

The TFIID subunit TAF4 regulates keratinocyte proliferation and has cell-autonomous and non-cell-autonomous tumour suppressor activity in mouse epidermis

Anas Fadloun¹, Dominique Kobi¹, Jean-Christophe Pointud^{1,*}, Arup Kumar Indra^{1,†}, Marius Teletin¹, Christine Bole-Feysot¹, Barbara Testoni², Roberto Mantovani², Daniel Metzger¹, Gabrielle Mengus¹ and Irwin Davidson^{1,‡}

The TAF4 subunit of transcription factor TFIID was inactivated in the basal keratinocytes of foetal and adult mouse epidermis. Loss of TAF4 in the foetal epidermis results in reduced expression of the genes required for skin barrier function, leading to early neonatal death. By contrast, TAF4 inactivation in adult epidermis leads to extensive fur loss and an aberrant hair cycle characterised by a defective anagen phase. Although the mutant epidermis contains few normal anagen-phase hair follicles, many genes expressed at this stage are strongly upregulated indicating desynchronised and inappropriate gene expression. The TAF4 mutant adult epidermis also displays interfollicular hyperplasia associated with a potent upregulation of several members of the EGF family of mitogens. Moreover, loss of TAF4 leads to malignant transformation of chemically induced papillomas and the appearance of invasive melanocytic tumours. Together, our results show that TAF4 is an important regulator of keratinocyte proliferation and has cell-autonomous and non-cell-autonomous tumour suppressor activity.

KEY WORDS: Alopecia, Hair cycle, Growth factors, EGF, TPA, Wnt pathway, Homologous recombination

INTRODUCTION

The general transcription factor TFIID is composed of the TATA-binding protein (TBP) and 13–14 evolutionary conserved TBP-associated factors (TAFs) (Tora, 2002). These factors assemble to form a large multiprotein complex comprising three large lobes around a central cavity (Grob et al., 2006; Leurent et al., 2004; Matangkasombut et al., 2004). TAF4 (also known as TAF4a) is a ubiquitously expressed TFIID subunit, also present in TFTC (Brand et al., 1999; Mengus et al., 1997; Tanese et al., 1996; Thuault et al., 2002), that is essential in yeast and plays a general role in transcription in *C. elegans* embryos (Sanders and Weil, 2000; Walker et al., 2001). The TAF4 family also comprises TAF4b, which is required for testis and ovary function (Falender et al., 2005; Freiman et al., 2001).

We previously characterised *Taf4*^{-/-} embryonic fibroblasts, in which loss of TAF4 promotes a positive autoregulatory feedback loop of transforming growth factor (TGF β) signalling leading to serum-independent autocrine growth (Mengus et al., 2005). More than 1000 genes are deregulated, including those encoding TGF β 1 and β 3 as well as connective tissue growth factor (CTGF) and factors that activate latent TGF β (for a review, see Shi and Massague, 2003). We also showed that TAF4 is a coactivator for the RAR [all-trans retinoic acid (T-RA) receptor] (Gangloff et al., 2000; Mengus et al., 1997), as the transcriptional activity of transfected RAR is strongly diminished in *Taf4*^{-/-} cells (Mengus et al., 2005).

As TAF4 controls signalling pathways and cell proliferation in vitro, we sought to test its role in physiological processes in vivo. In this context, the development and homeostasis of the epidermis constitutes an attractive model. The epidermis consists of a single layer of proliferating basal keratinocytes that differentiate into spinal and granular suprabasal layers, then into the flattened and enucleated corneocytes that provide the physical barrier against the environment. Epidermal morphogenesis begins at E8.5 as a single layer, followed by stratification at E9.5 and differentiation and formation of the skin barrier by E18.5 (Fuchs and Raghavan, 2002; Hardman et al., 1998). Several of the pathways regulated by TAF4 play important roles in epidermal homeostasis. The TGF β pathway restricts keratinocyte proliferation in the adult epidermis (Wang, 2001) and T-RA is an important regulator of keratinocyte function – T-RA and its derivatives being used to treat a variety of skin disorders (Futoryan and Gilchrist, 1994; Zouboulis, 2001).

To determine whether TAF4 plays a role in keratinocyte proliferation and differentiation, we used mice expressing the Cre recombinase or the tamoxifen (Tam)-dependent Cre-ER^{T2} under the control of the keratin 14 (*K14*; also known as *Krt14* – Mouse Genome Informatics) promoter to inactivate TAF4 in basal keratinocytes in the foetal and adult epidermis. Our results indicate that TAF4 plays distinct but crucial roles in normal foetal and adult epidermal development and homeostasis. Loss of TAF4 also provokes enhanced formation and malignant transformation of chemically induced epidermal papillomas and, unexpectedly, invasive non-cell-autonomous melanocytic tumours.

MATERIALS AND METHODS

Mice

The *Taf4*^{lox/lox} and *K14-Cre* and *K14-Cre-ER^{T2}* strains have been described previously (Li et al., 2001; Mengus et al., 2005). Animals were handled according to institutional guidelines and policies. 100 μ l of oil (olive oil) and Tam (50 μ g dissolved in olive oil) were administered by

¹Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/UJLP, 1 Rue Laurent Fries, 67404 Illkirch Cédex, France. ²Dipartimento di Scienze Biomolecolari e Biotecnologie, U. di Milano, Milano, Italy.

*Present address: UMR 6547, Genetiques des Eucaryotes et Endocrinologie Moléculaire, Complexe Universitaire des Cezeaux, 67133, Aubiere, France

†Present address: Department of Pharmaceutical Sciences, Program in Molecular and Cellular Biology, Oregon State University, Corvallis, OR 97331, USA

‡Author for correspondence (e-mail: irwin@titus.u-strasbg.fr)

intraperitoneal injection daily for 5 days. DMBA and TPA were resuspended in acetone. A single 50 µg DMBA application was followed by twice weekly 5 µg TPA applications for 35 weeks. For the double DMBA application, 100 µg were applied twice with a 1-week interval. For T-RA treatment, 80 nmol of T-RA in 200 µl acetone, or acetone alone as control, was applied daily for 5 days. For calcipotriol (Daivonex) treatment, 2.5 µg in 50 µl were applied daily for 5 days. All treatments were performed on a shaved region of the back.

Immunostaining and histological analysis

Skin biopsies from age- and sex-matched animals were taken from comparable locations and fixed with 4% paraformaldehyde/1×PBS for 12 hours at 4°C and embedded in paraffin. 5 µm sections were cut and immunofluorescence was performed essentially as described (Indra et al., 2005) using the anti-TAF4 antibody and commercial antibodies against Ki-67 and keratin 6. Nuclei were counterstained with Hoechst. For histological analysis, sections were stained with Haematoxylin and Eosin.

Skin permeability and transepidermal water loss (TEWL) assays

The X-Gal permeability assay was performed as described (Indra et al., 2005). E18.5 pups were rinsed in PBS for 1 hour and immersed in an X-Gal solution (pH 4.5) at 37°C for 8 hours. Following incubation, they were briefly washed in PBS and photographed. TEWL from foetal dorsal and ventral E18.5 skin was measured with a Tewameter (Courage and Kazaka TM210) and from the adults using the Dermalab System (Cortex Technology, Denmark) equipped with a TEWL probe as described (Indra et al., 2005). Mean values of six measurements from each animal were determined. Data are expressed as mean±s.d.

RT-PCR and microarray analyses

Total RNA was isolated from skin biopsies using the RNeasy Midi Kit (Qiagen). RT-PCR was performed essentially as previously described (Mengus et al., 2005). Q-PCR was performed using a Light Cycler (Roche) and the Cyber Green Kit (Qiagen). The primer sequences are listed in Table 1.

Microarray analyses were performed as previously described (Frontini et al., 2005) on a mouse oligonucleotide microarray covering 25,000 transcripts. The oligonucleotide collection is from a public resource – Réseau National des Génopoles, France and MRC, UK (Le Brigand et al., 2006). More information about these oligonucleotides is available at <http://www.microarray.fr:8080/merge/index>. Oligonucleotides (synthesized by Proligo) were diluted to 50 µM in 50% dimethyl sulphoxide and 100 mM phosphate buffer and spotted using a µGridII arrayer (BioRobotics) onto hydrogel-coated slides (H slides, Schott).

Total RNA (200 ng) prepared from Tam- or oil-injected *K14-CreER^{T2}/Taf^{4lox/lox}* animals was amplified by linear PCR, and the amplification products were labelled with Cy5 and purified using Nucleospin Extract II columns (Macherey Nagel). The RNA from one oil-injected animal was similarly amplified, labelled with Cy3 and used as a reference probe for the hybridisation. Labelled cDNAs (sample labelled with Cy5 and reference with Cy3) were hybridized on the oligonucleotide microarray in a Discovery Station using ChipHybe 80 hybridisation buffer at 42°C for 12 hours without any final stringency washes (Ventana Medical System hybridisation automate, reagents and microarray hybridisation procedure). All the protocols used are available at <http://www-microarrays.u-strasbg.fr/>. After slide scanning (ScanArray4000, PerkinElmer), the TIF images obtained were quantified using Imagene 5 (BioDiscovery) and the raw data normalised using the quantile method.

Table 1. Primers used in Q-PCR

Gene	Forward primer	Reverse primer
<i>Klf4</i>	CATTATCAAGAGCTCATGCCA	GTCACAGTGGTAAGGTTTCTC
<i>Cldn1</i>	GTGGAAGATTTACTCCTATGC	CAG-GATGCCAATTACCATCAA
<i>Tgm1</i>	GATGCCTGCCTGTACATCCT	GCAGAGACAACCCGAGAGAC
<i>Tgm3</i>	GCTCACGACACAGATCGAAA	CGCTATCACTGCCTTTCTCC
<i>TAp63</i>	CGAAAATGGTGCAACAAACA	GATGGAGAGAGGGGCATCAA
<i>DNP63</i>	GGAAAACAATGCCAGAGATC	GATGGAGAGAGGGGCATCAA
<i>Cdh1</i>	CAAGGACAGCCTTCTTTTCG	TGGACTTCAGCGTCACTTTG
<i>Cdh3</i>	GATTTTGAGGCTCAGGACCA	GACCTTGGAAAGGTGGAACAA
<i>Lor</i>	TCCTCTCAGCAGACCAGTCA	CACCTCCACAGCTACCACCT
<i>Flg</i>	GGGAGGCAAGCTACAACAAC	CCTGCCTCCTTCAGAGTCAC
<i>Ixl</i>	TATGGCAGGGGATCAGAAAG	GGGTCAAGGTGACTCCTGGTA
<i>Hprt</i>	GTAATGATCAGTCAACGGGGGAC	CCAGCAAGCTTGAACCTTAACCA
<i>Hbegf</i>	GACCCATGCCTCAGGAAATA	TGAGAAGTCCCACGATGACA
<i>Ahf</i>	AACAAGAGCTCCGTCAGGAA	CAGTGCTCTTCTCCAGTC
<i>Msx2</i>	CATCCAGCTTCTAGCCTTG	GCAGCCATTTTCAGCTTTTC
<i>Lrrc15</i>	GCTGACGAATCTCCGAGAAC	CATGAGACCATTGACGTTGG
<i>Gjb2</i>	ATGCTACGACCACCACTTCC	TACGGACCTTCTGGGTTTTG
<i>Rxra</i>	GTCGAGCCCAAGACTGAGAC	TCTAGGGGCGAGCTCAGAAAA
<i>Rxrb</i>	CTGCAAGGGTTTCTCAAGC	ATCCTGTCCACAGGCATCTC
<i>Sfrp4</i>	ACCCTGGCAACATACCTGAG	ATCATCCTTGAACGCCACTC
<i>Sfrp2</i>	ACGACAACGACATCATGGAA	CAGCACGGATTCTTCAGGT
<i>Dab2</i>	CGCTTTCACCTGGCTTAGACC	GTAAGTGGAGAAGGCGCTTG
<i>β-actin</i>	CTTCTCCCTGGAGAAGAGC	AAGGAAGGCTGGAAAAGAGC
<i>Scf</i>	TCCGAAGAGGCCAGAAACTA	TCAGATGCCACCATAAAGTCC
<i>Edn1</i>	ACTTCTGCACCTGGACATC	GTCTTTCAAGGAACGCTTGG
<i>Edn3</i>	ACACGCTTGCGTTGACTTG	TTTCTGCTCTCCCGGAATAA
<i>Ednrb</i>	CAAAGATTGGTGGCTGTTCA	GCCACTTCTCGTCTGCTT
<i>Mitf</i>	TGAAGCAAGAGCATTGGCTA	TAGCTCCTTAATGCGGTCTG
<i>Fgf2</i>	CGGTCACGGAAATACTCCAG	TATGGCCTTCTGTCCAGGTC
<i>Pomc</i>	GTACCCCAACGTTGCTGAGA	GACCTGCTCCAAGCCTAATG
<i>Mc1r</i>	ACTCCAATGCCACCTCTCAC	AGCCCTAGGCTGAGGAAGAG
<i>Dct</i>	AACAACCCTTCCACAGATGC	TAGTACCCGGTGGGAAGAAG
<i>Tyr</i>	TACTCAGCCACGATCCTT	TCAGGTGTTCCATCGCATAA
<i>Trp1</i>	TCTGGCCTCCAGTTACCAAC	GGCTTCATTCTGGTGCTTC

Sequences are shown 5' to 3'.

In order to determine genes differentially expressed between the two groups (five samples from Tam injection and three from oil injection), we performed a Student's *t*-test using the Cy3/Cy5 log₂ ratios for the oil- and Tam-injected samples. To limit the error resulting from multiple tests, we used the false discovery rate control procedure (Reiner et al., 2003). Genes with a *P*-value of less than 0.01 were then selected as significant. Moreover, we filtered out all genes having a log₂ signal lower than 9 (value corresponding to the first quartile). Finally, we selected deregulated genes with a log₂ change above 1 and below -1 [log change=M1-M2, calculated by using the mean (M1) of log₂ ratio for Tam-Cy3/oil-reference-Cy5 and the mean (M2) of log₂ ratio for oil-Cy3/oil-reference-Cy5].

Extract preparation and immunoblotting

Protein extracts were made from oil- and Tam-injected animals as follows. The epidermis was separated from the dermis and soluble extracts were made by crushing the epidermis in buffer containing 50 mM Tris-HCl (pH 7.9), 10% glycerol, 1 mM DTT, 1 mM EDTA, 0.2% NP40 and 0.5 M KCl, followed by three cycles of freeze-thaw, incubation for 30 minutes at 4°C and finally microfuge centrifugation at 13,000 rpm (10,000 *g*) for 10 minutes. CDKN1A, cyclin D1 and p53 were detected using antibodies SC 6246, 718 and 126 (Santa Cruz), respectively. Alternatively, for analysis of phosphorylated proteins, the epidermis was crushed directly in Laemmli loading buffer, boiled and aliquots were separated by SDS-PAGE followed by Coomassie Blue staining for quantitation. Equal amounts were then loaded on a second SDS-PAGE gel and probed with antibodies against phospho-p38 and phospho-CREM/ATF1 (9211 and 9191, both from Cell Signalling).

RESULTS

TAF4 is required for proper differentiation of the foetal epidermis and acquisition of skin barrier function

To investigate the role of TAF4 in epidermal development, mice bearing the previously described floxed alleles of *Taf4* (Mengus et al., 2005) were crossed with mice expressing the Cre recombinase under the control of the *K14* promoter to obtain *K14-Cre/Taf4^{lox/+}* mice. This transgene drives Cre expression in basal keratinocytes from E9.5 (Indra et al., 1999; Li et al., 2000). Following intercrossings, we obtained *K14-Cre/Taf4^{lox/+}* offspring, but no viable *K14-Cre/Taf4^{lox/lox}* mice were detected amongst the progeny (data not shown). These mice should lack TAF4 in basal keratinocytes owing to the Cre-mediated deletion of the floxed *Taf4* alleles, whereas the *K14-Cre/Taf4^{lox/+}* mice would continue to express TAF4 from the wild-type allele. We observed dead newborn *K14-Cre/Taf4^{lox/lox}* animals, suggesting that Cre-mediated inactivation of TAF4 in the foetal epidermis provokes early post-natal death.

To investigate this, gestating female mice were sacrificed at E18.5 and the foetuses recovered. Genotyping indicated the expected mendelian frequency of *K14-Cre/Taf4^{lox/lox}* animals (data not shown). Visual inspection showed that a proportion of the foetuses had shiny transparent skin (Fig. 1A), correlating with a *K14-Cre/Taf4^{lox/lox}* genotype.

Selective loss of TAF4 in the epidermis of the *K14-Cre/Taf4^{lox/lox}* foetuses was seen by immunofluorescence with an anti-TAF4 monoclonal antibody. TAF4 was strongly expressed in the nuclei of dermal cells and of the basal and suprabasal keratinocytes in the epidermis of *K14-Cre/Taf4^{lox/+}* mice (Fig. 1C). By contrast, TAF4 was absent from the epidermis of the *K14-Cre/Taf4^{lox/lox}* mice, but expressed in dermal cells. Histological staining showed that the epidermis of the *Taf4^{ep-/-}* (in which TAF4 was inactivated in the epidermis) animals was significantly thinner than that of the *Taf4^{eplox/+}* animals and displayed less surface keratin (Fig. 1D). Thus, selective inactivation of TAF4 in the epidermis results in altered skin appearance and histology.

The abnormal appearance of the TAF4 mutant epidermis suggested defective skin barrier function leading to enhanced water loss and early post-natal death. Wholemount assays revealed increased skin permeability of these foetuses, as evidenced by the extensive blue staining produced upon the metabolism by endogenous β-galactosidase of X-Gal that had efficiently penetrated the skin (Fig. 1B). Measurement of transepidermal water loss in E18.5 caesarean-delivered pups revealed an almost 10-fold increase in the *Taf4^{ep-/-}* foetuses as compared with *Taf4^{eplox/+}* (Fig. 1E). Increased water loss led to a rapid and significant weight loss (Fig. 1F) and is the likely cause of the inability of these animals to survive after birth.

Skin barrier formation requires expression of the KLF4 transcription factor, the tight junction protein claudin 1 (CLDN1), the cross-linking transglutaminase enzymes TGM1 and TGM3, and cadherin 1 (CDH1) (Dai and Segre, 2004; Furuse et al., 2002; Steinert, 2000; Zeeuwen, 2004). Quantitative RT-PCR analysis on RNA from the skin of three independent E18.5 *Taf4^{ep-/-}* and *Taf4^{eplox/+}* foetuses showed a marked reduction of *Cldn1* expression in *Taf4^{lox/lox}* animals, a lesser but significant reduction in *Klf4* and *Tgm1* expression, but little effect on *Tgm3* expression (Fig. 1G). Downregulation of *Cdh1* and *Cdh3* was observed along with that of the late differentiation markers loricrin (*Lor*) and filaggrin (*Flg*), although involucrin (*Ivl*) expression was unchanged. Together, these results indicate that TAF4 is essential for the normal expression of a series of genes required for proper development of the foetal epidermis and acquisition of skin barrier function.

Loss of TAF4 in the adult epidermis leads to a defective anagen phase of the hair cycle

We next investigated the role of TAF4 in homeostasis of the adult epidermis. Mice expressing a Tam-dependent Cre recombinase under the control of the *K14* promoter, *K14-CreER^{T2}*, were bred with the *Taf4^{lox/lox}* mice to generate *K14-CreER^{T2}/Taf4^{lox/lox}* mice. At 6-8 weeks after birth, these animals were injected daily for 5 days with 50 μg Tam or olive oil as control. After 2 weeks, a skin biopsy was taken from the tail and epidermal-specific Tam-induced recombination of the floxed *Taf4* alleles was observed by PCR (Fig. 2A). By immunofluorescence, strong TAF4 expression was seen in all basal keratinocytes in oil-injected animals, (Fig. 2B, upper). By contrast, in Tam-injected animals, TAF4 expression was lost from all basal keratinocytes (Fig. 2B, lower). Injection of Tam therefore results in a rapid and complete loss of epidermal TAF4 expression.

Three to four weeks after injection, significant loss of fur was detected in the Tam-injected, but not oil-injected, mice (see Fig. S1A,B in the supplementary material). This phenotype was 100% penetrant in recombined animals and was first evident around the snout, and by 4-6 weeks large areas of the body were affected (Fig. 3A-C). Fur loss was not homogenous, but patchy, each animal showing a localised fur loss either on the ventral face, the flanks or the back. Following the initial loss, fur regrew in the affected areas, but further loss took place at neighbouring locations (compare Fig. 3B with C). Upon renewal, however, the fur was much less dense than the original coat. After several cycles of localised loss and regrowth taking place over a 2-3 month period, the animals acquired a more homogenous sparse coat (Fig. 3D and see Fig. S1C in the supplementary material). TAF4 is therefore essential for a normal hair cycle.

The above results show that the Tam-injected animals are unable to compensate for the normal loss of fur by the growth of a new coat, suggesting an inability to properly enter the anagen phase. Depilation by wax stripping can be used to synchronize the anagen

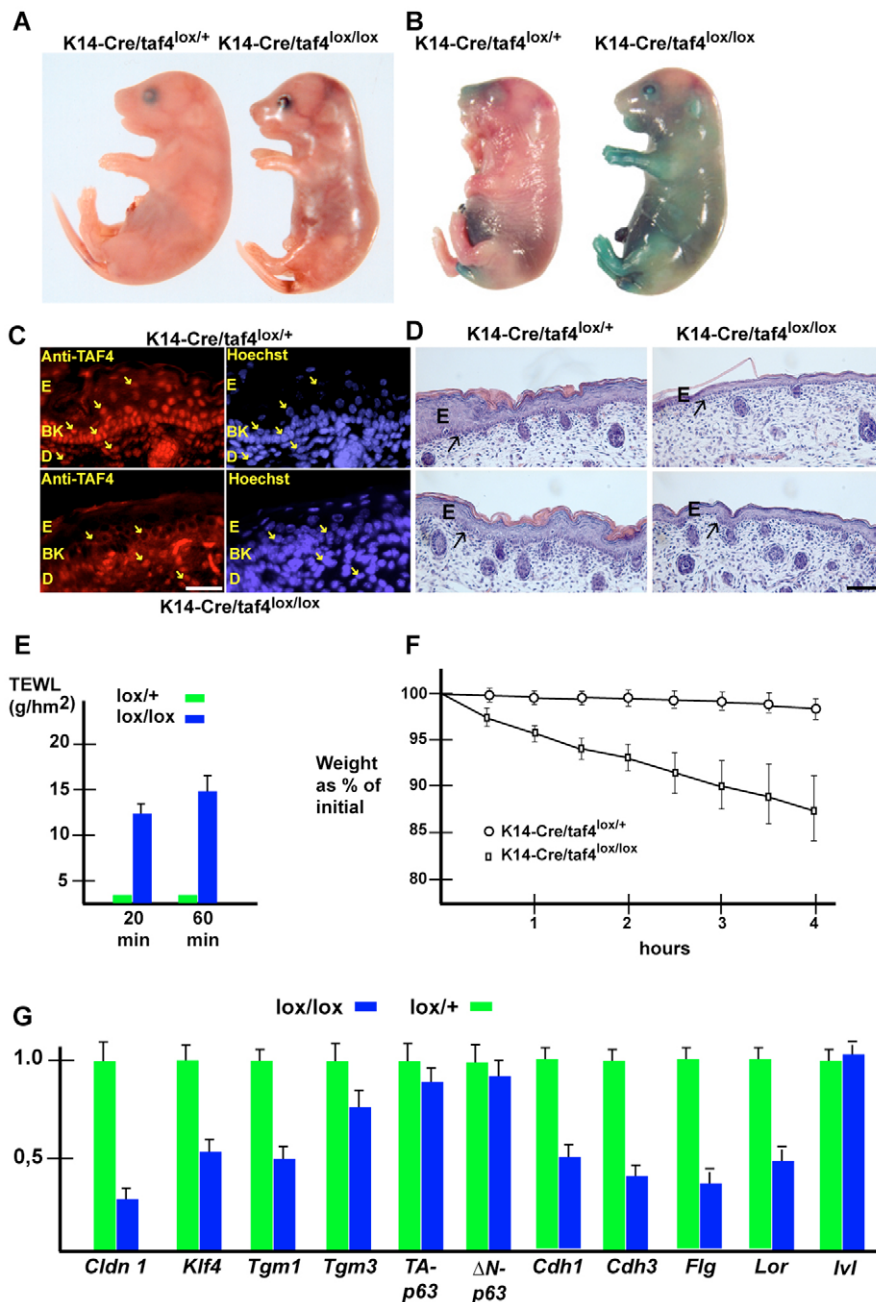


Fig. 1. Inactivation of TAF4 in foetal epidermis. (A, B) Gross morphology and X-Gal dye diffusion assay of E18.5 mice of the indicated genotypes. (C) Immunostaining of skin biopsies of E18.5 animals of the indicated phenotypes with anti-TAF4 antibody. The epidermis (E), dermis (D) and basal keratinocyte layer (BK) are indicated. Arrows indicate representative nuclei. Left panel, immunostaining; right panel, Hoechst stain. (D) Histological analysis of representative Haematoxylin-Eosin-stained paraffin sections from dorsal or ventral (upper and lower panels, respectively) regions of control and mutant foetuses at E18.5. The epidermis is indicated and the arrows indicate the location of the basal membrane. (E) Transepidermal water loss (TEWL) of three independent E18.5 foetuses of each genotype after 20 or 60 minutes as indicated. (F) Weight loss of three independent E18.5 foetuses of each genotype after the indicated times. (G) Quantitative RT-PCR on RNA from three independent E18.5 foetuses of each genotype. Duplicate reactions were performed for each RNA preparation. In each case, the expression in the *Taf4*^{lox/+} animals is set as 1. Scale bars: 30 μ m in C; 50 μ m in D.

phase of the hair cycle. The backs of oil and Tam-injected animals were depilated and fur growth was monitored over the following 20 days. The fur of oil-injected animals reappeared by 10 days and was reconstituted by 20 days (Fig. 3E). By contrast, little or no fur growth was seen in the Tam-injected animals (Fig. 3F) showing that anagen phase induction was severely impaired by TAF4 inactivation. This was confirmed by histological analysis, in which 6-10 days after depilation a large number of anagen-phase hair follicles were observed in the oil-injected but not Tam-injected animals (see Fig. S2 in the supplementary material).

Hyperplasia of TAF4 mutant epidermis

Immunostaining of the Tam-injected animals revealed large numbers of suprabasal keratinocytes (see Hoechst panel in Fig. 2B) suggesting that TAF4 inactivation induces epidermal hyperplasia. Histological analysis confirmed the thickening of the

interfollicular epidermis 2 and 3 weeks after Tam injection, seen on both dorsal and ventral biopsies and irrespective of the presence of fur (Fig. 2E and see Fig. S2 in the supplementary material). Rather than the 1-2 layers of suprabasal keratinocytes seen in control animals, the TAF4 mutant epidermis typically comprised 4-6 layers. Immunostaining with the proliferation marker Ki-67 (also known as MKi67 – Mouse Genome Informatics) labelled isolated basal keratinocytes in the control epidermis, but all basal keratinocytes in the mutant epidermis (Fig. 2C). Hair follicles also showed abnormal histology – the shafts were shorter, projecting less deeply within the dermis, and often had poorly defined morphology owing to hyperproliferation of the outer root sheath. Hyperplasia persisted throughout the life of the animal and, at late stages, the TAF4 mutant epidermis was further characterised by numerous utriculi and round, closed dermal cysts (see Fig. S2 in the supplementary material).

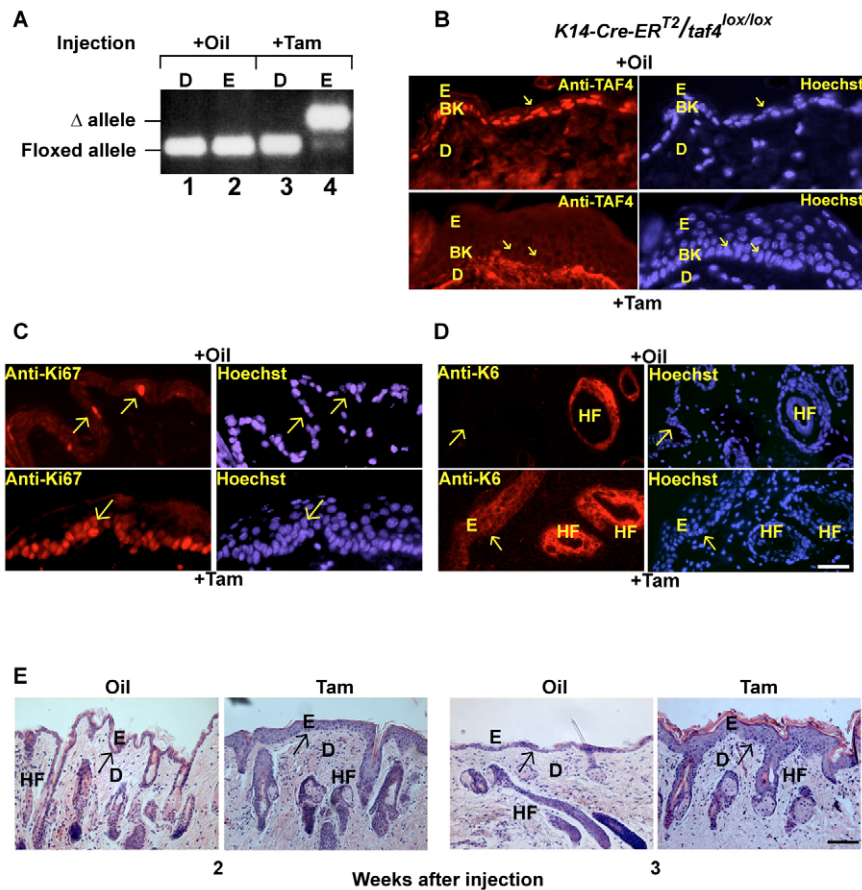


Fig. 2. Inactivation of TAF4 in adult epidermis. (A) Ethidium bromide-stained agarose gel of products of a triplex PCR. The locations of the floxed and deleted fragments amplified from the dermis (D) or epidermis (E) 2 weeks after oil or Tam injection are shown. (B) Immunostaining of skin biopsies 2 weeks after oil (above) or Tam (below) injection with anti-TAF4 antibody. Abbreviations as described in Fig. 1. (C) Immunostaining of skin biopsies 2 weeks after oil or Tam injection with anti-Ki-67 antibody. Arrows indicate representative positive staining nuclei. (D) Immunostaining of skin biopsies with anti-keratin 6 antibody. The arrows indicate the interfollicular epidermis. (E) Histological analysis of representative Haematoxylin-Eosin-stained paraffin sections from dorsal or ventral regions of oil- or Tam-injected mice. Hair follicles (HF) are indicated and arrows indicate the location of the basal membrane. Scale bar: 25 μm in D; 50 μm in E.

We also labelled the epidermis with antibodies against keratin 6 (K6; also known as KRT6 – Mouse Genome Informatics), which is normally expressed in the hair follicles, but not in the interfollicular epidermis. As expected, K6 staining was seen only in the hair follicle in the *Taf4^{eplox/lox}* animals, but in both hair follicle and interfollicular epidermis in *Taf4^{ep-/-}* animals (Fig. 2D). The skin of the *Taf4^{ep-/-}* animals had a dry and crinkled appearance with weak scaling, except for the footpads which were inflamed and subject to extensive scaling. Hyperkeratinisation of the tail was observed, leading to a drying, kinking and in some cases breakage (see Fig. S1D-F in the supplementary material). An increased transepidermal water loss was also observed in the mutant animals (see Fig. S1I in the supplementary material). Together, these results show that loss of TAF4 results in enhanced proliferation and aberrant differentiation of adult basal keratinocytes.

The *K14* promoter used to drive Cre expression is also active in the tongue (Li et al., 2001) (and data not shown). Histological analysis of the tongues from oil- or Tam-injected animals did not however reveal a significant increase in the thickness of the tongue epithelium or increased keratinisation (see Fig. S1G,H in the supplementary material). Thus, although TAF4 inactivation leads to hyperproliferation of the epidermis, it does not appear to have a proliferative effect in the tongue epithelium.

Induction of EGFs in TAF4 mutant epidermis

To better understand the molecular events responsible for this phenotype, we isolated RNA from skin biopsies from five independent Tam-injected and four oil-injected animals 3 weeks after treatment. The RNAs were hybridised on a microarray

detecting 25,000 transcripts from more than 20,000 genes, and transcripts that were consistently up- or downregulated in the mutant animals compared with the wild-type were identified (see Table S1 in the supplementary material).

In mutant animals, 353 genes were consistently upregulated more than 2-fold ($\log_2 1$), whereas 500 genes were downregulated (a list of some relevant genes is shown in Fig. 4A,B; for the full table, see Table S1 in the supplementary material). The expression of several relevant genes was verified by RT-PCR on two independent RNAs from wild-type or mutant epidermis (Fig. 4C).

Several members of the EGF family of mitogens, known to promote epidermal proliferation, were potently upregulated. Genes encoding heparin-binding epidermal growth factor (HBEGF), amphiregulin (AREG) and epithelial mitogen (EPGN), SCUBE1 a protein comprising multiple EGF domains expressed in the hair follicle (Grimmond et al., 2000) and the cognate EGF receptor (EGFR) were all strongly induced. Genes encoding several downstream MAP kinase pathway components, including BRAF and MAP3K6, were also upregulated, along with PBK (PDZ binding kinase) that interacts with RAF proteins and has oncogenic properties. PBK is a MAP3K upstream of p38 (MAPK14 – Mouse Genome Informatics) (Abe et al., 2000) and also been shown to phosphorylate histone H3 at serine 10 during mitosis (Park et al., 2006). Also upregulated was the gene encoding TAO2 (also known as TAOK2), a MAP3K that interacts with and activates MEK6 (also known as MAP2K6) (Zhou et al., 2004).

Not only were EGFs induced, but genes encoding several MAP kinase inhibitors were downregulated. DAB2 (disabled homolog 2) is a multifunctional tumour suppressor protein that forms a complex

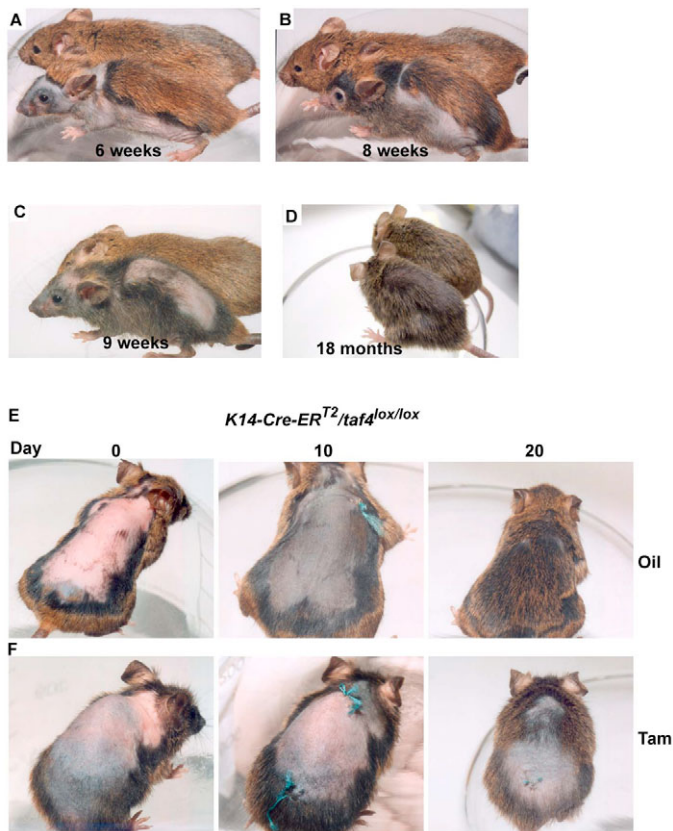


Fig. 3. Hair cycle defects in TAF4 mutant epidermis. (A-D) Animals were injected with oil or Tam as described in Materials and methods and two representative examples photographed at the indicated times post-injection. Reversible hair loss in the same Tam (foreground), but not oil (background) -injected animal is observed. **(E,F)** Defective depilation-induced anagen. Animals were injected with oil or Tam as indicated and depilated by wax-stripping 6 weeks after injection. Images show mice at the day of depilation and after 10 and 20 days.

with DAB2IP [DAB2 interacting protein (Wang et al., 2002)] and inhibits MAP kinase signalling (Zhou and Hsieh, 2001). DAB2 also antagonises the Wnt signalling that promotes the growth phase of the hair cycle and the proliferation and differentiation of keratinocyte stem cells (Hocevar et al., 2003). Interestingly, genes encoding SFRP2 and SFRP4, two further inhibitors of Wnt signalling, were downregulated, suggesting that this pathway is deregulated in the TAF4 mutant epidermis. By contrast, DAB2 acts as a positive cofactor for TGF β signalling that restricts basal keratinocyte proliferation (Hocevar et al., 2001). Together, these results show important changes in gene expression consistent with activation of EGF signalling and proliferation.

We investigated the phosphorylation of downstream targets of the MAP kinase pathways. Western blot analysis of epidermal extracts showed a strong increase in phosphorylation of the p38 MAP kinase and CREB/ATF1 in Tam-injected mice (Fig. 4E). Increased CREB/ATF1 phosphorylation could also be observed by immunofluorescence, where CREB/ATF1 labelling was limited to isolated basal keratinocytes in the interfollicular epidermis and in the hair follicle after oil injection, whereas after Tam injection, strong labelling of almost all interfollicular basal keratinocytes and some suprabasal cells, as well as of the outer root sheath of hair

follicle, was observed (see Fig. S3 in the supplementary material). These observations support the idea that TAF4 inactivation leads to activation of EGF signalling, stimulation of the MAP kinase pathway(s) and phosphorylation of downstream transcription factors.

We further investigated the expression of crucial cell cycle regulators, and observed a strong reduction of CDKN1A and an increase in cyclin D1 levels, whereas p53 (also known as TRP53 – Mouse Genome Informatics) expression was unchanged (Fig. 4E). As the transcription of *Cdkn1a* and cyclin D1 were not affected by TAF4 inactivation, these changes correspond to post-transcriptional events and correlate with increased proliferation.

Loss of TAF4 leads to important changes in cell adhesion and communication. The most strongly upregulated gene was *Lrrc15* [leucine rich repeat containing 15; also known as LRR-induced by β -amyloid (LIB)], which encodes a transmembrane protein of unknown function that localises to the leading edge of migrating cells (Reynolds et al., 2003; Satoh et al., 2002). It is interesting to note the upregulation of genes encoding ABL2, a tyrosine kinase implicated in cell motility (Woodring et al., 2003), ABI1 (ABL-interactor 1) and MAP4K4, a promigratory kinase (Collins et al., 2006). By contrast, *Tjp3* and claudin 23, which encode two tight junction proteins whose loss is associated with proliferation and tumour progression, were downregulated. The gap junction protein GJB2 (connexin 26) was strongly induced (reviewed by Richard, 2000), but the related GJB5 (connexin 31.1) was downregulated.

Amongst the other strongly upregulated genes were those encoding keratins such as K16 (KRT16), keratin associated proteins (KAPs, or KRTAPs) of the KAP3, 5 and 16 families, and trichohyalin [TCHH, also known as anagen-specific hair follicle (AHF)], a specific anagen phase marker (Takebe et al., 2003). The strong induction of trichohyalin is paradoxical, given the impaired anagen phase of the mutant animals. Expression of *Cd34*, a marker for keratinocyte stem cells (Trempe et al., 2003), was downregulated, suggesting depletion or modification of the stem cell population. Genes encoding several transcription factors were upregulated, including MSX2 and RUNX1, which have been shown previously to regulate epidermal proliferation and hair cycle (Wang et al., 1999).

We investigated whether some of the genes affected by TAF4 inactivation in adult epidermis were affected by loss of TAF4 in the foetal epidermis. Whereas genes such as *Hbegf* and *Lrrc15* were strongly induced by TAF4 inactivation in the adult, no comparable effect was observed in the foetal epidermis (Fig. 4D and data not shown). By contrast, although we observed no significant changes in expression of p63 (TRP63 – Mouse Genome Informatics) – whose various isoforms play a crucial role in the development and homeostasis of the epidermis (Koster et al., 2004; Koster and Roop, 2004) – in the foetal epidermis, the TAp63 isoforms were selectively downregulated in the adult (Fig. 4C). A balance between the TA and Δ Np63 isoforms may regulate proliferation versus differentiation of basal keratinocytes, where Δ Np63 maintains proliferation potential and is frequently upregulated during oncogenic transformation.

Similarly, TGM3 was unaffected in the foetus, but strongly induced in the adult, whereas no significant changes in expression of KLF4, CLDN1 and TGM1 were seen in adult epidermis (Fig. 1G and Fig. 4C and data not shown). These observations highlight the differential regulation of gene expression underlying the distinct phenotypes induced by TAF4 inactivation in the foetal and adult epidermis.

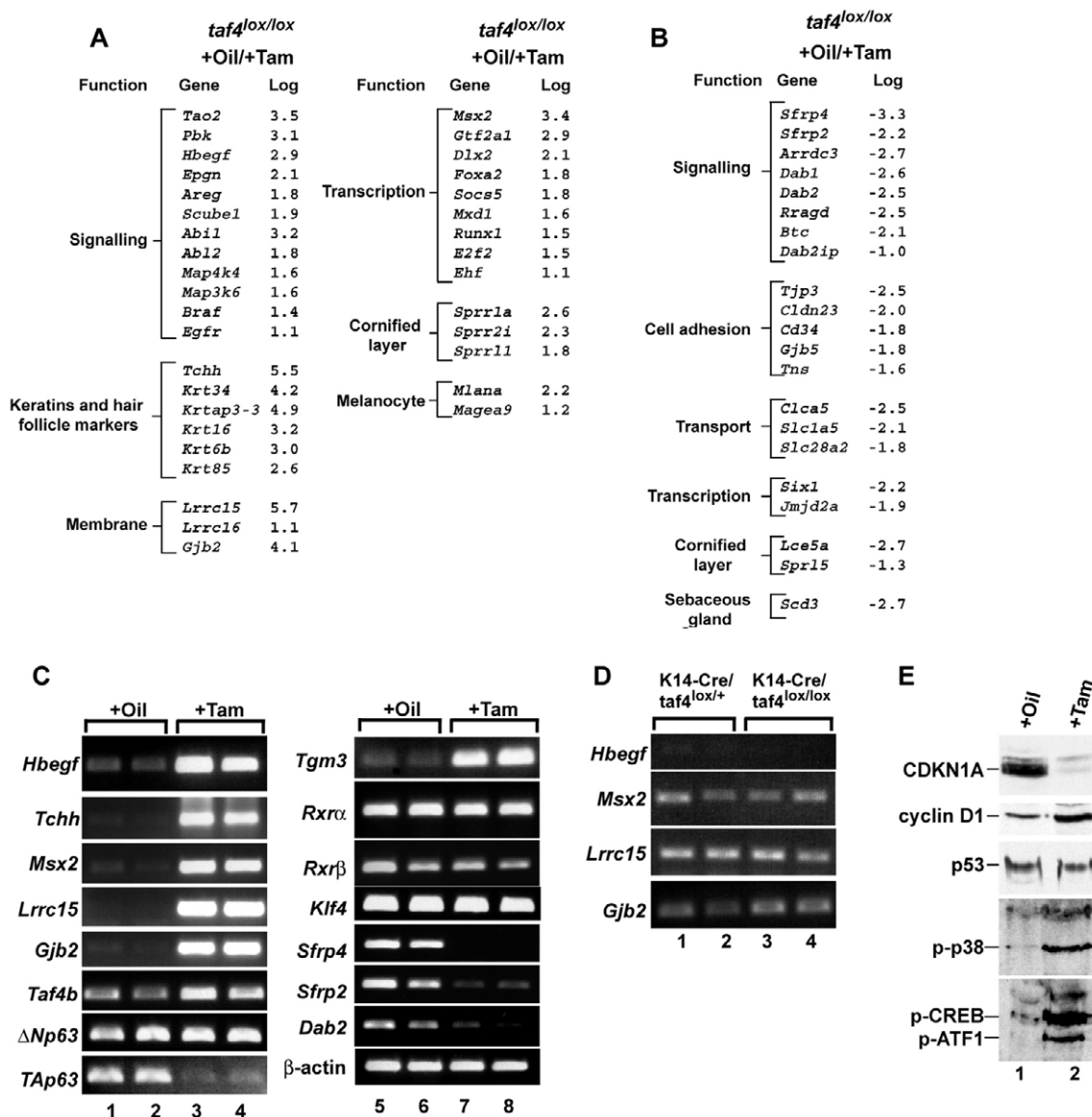


Fig. 4. Changes in gene expression upon TAF4 inactivation. (A,B) A restricted list of up- (A) or down- (B) regulated genes are shown along with their log₂ values classified by their function or intracellular location. (C) RT-PCR analysis of gene expression of the indicated genes in RNAs from skin biopsies from two independent oil- or Tam-injected mice. (D) RT-PCR analysis of gene expression of the indicated genes in RNAs from skin biopsies of two independent E18.5 fetuses. (E) Immunoblot analysis of CDKN1A and cyclin D1 expression in epidermal extracts. 20 μ g of total extract was loaded in each lane and the presence of CDKN1A, cyclin D1 and p53 revealed using the appropriate antibodies. In the lower part of the panel, phosphorylated p38 and CREB/ATF1 were revealed in total epidermal extracts prepared and quantified as described in Materials and methods.

Enhanced chemically induced carcinogenesis in TAF4 mutant epidermis

Although TAF4 inactivation upregulates EGF signalling, inducing hyperproliferation of basal keratinocytes associated with induction of markers correlating with oncogenic transformation, mutant mice do not spontaneously develop skin tumours (data not shown). We therefore asked whether *Taf4^{EP-/-}* mice were more sensitive to chemically induced carcinogenesis using the dimethyl-1-2 benzantracene/12-O-tetradecanoylphorbol-13 acetate (DMBA/TPA) protocol.

Mice were injected with oil or Tam and after 3 weeks were further treated with a single dose of the mutagen DMBA and then twice weekly with TPA. Using this protocol, Tam-injected mice developed twice as many tumours as the oil-injected controls (Fig. 5A). These

tumours were also on average larger than those in the control animals (Fig. 5B,E). No tumours were seen using TPA alone (data not shown).

In a second experiment, we initiated tumour formation with DMBA and TPA for 15 weeks before injection of oil or Tam. This protocol did not significantly affect the number of tumours present on oil-injected animals, but led to an increase in the number of tumours in the Tam-injected animals (Fig. 5A,C). Thus, loss of TAF4 stimulates tumour formation during the initiation and/or promotion phases.

TPA, like TAF4 inactivation, acts to promote epidermal hyperplasia. We therefore asked whether DMBA treatment alone would lead to efficient tumour formation in the mutant animals. Oil- or Tam-injected mice were treated twice with DMBA, but without

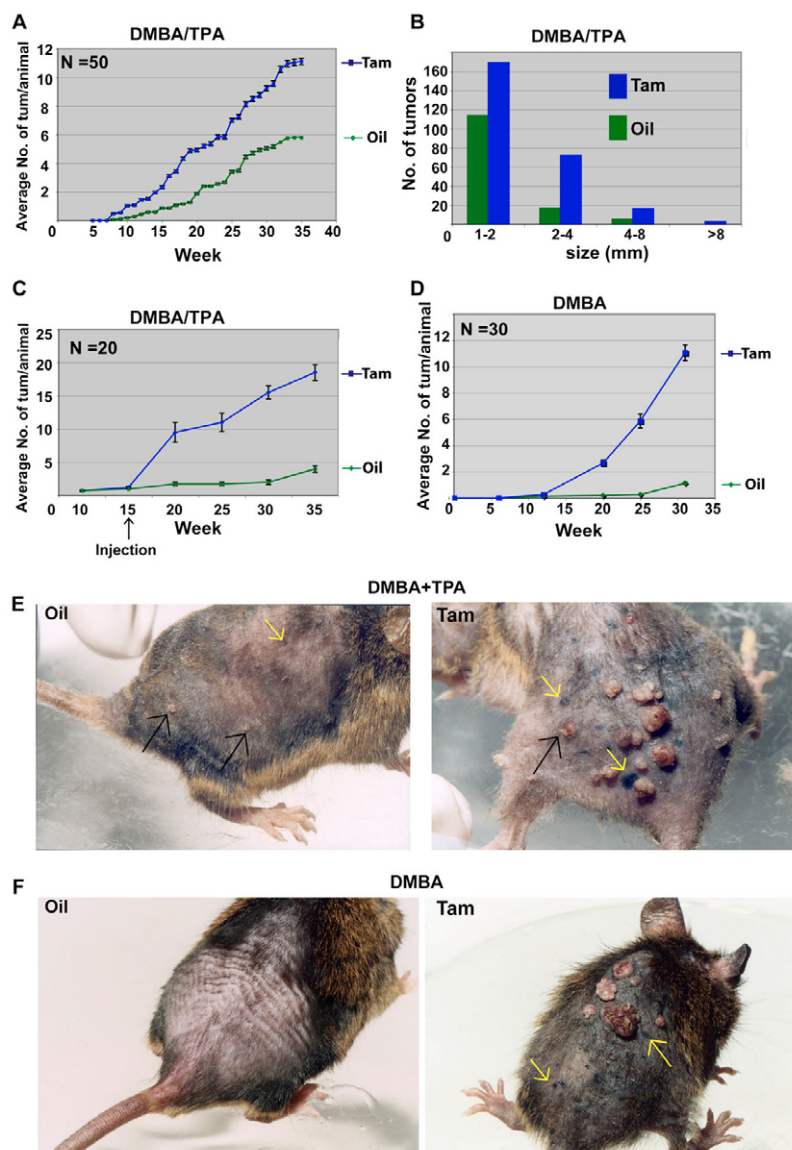


Fig. 5. TAF4 has tumour suppressor activity.

(A) Quantitation of tumours in oil- and Tam-injected mice after DMBA/TPA treatment. N, number of animals per group. (B) Number and size of tumours from oil- or Tam-injected animals after 26 weeks of treatment.

(C) Quantitation of tumours in animals treated with DMBA and TPA for 15 weeks prior to oil or Tam injection.

(D) Quantitation of tumours in oil- and Tam-injected animals after two sequential DMBA treatments.

(E) Representative animals after 20 weeks of DMBA/TPA treatment. Black arrows, epidermal tumours; yellow arrows, melanocytic growths. (F) Representative animals after 20 weeks of DMBA treatment.

subsequent TPA treatment. After 30 weeks, only rare tumours were observed in the oil-injected animals, whereas many large tumours were observed in the Tam-injected animals (Fig. 5D,F). Thus, loss of TAF4 mimics TPA treatment to promote efficient tumour formation.

Histological examination of the tumours showed that in control DMBA-treated animals, the majority of the tumours were benign papillomas (see Fig. S4A in the supplementary material). Only a small number of in situ carcinomas and rare squamous cell carcinomas were observed (Table 2). In Tam-injected animals, many more squamous cell carcinomas were observed (Table 2). Numerous moderately and well differentiated, locally invasive squamous cell carcinomas were observed in the Tam-injected animals (Fig. 6A,B,E and see Fig. S4B,C in the supplementary material). Loss of TAF4 thus leads to an increased number of tumours and enhances their malignant transformation.

Melanocytic tumours in TAF4 mutant skin

While observing the skin of animals treated with DMBA/TPA or DMBA alone, we noted, in addition to epidermal tumours, the presence of large melanocytic tumours in Tam-injected animals

which had no equivalent in the oil-injected controls (Fig. 5E,F, yellow arrows; Table 2). Upon histological analysis, the pigmented tumours in the Tam-injected animals showed the presence of densely packed melanocytes extending deep into the dermis and invading the underlying muscle and adipocyte layers (Fig. 6C,D). In other instances, clusters of melanocytes could be seen intermingled with the squamous cell carcinomas (Fig. 6E and see Fig. S4D in the supplementary material). In addition, we observed invasive melanocytes in lymph nodes close to pigmented tumours (Fig. 6F and see Fig. S4E in the supplementary material). In both the epidermis and lymph node, the pigmented cells showed speckled cytoplasmic staining with the PNL2 antibody (Rochaix et al., 2003), confirming their identity as melanocytes (see Fig. S5A,B in the supplementary material).

By immunolabelling, TAF4 expression could be clearly seen in the nuclei of epidermal tumours from oil-, but not Tam-injected animals (see Fig. S5C in the supplementary material). By contrast, TAF4 expression could be detected in the nuclei of melanocytes from Tam-injected animals (see Fig. S5D in the supplementary material). Hence, as expected, Tam injection leads to the loss of TAF4 in keratinocytes, but does not affect its expression in melanocytes.

Table 2. Malignant transformation of epidermal tumours in DMBA-treated mice

	<i>Taf4^{eplox/lox}</i>		<i>Taf4^{ep-/-}</i>	
	Mice	Tumours	Mice	Tumours
Number of mice	8		11	
Number of tumours	9		24	
Squamous cell hyperplasia	5	5	1	1
Squamous cell papilloma	3	3	5	10
Squamous cell carcinoma	1	1	7	12
Melanocytic tumour	0	0	4	12

DMBA-treated oil- or Tam-injected animals were sacrificed after 35 weeks and biopsies of tumours subjected to histological examination. The total number of sacrificed mice and of epidermal tumours analysed in each group are indicated at the top of the table. Below, the frequency of the different types of tumours is indicated for each group.

Upon RXR α inactivation in keratinocytes, epidermal hyperplasia, enhanced formation of epidermal tumours and non-cell-autonomous melanoma were all observed (Indra et al., 2007; Li et al., 2001). We therefore asked whether loss of TAF4 leads to a downregulation of RXR α or RXR β , the two family members expressed in the epidermis. RT-PCR showed no significant downregulation of these RXRs (Fig. 4C) and they did not register as downregulated in the microarrays.

Examination of the microarray data did however show that loss of TAF4 led to an upregulation of genes encoding melan-A and MAGEA9, two melanocyte-specific antigens associated with melanoma (Fig. 4A). To further examine potential changes in keratinocyte-melanocyte communication, we used Q-PCR to examine the expression of genes such as endothelin 1 and 3 (*Edn1*, *Edn3*), stem cell factor (*Scf*; also known as *Kitl* – Mouse Genome Informatics) and *Fgf2*, which encode factors secreted by keratinocytes that promote melanocyte growth. No significant change in expression of *Scf* and *Edn1* were seen and a 3-fold repression of *Fgf2* was observed (see Fig. S6 in the supplementary material). By contrast, *Edn3* expression increased 4-fold, but that of the cognate *Ednrb* receptor expressed in melanocytes was unchanged. Furthermore, expression of the gene encoding the melanocyte-specific MITF transcription factor was unaffected, but potent increases in expression of other melanocyte-specific genes, tyrosinase (*Tyr*), tyrosinase-related protein 1 (*Trp1*; also known as *Tyrp1* – Mouse Genome Informatics) and dopachrome tautomerase (*Dct*), were observed (see Fig. S6 in the supplementary material). These genes are downstream of the α MSH-MCR1 signalling pathway and both *Pomc1*, which encodes the α MSH precursor, and *Mclr*, which encodes the cognate melanocortin receptor 1, were also potentially induced. Loss of TAF4 in keratinocytes therefore affects the expression of genes involved in signalling to melanocytes and consequently modifies melanocyte gene expression.

Diminished retinoic acid-induced hyperplasia in TAF4 mutant epidermis

We have previously shown that TAF4 is required for activation of transcription by RAR in mouse embryonic fibroblasts (Mengus et al., 2005). To examine whether TAF4 is also required for the T-RA response of the epidermis, oil- or Tam-injected animals were treated ectopically with T-RA daily for 1 week. In oil-injected animals, T-RA induced a pronounced epidermal hyperplasia (Fig. 7A). By contrast, with the TAF4 mutant epidermis, treatment with T-RA at best led to only one additional layer of keratinocytes. No cumulative increase due to TAF4 inactivation and T-RA treatment was observed, indicating a strongly diminished T-RA response.

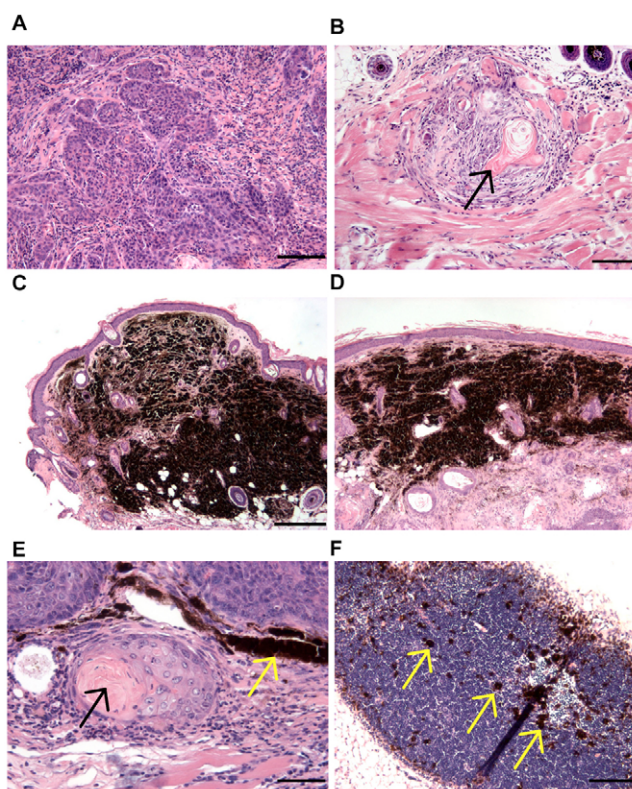


Fig. 6. Histological analysis of tumours. Twenty-six weeks after DMBA treatment of Tam-injected mice, epidermal and melanocytic tumours were excised, fixed in paraffin, sectioned and stained with Haematoxylin-Eosin for histological analysis. (A) Representative section from a moderately differentiated squamous cell carcinoma. (B) An invasive carcinoma amongst muscle tissue. Note the irregular, elongated cell morphology and the keratin pearl (arrow). (C,D) Large melanocytic tumours invading deeply into the muscle and adipocyte layers of the dermis. (E) Section of a locally invasive squamous cell carcinoma infiltrated by melanocytes. Black arrow, a keratin pearl; yellow arrow, melanocytes. (F) Section of a lymph node from a DMBA-treated Tam-injected animal. Yellow arrows, representative melanocytes. Scale bar: 100 μ m in A,B; 240 μ m in C; 50 μ m in E; 100 μ m in F.

We also verified the ability of the TAF4 mutant epidermis to respond to vitamin D3 receptor (VDR) signalling using the VDR agonist calcipotriol. After daily treatment with calcipotriol, a potent hyperplasia was observed in both the oil- and Tam-injected animals (Fig. 7B). Thus, although loss of TAF4 abolishes the proliferative effect of T-RA, the mutant epidermis retains the ability to respond to calcipotriol. This experiment also shows that the diminished proliferative response in the presence of T-RA is not due to an intrinsic limitation in the ability of the mutant epidermis to undergo additional thickening.

DISCUSSION

Contrasting functions of TAF4 in the foetal and adult epidermis

In this study, we show that TAF4 inactivation in the foetal and adult epidermis leads to distinct phenotypes. During foetal development, TAF4 inactivation leads to a loss of barrier function, increased epidermal water loss and early post-natal death. This phenotype is

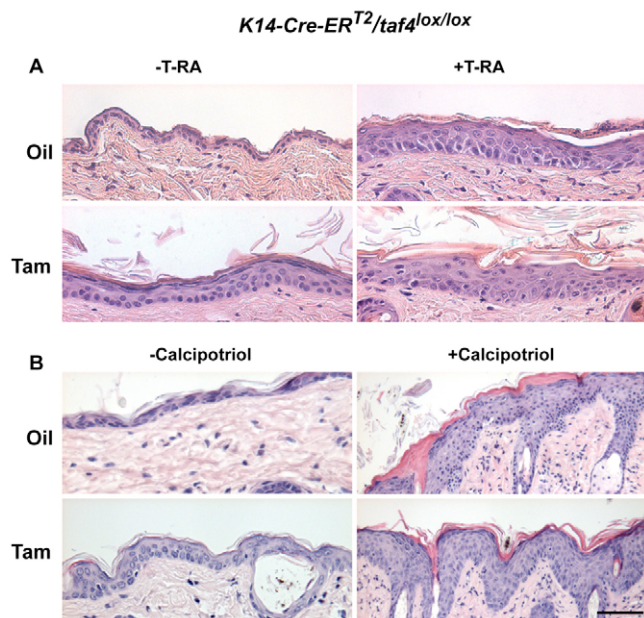


Fig. 7. Diminished T-RA response in TAF4 mutant epidermis.

(A) Haematoxylin-Eosin-stained sections from epidermis of oil- or Tam-injected animal after treatment with T-RA or acetone for 5 days.

(B) Epidermal sections from mice treated with calcipotriol for 5 days.

Scale bar: 50 μ m.

associated with a thinning and abnormal appearance of the epidermis and downregulation of a series of genes involved in barrier formation.

Upon TAF4 inactivation in the adult epidermis, two striking phenotypes are observed, a deregulated hair cycle and epidermal hyperplasia. The deregulated hair cycle is initially characterised by a total loss of fur, which initially affects large patches of skin with a locally synchronised cycle. This suggests that naturally occurring hair loss is not compensated by a normal cycle of regrowth. At later times, when the hair cycle is asynchronous, the *Taf4^{ep-/-}* animals have a sparse coat, again consistent with the idea that loss of fur is not compensated by normal regrowth. Histological analysis showed aberrant hair follicles with a reduced number in anagen phase and depilation failed to induce anagen phase in the mutant epidermis. It is also worth noting that the sparse coat characteristic of mutant animals remains rather constant over time. Together, these observations suggest that TAF4 inactivation induces a permanent cycling defect in the anagen phase, but there is no long-term degeneration and loss of hair follicles.

Despite the above observations, analysis of gene expression shows a strong upregulation of trichohyalin, an anagen phase marker in the mutant epidermis. Similarly, upregulation K16, also implicated in promoting the anagen phase (Tong and Coulombe, 2006), and of several KAPs normally transcribed during anagen, is observed. Two further observations might be directly relevant to the hair cycling defects. SFRP2, SFRP4 and DAB2 are antagonists of the Wnt pathway that promotes the anagen phase of the hair cycle and the differentiation of stem cells into follicular keratinocytes (Huelsken et al., 2001; Van Mater et al., 2003; Fuchs, 2001). These proteins are strongly downregulated, suggesting a chronic activation of the Wnt pathway in the TAF4 mutant epidermis. This would be consistent with the observed induction of anagen phase markers. Nevertheless, we do not observe normal anagen-phase hair follicles.

MSX2, a crucial transcription factor regulating the hair cycle and a potential Wnt-regulated gene, is strongly overexpressed in the mutant epidermis. Although loss of MSX2 leads to cyclic alopecia (Ma et al., 2003), its overexpression in transgenic animals leads to abnormal shrunken hair follicles and reduced hair growth as well as epidermal hyperplasia (Jiang et al., 1999), defects similar to those seen here. We suggest, therefore, that rather than receiving an ordered set of signals during hair cycle progression, hair follicles in the mutant epidermis are subject to chronic conflicting signals (including EGF stimulation, see below) leading to asynchronous and inappropriate gene expression and disruption of the normal cycle.

In addition to the abnormal hair cycle, loss of TAF4 leads to interfollicular hyperplasia. This is associated with a potent induction of HBEGF, AREG and EPGN, all of which have been previously shown to be major regulators of keratinocyte proliferation. Moreover, several components of the downstream MAP kinase pathway are induced, whereas DAB2 and DAB2IP, negative regulators of this pathway, are diminished and increased phosphorylation of downstream transcription factors such as CREB and ATF1 is seen. These observations strongly suggest that TAF4 regulates keratinocyte proliferation through control of EGF expression.

This observation might also be related to the diminished T-RA-induced hyperplasia of the TAF4 mutant epidermis. Several studies have shown that T-RA promotes epidermal hyperplasia through induction of HBEGF and AREG (Chapellier et al., 2002; Rittie et al., 2006; Stoll and Elder, 1998; Xiao et al., 1999). As these genes are induced by loss of TAF4, the mutant epidermis may be refractory to subsequent T-RA treatment. Thus, it is not that loss of TAF4 impairs the T-RA proliferative response per se, it is rather that these two stimuli act through a common pathway.

TAF4 regulates keratinocyte and fibroblast proliferation through distinct pathways

The phenotype observed upon loss of TAF4 in foetal epidermis is analogous to that seen upon inactivation of TAF10, another TFIID subunit, which also leads to a defective skin barrier function associated with downregulation of claudin 1 and KLF4 (Indra et al., 2005). However, in contrast to TAF4, loss of TAF10 has no effect in the adult epidermis (Indra et al., 2005). Thus, although TAF4 and TAF10 similarly regulate genes required for the proper development of the foetal epidermis, they have radically different functions in adult keratinocytes. In addition, we also show that TAF4b is expressed in the adult epidermis (Fig. 4C). There is no indication that TAF4b has a role in epidermis development as *Taf4b*-null mice are reported to appear normal (Freiman et al., 2001). Hence, whereas TAF4 may substitute for loss of TAF4b, the converse is not the case. A further study with the floxed *Taf4* alleles on the *Taf4b*-null background would help to reveal redundant and specific functions of these two related factors in the epidermis.

We have previously identified genes whose expression is deregulated by TAF4 inactivation in fibroblasts (Mengus et al., 2005). A comparison with the present study shows that the repertoire of genes affected by TAF4 inactivation in keratinocytes is distinct from that seen in embryonic fibroblasts. Only a few genes show similar regulation in both cell types, amongst which is *Hbegf*, which is strongly induced in each case. However, the results obtained in the adult epidermis are analogous to those seen in embryonic fibroblasts as TAF4 inactivation results in enhanced proliferation of both cell types (Mengus et al., 2005). In fibroblasts, the TGF β pathway is induced, whereas in keratinocytes enhanced EGF signalling is

observed. Together, these results show that TAF4 is a major regulator of proliferation, controlling expression of two distinct families of growth factors.

TAF4 inactivation highlights the ability of keratinocytes to regulate melanocyte proliferation

Despite the deregulated proliferation, *Taf4^{ep-/-}* mice do not spontaneously develop skin tumours. Nevertheless, enhanced DMBA-induced tumour formation is observed, even in the absence of TPA treatment where loss of TAF4 provides tumour-promoting activity. The tumours in the mutant epidermis also undergo increased malignant transformation.

Although this result can be explained by the loss of TAF4 in keratinocytes, we also observe melanocytic tumours in *Taf4^{ep-/-}* animals after DMBA-induced carcinogenesis unrelated to loss of TAF4 expression in this population. The large, locally invasive dermal melanocytic tumours can also invade neighbouring lymph nodes. In agreement with previous observations, no such invasive melanocytic tumours were seen in control DMBA-treated mice (Husain et al., 1991). This suggests that TAF4 inactivation in keratinocytes leads to altered communication with the melanocyte population. Further evidence for this comes from the observed changes in expression of melanocyte-specific genes upon TAF4 inactivation in keratinocytes.

How does loss of TAF4 in epidermal keratinocytes promote formation of melanocytic tumours? In adult mice, only a few melanocytes are present in the interfollicular epidermis and in the dermis – the majority are located in the hair follicle, where they are in close contact with the keratinocytes. Abnormal paracrine/juxtacrine communication with the TAF4 mutant keratinocytes perhaps leads to epigenetic changes and deregulated autocrine melanocyte growth followed by invasion of the dermis. In addition, we often observe melanocytes intermingled with keratinocyte-derived tumours, although not all melanocytic tumours are located close to epidermal tumours. Further studies will be required to identify the molecular paracrine/juxtacrine signals involved in initially promoting melanocyte proliferation.

Similar to TAF4, inactivation of RXR α in keratinocytes leads to epidermal hyperplasia, enhanced formation of epidermal tumours and non-cell-autonomous melanoma (Indra et al., 2007; Li et al., 2001). We have excluded the possibility that TAF4 inactivation leads to a loss of epidermal RXR α expression. In the epidermis, RXR α acts in heterodimers with RAR γ , VDR or PPAR γ (δ). We cannot therefore exclude the possibility that some effects of TAF4 inactivation reflect loss of function of one or several of these RXR α heterodimers. It remains to be determined, for example, whether TAF4 and RXR α act through the same or different paracrine/juxtacrine pathways to regulate melanocyte proliferation.

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Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/134/16/2947/DC1>

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