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Forward genetics identifies *Kdf1/1810019J16Rik* as an essential regulator of the proliferation–differentiation decision in epidermal progenitor cells

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A B S T R A C T

Cell fate decisions during embryogenesis and adult life govern tissue formation, homeostasis and repair. Two key decisions that must be tightly coordinated are proliferation and differentiation. Overproliferation can lead to hyperplasia or tumor formation while premature differentiation can result in a depletion of proliferating cells and organ failure. Maintaining this balance is especially important in tissues that undergo rapid turnover like skin however, despite recent advances, the genetic mechanisms that balance cell differentiation and proliferation are still unclear. In an unbiased genetic screen to identify genes affecting early development, we identified an essential regulator of the proliferation–differentiation balance in epidermal progenitor cells, the *Keratinocyte differentiation factor 1* (*Kdf1; 1810019J16Rik*) gene. Kdf1 is expressed in epidermal cells from early stages of epidermis formation through adulthood. Specifically, Kdf1 is expressed both in epidermal progenitor cells where it acts to curb the rate of proliferation as well as in their progeny where it is required to block proliferation and promote differentiation. Consequently, *Kdf1* mutants display both uncontrolled cell proliferation in the epidermis and failure to develop terminal fates. Our findings reveal a dual role for the novel gene Kdf1 both as a repressive signal for progenitor cell proliferation through its inhibition of p63 and a strong inductive signal for terminal differentiation through its interaction with the cell cycle regulator Stratifin.

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Introduction

The appropriate balance between cell division and differentiation is crucial for organ formation during development as well as for tissue maintenance postnatally. Excessive proliferation or defective differentiation can lead to tumor formation, while the inverse scenario can result in hypoplastic organs or defects in tissue repair. Stem cells are key to maintaining tissue integrity, acting as a reservoir to produce new progeny cells that will differentiate to replace damaged or lost tissue. Adult mammalian skin is the largest organ in the body and comprises a thin, external epidermis and an underlying, thicker dermis. The epidermis has been an excellent model to define the cellular interactions and genetic factors regulating this delicate balance of stem cell proliferation and differentiation, as this tissue is turned over approximately every 4–6 weeks in humans and between 1.5 and 2 million skin cells are sloughed off every hour (Milstone, 2004). Without replacement, the epidermis and the barrier it provides would rapidly be depleted. Establishment of the epidermis occurs during embryonic development, where epidermal progenitor cells give rise to a fully functional epidermis by birth. Thus, the coordination of proliferation and differentiation within the epidermal progenitor cells is also essential for epidermis formation and function.

During embryonic development, the undifferentiated ectoderm becomes committed to the epithelial lineage by embryonic day (e) 8.5 (Byrne et al., 1994). A few days later, (around e13.5) stratification begins, giving rise to a distal layer of epidermal cells (keratinocytes) called intermediate cells that continue to proliferate but also begin to express the first markers of differentiation (Byrne et al., 1994; Lechler and Fuchs, 2005; Smart, 1970). Intermediate cells are a transient embryonic epidermal population that retain the ability to divide, but later differentiate into non-proliferative spinous cells by e15.5 (Koster et al., 2007; Weiss and Zelickson, 1975). From this stage onward, proliferation is restricted to the basal layer of the epidermis (Byrne et al., 2003; Fuchs, 2008; Koster and Roop, 2007). As cells leave the basal layer, they undergo sequential differentiation to produce spinous, granular and cornified layers, which are eventually shed via

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desquamation (Fuchs and Horsley, 2008; Milstone, 2004). Disruption in the formation of these layers or connections between them can lead to a host of defects including lethal dehydration and microbial infection. Despite recent advances in epidermal biology, our understanding of epidermis formation and homeostasis, particularly in regards to the genes that regulate epidermal progenitor cell proliferation and differentiation is quite limited.

One factor that is essential for normal stratification of the epidermis is the cell cycle regulator Stratifin (14-3-3σ, Sfn). Although initially studied for its role in DNA damage response (Chan et al., 1999, 2000), Sfn was later shown to promote differentiation of suprabasal cells. Mouse embryos homozygous for a mutation in *Sfn* develop a thick, hyperplastic epidermis and keratinocytes fail to differentiate into granular or cornified cells (Guenet et al., 1979; Herron et al., 2005; Li et al., 2005). Instead, all suprabasal layers express spinous markers while remaining proliferative, suggesting that these cells are locked into the intermediate fate. A key regulator of *Sfn* is p63 (Dohn et al., 2001; Westfall et al., 2003), the first transcription factor expressed specifically in epithelial cells that have adopted an epidermal fate. At later stages, p63 is expressed primarily by basal keratinocytes and plays multiple roles during epidermis development (Green et al., 2003; Koster et al., 2004). p63 inhibits the expression of Sfn in basal cells and also promotes their proliferation such that loss of p63 results in a thin but differentiated epidermis (Mills et al., 1999; Yang et al., 1999). However, only a handful of factors that interact with Sfn or p63 to control proliferation and differentiation during embryonic epidermis formation are known.

Forward genetics is a powerful, unbiased tool to identify novel genes with essential roles in biological processes. Using this approach, we uncover a previously unknown but essential regulator of the proliferation–differentiation balance in epidermis, the *Keratinocyte Differentiation Factor 1* (*Kdf1; 1810019J16Rik*) gene. Here we demonstrate that mice carrying mutations in *Kdf1* display uncontrolled proliferation of basal progenitor cells and a failure of their progeny to differentiate. This results in a thickened, but non-functional epidermis. Our results further reveal that *Kdf1* represses basal cell proliferation by inhibiting p63 and also closely interacts with the cell cycle regulator *Sfn* to induce terminal differentiation in suprabasal cells. These results identify Kdf1 as a critical negative regulator of keratinocyte proliferation during epidermis development and an essential factor in the differentiation of epidermal progenitor cell progeny.

Materials and methods

Mouse strains

Sfn^{Er} (Guenet et al., 1979) mice were kindly provided by M. Karin. The $p63^{Brdm2}$ line was obtained from JAX. We genotyped mutant alleles as previously described (Herron et al., 2005; Li et al., 2005; Mills et al., 1999) and see Supplementary Fig. 5. We obtained ES cells harboring a targeted allele of *Kdf1* ($1810019J16Rik^{tm1a(EUCOMM)Wtsi}$) from the International Knockout Mouse Consortium in order to generate the *Kdf1* gene trap line ($Kdf1^{GT}$). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by Institutional animal care and use committee (IACUC protocol number 2010–11172).

Mutagenesis screen

C57BL/6J and C3HeB/FeJ mice were obtained from The Jackson Laboratory. ENU mutagenesis was performed essentially as described (Kasarskis et al., 1998). 10 mg/ml ENU in phosphate–citrate buffer was injected intraperitoneally into male mice 9–10 weeks old at the time of the first injection. C57BL/6J males received ENU in three doses of 100 mg/kg body weight administered once a week for 3 weeks. These males were used to establish lines by crossing to C3HeB/FeJ females. Each resulting F1 male was mated to two C3HeB/FeJ females, to generate second-generation (G2) females. Four to 10 G2 females per line were mated to the F1 male; lines with two or more litters containing embryos with similar abnormal morphology were considered potentially mutant. G2 males and females from these lines were produced and intercrossed, and litters were screened at 12.5 days post coitum to confirm the heritability of the phenotype and to identify G2 males that carried the mutation. Lines were maintained initially by outcrossing the carrier males to C3HeB/FeJ, intercrossing the resulting progeny, and examining embryos to identify new male carriers. After the mutation responsible for the phenotype was initially mapped, carriers of both sexes were identified by PCR as those carrying the C57BL/6J alleles of markers flanking the induced mutation.

Mapping of shd

The *shd* mutant was initially identified based on a short limb/snout phenotype at e12.5. Initial mapping of the *shd* mutation utilized two to five simple-sequence length polymorphism markers per chromosome to identify linkage between the epidermis phenotype and C57BL/6J DNA polymorphisms. Linkage to Chromosome 4 was established and subsequently the *shd* interval was narrowed to between markers D4Mit204 (132.98 Mb) and D4SKI3 (134.16 Mb; https://mouse.mskcc.org). The *shd* mutation was crossed > 11 generations onto the C3HeB/FeJ background, which removed more than 99.9% of the original mutagenized C57BL/6J background, supporting the idea that the *shd* phenotype is monogenic. Characterization of the *shd* phenotype occurred on a mixed C57BL/6J–C3HeB/FeJ background.

Sequence capture

The basic principle of Sequence Capture has been previously described (Hodges et al., 2007; Olson, 2007). In our study, a Nimblegen mouse Sequence Capture 385K array was designed to contain oligos complementary to all exons and splice sites within the *shd* interval. Genomic DNA was isolated from an e11.5 *shd* homozygote, and then sheared by sonication and adaptors were ligated to the resulting fragments. The adaptor-ligated templates were fractionated by agarose gel electrophoresis and fragments of the desired size were excised. Extracted DNA was amplified by ligation-mediated PCR, purified, and hybridized to the capture array. The array was washed, and bound DNA was eluted, purified, and amplified by ligation-mediated PCR (similar to methods employed in Choi et al. (2009)). The capture and sequencing experiments were performed at the W.M. Keck Foundation for Biotechnology Resources at Yale. This array also contained sequences from Chromosomes 11 & 7, unrelated to *shd*. For details about these sequences, please contact the authors.

Sequencing and mutation analysis

Captured libraries were sequenced on an Illumina Genome Analyzer II as single-end, 75-bp reads, as previously described (Choi et al., 2009). Illumina reads were first trimmed based on their quality scores to remove low-quality regions using the program Btrim (Kong, 2011). A cutoff of 20 for average quality scores within a moving window of size 5-bp was used. Minimum acceptable read length was set to 25-bp. Other parameters of Btrim were set to defaults. The pre-processed reads were then