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Cell Cycle 10:6, 866-867; March 15, 2011; © 2011 Landes Bioscience

E4F1 connects the Bmi1-ARF-p53 pathway to epidermal stem cell-dependent skin homeostasis

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Skin homeostasis relies on epidermal stem cells (ESC) that are mobilized from their niche located in the basal layer of the interfollicular epithelium (IFE) and the bulge of hair follicles (HF) to fuel highly proliferative transit amplifying compartments (TAC). Differentiation programs then generate the spinous, granular and cornified layers of the IFE or the different lineages of the mature HF.[1] ESC maintenance is orchestrated by a complex signalling network that remains incompletely characterized, including p63-, BMP-, TGFβ-, Wnt/β-catenin-, Rac1- and Notch-initiated signalling cascades.[1] A recent work by Lacroix, Caramel et al. reveals that E4F1 and the Bmi1-Ink4a/Arf-p53 axis define a novel layer of regulation controlling ESC-dependent skin homeostasis (**Fig. 1**).[2]

E4F1 is an ubiquitously expressed multifunctional protein, originally identified as a transcription factor targeted by the adenoviral oncoprotein E1A. E4F1 also acts as an atypical ubiquitin E3-ligase for p53,[3] and interacts with several components of the p53 pathway, including $p14^{ARF}$ and the polycomb member Bmi1.[4]

We recently generated *E4F1* conditional knock-out (KO) mice to circumvent the early embryonic developmental failure of *E4F1* KO embryos[5] and address *E4F1* functions in adult tissues. *E4F1* inactivation in adult skin first induces a transient hyperplasia of the epidermis associated with increased proliferation of keratinocytes with basal/TAC properties, followed by severe ulcerative lesions, broad disorganisation of the IFE, massive hyperkeratosis and ultimately, a permanent loss of cellularity of the epidermis and alopecia. *E4F1* inactivation during epidermal embryonic development also resulted in similar progressive neo-natal skin defects, leading to dehydration and death of animals 3 to 4 days after birth.[2]

Strikingly, *E4F1 KO* skin defects result from cell autonomous perturbations in resident stem cells. Hence, various ESC markers were strongly downregulated in *E4F1* KO epidermis [$\alpha6^{high}$/$CD34^{+}$, $K15^{+}$, BrdU Long-term Retaining Cells (LRC)] and *E4F1* KO keratinocytes exhibited a dramatically impaired clonogenic potential ex vivo. These defects correlated with deregulated expression of the *Ink4a/Arf* locus in ESC clones and accordingly, *E4F1* KO skin phenotypes were delayed in mouse with *Ink4a/Arf* and *E4F1* compound gene deficiencies. Inactivation of the *Ink4a/Arf* locus or ectopic expression of the *Ink4a/Arf* locus transcriptional repressor Bmi1 or depletion of its downstream target, p53, all partly rescued the ex vivo clonogenic potential of *E4F1* KO ESC.[2] These results reveal a new regulatory network involved in ESC maintenance and proper skin homeostasis, implicating E4F1, Bmi1, the *Ink4a/Arf* locus and p53.

However, several questions remain unanswered. For instance, why are skin defects only developing after birth, although Cre-mediated recombination of *E4F1* occurs much earlier during embryonic development? Among many possible scenari, Bmi1 and its downstream effectors might impact on ESC maintenance only at post-natal stages, as already suggested for hematopoietic stem cells (HSC). Interestingly, E4F1 has been previously shown to interfere with this HSC program.[4] However, in this case, the shRNA-mediated depletion of E4F1 rescues *Bmi1* KO HSC exhaustion,[4] suggesting that the role of the E4F1-Bmi1 axis in stem cells maintenance differs from one tissue to another. Moreover, while the E4F1-Bmi1 genetic interaction identified in HSC was claimed to be independent of p53,[4] we found that p53 is clearly involved in *E4F1* KO ESC phenotypes. The later observation raises queries about the exact function of the E4F1-Bmi1-Ink4a/Arf-p53 network in stem cells homeostasis. In ESC, this pathway might play its usual "gate-keeper" function in response to genotoxic or developmental stresses, but it might also directly regulate normal programs of ESC self-renewal, as suggested in mammary gland progenitors[10] where p53 impacts on asymmetric cell divisions.

Finally, given the multiple roles of E4F1, it will be necessary to evaluate the relative importance of E4F1-associated transcriptional and E3-ligase activities in ESC functions. Ongoing studies should clarify these points.

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Submitted: 01/20/11; Accepted: 01/28/11
DOI: 10.4161/cc.10.6.14974
Comment on: Lacroix M, et al. Proc Natl Acad Sci USA 2010; 107:21076–81.

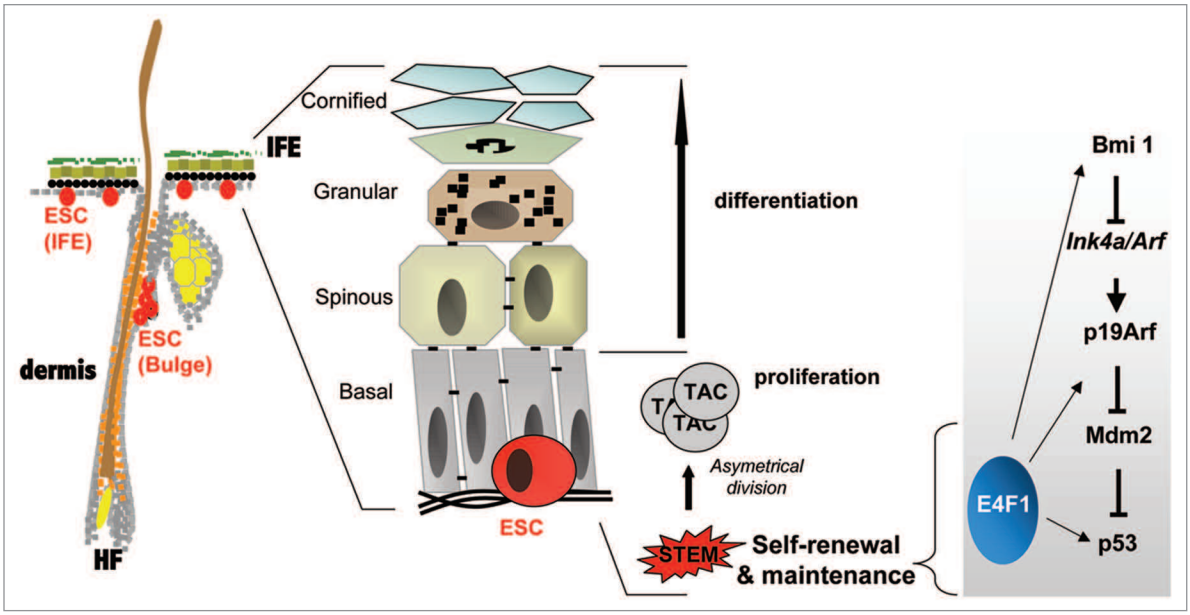

Figure 1. E4F1, through its connection with the Bmi1-ARF-p53 axis, regulates epidermal stem cell (ESC) maintenance. Constant renewal of the Interfollicular Epithelium (IFE) and of Hair Follicles (HF) relies on the recruitment of epidermal stem cells (ESC) located in the basal layer of IFE and in the bulge region of HF, respectively. ESC fuel the highly proliferative transit amplifying compartments (TAC) in the basal layer of IFE and in the bulb of HF. TAC cells then embark on differentiation programs to generate the spinous, granular and cornified layers in IFE or the different lineages of mature HF. *E4F1* inactivation in the entire skin or in the basal compartment of the epidermis induces skin homeostasis defects that result from cell autonomous alterations in ESC maintenance.[2] These ESC defects are partially restored upon inactivation of the Bmi-Arf-Mdm2-p53 pathway, several components (p53, Bmi1 and Arf) of which have been described to physically and functionally interact with E4F1.[3,4]