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Developing stratified epithelia: Lessons from the epidermis and thymus

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Abstract

Stratified squamous epithelial cells are found in a number of organs, including the skin epidermis and the thymus. The progenitor cells of the developing epidermis form a multilayered epithelium and appendages, like the hair follicle, to generate an essential barrier to protect against water loss and invasion of foreign pathogens. In contrast, the thymic epithelium forms a three-dimensional mesh of keratinocytes that are essential for positive and negative selection of self-restricted T cells. While these distinct stratified epithelial tissues derive from distinct embryonic germ layers, both tissues instruct immunity, and the epithelial differentiation programs and molecular mechanisms that control their development are remarkably similar. In this review, we aim to highlight some of the similarities between the thymus and the skin epidermis and its appendages during developmental specification.

Keywords

Epithelium; Skin; Thymus; Immunity; Development

Introduction

The organization of the mature epidermis generates an essential barrier that is vital for animals to survive the external environment throughout life¹ (Figure 1A). The epithelium is multi-layered with each layer displaying morphological and biochemical differences in protein expression, including keratins². The inner-most basal layer is composed of proliferative keratin 14 (K14) expressing cells that are intermixed with minor populations of sensory Merkel cells, melanocytes and leukocytes^{2–4}. The basal cells secrete components of the extracellular matrix that forms the basement membrane, a separation between the epidermis and the underlying dermis. Basal cells differentiate upward to form the spinous layer that expresses K1/K10, which is tightly adherent to the basal layer and other surrounding keratinocytes through adherens junctions and desmosomes. An additional suprabasal layer, the granular layer, contains keratinocytes that express loricrin, filaggrin and involucrin proteins, begin to lose their nuclei and generate membrane coating lipid-filled granules called lamellar bodies. Finally, the outermost cornified layer is composed of

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anucleated polyhedral cells that are surrounded by a lipid matrix, which generates a functional barrier. The embryonic basal keratinocytes also form placodes and generate epidermal appendages such as the hair follicle.

In contrast to the epidermis where the distinct epithelial layers are organized on top of a basement membrane⁵, the epithelial cell compartment of the thymus is structured in a three-dimensional network (Figure 1B) that is essential for homeostatic maintenance of the peripheral immune system. In its entirety, the thymus is an encapsulated primary lymphoid organ situated above the heart that is comprised of two types of epithelial cells: medullary thymic epithelial cells (mTEC) that express K14 and K5 and cortical thymic epithelial cells (cTEC) that express predominantly K18 and K8⁶⁻⁸ (Figure 1B). The cortex fills the outer regions of the thymus and supports T cell receptor rearrangement and positive selection of T cells^{6, 9, 10}. In contrast, mTECs reside in the central medulla, and terminally differentiate by progressively expressing the transcriptional regulator, autoimmune regulator (AIRE) followed by involucrin. Involucrin expressing cells form swirled epithelial structures known as Hassall's corpuscles^{11, 12}.

Despite the differences in the overall organization of the mature epidermal and thymic tissues, several similarities exist between epidermal and thymic epithelium. Similarities were clearly identified by *in vitro* analysis and comparison of gene and protein expression between human thymic epithelial cells and human keratinocytes^{13, 14}. Furthermore, the developmental program that allows mTECs to form Hassall's corpuscles is analogous to that of skin epidermal basal cells as they form cornified cells¹⁴ (Figures 1 and 2). In fact, the swirled Hassall's corpuscles contain markers similar to differentiated keratinocytes of the epidermis and resemble the keratin pearls in the disorganized squamous cell carcinomas of the epidermis. The differences in organization may arise from the interaction of the thymocytes with TECs during embryonic development since in the absence of thymocytes, TECs reorganize themselves, aligning along the capsule as if it were a basement membrane⁵.

Additional similarities between keratinocytes formed in these two organs are also highlighted by the responsiveness of thymic epithelial cells to the skin tissue environment. Transplantation of proliferative thymic epithelial cells derived from rats into the skin, permitted these cells to form epidermis and skin appendages such as the sebaceous gland and hair follicle². Thus, thymic keratinocytes display plasticity when subjected to an alternative microenvironment¹⁵.

While the functions of skin and thymic keratinocytes broadly seem quite distinct, both tissues have primary roles in establishing immunity. Thymic epithelial cells create an environment that promotes the expansion, maturation, and specification of immature T cells. Adhesive contacts between these two cell types provides growth factors to developing T cells and in turn, the T cell precursors deliver signals that encourage the maturation and differentiation of the epithelial cells. Thus, the development of thymocytes and thymic epithelial cells (TECs) are interdependent processes and this notion of reciprocal signaling has been termed "thymus cross-talk".

Epidermal keratinocytes are also essential for driving the activation of the innate and adaptive immune system. Keratinocytes produce cytokines that activate different lymphocyte populations collectively known as the “epimmunome”¹⁶. Furthermore, tissue injury leads to the induction of keratinocyte ‘stress-associated’ genes such as ribonucleic acid export 1 (*Rae1*)¹⁷ and *H60*¹⁸. These stress antigens engage NKG2D (CD314), an immunoreceptor expressed by cells of the innate (natural killer cells and dendritic epidermal T cells) and adaptive (activated CD8⁺ T cells, and natural killer T cells) immune system, activating a lymphoid stress-surveillance response¹⁹. Furthermore, tissue resident memory T cells develop within the skin where they require the action of the cytokines, IL15 and Tgfb that are produced by keratinocytes²⁰. These cytokines, not only control their development but also promote the long-term maintenance of this T cell population. In addition, co-culture of human keratinocytes and naïve T cells can produce functional self-restricted T cells²¹, suggesting that skin keratinocytes can promote T cell development like thymic epithelial cells, though at a low efficiency. Thus, by exploring the shared and distinct mechanisms that instruct the development of these epithelial tissues, this review aims to gain a picture of stratified epithelial formation and how shared cellular and molecular mechanisms may influence the formation of epithelia essential for immunity and barrier function.

Development of the interfollicular epidermis

During embryogenesis, the mature skin epidermis is established from the developing ectoderm that generates the multi-layered epithelium following a complex and precisely coordinated stratification program². Following gastrulation, a single layer of epidermal cells is generated known as the primitive ectoderm. This undifferentiated, multipotent layer expresses the keratins characteristic of simple epithelia, K8 and K18^{1, 22}. While regional differences exist in the timing of keratinocyte specification, after embryonic day (E) 8.5, the murine ectoderm is specified to an epidermal cell fate, indicated by the expression of the transcription factor, p63²³. After E9.5 in the mouse, the epidermal cells commit to a complex epithelial fate, indicated by the expression of K5, K14 and K15.

After this initial commitment step, keratinocytes in the newly established embryonic basal layer initiate the process of stratification at E10.5 giving rise to a second layer of cells, the periderm^{2, 24}. The periderm is thought to act as a transient barrier until the cornified layer is formed, since the periderm is shed before birth as the epidermal barrier is acquired^{2, 25}.

The maturation of the epidermis occurs as the cells asymmetrically divide, stratify and terminally differentiate, culminating in the formation of a multi-layered epidermis^{4, 22, 26, 27}. The first layer of intermediate cells initially maintain proliferative capacity prior to exiting the cell cycle, and form differentiated spinous cells with strong expression of K1 and K10. These keratins generate a robust intermediate filament network interlinked with desmosomes^{1, 2, 4}. By E16.5 additional layers of differentiated cells including the granular layer beneath the periderm are generated by the epidermis⁴. Further differentiation is evident at E17.5 by the appearance of filaggrin and the generation of acellular corneocytes, reminiscent of cornified epithelial cells, which create the epithelial barrier. Consequently, by E18.5 the murine epidermis has complete barrier function and is a fully stratified squamous epithelium⁴. Barrier formation is characterized by the establishment of cornified cell

envelopes that are composed of a rigid scaffold created by the bundling of keratins. These bundles are crosslinked with filaggrin and other structural proteins such as involucrin and loricrin, and surrounded by an insoluble lipid covered exterior^{4, 28}.

Multipotent epithelial progenitors in the epidermis

The epithelial progenitor cells of the epidermis are multipotent, giving rise to several lineages including appendages such as the hair follicle, sweat glands and sensory Merkel cells. Prior to stratification of the interfollicular epidermis, epidermal progenitors initiate hair follicle development in response to inductive signals from the mesenchyme. The instructed epithelium generates hair placodes as the underlying mesenchymal cells compact to form dermal condensates, precursors for the dermal papillae that remain associated with the hair follicle. The placodes subsequently grow down into the dermis and generate the multiple lineages of the hair follicle and sebaceous glands, which generate the mature pilosebaceous unit²⁹.

An additional lineage derives from epidermal progenitors during development, such as Merkel cells, the sensory cells that aid in the perception of touch. These cells express intermediate filament proteins associated with primitive and simple epithelia, K8 and K18, arise between E15 and E17 in mouse skin and are generated by keratin 14 expressing cells^{30–32}. The recent identification of the epithelial origin of Merkel cells has initiated the identification of molecular mechanisms that regulate the formation of this lineage including polycomb epigenetic modulators and the transcription factor SRY (sex determining region Y)-box 2 (SOX2)^{33,34}. Future work further clarifying how epidermal progenitors specify Merkel cells may have implications for the formation of Merkel cell carcinomas.

Thymic epithelial development

From an entirely distinct developmental origin, thymic epithelial cells arise from the endoderm of the anterior foregut³⁵. In a process similar to hair follicle placode formation, the endodermal progenitor cells grow down into the underlying mesenchyme to form the thymic rudiment (Figure 3). The first signs of the budding and outgrowth of the thymic rudiment are morphologically detectable by embryonic day 10–11 (E10–11) in mice, which coincides with the expression of the transcription factor, Forkhead box protein N1 (FOXP1), an essential transcription factor for thymic epithelial development^{36–38}. At about E12, the thymic primordium separates from the endodermal surface of the foregut and begins to migrate towards the anterior chest cavity. At this stage, the epithelial cells express high levels of K8 and the transcription factor, p63.

At around E12.5, coinciding with hematopoietic cell colonization and prior to vascularization, the immature thymus undergoes further patterning and differentiation⁷. This phase initiates the first signs of morphological medulla-cortex separation as the discrete cortical and medullary areas contain increasingly defined subsets of epithelial cells marked by differential keratin expression^{39, 40}.

Bipotent Thymic Epithelial Progenitors

Defined cortical and medullary epithelial lineages arise from a common progenitor population that forms early during thymic development^{41, 42}. Ectopic transplantation of isolated E12 thymus lobes demonstrated that progenitor cells exist within the thymic primordium, which have the capacity to generate both cortical and medullary epithelial cells^{43, 44}. Furthermore, clonal analysis of individual TECs in chimeric mice, in transplantation studies, or with genetic lineage tracing of K14⁺ cells, has demonstrated the presence of a common epithelial precursor with the capacity to generate both cortical and epithelial lineages^{45, 46, 43, 42}. Additionally, random reactivation of *Foxn1* in individual TECs in *Foxn1* null mice, further supported the idea that a single progenitor cell has the ability to generate both cortical and medullary epithelial cells in the postnatal thymus⁴². While bipotent epithelial progenitor cells co-express K5 and K8^{44, 47, 48} during thymic development, careful analysis of the timing of epithelial cell emergence revealed that cortical epithelial cells are generated prior to medullary epithelial cells and that CD205⁺ cells have the capacity to generate both cortical and medullary epithelial cells in transplantation experiments⁴⁹. While TECs are able to regenerate postnatally, albeit with diminished efficiency⁴¹, the lineage relationship between cortical and medullary epithelial progenitors postnatally is not known. Identification of appropriate phenotypic markers is required for the further elucidation of TEC progenitor biology^{42, 50}.

The generation of mature mTECs during development closely resembles the differentiation program of the interfollicular epidermis (Figure 2). Like basal epidermal keratinocytes, mTEC progenitor cells maintain expression of p63 and K14, while the majority of mTECs downregulate K8 expression. These proliferative precursor cells generate postmitotic cells that express the transcriptional regulator AIRE and elevate CD80 and MHC class II expression⁵¹. AIRE expression leads to the ability of mTECs to activate the expression of proteins found in peripheral tissues and to eliminate T cells that associate with these “self-peptides” through negative selection⁵². AIRE is also required for the generation of involucrin and filaggrin expressing cells within Hassall’s corpuscle-like structures in the medulla⁵³. These differentiated epidermal genes are located in a gene complex, and since their expression in the epidermis is controlled by the histone demethylase jumonji domain containing 3 (JMJD3) and the polycomb repressive complex enhancer of zeste homolog 2 (EZH2), AIRE may coordinate with these factors^{54, 55}. Furthermore, while AIRE expression has been reported in cultured human keratinocytes²¹, whether AIRE acts in epidermal keratinocytes to control epithelial function or differentiation is not known.

Molecular regulation of stratified epithelial development

The molecular control of the development of the epidermis and its appendages or the thymus, individually, has been covered in depth by several recent reviews^{29, 30, 56–58}. Here, we focus on transcriptional regulators and signaling pathways that impact epidermal and thymic epithelial tissues, highlighting similarities and differences in the two epithelial lineages (Figure 3).

Transcriptional regulation

p63: essential regulator of stratified epithelium—The development of both epidermal and thymic epithelium requires the expression of the p53 family transcription factor, p63^{2, 59, 60}, which is expressed early in the development of both epithelial lineages^{23, 60, 61}. Epidermal development is severely abrogated in loss of function mouse models for *p63* with few keratinocytes and lack of stratification resulting in rapid dehydration and early postnatal lethality of *p63*^{−/−} mice^{62, 63}. A similar epithelial phenotype occurs in the thymus of *p63*^{−/−} mice, which displayed thymic atrophy due to defects in the proliferative rate of thymic epithelial cells. While alternative promoter usage at the p63 locus, produces two isoforms of p63, a transactivating form (TAp63) and an isoform lacking the N terminal domain (Np63), isoform-specific knockout mice have revealed that Np63 is the major isoform responsible for maintenance of epidermal epithelium^{64, 65}. Complementation of *p63*^{−/−} mice with mice expressing Np63 driven by the K5 promoter partially increased thymic cellularity, implicating Np63 as a major isoform in the thymus as well. Since p63 is expressed prior to K14 expression and regulates K14 expression^{23, 66} the function of p63 prior to K5 expression may be required for thymic function.

The precise role of p63 in epidermal development is likely complex and is still not fully understood. Clues from both epidermal and thymic epithelial studies have implicated FGF and Notch signaling downstream of p63⁶⁷. Several human patients with ectodermal dysplasias have mutations in the *p63* gene and knockin mouse models harboring a missense mutation in the p63 SAM domain display skin and cleft palate phenotypes⁶⁸. Mice lacking *Fgfr2β* display similar ectodermal phenotypes as human patients with ectodermal dysplasias⁶⁹ as well as defects in thymic development, which is halted at E12⁷⁰. Indeed, *p63* null embryos lack FGF receptor 2β (FGFR2β) in the epidermis⁷¹ and thymus⁶⁷, suggesting that FGF signaling in part is a mechanism by which p63 controls stratified epithelial development by promoting proliferation of progenitor cells. Additional mechanisms by which FGF signaling regulates the development of these epithelia are discussed later in this review.

FOXN1: differential roles in stratified epithelial cells—Another key transcription factor for both epidermal and thymic epithelial development is FOXN1, a member of the Fox family of transcription factors that are involved in development, metabolism and aging. FOXN1 is expressed in the mature thymic and skin epithelia; however, it seems to have differential roles in these tissues. In both humans and mice, the *Foxn1* gene is comprised of eight coding exons and utilizes two alternative first exons in a tissue-specific fashion^{72, 73}. Although both promoters appear to be active in keratinocytes of the skin (albeit at different levels), only the most upstream element is active in the thymus, which may explain some of the differential roles this gene plays within these two tissues.

In the epidermis, FOXN1 is expressed within differentiated epidermal and follicular cells. Mice lacking *Foxn1* display defects in hair follicle differentiation, resulting in alopecia as well as defects in epidermal keratinocyte differentiation, where keratinocytes in vitro differentiate spontaneously in proliferative conditions^{74, 75}.

In contrast, FOXP1 acts early in thymus development where it promotes TEC progenitor proliferation and directs specification of thymic epithelial precursor cells to cortical and medullary lineages^{76–78}. In the absence of *Foxp1* expression, the development of early epithelial precursor cells is reversibly arrested, while restoration of FOXP1 expression can induce normal differentiation⁴². While the precise mechanisms by which FOXP1 promotes thymic epithelial differentiation are unknown, this transcription factor has been implicated in controlling expression of several other transcription factors important for TEC development including paired box protein 1 (PAX1), PAX9 and homeobox A3 (HOXA3)⁷⁹. Interestingly, it has been hypothesized that HOXA3 acts upstream of PAX transcription factors and may control the separation of the thymus primordia from the pharynx and their subsequent migration towards the mediastinum^{80, 81}. *Hoxa3* is required for the development of structures in the pharyngeal region, leading to a loss of thymus formation in *Hoxa3* null mice⁸². In epidermal keratinocytes, HOXA3 promoted the migration of keratinocytes during wound healing⁸³.

The regulation of FOXP1 expression by the retinoblastoma (RB) family of proteins (RB, p107 and p130) revealed a role for FOXP1 in the regulation of TEC proliferation⁷⁸. Genetic deletion of *RB* leads to increased TEC proliferation, resulting in enlarged thymic lobes and inhibition of thymic atrophy associated with age. This phenotype is similar to mice lacking both *RB* and *p107*, which displayed hyperplasia of the epidermis due to increased proliferation and differentiation⁸⁴. In the thymus, RB promotes the transcription of *Foxp1* expression, which is required for TEC expansion in *RB* mutant mice *in vivo*. These data are consistent with the requirement of FOXP1 for homeostatic control of postnatal TECs^{85, 86}, and indicate that FOXP1 promotes both proliferation and differentiation of TEC progenitors. It is unknown whether RB family members regulate *Foxp1* expression in epidermal keratinocytes to control epidermal homeostasis.

TBX1: development and regeneration of epithelial lineages—The T box gene *Tbx1*, is another transcription factor that is shared between epidermal progenitor cells in the epidermis and thymus. In the epidermis, TBX1 is highly expressed within the developing hair placode and becomes restricted to hair follicle stem cells and their progeny⁸⁷. Conditional deletion of *Tbx1* in K14⁺ cells resulted in loss of hair follicle stem cell renewal following several rounds of depilation. During thymus development, *Tbx1* is expressed early in the pharyngeal endoderm and mesoderm⁸⁸. Complete loss of *Tbx1* results in a loss of thymic epithelial development, which phenocopies DiGeorge syndrome^{89–91}, a genetic disease caused by heterozygous monoallelic microdeletion within human chromosome 22q11, clinically recognized by malformations of pharyngeal arch arteries and heart, parathyroid hypoplasia, and absence or ectopic location of the thymus⁹². TBX1 seems to be necessary in both the endoderm and the mesoderm to control thymic development. Furthermore, microarray analysis on E9.5 *Tbx1* null embryos identified several putative *Tbx* target genes including *Fgf8*⁹³ and *Pax9*⁹⁴. Whether like in the epidermis, TBX1 regulates TEC maintenance postnatally remains to be determined.

Regulation by signaling pathways

FGF signaling: building epithelial buds—A major regulator of both hair follicle placode formation and thymic epithelial development is FGF signaling. In both tissues, FGF7 and FGF10 are expressed by the surrounding mesenchyme^{70,95}. Deletion of the receptor for these ligands, FGFR2 isoform IIIb resulted in a block in thymic epithelium development after rudiment formation. These defects were similar to the phenotype of *Fgfr2IIIb* null mice in the thymus, which displayed a hypoplastic interfollicular epidermis and fewer and retarded hair follicle placode formation^{96, 97}. Unlike the inability of the thymus to progress beyond a rudiment, hair follicles formed in grafted skin of *Fgfr2IIIb* null mice, indicating that the hair defect was not completely abrogated. These studies revealed the importance of mesenchymal expression of FGFs to promote epithelial proliferation and invagination to generate mature thymic rudiments and epidermal hair placodes.

WNT signaling: promoting epithelial progenitor proliferation and fate determination—WNT signaling regulates development of several tissues and is involved in tumor formation in several epithelial tissues⁹⁸. When WNT ligands bind to frizzled receptors, in coordination with low-density lipoprotein receptor related proteins (LRPs), they transmit intercellular signals which ultimately stabilize cytoplasmic β -catenin (CTNNB1), allowing β -catenin to translocate to the nucleus where it complexes with the lymphoid enhancing factor/T cell factor (LEF/TCF) family transcription factors, to regulate gene expression.

In the epidermis, WNT signaling is activated in the IFE, placodes during hair follicle formation and during hair shaft differentiation^{99–101}. During embryogenesis, WNT signaling specifies epidermal fate¹⁰² and hair follicle placode initiation. In the postnatal/adult setting several studies have implicated WNT signaling in the control of hair follicle formation. Deletion of *Ctnnb1* or expression of Wnt inhibitor, DKK1 in an epithelial specific manner resulted in a lack of several epidermal appendages including hair follicles^{103, 104}. Furthermore, expression of a dominant active form of β -catenin in basal keratinocytes induced ectopic hair follicle formation¹⁰⁵. Deletion of *Lef1* in mice reduced hair follicle formation but not a complete ablation¹⁰⁶, suggesting that β -catenin may act with other factors to fully control hair follicle formation and development.

In addition to its role in the hair follicle, recent work suggested that WNT signaling controls IFE proliferation^{100, 101}. Mice, in which *Ctnnb1* was deleted in a subset of epidermal cells, using an inducible form of Cre recombinase driven by the *Axin2* promoter, displayed a thinner epidermal compartment due to defects in proliferation of IFE progenitor cells. This function of WNT signaling is similar to its role in hair follicle progenitor cells, where *Ctnnb1* is required for their proliferation during hair growth¹⁰⁰.

Less is understood about the role of WNT signaling in thymic epithelial development. TECs and T cells express WNT ligands during thymic development where they can activate FOXP1 expression in cultured TECs¹⁰⁷. Intriguingly, abnormal cortical TEC architecture has been identified in mice lacking *kringle containing transmembrane protein 1* (*Kremen1*), a negative regulator of WNT signaling. In addition, cortical TECs have been shown to express a range of WNT molecules, including WNT4 and WNT10b^{108, 109}. Additionally,

mice lacking *adenomatous polyposis coli* (*Apc*), a major component of the protein complex that degrades β -catenin in the absence of WNT ligands, in K14-expressing keratinocytes displayed enhanced WNT signaling in the thymus and developed smaller thymic lobes with reduced epithelial proliferation and increased numbers of K14 expressing epithelial cells¹¹⁰. Whether this thymic phenotype is solely due to the action of WNT signaling in the thymus or other roles of APC is not known. Furthermore, whether WNT signaling promotes proliferation of TECs and/or terminal differentiation similar to its role in the IFE and hair follicle will be an interesting area of future investigation.

Eph/ephrin signaling: organizing epithelia—Eph receptors, which bind to membrane bound ephrins, represent the largest group of receptor tyrosine kinases in mammals^{111, 112}. Ephs are classified into two subfamilies, EphA and EphB, depending on their binding preference to glycosylphosphatidylinositol (GPI)-anchored ephrins A or transmembrane ephrins B ligands, respectively^{111, 113}. In both the epidermis and thymus, Eph receptors and ephrins are compartmentalized with differential expression in distinct cell subsets¹¹⁴. In the interfollicular epidermis, ephrinA proteins are concentrated in basal keratinocytes while EphA receptors can be found within all viable layers of the epidermis¹¹⁵. Inhibition of Eph/ephrin signaling complexes may negatively regulate keratinocyte proliferation¹¹⁶, which may be relevant for tumorigenesis in the skin given the enhanced tumorigenesis of *EphA2* null mice¹¹⁷. Furthermore, EphA2 has been implicated in the terminal differentiation of epidermal keratinocytes¹¹⁵ perhaps by promoting desmosome maturation^{115, 118}. While the function of EphA2 has not been explored in thymic development, *EphA4*^{-/-} mice have reduced TEC cell numbers and a smaller and disorganized thymic cortex and medulla¹¹⁹, suggesting a role in proliferation. Whether EphA4 alters desmosome formation or other adhesion or differentiation mechanisms to control TEC organization would be an interesting future area of study.

EphB family members also regulate epidermal and thymic epithelial development. EphB2 and EphB3 and their ligands ephrinB1 and ephrinB2 are expressed on both thymic epithelial cells and thymocytes¹²⁰. Conditional deletion of *ephrinB1* and *ephrinB2* in epithelial cells led to alterations in skin development including short hair and premature eye opening¹¹². Furthermore, these mice exhibited abnormal thymic epithelial architecture, characterized by a reduced size and a higher proportion of K5⁺K8⁺ presumptive thymic epithelial progenitor cells and thymic epithelial cysts¹¹². Since EphA4 can bind to ephrinB ligands¹²¹, it is possible that some of the thymic phenotypes in the *ephrinB1/B2* and *EphA4* null mice may be shared.

BMP signaling: determining epithelial fate—Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGF β) superfamily that play roles in cell-fate determination and developmental patterning of many tissues^{122, 123}. BMPs bind to heterodimeric receptors and transmit signals through SMAD transcriptional complexes. In epidermal development, BMPs act early in the ectoderm to drive specification to the epidermal keratinocyte lineage^{2, 124, 125}. Later in epidermal appendage development, inhibition of BMP signaling promotes hair placode formation¹²⁶. Noggin, a BMP antagonist, is expressed by mesenchymal cells and is thought to decrease BMP signaling, which

activates the WNT responsive transcription factor LEF1¹²⁷ and induces hair follicle fate. Later in hair follicle formation, differentiation of hair follicle matrix cells requires active BMP signaling¹²⁸.

In the thymus, BMP signaling is required for proper epithelial development as mice expressing noggin in FOXN1 expressing progenitor cells, formed small thymic lobes with reduced epithelial cell number and mislocalization in the neck, rather than above the heart¹²⁹. The expression of *Bmp4* in the thymic anlage and mesenchyme suggests bidirectional signaling between epithelial mesenchyme^{129, 130}. Whether BMPs control thymic anlage development into the mesenchyme, similar to its role in the epidermal placode formation, or later stages of epithelial development is not known.

Notch signaling: determining epithelial fate—The canonical Notch signaling pathway regulates several steps of epidermal specification. During ectoderm specification, Notch signaling suppresses epidermal fate by blocking p63 expression²³. Later during IFE differentiation, Notch signaling is activated in spinous cells and conditional deletion of the transcription factor downstream of Notch signaling, *Rbpj* in the skin, blocked spinous cell formation¹³¹. A similar phenotype occurred in mice lacking *Hes1*, a Notch target gene¹³². Furthermore, *Notch1*-null mice displayed abnormal epidermal proliferation and differentiation¹³³. While Notch signaling between TECs and T cells can regulate T cell development¹³⁴, whether intrinsic Notch signaling regulates mTEC differentiation in a similar manner, has not been explored to date.

Conclusion

The epidermis and its appendages have become an excellent model system to explore the cellular and molecular mechanisms by which epithelial tissues develop and regenerate²⁹. In this review, we highlight significant advances that emphasize similarities between the development of epidermal and thymic epithelial tissues. Two major themes emerge from these comparisons: first, the similarities between hair follicle placode morphogenesis and that of the early thymic anlage development (Figure 3). These two structures grow down into the underlying mesenchyme, express similar transcriptional regulators and require cues from mesenchymal cells to drive morphogenesis (Figure 3A). A second theme emerges in the shared cell specification and differentiation programs that drive the formation of epithelial cells in the skin and thymus (Figure 3B).

In the future, it will be interesting to determine if parallels exist between the postnatal maintenance of epithelial progenitor cells in the epidermis and thymus. Recent work has highlighted the importance of epithelial stem cells in the maintenance of the interfollicular epidermis and hair follicles¹³⁵. However, the identity of such a cell in the thymus has remained elusive. Since age-associated thymic atrophy leads to a decline in naïve T cell output to the periphery and a reduced ability of the adult immune system to respond to infection and vaccination¹³⁶, it will be interesting to understand if mechanisms that maintain epidermal stem cells in the skin display altered function in thymic epithelial progenitor cells. In particular, the role of epigenetic regulation of chromatin and histones within the

epidermis has been shown to be required for maintenance of the epidermis^{137, 138} but their role in the thymus has not been explored to date.

Another interesting avenue for future investigation will be the role of the mesenchyme in controlling epithelial development and regeneration in both tissues. As discussed above, several studies have highlighted the importance of mesenchymal-epithelial interactions during thymic and hair follicle development and regeneration including WNT and FGF signaling. While recent work suggested that epidermal fibroblasts share a common progenitor during development¹³⁹, the mechanisms that control development of fibroblast lineages in both the epidermis and thymus are largely unknown. Since epithelial function can be disrupted by skin fibrosis and fibroadipogenic cells in the thymus with disease or age, the generation of genetic tools (e.g. Cre-loxP technology) that permit the study of dermal cell populations will facilitate our understanding of these changes in epithelial mesenchymal composition and how they alter epithelial tissues.

In the last several years, skin and thymic tissue development have been fruitful systems for understanding how epithelial cells develop and the molecular mechanisms that drive these processes. Advancing our understanding of epithelial development will help design effective molecular and cellular methods to treat skin and immune disorders, where the epithelium is dysfunctional.

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Figure 1. Schematic Representation of the Histology of the Skin and Thymus Epithelium

A) The skin epithelium is comprised of the interfollicular epidermis and contains epidermal appendages, such as the hair follicle. Multiple stratified layers of differentiated keratinocytes with different characteristics create an intricate barrier. B) The thymus is a bi-lobed organ that contains two major compartments of epithelial cells, the outer cortex (blue cells) and medulla (pink cells) that form a three-dimensional organ.

Figure 2. Model Of Skin Epithelium and Thymic Epithelium Development

A) Schematic illustrating the serial progression of epidermal keratinocytes from prekeratinocyte progenitor cells (preKC) to keratinocyte progenitor cells (KCp), which generate epidermal lineages, pilosebaceous progenitors and Merkel cells. B) Schematic illustrating thymic epithelial development. Bipotent thymic epithelial cells (bTECp) generate a progenitor that has greater cortical features (tTECp) that form cortical TEC progenitors (cTECp) and medullary TEC progenitors (mTECp). Both of these progenitors form immature and mature TECs of their lineage. Mature mTECs form Hassle's corpuscles in their final differentiation step. Circular arrows represent self-renewal. Shared markers are illustrated at the bottom.

Figure 3. Comparison Of Skin and Thymic Epithelial Molecular Mechanisms

A) Common signaling pathways utilized by both the thymus rudiment and hair placode during morphogenesis are indicated in Red. B) Cell specification utilizes some common signaling pathways as morphogenesis. Signaling pathways important at specific stages are indicated. SC, stem cell. TA, transit amplifying cell. Diff, differentiated cell.