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博士の専攻分野の名称 博士（医学）　　　　氏 名 Yunan Wang
（Degree conferred: Doctor of Philosophy）　　（Name）

学 位 論 文 題 名（Dissertation Title ）

Type XVII collagen is a deterministic factor of epidermal patterning

17 型コラーゲンは表皮パターンの決定因子である。

【Background and Objectives】 Vertebrates exhibit patterned epidermis. In some animals, these are visible through melanin distribution in the skin; in others, they are reflected by the allocation of skin components (e.g. hair follicles and fish scales). Human skin is characterized by fingerprints and similar structures, in which cristae cutis and sulci cutis are found alternately in the epidermis. Although skin patterns have been described in many creatures, the mechanisms underlying the development of these phenotypes are poorly understood. Mouse tail skin serves as a robust model for examining pattern formation, because the epidermis consists of scale (parakeratotic) areas and interscale (orthokeratotic) areas. Tail scale and interscale are distinguished by the expression of keratin 10 (K10) and keratin 31 (K31): scale is K10-/K31+, whereas the interscale epidermis is K10+/K31-. Lineage-tracing experiments have revealed that K10+ interscale epidermis is slow-cycling, whereas K31+ scale areas are fast-cycling.

The epidermis is maintained by the fine-tuned balance between the proliferation and differentiation of epidermal stem cell (SC) progeny. Epidermal SCs need niches to function properly. Integrins (e.g., β1 and α6) and type XVII collagen (COL17) have been implicated as niche proteins for epidermal SCs through colony formation experiments, animal studies, and mathematical modeling. In accordance with this, mice deficient in β1 integrin or COL17 show transient hyperproliferation in the epidermis. Their expression is enriched in human epidermis facing the dermal protrusion but not in the epidermal rete ridges. Interestingly, epidermal scale and interscale areas are not apparent in β1 integrin-null tail epidermis. These previous studies have implicated the involvement of SC niche proteins in the epidermal pattern formation. However, whether these SC niche proteins indeed regulate the epidermal patterns and its underlying mechanisms are unknown.

【Materials and Methods】 C57BL/6, *Col17a1-/-*, K14-hCOL17, hCOL17+; *Col17a1-/-*, K5-Cre;*aPKCλ*$^{\Delta E5/\Delta E5}$ (aPKCλ eKO), and *Prkcz*$^{-/-}$ (aPKCζ KO) were used in this study. Tail samples at the mouse age of 4 weeks were obtained after the mice were euthanized. Unstained or stained skin samples were observed by light microscopy or confocal microscopy. Immunofluorescence staining was performed to examine the expression of keratins, COL17 and proliferation markers. When necessary, the obtained images were analyzed by ImageJ software. Quantitative PCR was performed to analyze the keratin gene expression levels. For scale regeneration experiments, the surface of the tail skin was removed by scalpel from C57BL/6 adults. Healed skin samples were obtained 4 to 6 weeks after wounding. Three revertant mosaicism spots and three adjacent diseased skin areas from the upper arm of a junctional epidermolysis bullosa patient were analyzed. The characteristic direction of the epidermal pattern was analyzed by autocorrelation function.

【Results】 1) COL17 is preferentially expressed in tail scale epidermis. At P14 and 1-month-old (1MO), the K31 pattern in the scale epidermis is apparent, and COL17 expression extends to both DEJ and K31-positive suprabasal layers. Suprabasal COL17 is present in the cytoplasm but not in α-

catenin-labeled intercellular locations, suggesting that suprabasal COL17 is non-functional and possibly degraded. 2) COL17 deficiency leads to more slender scales in the tail skin. Transgenic rescue by the expression of human COL17 (hCOL17) under the keratin 14 (K14) promoter in *Col17a1-/-* mice reversed the slender scale phenotype. 3) Whole-mount imaging showed that the scale size is smaller in aPKCλ eKO than in littermate controls, but its scale proportion (width/length ratio) is wider than that of controls, which is in contrast to the slender scales of *Col17a1-/-* mice. aPKCζ knockout (aPKCζ KO, *Prkcz-/-*) mice, which have no apparent skin phenotype, showed slightly larger scales, while the width/length ratio did not exhibit significant change. These results show that aPKC deregulation does not phenocopy *Col17a1-/-* scale shape. 4) Expression of wound-induced keratins is pronounced in *Col17a1-/-* tail epidermis. In contrast, normal human epidermal keratinocytes treated with COL17A1 siRNAs did not result in the expression of wound-induced keratins, suggesting that this phenotype is dependent on in vivo settings. 5) Scale shape becomes slender after skin regeneration, and hCOL17 overexpression rescues the phenotype. 6) Taking advantage of the revertant mosaicism in epidermolysis bullosa (EB), in which the mutated genes are corrected spontaneously, COL17-negative (diseased) and COL17-positive (revertant) skin are compared from a junctional EB (JEB) patient with *COL17A1* mutations. The diseased skin surface appears coarse, while the revertant skin is smooth. The autocorrelation functions of the skin images show that the diseased skin harbors a distinct pattern from the revertant skin. These date demonstrate that COL17 influences human skin microtopography.

【Discussion】COL17 is a hemidesmosomal protein that anchors basal keratinocytes to the dermis. As a consequence, COL17 deficiency results in epidermolysis bullosa. Recently, COL17 has also been highlighted as an SC niche protein of hair follicles and epidermis, and its deficiency destabilizes epithelial SC maintenance. Our study provides new insights into COL17 biology. *Col17a1-/-* mice have slender tail scales and are characterized by the expression of wound-induced keratins in the epidermis. In line with this, the regenerated epidermis after wounding shows slender tail scales. hCOL17 overexpression reverses the alteration of scale shapes upon wounding.

Although COL17 interacts with the aPKC complex and helps maintain epidermal cell polarity, *Col17a1-/-* does not exhibit proportionally small scales as seen in aPKCλ eKO mice. This phenotypical difference indicates that the slender scale phenotype in *Col17a1-/-* mice is independent of aberrant cell polarity. The slender scales in *Col17a1-/-* mice most likely represent wound-related skin changes that involve the expression of wound-induced keratins in *Col17a1-/-* epidermis. However, the mechanisms by which wound-related skin changes affect epidermal patterning need further investigation.

Our study has also demonstrated that the presence of COL17 alters human skin microtopography. These facts corroborate the role of COL17 in epidermal patterning and highlight COL17 as a therapeutic target for wound-induced skin deformations.

【Conclusion】Our study suggests that epidermal patterning reflects the behavior of epidermal SCs as well as the cellular cytoskeletal organization, both of which might be altered by dysfunctional niche proteins and minor injury. Our study sheds light on the role of the SC niche in tissue pattern formation. In conclusion, our study highlights the unrecognized role of COL17 in epidermal patterning. We propose that COL17 modulation can be utilized to prevent epidermal deformation upon wounding.