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IKKα, a critical regulator of epidermal differentiation and a suppressor of skin cancer

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IκB kinase α (IKKα), one of the two catalytic subunits of the IKK complex involved in nuclear factor κB (NF-κB) activation, also functions as a molecular switch that controls epidermal differentiation. This unexpected function requires IKKα nuclear translocation but does not depend on its kinase activity, and is independent of NF-κB signalling. *Ikkα*$^{-/-}$ mice present with a hyperproliferative and undifferentiated epidermis characterized by complete absence of a granular layer and *stratum corneum*. *Ikkα*-deficient keratinocytes do not express terminal differentiation markers and continue to proliferate even when subjected to differentiation-inducing stimuli. This antiproliferative function of IKKα is also important for the suppression of squamous cell carcinogenesis. The exact mechanisms by which nuclear IKKα controls keratinocyte proliferation and differentiation remained mysterious for some time. Recent studies, however, have revealed that IKKα is a major cofactor in a TGFβ–Smad2/3 signalling pathway that is Smad4 independent. This pathway controls cell cycle withdrawal during keratinocyte terminal differentiation. Although these are not the only functions of nuclear IKKα, this multifunctional protein is a key regulator of keratinocyte and epidermal differentiation and a critical suppressor of skin cancer.

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Introduction

The epidermis, the outermost part of the skin, is a stratified and keratinized squamous epithelium mainly composed of keratinocytes, which forms a protective barrier. Epidermal differentiation, which starts in the mouse at embryonic day (E) 12, leads to formation of several distinct cell layers characterized by their ultrastructure, mitotic state and expression of specific molecular markers (Fuchs and Byrne, 1994; Koster and Roop, 2007). The basal layer develops from the surface ectoderm at approximately E9.5 in the mouse. The *p63* gene, which specifies different isoforms of a transcription factor related to tumour suppressor p53, controls basal layer formation and maintenance as well as IκB kinase α (IKKα) expression (Candi *et al*, 2007; Koster *et al*, 2007). Basal keratinocytes, including epidermal stem cells and transit-amplifying cells, are cuboidal, express cytokeratins (CKs) 5 and 14 and have a high proliferative potential (Koster and Roop, 2007). These cells form the embryonic periderm (M'Boneko and Merker, 1988), which is lost on establishment of the epidermal barrier. At E12 in the mouse, the basal cells give rise to the intermediate cell layer located between the embryonic basal layer and the periderm (Smart, 1970; Weiss and Zelickson, 1975). The intermediate cells divide several times before they withdraw from cell cycle and mature into postmitotic spinous cells (Smart, 1970; Koster and Roop, 2007). By contrast, adult basal keratinocytes directly become spinous cells when terminal differentiation is initiated, without involvement of an intermediate cell type (Koster and Roop, 2007). The spinous layer is characterized by a switch in keratin expression, from CK5 and CK14 to CK1 and CK10 (Fuchs and Green, 1980). Involucrin, a marker of early terminal differentiation is also synthesized in the upper part of this layer. The spinous cells continue their differentiation and maturation to form the granular layer, which is characterized by keratohyalin granules and expression of the late differentiation markers loricrin and filaggrin (Candi *et al*, 2005). Terminal differentiation gives rise to the cornified layer (*stratum corneum*), which consists of extremely flat, keratin-filled and anucleated keratinocytes, called corneocytes, which are mummified within a lipid matrix (Candi *et al*, 2005; Segre, 2006). The *stratum corneum* is primarily responsible for the barrier function of the skin (Elias, 2004; Segre, 2006), which is established around E17.5 in mouse (Hardman *et al*, 1998). Although major progress has been made in understanding the molecular changes that characterize epidermal differentiation, less is known about the signalling

pathways that control these events. Here, we provide an overview of the current understanding of the central function of IKKα in the control of epidermal differentiation, homoeostasis and tumorigenesis.

Defective epidermal morphogenesis in *Ikk*α-deficient mice

The oligomeric IKK complex is composed of two catalytic subunits, IKKα and IKKβ (DiDonato *et al*, 1997; Mercurio *et al*, 1997; Woronicz *et al*, 1997; Zandi *et al*, 1997), and a regulatory subunit named IKKγ or NEMO (nuclear factor κB (NF-κB) essential modulator) (Rothwarf *et al*, 1998; Yamaoka *et al*, 1998). This complex is the key mediator of NF-κB activation in response to proinflammatory and innate immune challenges (Rothwarf *et al*, 1998). Although IKKα and IKKβ share considerable sequence identity, it is IKKβ that usually serves the more critical function in the activation of classical NF-κB signalling (Tanaka *et al*, 1999; Li *et al*, 1999b, c). To determine the unique functions of IKKα, several groups have disrupted the *Ikk*α locus in mice and were surprised to find that it has an essential function in epidermal differentiation and morphogenesis (Hu *et al*, 1999; Li *et al*, 1999a; Takeda *et al*, 1999). Newborn *Ikk*$\alpha^{-/-}$ mice present with multiple morphological defects, including shiny and translucent skin, absence of erupted whiskers, shortened limbs and truncated snout and tail (Figure 1) (Hu *et al*, 1999; Takeda *et al*, 1999; Yoshida *et al*, 2000). These mice develop to term but die shortly after birth, probably as a consequence of a major skin barrier defect that results in severe dehydration.

The *Ikk*$\alpha^{-/-}$ epidermis is characterized by the presence of basal and suprabasal layers, both in a highly proliferative state, and complete absence of the granular and the cornified layers (Figure 2A) (Hu *et al*, 1999; Takeda *et al*, 1999). At the molecular level, these anomalies are characterized by the expression of CK5 and CK14 as well as proliferating cell markers, such as CK6, PCNA (proliferating cell nuclear antigen) and Ki67 in basal and suprabasal layers (Hu *et al*, 1999; Takeda *et al*, 1999). In contrast, CK1 and CK10 are appropriately expressed in the first suprabasal layers of the *Ikk*$\alpha^{-/-}$ epidermis (Hu *et al*, 1999; Takeda *et al*, 1999). Involucrin is also synthesized in the upper part of the suprabasal layer of these mice (Takeda *et al*, 1999), indicating that an early step in the differentiation process still takes place, although late terminal differentiation markers, including loricrin and filaggrin, are not expressed (Hu *et al*, 1999; Takeda *et al*, 1999) (Figure 2B). It was suggested that the highly proliferative suprabasal cells of the *Ikk*$\alpha^{-/-}$ epidermis, which express CK1 and CK10, are reminiscent of intermediate cells rather than spinous cells (Koster and Roop, 2007). Hence, the most critical function of IKKα may be induction of cell cycle exit needed for converting basal and intermediate keratinocytes to spinous cells. When this step fails, all subsequent differentiation states are aborted (Figure 3).

Nuclear IKKα controls terminal differentiation of keratinocytes

Control of epidermal proliferation and differentiation by IKKα does not involve its protein kinase function and is completely independent from NF-κB activation (Hu *et al*, 2001). *Ikk*$\alpha^{-/-}$ keratinocytes do not exhibit a primary defect in NF-κB activation (Hu *et al*, 1999; Takeda *et al*, 1999). In contrast, *Ikk*$\alpha^{-/-}$ keratinocytes display higher IKK and NF-κB activities than wild-type (WT) cells after incubation with either tumour necrosis factor-α or interleukin-1 (Hu *et al*, 2001). The observations that transgenic mice overexpressing a dominant inhibitor of NF-κB function (IκBαM) in the epidermis or mice lacking both RelA and c-Rel display epidermal hyperplasia that does not disrupt terminal differentiation and *stratum corneum* formation (Seitz *et al*, 1998; Gugasyan *et al*, 2004; Zhang *et al*, 2004), are consistent with the NF-κB-independent action of IKKα in the epidermis.

As observed *in vivo*, isolated *Ikk*$\alpha^{-/-}$ keratinocytes are hyperproliferative and do not respond to differentiation-inducing signals such as confluence or high Ca^{2+} (Hu *et al*, 1999, 2001). *Ikk*$\alpha^{-/-}$ keratinocytes, however, do differentiate *in vitro* when transduced by an adenovirus expressing a 'kinase-dead' form of IKKα, indicating that the kinase function is dispensable for keratinocyte differentiation (Hu *et al*, 2001). Instead, IKKα needs to enter the nucleus to induce keratinocyte cell cycle arrest and terminal differentiation (Sil *et al*, 2004). Nuclear entry depends on a nuclear localization sequence (NLS) within the IKKα kinase domain, the disruption of which prevents the induction of keratinocyte

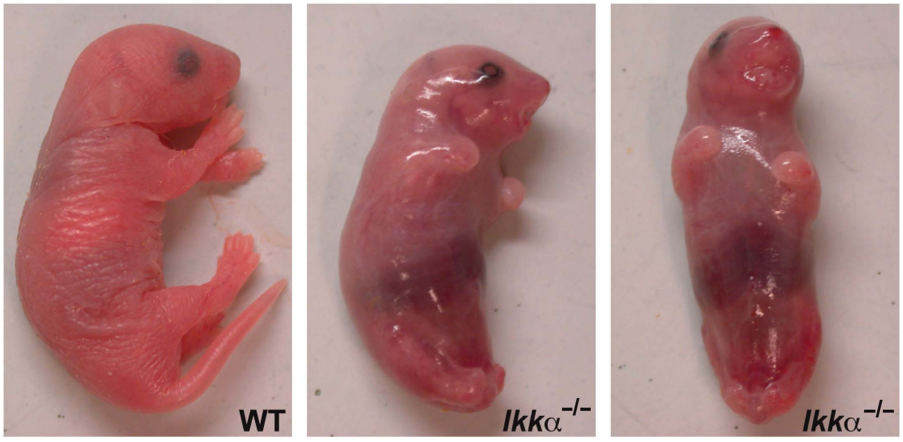

Figure 1 Macroscopical presentation of WT and *Ikk*$\alpha^{-/-}$ mice.

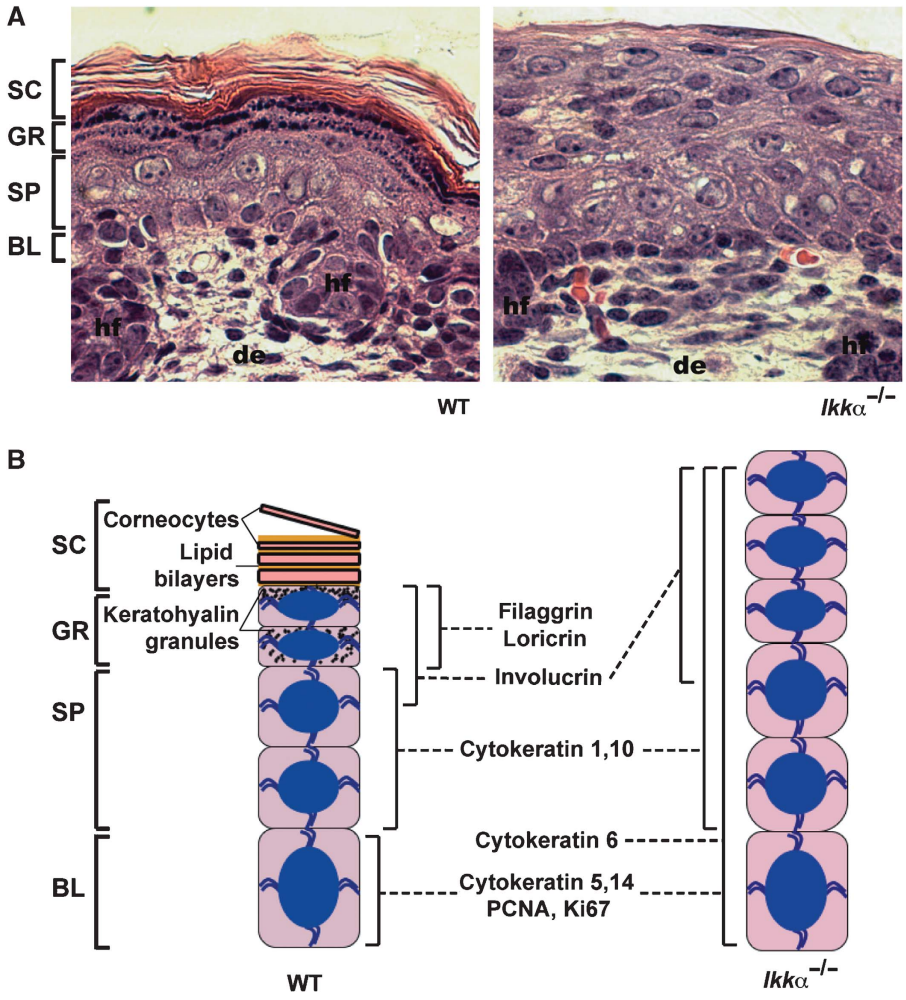

Figure 2 (**A**) Haematoxylin and eosin staining of skin sections from WT and *Ikkα*$^{-/-}$ mice. (**B**) Schematic representation of normal and *Ikkα*-deficient epidermis with expression profile of molecular markers of proliferation and differentiation (BL: basal layer; SP: spinous layer; GR: granular layer; SC: *stratum corneum*; hf: hair follicle; de: dermis; magnification × 100 in (A)).

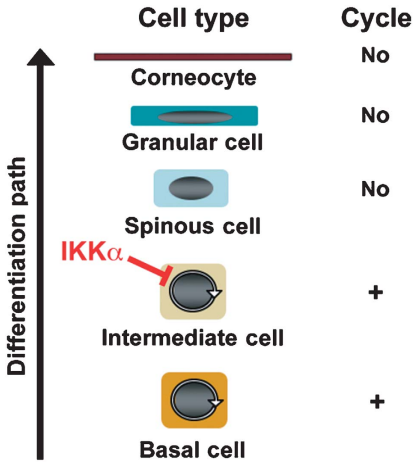

Figure 3 Schematic representation of epidermal development stages. We propose that the main function of IKKα is to induce cell cycle exit of intermediate keratinocytes when they mature into spinous cells.

differentiation (Sil *et al*, 2004). An NLS is absent from IKKβ, which cannot substitute for IKKα in keratinocyte differentiation and growth arrest. IKKα is also nuclear in basal and suprabasal cells of the epidermis (Descargues *et al*, 2008) and the oral epithelium (Maeda *et al*, 2007), findings that are consistent with the cell culture results.

The function of IKKα in the keratinocyte nucleus is linked to the production of a yet-to-be identified soluble factor or group of factors termed keratinocyte differentiation-inducing factor (kDIF) that can induce the expression of terminal differentiation markers, even in *Ikkα*$^{-/-}$ cells (Hu *et al*, 2001). Consistent with the existence of kDIF, transplantation of *Ikkα*$^{-/-}$ skin onto the back of immuodeficient WT mice allows the *Ikkα*-deficient epidermis to undergo normal differentiation, suggesting that the requirement for IKKα can be bypassed by factors produced by normal skin (Hu *et al*, 2001). These experiments do not exclude the possibility that another important source of kDIF or similarly acting factors are dermal fibroblasts, which are well known to produce factors that control epidermal morphogenesis (Wessells, 1977). In support of this hypothesis, a keratinocyte-specific *Ikkα* disruption results in a less severe epidermal differentiation defect with altered skin barrier function than the total *Ikkα* knockout (Gareus *et al*, 2007). These results were interpreted to suggest that Ikkα functions non-autonomously in the dermis to control epidermal differentiation. However, *Ikkα*-deficient keratinocytes from the epidermal-specific knockout mouse still fail to differentiate *in vitro*

(Gareus *et al*, 2007), similar to keratinocytes from total *Ikkα* knockout mice (Hu *et al*, 2001). Collectively, these results indicate that IKKα functions within epidermal keratinocytes and probably in dermal fibroblasts to induce keratinocyte differentiation. As keratinocyte-restricted expression of IKKα, unlike the keratinocyte-specific knockout, does not result in a differentiation defect (Sil *et al*, 2004), it appears that the keratinocyte is the major site of IKKα action with respect to keratinocyte differentiation and epidermal-directed morphogenetic events.

IKKα is a critical component of a Smad4-independent TGFβ–Smad2/3 signalling pathway

The most immediate effect of IKKα re-expression in *Ikkα*$^{-/-}$ keratinocytes is cell cycle withdrawal, which precedes the expression of differentiation markers (Hu *et al*, 2001). Thus, to understand the molecular function of IKKα, we searched for cell cycle-related target genes, the expression of which is IKKα dependent. This search netted several genes encoding negative cell cycle regulators, the expression of which is downregulated in *Ikkα*$^{-/-}$ keratinocytes and epidermis, including *Mad1* and *Ovol1* (Descargues *et al*, 2008), which encode negative regulators of Myc. The *c-Myc* oncogene is thought to influence the balance between keratinocyte proliferation and differentiation, depending on the intensity and timing of its activity (Watt *et al*, 2008). Mad1 is a basic region/helix-loop-helix/leucine zipper transcriptional regulator that dimerizes with Max to form Mad:Max heterodimers that antagonize the transcriptional function of Myc:Max dimers (Ayer *et al*, 1993; Grandori *et al*, 2000). *Mad1*$^{-/-}$ mice, however, are viable, phenotypically normal (Foley *et al*, 1998; Grandori *et al*, 2000) and do not show any epidermal defect. Most likely, other *Mad* genes, including *Mad2*, *Mad3* and *Mad4*, are functionally redundant with *Mad1* and compensate for its loss. Indeed *Mad2* and *Mad3* are also induced in keratinocytes in an IKKα-dependent manner (unpublished data). Ovol1 is a zinc-finger-containing transcription factor, which, similar to Mad1, is also expressed in differentiating suprabasal keratinocytes (Dai *et al*, 1998; Descargues *et al*, 2008). *Ovol1*$^{-/-}$ adult mice present with aberrant hair formation but normal epidermal differentiation (Dai *et al*, 1998). However, the suprabasal epidermis of *Ovol1*$^{-/-}$ embryos shows increased proliferation and *in vitro*, *Ovol1*$^{-/-}$ keratinocytes fail to exit the cell cycle in response to growth-inhibitory signals, such as high Ca^{2+} or TGFβ (Nair *et al*, 2006). This defect may be explained in part by abnormal upregulation of *c-Myc*, which is a direct target of Ovol1 in keratinocytes (Nair *et al*, 2006). Thus, IKKα controls keratinocyte proliferation and cycling through the regulation of several Myc antagonists to allow keratinocytes to embark on their differentiation pathway (Figure 4). Interestingly, another TGFβ-related mechanism involving Smad3/4 and E2F4/5 transcription factors has been shown earlier to directly inhibit *c-Myc* expression in keratinocytes (Chen *et al*, 2002) (Figure 4). Although this signalling pathway triggers antiproliferative effects of TGFβ in keratinocytes, its impact on keratinocyte differentiation is unknown.

Mad1 and Ovol1 are involved in the inhibition of keratinocyte proliferation induced by TGFβ family members

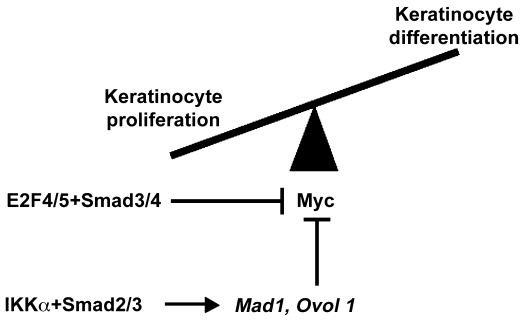

Figure 4 TGFβ-related signalling pathway controlling Myc activity in keratinocytes. The IKKα–Smad2/3 axis induces *Mad1* and *Ovol 1* expression on TGFβ stimulation. These proteins may inhibit the activity and expression of Myc, inducing in turn keratinocyte cycle exit and differentiation. Interestingly, a TGFβ–Smad3/4 signalling pathway, which is not associated with IKKα, but functions in cooperation with E2F4/5 transcription factors, has also been shown to negatively control *c-Myc* expression in keratinocytes (Chen *et al*, 2002).

(Vastrik *et al*, 1995; Gomis *et al*, 2006), suggesting a link between IKKα and the TGFβ signalling pathway. TGFβ family members, including TGFβs, activins and BMPs, are cytokines that control cell growth, differentiation and deposition of extracellular matrix through binding to heterodimeric cell surface receptor complexes composed of type I and II subunits, and intracellular Smad transcription factors (Shi and Massague, 2003; Feng and Derynck, 2005; Schmierer and Hill, 2007). The eight mammalian Smad proteins are divided into three distinct groups: receptor-activated Smads (R-Smads: Smad1, Smad2, Smad3, Smad5 and Smad8), a unique common Smad mediator (Co-Smads: Smad4) and inhibitory Smads (i-Smads: Smad6 and Smad7). On ligand binding, the type II receptor activates the type I receptor through its kinase domain, and the type I receptor in turn phosphorylates R-Smads. The activated R-Smads form heterodimeric complexes with Smad4, which accumulate in the nucleus and directly repress or activate specific target genes (Shi and Massague, 2003; Feng and Derynck, 2005; Schmierer and Hill, 2007).

TGFβ family members, their receptors and Smad transcription factors are abundantly expressed in epidermal keratinocytes, suggesting homoeostatic and regulatory functions (He *et al*, 2001; Li *et al*, 2003). Phosphorylated Smad2 and Smad3 proteins are concentrated in the nuclei of basal and suprabasal keratinocytes, whereas nuclear Smad4 staining is more preeminent in basal cells (Descargues *et al*, 2008). Nonetheless, alterations of TGFβ signalling in transgenic/knockout mice have often resulted only in minor epidermal defects, thereby obscuring its exact functions in this tissue (Li *et al*, 2003). We recently found that IKKα interacts strongly with Smad3 and weakly with Smad2, but does not bind Smad4 (Descargues *et al*, 2008). IKKα associates with the C-terminal MH2 domain of Smad3 through its kinase domain (unpublished observations). The R-Smad MH2 domain is known to be essential for *trans*-activation, phosphorylation by type I receptors and Smad4 binding (Massague, 2000; Feng and Derynck, 2005). On stimulation by TGFβ1, IKKα and Smad3 form a transcriptional complex that accumulates in the keratinocyte nucleus to directly control the transcription of *Mad1* (Descargues *et al*, 2008). Similar findings were made

for *Ovol1* (unpublished data). In *Ikkα*$^{-/-}$ keratinocytes stimulated with TGFβ1, Smad3 is no longer recruited to the *Mad1* regulatory region despite its normal association with Smad4. Furthermore, nuclear staining for activated Smad2 and Smad3 is dramatically diminished in the *Ikkα*$^{-/-}$ epidermis (Descargues *et al*, 2008). Taken together, these results indicate that IKKα is required for nuclear accumulation and chromatin recruitment of phosphorylated Smad2 and Smad3 to IKKα-regulated genes in response to TGFβ1. Interestingly, activin A, an important regulator of epidermal differentiation (Owens *et al*, 2008), is involved in the induction of *Mad1* expression in keratinocytes (Werner *et al*, 2001). As a result, one may speculate that the IKKα–Smad2/3 complex forms and activates anti-Myc genes, including *Mad1*, on activin A signals. As activin A also downregulates the *Id1*, *Id2* and *Id3* genes in keratinocytes (Rotzer *et al*, 2006), it could also be interesting to analyse whether this signalling pathway depends on IKKα. Finally, we found that kDIF functions downstream of the IKKα–Smad2/3 signalling pathway as it can induce differentiation of *Ikkα*$^{-/-}$ keratinocytes without inducing *Mad1* expression (Figure 5). Exactly how kDIF functions and what it is composed of remain to be determined.

The results mentioned above provided the first clear evidence for a critical function for Smad transcription factors in epidermal differentiation. Although Smad3 deficiency alone does not result in any cutaneous defect (Zhu *et al*, 1998; Datto *et al*, 1999; Yang *et al*, 1999) and the loss of Smad2 leads to early embryonic lethality (Nomura and Li, 1998; Waldrip *et al*, 1998; Weinstein *et al*, 1998; Heyer *et al*, 1999), loricrin and filaggrin expression is barely detectable in E15.5 embryos lacking both *Smad3* alleles and one *Smad2* allele in their epidermis (Descargues *et al*, 2008). Similarly, siRNA-mediated Smad2 knockdown in Smad3-deficient keratinocytes inhibited the expression of loricrin and filaggrin, as well as Mad1, in response to high Ca^{2+} (Descargues *et al*, 2008). These results suggest that Smad2 and Smad3 are functionally redundant. The importance of the TGFβ–Smad2/3–IKKα axis for proper epidermal differentiation is also underscored by the analysis of transgenic mice in which the i-Smad Smad7 is inducibly expressed in the epidermis, resulting in defective expression of loricrin and filaggrin and failed *stratum corneum* formation (Descargues *et al*, 2008). Consequently, these mutant mice display epidermal hyperplasia due to abnormal proliferation of suprabasal keratinocytes and loss of nuclear IKKα and Mad1 (Descargues *et al*, 2008).

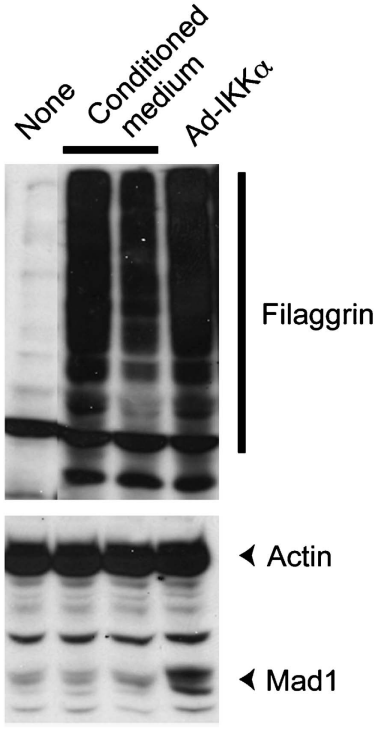

Figure 5 Mad1 expression is not induced by kDIF-mediated keratinocyte differentiation. Conditioned medium from WT keratinocytes, which contains kDIF as shown earlier (Hu *et al*, 2001), failed to induce Mad1 expression in *Ikkα*$^{-/-}$ keratinocytes while leading to keratinocyte differentiation as indicated by filaggrin expression. Only the re-expression of IKKα in *Ikkα*$^{-/-}$ keratinocytes infected with adenovirus encoding this protein (Ad-IKKα) induces Mad1 expression.

Surprisingly, this new TGFβ response pathway centred around Smad2/3–IKKα complex formation is independent of Smad4. Smad4-deficient keratinocytes display normal Mad1 expression (Descargues *et al*, 2008) and undergo terminal differentiation in response to high Ca^{2+} (unpublished data). These results are consistent with the phenotype of mice with epidermal-specific deletion of *Smad4* (*Smad4*$^{\Delta/\Delta}$ mice). These mice present with degeneration of hair follicles and dermal cysts that progress to skin tumours in old animals, but do not show any perturbated epidermal differentiation and *stratum corneum* formation (Yang *et al*, 2005; Qiao *et al*, 2006). Furthermore, activated Smad2 and Smad3, as well as IKKα, are normally localized in the nuclei of *Smad4*$^{\Delta/\Delta}$ keratinocytes, which display normal Mad1 expression (Descargues *et al*, 2008). Taken together, these results strongly indicate that Smad4 is not required for epidermal differentiation. The Smad4 independence of the TGFβ–Smad2/3–IKKα signalling pathway is reminiscent of another TGFβ signalling operative during erythroid development in which TIF1γ (also called TRIM33 or ectodermin) replaces Smad4 (He *et al*, 2006). However, TIF1γ is a RING-type ubiquitin ligase that can target Smad4 to degradation and can therefore function as a negative regulator of Smad4-dependent TGFβ signalling (Dupont *et al*, 2005). Hence the exact function of TIF1γ in TGFβ signalling is not fully understood, and it is not known whether it affects the IKKα-dependent pathway.

IKKα and squamous cell carcinoma

IKKα was recently identified as a tumour suppressor in squamous cell carcinoma (SCC) (Liu *et al*, 2006; Maeda *et al*, 2007). SCC is a cancer derived from squamous epithelia of the skin (epidermis), head and neck tissues (mouth, throat, oral and nasal cavities, esophagus) as well as other sites. SCC is the second most common skin cancer in Caucasians with an estimated incidence of 100 000–150 000 new cases per year in the United States (Johnson *et al*, 1992). Sun exposure and immune suppression increase the risk of SCC development and so does tobacco use (Rudolph and Zelac, 2004; Hampton, 2005). SCC of the oral cavity is one of the most prevalent cancers of the head and neck region with a worldwide incidence of 300 000 new cases per year, the

occurrence of which is linked to tobacco use and betel nut chewing (Silverman, 2001). SCCs of the oral cavity are more aggressive than those developing from the skin, and are associated with a 5-year survival rate of about 50–55% (Silverman, 2001).

Molecular changes in SCCs are characterized by a marked heterogeneity and include activation of oncogenes, such as *RAS*, *MYC*, *EGFR* and *Cyclin D1*, as well inactivation of tumour suppressors, including *p53* and *p16* (Hardisson, 2003). These genetic alterations are thought to influence malignant keratinocyte behaviour and tumour progression, but the precise molecular pathogenesis of SCC is poorly understood. Interestingly, mutations in exon 15 of the *IKKα* locus were described in a few high-grade and poorly differentiated human SCCs of the skin and were shown to be associated with reduced *IKKα* expression (Liu *et al*, 2006). However, it seems that downregulation of IKKα due to epigenetic silencing of the *IKKα* locus is a more common occurrence seen in close to 30% of invasive oral SCCs (Maeda *et al*, 2007). It was also reported that overexpression of IKKα in the suprabasal compartment, which results in increased epidermal differentiation and reduced keratinocyte proliferation, inhibits chemically induced SCC formation and progression in mice (Liu *et al*, 2006). These results, together with enhanced SCC incidence in $Ikk\alpha^{+/-}$ mice subjected to two-stage skin carcinogenesis and loss of *Ikkα* heterozygosity in the tumours, provide evidence that IKKα is a tumour suppressor in the epidermis (Liu *et al*, 2006; Park *et al*, 2007). This function of IKKα is probably mediated through the control of keratinocyte proliferation. The loss of nuclear IKKα contributes to malignant conversion of keratinocytes into less differentiated and proliferative carcinoma cells. However, a recent study has suggested that increased IKKα may be found in acantholytic SCC (ASCC) (Moreno-Maldonado *et al*, 2008), an histologic variant of SCC showing positive staining for CKs (Rinker *et al*, 2001). Unfortunately, the authors of that study have not carefully analysed whether ASCCs present with loss of nuclear IKKα, which would make their results more consistent with other studies mentioned above.

SCC and other carcinoma cells are known to overproduce TGFβ1 to modify their microenvironment through local immunosupression, extracellular matrix remodelling and neoangiogenesis, and these changes are required for tumour progression and invasiveness (Oft *et al*, 1996, 1998; Siegel and Massague, 2003; Li *et al*, 2005a, 2006). At the same time, carcinoma cells become resistant to TGFβ-induced growth arrest (Siegel and Massague, 2003; Li *et al*, 2005a, 2006). Altered IKKα function may contribute, at least in part, to acquired resistance to TGFβ1-induced growth arrest.

Conclusions and future directions

Although IKKα was first identified as a catalytic subunit of the IKK complex, which mediates NF-κB activation (DiDonato *et al*, 1997; Mercurio *et al*, 1997; Regnier *et al*, 1997; Zandi *et al*, 1997), it has quickly emerged as a multifunctional protein with several unexpected and surprising activities. In

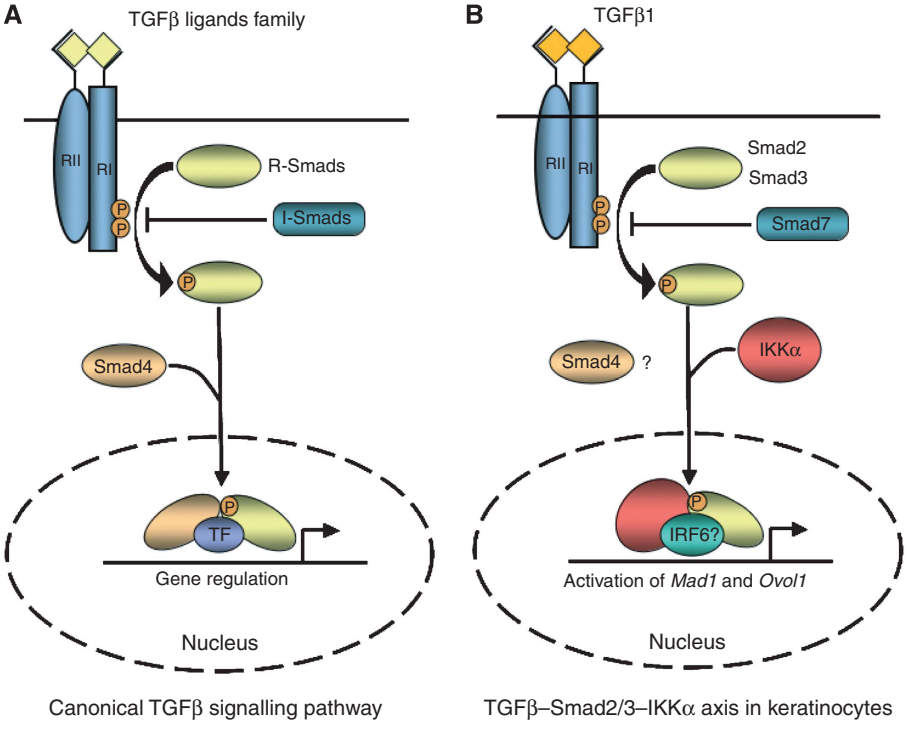

Figure 6 (**A**) In the canonical TGFβ signalling pathway, ligands signal through type I and II transmembrane protein kinase receptors. After TGFβ binding, type II receptor recruits and phosphorylates type I receptor, which in turn activates R-Smads. Phosphorylated R-Smads oligomerize with the co-Smad Smad4 and accumulate in the nucleus where they interact with DNA and transcription factors to regulate the expression of target genes. (**B**) During epidermal terminal differentiation, TGFβ1 stimulation of keratinocytes induces the formation of a complex between activated Smad2/3 and IKKα. This complex accumulates in keratinocyte nuclei independently of the presence of Smad4 and controls the transcriptional activation of *Mad1* and *Ovol1* probably with the cooperation of other transcription factors such as IRF6. The Smad4-independent TGFβ–Smad2/3–IKKα axis is required for cell cycle exit and induction of terminal differentiation of keratinocytes.

the epidermis, IKKα has turned out to be a critical regulator of keratinocyte proliferation, differentiation and oncogenic transformation, and this function is completely unrelated to its protein kinase activity or NF-κB signalling. Instead, IKKα functions as a cofactor for Smad2/3 in a Smad4-independent pathway that inhibits keratinocytes proliferation (Descargues *et al*, 2008) (Figure 6). In this capacity, IKKα is required for the induction of a specific subset of TGFβ-responsive genes that include the Myc antagonists *Mad1* and *Ovol1*, but is not needed for other well-known TGFβ target genes, such as *p21*, *p15* and *p27*, encoding inhibitors of cyclin-dependent kinases (CDKs) (Descargues *et al*, 2008). This is reminiscent of the two different classes of antiproliferative gene responses: Myc repression and inhibition of CDKs, respectively, that are induced during TGFβ-mediated cell cycle arrest (Massague *et al*, 2000). It is attractive to speculate that the tumour suppressive function of IKKα is exerted through this pathway as well.

Other proteins may be part of the TGFβ–Smad2/3–IKKα signalling pathway, as revealed by two mouse models with functional alterations of 14-3-3σ (repeated epilation mutant mice) and IRF6, the disruption of which faithfully mimics the phenotype of *Ikkα*$^{-/-}$ mice (Herron *et al*, 2005; Li *et al*, 2005b; Ingraham *et al*, 2006; Richardson *et al*, 2006). 14-3-3σ belongs to a family of adaptors that can interact with target proteins in a sequence-specific manner, although its exact function is poorly understood (Mhawech, 2005). It was reported that IKKα may protect the *14-3-3σ* locus from hypermethylation in keratinocytes by interacting with histone H3 (Zhu *et al*, 2007). In that study, the authors showed that *14-3-3σ* is downregulated in *Ikkα*$^{-/-}$ keratinocytes, suggesting that this gene is a downstream target of IKKα (Zhu *et al*, 2007). The human *IRF6* locus is defective in Van der Woude (VWS, OMIM: 119300) and popliteal pterygium (PPS, OMIM: 11500) syndromes, which are characterized by orofacial defects such as cleft lip and palate (Kondo *et al*, 2002). IRF6 belongs to a family of transcription factors that share a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain. Interestingly, this protein-binding domain is related to the C-terminal MH2 domain of Smad proteins and has been referred to SMIR (Smad and IRF) domain (Eroshkin and Mushegian, 1999). As DNA binding by Smad transcription factors depends on their association with other DNA-bound transcription factors (Derynck and Zhang, 2003; ten Dijke and Hill, 2004), one can speculate that IRF6 may be a component of the Smad2/3–IKKα transcriptional complex that accumulates in the keratinocyte nucleus to induce the obligatory cell cycle exit that precedes terminal differentiation (Figure 6). In addition, IKKα may also interact with other transcription factors, such as RARs to control epidermal barrier formation (Gareus *et al*, 2007). The identification of other IKKα-interacting proteins and additional IKKα target genes will provide an ever better understanding of how this critical regulator of epidermal proliferation and differentiation carries out its daily work.

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