INTRODUCTION

Molecules in the collagen family harbour one or more collagenous domains in their protein sequences and account for 30% of total protein mass in mammals. Of the 28 collagens known to be expressed in mammals, type XVII collagen (COL17) is known to have the following characteristics: (i) It is expressed in epidermal basal keratinocytes, and (ii) it is defective in junctional epidermolysis bullosa (EB) and (iii) it is an autoantigen in bullous pemphigoid (BP). COL17, a transmembrane collagen, is distinct from fibrillar collagens, which are generally secreted into extracellular spaces, such as type VII collagen (COL7). Recently, there have been many excellent review papers on junctional EB and BP. Therefore, the current review will only briefly address these diseases. Instead, this review paper will mainly focus on the physiological properties of COL17 in the epidermis, its role in maintaining stem cells and its association with signalling pathways. We propose possible solutions to unanswered questions in this field.

PHYSIOLOGICAL PROPERTIES OF COL17

Discovery of COL17

More than half a century ago, researchers started to characterize the ultrastructure of tissues, including the skin. Electron microscopy (EM) shows the dermo-epidermal junction (DEJ) and the epidermal basement membrane zone (BMZ) to have multiple structural components, including hemidesmosomes (inner plaques and outer plaques), the lamina lucida, the lamina densa and anchoring fibrils. Destroyed or blurred BMZ structural components in the skin of epidermolysis bullosa (EB) patients highlighted epidermal BMZ as being essential to epidermal-dermal attachment. Recently, in addition to facilitating epidermal-dermal attachment, COL17 has been reported to serve as a niche for hair follicle stem cells, to regulate proliferation in the interfollicular epidermis and to be present along the non-hemidesmosomal plasma membrane of epidermal basal keratinocytes. This review focuses on the physiological properties of COL17 in the epidermis, its role in maintaining stem cells and its association with signalling pathways. We propose possible solutions to unanswered questions in this field.
according to molecular weight: a 230-kDa protein (BP230 or BP antigen 1 (BPAG1)) and a 180-kDa protein (BP180 or BP antigen 2 (BPAG2)). In the early 1990s, molecular cloning techniques identified the sequences of BP230 and BP180. As collagen repeats (Gly-X-Y) were detected in BP180, BP180 was relabelled as type XVII collagen (COL17). COL17 is a type II transmembranous protein whose N-terminus is located in the cytoplasm while C-terminus is in the extracellular space. COL17, rather than BP230, has been regarded as the pathogenic BP autoantigen, because immuno-EM demonstrated COL17 as lying along the plasma membrane, whereas BP230 is located in the cytoplasm of epidermal keratinocytes. In addition, mutations in COL17A1, encoding COL17, were detected in junctional EB, generalized intermediate subtype (formerly called non-Herlitz junctional EB). The blistering phenotypes of BP and junctional EB have highlighted COL17 as an anchor between epidermis and dermis. However, COL17 labelling in human and murine epidermis is found not only in the DEJ but also in the apicolateral plasma membrane of epidermal basal keratinocytes. COL17 does not colocalize with desmosomal proteins, which are involved in epidermal-dermal attachment, non-hemidesmosomal COL17 might play other roles in keratinocyte physiology. Recently, physiological or organismal ageing has been reported to reduce non-hemidesmosomal COL17 in the interfollicular epidermis (IFE) (Figure 2). Epidermal polarity is maintained by the fine balance between symmetrical cell division (SCD) and asymmetrical cell division (ACD). Physiological ageing increases the ratio of ACD to SCD in the IFE, which is consistent with the phenotypes of premature ageing in mice with the epidermal-specific deletion of atypical PKCλ (aPKCλ), a key molecule that regulates cell polarity. This is corroborated by the reduction in non-hemidesmosomal COL17 in 3D-reconstructed epidermis that is treated with aPKC inhibitors. These results suggest that the COL17 distribution in a cell is regulated in the context of cell polarity. It is also noted that COL17A1 gene expression in the IFE does not alter with physiological ageing, whereas other collagens and laminins are generally reduced at the transcriptional level. The main question in this field is how polarity-related molecules affect COL17 distribution in the cells.

2.2 | Hemidesmosomal COL17

The hemidesmosomes of epidermal keratinocytes are divided by EM into inner and outer plaques. Inner plaques harbour plectin and BP230, both of which are plakin family proteins. Plectin serves as a linker protein between intermediate filaments and hemidesmosomes. In contrast, outer plaques are composed of COL17 and integrin α6/β4. The N-terminus of COL17 is located in the outer plaque of hemidesmosomes, whereas its extracellular domain extends from the lamina lucida to the lamina densa. Rotary-shadowed images of COL17 show a molecular shape mimicking a quaver (an eighth note, in musical notation). Many cell lines have been used to recapitulate hemidesmosomes in vitro. As expected, COL17 is present in hemidesmosome-like structures in cultured cells. However, it is noteworthy that hemidesmosome-like structures are not clearly seen in normal human epidermal keratinocytes (NHEKs). Hemidesmosome-rich fractions, which are extracted from DJM-1 cells, also contain COL17.

2.3 | Non-hemidesmosomal COL17

Although COL17 has been characterized as a hemidesmosomal component, COL17 labelling in skin shows COL17 not only at the DEJ but also at the intercellular spaces between basal keratinocytes (Figure 1). The fact that hemidesmosomes are not observed at the cell-junction of keratinocytes indicates the presence of non-hemidesmosomal COL17. Non-hemidesmosomal COL17 is triton-X100 soluble and is distinct from hemidesmosomal COL17, which is triton-X100 insoluble. Non-hemidesmosomal COL17 does not colocalize with desmosomal proteins, which are compatible with detergent-solubility. As it does not appear to be involved in epidermal-dermal attachment, non-hemidesmosomal COL17 might play other roles in keratinocyte physiology. Recently, physiological or organismal ageing has been reported to reduce non-hemidesmosomal COL17 in the interfollicular epidermis (IFE) (Figure 2). Epidermal polarity is maintained by the fine balance between symmetrical cell division (SCD) and asymmetrical cell division (ACD). Physiological ageing increases the ratio of ACD to SCD in the IFE, which is consistent with the phenotypes of premature ageing in mice with the epidermal-specific deletion of atypical PKCλ (aPKCλ), a key molecule that regulates cell polarity. This is corroborated by the reduction in non-hemidesmosomal COL17 in 3D-reconstructed epidermis that is treated with aPKC inhibitors. These results suggest that the COL17 distribution in a cell is regulated in the context of cell polarity. It is also noted that COL17A1 gene expression in the IFE does not alter with physiological ageing, whereas other collagens and laminins are generally reduced at the transcriptional level. The main question in this field is how polarity-related molecules affect COL17 distribution in the cells.

2.4 | Binding partners

As the N-terminus of hemidesmosomal COL17 is localized in the cytoplasm of epidermal basal keratinocytes and its collagenous domains are present in the extracellular spaces, COL17 binds to hemidesmosomal proteins and to extracellular matrix (ECM) proteins in the epidermal BMZ.

BP230, the other bullous pemphigoid antigen, is located in the inner plaque of the hemidesmosomes. Yeast two-hybrid assays and protein-protein binding assays have demonstrated direct interaction between BP230 and COL17 at their N-termini. Plectin is also in the inner plaque of the hemidesmosomes and...
collagen (COL4) is a major component of the lamina densa (basal lamina) in the epidermal BMZ. COL17 at the skin wounding edge is upregulated or downregulated depending on the context. Ectodomain shedding induces conformational changes in COL17, which probably induce the antigenicity of the protein in LAD. The shed ectodomain, which is found in the ECM, impedes cell motility in the scratch-wounding assay in vitro. It is not clear whether in vivo ectodomain shedding of COL17 at the skin wounding edge is upregulated or downregulated. Knock-in mice with 41 amino acid deletions at ADAM cleavage site of COL17, in which COL17 ectodomain shedding is impaired, show accelerated wound healing compared with controls, possibly through the activation of mTOR (mammalian target of rapamycin) signalling. However, no other phenotypes are apparent in non-shedding knock-in mice.

The biological significance of this ectodomain shedding has been discussed since its discovery. Ectodomain shedding induces conformational changes in COL17, which probably induce the antigenicity of the protein in LAD. The shed ectodomain, which is found in the ECM, impedes cell motility in the scratch-wounding assay in vitro. It is not clear whether in vivo ectodomain shedding of COL17 at the skin wounding edge is upregulated or downregulated. Knock-in mice with 41 amino acid deletions at ADAM cleavage sites of COL17, in which COL17 ectodomain shedding is impaired, show accelerated wound healing compared with controls, possibly through the activation of mTOR (mammalian target of rapamycin) signalling. However, no other phenotypes are apparent in non-shedding knock-in mice.

2.5 | Ectodomain shedding

Linear IgA dermatosis (LAD), an autoimmune subepidermal blistering disease, has been known to have two autoantigens: the 120 kDa protein LAD-1 and the 97 kDa protein LABD97, both of which are produced by epidermal keratinocytes. These two proteins are shed ectodomains of COL17 released from keratinocytes. In accordance with this, LAD antibodies fail to react with the skin specimens of junctional EB patients who carry COL17A1 mutations. The ectodomain cleavage occurs within the non-collagenous (NC) 16A domain of COL17 to yield a 120 kDa form, and processing into the 97 kDa form requires further cleavage in the C-terminus. The ectodomain shedding includes lipid rafts on the plasma membrane, the extracellular phosphorylation of COL17, ECM composition that faces keratinocytes and the p.Arg1303Gln mutation in COL17.

Multiple proteases have been implicated in the process of COL17 ectodomain shedding. A disintegrin and metalloprotease (ADAM) 9 and ADAM10 directly shed COL17 into a 120 kDa form, whereas ADAM17 indirectly contributes to this process. Plasmin digests COL17 into a 97 kDa form independently of ADAMs. In addition to the proteases, the factors that are known to regulate or alter COL17 ectodomain shedding include lipid rafts on the plasma membrane, the extracellular phosphorylation of COL17, ECM composition that faces keratinocytes and the p.Arg1303Gln mutation in COL17.

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3 | Niche for Stem Cells (SCs)

3.1 | Hair follicles

The fact that Col17a1−/− mice and JEB patients with COL17A1 mutations show hair loss and hair greying has drawn attention to the role of COL17 in hair follicles (HFs) as well as in melanocytic SC maintenance. In addition to being present in the DEJ of the interfollicular...
epidermis (IFE), COL17 is found in HF stem cells (HFSCs), which are located in the HF bulge region. In contrast, melanocytes and their SCs do not express COL17. Col17a1−/− mice show normal hair development but immature 2nd anagen entry, eventually leading to hair loss and hair greying. Hair loss is explained by SC exhaustion due to the loss of COL17 as a niche for HFSCs, while hair greying is thought to represent aberrant melanocyte SC maintenance because of HFSC exhaustion. Hair loss with physiological ageing is also accounted for by the proteolytic degradation of COL17 in HFSCs and can be reversed with the overexpression of COL17. Together with the depletion of non-hemidesmosomal COL17 in the aged IFE as described above, the pharmacological modulation of COL17 could be a promising candidate therapy for skin ageing, including for age-related alopecia.

3.2 | Interfollicular epidermis (IFE)

Although the pivotal role of COL17 in regulating HF is well characterized, mostly based on the phenotypes of Col17a1−/− mice and patients with JEB who carry COL17A1 mutations, it had not been known whether COL17 is involved in maintaining IFE homeostasis. Recently, neonatal Col17a1−/− IFE was reported to show transient hyperproliferation (Figure 2), which is consistent with similar phenotypes of mice with the epidermal deletion of α6 or β1 integrin. This hyperproliferative phenotype of neonatal Col17a1−/− IFE disappears at P20. Mathematical modelling suggests that reduced attachment of the epidermis to the dermis in Col17a1−/− skin might destabilize IFE stem cell (IFESC) maintenance and be responsible for the increases in transient-amplifying cells (TACs), leading to temporary epidermal hypertrophy followed by the reduction in differentiated cells once TACs complete their limited number of cell divisions. In other words, COL17 might maintain the quiescence of IFESCs. This hypothesis could be evaluated by in vivo clonal assays using lineage-tracing techniques.

It has been controversial whether the presence of COL17 affects the cell growth of cultured keratinocytes. Murine keratinocytes derived from Col17a1−/− mice have shown less colony-forming ability than those of control mice, while it has been reported that human keratinocytes with COL17A1 mutations outgrow keratinocytes with revertant mosaicism (natural gene correction) from the same individual. More recently, normal human keratinocytes with COL17 knock-down have revealed a colony-forming ability and growth curves similar to those of cells treated with mock siRNA. These discrepancies might be attributable to the different experimental design of each experiment.

4 | ASSOCIATION WITH SIGNALLING PATHWAYS

4.1 | TGF-β

TGF-β (transforming growth factor-β) is a cytokine that regulates cellular proliferation and differentiation in many organs. The TGF-β pathway is implicated in COL17-related HFSC maintenance, as TGF-β signalling is downregulated in Col17a1−/− mice and TGF-β receptor-null mice show grey hair, similar to Col17a1−/− mice. This is in contrast to Col17a1−/− IFE, in which TGF-β signalling may not be significantly altered. TGF-β presumably acts to keep HFSC maintenance in an autocrine/paracrine way.

4.2 | Wnt

The Wnt pathway plays an active role in cell proliferation, differentiation, and migration, regulating development and SC fate mostly during tissue morphogenesis. The pathway has received focus for its governing of epidermal SC dynamics for decades. The relationship between COL17 and Wnt signalling is implicated by the gene expression profiles of the IFE of neonatal Col17a1−/− mice, whose expression of Wnt-related genes is altered. In line with the gene expression profiles, Col17a1−/− IFE harbours fewer Lef1 and nuclear β-catenin-labelled cells, K14-ΔNleF mice, in which dominant negative Lef1 suppresses Wnt signalling, and mice treated with Wnt antagonists show hyperproliferation in the neonatal IFE, which mirrors the Col17a1−/− phenotype. Reporter assays further confirm that the presence of COL17 augments Wnt activities in vitro and that COL17 deletion reduces its activity in the IFE in vivo. The transgenic rescue of neonatal Col17a1−/− by overexpression of human COL17 restores the expression of Wnt-related genes and the labelling of Lef1 and nuclear β-catenin in basal epidermal keratinocytes. These results clearly indicate that COL17 stabilizes Wnt signalling.

4.3 | STAT3

STAT3 (信号转导和激活子3) is a transcription factor that is localized in the cytoplasm at the steady state and is translocated into the cell nucleus upon phosphorylation by JAK (Janus kinase). STAT3 acts as a transcription activator in the nucleus, contributing to the regulation of cell growth and apoptosis. Tumor-initiating cells (TICs) or cancer SCs can be maintained and enriched with spheroid culture condition in vitro. The phosphorylation of STAT3 at Ser727 has been shown to mediate the survival ability of spheroid-enriched TICs. Gene expression screening has identified COL17 as a target of Ser727-phosphorylated STAT3 and as being essential to the maintenance of TIC survival. These results are in line with the correlation between COL17 expression and higher tumor stages in colorectal cancer. Whether COL17 is associated with STAT3 in the epidermis is not known.

4.4 | Hippo

The Hippo signalling pathway regulates cell proliferation and apoptosis to determine organ size and is involved in cell contact inhibition. Hippo activation phosphorylates YAP1/TAZ, key transcription co-activator of this pathway, and retains them in the cytoplasm. When YAP1/TAZ is dephosphorylated, they enter the nucleus and interact with transcription factors to promote cell proliferation.
and inhibit apoptosis. Recently, the binding of YAP to the Col17a1 promoter together with the formation of the YAP and NKX2.1 complex, a lung-specific transcription factor, has been reported to downregulate Col17a1 gene expression levels in lung epithelial cells. However, Col17a1 downregulation upon the translocation of YAP into the nucleus is specific to lung tissue and is not observed in epidermal keratinocytes. This divergence might be explained by the preferential expression of NKX2.1 in lung tissue.

4.5 | Rac1 and p38MAPK

When cells migrate, they need to change their morphology such as to have leading and trailing edges. The lamellipodium is a sheet-like extension that is seen at the leading edge, and its formation requires actin cytoskeleton rearrangement. Rac1 (RAS-related C3 botulinus toxin substrate 1) is a small GTPase of the Rho family that regulates lamellipodium formation. COL17 knock-down in human epidermal keratinocytes reduces Rac activity as well as lamellipodia persistence and extension distances. As Rac is involved in the regulation of the p38MAPK pathway, these results are consistent with the impairment in p38MAPK activity of HaCaT cells with COL17 knock-down.

4.6 | p53

p53 is a well-known tumor suppressor whose dysfunction leads to aggressive tumor phenotypes. Screening of the p53 target in mammary epithelial cancer cell lines has identified COL17 as a novel direct target of p53. p53 binds to the COL17A1 promoter to regulate its gene expression, and the downregulated COL17 expression correlates with poor prognosis in breast cancer, which is in line with p53 dysfunction. The relationship between p53 and COL17 has not been reported with regard to the epidermis.

5 | FUTURE PERSPECTIVES

Table 1 lists unanswered questions in this field. Although non-hemidesmosomal COL17 expression is diminished with physiological ageing in association with altered epidermal cell polarity, the way in which the differential expression of non-hemidesmosomal and hemidesmosomal COL17 is maintained needs further investigation. This differential expression might be related to the intracellular trafficking of collagens. Coat protein complex II (COPII)-coated vesicles are used for the intracellular trafficking of collagens from the endoplasmic reticulum (ER). COL7, another collagen in the epidermal BMZ, binds to TANGO1 (transport and Golgi organization 1), which facilitates the loading of secretory proteins including COL7 into COPII. However, the intracellular trafficking of COL17, which is a transmembrane protein but not a secreted protein, has not been well studied. As highlighted in this review, various signalling pathways including TGF-β and Wnt are affected by COL17 modulation, although the mechanisms underlying these associations have been unclear. In addition, whether these signalling pathways are related specifically to COL17 or to disturbed dermo-epidermal adhesion remains unknown.

The incidence of skin cancers in junctional EB patients with the generalized intermediate subtype (mostly carrying mutations in LAMB3, encoding laminin β3) has been argued as being high, although only a few junctional EB patients with COL17A1 mutations have been reported to develop skin cancer. It remains unclear whether COL17A1 mutations are associated with a greater risk of skin cancer compared with the general population. This stands in contrast to COL7-deficient recessive dystrophic EB patients, whose incidence of cutaneous squamous cell carcinoma (SCC) is known to exceed 80% by the age of 45. It is of note that the increased expression of COL17 has been reported in SCC, colorectal cancer, colorectal cancer, colorectal cancer, colorectal cancer, and lung cancer. Recently, the depletion of COL17 has been shown to suppress SCC cell growth through in vitro and in vivo xenograft experiments. Intriguingly, the prevention of COL17 ectodomain shedding disturbs in vitro SCC growth and invasion. To further confirm these results, it may be of interest to observe carcinogenesis experiments on Col17a1−/− mice or on non-shedding COL17 knock-in mice.

We hope that the answers to these questions will pave a path to understanding the pathomechanisms of blistering diseases and towards the development of new therapeutics for these diseases.

AUTHOR CONTRIBUTION

K.N. wrote the original draft. M.W., W.N. and H.S. reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

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