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Integration of the Transcription Factor-Regulated and Epigenetic Mechanisms in the Control of Keratinocyte Differentiation

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Abstract

The epidermal differentiation program is regulated at several levels including signaling pathways, lineage-specific transcription factors, and epigenetic regulators that establish well-coordinated process of terminal differentiation resulting in formation of the epidermal barrier. The epigenetic regulatory machinery operates at several levels including modulation of covalent DNA/histone modifications, as well as through higher-order chromatin remodeling to establish long-range topological interactions between the genes and their enhancer elements. Epigenetic regulators exhibit both activating and repressive effects on chromatin in keratinocytes (KCs): whereas some of them promote terminal differentiation, the others stimulate proliferation of progenitor cells, as well as inhibit premature activation of terminal differentiation-associated genes. Transcription factor-regulated and epigenetic mechanisms are highly connected, and the p63 transcription factor has an important role in the higher-order chromatin remodeling of the KC-specific gene loci via direct control of the genome organizer *Satb1* and ATP-dependent chromatin remodeler *Brg1*. However, additional efforts are required to fully understand the complexity of interactions between distinct transcription factors and epigenetic regulators in the control of KC differentiation. Further understanding of these interactions and their alterations in different pathological skin conditions will help to progress toward the development of novel approaches for the treatment of skin disorders by targeting epigenetic regulators and modulating chromatin organization in KCs.

INTRODUCTION

Establishment of the functional epidermal barrier is one of the major goals of the epidermal differentiation program, which includes a tightly regulated process of keratinocyte (KC) proliferation, terminal differentiation, apoptosis, and shedding. The program of epidermal development and KC differentiation is governed by coordinated involvement of several transcription factors (p63, AP-1, Klf4, Arnt, and so on), signaling pathways (Wnt, Bmp, Hedgehog, EGF, Notch, FGF, and so on) and epigenetic regulators (DNA/histone-modifying

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enzymes, Polycomb genes, higher-order and ATP-dependent chromatin remodelers, and non-coding RNAs) that control expression of lineage-specific genes (reviewed in Botchkarev *et al.*, 2012; Frye and Benitah, 2012; Perdigoto *et al.*, 2014).

MOLECULAR CONTROL OF CELL DIFFERENTIATION

The p63 transcription factor operates as master regulator of this program, and subsequently and stage-dependently induces in the epidermal progenitor cells expression of several groups of genes encoding the essential components of the cytoskeleton (keratins 5 and 14), cell adhesion molecules (P-cadherin, integrin-α3, Perp, dystonin, and so on), cell matrix regulators (Fras-1), transcription factors (AP-2γ, IKKα, IRF6), and epigenetic regulators (Satb1, Brg1) involved in the control of epidermal differentiation (Koster and Roop, 2007; Vanbokhoven *et al.*, 2011; Botchkarev and Flores, 2014).

Genetic p63 ablation in mice results in the failure of stratification of the epidermis and other squamous epithelia, lack of the formation of epidermal appendages (hair follicles, glands, teeth) and in severe abnormalities in the development of limbs and external genitalia (Mills *et al.*, 1999; Yang *et al.*, 1999; Ince *et al.*, 2002). Moreover, heterozygous mutations in the human p63 gene underlie several ectodermal dysplasia syndromes, also characterized by abnormalities in the development of digits, teeth, hairs, nails, and sweat glands (reviewed in Rinne *et al.*, 2007; Koster, 2010).

p63 has multiple isoforms, including TAp63 and ΔNp63, which show distinct, although partially overlapping, roles in the control of epidermal differentiation and stratification (Koster and Roop, 2007; Vanbokhoven *et al.*, 2011; Botchkarev and Flores, 2014). The ΔNp63 isoform is more abundant in the epidermis compared with TAp63, is strongly expressed in basal epidermal KCs, and is markedly downregulated in the spinous layer (Romano *et al.*, 2012). ΔNp63 has a major role in mediating the effects of p63 on epidermal development, whereas TAp63 protects KCs from senescence and suppress tumorigenesis in postnatal epidermis (Guo *et al.*, 2009; Romano *et al.*, 2012).

EPIGENETIC MECHANISMS

Epigenetic mechanisms have an important role in the control of cellular functions in living organisms and are considered as a driving force of the phenotypic plasticity and evolutionary adaptation (Feinberg, 2007). Epigenetic regulatory machinery operates at several levels including modulation of covalent DNA/histone modifications, as well as through higher-order chromatin remodeling to establish long-range topological interactions between the genes and their enhancer elements in three-dimensional (3D) nuclear space (Ho and Crabtree, 2010; Bickmore and van Steensel, 2013).

Epigenetic regulators exhibit both activating and repressive effects on chromatin in KCs: histone demethylase Jmjd3, ATP-dependent chromatin remodeler Brg1, and genome organizer Satb1 promote terminal KC differentiation, whereas DNA methyltransferase (DNMT1), histone deacetylases (HDAC1/2), and polycomp components (Bmi1 and Ezh1/2) stimulate proliferation of progenitor cells via repression of the genes encoding cell-cycle inhibitors, as well as inhibit premature activation of terminal differentiation-associated genes

(reviewed in Eckert *et al.*, 2011; Botchkarev *et al.*, 2012; Frye and Benitah, 2012; Perdigoto *et al.*, 2014). In addition, epigenetic regulators might control expression of transcription factors in KCs via formation of the active or repressive local chromatin structure at their promoter regions: for instance, p63 expression in KCs is inhibited by histone methyltransferase Setd8 and HDAC2, which interfere with the ΔNp63-regulated gene expression program in KCs (LeBoeuf *et al.*, 2010; Driskell *et al.*, 2012).

The KC nucleus is a complex and highly compartmentalized organelle, whose 3D organization undergoes major changes during terminal differentiation in the epidermis (Gdula *et al.*, 2013). The process of terminal differentiation in epidermal cells is accompanied by sequential changes of gene expression in the keratin type I/II loci, followed by the onset of expression of the epidermal differentiation complex (EDC) locus genes encoding the essential components of the epidermal barrier (Fuchs and Horsley, 2011).

Our recent studies revealed that transcription factor-dependent and epigenetic regulatory mechanisms in KCs are highly connected, and p63 has a hitherto unrecognized role in the higher-order chromatin remodeling of the EDC locus via direct control of the genome organizer *Satb1* and ATP-dependent chromatin remodeler *Brg1* (Fessing *et al.*, 2011; Mardaryev *et al.*, 2014).

Among different epigenetic regulators, Satb1 has a unique role in the execution of lineage-specific gene expression programs by integrating high-order chromatin organization with regulation of gene expression (Cai *et al.*, 2006). Satb1 binds specialized DNA regions with an ATC-sequence context and folds chromatin into loops involving tissue-specific gene loci (T_{H2}-cytokine and MHC class I loci, globin locus, and so on), as well as targets chromatin remodelers/transcription factors to gene loci (Kumar *et al.*, 2007). Satb1 also contributes to the progression of tumors and promotes reprogramming of the genome of breast cancer cells towards metastasis (Kohwi-Shigematsu *et al.*, 2013).

Satb1 is expressed in basal epidermal KCs and promotes cell differentiation via establishment of specific conformation of the EDC locus, whereas its ablation in mice results in the marked elongation of the EDC central domain associated with alterations in expression of the EDC genes and in epidermal morphology (Fessing *et al.*, 2011).

ATP-dependent chromatin remodeler *Brg1*, on the other hand, promotes developmentally regulated relocation of the EDC locus from the nuclear periphery towards nuclear interior into the compartment enriched by nuclear speckles, which is associated with marked increase in expression of the EDC genes (Mardaryev *et al.*, 2014). Importantly, conditional ablation of Brg1 in the epidermis results in failure to form a functional barrier, thus partially resembling the phenotype of p63 knock-out mice (Indra *et al.*, 2005). These data suggest that chromatin remodeling genes represent a novel cohort of p63 targets that mediate its effects on execution of lineagespecific gene expression program in KCs (Botchkarev *et al.*, 2012; Fessing, 2014).

CONCLUSIONS AND FUTURE DIRECTIONS

However, additional efforts are required to fully understand the complexity of interactions between distinct transcription factors and epigenetic regulatory machinery in the control of epidermal development, regeneration, and stem cell activity. Recently, a number of molecules that are capable of modulating distinct components of the epigenetic machinery have been developed, and some of them are already approved for treatment of the distinct neoplastic conditions or under clinical trials (Heightman, 2011). Thus, understanding of epigenetic mechanisms controlling epidermal differentiation and skin stem cell activity and their alterations in different pathological skin conditions will help to develop a novel cohort of epigenetic drugs for the treatment of skin disorders.

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Abbreviations

KC	keratinocyte
3D	three-dimensional
EDC	epidermal differentiation complex

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