Stabilization of Hemidesmosomal Proteins: A Possible Key Contributor to Wnt/β-Catenin Pathway Action in the Skin

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Basal keratinocytes (KCs) attach to the extracellular matrix (ECM) through complexes that include hemidesmosomes (HDs) (Tsuruta et al., 2011). In the skin, HDs consist of transmembrane proteins (β6β4 integrin, collagen XVII [COL17], CD151), matrix proteins (laminin 332), and cytoplasmic proteins (plectin, BP230) and connect keratin filaments and ECM (Figure 1). In homeostatic conditions, HDs connect the dermis and epidermis, and variants of genes encoding HD components result in congenital blistering diseases termed epidermolysis bullosa. It has been long believed that the function of HDs relates exclusively to attachment of the epidermis to the dermis, but recent studies have revealed that HD components, especially COL17, are essential for normal motility, polarity, proliferation, and development.

COL17, a transmembrane protein also known as BP180 and BPAG2, is a major target protein for autoantibodies in the autoimmune subepidermal blistering disease bullous pemphigoid, and deficiency of COL17 in humans results in epidermolysis bullosa. In vitro, loss of COL17 decreases cell–matrix attachment strength and also affects cell migration with disturbances of actin dynamics at the lamellae (Hiroyasu et al., 2016). In collective cell migration, COL17 is required for KC locomotion, which creates spaces for cell proliferation in the basal layer, thereby increasing the proliferative capacity in KC stem cells (Nanba et al., 2021). COL17 is also highly expressed in hair follicle stem cells (HFSCs), where it exhibits an essential role in the maintenance of both HFSCs and melanocyte stem cells (MSCs) because loss of COL17 results in both hair loss and hair graying (Tanimura et al., 2011). COL17 in HFSCs has been suggested to stabilize the cells in hair bulges, contributing to a functional niche for MSCs. Thus, COL17 in KCs and HFSCs exhibits multiple roles in attachment, cell motility, polarity, proliferation, and development. Although the functions of HD components have been long studied in cell attachment, the involvement of HD proteins in skin wound healing, skin development, and hair differentiation should also be evaluated in various physiological and pathological conditions.

Wnt/β-catenin signaling has multiple important roles in organ development (Veltri et al., 2018). In the skin, Wnt signaling contributes to the development of placodes, hair, and epidermal stratification. Although the roles of Wnt/β-catenin signaling in skin development and differentiation have been well-recognized, recent studies indicated that Wnt signaling also contributes to other processes, including wound healing and tumorigenesis. Although both Wnt signaling and COL17 contribute to overlapping processes in the skin, such as wound healing and development of skin and hair, interactions of Wnt/β-catenin signaling and COL17 have not been studied before.

In their new article in the Journal of Investigative Dermatology, Kosumi et al. (2021) elucidated a link between the Wnt/β-catenin pathway and the HD components plectin and COL17. In this paper, inhibition of the Wnt/β-catenin pathway reduced HD-like structures in cultured epidermal KCs. Decreased HD-like structures corresponded to the altered localization of both plectin and COL17 from basal sides to the lateral sides of KCs both in vivo and in vitro. Because variants of plectin-encoding genes led to the reduction of COL17 at the basal side, the observed decreases of COL17 in HDs with Wnt inhibition were likely to be induced by displacement of plectin from HD. Finally, inhibition of atypical protein kinase C (aPKC) reversed the phenotype in Wnt-inhibited KCs, suggesting that Wnt signaling stabilizes plectin in HD through the inhibition of aPKC. Although previous literature indicated that HD components are upstream modulators of Wnt signaling (Watanabe et al., 2017), this study reported that Wnt signaling can be an upstream signal that stabilizes HD components. The authors proposed that stabilizing HD through Wnt signaling activation may be a treatment option for epidermolysis bullosa. In addition, as mentioned earlier, because recent studies...
revealed that COL17 contributes to cell attachment, motility, polarity, proliferation, and differentiation, various other events that are regulated by Wnt signaling, such as wound healing, skin development, and hair differentiation, might be induced through the regulation of COL17 localization.

In skin wound healing, Wnt/β-catenin signaling is activated soon after injury. Although Wnt signaling is well-recognized as an essential contributor to the regeneration of appendages such as hair and sebaceous glands in wound healing, it also increases wound healing capacity by increasing the proliferation of epidermal stem cells (Shi et al., 2015). Although upregulation of c-Myc has been indicated to be an inducer of escalating proliferation, other mechanisms have not been excluded. In light of the recent report indicating that COL17 promotes both motility and proliferation in KCs (Nanba et al., 2021), the data by Kosumi et al. (2021) may explain how Wnt signals possibly influence the proliferation of KCs stem cells independent from c-Myc. Instead, activated Wnt signaling may stabilize COL17 in HDs to increase motility, hence escalating the proliferation of the KCs in the wound edge.

This mechanism may also be operative while Wnt signaling pathway contributes to epidermal development. Wnt signaling-induced stabilization of COL17 may increase motility and cell proliferation and may hence contribute to skin development. Indeed, the study mentioned earlier showed that increased motility and proliferation in basal KCs promoted uniform stratification of KC sheets and contributed to epidermal development (Nanba et al., 2021). One conundrum regarding Wnt signaling in epidermal development is that Wnt/β-catenin depletion in the skin displays discrepant phenotypes in different model systems. Whereas keratin 14 Cre–driven β-catenin depletion shows hyperproliferation in mouse skin, Axin2 Cre–driven β-catenin depletion displays severe hypoproliferation (Veltri et al., 2018). Interestingly, COL17 in KCs has also been reported to display discrepant patterns in motility. Whereas multiple reports have indicated that KCs that are COL17 deficient exhibited decreased directional migration, locomotive speed, and wound closure speed (Hiroyasu et al., 2016; Nanba et al., 2021), other studies showed that COL17-deficient KCs move faster during an in vitro motility assay (Tasanen et al., 2004). These discrepant results suggest that the roles of COL17 in the motility and proliferation in KCs depend on the models and contexts that are being considered. The discrepant results in motility with COL17 deficiencies may relate to the discrepant phenotypes in skin development in Wnt/β-catenin–deficient models.

In contrast to the sustained proliferation of KCs during epidermal development, hair progresses through multiple cycles that are tightly regulated by a balance of BMP and Wnt/β-catenin signaling. At the onset of hair follicle regeneration (anagen phase), Wnt/β-catenin signaling is activated in HFSCs to induce the proliferation of HFSCs (Rabbani et al., 2011). Activation of the Wnt pathway in HFSCs also induces the secretion of Wnt ligands that activate the Wnt pathway in neighboring MSCs, stimulating MSCs to proliferate and differentiate. In the telogen phase, low levels of Wnt/β-catenin signaling are maintained, and this is critical for HFSCs to maintain their ability to differentiate into hair follicle lineage cells (Veltri et al., 2018). Coincidentally, COL17 in HFSCs is required for the maintenance of immaturity in both HFSCs and MSCs (Tanimura et al., 2011). Although the regulatory mechanisms underlying the hair cycle are too complicated to be adequately explained by a single factor, Wnt/β-catenin signaling may influence the characteristics of HFSCs through the regulation of COL17 stabilization.

**Clinical Implications**
- Wnt/β-catenin signaling stabilizes plectin and collagen XVII (COL17) in hemidesmosomes.
- Wnt/β-catenin pathway activation is proposed as a treatment option for epidermolysis bullosa.
- Wnt/β-catenin pathway action in the skin/hair may involve COL17 stabilization.

**Figure 1. Inhibition of Wnt/β-catenin signaling displaces plectin and COL17 from hemidesmosomes.** In the skin, hemidesmosomes consist of α6β4 integrin, COL17, CD151, laminin 332, plectin, and BP230, and they connect keratin filaments and extracellular matrix that includes COL7. Kosumi et al. (2021) show that plectin and COL17 are displaced from hemidesmosomes with inhibition of Wnt/β-catenin signaling. COL7, collagen VII; COL17, collagen XVII.
In summary, Kosumi et al. (2021) have identified COL17 displacement from HDs that occurs with inhibition of Wnt/β-catenin signaling. This phenotype was concurrent with αPKC-induced reduction of plectin in HDs. Although causality remains to be conclusively demonstrated, existing literature suggests that several physiological and pathological events can be influenced by Wnt/β-catenin signaling—induced COL17 stabilization in the epidermis. Because recent studies have elucidated the novel functions of COL17, exploring upstream signaling pathways that regulate COL17 localization or expression is gaining importance. Kosumi et al. (2021) propose a possible upstream pathway of COL17 regulation that may be involved in physiological or pathological events in the skin.

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CONFLICT OF INTEREST
The authors state no conflicts of interest.

REFERENCES

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Dermal Hedgehog Signaling in Papillary Fibroblasts: An Emerging Key Player in Skin Regeneration

Emanuel Rognoni


Skin morphogenesis and regeneration require the coordinated function of two layers: the outer epidermis and the underlying dermis. The epidermis is built of tightly packed keratinocytes, and the most common cells in the dermis are fibroblasts that are essential for structural integrity and are best known for their ability to deposit and remodel the extracellular matrix (ECM). Recent advances in single-cell RNA sequencing (scRNA-seq) and lineage tracing technologies have revealed impressive fibroblast heterogeneity in the skin and other organs (Shaw and Rognoni, 2020). However, our understanding of the functions of these subpopulations and how they are regulated by different signaling pathways is largely unknown.

During skin development, multipotent fibroblasts differentiate into distinct subpopulations that create the dermal sublayers: papillary dermis, reticular dermis, and dermal white adipose tissue (DWAT) (Rognoni and Watt, 2018). These fibroblast subpopulations differ in location and function, and their cell identity and composition change with age. Although papillary fibroblasts immediately beneath the basement membrane have an active Wnt signaling signature, show high Hedgehog (Hh) target gene expression, and are required for hair follicle (HF) formation, fibroblasts in the reticular dermal layer express genes associated with ECM and immune signaling at high levels and mediate the initial phase of wound repair. Dermal maturation is governed by a tight balance of fibroblast proliferation, quiescence, and ECM deposition during development. Notably, there is a coordinated switch in fibroblast behavior from highly proliferative in embryonic development to postnatal quiescence, allowing...