

Review

Mechanisms regulating epidermal stem cells

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The skin epidermis contains different appendages such as the hair follicle and the sebaceous glands. Recent studies demonstrated that several types of stem cells (SCs) exist in different niches within the epidermis and maintain discrete epidermal compartments, but the exact contribution of each SC populations under physiological conditions is still unclear. In addition, the precise mechanisms controlling the balance between proliferation and differentiation of epidermal SC still remain elusive. Recent studies provide new insights into these important questions by showing the contribution of hair follicle SC to the sebaceous lineage and the importance of chromatin modifications and micro-RNAs (miRs) in regulating epidermal SCs renewal and differentiation. In this review, we will discuss the importance of these papers to our understanding of the mechanisms that control epidermal SC functions.

The EMBO Journal advance online publication, 20 March 2012; doi:10.1038/emboj.2012.67

Subject Categories: development

Keywords: epigenetics; keratinocytes; lineage tracing; micro-RNA; stem cells

Introduction

The skin epidermis is a stratified epithelium that constitutes a barrier protecting the animals against microorganisms, external stress and water loss. To ensure these vital functions, the skin epidermis must replace the cells that are naturally lost and must be able to repair the tissue following injuries. The skin epidermis contains different appendages such as the hair follicle, the sweat gland and the sebaceous gland (SG), which ensure protection, camouflage and thermoregulation. Different stem cells (SCs) present in the skin epidermis regulated the maintenance and repair of the different part of the skin epidermis during physiological conditions (Blanpain *et al.*, 2007).

During embryonic development, epidermis originates from a single layer of ectodermal cells that will undergo a stratification process allowing the formation of the different layers of differentiated cells required to fulfil its barrier function

(basal, spinous and granular layers). The basal layer cells express high level of basal integrins, keratin 5 (K5) and K14, while the spinous layers express K1 and K10 and the granular layers accomplish the terminal differentiation process and expressed filaggrin and loricrin. P63, a gene of the p53 family, is expressed in basal cells of all stratified epithelia and is thought to represent a master regulator of the stratification process (Blanpain and Fuchs, 2007). In the absence of p63, epidermis indeed fails to form a stratified epithelium (Mills *et al.*, 1999; Yang *et al.*, 1999). Different theories have been proposed to account for the critical p63 function during epidermal morphogenesis. p63 has been proposed to be required for the initiation of the stratification programme (Mills *et al.*, 1999), for epidermal SC renewal (Yang *et al.*, 1999; Senoo *et al.*, 2007) or both processes (Truong *et al.*, 2006).

HF morphogenesis begins during embryonic development through a series of epithelial-to-mesenchymal interactions and it is complete by postnatal day 8 in mice. After reaching a critical size, the lower part of the HF degenerates in a process called catagen. The permanent portion of the HF called the bulge region enters a resting stage (telogen) before entering in a new growing stage (anagen) (Paus *et al.*, 1999). Throughout the life of the animals, HF will alternate cycle of growth and degeneration called the hair cycle. The cyclic HF regeneration is mediated by the presence of multipotent SC located in the bulge region. The first defined characteristic of bulge SC that has been identified is their relative quiescence, which can be visualized by their ability to retain nucleotide analogues during pulse chase experiments and called for this reason labelled retaining cells or LRCs (Cotsarelis *et al.*, 1990; Morris and Potten, 1999). Bulge SC have been purified based on their slow cycling property (Tumbar *et al.*, 2004) or the expression of several markers including CD34/CD49f ($\alpha 6$ integrin) (Trempeus *et al.*, 2003; Blanpain *et al.*, 2004), keratin 15 (Morris *et al.*, 2004), Lgr5 (Jaks *et al.*, 2008). While bulge SC are able to differentiate into all epidermal lineage upon transplantation into immunodeficient mice, adult bulge SC lineage tracing experiments demonstrated that bulge SCs participate to the homeostasis of all HF cells below the SGs but not contribute to SG and IFE under physiological conditions (Morris *et al.*, 2004; Levy *et al.*, 2005). However, during pathological conditions such as during wounding, bulge SC are rapidly mobilized and participate into the repair of the IFE (Ito *et al.*, 2005; Jaks *et al.*, 2008; Nowak *et al.*, 2008).

The continuous replacement of terminally differentiated cells that are shed from the skin surface is mediated by the presence of multiple small epidermal proliferation units (EPUs) scattered all along the IFE that contain one SCs or progenitor cells (Ghazizadeh and Taichman, 2005). Upon division, these SC can undergo three fates: most of the divisions are asymmetric, giving rise to one SC and one

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Received: 2 February 2012; accepted: 27 February 2012

committed cell, the remaining divisions are symmetric (renewal or differentiation). Based on clonal analysis and mathematical modelling, it has been recently proposed that the homeostasis of the IFE can be maintained by the presence of only one type of progenitors that stochastically choose between these three different fates with a fixed probability (Clayton *et al.*, 2007; Doupe *et al.*, 2010).

Recent studies demonstrated that even more progenitors and SC exist in the epidermis and contribute to the maintenance of the other epidermal compartments such as the isthmus, the junctional zone, the infundibulum (Nijhof *et al.*, 2006; Jensen *et al.*, 2008; Jensen *et al.*, 2009; Raymond *et al.*, 2010; Snippert *et al.*, 2010). But it is not clear what is the precise contribution of each SC populations under physiological conditions and how the compartmentalization of the epidermis is orchestrated. In addition, while different signalling pathways regulating epidermal SC functions have been identified, the precise mechanisms controlling the balance between proliferation and differentiation of epidermal SC still remain elusive. Especially, the impact of chromatin modifications and micro-RNAs (miRs) expression on epidermal SCs renewal and differentiation is also still poorly understood.

Recently, a flurry of papers published in the *EMBO Journal* provides new insights into these outstanding questions (Chikh *et al.*, 2011; Driskell *et al.*, 2011; Mejetta *et al.*, 2011; Petersson *et al.*, 2011). In this review, we discuss the importance of these papers to the understanding of the heterogeneity of skin SCs, and the role of miR and histone modifications on epidermal SC functions.

Contribution of bulge SC to the sebaceous gland lineage

SG is a part of the pilosebaceous unit that through holocrine differentiation of the sebocytes secretes the sebum, which maintain the waterproof nature of the fur. The important cellular turnover within the SG is thought to be fuelled by the presence of SCs. Until recently, very little was known about the cellular and molecular mechanisms that control SG morphogenesis and homeostasis. Lineage tracing performed during epidermal development demonstrate that SG originates from bipotent embryonic progenitors common for both HF and SG lineages that expressed *Shh* and *Sox9* at one time of their specification (Levy *et al.*, 2005; Nowak *et al.*, 2008). Lineage tracing of adult bulge SC was performed using different inducible CRE expressed under the regulatory region of *K15*, *Lgr5* and *K19*, which are expressed specifically in bulge SC during telogen (Morris *et al.*, 2004; Ito *et al.*, 2005; Levy *et al.*, 2005; Youssef *et al.*, 2010). *K19CREER* and *Lgr5CREER* are knockin mice, in which the CREER is inserted into the endogenous locus of these genes (Barker *et al.*, 2007; Means *et al.*, 2008), whereas *K15CREPR* are transgenic mice made by random integration of fragment of the *K15* promoter (Morris *et al.*, 2004). In mice expressing one of these inducible CRE together with a reporter gene (e.g., *Rosa/YFP* or *Rosa/LacZ*), tamoxifen (TAM) or RU486 administration induces the activity of the inducible CRE, allowing the excision of a stop cassette leading to the reporter gene expression in bulge SC and all their future progeny. These experiments showed that only rarely were SG cells labelled using these different bulge specific inducible CRE, suggesting that the SG is mainly maintained independently of bulge SC during physiological

condition. The transcriptional repressor *Blimp1* is expressed during morphogenesis in the upper HF adjacent to the emergence of the newly formed SGs. *Blimp1* expressing cells encompass progenitors committed to SG lineage as demonstrated by lineage tracing using *Blimp1CRE* mice. The persistence of *Blimp1* expressing cells in adult HF suggested that the SG could be maintained by unipotent progenitors during adult homeostasis. In the absence of *Blimp1*, increased bulge SC proliferation is observed, possibly through a derepression of *c-Myc* mediated by *Blimp1* (Horsley *et al.*, 2006). Two independent groups have shown that cells located in the upper follicle called the upper isthmus (UI), expressing high level of *MTS24* or low level of *Sca1*, are highly clonogenic *in vitro* and multipotent *in vivo*, suggesting that this region of the HF might contain a subset of SCs that sustain SG homeostasis (Nijhof *et al.*, 2006; Jensen *et al.*, 2008). Another population of cells located at the junction zone (JZ) between UI and IFE, expressing *Lrig1* and enriched for *Blimp1* transcript, contain multipotent SC as demonstrated by transplantation assays (Jensen *et al.*, 2009). Finally, a recent study using *Lgr6CREER*, which is expressed in the UI, demonstrated the presence of unipotent SG SCs responsible for the long-term labelling of the SG and the surrounding UI HF (Snippert *et al.*, 2010). It is still unclear whether SGs are only maintained by *Lgr6+* SCs, a subset of which expressed *MTS24* and possibly *Blimp1*, or that several population of SCs, including HF SCs, are mobilized and contribute to the SG turnover during adult life.

In a recent study, Petersson *et al.* (2011) used new bulge SC lineage tracing to show that a subpopulation of bulge SCs may contribute to the homeostasis of SG. Using new *K15CreERT2* transgenic mice crossed with the *Rosa26-YFP* or *Rosa-LacZ* reporter mice, the authors performed lineage tracing of bulge SCs. TAM administration labels cells preferentially in the bulge region (90% of *LacZ+* cells) while the remaining labelled cells are already localized in the SG and its duct. Clonal analysis showed that a subset of *YFP+* cells apparently coming from the bulge migrates to the UI and the SG 5 days later, as suggested by the increased number of the SG labelled. These *K15CREER*-labelled cells then locally self-renew and continue to contribute to SGs homeostasis up to 6 months after TAM administration.

K15CREER-labelled cells also renew and expand overtime within the bulge region, consistent with the notion that some of the bulge SCs undergo symmetric self-renewing division within their niche, as previously suggested (Waghmare *et al.*, 2008; Zhang *et al.*, 2009, 2010). The absence of contiguous trail of *YFP+* cells between the bulge and the SGs suggests that the *K15*-derived SG progenitors arising from the bulge are not bipotent at the clonal level, and leave the bulge niche once committed to the SG lineage. The apparent contribution of bulge SC to the SG homeostasis is not limited to a particular stage of the hair cycle, although the flux of bulge SC to the SG seems to increase during the activation stage of bulge SC that occurs during the initiation of HF regeneration.

The migration of bulge SC to the SG has been substantiated by two different experimental approaches. The level of expression of SG progenitor markers (*Lgr6*, *Lrig1* and *Blimp1*) in *YFP*-labelled cells increases with time after TAM administration, consistent with a progressive enrichment of SG progenitors within the progeny of *K15CREER*-derived cells. The authors also developed a new method to perform

ex vivo live cell imaging of whole mount preparation of the skin epidermis in order to visualize the migration of bulge cells in real time. This elegant approach allowed the authors to follow the fate of individual bulge SC during 22 h. As expected, most of the labelled bulge SC remained quiescent during the observation but a fraction of those cells divide and migrate locally, downward but also upward towards the UI and SG (Figure 1). However, the whole mount preparation could not be imaged long enough to visualize the whole process of bulge SC migration to the SG and their subsequent contribution to the sebocyte differentiation.

It has been previously demonstrated that overexpression of a mutant of Lef1 that cannot bind β -catenin (K14 Δ NLef1) leads to development of ectopic SGs, suggesting that β -catenin/Lef1 signalling regulates SG differentiation (Merrill *et al*, 2001; Niemann *et al*, 2002, 2007). To determine the cellular origin of the *de novo* SGs in K14 Δ NLef1, the authors performed lineage tracing experiments using K15CreERT2/Rosa-YFP in K14 Δ NLef1 mice. The presence of YFP-labelled cells in *de novo* SG above and below the bulge demonstrates that these lesions arise from the bulge SC that progressively expressed markers of the SG progenitors (Lgr6, Lrig1, Blimp1 and Plet1) ectopically. Interestingly, while *de novo* SG arise from cells below and above the bulge region in K14 Δ NLef1, *de novo* SGs arise only in their normal niche when Δ NLef1 is preferentially expressed in the bulge region (K15 Δ NLef1 transgenic mice).

These new results are very interesting and intriguing. Indeed, while it has been previously reported that bulge SC can contribute to the SG lineage using K15CrePR and Lgr5CreER lineage tracing, but the contribution of the SG was reported to be limited to only rare follicles (Morris *et al*, 2004; Jaks *et al*, 2008). One possibility is that these new CREER mice target a subpopulation of the upper bulge more prone to give rise to the SG lineages than the previously described bulge specific inducible CRE (Morris *et al*, 2004; Levy *et al*, 2005; Jaks *et al*, 2008; Youssef *et al*, 2010) or that these new K15CREER also targeted a rare subpopulation of SG progenitors known to participate to both isthmus and SG maintenance such as Lgr6+ cells (Snippert *et al*, 2010), Lrig1+ cells (Jensen *et al*, 2009) or MTS24+ cells (Nijhof *et al*, 2006). The long-term labelling of SG by resident progenitors observed using Lgr6CREER (Snippert *et al*, 2010) or K15CreER in this study (Pettersson *et al*, 2011), demonstrates that the SG contain resident progenitor that locally self-renew and that not all SGs are constantly renewed through a continuous flux of bulge SC. It will be important to determine what mechanisms regulate the asymmetric cell fate decision of bulge SCs during the specification of SG progenitors and what regulate the flux of bulge SC towards the SG.

Regulation of p63 expression by an autoregulatory loop involving miRs

p63, a member of the p53 family of protooncogenes, has been identified as a key regulator of stratified epithelium development. Mice lacking p63 are born alive but lack all major stratified epithelia including the epidermis (Mills *et al*, 1999; Yang *et al*, 1999). p63 has several isoforms depending on the transcription site and alternative splicing (Yang *et al*, 1998). Δ Np63 is the major isoform express in the epidermis and is expressed mainly in the basal layer of the epidermis (Koster

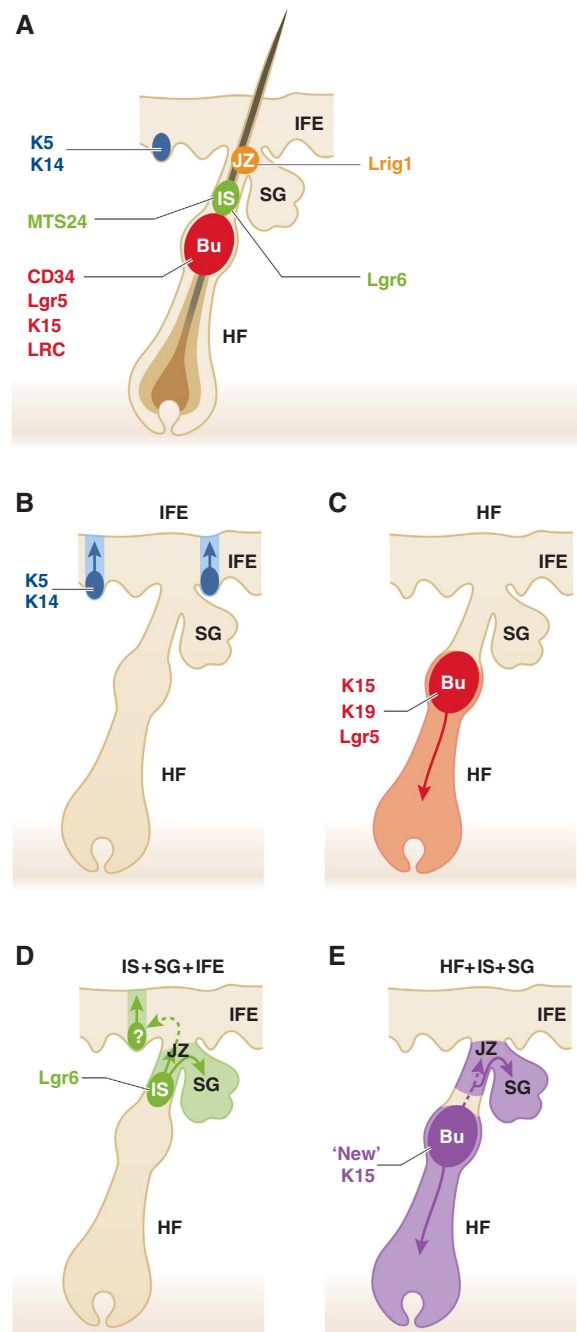


Figure 1 Bulge SC contribution to the sebaceous lineage. (A) Scheme highlighting the different epidermal SC populations found in the skin epidermis: the IFE SC (shown in blue), the bulge SC (Bu in red), the isthmus SC (Is in green) and the junctional SC (JZ in yellow). (B) Lineage tracing experiments have shown that IFE is maintained independently of the HF by the presence of number small unit of proliferation. (C) Lineage tracing of bulge SC using K15CrePR, Lgr5CreER and K19CreER demonstrate their ability to contribute to the homeostasis of the hair follicle but not the SG or the IFE. (D) Lgr6 lineage tracing show the long-term renewal of Lgr6+ isthmus cells and their contribution to the sebaceous lineage. (E) The recent study of Pettersson and colleagues using a new K15CreER shows the potential contribution of bulge SCs to the SG homeostasis.

et al, 2004; Laurikkala *et al*, 2006). Subsequent studies suggest that p63 can regulate renewal and differentiation of the epidermal progenitors (Truong *et al*, 2006; Senoo *et al*,

2007) but the mechanisms regulating p63 expression in epidermal cells remain elusive.

miRNAs provide an additional layer of complexity to the transcriptional regulatory switch of p63 during epidermal differentiation. miR-203 is upregulated during epidermal stratification suprabasally at E15.5 and was shown to directly represses p63 expression in the suprabasal differentiated cells to promote differentiation and stratification of the epidermis (Lena *et al*, 2008; Yi *et al*, 2008).

In a recent study published in the *EMBO Journal*, Chikh *et al* (2011) showed that iASPP regulates p63 expression in the epidermis through the regulation of miRNA expression. Apoptosis stimulating proteins of p53 (ASPP) are a family of proteins that regulates p53-mediated apoptosis. ASPP1, ASPP2 potentiate the pro-apoptotic function of p53, while the third member iASPP inhibits this function (Bergamaschi *et al*, 2003). iASPP is mostly expressed in epithelial cells from several tissues including skin (Herron *et al*, 2005) and is frequently mutated in human cancers (Bergamaschi *et al*, 2003). Structural studies predicted that iASPP preferentially binds to p63, suggesting that iASPP could be involved in the regulation of p63 expression in the skin (Robinson *et al*, 2008).

In this new study, the authors showed that iASPP is expressed in the early developing mouse epidermis and colocalizes with p63 in the nucleus of basal epidermal cells at E15.5 and thereafter. The same pattern of iASPP expression is found in human epidermis. Stimulation of human keratinocyte differentiation decreases both Δ Np63 and iASPP expression, suggesting that iASPP might be involved in p63 downregulation. The authors performed CHIP experiments to demonstrate that p63 directly binds to iASPP promoter *in vivo*. Overexpression of the two p63 isoforms stimulates iASPP expression, while their knockdown by siRNA downregulates iASPP, thus functionally demonstrating that p63 regulates iASPP *in vitro*. Reciprocally, downregulation of iASPP decreases expression of TAp63 and Δ Np63 at protein level, demonstrating that iASPP and p63 form a positive autoregulatory feedback loop. Intriguingly, iASPP knockdown does not regulate p63 mRNA expression, suggesting that iASPP

may regulate the expression of p63 protein through a post-transcriptional or a postranslational mechanism. By profiling miR expression after iASPP KD, the authors found that miR-574-3p and miR-720, two miRNAs predicted to target p63, were upregulated following iASPP silencing. Antagomirs against these miRNAs restored the expression of p63 in the context of iASPP knockdown, suggesting that iASPP represses miR-574-3p and miR-720 expression, which in turn promote p63 expression.

To determine the role of iASPP in keratinocytes, the authors used transcriptional profiling by microarray of iASPP-depleted cells. This experiment showed that iASPP is involved in the regulation of several members of the desmosomal complexes, tight junction and gap junction components, and a decrease in β 1 integrin expression. Cells depleted for iASPP acquire a phenotype of differentiated cells with reduced proliferation. Human skin equivalent using organotypic culture of iASPP-depleted keratinocytes are thicker, less proliferative and present premature differentiation characterized by the expression of K1, involucrin and loricrin-positive cells within the basal compartment. This phenotype was partially rescued by specific antagomirs against miR-574-3p and miR-720, suggesting that iASPP promotes self-renewal and inhibits differentiation by repressing miR-574-3p and miR-720 expression (Figure 2).

This study shows that p63, a major regulator of epidermal morphogenesis and differentiation, is indirectly regulated by iASPP through the modulation of miR expression. While miR-574-3p and miR-720 regulate p63 expression, iASPP KD does not exactly phenocopy p63 KD (Carroll *et al*, 2006; Truong *et al*, 2006). Indeed, while iASPP-depleted keratinocytes show a decrease of β 1 integrin and an increase of α 3 integrin expression without modifications of β 4 integrin; p63 knockdown induces a strong decrease in the expression of β 1, β 4 and α 3 integrins at mRNA level (Carroll *et al*, 2006). In addition, iASPP KD impairs cell proliferation similarly as p63 KD, but also induces a premature differentiation of skin equivalent, which is inhibited upon p63 silencing (Truong *et al*, 2006), suggesting that iASPP may regulate other signaling pathways in addition of p63. Future studies will be

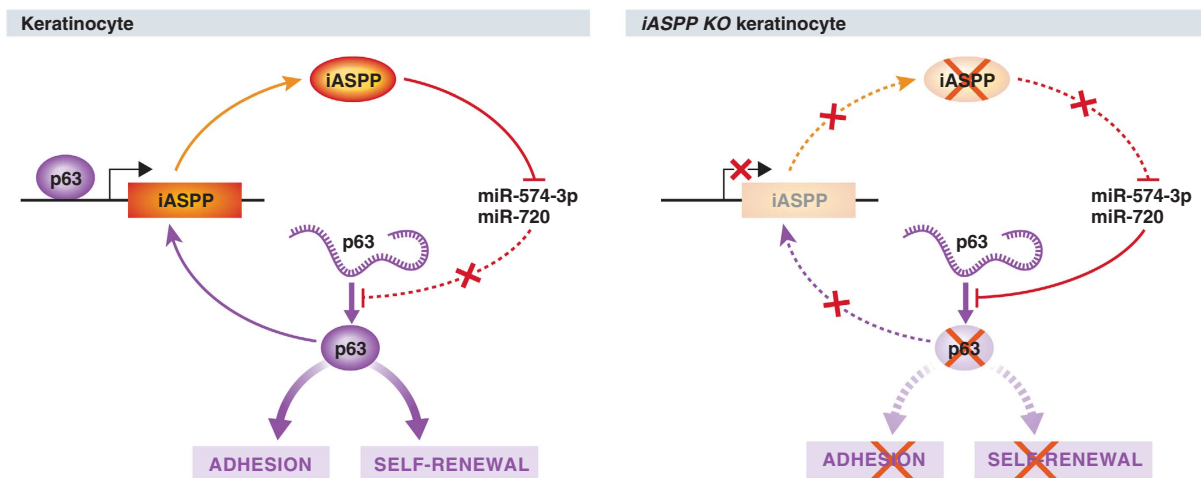


Figure 2 iASPP regulates keratinocyte renewal and differentiation by regulating miR expression. Under physiological conditions, p63 regulates iASPP expression, which in turn, represses miR-574-3p and miR-720. Upon iASPP knockdown in human keratinocytes, these miRNAs are upregulated and inhibit p63 expression, leading to defect in keratinocyte adhesion, renewal and differentiation (Chikh *et al*, 2011).

required to clarify the discrepancy between iASPP and p63 KD, and determine what could be the other targets regulated by miR-574-3p and miR-720.

Role of the polycomb repressor complex in the regulation of epidermal SCs

The polycomb group proteins (PcG) constitute a chromatin remodelling complex referred to as Polycomb Repressor Complexes (PRC). PRC2 is composed of Ezh2 (or Ezh1), Eed, Suz12 and RBAP46/48 (also known as Rbbp7/4) (Margueron and Reinberg, 2011). This complex is recruited to chromatin and Ezh2 mediates the trimethylation of histone H3 on Lysine 27 (H3K27me3). This histone mark induces recruitment of PRC1, which participates to repress adjacent locus. PRC1 complexes are more variable and are composed of Ring1a/b together with Bmi1, Cbx and PH proteins that mediate the monoubiquitination of H2A at Lys119, which induces gene silencing (Simon and Kingston, 2009).

Deletion of PRC2 complexes subunit leads to the upregulation of lineage commitment genes normally silenced in embryonic SCs that lead to early embryonic lethality (O'Carroll *et al.*, 2001; Voncken *et al.*, 2003). In the skin, loss of Ezh2 impaired proliferation and induced the premature differentiation of the epidermis during embryonic development but this phenotype disappeared postnatally (Ezhkova *et al.*, 2009). Combined deletion of Ezh1 and Ezh2 leads to hair loss and hyperproliferation of IFE cells (Ezhkova *et al.*, 2011). In HF lineages, Ezh1/Ezh2 cKO induces upregulation of Ink4b/Ink4a/Arf and subsequent overexpression of p16/p19 (Ezhkova *et al.*, 2011). Since Ink4b/Ink4a/Arf shRNA restore HF cells self-renewal *in vitro*, these results suggest that Ezh1/Ezh2 regulate HF SCs proliferation mostly by repressing Ink4b/Ink4a/Arf locus, similarly to Bmi1, another PRC2 subunit (Jacobs *et al.*, 1999; Cordisco *et al.*, 2010). In addition, Jmjd3 histone demethylase, which is involved in H3K27me3 demethylation, has been shown to be upregulated following keratinocyte differentiation and its overexpression in basal epidermal cells induces premature differentiation. This effect is associated to the derepression of differentiation-related genes such as *krt1* and *S100A8* and ectopic expression of *krt1*, *krt10*, filaggrin and involucrin. Inhibition of Jmjd3 demethylase activity by point mutation blocked its promoting effect on differentiation, suggesting that jmjd3 stimulates epidermal differentiation by the demethylation of H3 at several loci (Sen *et al.*, 2008). Jarid2, a jumonji/JmjC domain-containing protein, has been shown to help the recruitment of PRC2 to the promoter of repressed genes in ESC (Peng *et al.*, 2009; Shen *et al.*, 2009; Landeira *et al.*, 2010). However, conflicting results have been published concerning the role of Jarid2 in regulating the methyltransferase activity of PRC2 (Herz and Shilatifard, 2010; Landeira and Fisher, 2011). Little is known about the mechanisms that contribute to the recruitment of PRC2 complex to the promoter of repressed genes in adult tissue.

A recent study by Mejetta *et al.* (2011) published in *EMBO Journal* showed that Jarid2 regulates epidermal progenitor proliferation and differentiation in the mouse skin epidermis. Moreover, Jarid2 also recruits PRC1 to the promoters of lineage-specific genes in embryonic SCs (Landeira *et al.*, 2010). Conditional deletion of Jarid2 in epidermal cells during embryonic development induced no initial defect of

proliferation and differentiation. However, after birth, Jarid2 null epidermis is characterized by an increased number of differentiated cells as well as a decrease in the proliferation of basal epidermal progenitors. Jarid2 null keratinocytes present fewer proliferative colonies than control keratinocytes and present characteristics of terminally differentiated keratinocytes *in vitro*. While HF morphogenesis is apparently unaffected in the absence of Jarid2, there is a delay in the initiation of HF regeneration, despite the similar number of adult HF bulge SC. HFs present a delay in anagen re-entry, but they eventually resume hair cycle and regenerate new hairs. In addition, Jarid2 null HFs are less responsive to TPA-induced bulge SC proliferation, and their epidermis presented increased differentiation upon TPA treatment.

The authors then showed by co-immunoprecipitation that Jarid2 interacts with PRC2 components Ezh2 and Suz12 in mouse keratinocytes, as it does in ESC. By performing ChIP experiments, they showed that Jarid2 localized to the promoter of several epidermal and non-epidermal target genes of the PRC2 complex previously shown to be occupied by Ezh2 in the skin epidermis (Ezhkova *et al.*, 2009). In the absence of Jarid2, there is a small but significant decrease in the occupancy of these target genes by Suz12 as well as a decrease in the level of H3 trimethylation at these loci. These results are consistent with the notion that Jarid2 helps the recruitment of PRC2 and positively regulates the methyl transferase activity of the PRC2 complex at these loci. Accordingly, these PRC2 target genes including genes involved in keratinocyte differentiation, as well as p16, a gene that negatively regulates cell-cycle progression were upregulated in the absence of Jarid2 in newborn skin epidermis. In contrast, in the adult epidermis, the absence of Jarid2 has no effect on the expression of differentiated genes but still present a decrease in the recruitment of Suz12 at the *Ink4a* promoter, leading to an increase of p16 expression (Figure 3).

These results highlight the complexity of the regulation of epidermal SCs by histone modification. While Jarid2 and Ezh2 null epidermis present some similarities (e.g., increase of p16), there are clear differences in the timing and the extent of their phenotypes (Ezhkova *et al.*, 2009; Mejetta *et al.*, 2011). These apparent discrepancies between the two models suggest that Jarid2 and Ezh2 functions have only partially overlapping functions and other factors are probably involved in the regulation of gene expression mediated by these two proteins. Consistent with this possibility, it has been shown that Ezh2 regulates differentiation by inhibiting AP1 (c-Jun/c-Fos) recruitment to the promoter of genes involved in epidermal differentiation (Ezhkova *et al.*, 2009). It will be interesting to determine whether the difference between Ezh2 and Jarid2 functions is at least partially related to their ability to modulate AP1 function. Moreover, the modest reduction in the level of H3K27me3 in the absence of Jarid2 suggests that some functions of Jarid2 in the skin epidermis may be not related to its ability to stimulate the histone methylation but potentially serves to recruit other proteins that mediate distinct functions. Furthermore, the work of Ezhkova *et al.* (2011) had shown that despite the complete absence of H3K27me3 marks in Ezh1/2 null keratinocytes, only few genes bound by PRC2 are actually upregulated in the absence of Ezh1/2, suggesting that the repression of differentiation genes is not only mediated by the trimethylation of H3 at K27.

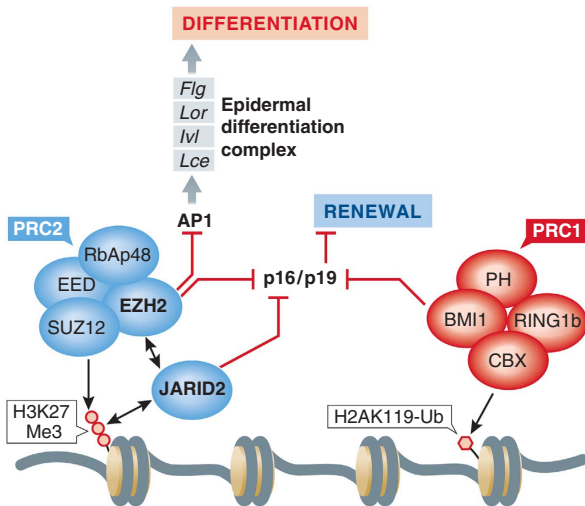


Figure 3 Jarid2 regulates epidermal SC proliferation and differentiation. Jarid2 modulates the recruitment of the PRC2 complex and the trimethylation of lysine 27 Histone H3 (H3K27Me3). This epigenetic mark represses the *Arf4* locus, leading to repression of p16/p19. In Jarid2 cKO mice (K14Cre::Jarid2^{fl/fl}), trimethylation of H3K27 is decreased and subsequently, p16 is upregulated leading to a delay in bulge SC activation and increased IFE differentiation. This effect could be related, at least partially, by a decrease in the recruitment of Ezh2, which that is involved in repressing of p16 and epidermal differentiation complex encompassing Filaggrin (Flg), Loricrin (Lor), Involucrin (Iv1) and late cornified envelope (Lce) genes in an AP1-dependent manner (Ezhkova *et al*, 2009). In the absence of Jarid2, cultured keratinocytes present a decrease in their clonogenic potential, suggesting that Jarid2 controls epidermal SC renewal.

The notion that the recruitment of specific chromatin-associated protein can function independently of their catalytic activity is reinforced by a recent study that showed that Cbx4, a PRC1-associated protein, controls proliferation and differentiation of human epidermal SCs by distinct mechanisms (Luis *et al*, 2011). Surprisingly, both gain and loss of function for cbx4 inhibit the clonogenicity of epidermal SC *in vitro*. While Cbx4 overexpression inhibits cell proliferation without modifying differentiation, Cbx4 KD promotes differentiation. Overexpression of a chromodomain mutant of Cbx4 that can no longer interact with PRC1 induces senescence by upregulating *Ink4a* locus (p16) and p57 without modifying differentiation, while overexpression of a Cbx4 SUMO-U3 ligase activity mutant stimulates differentiation.

The histone H4 monomethyltransferase Setd8 is required for epidermal SC maintenance

Setd8 is the sole enzyme responsible for the monomethylation of histone H4 at lysine 20 (H4K20me1) (Xiao *et al*, 2005). This methylation of histone H4 has been reported to be essential for cell-cycle progression *in vitro* (Jorgensen *et al*, 2007; Tardat *et al*, 2007) and its deletion is lethal in flies and during early embryonic development in mice (Nishioka *et al*, 2002; Karachentsev *et al*, 2005; Oda *et al*, 2009). Deletion of Setd8 in ESC induces DNA damage response, genomic instability and cell-cycle arrest (Oda *et al*, 2009). While *in vitro* studies suggest a critical role of Setd8 in cell-cycle progression *in vitro* and early embryonic development, little is known about the role of Setd8 during the later stage of

embryogenesis and adult renewing tissue. In the epidermis, it has been previously shown that Myc regulated the methylation of histone H4 (H4K20me1) by an HDAC-dependent mechanism, and this chromatin modifications was associated with differentiation (Frye *et al*, 2007). A recent study of Driskell *et al* (2011) published in the *EMBO Journal* demonstrated the essential role of Setd8 in skin development. In epidermis, H4K20me1 is found in basal undifferentiated cells of IFE, SG and anagen hair follicle and is mostly excluded from cells in S-phase, although rare cells positive for BrdU and H4K20me1 is seen in HF-committed cells. While no antibodies recognized specifically Setd8 in the epidermis, reporter mouse expressing β -galactosidase under the regulatory region of the Setd8 locus (Huen *et al*, 2008) indicated that the strongest setd8 expression in the epidermis is found in the developing epidermis, adult SG and HF matrix cells. Lower and patchy expression of the Setd8 reporter activity is seen in the adult IFE.

The authors first investigated the role of Setd8 during morphogenesis using conditional deletion of Setd8 using the K14CRE mice, which is expressed in the early stage of mouse epidermal development (around E12). Mice deficient for Setd8 are born alive but they lack limbs and skin and die immediately after birth, a phenotype reminiscent of the p63 null mice (Mills *et al*, 1999; Yang *et al*, 1999). Indeed, p63 was severely reduced in Setd8 null epidermis, which is composed of a single layer of K8 expressing cells that failed to renew and stratify and which are progressively lost from the skin surface, identically to p63 null mice.

To determine the role of Setd8 in adult epidermis, the authors performed the conditional deletion of Setd8 using the K14CREER. Setd8 deletion in adult epidermis results in decrease of IFE progenitors proliferation, increase in progenitor apoptosis and decrease in the expression of progenitor markers including $\alpha 6$ integrin and p63, as well as a decrease in the expression of differentiation markers. Concomitant expression of YFP and deletion of Setd8 demonstrated that Setd8-deficient cells are progressively lost, which induced a wounding-like stimulus that simulated the non-deleted Setd8 HF cells (due to the poor efficiency of HF targeting by K14CREER) to replace the Setd8 YFP+ deficient IFE cells. Similarly, homeostasis of the SG was also severely affected following the conditional deletion of Setd8 in the adult epidermis that can also recover through the repopulation of the SG by Setd8 HF SCs.

c-Myc overexpression stimulates epidermal cell proliferation and differentiation (Waikel *et al*, 1999, 2001; Arnold and Watt, 2001), as well as monomethylation of H4K20me1 (Frye *et al*, 2007). Driskell and colleagues have now demonstrated that Myc directly binds to the promoter of Setd8 and stimulates its expression. Deletion of Setd8 rescues the increase in cell proliferation and differentiation associated with c-Myc overexpression, suggesting that Setd8 is required to mediate some Myc-associated functions.

The authors showed that p53 expression was majorly increased upon Setd8 deletion in the epidermis. Combined deletion of p53 and Setd8, rescues the proliferation defect, p63 expression and the differentiation defect seen in Setd8 null epidermis *in vivo*, and overexpression of p63 rescues the proliferation defect of Setd8 null keratinocytes *in vitro*. The authors proposed a model by which Setd8 represses p53 and stimulates p63 to allow epidermal renewal and differentiation

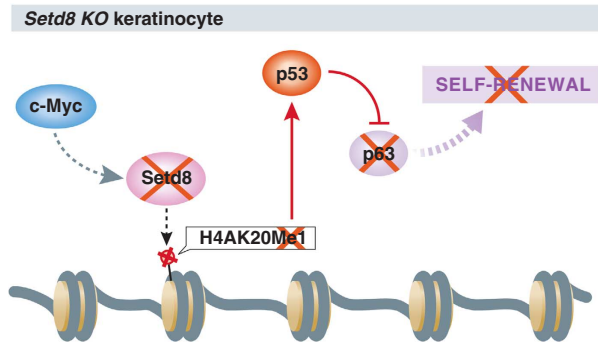


Figure 4 The Histone monomethyltransferase Setd8 is required for human keratinocytes self-renewal. The recent study of Driskell and colleagues showed that Setd8 KO leads to the loss of H4K20Me1 imprint in keratinocytes and impairment of self-renewal due to the loss of p63 (Driskell *et al.*, 2011). Intriguingly, loss of H4K20Me1 leads to the stabilization of p53 and deletion of p53 rescues the phenotype of Setd8 cKO mice by restoring the level of p63. It is still unclear what exactly regulates Setd8 expression in keratinocytes albeit it is known that it is a target gene of c-Myc. Moreover, the mechanism of stabilization of p53 in the absence of the H4K20Me1 imprint is still unknown.

(Figure 4). An interesting parallel with these findings is the recent study reporting that epidermal deletion of HDAC1/2, which normally remove the histone acetylation marks important for gene repression, also induces a phenotype reminiscent of the p63 null mice, presenting a major defect in epidermal differentiation (LeBoeuf *et al.*, 2010). In contrast to Setd8 deficiency, which leads to a decrease of p63 expression, the absence of HDAC1/2 did not modify p63 expression but impairs its ability to repress gene expression including p16 (Su *et al.*, 2009; LeBoeuf *et al.*, 2010), which in turn induces cell-cycle arrest. In addition, HDAC1/2 deficiency also lead to increased p53 acetylation, increasing its transcriptional activity leading to p21 overexpression, which also concurs to inhibit epidermal cell proliferation (LeBoeuf *et al.*, 2010).

It is still not clear why exactly p53 is massively increased upon loss of Setd8. As DNA damage is one of the major trigger of p53 stabilization, it would be interesting to determine whether Setd8 prevent DNA damage and consequently p53 activation? Or does H4K20me1 methylation regulate p53 activation? Do other histone modifications regulate directly and/or indirectly p53 and p63 expression?

Conclusions and perspectives

These recent studies published in the *EMBO Journal* stress the complexity of the regulation of the skin epidermis

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maintenance. Recent lineage tracing experiments has demonstrated the heterogeneity of bulge SC and suggested that bulge may directly participate into SG homeostasis during epidermal homeostasis (Petersson *et al.*, 2011). More studies will be required to define more precisely the mechanisms that regulate the balance between asymmetric and symmetric cell division within the bulge SC, the mechanisms that trigger SG commitment of bulge SC and that regulate their migration towards the SG as well as the mechanisms that prevent migration of bulge SC towards the IFE in the absence of wounding.

miRs appeared as important regulators of the main signalling pathways that regulated epidermal identity, proliferation and differentiation. It will be important to determine in the future whether similar mechanisms are also impaired in skin cancers in which epithelial cells are characterized by increased proliferation and defect of differentiation. It will be also crucial to determine more precisely what regulate the expression of these different miRs in the skin epidermis.

Many studies have now demonstrated the crucial role of epigenetic and histone modifications in the regulation of SC proliferation and differentiation (Frye *et al.*, 2007; Ezhkova *et al.*, 2009, 2011; Sen *et al.*, 2010; Driskell *et al.*, 2011; Mejetta *et al.*, 2011). While the deletion of the different members of the PRC2 complex in the skin epidermis lead to similar but also distinct phenotypes (Ezhkova *et al.*, 2009, 2011; Mejetta *et al.*, 2011), they all lead to the repression of *Ink4a* locus encoding p16, preventing cellular senescence as it was initially discovered for Bmi1 deficiency in fibroblasts (Jacobs *et al.*, 1999) as well as in haematopoietic and leukaemic SCs (Lessard *et al.*, 1999). Clearly, more studies are needed to fully understand the functions of epigenetic modifications in the regulation of skin SCs. Does DNA methylation also play an important function in regulating proliferation and differentiation at it was recently suggested in human epidermal SC (Sen *et al.*, 2010) and how are DNA modifications connected to histone modifications? How are these different epigenetic marks established and dynamically regulated in the different stages of SC ontogeny and activation? How exactly do they control gene expression and DNA damage? The answers to these questions and the role of these processes during diseases such as in cancer will constitute important challenges for future research.

Conflict of interest

The authors declare that they have no conflict of interest.

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