymers*, *99*(2), 155–163. https://doi.org/10.1002/bip.22127

Blanco, S., Bandiera, R., Popis, M., Hussain, S., Lombard, P., Aleksic, J., Sajini, A., Tanna, H., Cortés-Garrido, R., Gkatza, N., Dietmann, S., & Frye, M. (2016). Stem cell function and stress response are controlled by protein synthesis. *Nature*, *534*(7607), 335–340. https://doi.org/10.1038/nature18282

Blanpain, C., Lowry, W. E., Pasolli, H. A., & Fuchs, E. (2006). Canonical notch signaling functions as a commitment switch in the epidermal lineage. *Genes & Development*, *20*(21), 3022–3035. https://doi.org/10.1101/gad.1477606

Blick, T., Hugo, H., Widodo, E., Waltham, M., Pinto, C., Mani, S. A., Weinberg, R. A., Neve, R. M., Lenburg, M. E., & Thompson, E. W. (2010). Epithelial Mesenchymal Transition Traits in Human Breast Cancer Cell Lines Parallel the CD44hi/CD24lo/- Stem Cell Phenotype in Human Breast Cancer. *Journal of Mammary Gland Biology and Neoplasia*, *15*(2), 235–252. https://doi.org/10.1007/s10911-010-9175-z

Botchkarev, V. A., Botchkareva, N. V, Roth, W., Nakamura, M., Chen, L. H., Herzog, W., Lindner, G., McMahon, J. A., Peters, C., Lauster, R., McMahon, A. P., & Paus, R. (1999). Noggin is a mesenchymally derived stimulator of hair-follicle induction. *Nature Cell Biology*, *1*(3), 158–164. https://doi.org/10.1038/11078

Botchkarev, V. A., & Flores, E. R. (2014). p53/p63/p73 in the Epidermis in Health and Disease. *Cold Spring Harbor Perspectives in Medicine*, *4*(8), a015248. https://doi.org/10.1101/cshperspect.a015248

Brahmajosyula, M., & Miyake, M. (2013a). Localization and expression of peptidylarginine deiminase 4 (PAD4) in mammalian oocytes and preimplantation embryos. *Zygote (Cambridge, England)*, *21*(4), 314–324. https://doi.org/10.1017/S0967199411000633

Brahmajosyula, M., & Miyake, M. (2013b). Localization and expression of peptidylarginine deiminase 4 (PAD4) in mammalian oocytes and preimplantation embryos. *Zygote (Cambridge, England)*, *21*(4), 314–324. https://doi.org/10.1017/S0967199411000633

Brahmajosyula, M., & Miyake, M. (2013c). Role of peptidylarginine deiminase 4 (PAD4) in pig parthenogenetic preimplantation embryonic development. *Zygote*, *21*(4), 385–393. https://doi.org/DOI: 10.1017/S0967199412000160

Brahmajosyula, M., Wakayama, T., & Miyake, M. (2010). Role of Peptidylarginine Deiminase 4 in Mammalian Preimplantation Embryonic Development. *Biology of Reproduction*, *83*(Suppl_1), 253. https://doi.org/10.1093/biolreprod/83.s1.253

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., & Zychlinsky, A. (2004). Neutrophil Extracellular Traps Kill Bacteria. *Science*, *303*(5663), 1532 LP – 1535. https://doi.org/10.1126/science.1092385

Cai, E. Y., Kufeld, M. N., Schuster, S., Arora, S., Larkin, M., Germanos, A. A., Hsieh, A. C., & Beronja, S. (2020). Selective Translation of Cell Fate Regulators Mediates Tolerance to Broad Oncogenic Stress. *Cell Stem Cell*, *27*(2), 270-283.e7. https://doi.org/https://doi.org/10.1016/j.stem.2020.05.007

Candi, E., Schmidt, R., & Melino, G. (2005). The cornified envelope: a model of cell death in the skin. *Nature Reviews Molecular Cell Biology*, *6*(4), 328–340. https://doi.org/10.1038/nrm1619

Caprariello, A. V, Rogers, J. A., Morgan, M. L., Hoghooghi, V., Plemel, J. R., Koebel, A., Tsutsui, S., Dunn, J. F., Kotra, L. P., Ousman, S. S., Wee Yong, V., & Stys, P. K. (2018). Biochemically altered myelin triggers autoimmune demyelination. *Proceedings of the National Academy of Sciences*, *115*(21), 5528–5533. https://doi.org/10.1073/pnas.1721115115

Carrillo-Vico, A., Leech, M. D., & Anderton, S. M. (2010). Contribution of Myelin Autoantigen Citrullination to T Cell Autoaggression in the Central Nervous System. *The Journal of Immunology*, *184*(6), 2839 LP – 2846. https://doi.org/10.4049/jimmunol.0903639

Casanova, V., Sousa, F. H., Shakamuri, P., Svoboda, P., Buch, C., D'Acremont, M., Christophorou, M. A., Pohl, J., Stevens, C., & Barlow, P. G. (2020). Citrullination Alters the Antiviral and Immunomodulatory Activities of the Human Cathelicidin LL-37 During Rhinovirus Infection . In *Frontiers in Immunology* (Vol. 11, p. 85). https://www.frontiersin.org/article/10.3389/fimmu.2020.00085

Cau, L., Méchin, M.-C., & Simon, M. (2018). Peptidylarginine deiminases and deiminated proteins at the epidermal barrier. *Experimental Dermatology*, *27*(8), 852–858. https://doi.org/10.1111/exd.13684

Chaerkady, R., Zhou, Y., Delmar, J. A., Weng, S. H. S., Wang, J., Awasthi, S., Sims, D., Bowen, M. A., Yu, W., Cazares, L. H., Sims, G. P., & Hess, S. (2021). Characterization of Citrullination Sites in Neutrophils and Mast Cells Activated by Ionomycin via Integration of Mass Spectrometry and Machine Learning. *Journal of Proteome Research*, *20*(6), 3150–3164. https://doi.org/10.1021/acs.jproteome.1c00028

Chai, Z., Wang, L., Zheng, Y., Liang, N., Wang, X., Zheng, Y., Zhang, Z., Zhao, C., Zhu, T., & Liu, C. (2019). PADI3 plays an antitumor role via the Hsp90/CKS1 pathway in colon cancer. *Cancer Cell International*, *19*, 277. https://doi.org/10.1186/s12935-019-0999-3

Chang, H.-H., Dwivedi, N., Nicholas, A. P., & Ho, I.-C. (2015). The W620 Polymorphism in PTPN22 Disrupts Its Interaction With Peptidylarginine Deiminase Type 4 and Enhances Citrullination and NETosis. *Arthritis & Rheumatology*, *67*(9), 2323–2334. https://doi.org/10.1002/art.39215

Chang, X., Chai, Z., Zou, J., Wang, H., Wang, Y., Zheng, Y., Wu, H., & Liu, C. (2019). PADI3 induces cell cycle arrest via the Sirt2/AKT/p21 pathway and acts as a tumor suppressor gene in colon cancer. *Cancer Biology & Medicine*, *16*(4), 729–742. https://doi.org/10.20892/j.issn.2095-3941.2019.0065

Chang, X., Yamada, R., Suzuki, A., Sawada, T., Yoshino, S., Tokuhiro, S., & Yamamoto, K. (2004). Localization of peptidylarginine deiminase 4 (PADI4) and citrullinated protein in synovial tissue of rheumatoid arthritis. *Rheumatology*, *44*(1), 40–50. https://doi.org/10.1093/rheumatology/keh414

Chapman, E. A., Lyon, M., Simpson, D., Mason, D., Beynon, R. J., Moots, R. J., & Wright, H. L. (2019). Caught in a Trap? Proteomic Analysis of Neutrophil Extracellular Traps in Rheumatoid Arthritis and Systemic Lupus Erythematosus. *Frontiers in Immunology*, *10*, 423. https://doi.org/10.3389/fimmu.2019.00423

Chavanas, S., Méchin, M.-C., Takahara, H., Kawada, A., Nachat, R., Serre, G., & Simon, M. (2004a). Comparative analysis of the mouse and human peptidylarginine deiminase gene clusters reveals highly conserved non-coding segments and a new human gene, PADI6. *Gene*, *330*, 19–27. https://doi.org/https://doi.org/10.1016/j.gene.2003.12.038

Chavanas, S., Méchin, M.-C., Takahara, H., Kawada, A., Nachat, R., Serre, G., & Simon, M. (2004b). Comparative analysis of the mouse and human peptidylarginine deiminase gene clusters reveals highly conserved non-coding segments and a new

human gene, PADI6. *Gene*, *330*, 19–27. https://doi.org/https://doi.org/10.1016/j.gene.2003.12.038

Cherrington, B. D., Morency, E., Struble, A. M., Coonrod, S. A., & Wakshlag, J. J. (2010a). Potential role for peptidylarginine deiminase 2 (PAD2) in citrullination of canine mammary epithelial cell histones. *PLoS One*, *5*. https://doi.org/10.1371/journal.pone.0011768

Cherrington, B. D., Morency, E., Struble, A. M., Coonrod, S. A., & Wakshlag, J. J. (2010b). Potential role for peptidylarginine deiminase 2 (PAD2) in citrullination of canine mammary epithelial cell histones. *PLoS One*, *5*. https://doi.org/10.1371/journal.pone.0011768

Cherrington, B. D., Zhang, X., McElwee, J. L., Morency, E., Anguish, L. J., & Coonrod, S. A. (2012a). Potential role for PAD2 in gene regulation in breast cancer cells. *PloS One*, *7*(7), e41242–e41242. https://doi.org/10.1371/journal.pone.0041242

Cherrington, B. D., Zhang, X., McElwee, J. L., Morency, E., Anguish, L. J., & Coonrod, S. A. (2012b). Potential role for PAD2 in gene regulation in breast cancer cells. *PloS One*, *7*(7), e41242–e41242. https://doi.org/10.1371/journal.pone.0041242

Chovatiya, R., & Paller, A. S. (2021). JAK inhibitors in the treatment of atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *148*(4), 927–940. https://doi.org/10.1016/j.jaci.2021.08.009

Christensen, A. O., Li, G., Young, C. H., Snow, B., Khan, S. A., DeVore, S. B., Edwards, S., Bouma, G. J., Navratil, A. M., Cherrington, B. D., & Rothfuss, H. M. (2022). Peptidylarginine deiminase enzymes and citrullinated proteins in female reproductive physiology and associated diseases†. *Biology of Reproduction*, *107*(6), 1395–1410. https://doi.org/10.1093/biolre/ioac173

Christophorou, M. A. (2022). The virtues and vices of protein citrullination. *Royal Society Open Science*, *9*(6), 220125. https://doi.org/10.1098/rsos.220125

Christophorou, M. A., Castelo-Branco, G., Halley-Stott, R. P., Slade Oliveira, C., Loos, R., Radzisheuskaya, A., Mowen, K. A., Bertone, P., Silva, J., Zernicka-Goetz, M., Nielsen, M. L., Gurdon, J., & Kouzarides, T. (2014a). Citrullination regulates pluripotency and histone H1 binding to chromatin. In *Nature* (Vol. 507, Issue 7490, pp. 104–108). https://doi.org/10.1038/nature12942

Christophorou, M. A., Castelo-Branco, G., Halley-Stott, R. P., Slade Oliveira, C., Loos, R., Radzisheuskaya, A., Mowen, K. A., Bertone, P., Silva, J., Zernicka-Goetz, M., Nielsen, M. L., Gurdon, J., & Kouzarides, T. (2014b). Citrullination regulates pluripotency and

histone H1 binding to chromatin. In *Nature* (Vol. 507, Issue 7490, pp. 104–108). https://doi.org/10.1038/nature12942

Clancy, K. W., Russell, A.-M., Subramanian, V., Nguyen, H., Qian, Y., Campbell, R. M., & Thompson, P. R. (2017). Citrullination/Methylation Crosstalk on Histone H3 Regulates ER-Target Gene Transcription. *ACS Chemical Biology*, *12*(6), 1691–1702. https://doi.org/10.1021/acschembio.7b00241

Clavel, C., Grisanti, L., Zemla, R., Rezza, A., Barros, R., Sennett, R., Mazloom, A. R., Chung, C.-Y., Cai, X., Cai, C.-L., Pevny, L., Nicolis, S., Ma'ayan, A., & Rendl, M. (2012a). Sox2 in the dermal papilla niche controls hair growth by fine-tuning BMP signaling in differentiating hair shaft progenitors. *Developmental Cell*, *23*(5), 981–994. https://doi.org/10.1016/j.devcel.2012.10.013

Clavel, C., Grisanti, L., Zemla, R., Rezza, A., Barros, R., Sennett, R., Mazloom, A. R., Chung, C.-Y., Cai, X., Cai, C.-L., Pevny, L., Nicolis, S., Ma'ayan, A., & Rendl, M. (2012b). Sox2 in the dermal papilla niche controls hair growth by fine-tuning BMP signaling in differentiating hair shaft progenitors. *Developmental Cell*, *23*(5), 981–994. https://doi.org/10.1016/j.devcel.2012.10.013

Clayton, E., Doupé, D. P., Klein, A. M., Winton, D. J., Simons, B. D., & Jones, P. H. (2007). A single type of progenitor cell maintains normal epidermis. *Nature*, *446*(7132), 185–189. https://doi.org/10.1038/nature05574

Clayton, K., Vallejo, A. F., Davies, J., Sirvent, S., & Polak, M. E. (2017). Langerhans Cells-Programmed by the Epidermis. *Frontiers in Immunology*, *8*, 1676. https://doi.org/10.3389/fimmu.2017.01676

Cockburn, K., Annusver, K., Gonzalez, D. G., Ganesan, S., May, D. P., Mesa, K. R., Kawaguchi, K., Kasper, M., & Greco, V. (2022). Gradual differentiation uncoupled from cell cycle exit generates heterogeneity in the epidermal stem cell layer. *Nature Cell Biology*, *24*(12), 1692–1700. https://doi.org/10.1038/s41556-022-01021-8

Corsiero, E., Bombardieri, M., Carlotti, E., Pratesi, F., Robinson, W., Migliorini, P., & Pitzalis, C. (2016). Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Annals of the Rheumatic Diseases*, *75*(10), 1866–1875. https://doi.org/10.1136/annrheumdis-2015-208356

Crisp, A., Boschetti, C., Perry, M., Tunnacliffe, A., & Micklem, G. (2015). Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biology*, *16*(1), 50. https://doi.org/10.1186/s13059-015-0607-3

Cui, Y. ying, Yan, L., Zhou, J., Zhao, S., Zheng, Y. bing, Sun, B. hui, Lv, H. tao, Rong, F. nian, & Chang, X. tian. (2016). The role of peptidylarginine deiminase 4 in ovarian cancer cell tumorigenesis and invasion. *Tumor Biology*, *37*(4), 5375–5383. https://doi.org/10.1007/s13277-015-4363-5

Cummings, T. F. M., Gori, K., Sanchez-Pulido, L., Gavriilidis, G., Moi, D., Wilson, A. R., Murchison, E., Dessimoz, C., Ponting, C. P., & Christophorou, M. A. (2022). Citrullination Was Introduced into Animals by Horizontal Gene Transfer from Cyanobacteria. *Molecular Biology and Evolution*, *39*(2), msab317. https://doi.org/10.1093/molbev/msab317

Curtis, C., Shah, S. P., Chin, S.-F., Turashvili, G., Rueda, O. M., Dunning, M. J., Speed, D., Lynch, A. G., Samarajiwa, S., Yuan, Y., Gräf, S., Ha, G., Haffari, G., Bashashati, A., Russell, R., McKinney, S., Caldas, C., Aparicio, S., Curtis†, C., … subgroup:, D. analysis. (2012). The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, *486*(7403), 346–352. https://doi.org/10.1038/nature10983

Cuthbert, G. L., Daujat, S., Snowden, A. W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P. D., Tempst, P., Bannister, A. J., & Kouzarides, T. (2004a). Histone deimination antagonizes arginine methylation. *Cell*, *118*(5), 545–553. https://doi.org/10.1016/j.cell.2004.08.020

Cuthbert, G. L., Daujat, S., Snowden, A. W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P. D., Tempst, P., Bannister, A. J., & Kouzarides, T. (2004b). Histone deimination antagonizes arginine methylation. *Cell*, *118*(5), 545–553. https://doi.org/10.1016/j.cell.2004.08.020

Damen, M., Wirtz, L., Soroka, E., Khatif, H., Kukat, C., Simons, B. D., & Bazzi, H. (2021). High proliferation and delamination during skin epidermal stratification. *Nature Communications*, *12*(1), 3227. https://doi.org/10.1038/s41467-021-23386-4

Damgaard, D., Bjørn, M. E., Jensen, P. Ø., & Nielsen, C. H. (2017). Reactive oxygen species inhibit catalytic activity of peptidylarginine deiminase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 1203–1208. https://doi.org/10.1080/14756366.2017.1368505

Damgaard, D., Bjørn, M. E., Steffensen, M. A., Pruijn, G. J. M., & Nielsen, C. H. (2016). Reduced glutathione as a physiological co-activator in the activation of peptidylarginine deiminase. *Arthritis Research & Therapy*, *18*(1), 102. https://doi.org/10.1186/s13075-016-1000-7

Darrah, E., Giles, J. T., Ols, M. L., Bull, H. G., Andrade, F., & Rosen, A. (2013). Erosive Rheumatoid Arthritis Is Associated with Antibodies That Activate PAD4 by

Increasing Calcium Sensitivity. *Science Translational Medicine*, *5*(186), 186ra65-186ra65. https://doi.org/10.1126/scitranslmed.3005370

DasGupta, R., & Fuchs, E. (1999). Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development*, *126*(20), 4557 LP – 4568. http://dev.biologists.org/content/126/20/4557.abstract

De Rosa, L., Secone Seconetti, A., De Santis, G., Pellacani, G., Hirsch, T., Rothoeft, T., Teig, N., Pellegrini, G., Bauer, J. W., & De Luca, M. (2019). Laminin 332-Dependent YAP Dysregulation Depletes Epidermal Stem Cells in Junctional Epidermolysis Bullosa. *Cell Reports*, *27*(7), 2036-2049.e6. https://doi.org/https://doi.org/10.1016/j.celrep.2019.04.055

Dekoninck, S., Hannezo, E., Sifrim, A., Miroshnikova, Y. A., Aragona, M., Malfait, M., Gargouri, S., de Neunheuser, C., Dubois, C., Voet, T., Wickström, S. A., Simons, B. D., & Blanpain, C. (2020). Defining the Design Principles of Skin Epidermis Postnatal Growth. *Cell*, *181*(3), 604-620.e22. https://doi.org/https://doi.org/10.1016/j.cell.2020.03.015

Demoruelle, M. K., Wang, H., Davis, R. L., Visser, A., Hoang, J., Norris, J. M., Holers, V. M., Deane, K. D., & Darrah, E. (2021). Anti-peptidylarginine deiminase-4 antibodies at mucosal sites can activate peptidylarginine deiminase-4 enzyme activity in rheumatoid arthritis. *Arthritis Research & Therapy*, *23*(1), 163. https://doi.org/10.1186/s13075-021-02528-5

Deng, Z., Lei, X., Zhang, X., Zhang, H., Liu, S., Chen, Q., Hu, H., Wang, X., Ning, L., Cao, Y., Zhao, T., Zhou, J., Chen, T., & Duan, E. (2015). mTOR signaling promotes stem cell activation via counterbalancing BMP-mediated suppression during hair regeneration. *Journal of Molecular Cell Biology*, *7*(1), 62–72. https://doi.org/10.1093/jmcb/mjv005

Deplus, R., Denis, H., Putmans, P., Calonne, E., Fourrez, M., Yamamoto, K., Suzuki, A., & Fuks, F. (2014). Citrullination of DNMT3A by PADI4 regulates its stability and controls DNA methylation. *Nucleic Acids Research*, *42*(13), 8285–8296. https://doi.org/10.1093/nar/gku522

Díez-Del-Molino, D., Dehasque, M., Chacón-Duque, J. C., Pečnerová, P., Tikhonov, A., Protopopov, A., Plotnikov, V., Kanellidou, F., Nikolskiy, P., Mortensen, P., Danilov, G. K., Vartanyan, S., Gilbert, M. T. P., Lister, A. M., Heintzman, P. D., van der Valk, T., & Dalén, L. (2023). Genomics of adaptive evolution in the woolly mammoth. *Current Biology : CB*, *33*(9), 1753-1764.e4. https://doi.org/10.1016/j.cub.2023.03.084

Ding, X., Bloch, W., Iden, S., Rüegg, M. A., Hall, M. N., Leptin, M., Partridge, L., & Eming, S. A. (2016). mTORC1 and mTORC2 regulate skin morphogenesis and epidermal barrier formation. *Nature Communications*, *7*(1), 13226. https://doi.org/10.1038/ncomms13226

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. *International Journal of Molecular Sciences*, *14*(6), 12222–12248. https://doi.org/10.3390/ijms140612222

Doupé, D. P., Klein, A. M., Simons, B. D., & Jones, P. H. (2010). The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate. *Developmental Cell*, *18*(2), 317–323. https://doi.org/10.1016/j.devcel.2009.12.016

Elia, A., Constantinou, C., & Clemens, M. J. (2008). Effects of protein phosphorylation on ubiquitination and stability of the translational inhibitor protein 4E-BP1. *Oncogene*, *27*(6), 811–822. https://doi.org/10.1038/sj.onc.1210678

Elkenani, M., Nyamsuren, G., Raju, P., Liakath-Ali, K., Hamdaoui, A., Kata, A., Dressel, R., Klonisch, T., Watt, F. M., Engel, W., Thliveris, J. A., Krishna Pantakani, D. V, & Adham, I. M. (2016). Pelota Regulates Epidermal Differentiation by Modulating BMP and PI3K/AKT Signaling Pathways. *Journal of Investigative Dermatology*, *136*(8), 1664–1671. https://doi.org/https://doi.org/10.1016/j.jid.2016.04.020

Ellis, T., Gambardella, L., Horcher, M., Tschanz, S., Capol, J., Bertram, P., Jochum, W., Barrandon, Y., & Busslinger, M. (2001). The transcriptional repressor CDP (Cutl1) is essential for epithelial cell differentiation of the lung and the hair follicle. *Genes & Development* , *15*(17), 2307–2319. https://doi.org/10.1101/gad.200101

El-Sayed, A. S. A., Shindia, A. A., AbouZaid, A. A., Yassin, A. M., Ali, G. S., & Sitohy, M. Z. (2019). Biochemical characterization of peptidylarginine deiminase-like orthologs from thermotolerant Emericella dentata and Aspergillus nidulans. *Enzyme and Microbial Technology*, *124*, 41–53. https://doi.org/https://doi.org/10.1016/j.enzmictec.2019.02.004

Esposito, G., Vitale, A. M., Leijten, F. P., Strik, A. M., Koonen-Reemst, A. M., Yurttas, P., Robben, T. J., Coonrod, S., & Gossen, J. A. (2007). Peptidylarginine deiminase (PAD) 6 is essential for oocyte cytoskeletal sheet formation and female fertility. *Mol Cell Endocrinol*, *273*. https://doi.org/10.1016/j.mce.2007.05.005

Estrach, S., Ambler, C. A., Lo Celso, C. Lo, Hozumi, K., & Watt, F. M. (2006). Jagged 1 is a β-catenin target gene required for ectopic hair follicle formation in adult epidermis. *Development*, *133*(22), 4427–4438. https://doi.org/10.1242/dev.02644

Evans, R. D., Perkins, V. C., Henry, A., Stephens, P. E., Robinson, M. K., & Watt, F. M. (2003). A tumor-associated beta 1 integrin mutation that abrogates epithelial differentiation control. *The Journal of Cell Biology*, *160*(4), 589–596. https://doi.org/10.1083/jcb.200209016

Falcão, A. M., Meijer, M., Scaglione, A., Rinwa, P., Agirre, E., Liang, J., Larsen, S. C., Heskol, A., Frawley, R., Klingener, M., Varas-Godoy, M., Raposo, A. A. S. F., Ernfors, P., Castro, D. S., Nielsen, M. L., Casaccia, P., & Castelo-Branco, G. (2019a). PAD2-Mediated Citrullination Contributes to Efficient Oligodendrocyte Differentiation and Myelination. *Cell Reports*, *27*(4), 1090-1102.e10. https://doi.org/10.1016/j.celrep.2019.03.108

Falcão, A. M., Meijer, M., Scaglione, A., Rinwa, P., Agirre, E., Liang, J., Larsen, S. C., Heskol, A., Frawley, R., Klingener, M., Varas-Godoy, M., Raposo, A. A. S. F., Ernfors, P., Castro, D. S., Nielsen, M. L., Casaccia, P., & Castelo-Branco, G. (2019b). PAD2-Mediated Citrullination Contributes to Efficient Oligodendrocyte Differentiation and Myelination. *Cell Reports*, *27*(4), 1090-1102.e10. https://doi.org/https://doi.org/10.1016/j.celrep.2019.03.108

Fan, X., Wang, D., Burgmaier, J. E., Teng, Y., Romano, R.-A., Sinha, S., & Yi, R. (2018). Single Cell and Open Chromatin Analysis Reveals Molecular Origin of Epidermal Cells of the Skin. *Developmental Cell*, *47*(1), 21-37.e5. https://doi.org/10.1016/j.devcel.2018.08.010

Farrelly, L. A., Thompson, R. E., Zhao, S., Lepack, A. E., Lyu, Y., Bhanu, N. V, Zhang, B., Loh, Y.-H. E., Ramakrishnan, A., Vadodaria, K. C., Heard, K. J., Erikson, G., Nakadai, T., Bastle, R. M., Lukasak, B. J., Zebroski, H., Alenina, N., Bader, M., Berton, O., … Maze, I. (2019). Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. *Nature*, *567*(7749), 535–539. https://doi.org/10.1038/s41586-019-1024-7

Ferby, I., Reschke, M., Kudlacek, O., Knyazev, P., Pantè, G., Amann, K., Sommergruber, W., Kraut, N., Ullrich, A., Fässler, R., & Klein, R. (2006). Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. *Nature Medicine*, *12*(5), 568–573. https://doi.org/10.1038/nm1401

Ferreira, I. G., Weber, M. B., & Bonamigo, R. R. (2021). History of dermatology: the study of skin diseases over the centuries. *Anais Brasileiros de Dermatologia*, *96*(3), 332–345. https://doi.org/10.1016/j.abd.2020.09.006

Fert-Bober, J., Darrah, E., & Andrade, F. (2020). Insights into the study and origin of the citrullinome in rheumatoid arthritis. *Immunological Reviews*, *294*(1), 133–147. https://doi.org/10.1111/imr.12834

Fert-Bober, J., Venkatraman, V., Hunter, C. L., Liu, R., Crowgey, E. L., Pandey, R., Holewinski, R. J., Stotland, A., Berman, B. P., & Van Eyk, J. E. (2019a). Mapping Citrullinated Sites in Multiple Organs of Mice Using Hypercitrullinated Library. *Journal of Proteome Research*, *18*(5), 2270–2278. https://doi.org/10.1021/acs.jproteome.9b00118

Fert-Bober, J., Venkatraman, V., Hunter, C. L., Liu, R., Crowgey, E. L., Pandey, R., Holewinski, R. J., Stotland, A., Berman, B. P., & Van Eyk, J. E. (2019b). Mapping Citrullinated Sites in Multiple Organs of Mice Using Hypercitrullinated Library. *Journal of Proteome Research*, *18*(5), 2270–2278. https://doi.org/10.1021/acs.jproteome.9b00118

Fert-Bober, J., Venkatraman, V., Hunter, C. L., Liu, R., Crowgey, E. L., Pandey, R., Holewinski, R. J., Stotland, A., Berman, B. P., & Van Eyk, J. E. (2019c). Mapping Citrullinated Sites in Multiple Organs of Mice Using Hypercitrullinated Library. *Journal of Proteome Research*, *18*(5), 2270–2278. https://doi.org/10.1021/acs.jproteome.9b00118

Festa, E., Fretz, J., Berry, R., Schmidt, B., Rodeheffer, M., Horowitz, M., & Horsley, V. (2011). Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell*, *146*(5), 761–771. https://doi.org/10.1016/j.cell.2011.07.019

Fischer, J. J., Toedling, J., Krueger, T., Schueler, M., Huber, W., & Sperling, S. (2008). Combinatorial effects of four histone modifications in transcription and differentiation. *Genomics*, *91*(1), 41–51. https://doi.org/https://doi.org/10.1016/j.ygeno.2007.08.010

Folgueras, A. R., Guo, X., Pasolli, H. A., Stokes, N., Polak, L., Zheng, D., & Fuchs, E. (2013). Architectural niche organization by LHX2 is linked to hair follicle stem cell function. *Cell Stem Cell*, *13*(3), 314–327. https://doi.org/10.1016/j.stem.2013.06.018

Fuchs, E. (2007a). Scratching the surface of skin development. *Nature*, *445*(7130), 834–842. https://doi.org/10.1038/nature05659

Fuchs, E. (2007b). Scratching the surface of skin development. *Nature*, *445*(7130), 834–842. https://doi.org/10.1038/nature05659

Fuhrmann, J., & Thompson, P. R. (2016). Protein Arginine Methylation and Citrullination in Epigenetic Regulation. In *ACS Chemical Biology* (Vol. 11, Issue 3, pp. 654–668). https://doi.org/10.1021/acschembio.5b00942

FUJISAKI, M., & SUGAWARA, K. (1981). Properties of Peptidylarginine Deiminase from the Epidermis of Newborn Rats1. *The Journal of Biochemistry*, *89*(1), 257–263. https://doi.org/10.1093/oxfordjournals.jbchem.a133189

Füllgrabe, A., Joost, S., Are, A., Jacob, T., Sivan, U., Haegebarth, A., Linnarsson, S., Simons, B. D., Clevers, H., Toftgård, R., & Kasper, M. (2015). Dynamics of Lgr6^{+} Progenitor Cells in the Hair Follicle, Sebaceous Gland, and Interfollicular Epidermis. *Stem Cell Reports*, *5*(5), 843–855. https://doi.org/10.1016/j.stemcr.2015.09.013

Garcia-Romo, G. S., Caielli, S., Vega, B., Connolly, J., Allantaz, F., Xu, Z., Punaro, M., Baisch, J., Guiducci, C., Coffman, R. L., Barrat, F. J., Banchereau, J., & Pascual, V. (2011). Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus

erythematosus. *Science Translational Medicine*, *3*(73), 73ra20. https://doi.org/10.1126/scitranslmed.3001201

Garrod, D., & Chidgey, M. (2008a). Desmosome structure, composition and function. In *Biochimica et Biophysica Acta - Biomembranes* (Vol. 1778, Issue 3, pp. 572–587). https://doi.org/10.1016/j.bbamem.2007.07.014

Garrod, D., & Chidgey, M. (2008b). Desmosome structure, composition and function. In *Biochimica et Biophysica Acta - Biomembranes* (Vol. 1778, Issue 3, pp. 572–587). https://doi.org/10.1016/j.bbamem.2007.07.014

Genander, M., Cook, P. J., Ramsköld, D., Keyes, B. E., Mertz, A. F., Sandberg, R., & Fuchs, E. (2014). BMP signaling and its pSMAD1/5 target genes differentially regulate hair follicle stem cell lineages. *Cell Stem Cell*, *15*(5), 619–633. https://doi.org/10.1016/j.stem.2014.09.009

Geyfman, M., Plikus, M. V, Treffeisen, E., Andersen, B., & Paus, R. (2015). Resting no more: re-defining telogen, the maintenance stage of the hair growth cycle. *Biological Reviews*, *90*(4), 1179–1196. https://doi.org/10.1111/brv.12151

Ghahramani, A., Donati, G., Luscombe, N. M., & Watt, F. M. (2018). Epidermal Wnt signalling regulates transcriptome heterogeneity and proliferative fate in neighbouring cells. *Genome Biology*, *19*(1), 3. https://doi.org/10.1186/s13059-017-1384-y

Ghari, F., Quirke, A.-M., Munro, S., Kawalkowska, J., Picaud, S., McGouran, J., Subramanian, V., Muth, A., Williams, R., Kessler, B., Thompson, P. R., Fillipakopoulos, P., Knapp, S., Venables, P. J., & La Thangue, N. B. (2016). Citrullination-acetylation interplay guides E2F-1 activity during the inflammatory response. *Science Advances*, *2*(2), e1501257. https://doi.org/10.1126/sciadv.1501257

Ghazizadeh, S., & Taichman, L. B. (2001). Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. *The EMBO Journal*, *20*(6), 1215–1222. https://doi.org/10.1093/emboj/20.6.1215

Ghuwalewala, S., Lee, S. A., Jiang, K., Baidya, J., Chovatiya, G., Kaur, P., Shalloway, D., & Tumbar, T. (2022). Binary organization of epidermal basal domains highlights robustness to environmental exposure. *The EMBO Journal*, *41*(18), e110488. https://doi.org/10.15252/embj.2021110488

Goëb, V., Thomas-L'Otellier, M., Daveau, R., Charlionet, R., Fardellone, P., Le Loët, X., Tron, F., Gilbert, D., & Vittecoq, O. (2009). Candidate autoantigens identified by mass spectrometry in early rheumatoid arthritis are chaperones and citrullinated glycolytic enzymes. *Arthritis Research & Therapy*, *11*(2), R38. https://doi.org/10.1186/ar2644

Golenberg, N., Squirrell, J. M., Bennin, D. A., Rindy, J., Pistono, P. E., Eliceiri, K. W., Shelef, M. A., Kang, J., & Huttenlocher, A. (2020). Citrullination regulates wound responses and tissue regeneration in zebrafish. *The Journal of Cell Biology*, *219*(4). https://doi.org/10.1083/jcb.201908164

Gomez, C., Chua, W., Miremadi, A., Quist, S., Headon, D. J., & Watt, F. M. (2013). The Interfollicular Epidermis of Adult Mouse Tail Comprises Two Distinct Cell Lineages that Are Differentially Regulated by Wnt, Edaradd, and Lrig1. *Stem Cell Reports*, *1*(1), 19–27. https://doi.org/10.1016/j.stemcr.2013.04.001

Gonzales, K. A. U., & Fuchs, E. (2017). Skin and Its Regenerative Powers: An Alliance between Stem Cells and Their Niche. *Developmental Cell*, *43*(4), 387–401. https://doi.org/10.1016/j.devcel.2017.10.001

Goulas, T., Mizgalska, D., Garcia-Ferrer, I., Kantyka, T., Guevara, T., Szmigielski, B., Sroka, A., Millán, C., Usón, I., Veillard, F., Potempa, B., Mydel, P., Solà, M., Potempa, J., & Gomis-Rüth, F. X. (2015). Structure and mechanism of a bacterial host-protein citrullinating virulence factor, Porphyromonas gingivalis peptidylarginine deiminase. *Scientific Reports*, *5*(1), 11969. https://doi.org/10.1038/srep11969

Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., dela Cruz-Racelis, J., & Fuchs, E. (2009a). A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. *Cell Stem Cell*, *4*(2), 155–169. https://doi.org/https://doi.org/10.1016/j.stem.2008.12.009

Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., dela Cruz-Racelis, J., & Fuchs, E. (2009b). A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. *Cell Stem Cell*, *4*(2), 155–169. https://doi.org/https://doi.org/10.1016/j.stem.2008.12.009

Guerrin, M., Ishigami, A., Mechin, M.-C., Nachat, R., Valmary, S., Sebbag, M., Simon, M., Senshu, T., & Serre, G. (2003). cDNA cloning, gene organization and expression analysis of human peptidylarginine deiminase type I. *The Biochemical Journal*, *370*(Pt 1), 167–174. https://doi.org/10.1042/BJ20020870

Guertin, M. J., Zhang, X., Anguish, L., Kim, S., Varticovski, L., Lis, J. T., Hager, G. L., & Coonrod, S. A. (2014). Targeted H3R26 Deimination Specifically Facilitates Estrogen Receptor Binding by Modifying Nucleosome Structure. *PLOS Genetics*, *10*(9), e1004613. https://doi.org/10.1371/journal.pgen.1004613

Guo, Q., Bedford, M. T., & Fast, W. (2011). Discovery of peptidylarginine deiminase-4 substrates by protein array: antagonistic citrullination and methylation of human ribosomal protein S2. *Molecular BioSystems*, *7*(7), 2286–2295. https://doi.org/10.1039/C1MB05089C

Guo, Q., & Fast, W. (2011). Citrullination of Inhibitor of Growth 4 (ING4) by Peptidylarginine Deminase 4 (PAD4) disrupts the interaction between ING4 and p53. *Journal of Biological Chemistry*, *286*(19), 17069–17078. https://doi.org/10.1074/jbc.M111.230961

Györffy, B., Lanczky, A., Eklund, A. C., Denkert, C., Budczies, J., Li, Q., & Szallasi, Z. (2010). An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Research and Treatment*, *123*(3), 725–731. https://doi.org/10.1007/s10549-009-0674-9

Haensel, D., Jin, S., Sun, P., Cinco, R., Dragan, M., Nguyen, Q., Cang, Z., Gong, Y., Vu, R., MacLean, A. L., Kessenbrock, K., Gratton, E., Nie, Q., & Dai, X. (2020). Defining Epidermal Basal Cell States during Skin Homeostasis and Wound Healing Using Single-Cell Transcriptomics. *Cell Reports*, *30*(11), 3932-3947.e6. https://doi.org/10.1016/j.celrep.2020.02.091

Hagiwara, T., Nakashima, K., Hirano, H., Senshu, T., & Yamada, M. (2002). Deimination of arginine residues in nucleophosmin/B23 and histones in HL-60 granulocytes. *Biochemical and Biophysical Research Communications*, *290*(3), 979–983. https://doi.org/10.1006/bbrc.2001.6303

Hänel, K. H., Cornelissen, C., Lüscher, B., & Baron, J. M. (2013). Cytokines and the Skin Barrier. In *International Journal of Molecular Sciences* (Vol. 14, Issue 4, pp. 6720–6745). https://doi.org/10.3390/ijms14046720

Hao, G., Wang, D., Gu, J., Shen, Q., Gross, S. S., & Wang, Y. (2009). Neutral loss of isocyanic acid in peptide CID spectra: A novel diagnostic marker for mass spectrometric identification of protein citrullination. *Journal of the American Society for Mass Spectrometry*, *20*(4), 723–727. https://doi.org/10.1021/jasms.8b03443

He, H., Bissonnette, R., Wu, J., Diaz, A., Saint-Cyr Proulx, E., Maari, C., Jack, C., Louis, M., Estrada, Y., Krueger, J. G., Zhang, N., Pavel, A. B., & Guttman-Yassky, E. (2021). Tape strips detect distinct immune and barrier profiles in atopic dermatitis and psoriasis. *Journal of Allergy and Clinical Immunology*, *147*(1), 199–212. https://doi.org/10.1016/j.jaci.2020.05.048

Hemmers, S., Teijaro, J. R., Arandjelovic, S., & Mowen, K. A. (2011). PAD4-mediated neutrophil extracellular trap formation is not required for immunity against influenza infection. *PloS One*, *6*(7), e22043–e22043. https://doi.org/10.1371/journal.pone.0022043

Hermansson, M., Artemenko, K., Ossipova, E., Eriksson, H., Lengqvist, J., Makrygiannakis, D., Catrina, A. I., Nicholas, A. P., Klareskog, L., Savitski, M., A. Zubarev, R., & Jakobsson, P.-J. (2010). MS analysis of rheumatoid arthritic synovial tissue identifies specific

citrullination sites on fibrinogen. *PROTEOMICS – Clinical Applications*, *4*(5), 511–518. https://doi.org/10.1002/prca.200900088

Hinnebusch, A. G. (2012). Translational homeostasis via eIF4E and 4E-BP1. *Molecular Cell*, *46*(6), 717–719. https://doi.org/10.1016/j.molcel.2012.06.001

Holmes, C. L., Shim, D., Kernien, J., Johnson, C. J., Nett, J. E., & Shelef, M. A. (2019). Insight into Neutrophil Extracellular Traps through Systematic Evaluation of Citrullination and Peptidylarginine Deiminases. *Journal of Immunology Research*, *2019*, 2160192. https://doi.org/10.1155/2019/2160192

Hsu, C.-Y., Gasc, G., Raymond, A.-A., Burlet-Schiltz, O., Takahara, H., Serre, G., Méchin, M.-C., & Simon, M. (2017). Deimination of Human Hornerin Enhances its Processing by Calpain-1 and its Cross-Linking by Transglutaminases. *Journal of Investigative Dermatology*, *137*(2), 422–429. https://doi.org/https://doi.org/10.1016/j.jid.2016.09.030

Hsu, C.-Y., Henry, J., Raymond, A.-A., Mechin, M.-C., Pendaries, V., Nassar, D., Hansmann, B., Balica, S., Burlet-Schiltz, O., Schmitt, A.-M., Takahara, H., Paul, C., Serre, G., & Simon, M. (2011). Deimination of human filaggrin-2 promotes its proteolysis by calpain 1. *The Journal of Biological Chemistry*, *286*(26), 23222–23233. https://doi.org/10.1074/jbc.M110.197400

Hsu, Y. C., Li, L., & Fuchs, E. (2014a). Emerging interactions between skin stem cells and their niches. In *Nature Medicine* (Vol. 20, Issue 8, pp. 847–856). https://doi.org/10.1038/nm.3643

Hsu, Y. C., Li, L., & Fuchs, E. (2014b). Emerging interactions between skin stem cells and their niches. In *Nature Medicine* (Vol. 20, Issue 8, pp. 847–856). https://doi.org/10.1038/nm.3643

Hsu, Y.-C., Li, L., & Fuchs, E. (2014). Transit-Amplifying Cells Orchestrate Stem Cell Activity and Tissue Regeneration. *Cell*, *157*(4), 935–949. https://doi.org/10.1016/j.cell.2014.02.057

Hsu, Y.-C., Pasolli, H. A., & Fuchs, E. (2011a). Dynamics between Stem Cells, Niche, and Progeny in the Hair Follicle. *Cell*, *144*(1), 92–105. https://doi.org/https://doi.org/10.1016/j.cell.2010.11.049

Hsu, Y.-C., Pasolli, H. A., & Fuchs, E. (2011b). Dynamics between Stem Cells, Niche, and Progeny in the Hair Follicle. *Cell*, *144*(1), 92–105. https://doi.org/https://doi.org/10.1016/j.cell.2010.11.049

Hu, M. S., Borrelli, M. R., Hong, W. X., Malhotra, S., Cheung, A. T. M., Ransom, R. C., Rennert, R. C., Morrison, S. D., Lorenz, H. P., & Longaker, M. T. (2018). Embryonic skin

development and repair. *Organogenesis*, *14*(1), 46–63. https://doi.org/10.1080/15476278.2017.1421882

Humbert, P., Maibach, H. I., Fanian, F., & Agache, P. (2017). Agache's Measuring the Skin: Non-invasive Investigations, Physiology, Normal Constants: Second Edition. *Agache's Measuring the Skin: Non-Invasive Investigations, Physiology, Normal Constants: Second Edition*, *April*, 1–1651. https://doi.org/10.1007/978-3-319-32383-1

Ishida-Yamamoto, A., Senshu, T., Eady, R. A. J., Takahashi, H., Shimizu, H., Akiyama, M., & Iizuka, H. (2002a). Sequential Reorganization of Cornified Cell Keratin Filaments Involving Filaggrin-Mediated Compaction and Keratin 1 Deimination. *Journal of Investigative Dermatology*, *118*(2), 282–287. https://doi.org/https://doi.org/10.1046/j.0022-202x.2001.01671.x

Ishida-Yamamoto, A., Senshu, T., Eady, R. A. J., Takahashi, H., Shimizu, H., Akiyama, M., & Iizuka, H. (2002b). Sequential Reorganization of Cornified Cell Keratin Filaments Involving Filaggrin-Mediated Compaction and Keratin 1 Deimination. *Journal of Investigative Dermatology*, *118*(2), 282–287. https://doi.org/https://doi.org/10.1046/j.0022-202x.2001.01671.x

Ishida-Yamamoto, A., Takahashi, H., Iizuka, H., Senshu, T., Akiyama, K., & Nomura, K. (2000). Decreased Deiminated Keratin K1 in Psoriatic Hyperproliferative Epidermis. *Journal of Investigative Dermatology*, *114*(4), 701–705. https://doi.org/https://doi.org/10.1046/j.1523-1747.2000.00936.x

Jaiswal, A., & Singh, R. (2022). Homeostases of epidermis and hair follicle, and development of basal cell carcinoma. *Biochimica et Biophysica Acta. Reviews on Cancer*, *1877*(5), 188795. https://doi.org/10.1016/j.bbcan.2022.188795

Jamora, C., DasGupta, R., Kocieniewski, P., & Fuchs, E. (2003). Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature*, *422*(6929), 317–322. https://doi.org/10.1038/nature01458

Jensen, K. B., Collins, C. A., Nascimento, E., Tan, D. W., Frye, M., Itami, S., & Watt, F. M. (2009). Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell*, *4*(5), 427–439. https://doi.org/10.1016/j.stem.2009.04.014

Jensen, U. B., Lowell, S., & Watt, F. M. (1999). The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. *Development (Cambridge, England)*, *126*(11), 2409–2418. https://doi.org/10.1242/dev.126.11.2409

Jensen, U. B., Yan, X., Triel, C., Woo, S. H., Christensen, R., & Owens, D. M. (2008). A distinct population of clonogenic and multipotent murine follicular keratinocytes residing in the upper isthmus. *Journal of Cell Science*, *121*(5), 609–617. https://doi.org/10.1242/jcs.025502

Jenuwein, T., & Allis, C. D. (2001). Translating the Histone Code. *Science*, *293*(5532), 1074 LP – 1080. https://doi.org/10.1126/science.1063127

Jobert, L., Argentini, M., & Tora, L. (2009). PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function. *Experimental Cell Research*, *315*(7), 1273–1286. https://doi.org/10.1016/j.yexcr.2008.12.008

Jones, P. H., Harper, S., & Watt, F. M. (1995). Stem cell patterning and fate in human epidermis. *Cell*, *80*(1), 83–93. https://doi.org/10.1016/0092-8674(95)90453-0

Joost, S., Annusver, K., Jacob, T., Sun, X., Dalessandri, T., Sivan, U., Sequeira, I., Sandberg, R., & Kasper, M. (2020a). The Molecular Anatomy of Mouse Skin during Hair Growth and Rest. *Cell Stem Cell*, *26*(3), 441-457.e7. https://doi.org/10.1016/j.stem.2020.01.012

Joost, S., Annusver, K., Jacob, T., Sun, X., Dalessandri, T., Sivan, U., Sequeira, I., Sandberg, R., & Kasper, M. (2020b). The Molecular Anatomy of Mouse Skin during Hair Growth and Rest. *Cell Stem Cell*, *26*(3), 441-457.e7. https://doi.org/10.1016/j.stem.2020.01.012

Joost, S., Jacob, T., Sun, X., Annusver, K., La Manno, G., Sur, I., & Kasper, M. (2018a). Single-Cell Transcriptomics of Traced Epidermal and Hair Follicle Stem Cells Reveals Rapid Adaptations during Wound Healing. *Cell Reports*, *25*(3), 585-597.e7. https://doi.org/https://doi.org/10.1016/j.celrep.2018.09.059

Joost, S., Jacob, T., Sun, X., Annusver, K., La Manno, G., Sur, I., & Kasper, M. (2018b). Single-Cell Transcriptomics of Traced Epidermal and Hair Follicle Stem Cells Reveals Rapid Adaptations during Wound Healing. *Cell Reports*, *25*(3), 585-597.e7. https://doi.org/https://doi.org/10.1016/j.celrep.2018.09.059

Joost, S., Zeisel, A., Jacob, T., Sun, X., La Manno, G., Lönnerberg, P., Linnarsson, S., & Kasper, M. (2016). Single-Cell Transcriptomics Reveals that Differentiation and Spatial Signatures Shape Epidermal and Hair Follicle Heterogeneity. *Cell Systems*, *3*(3), 221-237.e9. https://doi.org/10.1016/j.cels.2016.08.010

Kahata, K., Dadras, M. S., & Moustakas, A. (2018). TGF-β Family Signaling in Epithelial Differentiation and Epithelial-Mesenchymal Transition. *Cold Spring Harbor Perspectives in Biology*, *10*(1). https://doi.org/10.1101/cshperspect.a022194

Kan, R., Jin, M., Subramanian, V., Causey, C. P., Thompson, P. R., & Coonrod, S. A. (2012a). Potential role for PADI-mediated histone citrullination in preimplantation development. *BMC Developmental Biology*, *12*(1), 19. https://doi.org/10.1186/1471-213X-12-19

Kan, R., Jin, M., Subramanian, V., Causey, C. P., Thompson, P. R., & Coonrod, S. A. (2012b). Potential role for PADI-mediated histone citrullination in preimplantation development. *BMC Developmental Biology*, *12*(1), 19. https://doi.org/10.1186/1471-213X-12-19

Kan, R., Yurttas, P., Kim, B., Jin, M., Wo, L., Lee, B., Gosden, R., & Coonrod, S. A. (2011). Regulation of mouse oocyte microtubule and organelle dynamics by PADI6 and the cytoplasmic lattices. *Dev Biol*, *350*. https://doi.org/10.1016/j.ydbio.2010.11.033

Kandyba, E., Leung, Y., Chen, Y.-B., Widelitz, R., Chuong, C.-M., & Kobielak, K. (2013). Competitive balance of intrabulge BMP/Wnt signaling reveals a robust gene network ruling stem cell homeostasis and cyclic activation. *Proceedings of the National Academy of Sciences*, *110*(4), 1351–1356. https://doi.org/10.1073/pnas.1121312110

Kanno, T., Kawada, A., Yamanouchi, J., Yosida-Noro, C., Yoshiki, A., Shiraiwa, M., Kusakabe, M., Manabe, M., Tezuka, T., & Takahara, H. (2000). Human peptidylarginine deiminase type III: Molecular cloning and nucleotide sequence of the cDNA, properties of the recombinant enzyme, and immunohistochemical localization in human skin. *Journal of Investigative Dermatology*, *115*(5), 813–823. https://doi.org/10.1046/j.1523-1747.2000.00131.x

Kaufman, C. K., Zhou, P., Pasolli, H. A., Rendl, M., Bolotin, D., Lim, K. C., Dai, X., Alegre, M. L., & Fuchs, E. (2003). GATA-3: An unexpected regulator of cell lineage determination in skin. *Genes and Development*, *17*(17), 2108–2122. https://doi.org/10.1101/gad.1115203

Keyes, B. E., Segal, J. P., Heller, E., Lien, W. H., Chang, C. Y., Guo, X., Oristian, D. S., Zheng, D., & Fuchs, E. (2013). Nfatc1 orchestrates aging in hair follicle stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(51). https://doi.org/10.1073/pnas.1320301110

Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J. S., Friday, S., Li, S., Patel, R. M., Subramanian, V., Thompson, P., Chen, P., Fox, D. A., Pennathur, S., & Kaplan, M. J. (2013a). NETs Are a Source of Citrullinated Autoantigens and Stimulate Inflammatory Responses in Rheumatoid Arthritis. *Science Translational Medicine*, *5*(178), 178ra40 LP-178ra40. https://doi.org/10.1126/scitranslmed.3005580

Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J. S., Friday, S., Li, S., Patel, R. M., Subramanian, V., Thompson, P., Chen, P., Fox, D. A., Pennathur, S., & Kaplan, M. J. (2013b). NETs Are a Source of Citrullinated Autoantigens and Stimulate Inflammatory Responses in Rheumatoid Arthritis. *Science Translational Medicine*, *5*(178), 178ra40 LP-178ra40. https://doi.org/10.1126/scitranslmed.3005580

Kilsgård, O., Andersson, P., Malmsten, M., Nordin, S. L., Linge, H. M., Eliasson, M., Sörenson, E., Erjefält, J. S., Bylund, J., Olin, A. I., Sørensen, O. E., & Egesten, A. (2012). Peptidylarginine Deiminases Present in the Airways during Tobacco Smoking and Inflammation Can Citrullinate the Host Defense Peptide LL-37, Resulting in Altered Activities. *American Journal of Respiratory Cell and Molecular Biology*, *46*(2), 240–248. https://doi.org/10.1165/rcmb.2010-0500OC

Kimura-Ueki, M., Oda, Y., Oki, J., Komi-Kuramochi, A., Honda, E., Asada, M., Suzuki, M., & Imamura, T. (2012). Hair Cycle Resting Phase Is Regulated by Cyclic Epithelial FGF18 Signaling. *Journal of Investigative Dermatology*, *132*(5), 1338–1345. https://doi.org/https://doi.org/10.1038/jid.2011.490

Kizawa, K., Fujimori, T., & Kawai, T. (2017). Arachidonate 12-Lipoxygenase Inhibitors Promote S100A3 Citrullination in Cultured SW480 Cells and Isolated Hair Follicles. *Biological and Pharmaceutical Bulletin*, *40*(4), 516–523. https://doi.org/10.1248/bpb.b16-00954

Klopf, J., Brostjan, C., Eilenberg, W., & Neumayer, C. (2021). Neutrophil Extracellular Traps and Their Implications in Cardiovascular and Inflammatory Disease. *International Journal of Molecular Sciences*, *22*(2), 559. https://doi.org/10.3390/ijms22020559

Knight, J. S., Subramanian, V., O'Dell, A. A., Yalavarthi, S., Zhao, W., Smith, C. K., Hodgin, J. B., Thompson, P. R., & Kaplan, M. J. (2015). Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. *Annals of the Rheumatic Diseases*, *74*(12), 2199–2206. https://doi.org/10.1136/annrheumdis-2014-205365

Knuckley, B., Causey, C. P., Jones, J. E., Bhatia, M., Dreyton, C. J., Osborne, T. C., Takahara, H., & Thompson, P. R. (2010). Substrate specificity and kinetic studies of PADs 1, 3, and 4 identify potent and selective inhibitors of protein arginine deiminase 3. *Biochemistry*, *49*(23), 4852–4863. https://doi.org/10.1021/bi100363t

Kobielak, K., Pasolli, H. A., Alonso, L., Polak, L., & Fuchs, E. (2003). Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA. *The Journal of Cell Biology*, *163*(3), 609–623. https://doi.org/10.1083/jcb.200309042

Koboldt, D. C., Fulton, R. S., McLellan, M. D., Schmidt, H., Kalicki-Veizer, J., McMichael, J. F., Fulton, L. L., Dooling, D. J., Ding, L., Mardis, E. R., Wilson, R. K., Ally, A., Balasundaram, M., Butterfield, Y. S. N., Carlsen, R., Carter, C., Chu, A., Chuah, E., Chun, H.-J. E., … Center, M. D. A. C. (2012). Comprehensive molecular portraits of human breast tumours. *Nature*, *490*(7418), 61–70. https://doi.org/10.1038/nature11412

Kolodziej, S., Kuvardina, O. N., Oellerich, T., Herglotz, J., Backert, I., Kohrs, N., Buscató, Estel. la, Wittmann, S. K., Salinas-Riester, G., Bonig, H., Karas, M., Serve, H., Proschak, E., & Lausen, J. (2014). PADI4 acts as a coactivator of Tal1 by counteracting repressive histone arginine methylation. *Nature Communications*, *5*, 3995.

Koster, M. I., Dai, D., Marinari, B., Sano, Y., Costanzo, A., Karin, M., & Roop, D. R. (2007). p63 induces key target genes required for epidermal morphogenesis. *Proceedings of the National Academy of Sciences*, *104*(9), 3255–3260. https://doi.org/10.1073/pnas.0611376104

Koster, M. I., Kim, S., Mills, A. A., DeMayo, F. J., & Roop, D. R. (2004a). p63 is the molecular switch for initiation of an epithelial stratification program. *Genes & Development*, *18*(2), 126–131. https://doi.org/10.1101/gad.1165104

Koster, M. I., Kim, S., Mills, A. A., DeMayo, F. J., & Roop, D. R. (2004b). p63 is the molecular switch for initiation of an epithelial stratification program. *Genes & Development*, *18*(2), 126–131. https://doi.org/10.1101/gad.1165104

Koster, M. I., & Roop, D. R. (2007a). Mechanisms Regulating Epithelial Stratification. *Annual Review of Cell and Developmental Biology*, *23*(1), 93–113. https://doi.org/10.1146/annurev.cellbio.23.090506.123357

Koster, M. I., & Roop, D. R. (2007b). Mechanisms Regulating Epithelial Stratification. *Annual Review of Cell and Developmental Biology*, *23*(1), 93–113. https://doi.org/10.1146/annurev.cellbio.23.090506.123357

Kowalczyk, A. P., & Green, K. J. (2013a). Chapter Five - Structure, Function, and Regulation of Desmosomes. In F. B. T.-P. in M. B. and T. S. van Roy (Ed.), *The Molecular Biology of Cadherins* (Vol. 116, pp. 95–118). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-394311-8.00005-4

Kowalczyk, A. P., & Green, K. J. (2013b). Chapter Five - Structure, Function, and Regulation of Desmosomes. In F. B. T.-P. in M. B. and T. S. van Roy (Ed.), *The Molecular Biology of Cadherins* (Vol. 116, pp. 95–118). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-394311-8.00005-4

Krishnamurthy, A., Ytterberg, A. J., Sun, M., Sakuraba, K., Steen, J., Joshua, V., Tarasova, N. K., Malmström, V., Wähämaa, H., Réthi, B., & Catrina, A. I. (2019). Citrullination Controls

Dendritic Cell Transdifferentiation into Osteoclasts. *The Journal of Immunology*, *202*(11), 3143 LP – 3150. https://doi.org/10.4049/jimmunol.1800534

Lande, R., Palazzo, R., Gestermann, N., Jandus, C., Falchi, M., Spadaro, F., Riccieri, V., James, E. A., Butera, A., Boirivant, M., Feldmeyer, L., Surbeck, I., Di Lucca, J., Stuber, F., Spinelli, F. R., Botti, E., Marinari, B., Bianchi, L., Pica, R., … Frasca, L. (2020). Native/citrullinated LL37-specific T-cells help autoantibody production in Systemic Lupus Erythematosus. *Scientific Reports*, *10*(1), 5851. https://doi.org/10.1038/s41598-020-62480-3

Langbein, L., Yoshida, H., Praetzel-Wunder, S., Parry, D. A., & Schweizer, J. (2010a). The keratins of the human beard hair medulla: The riddle in the middle. *Journal of Investigative Dermatology*, *130*(1), 55–73. https://doi.org/10.1038/jid.2009.192

Langbein, L., Yoshida, H., Praetzel-Wunder, S., Parry, D. A., & Schweizer, J. (2010b). The keratins of the human beard hair medulla: The riddle in the middle. *Journal of Investigative Dermatology*, *130*(1), 55–73. https://doi.org/10.1038/jid.2009.192

Lechler, T., & Fuchs, E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature*, *437*(7056), 275–280. https://doi.org/10.1038/nature03922

Lee, C. Y., Wang, D., Wilhelm, M., Zolg, D. P., Schmidt, T., Schnatbaum, K., Reimer, U., Pontén, F., Uhlén, M., Hahne, H., & Kuster, B. (2018). Mining the human tissue proteome for protein citrullination. *Molecular and Cellular Proteomics*, *17*(7), 1378–1391. https://doi.org/10.1074/mcp.RA118.000696

Lee, C.-Y., Lin, C.-C., Liu, Y.-L., Liu, G.-Y., Liu, J.-H., & Hung, H.-C. (2017). Molecular Interplay between the Dimer Interface and the Substrate-Binding Site of Human Peptidylarginine Deiminase 4. *Scientific Reports*, *7*(1), 42662. https://doi.org/10.1038/srep42662

Lee, K. H., Kronbichler, A., Park, D. D.-Y., Park, Y., Moon, H., Kim, H., Choi, J. H., Choi, Y., Shim, S., Lyu, I. S., Yun, B. H., Han, Y., Lee, D., Lee, S. Y., Yoo, B. H., Lee, K. H., Kim, T. L., Kim, H., Shim, J. S., … Shin, J. Il. (2017). Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. *Autoimmunity Reviews*, *16*(11), 1160–1173. https://doi.org/https://doi.org/10.1016/j.autrev.2017.09.012

Lee, Y. H., Bae, S.-C., Choi, S. J., Ji, J. D., & Song, G. G. (2012). Genome-wide pathway analysis of genome-wide association studies on systemic lupus erythematosus and rheumatoid arthritis. *Molecular Biology Reports*, *39*(12), 10627–10635. https://doi.org/10.1007/s11033-012-1952-x

Lee, Y.-H., Coonrod, S. A., Kraus, W. L., Jelinek, M. A., & Stallcup, M. R. (2005). Regulation of coactivator complex assembly and function by protein arginine methylation and demethylimination. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(10), 3611 LP – 3616. https://doi.org/10.1073/pnas.0407159102

Lefort, K., Mandinova, A., Ostano, P., Kolev, V., Calpini, V., Kolfschoten, I., Devgan, V., Lieb, J., Raffoul, W., Hohl, D., Neel, V., Garlick, J., Chiorino, G., & Dotto, G. P. (2007). Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKalpha kinases. *Genes & Development*, *21*(5), 562–577. https://doi.org/10.1101/gad.1484707

Leishman, E., Howard, J. M., Garcia, G. E., Miao, Q., Ku, A. T., Dekker, J. D., Tucker, H., & Nguyen, H. (2013). Foxp1 maintains hair follicle stem cell quiescence through regulation of Fgf18. *Development (Cambridge, England)*, *140*(18), 3809–3818. https://doi.org/10.1242/dev.097477

Lewallen, D. M., Bicker, K. L., Subramanian, V., Clancy, K. W., Slade, D. J., Martell, J., Dreyton, C. J., Sokolove, J., Weerapana, E., & Thompson, P. R. (2015). Chemical Proteomic Platform To Identify Citrullinated Proteins. *ACS Chemical Biology*, *10*(11), 2520–2528. https://doi.org/10.1021/acschembio.5b00438

Lewis, H. D., Liddle, J., Coote, J. E., Atkinson, S. J., Barker, M. D., Bax, B. D., Bicker, K. L., Bingham, R. P., Campbell, M., Chen, Y. H., Chung, C., Craggs, P. D., Davis, R. P., Eberhard, D., Joberty, G., Lind, K. E., Locke, K., Maller, C., Martinod, K., … Wilson, D. M. (2015). Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nature Chemical Biology*, *11*(3), 189–191. https://doi.org/10.1038/nchembio.1735

Li, P., Li, M., Lindberg, M. R., Kennett, M. J., Xiong, N., & Wang, Y. (2010a). PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *Journal of Experimental Medicine*, *207*(9), 1853–1862. https://doi.org/10.1084/jem.20100239

Li, P., Li, M., Lindberg, M. R., Kennett, M. J., Xiong, N., & Wang, Y. (2010b). PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *Journal of Experimental Medicine*, *207*(9), 1853–1862. https://doi.org/10.1084/jem.20100239

Li, P., Wang, D., Yao, H., Doret, P., Hao, G., Shen, Q., Qiu, H., Zhang, X., Wang, Y., Chen, G., & Wang, Y. (2010). Coordination of PAD4 and HDAC2 in the regulation of p53-target gene expression. *Oncogene*, *29*(21), 3153–3162. https://doi.org/10.1038/onc.2010.51

Li, P., Yao, H., Zhang, Z., Li, M., Luo, Y., Thompson, P. R., Gilmour, D. S., & Wang, Y. (2008). Regulation of p53 target gene expression by peptidylarginine deiminase 4. *Mol Cell Biol*, *28*. https://doi.org/10.1128/MCB.01747-07

Li, Y.-H., Zhang, K., Yang, K., Ye, J.-X., Xing, Y.-Z., Guo, H.-Y., Deng, F., Lian, X.-H., & Yang, T. (2013). Adenovirus-Mediated Wnt10b Overexpression Induces Hair Follicle Regeneration. *Journal of Investigative Dermatology*, *133*(1), 42–48. https://doi.org/10.1038/jid.2012.235

Liakath-Ali, K., Mills, E. W., Sequeira, I., Lichtenberger, B. M., Pisco, A. O., Sipilä, K. H., Mishra, A., Yoshikawa, H., Wu, C. C.-C., Ly, T., Lamond, A. I., Adham, I. M., Green, R., & Watt, F. M. (2018). An evolutionarily conserved ribosome-rescue pathway maintains epidermal homeostasis. *Nature*, *556*(7701), 376–380. https://doi.org/10.1038/s41586-018-0032-3

Lien, W.-H., & Fuchs, E. (2014). Wnt some lose some: transcriptional governance of stem cells by Wnt/β-catenin signaling. *Genes & Development*, *28*(14), 1517–1532. https://doi.org/10.1101/gad.244772.114

Lien, W.-H., Guo, X., Polak, L., Lawton, L. N., Young, R. A., Zheng, D., & Fuchs, E. (2011). Genome-wide Maps of Histone Modifications Unwind In Vivo Chromatin States of the Hair Follicle Lineage. *Cell Stem Cell*, *9*(3), 219–232. https://doi.org/10.1016/j.stem.2011.07.015

Lim, X., & Nusse, R. (2013). Wnt Signaling in Skin Development, Homeostasis, and Disease. *Cold Spring Harbor Perspectives in Biology* , *5*(2). https://doi.org/10.1101/cshperspect.a008029

Lin, Z., Jin, S., Chen, J., Li, Z., Lin, Z., Tang, L., Nie, Q., & Andersen, B. (2020). Murine interfollicular epidermal differentiation is gradualistic with GRHL3 controlling progression from stem to transition cell states. *Nature Communications*, *11*(1), 5434. https://doi.org/10.1038/s41467-020-19234-6

Linsky, T., & Fast, W. (2010). Mechanistic similarity and diversity among the guanidine-modifying members of the pentein superfamily. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1804*(10), 1943–1953. https://doi.org/https://doi.org/10.1016/j.bbapap.2010.07.016

Liu, C., Tang, J., Li, C., Pu, G., Yang, D., & Chang, X. (2019). PADI4 stimulates esophageal squamous cell carcinoma tumor growth and up-regulates CA9 expression. *Molecular Carcinogenesis*, *58*(1), 66–75. https://doi.org/10.1002/mc.22907

Liu, G. Y., & Sabatini, D. M. (2020). mTOR at the nexus of nutrition, growth, ageing and disease. *Nature Reviews Molecular Cell Biology*, *21*(4), 183–203. https://doi.org/10.1038/s41580-019-0199-y

Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, *2*(1), 17023. https://doi.org/10.1038/sigtrans.2017.23

Lo Celso, C., Prowse, D. M., & Watt, F. M. (2004). Transient activation of beta-catenin signalling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours. *Development (Cambridge, England)*, *131*(8), 1787–1799. https://doi.org/10.1242/dev.01052

Lo, J. A., & Fisher, D. E. (2014). The melanoma revolution: From UV carcinogenesis to a new era in therapeutics. *Science*, *346*(6212), 945 LP – 949. https://doi.org/10.1126/science.1253735

Lopez, R. G., Garcia-Silva, S., Moore, S. J., Bereshchenko, O., Martinez-Cruz, A. B., Ermakova, O., Kurz, E., Paramio, J. M., & Nerlov, C. (2009). C/EBPα and β couple interfollicular keratinocyte proliferation arrest to commitment and terminal differentiation. *Nature Cell Biology*, *11*(10), 1181–1190. https://doi.org/10.1038/ncb1960

Lowry, W. E., Blanpain, C., Nowak, J. A., Guasch, G., Lewis, L., & Fuchs, E. (2005). Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. *Genes & Development*, *19*(13), 1596–1611. https://doi.org/10.1101/gad.1324905

Luo, Y., Arita, K., Bhatia, M., Knuckley, B., Lee, Y., Stallcup, M. R., Sato, M., & Thompson, P. R. (2006). Inhibitors and Inactivators of Protein Arginine Deiminase 4 : Functional and. *Biochemistry*, *45*(39), 11727–11736.

Mackay, A., Tamber, N., Fenwick, K., Iravani, M., Grigoriadis, A., Dexter, T., Lord, C. J., Reis-Filho, J. S., & Ashworth, A. (2009). A high-resolution integrated analysis of genetic and expression profiles of breast cancer cell lines. *Breast Cancer Research and Treatment*, *118*(3), 481–498. https://doi.org/10.1007/s10549-008-0296-7

Mackenzie, I. C. (1997). Retroviral transduction of murine epidermal stem cells demonstrates clonal units of epidermal structure. *The Journal of Investigative Dermatology*, *109*(3), 377–383. https://doi.org/10.1111/1523-1747.ep12336255

Malki, L., Sarig, O., Romano, M.-T., Méchin, M.-C., Peled, A., Pavlovsky, M., Warshauer, E., Samuelov, L., Uwakwe, L., Briskin, V., Mohamad, J., Gat, A., Isakov, O., Rabinowitz, T., Shomron, N., Adir, N., Simon, M., McMichael, A., Dlova, N. C., … Sprecher, E. (2019). Variant PADI3 in Central Centrifugal Cicatricial Alopecia. *New England Journal of Medicine*, *380*(9), 833–841. https://doi.org/10.1056/NEJMoa1816614

Mammucari, C., Tommasi di Vignano, A., Sharov, A. A., Neilson, J., Havrda, M. C., Roop, D. R., Botchkarev, V. A., Crabtree, G. R., & Dotto, G. P. (2005). Integration of Notch 1 and calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation

control. *Developmental Cell*, *8*(5), 665–676. https://doi.org/10.1016/j.devcel.2005.02.016

Mardaryev, A. N., Meier, N., Poterlowicz, K., Sharov, A. A., Sharova, T. Y., Ahmed, M. I., Rapisarda, V., Lewis, C., Fessing, M. Y., Ruenger, T. M., Bhawan, J., Werner, S., Paus, R., & Botchkarev, V. A. (2011). Lhx2 differentially regulates Sox9, Tcf4 and Lgr5 in hair follicle stem cells to promote epidermal regeneration after injury. *Development*, *138*(22), 4843 LP – 4852. https://doi.org/10.1242/dev.070284

Marondedze, C., Elia, G., Thomas, L., Wong, A., & Gehring, C. (2021). Citrullination of Proteins as a Specific Response Mechanism in Plants . In *Frontiers in Plant Science* (Vol. 12). https://www.frontiersin.org/articles/10.3389/fpls.2021.638392

Mascré, G., Dekoninck, S., Drogat, B., Youssef, K. K., Brohée, S., Sotiropoulou, P. A., Simons, B. D., & Blanpain, C. (2012). Distinct contribution of stem and progenitor cells to epidermal maintenance. *Nature*, *489*(7415), 257–262. https://doi.org/10.1038/nature11393

Massarenti, L., Enevold, C., Damgaard, D., Ødum, N., Nielsen, C. H., & Jacobsen, S. (2019). Peptidylarginine deiminase-4 gene polymorphisms are associated with systemic lupus erythematosus and lupus nephritis. *Scandinavian Journal of Rheumatology*, *48*(2), 133–140. https://doi.org/10.1080/03009742.2018.1488273

Masson-Bessière, C., Sebbag, M., Durieux, J.-J., Nogueira, L., Vincent, C., Girbal-Neuhauser, E., Durroux, R., Cantagrel, A., & Serre, G. (2000). In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. *Clinical & Experimental Immunology*, *119*(3), 544–552. https://doi.org/10.1046/j.1365-2249.2000.01171.x

Mastronardi, F. G., Wood, D. D., Mei, J., Raijmakers, R., Tseveleki, V., Dosch, H.-M., Probert, L., Casaccia-Bonnefil, P., & Moscarello, M. A. (2006). Increased citrullination of histone H3 in multiple sclerosis brain and animal models of demyelination: a role for tumor necrosis factor-induced peptidylarginine deiminase 4 translocation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(44), 11387–11396. https://doi.org/10.1523/JNEUROSCI.3349-06.2006

Matos, I., Asare, A., Levorse, J., Ouspenskaia, T., de la Cruz-Racelis, J., Schuhmacher, L.-N., & Fuchs, E. (2020). Progenitors oppositely polarize WNT activators and inhibitors to orchestrate tissue development. *ELife*, *9*. https://doi.org/10.7554/eLife.54304

Matsui, T., & Amagai, M. (2015). Dissecting the formation, structure and barrier function of the stratum corneum. *International Immunology*, *27*(6), 269–280. https://doi.org/10.1093/intimm/dxv013

M'Boneko, V., & Merker, H. J. (1988). Development and morphology of the periderm of mouse embryos (days 9-12 of gestation). *Acta Anatomica*, *133*(4), 325–336. https://doi.org/10.1159/000146662

McElwee, J. L., Mohanan, S., Horibata, S., Sams, K. L., Anguish, L. J., McLean, D., Cvitaš, I., Wakshlag, J. J., & Coonrod, S. A. (2014). PAD2 Overexpression in Transgenic Mice Promotes Spontaneous Skin Neoplasia. *Cancer Research*, *74*(21), 6306–6317. https://doi.org/10.1158/0008-5472.CAN-14-0749

McNee, G., Eales, K. L., Wei, W., Williams, D. S., Barkhuizen, A., Bartlett, D. B., Essex, S., Anandram, S., Filer, A., Moss, P. A. H., Pratt, G., Basu, S., Davies, C. C., & Tennant, D. A. (2017). Citrullination of histone H3 drives IL-6 production by bone marrow mesenchymal stem cells in MGUS and multiple myeloma. *Leukemia*, *31*(2), 373–381. https://doi.org/10.1038/leu.2016.187

Méchin, M. C., Takahara, H., & Simon, M. (2020). Deimination and peptidylarginine deiminases in skin physiology and diseases. *International Journal of Molecular Sciences*, *21*(2), 1–15. https://doi.org/10.3390/ijms21020566

Méchin, M.-C., Sebbag, M., Arnaud, J., Nachat, R., Foulquier, C., Adoue, V., Coudane, F., Duplan, H., Schmitt, A.-M., Chavanas, S., Guerrin, M., Serre, G., & Simon, M. (2007). Update on peptidylarginine deiminases and deimination in skin physiology and severe human diseases. *International Journal of Cosmetic Science*, *29*(3), 147–168. https://doi.org/10.1111/j.1467-2494.2007.00377.x

Méchin, M.-C., Takahara, H., & Simon, M. (2020). Deimination and Peptidylarginine Deiminases in Skin Physiology and Diseases. In *International Journal of Molecular Sciences* (Vol. 21, Issue 2). https://doi.org/10.3390/ijms21020566

Merrill, B. J., Gat, U., DasGupta, R., & Fuchs, E. (2001). Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. *Genes & Development*, *15*(13), 1688–1705. https://doi.org/10.1101/gad.891401

Mesa, K. R., Kawaguchi, K., Cockburn, K., Gonzalez, D., Boucher, J., Xin, T., Klein, A. M., & Greco, V. (2018). Homeostatic Epidermal Stem Cell Self-Renewal Is Driven by Local Differentiation. *Cell Stem Cell*, *23*(5), 677–686.e4. https://doi.org/10.1016/j.stem.2018.09.005

Mikkola, M. L. (2007). Genetic basis of skin appendage development. *Seminars in Cell & Developmental Biology*, *18*(2), 225–236. https://doi.org/https://doi.org/10.1016/j.semcdb.2007.01.007

Ming Kwan, K., Li, A. G., Wang, X.-J., Wurst, W., & Behringer, R. R. (2004). Essential roles of BMPR-IA signaling in differentiation and growth of hair follicles and in skin

tumorigenesis. *Genesis (New York, N.Y. : 2000)*, *39*(1), 10–25. https://doi.org/10.1002/gene.20021

Miroshnikova, Y. A., Le, H. Q., Schneider, D., Thalheim, T., Rübsam, M., Bremicker, N., Polleux, J., Kamprad, N., Tarantola, M., Wang, I., Balland, M., Niessen, C. M., Galle, J., & Wickström, S. A. (2018). Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. *Nature Cell Biology*, *20*(1), 69–80. https://doi.org/10.1038/s41556-017-0005-z

Mohanan, S., Cherrington, B. D., Horibata, S., McElwee, J. L., Thompson, P. R., & Coonrod, S. A. (2012). Potential Role of Peptidylarginine Deiminase Enzymes and Protein Citrullination in Cancer Pathogenesis. *Biochemistry Research International*, *2012*, 895343. https://doi.org/10.1155/2012/895343

Mohanan, S., Horibata, S., Anguish, L. J., Mukai, C., Sams, K., McElwee, J. L., McLean, D., Yan, A., & Coonrod, S. A. (2017). PAD2 overexpression in transgenic mice augments malignancy and tumor-associated inflammation in chemically initiated skin tumors. *Cell and Tissue Research*, *370*(2), 275–283. https://doi.org/10.1007/s00441-017-2669-x

Moscarello, M. A., Lei, H., Mastronardi, F. G., Winer, S., Tsui, H., Li, Z., Ackerley, C., Zhang, L., Raijmakers, R., & Wood, D. D. (2013). Inhibition of peptidyl-arginine deiminases reverses protein- hypercitrullination and disease in mouse models of multiple sclerosis. *DMM Disease Models and Mechanisms*, *6*(2), 467–478. https://doi.org/10.1242/dmm.010520

Müller-Röver, S., Handjiski, B., Van Der Veen, C., Eichmüller, S., Foitzik, K., McKay, I. A., Stenn, K. S., & Paus, R. (2001). A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. In *Journal of Investigative Dermatology* (Vol. 117, Issue 1, pp. 3–15). Elsevier. https://doi.org/10.1046/j.0022-202X.2001.01377.x

Müller-Röver, S., Tokura, Y., Welker, P., Furukawa, F., Wakita, H., Takigawa, M., & Paus, R. (1999). E- and P-cadherin expression during murine hair follicle morphogenesis and cycling. *Experimental Dermatology*, *8*(4), 237–246. https://doi.org/10.1111/j.1600-0625.1999.tb00377.x

Musse, A. A., Li, Z., Ackerley, C. A., Bienzle, D., Lei, H., Poma, R., Harauz, G., Moscarello, M. A., & Mastronardi, F. G. (2008). Peptidylarginine deiminase 2 (PAD2) overexpression in transgenic mice leads to myelin loss in the central nervous system. *Disease Models & Mechanisms*, *1*(4–5), 229–240. https://doi.org/10.1242/dmm.000729

Nachat, R., Méchin, M. C., Takahara, H., Chavanas, S., Charveron, M., Serre, G., & Simon, M. (2005). Peptidylarginine deiminase isoforms 1-3 are expressed in the epidermis

and involved in the deimination of K1 and filaggrin. *Journal of Investigative Dermatology*, *124*(2), 384–393. https://doi.org/10.1111/j.0022-202X.2004.23568.x

Nachat, R., Mechin, M.-C., Charveron, M., Serre, G., Constans, J., & Simon, M. (2005a). Peptidylarginine deiminase isoforms are differentially expressed in the anagen hair follicles and other human skin appendages. *The Journal of Investigative Dermatology*, *125*(1), 34–41. https://doi.org/10.1111/j.0022-202X.2005.23763.x

Nachat, R., Mechin, M.-C., Charveron, M., Serre, G., Constans, J., & Simon, M. (2005b). Peptidylarginine deiminase isoforms are differentially expressed in the anagen hair follicles and other human skin appendages. *The Journal of Investigative Dermatology*, *125*(1), 34–41. https://doi.org/10.1111/j.0022-202X.2005.23763.x

Nachat, R., Mechin, M.-C., Takahara, H., Chavanas, S., Charveron, M., Serre, G., & Simon, M. (2005). Peptidylarginine deiminase isoforms 1-3 are expressed in the epidermis and involved in the deimination of K1 and filaggrin. *The Journal of Investigative Dermatology*, *124*(2), 384–393. https://doi.org/10.1111/j.0022-202X.2004.23568.x

Naineni, S. K., Robert, F., Nagar, B., & Pelletier, J. (2023). Targeting DEAD-box RNA helicases: The emergence of molecular staples. *WIREs RNA*, *14*(2), e1738. https://doi.org/https://doi.org/10.1002/wrna.1738

Nakashima, K., Arai, S., Suzuki, A., Nariai, Y., Urano, T., Nakayama, M., Ohara, O., Yamamura, K. I., Yamamoto, K., & Miyazaki, T. (2013a). PAD4 regulates proliferation of multipotent haematopoietic cells by controlling c-myc expression. *Nature Communications*, *4*(May), 1836–1838. https://doi.org/10.1038/ncomms2862

Nakashima, K., Arai, S., Suzuki, A., Nariai, Y., Urano, T., Nakayama, M., Ohara, O., Yamamura, K.-I., Yamamoto, K., & Miyazaki, T. (2013b). PAD4 regulates proliferation of multipotent haematopoietic cells by controlling c-myc expression. *Nature Communications*, *4*, 1836. https://doi.org/10.1038/ncomms2862

Nakashima, K., Hagiwara, T., Ishigami, a, Nagata, S., Asaga, H., Kuramoto, M., Senshu, T., & Yamada, M. (1999). Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1alpha,25-dihydroxyvitamin D(3). *The Journal of Biological Chemistry*, *274*(39), 27786–27792. https://doi.org/10.1074/jbc.274.39.27786

Nakashima, K., Hagiwara, T., & Yamada, M. (2002). Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. *The Journal of Biological Chemistry*, *277*(51), 49562–49568. https://doi.org/10.1074/jbc.M208795200

Neeli, I., Khan, S. N., & Radic, M. (2008a). Histone deimination as a response to inflammatory stimuli in neutrophils. *Journal of Immunology (Baltimore, Md. : 1950)*, *180*(3), 1895–1902. https://doi.org/10.4049/jimmunol.180.3.1895

Neeli, I., Khan, S. N., & Radic, M. (2008b). Histone deimination as a response to inflammatory stimuli in neutrophils. *Journal of Immunology (Baltimore, Md. : 1950)*, *180*(3), 1895–1902. https://doi.org/10.4049/jimmunol.180.3.1895

Neira, J. L., Rizzuti, B., Abián, O., Araujo-Abad, S., Velázquez-Campoy, A., & de Juan Romero, C. (2022). Human Enzyme PADI4 Binds to the Nuclear Carrier Importin α3. *Cells*, *11*(14). https://doi.org/10.3390/cells11142166

Nguyen, B.-C., Lefort, K., Mandinova, A., Antonini, D., Devgan, V., Della Gatta, G., Koster, M. I., Zhang, Z., Wang, J., Tommasi di Vignano, A., Kitajewski, J., Chiorino, G., Roop, D. R., Missero, C., & Dotto, G. P. (2006). Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes & Development*, *20*(8), 1028–1042. https://doi.org/10.1101/gad.1406006

Nguyen, H., Rendl, M., & Fuchs, E. (2006). Tcf3 governs stem cell features and represses cell fate determination in skin. *Cell*, *127*(1), 171–183. https://doi.org/10.1016/j.cell.2006.07.036

Nguyen, A. V, & Soulika, A. M. (2019). The Dynamics of the Skin's Immune System. *International Journal of Molecular Sciences*, *20*(8), 1811. https://doi.org/10.3390/ijms20081811

Nickoloff, B. J., Qin, J.-Z., Chaturvedi, V., Denning, M. F., Bonish, B., & Miele, L. (2002). Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-κB and PPARγ. *Cell Death & Differentiation*, *9*(8), 842–855. https://doi.org/10.1038/sj.cdd.4401036

Nowell, C., & Radtke, F. (2013). Cutaneous Notch signaling in health and disease. *Cold Spring Harbor Perspectives in Medicine*, *3*(12), a017772. https://doi.org/10.1101/cshperspect.a017772

Oki, M., Aihara, H., & Ito, T. (2007). Role of histone phosphorylation in chromatin dynamics and its implications in diseases. *Sub-Cellular Biochemistry*, *41*, 319–336.

Oshimori, N., & Fuchs, E. (2012). Paracrine TGF-β signaling counterbalances BMP-mediated repression in hair follicle stem cell activation. *Cell Stem Cell*, *10*(1), 63–75. https://doi.org/10.1016/j.stem.2011.11.005

Ouspenskaia, T., Matos, I., Mertz, A. F., Fiore, V. F., & Fuchs, E. (2016). WNT-SHH Antagonism Specifies and Expands Stem Cells prior to Niche Formation. *Cell*, *164*(1–2), 156–169. https://doi.org/10.1016/j.cell.2015.11.058

Padhi, A., Rekha, R. S., Benrejdal, L., Grundeken, M. E., Lourda, M., Ehrström, M., Eyerich, K., Páez, I. T., Johansson, E. K., Bradley, M., Bergman, P., & Lysell, J. (2023). Baricitinib Blocks Cytokine-Mediated Downregulation of PAD1 in Human Keratinocytes: A Possible Molecular Link to the Effects of JAK Inhibitors in Atopic Dermatitis. *Journal of Investigative Dermatology*, *143*(7), 1306-1309.e4. https://doi.org/10.1016/j.jid.2022.12.012

Padhi, A., Srivastava, A., Ramesh, A., Ehrström, M., Simon, M., Sonkoly, E., Eidsmo, L., Bergman, P., & Lysell, J. (2022). IL-22 Downregulates Peptidylarginine Deiminase-1 in Human Keratinocytes: Adding Another Piece to the IL-22 Puzzle in Epidermal Barrier Formation. *Journal of Investigative Dermatology*, *142*(2), 333-342.e6. https://doi.org/10.1016/j.jid.2021.07.155

Pálmer, H. G., Anjos-Afonso, F., Carmeliet, G., Takeda, H., & Watt, F. M. (2008). The Vitamin D Receptor Is a Wnt Effector that Controls Hair Follicle Differentiation and Specifies Tumor Type in Adult Epidermis. *PLOS ONE*, *3*(1), e1483. https://doi.org/10.1371/journal.pone.0001483

Pan, Y., Lin, M.-H., Tian, X., Cheng, H.-T., Gridley, T., Shen, J., & Kopan, R. (2004). gamma-secretase functions through Notch signaling to maintain skin appendages but is not required for their patterning or initial morphogenesis. *Developmental Cell*, *7*(5), 731–743. https://doi.org/10.1016/j.devcel.2004.09.014

Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology*, *18*(2), 134–147. https://doi.org/10.1038/nri.2017.105

Park, S. (2022). Hair Follicle Morphogenesis During Embryogenesis, Neogenesis, and Organogenesis . In *Frontiers in Cell and Developmental Biology* (Vol. 10). https://www.frontiersin.org/articles/10.3389/fcell.2022.933370

Passos, D. O., Bressan, G. C., Nery, F. C., & Kobarg, J. (2006). Ki-1/57 interacts with PRMT1 and is a substrate for arginine methylation. *The FEBS Journal*, *273*(17), 3946–3961. https://doi.org/10.1111/j.1742-4658.2006.05399.x

Pellegrini, G., Dellambra, E., Golisano, O., Martinelli, E., Fantozzi, I., Bondanza, S., Ponzin, D., McKeon, F., & De Luca, M. (2001). p63 identifies keratinocyte stem cells. *Proceedings of the National Academy of Sciences*, *98*(6), 3156–3161. https://doi.org/10.1073/pnas.061032098

Petiot, A., Conti, F. J. A., Grose, R., Revest, J.-M., Hodivala-Dilke, K. M., & Dickson, C. (2003). A crucial role for Fgfr2-IIIb signalling in epidermal development and hair follicle patterning. *Development*, *130*(22), 5493–5501. https://doi.org/10.1242/dev.00788

Piedrafita, G., Kostiou, V., Wabik, A., Colom, B., Fernandez-Antoran, D., Herms, A., Murai, K., Hall, B. A., & Jones, P. H. (2020). A single-progenitor model as the unifying paradigm of epidermal and esophageal epithelial maintenance in mice. *Nature Communications*, *11*(1), 1429. https://doi.org/10.1038/s41467-020-15258-0

Plikus, M. V, & Chuong, C.-M. (2014). Macroenvironmental regulation of hair cycling and collective regenerative behavior. *Cold Spring Harbor Perspectives in Medicine*, *4*(1), a015198. https://doi.org/10.1101/cshperspect.a015198

Plikus, M. V, Mayer, J. A., de la Cruz, D., Baker, R. E., Maini, P. K., Maxson, R., & Chuong, C.-M. (2008a). Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. *Nature*, *451*(7176), 340–344. https://doi.org/10.1038/nature06457

Plikus, M. V, Mayer, J. A., de la Cruz, D., Baker, R. E., Maini, P. K., Maxson, R., & Chuong, C.-M. (2008b). Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. *Nature*, *451*(7176), 340–344. https://doi.org/10.1038/nature06457

Potten, C. S. (1974). The epidermal proliferative unit: the possible role of the central basal cell. *Cell and Tissue Kinetics*, *7*(1), 77–88. https://doi.org/10.1111/j.1365-2184.1974.tb00401.x

Pummila, M., Fliniaux, I., Jaatinen, R., James, M. J., Laurikkala, J., Schneider, P., Thesleff, I., & Mikkola, M. L. (2007). Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. *Development*, *134*(1), 117–125. https://doi.org/10.1242/dev.02708

Qian, J., Nguyen, N. M. P., Rezaei, M., Huang, B., Tao, Y., Zhang, X., Cheng, Q., Yang, H., Asangla, A., Majewski, J., & Slim, R. (2018). Biallelic PADI6 variants linking infertility, miscarriages, and hydatidiform moles. *European Journal of Human Genetics*, *26*(7), 1007–1013. https://doi.org/10.1038/s41431-018-0141-3

Raijmakers, R., van Beers, J. J. B. C., El-Azzouny, M., Visser, N. F. C., Božič, B., Pruijn, G. J. M., & Heck, A. J. R. (2012). Elevated levels of fibrinogen-derived endogenous citrullinated peptides in synovial fluid of rheumatoid arthritis patients. *Arthritis Research & Therapy*, *14*(3), R114. https://doi.org/10.1186/ar3840

Raijmakers, R., Zendman, A. J. W., Egberts, W. V., Vossenaar, E. R., Raats, J., Soede-Huijbregts, C., Rutjes, F. P. J. T., van Veelen, P. A., Drijfhout, J. W., & Pruijn, G. J. M. (2007). Methylation of Arginine Residues Interferes with Citrullination by Peptidylarginine Deiminases in vitro. *Journal of Molecular Biology*, *367*(4), 1118–1129. https://doi.org/https://doi.org/10.1016/j.jmb.2007.01.054

Rebak, A. S., Hendriks, I. A., Buch-Larsen, S. C., Elsborg, J. D., Kirsch, R., Doncheva, N. T., Jensen, L. J., Christophorou, M., & Nielsen, M. L. (2022). A quantitative and site-

specific atlas of the in vivo citrullinome reveals widespread existence of citrullination. *BioRxiv*, 2022.12.22.521572. https://doi.org/10.1101/2022.12.22.521572

Restivo, G., Nguyen, B.-C., Dziunycz, P., Ristorcelli, E., Ryan, R. J. H., Özuysal, Ö. Y., Di Piazza, M., Radtke, F., Dixon, M. J., Hofbauer, G. F. L., Lefort, K., & Dotto, G. P. (2011). IRF6 is a mediator of Notch pro-differentiation and tumour suppressive function in keratinocytes. *The EMBO Journal*, *30*(22), 4571–4585. https://doi.org/10.1038/emboj.2011.325

Rhee, H., Polak, L., & Fuchs, E. (2006). Lhx2 maintains stem cell character in hair follicles. *Science (New York, N.Y.)*, *312*(5782), 1946–1949. https://doi.org/10.1126/science.1128004

Richardson, R. J., Hammond, N. L., Coulombe, P. A., Saloranta, C., Nousiainen, H. O., Salonen, R., Berry, A., Hanley, N., Headon, D., Karikoski, R., & Dixon, M. J. (2014). Periderm prevents pathological epithelial adhesions during embryogenesis. *The Journal of Clinical Investigation*, *124*(9), 3891–3900. https://doi.org/10.1172/JCI71946

Ro, S., & Rannala, B. (2004). A stop-EGFP transgenic mouse to detect clonal cell lineages generated by mutation. *EMBO Reports*, *5*(9), 914–920. https://doi.org/10.1038/sj.embor.7400218

Robichaud, N., Sonenberg, N., Ruggero, D., & Schneider, R. J. (2019). Translational Control in Cancer. *Cold Spring Harbor Perspectives in Biology*, *11*(7). https://doi.org/10.1101/cshperspect.a032896

ROGERS, G. E. (1958). Some observations on the proteins of the inner root sheath cells of hair follicles. *Biochimica et Biophysica Acta*, *29*(1), 33–43. https://doi.org/10.1016/0006-3002(58)90143-4

ROGERS, G. E. (1962). Occurrence of Citrulline in Proteins. *Nature*, *194*(4834), 1149–1151. https://doi.org/10.1038/1941149a0

Rogers, G. E., Harding, H. W., & Llewellyn-Smith, I. J. (1977). The origin of citrulline-containing proteins in the hair follicle and the chemical nature of trichohyalin, an intracellular precursor. *Biochimica et Biophysica Acta*, *495*(1), 159–175. https://doi.org/10.1016/0005-2795(77)90250-1

Rognoni, E., & Watt, F. M. (2018). Skin Cell Heterogeneity in Development, Wound Healing, and Cancer. *Trends in Cell Biology*, *28*(9), 709–722. https://doi.org/10.1016/j.tcb.2018.05.002

Rompolas, P., & Greco, V. (2014). Stem cell dynamics in the hair follicle niche. *Seminars in Cell & Developmental Biology*, *25–26*, 34–42. https://doi.org/https://doi.org/10.1016/j.semcdb.2013.12.005

Rompolas, P., Mesa, K. R., & Greco, V. (2013). Spatial organization within a niche as a determinant of stem-cell fate. *Nature*, *502*(7472), 513–518. https://doi.org/10.1038/nature12602

Rompolas, P., Mesa, K. R., Kawaguchi, K., Park, S., Gonzalez, D., Brown, S., Boucher, J., Klein, A. M., & Greco, V. (2016). Spatiotemporal coordination of stem cell commitment during epidermal homeostasis. *Science*, *352*(6292), 1471–1474. https://doi.org/10.1126/science.aaf7012

Roy, E., Neufeld, Z., Cerone, L., Wong, H. Y., Hodgson, S., Livet, J., & Khosrotehrani, K. (2016). Bimodal behaviour of interfollicular epidermal progenitors regulated by hair follicle position and cycling. *The EMBO Journal*, *35*(24), 2658–2670. https://doi.org/10.15252/embj.201693806

Ruggero, D. (2013). Translational control in cancer etiology. *Cold Spring Harbor Perspectives in Biology*, *5*(2). https://doi.org/10.1101/cshperspect.a012336

Rus'd, A. A., Ikejiri, Y., Ono, H., Yonekawa, T., Shiraiwa, M., Kawada, A., & Takahara, H. (1999). Molecular cloning of cDNAs of mouse peptidylarginine deiminase type I, type III and type IV, and the expression pattern of type I in mouse. *European Journal of Biochemistry*, *259*(3), 660–669. https://doi.org/10.1046/j.1432-1327.1999.00083.x

Saba, J. A., Liakath-Ali, K., Green, R., & Watt, F. M. (2021). Translational control of stem cell function. *Nature Reviews. Molecular Cell Biology*, *22*(10), 671–690. https://doi.org/10.1038/s41580-021-00386-2

Sada, A., Jacob, F., Leung, E., Wang, S., White, B. S., Shalloway, D., & Tumbar, T. (2016). Defining the cellular lineage hierarchy in the interfollicular epidermis of adult skin. *Nature Cell Biology*, *18*(6), 619–631. https://doi.org/10.1038/ncb3359

Saijo, S., Nagai, A., Kinjo, S., Mashimo, R., Akimoto, M., Kizawa, K., Yabe-Wada, T., Shimizu, N., Takahara, H., & Unno, M. (2016). Monomeric Form of Peptidylarginine Deiminase Type I Revealed by X-ray Crystallography and Small-Angle X-ray Scattering. *Journal of Molecular Biology*, *428*(15), 3058–3073. https://doi.org/https://doi.org/10.1016/j.jmb.2016.06.018

Salzberg, S. L. (2017). Horizontal gene transfer is not a hallmark of the human genome. *Genome Biology*, *18*(1), 85. https://doi.org/10.1186/s13059-017-1214-2

Sánchez-Danés, A., Hannezo, E., Larsimont, J.-C., Liagre, M., Youssef, K. K., Simons, B. D., & Blanpain, C. (2016). Defining the clonal dynamics leading to mouse skin tumour initiation. *Nature*, *536*(7616), 298–303. https://doi.org/10.1038/nature19069

Schepeler, T., Page, M. E., & Jensen, K. B. (2014). Heterogeneity and plasticity of epidermal stem cells. *Development*, *141*(13), 2559 LP – 2567. https://doi.org/10.1242/dev.104588

Schmidt-Ullrich, R., & Paus, R. (2005a). Molecular principles of hair follicle induction and morphogenesis. *BioEssays*, *27*(3), 247–261. https://doi.org/https://doi.org/10.1002/bies.20184

Schmidt-Ullrich, R., & Paus, R. (2005b). Molecular principles of hair follicle induction and morphogenesis. In *BioEssays* (Vol. 27, Issue 3, pp. 247–261). https://doi.org/10.1002/bies.20184

Schneider, M. R., Schmidt-Ullrich, R., & Paus, R. (2009a). The Hair Follicle as a Dynamic Miniorgan. *Current Biology*, *19*(3), R132–R142. https://doi.org/https://doi.org/10.1016/j.cub.2008.12.005

Schneider, M. R., Schmidt-Ullrich, R., & Paus, R. (2009b). The Hair Follicle as a Dynamic Miniorgan. *Current Biology*, *19*(3), R132–R142. https://doi.org/https://doi.org/10.1016/j.cub.2008.12.005

Schön, M. P., & Erpenbeck, L. (2018). The Interleukin-23/Interleukin-17 Axis Links Adaptive and Innate Immunity in Psoriasis. *Frontiers in Immunology*, *9*, 1323. https://doi.org/10.3389/fimmu.2018.01323

Segre, J. (2003). Complex redundancy to build a simple epidermal permeability barrier. *Current Opinion in Cell Biology*, *15*(6), 776–782. https://doi.org/https://doi.org/10.1016/j.ceb.2003.10.001

Senoo, M., Pinto, F., Crum, C. P., & McKeon, F. (2007). p63 Is Essential for the Proliferative Potential of Stem Cells in Stratified Epithelia. *Cell*, *129*(3), 523–536. https://doi.org/10.1016/j.cell.2007.02.045

Senshu, T., Kan, S., Ogawa, H., Manabe, M., & Asaga, H. (1996a). Preferential Deimination of Keratin K1 and Filaggrin during the Terminal Differentiation of Human Epidermis. *Biochemical and Biophysical Research Communications*, *225*(3), 712–719. https://doi.org/https://doi.org/10.1006/bbrc.1996.1240

Senshu, T., Kan, S., Ogawa, H., Manabe, M., & Asaga, H. (1996b). Preferential Deimination of Keratin K1 and Filaggrin during the Terminal Differentiation of Human Epidermis. *Biochemical and Biophysical Research Communications*, *225*(3), 712–719. https://doi.org/https://doi.org/10.1006/bbrc.1996.1240

Senshu, T., Sato, T., Inoue, T., Akiyama, K., & Asaga, H. (1992). Detection of citrulline residues in deiminated proteins on polyvinylidene difluoride membrane. *Analytical Biochemistry*, *203*(1), 94–100. https://doi.org/10.1016/0003-2697(92)90047-b

Sequeira, I., & Nicolas, J.-F. (2012). Redefining the structure of the hair follicle by 3D clonal analysis. *Development*, *139*(20), 3741 LP – 3751. https://doi.org/10.1242/dev.081091

Shapter, F. M., & Waters, D. L. E. (2014). *Genome Walking BT - Cereal Genomics: Methods and Protocols* (R. J. Henry & A. Furtado, Eds.; pp. 133–146). Humana Press. https://doi.org/10.1007/978-1-62703-715-0_12

Sharma, P., Azebi, S., England, P., Christensen, T., Moller-Larsen, A., Petersen, T., Batsche, E., & Muchardt, C. (2012). Citrullination of histone H3 interferes with HP1-mediated transcriptional repression. *PLoS Genetics*, *8*(9), e1002934. https://doi.org/10.1371/journal.pgen.1002934

Sharma, P., Lioutas, A., Fernandez-Fuentes, N., Quilez, J., Carbonell-Caballero, J., Wright, R. H. G., Di Vona, C., Le Dily, F., Schüller, R., Eick, D., Oliva, B., & Beato, M. (2019a). Arginine Citrullination at the C-Terminal Domain Controls RNA Polymerase II Transcription. *Molecular Cell*, *73*(1), 84-96.e7. https://doi.org/10.1016/j.molcel.2018.10.016

Sharma, P., Lioutas, A., Fernandez-Fuentes, N., Quilez, J., Carbonell-Caballero, J., Wright, R. H. G., Di Vona, C., Le Dily, F., Schüller, R., Eick, D., Oliva, B., & Beato, M. (2019b). Arginine Citrullination at the C-Terminal Domain Controls RNA Polymerase II Transcription. *Molecular Cell*, *73*(1), 84-96.e7. https://doi.org/10.1016/j.molcel.2018.10.016

Signer, R. A. J., Magee, J. A., Salic, A., & Morrison, S. J. (2014). Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature*, *509*(7498), 49–54. https://doi.org/10.1038/nature13035

Slack, J. L., Jones, L. E., Bhatia, M. M., & Thompson, P. R. (2011). Autodeimination of Protein Arginine Deiminase 4 Alters Protein–Protein Interactions but Not Activity. *Biochemistry*, *50*(19), 3997–4010. https://doi.org/10.1021/bi200309e

Slade, D. J., Fang, P., Dreyton, C. J., Zhang, Y., Fuhrmann, J., Rempel, D., Bax, B. D., Coonrod, S. A., Lewis, H. D., Guo, M., Gross, M. L., & Thompson, P. R. (2015a). Protein Arginine Deiminase 2 Binds Calcium in an Ordered Fashion: Implications for Inhibitor Design. *ACS Chemical Biology*, *10*(4), 1043–1053. https://doi.org/10.1021/cb500933j

Slade, D. J., Fang, P., Dreyton, C. J., Zhang, Y., Fuhrmann, J., Rempel, D., Bax, B. D., Coonrod, S. A., Lewis, H. D., Guo, M., Gross, M. L., & Thompson, P. R. (2015b). Protein Arginine Deiminase 2 Binds Calcium in an Ordered Fashion: Implications for Inhibitor Design. *ACS Chemical Biology*, *10*(4), 1043–1053. https://doi.org/10.1021/cb500933j

Snijders, A. P., Hautbergue, G. M., Bloom, A., Williamson, J. C., Minshull, T. C., Phillips, H. L., Mihaylov, S. R., Gjerde, D. T., Hornby, D. P., Wilson, S. A., Hurd, P. J., & Dickman, M. J. (2015). Arginine methylation and citrullination of splicing factor proline- and

glutamine-rich (SFPQ/PSF) regulates its association with mRNA. *RNA* , *21*(3), 347–359. https://doi.org/10.1261/rna.045138.114

Snippert, H. J., Haegebarth, A., Kasper, M., Jaks, V., van Es, J. H., Barker, N., van de Wetering, M., van den Born, M., Begthel, H., Vries, R. G., Stange, D. E., Toftgård, R., & Clevers, H. (2010a). Lgr6 Marks Stem Cells in the Hair Follicle That Generate All Cell Lineages of the Skin. *Science*, *327*(5971), 1385 LP – 1389. https://doi.org/10.1126/science.1184733

Snippert, H. J., Haegebarth, A., Kasper, M., Jaks, V., van Es, J. H., Barker, N., van de Wetering, M., van den Born, M., Begthel, H., Vries, R. G., Stange, D. E., Toftgård, R., & Clevers, H. (2010b). Lgr6 Marks Stem Cells in the Hair Follicle That Generate All Cell Lineages of the Skin. *Science*, *327*(5971), 1385 LP – 1389. https://doi.org/10.1126/science.1184733

Song, P., Yang, F., Jin, H., & Wang, X. (2021). The regulation of protein translation and its implications for cancer. *Signal Transduction and Targeted Therapy*, *6*(1), 68. https://doi.org/10.1038/s41392-020-00444-9

Spengler, J., Lugonja, B., Jimmy Ytterberg, A., Zubarev, R. A., Creese, A. J., Pearson, M. J., Grant, M. M., Milward, M., Lundberg, K., Buckley, C. D., Filer, A., Raza, K., Cooper, P. R., Chapple, I. L., & Scheel-Toellner, D. (2015). Release of Active Peptidyl Arginine Deiminases by Neutrophils Can Explain Production of Extracellular Citrullinated Autoantigens in Rheumatoid Arthritis Synovial Fluid. *Arthritis & Rheumatology*, *67*(12), 3135–3145. https://doi.org/https://doi.org/10.1002/art.39313

Stadler, S. C., Vincent, C. T., Fedorov, V. D., Patsialou, A., Cherrington, B. D., Wakshlag, J. J., Mohanan, S., Zee, B. M., Zhang, X., Garcia, B. A., Condeelis, J. S., Brown, A. M. C., Coonrod, S. A., & Allis, C. D. (2013). Dysregulation of PAD4-mediated citrullination of nuclear GSK3β activates TGF-β signaling and induces epithelial-to-mesenchymal transition in breast cancer cells. *Proceedings of the National Academy of Sciences*, *110*(29), 11851 LP – 11856. https://doi.org/10.1073/pnas.1308362110

Steinert, P. M., Parry, D. A. D., & Marekov, L. N. (2003). Trichohyalin mechanically strengthens the hair follicle: multiple cross-bridging roles in the inner root shealth. *The Journal of Biological Chemistry*, *278*(42), 41409–41419. https://doi.org/10.1074/jbc.M302037200

Sun, B., Dwivedi, N., Bechtel, T. J., Paulsen, J. L., Muth, A., Bawadekar, M., Li, G., Thompson, P. R., Shelef, M. A., Schiffer, C. A., Weerapana, E., & Ho, I.-C. (2017). Citrullination of NF-κB p65 promotes its nuclear localization and TLR-induced expression of IL-1β and TNFα. *Science Immunology*, *2*(12). https://doi.org/10.1126/sciimmunol.aal3062

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., … Yamamoto, K. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nature Genetics*, *34*(4), 395–402. https://doi.org/10.1038/ng1206

Tan, D. W. M., Jensen, K. B., Trotter, M. W. B., Connelly, J. T., Broad, S., & Watt, F. M. (2013). Single-cell gene expression profiling reveals functional heterogeneity of undifferentiated human epidermal cells. *Development*, *140*(7), 1433–1444. https://doi.org/10.1242/dev.087551

Tanikawa, C., Espinosa, M., Suzuki, A., Masuda, K., Yamamoto, K., Tsuchiya, E., Ueda, K., Daigo, Y., Nakamura, Y., & Matsuda, K. (2012). Regulation of histone modification and chromatin structure by the p53–PADI4 pathway. *Nature Communications*, *3*, 676.

Tanikawa, C., Ueda, K., Nakagawa, H., Yoshida, N., Nakamura, Y., & Matsuda, K. (2009). Regulation of Protein Citrullination through p53/PADI4 Network in DNA Damage Response. *Cancer Research*, *69*(22), 8761 LP – 8769. https://doi.org/10.1158/0008-5472.CAN-09-2280

Tanikawa, C., Ueda, K., Suzuki, A., Iida, A., Nakamura, R., Atsuta, N., Tohnai, G., Sobue, G., Saichi, N., Momozawa, Y., Kamatani, Y., Kubo, M., Yamamoto, K., Nakamura, Y., & Matsuda, K. (2018a). Citrullination of RGG Motifs in FET Proteins by PAD4 Regulates Protein Aggregation and ALS Susceptibility. *Cell Reports*, *22*(6), 1473–1483. https://doi.org/10.1016/j.celrep.2018.01.031

Tanikawa, C., Ueda, K., Suzuki, A., Iida, A., Nakamura, R., Atsuta, N., Tohnai, G., Sobue, G., Saichi, N., Momozawa, Y., Kamatani, Y., Kubo, M., Yamamoto, K., Nakamura, Y., & Matsuda, K. (2018b). Citrullination of RGG Motifs in FET Proteins by PAD4 Regulates Protein Aggregation and ALS Susceptibility. *Cell Reports*, *22*(6), 1473–1483. https://doi.org/10.1016/j.celrep.2018.01.031

Tarcsa, E., Marekov, L. N., Andreoli, J., Idler, W. W., Candi, E., Chung, S.-I., & Steinert, P. M. (1997). The Fate of Trichohyalin: SEQUENTIAL POST-TRANSLATIONAL MODIFICATIONS BY PEPTIDYL-ARGININE DEIMINASE AND TRANSGLUTAMINASES*. *Journal of Biological Chemistry*, *272*(44), 27893–27901. https://doi.org/https://doi.org/10.1074/jbc.272.44.27893

Tarcsa, E., Marekov, L. N., Mei, G., Melino, G., Lee, S. C., & Steinert, P. M. (1996a). Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. *The Journal of*

Biological Chemistry, *271*(48), 30709–30716. https://doi.org/10.1074/jbc.271.48.30709

Tarcsa, E., Marekov, L. N., Mei, G., Melino, G., Lee, S. C., & Steinert, P. M. (1996b). Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. *The Journal of Biological Chemistry*, *271*(48), 30709–30716. https://doi.org/10.1074/jbc.271.48.30709

Terakawa, H., Takahara, H., & Sugawara, K. (1991). Three Types of Mouse Peptidylarginine Deiminase: Characterization and Tissue Distribution1. *The Journal of Biochemistry*, *110*(4), 661–666. https://doi.org/10.1093/oxfordjournals.jbchem.a123636

Thiam, H. R., Wong, S. L., Qiu, R., Kittisopikul, M., Vahabikashi, A., Goldman, A. E., Goldman, R. D., Wagner, D. D., & Waterman, C. M. (2020). NETosis proceeds by cytoskeleton and endomembrane disassembly and PAD4-mediated chromatin decondensation and nuclear envelope rupture. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(13), 7326–7337. https://doi.org/10.1073/pnas.1909546117

Tilvawala, R., Nguyen, S. H., Maurais, A. J., Nemmara, V. V, Nagar, M., Salinger, A. J., Nagpal, S., Weerapana, E., & Thompson, P. R. (2018). The Rheumatoid Arthritis-Associated Citrullinome. *Cell Chemical Biology*, *25*(6), 691-704.e6. https://doi.org/10.1016/j.chembiol.2018.03.002

Tong, X., & Coulombe, P. A. (2006). Keratin 17 modulates hair follicle cycling in a TNFalpha-dependent fashion. *Genes & Development*, *20*(10), 1353–1364. https://doi.org/10.1101/gad.1387406

Too, C. L., Murad, S., Dhaliwal, J. S., Larsson, P., Jiang, X., Ding, B., Alfredsson, L., Klareskog, L., & Padyukov, L. (2012). Polymorphisms in peptidylarginine deiminase associate with rheumatoid arthritis in diverse Asian populations: evidence from MyEIRA study and meta-analysis. *Arthritis Research & Therapy*, *14*(6), R250. https://doi.org/10.1186/ar4093

Tour, O., Adams, S. R., Kerr, R. A., Meijer, R. M., Sejnowski, T. J., Tsien, R. W., & Tsien, R. Y. (2007). Calcium Green FlAsH as a genetically targeted small-molecule calcium indicator. *Nature Chemical Biology*, *3*(7), 423–431. https://doi.org/10.1038/nchembio.2007.4

Touz, M. C., Rópolo, A. S., Rivero, M. R., Vranych, C. V., Conrad, J. T., Svard, S. G., & Nash, T. E. (2008). Arginine deiminase has multiple regulatory roles in the biology of Giardia lamblia. *Journal of Cell Science*, *121*(17), 2930–2938. https://doi.org/10.1242/jcs.026963

Truong, A. B., Kretz, M., Ridky, T. W., Kimmel, R., & Khavari, P. A. (2006). p63 regulates proliferation and differentiation of developmentally mature keratinocytes. *Genes & Development , 20*(22), 3185–3197. http://genesdev.cshlp.org/content/20/22/3185.abstract

Tsourouktsoglou, T.-D., Warnatsch, A., Ioannou, M., Hoving, D., Wang, Q., & Papayannopoulos, V. (2020). Histones, DNA, and Citrullination Promote Neutrophil Extracellular Trap Inflammation by Regulating the Localization and Activation of TLR4. *Cell Reports*, *31*(5), 107602. https://doi.org/https://doi.org/10.1016/j.celrep.2020.107602

Tutturen, A. E. V, Fleckenstein, B., & de Souza, G. A. (2014). Assessing the citrullinome in rheumatoid arthritis synovial fluid with and without enrichment of citrullinated peptides. *Journal of Proteome Research*, *13*(6), 2867–2873. https://doi.org/10.1021/pr500030x

Ü. Basmanav, F. B., Cau, L., Tafazzoli, A., Méchin, M.-C., Wolf, S., Romano, M. T., Valentin, F., Wiegmann, H., Huchenq, A., Kandil, R., Garcia Bartels, N., Kilic, A., George, S., Ralser, D. J., Bergner, S., Ferguson, D. J. P., Oprisoreanu, A.-M., Wehner, M., Thiele, H., … Betz, R. C. (2016). Mutations in Three Genes Encoding Proteins Involved in Hair Shaft Formation Cause Uncombable Hair Syndrome. *The American Journal of Human Genetics*, *99*(6), 1292–1304. https://doi.org/https://doi.org/10.1016/j.ajhg.2016.10.004

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A.-K., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., … Pontén, F. (2015). Tissue-based map of the human proteome. *Science*, *347*(6220), 1260419. https://doi.org/10.1126/science.1260419

Van Beers, J. J. B. C., Schwarte, C. M., Stammen-Vogelzangs, J., Oosterink, E., Božič, B., & Pruijn, G. J. M. (2013). The rheumatoid arthritis synovial fluid citrullinome reveals novel citrullinated epitopes in apolipoprotein E, myeloid nuclear differentiation antigen, and β-actin. *Arthritis and Rheumatism*, *65*(1), 69–80. https://doi.org/10.1002/art.37720

Van Beers, J. J. B. C., Zendman, A. J. W., Raijmakers, R., Stammen-Vogelzangs, J., & Pruijn, G. J. M. (2013). Peptidylarginine deiminase expression and activity in PAD2 knock-out and PAD4-low mice. *Biochimie*, *95*(2), 299–308. https://doi.org/10.1016/j.biochi.2012.09.029

van Genderen, C., Okamura, R. M., Fariñas, I., Quo, R. G., Parslow, T. G., Bruhn, L., & Grosschedl, R. (1994). Development of several organs that require inductive

epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes & Development*, *8*(22), 2691–2703. https://doi.org/10.1101/gad.8.22.2691

Van Venrooij, W. J., Van Beers, J. J. B. C., & Pruijn, G. J. M. (2008). Anti-CCP Antibody, a Marker for the Early Detection of Rheumatoid Arthritis. *Annals of the New York Academy of Sciences*, *1143*(1), 268–285. https://doi.org/10.1196/annals.1443.013

Vasioukhin, V., Degenstein, L., Wise, B., & Fuchs, E. (1999). The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8551–8556. https://doi.org/10.1073/pnas.96.15.8551

Vassar, R., & Fuchs, E. (1991). Transgenic mice provide new insights into the role of TGF-alpha during epidermal development and differentiation. *Genes & Development* , *5*(5), 714–727. https://doi.org/10.1101/gad.5.5.714

Venkatesh, S., & Workman, J. L. (2015). Histone exchange, chromatin structure and the regulation of transcription. *Nature Reviews Molecular Cell Biology*, *16*(3), 178–189. https://doi.org/10.1038/nrm3941

Verheul, M. K., van Veelen, P. A., van Delft, M. A. M., de Ru, A., Janssen, G. M. C., Rispens, T., Toes, R. E. M., & Trouw, L. A. (2018). Pitfalls in the detection of citrullination and carbamylation. *Autoimmunity Reviews*, *17*(2), 136–141. https://doi.org/https://doi.org/10.1016/j.autrev.2017.11.017

Vidal, V. P. I., Chaboissier, M.-C., Lützkendorf, S., Cotsarelis, G., Mill, P., Hui, C.-C., Ortonne, N., Ortonne, J.-P., & Schedl, A. (2005). Sox9 Is Essential for Outer Root Sheath Differentiation and the Formation of the Hair Stem Cell Compartment. *Current Biology*, *15*(15), 1340–1351. https://doi.org/https://doi.org/10.1016/j.cub.2005.06.064

Villanueva-Cañas, J. L., Fernandez-Fuentes, N., Teyssier, C., Rogalska, M. E., Pérez, F. P., Oliva, B., Notredame, C., Beato, M., & Sharma, P. (2022). Evolutionary insights reveal a new role of PADI2 in transcriptional elongation. *BioRxiv*, 2022.09.19.508513. https://doi.org/10.1101/2022.09.19.508513

Violeta, R., Justyna, F.-B., A., N. P., Erika, D., J., H. U., M., L. D., Jennifer, van E., Antony, R., & Felipe, A. (2013). Immune-Mediated Pore-Forming Pathways Induce Cellular Hypercitrullination and Generate Citrullinated Autoantigens in Rheumatoid Arthritis. *Science Translational Medicine*, *5*(209), 209ra150-209ra150. https://doi.org/10.1126/scitranslmed.3006869

Volker, B., Ulrike, R., Christian, G., Beatrix, F., Yvonne, U., S., W. D., Yvette, W., & Arturo, Z. (2004). Neutrophil Extracellular Traps Kill Bacteria. *Science*, *303*(5663), 1532–1535. https://doi.org/10.1126/science.1092385

Vossenaar, E. R. (2004). Expression and activity of citrullinating peptidylarginine deiminase enzymes in monocytes and macrophages. *Annals of the Rheumatic Diseases*, *63*(4), 373–381. https://doi.org/10.1136/ard.2003.012211

Vossenaar, E. R., Zendman, A. J. W., Van Venrooij, W. J., & Pruijn, G. J. M. (2003a). PAD, a growing family of citrullinating enzymes: Genes, features and involvement in disease. In *BioEssays* (Vol. 25, Issue 11, pp. 1106–1118). https://doi.org/10.1002/bies.10357

Vossenaar, E. R., Zendman, A. J. W., Van Venrooij, W. J., & Pruijn, G. J. M. (2003b). PAD, a growing family of citrullinating enzymes: Genes, features and involvement in disease. In *BioEssays* (Vol. 25, Issue 11, pp. 1106–1118). https://doi.org/10.1002/bies.10357

Wang, F., Chen, F. F., Gao, W. B., Wang, H. Y., Zhao, N. W., Xu, M., Gao, D. Y., Yu, W., Yan, X. L., Zhao, J. N., & Li, X. J. (2016). Identification of citrullinated peptides in the synovial fluid of patients with rheumatoid arthritis using LC-MALDI-TOF/TOF. *Clinical Rheumatology*, *35*(9), 2185–2194. https://doi.org/10.1007/s10067-016-3247-4

Wang, S., & Wang, Y. (2013a). Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. In *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* (Vol. 1829, Issue 10, pp. 1126–1135). Elsevier B.V. https://doi.org/10.1016/j.bbagrm.2013.07.003

Wang, S., & Wang, Y. (2013b). Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. In *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* (Vol. 1829, Issue 10, pp. 1126–1135). Elsevier B.V. https://doi.org/10.1016/j.bbagrm.2013.07.003

Wang, X., Pasolli, H. A., Williams, T., & Fuchs, E. (2008). AP-2 factors act in concert with Notch to orchestrate terminal differentiation in skin epidermis . *Journal of Cell Biology*, *183*(1), 37–48. https://doi.org/10.1083/jcb.200804030

Wang, X., Zhu, H., He, Y., Zeng, J., Zhao, J., Xia, Q., Wu, L., Yao, Z., & Li, Y. (2022). A novel homozygous mutation in the PADI6 gene causes early embryo arrest. *Reproductive Health*, *19*(1), 190. https://doi.org/10.1186/s12978-022-01495-7

Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., Hayama, R., Leonelli, L., Han, H., Grigoryev, S. A., Allis, C. D., & Coonrod, S. A. (2009a). Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *Journal of Cell Biology*, *184*(2), 205–213. https://doi.org/10.1083/jcb.200806072

Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., Hayama, R., Leonelli, L., Han, H., Grigoryev, S. A., Allis, C. D., & Coonrod, S. A. (2009b). Histone hypercitrullination

mediates chromatin decondensation and neutrophil extracellular trap formation. *Journal of Cell Biology*, *184*(2), 205–213. https://doi.org/10.1083/jcb.200806072

Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.-H., Perlin, J. R., Leonelli, L., Sonbuchner, L. S., McDonald, C. H., Cook, R. G., Dou, Y., Roeder, R. G., Clarke, S., Stallcup, M. R., Allis, C. D., & Coonrod, S. A. (2004a). Human PAD4 Regulates Histone Arginine Methylation Levels via Demethylimination. *Science*, *306*(5694), 279 LP – 283. https://doi.org/10.1126/science.1101400

Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.-H., Perlin, J. R., Leonelli, L., Sonbuchner, L. S., McDonald, C. H., Cook, R. G., Dou, Y., Roeder, R. G., Clarke, S., Stallcup, M. R., Allis, C. D., & Coonrod, S. A. (2004b). Human PAD4 Regulates Histone Arginine Methylation Levels via Demethylimination. *Science*, *306*(5694), 279–283.

Watt, F. M. (1983). Involucrin and other markers of keratinocyte terminal differentiation. *The Journal of Investigative Dermatology*, *81*(1 Suppl), 100s–103s. https://doi.org/10.1111/1523-1747.ep12540786

Watt, F. M. (2002a). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO Journal*, *21*(15), 3919–3926. https://doi.org/10.1093/emboj/cdf399

Watt, F. M. (2002b). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO Journal*, *21*(15), 3919–3926. https://doi.org/10.1093/emboj/cdf399

Watt, F. M. (2014). Mammalian skin cell biology: At the interface between laboratory and clinic. *Science*, *346*(6212), 937 LP – 940. https://doi.org/10.1126/science.1253734

Watt, F. M., Estrach, S., & Ambler, C. A. (2008). Epidermal Notch signalling: differentiation, cancer and adhesion. *Current Opinion in Cell Biology*, *20*(2), 171–179. https://doi.org/10.1016/j.ceb.2008.01.010

Watt, F. M., & Fujiwara, H. (2011). Cell-Extracellular Matrix Interactions in Normal and Diseased Skin. *Cold Spring Harbor Perspectives in Biology* , *3*(4). https://doi.org/10.1101/cshperspect.a005124

Wegner, N., Lundberg, K., Kinloch, A., Fisher, B., Malmström, V., Feldmann, M., & Venables, P. J. (2010). Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. *Immunological Reviews*, *233*(1), 34–54. https://doi.org/10.1111/j.0105-2896.2009.00850.x

Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K.-A., Lundberg, K., Kinloch, A., Culshaw, S., Potempa, J., & Venables, P. J. (2010). Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase:

implications for autoimmunity in rheumatoid arthritis. *Arthritis and Rheumatism*, *62*(9), 2662–2672. https://doi.org/10.1002/art.27552

Wiese, M., Bannister, A. J., Basu, S., Boucher, W., Wohlfahrt, K., Christophorou, M. A., Nielsen, M. L., Klenerman, D., Laue, E. D., & Kouzarides, T. (2019). Citrullination of HP1γ chromodomain affects association with chromatin. *Epigenetics & Chromatin*, *12*(1), 21. https://doi.org/10.1186/s13072-019-0265-x

Winget, J. M., Finlay, D., Mills, K. J., Huggins, T., Bascom, C., Isfort, R. J., & Moritz, R. L. (2016). Quantitative Proteomic Analysis of Stratum Corneum Dysfunction in Adult Chronic Atopic Dermatitis. In *The Journal of investigative dermatology* (Vol. 136, Issue 8, pp. 1732–1735). https://doi.org/10.1016/j.jid.2016.03.037

Wood, D. D., Ackerley, C. A., Brand, B. van den, Zhang, L., Raijmakers, R., Mastronardi, F. G., & Moscarello, M. A. (2008a). Myelin localization of peptidylarginine deiminases 2 and 4: comparison of PAD2 and PAD4 activities. *Laboratory Investigation*, *88*(4), 354–364. https://doi.org/10.1038/labinvest.3700748

Wood, D. D., Ackerley, C. A., Brand, B. van den, Zhang, L., Raijmakers, R., Mastronardi, F. G., & Moscarello, M. A. (2008b). Myelin localization of peptidylarginine deiminases 2 and 4: comparison of PAD2 and PAD4 activities. *Laboratory Investigation*, *88*(4), 354–364. https://doi.org/10.1038/labinvest.3700748

Wright, P. W., Bolling, L. C., Calvert, M. E., Sarmento, O. F., Berkeley, E. V, Shea, M. C., Hao, Z., Jayes, F. C., Bush, L. A., Shetty, J., Shore, A. N., Reddi, P. P., Tung, K. S., Samy, E., Allietta, M. M., Sherman, N. E., Herr, J. C., & Coonrod, S. A. (2003). ePAD, an oocyte and early embryo-abundant peptidylarginine deiminase-like protein that localizes to egg cytoplasmic sheets. *Developmental Biology*, *256*(1), 74–89. https://doi.org/10.1016/S0012-1606(02)00126-4

Xiao, S., Lu, J., Sridhar, B., Cao, X., Yu, P., Zhao, T., Chen, C.-C., McDee, D., Sloofman, L., Wang, Y., Rivas-Astroza, M., Telugu, B. P. V. L., Levasseur, D., Zhang, K., Liang, H., Zhao, J. C., Tanaka, T. S., Stormo, G., & Zhong, S. (2017). SMARCAD1 Contributes to the Regulation of Naive Pluripotency by Interacting with Histone Citrullination. *Cell Reports*, *18*(13), 3117–3128. https://doi.org/10.1016/j.celrep.2017.02.070

Xie, W., Zhu, H., Zhao, M., Wang, L., Li, S., Zhao, C., Zhou, Y., Zhu, B., Jiang, X., Liu, W., & Ren, C. (2021). Crucial roles of different RNA-binding hnRNP proteins in Stem Cells. *International Journal of Biological Sciences*, *17*(3), 807–817. https://doi.org/10.7150/ijbs.55120

Xin, T., Gonzalez, D., Rompolas, P., & Greco, V. (2018). Flexible fate determination ensures robust differentiation in the hair follicle. *Nature Cell Biology*, *20*(12), 1361–1369. https://doi.org/10.1038/s41556-018-0232-y

Xu, Y., Shi, Y., Fu, J., Yu, M., Feng, R., Sang, Q., Liang, B., Chen, B., Qu, R., Li, B., Yan, Z., Mao, X., Kuang, Y., Jin, L., He, L., Sun, X., & Wang, L. (2016). Mutations in PADI6 Cause Female Infertility Characterized by Early Embryonic Arrest. *The American Journal of Human Genetics*, *99*(3), 744–752. https://doi.org/10.1016/j.ajhg.2016.06.024

Xu, Y., Wang, R., Pang, Z., Wei, Z., Sun, L., Li, S., Wang, G., Liu, Y., Zhou, Y., Ye, H., Jin, L., & Xue, S. (2022). Novel Homozygous PADI6 Variants in Infertile Females with Early Embryonic Arrest. *Frontiers in Cell and Developmental Biology*, *10*, 819667. https://doi.org/10.3389/fcell.2022.819667

Xu, Z., Wang, W., Jiang, K., Yu, Z., Huang, H., Wang, F., Zhou, B., & Chen, T. (2015). Embryonic attenuated Wnt/β-catenin signaling defines niche location and long-term stem cell fate in hair follicle. *ELife*, *4*, e10567. https://doi.org/10.7554/eLife.10567

Yanagiya, A., Suyama, E., Adachi, H., Svitkin, Y. V., Aza-Blanc, P., Imataka, H., Mikami, S., Martineau, Y., Ronai, Z. A., & Sonenberg, N. (2012). Translational Homeostasis via the mRNA Cap-Binding Protein, eIF4E. *Molecular Cell*, *46*(6), 847–858. https://doi.org/https://doi.org/10.1016/j.molcel.2012.04.004

Yang, A., Zhu, Z., Kapranov, P., McKeon, F., Church, G. M., Gingeras, T. R., & Struhl, K. (2006). Relationships between p63 Binding, DNA Sequence, Transcription Activity, and Biological Function in Human Cells. *Molecular Cell*, *24*(4), 593–602. https://doi.org/10.1016/j.molcel.2006.10.018

Yang, H., Adam, R. C., Ge, Y., Hua, Z. L., & Fuchs, E. (2017). Epithelial-Mesenchymal Micro-niches Govern Stem Cell Lineage Choices. *Cell*, *169*(3), 483-496.e13. https://doi.org/10.1016/j.cell.2017.03.038

Yao, H., Li, P., Venters, B. J., Zheng, S., Thompson, P. R., Pugh, B. F., & Wang, Y. (2008). Histone Arg modifications and p53 regulate the expression of OKL38, a mediator of apoptosis. *J Biol Chem*, *283*. https://doi.org/10.1074/jbc.M802940200

Yokouchi, M., Atsugi, T., Logtestijn, M. van, Tanaka, R. J., Kajimura, M., Suematsu, M., Furuse, M., Amagai, M., & Kubo, A. (2016a). Epidermal cell turnover across tight junctions based on Kelvin's tetrakaidecahedron cell shape. *ELife*, *5*. https://doi.org/10.7554/eLife.19593

Yokouchi, M., Atsugi, T., Logtestijn, M. van, Tanaka, R. J., Kajimura, M., Suematsu, M., Furuse, M., Amagai, M., & Kubo, A. (2016b). Epidermal cell turnover across tight junctions based on Kelvin's tetrakaidecahedron cell shape. *ELife*, *5*. https://doi.org/10.7554/eLife.19593

Young, C., Russell, J. R., Van De Lagemaat, L. N., Lawson, H., Mapperley, C., Kranc, K. R., & Christophorou, M. A. (2022). Intrinsic function of the peptidylarginine deiminase PADI4 is dispensable for normal haematopoiesis. *Biology Open*, *11*(6), bio059143. https://doi.org/10.1242/bio.059143

Yurttas, P., Vitale, A. M., Fitzhenry, R. J., Cohen-Gould, L., Wu, W., Gossen, J. A., & Coonrod, S. A. (2008). Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development*, *135*(15), 2627–2636. https://doi.org/10.1242/dev.016329

Yuzhalin, A. E., Gordon-Weeks, A. N., Tognoli, M. L., Jones, K., Markelc, B., Konietzny, R., Fischer, R., Muth, A., O'Neill, E., Thompson, P. R., Venables, P. J., Kessler, B. M., Lim, S. Y., & Muschel, R. J. (2018). Colorectal cancer liver metastatic growth depends on PAD4-driven citrullination of the extracellular matrix. *Nature Communications*, *9*(1), 4783. https://doi.org/10.1038/s41467-018-07306-7

Zecha, J., Satpathy, S., Kanashova, T., Avanessian, S. C., Kane, M. H., Clauser, K. R., Mertins, P., Carr, S. A., & Kuster, B. (2019). TMT Labeling for the Masses: A Robust and Cost-efficient, In-solution Labeling Approach. *Molecular & Cellular Proteomics : MCP*, *18*(7), 1468–1478. https://doi.org/10.1074/mcp.TIR119.001385

Zhai, Q., Wang, L., Zhao, P., & Li, T. (2017). Role of citrullination modification catalyzed by peptidylarginine deiminase 4 in gene transcriptional regulation. In *Acta Biochimica et Biophysica Sinica* (Vol. 49, Issue 7, pp. 567–572). https://doi.org/10.1093/abbs/gmx042

Zhang, X., Bolt, M., Guertin, M. J., Chen, W., Zhang, S., Cherrington, B. D., Slade, D. J., Dreyton, C. J., Subramanian, V., Bicker, K. L., Thompson, P. R., Mancini, M. A., Lis, J. T., & Coonrod, S. A. (2012). Peptidylarginine deiminase 2-catalyzed histone H3 arginine 26 citrullination facilitates estrogen receptor α target gene activation. *Proceedings of the National Academy of Sciences*, *109*(33), 13331–13336. https://doi.org/10.1073/pnas.1203280109

Zhang, X., Gamble, M. J., Stadler, S., Cherrington, B. D., Causey, C. P., Thompson, P. R., Roberson, M. S., Kraus, W. L., & Coonrod, S. A. (2011a). Genome-Wide analysis reveals PADI4 cooperates with Elk-1 to activate C-Fos expression in breast cancer cells. *PLoS Genetics*, *7*(6). https://doi.org/10.1371/journal.pgen.1002112

Zhang, X., Gamble, M. J., Stadler, S., Cherrington, B. D., Causey, C. P., Thompson, P. R., Roberson, M. S., Kraus, W. L., & Coonrod, S. A. (2011b). Genome-Wide analysis reveals PADI4 cooperates with Elk-1 to activate C-Fos expression in breast cancer cells. *PLoS Genetics*, *7*(6). https://doi.org/10.1371/journal.pgen.1002112

Zhang, X., Liu, X., Zhang, M., Li, T., Muth, A., Thompson, P. R., Coonrod, S. A., & Zhang, X. (2016a). Peptidylarginine deiminase 1-catalyzed histone citrullination is essential for early embryo development. *Scientific Reports*, *6*, 38727. http://dx.doi.org/10.1038/srep38727

Zhang, X., Liu, X., Zhang, M., Li, T., Muth, A., Thompson, P. R., Coonrod, S. A., & Zhang, X. (2016b). Peptidylarginine deiminase 1-catalyzed histone citrullination is essential for early embryo development. *Scientific Reports*, *6*, 38727.

Zhao, Q., Liu, S., Zhang, H., Li, N., Wang, X., Cao, Y., Ning, L., Duan, E., & Xia, G. (2015). Spatiotemporal Expression of p63 in Mouse Epidermal Commitment. In *International Journal of Molecular Sciences* (Vol. 16, Issue 12, pp. 29542–29553). https://doi.org/10.3390/ijms161226185

Zheng, Y., Zhao, G., Xu, B., Liu, C., Li, C., Zhang, X., & Chang, X. (2016). PADI4 has genetic susceptibility to gastric carcinoma and upregulates CXCR2, KRT14 and TNF-α expression levels. *Oncotarget*, *7*(38), 62159–62176. https://doi.org/10.18632/oncotarget.11398

Zhou, Y., Chen, B., Mittereder, N., Chaerkady, R., Strain, M., An, L.-L., Rahman, S., Ma, W., Low, C. P., Chan, D., Neal, F., Bingham, C. O., Sampson, K., Darrah, E., Siegel, R. M., Hasni, S., Andrade, F., Vousden, K. A., Mustelin, T., & Sims, G. P. (2017). Spontaneous Secretion of the Citrullination Enzyme PAD2 and Cell Surface Exposure of PAD4 by Neutrophils . In *Frontiers in Immunology* (Vol. 8, p. 1200). https://www.frontiersin.org/article/10.3389/fimmu.2017.01200

Zhou, Y., Mittereder, N., & Sims, G. P. (2018). Perspective on Protein Arginine Deiminase Activity-Bicarbonate Is a pH-Independent Regulator of Citrullination. *Frontiers in Immunology*, *9*, 34. https://doi.org/10.3389/fimmu.2018.00034

Zimmerman, A., Bai, L., & Ginty, D. D. (2014). The gentle touch receptors of mammalian skin. *Science*, *346*(6212), 950 LP – 954. https://doi.org/10.1126/science.1254229