Role of β -catenin in Epidermal Stem Cell Expansion, Lineage Selection, and Cancer

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The mammalian epidermis is an excellent model with which to analyze the factors that regulate adult stem cell renewal, lineage selection, and tumor formation. One of the key regulators of all three processes is β -catenin, the main cytoplasmic effector of the canonical Wnt signaling pathway. In this chapter, we review some of the ways in which β -catenin exerts its effects on cultured human epidermal cells and in genetically modified mice. We highlight the importance of the timing and level of activation and discuss some of the pathways activated downstream from β -catenin. Finally, we demonstrate the importance of Lef/Tcf-independent β -catenin signaling through interaction with the vitamin D receptor.

The epidermis provides a protective interface between the body and the environment. The main epidermal cell type is an epithelial cell, the keratinocyte. The major structures of the epidermis are the interfollicular epidermis (IFE), hair follicles (HFs), sweat glands, and sebaceous glands (SGs). In human epidermis, the sweat glands are present in both hair-bearing and hairless regions, whereas in mouse epidermis, the sweat glands are restricted to the paws. Because most *in vivo* studies are performed on the mouse, we will not refer to the sweat glands further in this chapter (Fig. 1).

To fulfill their functions, keratinocytes within the epidermis undergo terminal differentiation, resulting ultimately in cell death. Thus, the outermost, cornified, layers of the IFE, which form a physical barrier to penetration by microorganisms and prevent water loss, are anucleate cells

filled with heavily cross-linked proteins and lipids. The cells of the hair shaft are also dead cells that are replaced during the hair growth cycle. Furthermore, during terminal differentiation, SG cells burst, releasing their lipid content onto the hairs and the surface of the skin to provide lubrication.

Because the terminally differentiated cells of the epidermis are dead cells and are continually shed from the skin surface, it was appreciated many decades ago that the tissue must be maintained by proliferation of less-differentiated cells that both replenish themselves and the terminally differentiated cells. Thus, the epidermis is one of the tissues in which it has long been acknowledged that there resides a stem cell compartment (Hall and Watt 1989). Our current view is that there are distinct pools of stem cells in discrete locations within the HF, IFE, and SG

Figure 1. Organization of adult mouse epidermis. The epidermis is shown overlying the dermis and fat layers of the skin. (IFE) Interfollicular epidermis, comprising the BE, SBE, and CL. (BE) Basal epidermal layer; (SBE) suprabasal epidermal layers; (CL) cornified layers; (SG) sebaceous gland. Different regions of the HFs are shown, including the outer root sheath (ORS) and inner root sheath (IRS). (DP) Dermal papilla, the specialized mesenchymal cells at the base of the HFs.

(Owens and Watt 2003). When the epidermis is undamaged, each stem cell pool is responsible for maintaining a restricted number of differentiated lineages, such that IFE stem cells give rise to the differentiated lineage of the IFE, SG stem cells to the differentiated lineage of the SG, and HF stem cells to the eight lineages of the HF. However, when the epidermis is damaged or subject to genetic manipulation, stem cells in any location can give rise to any of the differentiated lineages of the epidermis (Watt et al. 2006; Jones et al. 2007).

The skin is an excellent tissue with which to investigate the factors that regulate adult stem cell renewal, lineage selection, and tissue assembly. In addition, it has long provided an experimental model for investigating cancer, from the earliest initiating events through to metastasis. In this chapter, we review the tools that are available to study these processes and some of the lessons that we have learned. One of the striking conclusions is that the principles of stem cell regulation in the epidermis can be applied much more widely to other adult and embryonic stem cells in a variety of organisms.

THE TOOL KIT

We have taken two complementary approaches to identify and manipulate epidermal stem cells: cultivation of human cells and generation of transgenic mice. The first approach is based on the discovery, in 1975, that cells isolated from human epidermis can be grown at clonal density and will form confluent multilayered sheets, in which proliferation occurs in the basal layer and IFE differentiation occurs in the suprabasal layers (Rheinwald and Green 1975; Watt 1998). Sheets of autologous cultured human epidermis can provide a permanent and functional IFE used to replace skin lost through burn injury. This led to the conclusion that clonal analysis of human epidermal cells could be used as a readout of stem cell activity (Barrandon and Green 1987; Jones and Watt 1993).

Different types of clonal growth assays are used, but in each case, stem cells are assigned to the category of cells capable of generating large numbers of both undifferentiated and differentiated progeny, whereas cells that show limited self-renewal ability and have a high probability of undergoing terminal differentiation are usually referred to as transit-amplifying cells (Jones et al. 2007). In vivo assays of function have included reconstitution of IFE following grafting of human keratinocytes into nude mice (Jones et al. 1995). The ability of the progeny of a single epidermal cell to reconstitute IFE, HF, and SG has been demonstrated for rodents, although not, so far, for humans (Blanpain et al. 2004; Claudinot et al. 2005). Retroviral vectors have been used to manipulate gene expression in human keratinocytes, making use of the high infection efficiency and minimal selection pressure involved (Gerrard et al. 1993; Levy et al. 1998).

One limitation of studies with cultured human keratinocytes has been that only differentiation along the IFE lineage can be examined. However, this is changing, with the discovery that cells immortalized from human SG can generate progeny that select the IFE and SG differentiation pathways in culture or after grafting into nude mice (Lo

Celso et al. 2008). Thus, in addition to investigating the factors that trigger exit from the stem cell compartment, we can begin to explore, at the single-cell level, the factors that regulate lineage selection (Lo Celso et al. 2008).

Studies of the stem cell compartment in mouse epidermis have been facilitated by characterization of promoters that drive transgene expression to different subpopulations of cells (Vassar et al. 1989; Carroll et al. 1993; Greenhalgh et al. 1993). The promoter that is used most frequently is the keratin 14 (K14) promoter, which targets basal layer cells, including stem cells, in the HF, SG, and IFE (Vassar et al. 1989). More recent refinements have been to selectively target the reservoir of stem cells in the HF bulge, via the K15 promoter (Liu et al. 2003) and to use inducible transgenes, so that the location and timing of transgene expression or deletion can be controlled, for example, by application of estrogen or progesterone analogs to the skin (Pelengaris et al. 1999; Vasioukhin et al. 1999; Wang et al. 1999). Recent studies show that it is possible to regulate the level of transgene expression by applying different concentrations of an inducing agent (Silva-Vargas et al. 2005).

Using keratinocyte cultures or transgenic mice as an assay system, a number of different markers for epidermal stem cells and the different differentiated lineages have been identified over the years. The first cell surface marker used to enrich for human epidermal stem cells was expression of high levels of integrin extracellular matrix receptors (Jones and Watt 1993; Jones et al. 1995); integrins now turn out to be a useful marker of stem cells in a range of other tissues, such as breast (Shackleton et al. 2006). The introduction of whole-mount preparations, for both human and mouse epidermis, has provided information about the spatial organization of cells in the different compartments and facilitates gathering of quantitative data (Jensen et al. 1999; Braun et al. 2003; Silva-Vargas et al. 2005). Finally, techniques for generating spontaneously immortalized mouse keratinocyte lines and for culturing primary adult mouse keratinocytes at clonal density have improved in recent years (Romero et al. 1999; Silva-Vargas et al. 2005; Wu and Morris 2005), providing the opportunity for direct comparisons between the behavior of mouse epidermal stem cells in culture and in vivo.

THE WNT PATHWAY

The pathway that is of central importance in regulating epidermal stem cell renewal and lineage selection is the Wnt pathway. The key components of the Wnt signaling pathway are summarized schematically in Figure 2 (Klaus and Birchmeier 2008). In the absence of a Wnt ligand, cytoplasmic β -catenin is rapidly degraded (Fig. 2a). However, when Wnt binds to cell surface receptors, the β -catenin destruction complex is inactivated. Thereafter, β -catenin accumulates in the cytoplasm and then translocates to the nucleus. In the nucleus, β -catenin forms a transcriptionally active complex with the Lef and Tcf transcription factors by displacing Groucho transcriptional repressors and interacting with coactivators such as CBP and Pygopus (Fig. 2b).

Within the epidermis, there are several ways in which the Wnt pathway can be regulated, one of which is via differential expression of pathway components. Different

a

b

Figure 2. Diagram of the canonical Wnt signaling pathway. (a) In the absence of Wnt, the pathway is inactive; (b) the pathway is activated when Wnt binds to its receptor complex. (Two parallel lines) Plasma membrane; (dotted line) nucleus. (DKK) Dickkopf; (LRP) LDL-related receptor protein; (SFRP) secreted Frizzled-related protein; (WIF) Wnt inhibitory factor 1; (DVL) Dishevelled; (β -TrCP) β -transducin repeat-containing protein; (CK1) casein kinase 1; (GSK3 β) glycogen synthase kinase 3 β ; (APC) adenomatous polyposis coli; (TCF) T-cell factor; (Lef) lymphoid enhancer factor; (Pygo) Pygopus; (CBP) CREB-binding protein; (P) phosphorylation; (Ub) ubiquitylation; (N) amino terminus; (C) carboxyl terminus. (Redrawn from Klaus and Birchmeier 2008.)

members of the Wnt family are expressed in specific subsets of cells in developing and adult epidermis; expression of *Frizzled* genes and Wnt antagonists is also dynamically regulated (Reddy et al. 2001, 2004; Sick et al. 2006). Lef1 and Tcf3, the main transcriptional effectors of the Wnt pathway in the epidermis, are expressed in different cell subpopulations (DasGupta and Fuchs 1999; Merrill et al. 2001). In the absence of Wnt, Tcf3 acts as a transcriptional repressor: Tcf3-repressed genes include transcriptional regulators of the IFE, SG, and HF lineages (Alonso and Fuchs 2003; Nguyen et al. 2006). Finally, in addition to its role as the key cytoplasmic effector of the Wnt pathway, β -catenin is a component of intercellular adhesive junctions, where it binds to the cytoplasmic domain of E-cadherin via the same amino acid residues as required for binding to adenomatous polyposis coli (APC) (Fig. 2) (Huelsen et al. 1994). Although β -catenin is not essential for adherens junction formation in the epidermis (Huelsen et al. 2001), due in part to coexpression with the related protein plakoglobin/ γ -catenin, the ability of E-cadherin to bind β -catenin can lead to depletion of the pool of β -catenin available for Wnt signaling (Zhu and Watt 1999).

β -CATENIN IN CULTURED KERATINOCYTES

Our laboratory's interest in β -catenin originated not from its role in the Wnt pathway, but from its role in intercellular adhesion. We had previously established that integrin-mediated cell-extracellular matrix adhesion is a negative regulator of epidermal terminal differentiation (Adams and Watt 1989; Watt 2002) and found that cell-cell adhesion influences integrin expression and localization (Hodivala and Watt 1994). We therefore became interested in whether cell-cell adhesion regulates terminal differentiation. To study this, we infected human keratinocytes with a retroviral vector encoding the extracellular domain of H-2Kd and the transmembrane and cytoplasmic domains of E-cadherin (H-2Kd-E-cad), in order to inhibit adherens junction assembly. As a control, we deleted the binding site for β -catenin and plakoglobin (H-2Kd-E-cad Δ C25) (Zhu and Watt 1996). As anticipated, cell-cell adhesion and stratification were inhibited. However, unexpectedly, the dominant-negative mutant had an inhibitory effect on keratinocyte proliferation and stimulated terminal differentiation. Terminal differentiation was stimulated even under conditions in which intercellular

adhesion was prevented by disaggregating keratinocytes and holding them in suspension (Fig. 3a) (Zhu and Watt 1996).

In an attempt to discover why overexpression of the E-cadherin cytoplasmic domain could stimulate differentiation of disaggregated cells, we began to study β -catenin. We found that the subpopulation of cultured human keratinocytes with high proliferative potential, the putative epidermal stem cells, had a larger pool of noncadherin-associated β -catenin than transit-amplifying cells (Zhu and Watt 1999). Retroviral expression of stabilized amino-terminally truncated β -catenin ($\Delta N\beta$ -catenin) increased the proportion of putative stem cells to almost 90% of the proliferative population in vitro, without blocking terminal differentiation or changing cell cycle kinetics (Zhu and Watt 1999). Furthermore, $\Delta N\beta$ -catenin expression rescued keratinocytes from the differentiation stimulatory effect of overexpressing the E-cadherin cytoplasmic domain (Fig. 3b) (Zhu and Watt 1999). Conversely, β -catenin lacking armadillo repeats acted as a dominant-negative mutant and stimulated exit from the stem cell compartment in culture. The positive and negative effects of the β -catenin mutants on proliferative potential were independent of effects on intercellular adhesion.

The concepts that stem cells have a higher pool of β -catenin available for Wnt signaling than their more differentiated progeny and that β -catenin activation can expand the stem cell pool have subsequently been confirmed in work on hematopoietic stem cells (Reya et al. 2003) and other tissues.

β -CATENIN IN MOUSE EPIDERMIS

While our lab was examining intercellular adhesion of cultured keratinocytes, it was reported that expression of amino-terminally truncated β -catenin under the control of the K14 promoter led to formation of additional HF in transgenic mice (Gat et al. 1998). This led us to wonder whether the phenotype was one of stem cell expansion, as would be predicted from our in vitro experiments (Zhu and Watt 1999). If so, inhibition of Wnt signaling should lead to a failure to maintain the epidermis through stem cell depletion. We tested this by using the K14 promoter to overexpress an amino-terminally truncated form of Lef1 that cannot bind β -catenin ($\Delta N\text{Lef1}$) and thereby acts as a dominant-negative inhibitor of canonical Wnt signaling (Niemann et al. 2002). The result was clear: Stem cell depletion did not occur; rather, the effect was to convert hair follicles into cysts of interfollicular epidermis with ectopic sebocytes. Our interpretation—that in vivo, β -catenin directed lineage selection rather than maintaining the stem cell compartment—was in agreement with other reports of the effects of overexpressing a different $\Delta N\text{Lef1}$ transgene (Merrill et al. 2001), of deleting β -catenin in the epidermis (Huelsen et al. 2001), or of inhibiting the pathway by overexpressing Dkk1 (Andl et al. 2002).

In subsequent work, we have examined, in some depth, the effect of selectively activating β -catenin in adult mouse epidermis. Our approach has been to overexpress $\Delta N\beta$ -catenin fused at the carboxyl terminus to the ligand-binding domain of a mutant estrogen receptor ($\Delta N\beta$ -cateninER) (Fig. 4) (Lo Celso et al. 2004). By topically applying the

Figure 3. β -catenin-induced stem cell expansion in cultured human epidermis. (a) Stimulation of terminal differentiation by dominant-negative E-cadherin in adherent postconfluent cultures and in single-cell suspensions. The proportion of involucrin-positive keratinocytes in pre-confluent cultures (Before suspension), confluent cultures and following 24 hours suspension in methyl cellulose (After suspension) is shown. Expression of the dominant-negative E-cadherin mutant H-2Kd-E-cad (E) stimulates differentiation relative to cells expressing a version of the construct in which the β -catenin-binding site is mutated, H-2Kd-E-cad $\Delta C25$ (Δ) (* $P < 0.005$). (K) Uninfected keratinocytes; (P) keratinocytes transduced with empty retroviral vector. Error bars represent standard deviation. (b) Rescue of growth by β -catenin. Keratinocytes expressing H-2Kd-E-cad (E) or H-2Kd-E-cad $\Delta C25$ (Δ) were doubly infected with a retroviral vector encoding stabilized β -catenin (T2) or empty vector (puro) and plated at equal density in 35-mm dishes. Triplicate dishes were harvested on the days shown and cell numbers were determined. Error bars represent standard deviation of the mean. (a, Reprinted, with permission, from Zhu and Watt 1996 [© Company of Biologists]; b, reprinted, with permission, from Zhu and Watt 1999 [© Company of Biologists].)

inducing agent 4-hydroxy-Tamoxifen (4OHT), we can control when, where, and how long β -catenin is activated. In addition, we can induce different levels of β -catenin-dependent transcriptional activity with different doses of

Figure 4. β -catenin-induced ectopic HF formation in adult mouse epidermis. (a) Nontransgenic tail skin treated with 4OHT; (b) K14 Δ N β -cateninER tail skin treated with 4OHT for 21 days. (SG) Sebaceous gland; (IFE) interfollicular epidermis; (EF) ectopic hair follicles. Bar, 100 μ m.

4OHT. Finally, by examining epidermal whole-mount preparations, we can obtain quantitative data about the number and location of the ectopic HFs that form in response to β -catenin activation (Silva-Vargas et al. 2005). From our studies, we conclude that different regions of the epidermis exhibit differential sensitivity to a given level of β -catenin induction and that optimal HF morphogenesis is not achieved with the highest level of β -catenin signal, because under those circumstances, proliferation tends to predominate (Silva-Vargas et al. 2005).

The K14 Δ N β -cateninER model has allowed us to demonstrate that β -catenin-induced ectopic follicles contain cells with characteristics of HF stem cells (CD34, K15, and high levels of α 6 integrin expression; clonal growth in vitro) and thus that, as in vitro (Zhu and Watt 1999), β -catenin activation can result in expansion of the stem cell compartment (Silva-Vargas et al. 2005). We have also shown, using lineage tracing, that ectopic follicles induced in the interfollicular epidermis are not obligatorily clonal in origin and are derived from the IFE, rather than the neighboring HF (Silva-Vargas et al. 2005). The latter finding has since been confirmed using a different experimental strategy (Ito et al. 2007).

The overall conclusion from our studies and those of other investigators is that β -catenin activation promotes differentiation of the HF lineages in embryonic and adult epidermis and can, under certain circumstances, expand the stem cell compartment (Alonso and Fuchs 2003; Ito et al. 2007; Närhi et al. 2008; Zhang et al. 2008). In addition to promoting the HF lineages, there are indications that β -catenin signaling negatively regulates SG differentiation (Huelsken et al. 2001; Merrill et al. 2001; Niemann et al. 2002; Lo Celso et al. 2004) and actively suppresses IFE differentiation in developing skin (Närhi et al. 2008; Zhang et al. 2008). This supports the concept that lineage selection involves both active transcription of the set of genes corresponding to the chosen lineage and repression of those in the lineage that has not been selected (Nguyen et al. 2006).

Although there is evidence that β -catenin signaling suppresses the IFE and SG lineages in vivo, this does not appear to be the case in culture. The expansion of the stem cell compartment that we observed in cultured human IFE keratinocytes was not correlated with impaired terminal differentiation, as evaluated by stratification and expression of involucrin, a marker of the differentiating layers of the IFE and the HF inner root sheath (Zhu and Watt 1999). Furthermore, activation of β -catenin in bipotential SG cells increases the proportion of cells that express involucrin, without decreasing the proportion that differentiate into mature sebocytes (Lo Celso et al. 2008). There are a number of potential reasons for the difference between the in vivo and in vitro results. One possibility is that the effects of β -catenin activation in vitro reflect the fact that the culture environment is not permissive for HF morphogenesis and that, as a result, the stem cell compartment expands. Another possibility is that the in vivo response to β -catenin is heavily dependent on reciprocal interactions with the underlying mesenchyme, which are not recreated in culture. These are interesting issues that remain to be explored.

HOW DOES β -CATENIN EXERT ITS EFFECTS ON ADULT EPIDERMIS?

To examine the pathways that act downstream from β -catenin to induce stem cell expansion and ectopic follicle formation, we performed Affymetrix microarray analysis using RNA isolated from total back skin of K14 Δ N β -cateninER mice treated for 7 days with 4OHT (Silva-Vargas et al. 2005). Bearing in mind that only the basal layer of the epidermis expresses the transgene, this search was definitely not restricted to direct β -catenin target genes. Nevertheless, in validation of the approach, we did identify a number of direct target genes, including many HF keratin genes (Zhou et al. 1995).

One of the pathways that was identified as up-regulated in response to β -catenin activation was the Hedgehog signaling pathway, with Sonic hedgehog (Shh) showing a 70-

fold increase in RNA level (Lo Celso et al. 2004; Silva-Vargas et al. 2005). To test the functional significance of this, we treated K14ΔNβ-cateninER epidermis with cyclopamine, a pharmacological inhibitor of Hedgehog signaling, at the same time as β-catenin was activated with 4OHT. The effect was to reduce the local increases in epidermal proliferation that occur on β-catenin activation. Cyclopamine treatment in combination with modest activation of β-catenin blocked ectopic HF formation and prevented existing follicles from entering anagen. However, in the presence of higher β-catenin activity, the effect of blocking Hedgehog signaling was to improve HF morphogenesis. The conclusion is that Shh signaling drives the local increases in proliferation required for ectopic HF formation but that excessive proliferation impairs morphogenesis (Silva-Vargas et al. 2005).

Another pathway that we identified as being induced on β-catenin activation is the Notch pathway. It has previously been well documented that this pathway is not required for HF development during embryogenesis; however, it is necessary for postnatal HF maintenance (Watt et al. 2008a). We identified one of the Notch ligands, *Jagged1*, as a direct β-catenin target gene. We further found that inhibition of the Notch pathway pharmacologically, with a γ-secretase inhibitor, or by genetic ablation of *Jagged1*, prevented β-catenin-induced ectopic HF formation (Estrach et al. 2006). By crossing K14ΔNβ-cateninER mice with mice in which Notch activation is inducible with 4OHT (K14N^{ICDΔOP}ER mice), we showed that the combined activation of both pathways did not give a sustained increase in HF formation relative to β-catenin alone. However, the follicles that did form in the double transgenics were more highly differentiated than in K14ΔNβ-cateninER single transgenics, as judged from their length and the markers of HF differentiation expressed (Estrach et al. 2006). As in the case of β-catenin activity, it appears that different levels of Notch activity result in different epidermal responses. In addition, non-cell-autonomous signaling between cells with different levels of Notch ligands can contribute both to exit from the stem cell compartment and to stem cell patterning (Watt et al. 2008a).

Several other important pathways that intersect with the Wnt pathway to regulate the epidermal stem cell compartment have been characterized (Fuchs 2008). However, one that stands out because it is so surprising involves the proto-oncogene c-Myc (Watt et al. 2008b). Myc is a β-catenin target gene and is the key effector of β-catenin-induced proliferation in intestine (Klaus and Birchmeier 2008). Nevertheless, in skin, β-catenin and Myc exert quite different effects. Whereas β-catenin can expand the stem cell compartment and induce ectopic follicle formation, Myc can deplete the stem cell compartment and promote IFE and SG differentiation (Watt et al. 2008b). In sebocyte cultures, β-catenin stimulates IFE differentiation, whereas Myc stimulates SG differentiation (Lo Celso et al. 2008). Myc, like β-catenin, can stimulate Hedgehog signaling; however, whereas β-catenin signaling increases Shh expression, Indian hedgehog (*Ihh*) is a direct Myc target gene (Lo Celso et al. 2008).

To examine whether activation of Myc and β-catenin are mutually antagonistic, we crossed K14ΔNβ-cateninER

transgenic mice with mice in which Myc is activated by 4OHT treatment (K14MycER; Arnold and Watt 2001). In these bitransgenic mice, there was inhibition of both β-catenin-induced ectopic follicle formation and Myc-induced SG differentiation (Lo Celso et al. 2008). Clearly, the key to understanding the different effects of β-catenin and Myc in the epidermis lies in defining the different effectors with which Myc and β-catenin interact. Nevertheless, our existing observations provide a very clear example of the context-dependent consequences of activating the same signaling pathways in different cell types.

TCF/LEF1-INDEPENDENT β-CATENIN SIGNALING

Although the classical transcriptional effector of the canonical Wnt pathway is the β-catenin/Tcf/Lef complex (see Fig. 2b), there is growing evidence that the Wnt pathway can activate Tcf/Lef-independent genes, as the following examples illustrate. Deletion of the Tcf/Lef-binding sites in the P-cadherin promoter does not prevent β-catenin from inducing P-cadherin gene transcription (Faraldo et al. 2007). β-catenin regulates the myogenic basic helix-loop-helix (bHLH) transcription factor MyoD via a Tcf/Lef-independent mechanism that involves β-catenin binding to MyoD and enhancing MyoD binding to E-box elements (Kim et al. 2008). During pituitary gland development, the paired-type homeodomain transcription factor *Prop1* forms a complex with β-catenin, leading to both transcriptional activation of the lineage-determining transcription factor *Pit1* and repression of the lineage-inhibiting transcription factor *Hesx1* (Olson et al. 2006). Finally, β-catenin is known to bind to and activate the vitamin D receptor (VDR), which acts via vitamin D response elements (Pálmer et al. 2001; Shah et al. 2006).

The VDR, like β-catenin, is essential for postnatal maintenance of HFs. VDR null mice fail to undergo the first postnatal hair cycle; instead, the HFs convert to cysts of IFE, thereby resembling the phenotype of K14ΔN^{Lef1} mice (Sakai et al. 2001; Niemann et al. 2002). When examining the epidermal genes that are up-regulated on 4OHT treatment of K14ΔNβ-cateninER mice, we found that many, including the bulge stem cell marker keratin 15, contain vitamin D response elements (VDREs) and that several are induced independently of Tcf/Lef (Pálmer et al. 2008a).

By crossing K14ΔNβ-cateninER mice with VDR null mice, we were able to show that the VDR is required for β-catenin-induced ectopic HF formation. Conversely, application of a vitamin D analog in combination with 4OHT stimulates the differentiation program within the HFs to a greater extent than activating β-catenin alone (Pálmer et al. 2008a).

Although it has been reported that VDR ablation results in gradual depletion of the HF stem cell pool (Cianferotti et al. 2007), in our hands, the degeneration of VDR null follicles does not reflect a loss of follicle stem cells (Pálmer et al. 2008b). Furthermore, in response to the phorbol ester TPA, (12*O*-tetradecanoylphorbol-13-acetate) VDR null bulge cells, like wild-type bulge cells, are competent to proliferate. We observed that in degener-

ating VDR null follicles, there was extensive internalization of the $\alpha 6 \beta 4$ integrin and that this correlated with reduced migration of VDR null keratinocytes in culture (Pálmer et al. 2008b). We therefore suggest that the failure of VDR null epidermis to maintain HF in adult life is due, at least in part, to a failure of the cells to migrate along the follicle during anagen.

Our model for the interactions among β -catenin, Tcf/Lef, and VDR is shown schematically in Figure 5. In wild-type epidermis, initiation of the growth phase of the HF cycle (anagen) is dependent on activation of Wnt signaling, and in the absence of that stimulus, the HFs are in the resting (telogen) phase. However, in the absence of the VDR, the hair growth cycle cannot be maintained and the follicles degenerate. A high level of Wnt signaling, in the presence of endogenous vitamin D or a topically applied vitamin D analog, not only triggers anagen, but can also induce ectopic HFs. In the absence of the VDR, Wnt activation is unable to induce differentiation of ectopic follicles.

β -CATENIN IN TUMORS

A final issue to consider is how the information we obtain about normal stem cell renewal and differentiation impacts our understanding of disease mechanisms. One clear message is that tumor type within the epidermis is determined, at least in part, by the same pathways that regulate normal differentiation (Table 1) (Owens and Watt 2003). Prolonged activation of β -catenin in transgenic mice

Table 1. Different skin tumor types associated with different types of genetic alteration

Genetic change	Tumor type
β -catenin activation	pilomatricoma
β -catenin activation in absence of VDR	basal cell carcinoma
Ras activation	papilloma, squamous cell carcinoma
Δ NLef1	sebaceous adenoma, seboma
Ras and Δ NLef1	sebaceous adenoma, seboma

is sufficient to induce benign HF tumors (pilomatricomas; Fig. 5) (Gat et al. 1998), although these regress when the pathway is no longer active (Lo Celso et al. 2004). The importance of β -catenin activation extends to human HF tumors, because human pilomatricomas have been found to harbor activating β -catenin mutations (Chan et al. 1999).

Wnt signaling is frequently activated inappropriately in tumors (Klaus and Birchmeier 2008), and, consistent with this, deletion of β -catenin renders the epidermis resistant to developing chemically induced tumors (Malanchi et al. 2008). It is therefore surprising that the K14 Δ NLef1 mice generated in our laboratory develop SG tumors with high frequency (Table 1) (Niemann et al. 2002). The tumor type is consistent with the ability of the Δ NLef1 transgene to promote sebocyte differentiation (Niemann et al. 2002). Furthermore, we have found that one third of human SG tumors that we examined has mutations in the amino terminus of LEF1, which block β -catenin binding (Takeda et al. 2006).

Figure 5. Interaction among β -catenin, Lef/Tcf transcription factors, and the VDR. The epidermal phenotypes and Wnt/VDR transcriptional activity of wild-type and VDR^{-/-} mice are compared in the presence or absence of β -catenin activation in 4OHT-treated K14 Δ N β -cateninER transgenic mice. (EF) Ectopic hair follicle; (BCC) basal cell carcinoma. Tcf/Lef and VDR are shown bound to DNA in the presence of transcriptional corepressors (R) or β -catenin. Vitamin D ligand is indicated by an asterisk. (Based on Pálmer et al. 2008a.)

Figure 6. Patched1 is expressed in basal cell carcinoma-like lesions induced by β -catenin in the absence of the VDR. (a) Bright- and (b) dark-field images of epithelial mass induced by 4OHT treatment of K14 $\Delta\beta$ -cateninER $\times$ VDR^{-/-} mouse skin. Radioactive in situ hybridization using the Ptc1 probe. Skin had been treated with 0.5 mg of 4OHT for 21 days. Bar, 100 μ m.

There is evidence that Δ NLeF1 not only determines tumor type, but also acts as a tumor promoter. In chemical carcinogenesis protocols, K14 Δ NLeF1 transgenics develop tumors in response to DMBA (dimethylbenz[a]anthracene) treatment (tumor initiator) alone, whereas wild-type mice do not (Niemann et al. 2007). One of the ways in which Δ NLeF1 acts is by blocking induction of the tumor suppressor gene *p53* (Niemann et al. 2007).

Evidence also exists that the intersection of the Wnt pathway with other pathways can determine tumor type (Table 1). During chemical carcinogenesis, DMBA is applied to induce Ha-Ras mutations, and this is followed by repeated applications of the phorbol ester tumor promoter TPA. DMBA/TPA treatment of wild-type mouse skin results in the development of papillomas and squamous cell carcinomas, which have the differentiation characteristics of IFE (Owens and Watt 2003). However, in the presence of the Δ NLeF1 transgene, the type of tumors that develop on DMBA-mediated Ras mutation are SG tumors (Table 1) (Niemann et al. 2007).

A further example is that prolonged activation of β -catenin in the absence of the VDR results in the development not of pilomatricomas but of undifferentiated tumors resembling basal cell carcinomas (Table 1; Fig. 5) (Pálmer et al. 2008a). The tumors show evidence of up-regulated Ptc1 expression, a feature of basal cell carcinomas (Fig. 6). Conversely, activation of β -catenin in the presence of a vitamin D analog prevents β -catenin-induced formation of pilomatricomas. We have also found that human trichofolliculomas, another benign HF tumor, have cells with high levels of nuclear β -catenin and VDR, whereas infiltrative human basal cell carcinomas have high β -catenin levels and low VDR levels (Pálmer et al. 2008a). The observations suggest that the anticancer activity of vitamin D analogs may reflect, at least in part, inhibition of inappropriate Wnt signaling (Deeb et al. 2007).

CONCLUSIONS

Mammalian epidermis provides a powerful experimental model not only for studying adult stem cells, but also for examining the origin of different tumor types. Insights obtained from studies of the epidermis turn out to be widely applicable to other tissues. Technical advances in imaging cells in vivo will offer even more possibilities for unraveling the properties of epidermal stem cells, in particular their interactions with their neighbors.

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