CANCER

Stem Cell Proliferation in the Skin: α -Catenin Takes Over the Hippo Pathway

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Epithelial tissues in the human body undergo constant renewal. For example, the skin is regenerated continuously through the periodic proliferation of normally quiescent stem cells in the basal compartment of the skin. Proper balance between stem cell quiescence and proliferation is maintained over the lifetime of the organism to preserve pools of stem cells required to maintain and repair tissues. However, mechanisms controlling the rate of stem cell renewal are poorly understood. Additionally, whether deregulation of these mechanisms within epidermal stem cells leads to skin cancer is not known. The adherens junction component α -catenin has been identified as a regulator of epidermal stem cell proliferation and as a suppressor of skin cancer through its inhibition of Yap, a transcriptional effector of the Hippo growth control pathway. Understanding the pathways that regulate the proliferation of stem cells in the skin holds promise for reversing the aging process and tumor development.

Precise control of organ growth and regenerative mechanisms that maintain tissue size and integrity are fundamental to human and animal biology; however, the mechanisms that control the extent of cell proliferation are surprisingly poorly understood (1). The Hippo signal transduction pathway is critical in controlling organ growth in *Drosophila* and vertebrates (2–4). The core of the Hippo pathway consists of a cascade that signals from the kinases Mst1 and Mst2 (Hippo in flies) to the kinases Lats1 and Lats2 (Warts in flies) to limit the activity of the Yes-associated protein (Yap; known as Yorkie in flies), a transcriptional coactivator that binds to the TEAD transcription factors to induce expression of target genes. Phosphorylation by Lats1/2 at Ser¹²⁷ causes cytoplasmic retention of Yap and thus inhibits its ability to induce transcription of target genes; mutation of this residue results in a form of Yap that constitutively localizes to the nucleus and is constitutively active (5, 6). In flies and mice, loss of

Hippo signaling or constitutive activation of Yorkie or Yap results in overgrowth of diverse tissues such as eyes and wings in flies and the liver and heart in mice (2–4, 7). Proper regulation of Yap is also critical for stem cell renewal and differentiation of cultured embryonic stem cells in vitro (8), and constitutive activation of Yap drives expansion of the stem and progenitor cells in the intestinal epithelium in mice (9). In the skin of newborn mice, the abundance and subcellular localization of Yap vary according to the proliferation status of the cell. Yap is present in the nuclei of most basal layer cells, which are actively proliferating, whereas in differentiating suprabasal cells, its abundance is reduced and its localization nonnuclear. Using mouse models to manipulate the extent of Yap activity in the skin, the Camargo and Fuchs groups found that increased activation of Yap was sufficient to drive increased proliferation of stem cells within the epidermis (10, 11). In *K14-Yap1* (S127A) transgenic mice, which express a mutant version of Yap with increased nuclear localization in K14-positive basal stem cells, proliferating basaloid epidermal cells were present suprabasally instead of being confined to the basal layer of the skin (10, 11). These results suggested, but did not directly demonstrate, that constitutively activated Yap induces proliferation of epidermal stem cells. To test this, Schlegelmilch *et al.* (10) cultured epidermal cells from the *K14-Yap1* (S127A) mice in vitro using a method that promotes epidermal stem cell renewal (12). Constitutively activated Yap triggered the epidermal stem cells to re-

new more frequently than in their wild-type counterparts. Conversely, mice deficient for *Yap* in K14-expressing cells exhibited fewer stem cells within the epidermis, had fewer hair follicles, and died shortly after birth owing to failure of the epidermal barrier, substantiating an essential role for *Yap* in skin stem cell proliferation. During epidermal differentiation, the daughters of epidermal stem cells in the hair bulge and basal layer migrate and stratify to form a layer of enucleated cells that create an impermeable membrane protecting the body (13). Yap (S127)—overexpressing epidermal stem cells not only continued to proliferate but also failed to initiate differentiation, as indicated by their expression of markers of stem cells rather than those of epidermal differentiation (10, 11). Therefore, Yap promotes the proliferation of epidermal stem and progenitor cells and maintains cells in an undifferentiated state, and down-regulation of Yap activity is required for terminal differentiation of epidermal cells.

The observed Yap gain- and loss-of-function phenotypes indicate that proper regulation of Yap activity is essential for normal skin development and maintenance. Yap is regulated by the Mst1/2 and Lats1/2 kinase cascade in other tissues, and a homologous cascade is conserved in flies. However, life is like a box of chocolates; you never know what you're gonna get. This was certainly true for the finding that Mst1/2 and possibly Lats1/2 are not essential to regulate stem cell proliferation and Yap activity in the skin (10), implying an alternative upstream regulator of Yap in epidermal stem cells. By mass spectrometry, they identified α -catenin, a component of adherens junctions that links the β -catenin–E-cadherin adhesion complex to the actin cytoskeleton, as a binding partner for Yap in high-density human keratinocytes. Under these conditions, Yap is inactive and nonnuclear, and some Yap colocalizes with α -catenin at the cell membrane and some localizes in the cytosol (10). α -Catenin bound indirectly to Yap through the adaptor protein 14-3-3, which was also identified in the Yap complexes by mass spectrometry, suggesting a tripartite complex composed of α -catenin, 14-3-3, and Yap. Knockdown of α -catenin in keratinocytes caused phosphorylation and nuclear localization of Yap but did not affect the activation status of Mst1/2 and Lats1/2, suggesting that α -catenin regulates Yap independently of these kinases. In vivo deletion of α -catenin in K14-positive

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cells within the epidermis and hair follicle similarly increased Yap localization in the nucleus, leading to an increased number of cells with high amounts of nuclear Yap, which sometimes persisted in cells within the suprabasal layers. Conversely, overexpression of α -catenin in low-density cultures led to the relocalization of Yap from

are induced to terminally differentiate, α -catenin is required for Yap translocation from the nucleus and for epidermal basal cells to exit the cell cycle and stratify (Fig. 1A). Phosphorylated Yap within a complex of 14-3-3 and α -catenin was protected from PP2A-mediated dephosphorylation, possibly by directly competing for binding.

often observed in human tumors (16, 17), suggesting that α -catenin might be a tumor suppressor. Silvis *et al.* conditionally deleted α -catenin in the bulge region and dermal papilla of the hair follicle, the niche for stem cells of the skin (14). Similar to the Camargo group, they found that these mice were more prone to developing squamous cell carcinomas (SSCs) because of an expansion of proliferating Sox9-positive stem cells and early progenitor cells (Fig. 1B). These data confirmed a tumor-suppressive role for α -catenin in the skin. They then isolated *Yap* in an RNA interference screen to identify genes required for proliferation of α -catenin-deficient keratinocytes in vitro. Like the Camargo group, the Vasioukhin laboratory found that Yap localization was predominantly nuclear in confluent α -catenin-deficient keratinocytes, whereas in wild-type cells, confluence triggers the export of Yap from the nucleus. Yap was found in a complex with α -catenin in keratinocytes, and overexpression of α -catenin reduced the transcriptional activity of Yap in kidney epithelial cells, whereas knockdown of α -catenin in keratinocytes resulted in increased Yap activity. These data indicate that α -catenin inhibits Yap activity in vitro and in vivo.

Thus, α -catenin is an upstream regulator of the Hippo pathway effector Yap in skin and suppresses the formation of squamous cell carcinoma through Yap. Moreover, regulation of Yap by α -catenin controls the proliferation and terminal differentiation of epidermal stem cells. These findings have important ramifications for our understanding of processes in the skin where the regulation of stem cell proliferation is critical, such as aging of the skin, wound healing, and skin cancers. Additionally, Yap may be regulated in a similar manner by α -catenin in other organs of epithelial origin. Stem cells within other epithelial tissues—such as the intestine, colon, lung, and mammary gland—have a life cycle similar to that of stem cells that reside in the skin (13, 18–20). The findings from the Camargo and Vasioukhin groups have provided a critical connection between α -catenin and the Hippo pathway that may be exploited to control the proliferation of epidermal stem cells in multiple diseases of the skin. It will be exciting to investigate whether α -catenin, adherens junction components, and other inhibitors of Yap in stem cells and cancer stem cells of epithelial tissues can be manipulated to suppress tumorigenesis.

Fig. 1. Colocalization of α -catenin and Yap is critical for terminal differentiation of keratinocytes and suppression of cutaneous squamous cell carcinoma. (A) Schematic of a normal stratified epidermis with Sox9-positive stem cells residing in the hair bulge and dermal papilla (red) and Sox9-negative stem cells in the basal layer (purple). Yap (yellow) is present in the nuclei of stem cells. As cells divide, stratify, and differentiate, Yap is sequestered in the cytosol in a process that requires interaction with α -catenin (blue cells). Such cells then differentiate into enucleated squames that make up the outermost layer of the skin (beige). (B) Squamous cell carcinoma forms in α -catenin-deficient skin. Loss of α -catenin from epidermal stem cells results in an expansion of Sox9-positive stem cells (red), which hyperproliferate in the hair bulge and basal layer.

the nucleus to the membrane. These data thus support a model in which α -catenin is required to recruit Yap to adherens junctions. However, knockdown of other adherens junction components, such as β -catenin and E-cadherin, did not affect the localization or activity of Yap. Therefore, the increased activation of Yap in α -catenin-deficient cells is not simply a consequence of loss of cell adhesion. Rather, α -catenin seems to be involved in a specific mechanism that operates at the adherens junctions to regulate Yap. Thus, as epidermal cells

Thus, α -catenin may regulate Yap activity by protecting the inactive, phosphorylated form of Yap from activation by PP2A. Whether the interaction between Yap and 14-3-3- α -catenin is modulated to regulate Yap activity is currently not known, but it is an intriguing possibility to be investigated in the future.

The Vasioukhin group also identified the α -catenin–Yap interaction in the context of tumor suppression (14). Loss of α -catenin results in loss of contact inhibition in keratinocytes cultured in vitro (15) and is

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