CHAPTER 4

MATRIPTASE-1 EXPRESSION IS LOST IN PSORIATIC SKIN LESIONS AND DOWN-REGULATED BY TNFα IN VITRO

Submitted manuscript

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Expression of the serine protease matriptase-1 is lost in psoriatic skin lesions and is down-regulated by TNF-alpha *in vitro*.

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SUMMARY

BACKGROUND: The type II serine protease matriptase-1 has been shown to be indispensable for regular terminal differentiation of epidermal keratinocytes.
OBJECTIVES: To investigated the expression and regulation of matriptase-1 in psoriatic skin and in keratinocytes (KC) *in vitro*.
METHODS: Matriptase-1 expression *in vivo* was examined by Western blot analysis and immunoflurescence staining of psoriatic and adjacent normal skin. Matriptase-1 activity was determined in epidermal lysates using Boc-Gln-Ala-Arg-AMC as substrate. To investigate the regulation of matriptase-1 *in vitro,* monolayer cultures and organotypic skin cultures of human primary KC were treated with TNFα and analyzed by RT-PCR, Western blotting and immunfluorescence staining. An involvement of the nuclear factor kappa B (NFκB) signaling pathway was investigated by adenoviral over-expression of a dominant negative form of IKK2.
RESULTS: Matriptase-1 expression was detected in the stratum granulosum of organotypic skin cultures *in vitro* and human skin *in vivo*. Western blot analysis, immunofluorescence staining and activity assays revealed that both matriptase-1 expression and enzymatic activity was strongly reduced in psoriatic skin lesions as compared to the uninvolved adjacent skin of the same donors. Exposure of KC to TNFα led to a strong down-regulation of matriptase-1 mRNA and protein production *in vitro*. Organotypic skin cultures treated with TNFα showed disturbed KC-differentiation, accompanied by complete loss of matriptase-1 expression. Inhibition of the NFκB signaling pathway completely blocked TNFα induced down-regulation of matriptase-1.
CONCLUSION: Since matriptase-1 is involved in regular terminal KC differentiation, its absence in psoriatic skin lesions might directly contribute to the barrier alterations in this disease. Blocking the IKK2-pathway might represent an interesting target for the treatment of psoriasis.

INTRODUCTION

Matriptase-1, also referred to as membrane-type serine protease-1 (MT-SP1) or tumor-associated differentially expressed gene-15 (TADG-15) is a member of the type 2 integral membrane serine protease family (Lin et al., 1999; Takeuchi et al., 1999; Tanimoto et al., 2005). Matriptase-1 was first described as an extracellular matrix degrading enzyme in human breast cancer cells (Shi et al., 1993; Lin et al., 1997), and was subsequently purified from human milk (Lin et al., 1999). Several biological substrates have so far been identified for matriptase-1 including urokinase-type plasminogen activator (uPA), hepatocyte growth factor (HGF), protease activated receptor-2 (PAR-2) (Takeuchi et al., 2000; Lee et al., 2000) and stromelysin (MMP-3) (Jin et al., 2006). However, since matriptase-1 is a trypsin-like protease, it is very likely that also additional substrates for this enzyme exist (Lin et al., 1999).

The fact that matriptase-1 deficient mice die within 48 hours after birth due to dehydration caused by defective skin barrier formation, demonstrates that this enzyme plays a crucial role in epidermal differentiation (List et al., 2002). In particular, matriptase-1 deficiency perturbs lipid matrix formation, cornified envelope morphogenesis and stratum corneum desquamation (List et al., 2003) and inactivation of matriptase-1 results in the loss of proteolytically processed filaggrin monomer units and the NH_2-terminal filaggrin S-100 regulatory protein (List et al., 2003). When skin of newborn mutant mice was transplanted onto SCID mice, the epidermis showed hyperproliferation, acanthosis, hyperkeratosis and parakeratosis, with formation of a thickened and compact stratum corneum (List et al., 2003). This *in vivo* murine phenotype could be partly reproduced in a human *in vitro* organotypic skin culture model. Knock down of matriptase-1 expression by RNAi technology results in a thickened stratum corneum and retention of nuclei within corneocytes (Mildner et al., 2006). The importance of matriptase-1 for KC terminal differentiation was further confirmed by the recent finding that a mutation within the matriptase-1 gene leads to an autosomal recessive ichthyosis with hypotrichosis in an Israeli-Arab family (Basel-Vanagaite et al., 2007) (Avrahami et al., 2008; Alef et al., 2009).

Here we have investigated the expression of matriptase-1 in psoriatic skin and report that expression of this enzyme is dramatically reduced in these lesions. Furthermore, we show that TNFα is able to down-regulate matriptase-1 expression in KC both in monolayer and organotypic skin cultures.

MATERIALS AND METHODS

Cell culture:

Human dermal fibroblasts were obtained from Lonza (Basel, Switzerland) and cultured in Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Gaithersburg, USA) supplemented with 10% fetal bovine serum (FBS, PAA, Linz, Austria), 25 mM L-glutamine (Gibco) and 1% penicillin/streptomycin (Gibco).

KC derived from neonatal foreskin of single donors were purchased from Lonza and cultured in KC growth medium (KGM, Lonza) which is a modified MCDB 153 medium supplemented with 0.1 ng/ml human recombinant epidermal growth factor, 5 µg/ml insulin, 0.5 µg/ml hydrocortisone, 0.4 % bovine pituitary extract, 50 µg/ml gentamycin and 50 ng/ml amphothericin B.

Preparation of organotypic skin cultures:

In vitro organotypic skin cultures were generated as described previously, with minor modification (Rendl et al., 2002). Briefly, a suspension of collagen type I (PureCol, Advanced BioMatrix, San Diego, CA, USA) containing $1x10^5$ fibroblasts per ml was poured into cell-culture inserts (3 µm pore size; BD Bioscience, Bedford, MA, USA) and allowed to gel for 2 hours at 37°C in a humidified atmosphere. The gels were then equilibrated with KGM for 2 hours, and $1.5x10^6$ KC, in a total volume of 2 ml KGM, were placed on the collagen gel. After overnight incubation, the medium was removed from both the inserts and external wells, and replaced in the external wells by serum-free KC defined medium (SKDM), consisting of KGM but without bovine pituitary extract and supplemented with 1.3 mM calcium (Sigma, Vienna, Austria), 10 µg/ml transferrin (Sigma), 50 µg/ml ascorbic acid (Sigma), and 0.1% bovine serum albumin (Sigma) for up to 7 days. The volume of culture medium was chosen so that KC were positioned at the air-liquid interface. SKDM was changed every second day. For TNFα stimulation experiments, organotypic skin cultures were treated with 5 and 25 ng/ml TNFα during the whole culture period. Matriptase-1 knock-down organotypic skin culture were generated according to a published protocol (Mildner et al., 2006). Briefly, third passage KC were grown to 70-80% confluency in a T75 culture flask. Lipofectamine 2000 (50 µl, Invitogen) was mixed with 65 µl of a 20 µM siRNA solution (Invitrogen) and 5 ml OPTI-MEM medium (Gibco). After 30 minutes at room temperature KGM (20 ml) was added and the solution was poured onto the KC monolayer (25 ml) for twenty four hours. After transfection KC were trypsinized and seeded onto a fibroblast collagen gel as described above.

Tissue samples and immunostaining:

Ten samples of each healthy skin and psoriasis were analyzed. Healthy skin samples were obtained from the department of plastic surgery with informed consent of the patients. The biopsies were fixed in formalin and embedded in paraffin. Paraffin samples of psoriasis specimens were from the histopathology files of the department of dermatology. Immunofluorescence staining was performed on 5 µm thick sections of formalin fixed, paraffin embedded tissues. After deparaffination and hydration, sections were pre-treated with protease XXIV (0,5mg/ml, Sigma) for 5 minutes at 37°C for matriptase-1 staining and with microwave for filaggrin staining. After three washes with PBS (Gibco) non-specific staining was blocked by incubation with 10% normal goat serum for 1 hour. The slides were subsequently incubated (overnight in a humidified chamber at 4°) with either a rabbit anti-matriptase-1 antiserum (1:3000; Bethyl Laboratories, Montgomery, Texas, USA) or a mouse anti-filaggrin monoclonal antibody (1:1000; Abnova, Heidelberg, Germany) in PBS containing 2% bovine serum albumin (BSA) and 2% goat serum. After washing with PBS, slides were incubated sequentially with an Alexa fluor 488 linked goat anti-rabbit IgG (1:300, Molecular Probes) or an Alexa fluor 633 goat anti-mouse IgG (1:300; Molecular Probes) in PBS containing BSA and 2% goat serum for 1 hour. For control immunostainings mouse IgG_1 or normal rabbit serum was used as a first step reagent in the same concentrations as the anti-filaggrin or anti-matriptase-1 antibodies, respectively. After washing, cell nuclear staining was performed by incubation with Hoechst dye (2µg/ml in PBS, Sigma) for 5 minutes. Slides were mounted with Fluoprep (bioMérieux, Marcy l´Etoile, France).

RNA isolation and cDNA preparation:

After lysis with TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) and RNA extraction according to the manufacturer's instructions, cDNAs were transcribed with the GeneAmp® Kit using MuLV-reverse transcriptase and Oligo dT primers (Applied Biosystems, Foster City, CA) as indicated in the instruction manual.

Quantitative real time PCR:

mRNA expression was quantified by real time PCR with LightCycler Fast Start DNA Master SYBR Green I (Roche Applied Science, Penzberg, Germany) according to the manufacturer's protocol. The primers for matriptase-1 (forward: 5´-AGGTGTTCCAATGGGAAGTG-3´, reverse: 5´-CGTCGCTACAGTCCTCCTTC-3´) and β-2-

microglobulin (B2M, forward: 5´-GATGAGTATGCCTGCCGTGTG-3´, reverse: 5´-CAATCCAAATGCGGCATCT-3´) were designed as described previously (Kadl et al, 2002). The relative expression of the target genes was calculated by comparison to the house keeping gene β2M using a formula described by Wellmann et al (Wellmann et al., 2001). The efficiencies of the primer pairs were determined as described in (Kadl et al., 2002).

Matriptase-1 activity-assay:

Skin shave biopsies from 3 patients with psoriasis were obtained after informed consent. Shave biopsies were floated on 2.4 U/ml Dispase (Roche, Vienna, Austria) in PBS for 30 minutes at 37°C (Tschachler et al., 2004). Dermis and epidermis were separated with watch-maker's forceps and epidermal sheets were extensively washed in PBS (Gibco) to remove excessive dispase. Lesional and paralesional epidermal sheets were lysed in 20 mM Tris buffer, pH 8.5 by sonication. Matriptase-1 activity was measured according to a published protocol {Lin, 1999 44 /id} using Boc-Gln-Ala-Arg-AMC (Bachem, Weil am Rhein, Germany) as substrate. Fluorescence was measured on the FLUOstar OPTIMA (BMG Labtech, Vienna, Austria) using 355 nm excitation and 460 nm emission wavelength.

Transduction of human KC with recombinant adenovirus:

Adenovirus expressing either GFP (AdV-GFP) or human dominant negative IKK2 (AdV-dnIKK2) was generated as describe previously (deMartin et al., 1997; Oitzinger et al., 2001). Preconfluent KC were washed with PBS and incubated with AdV-dnIKK2 or control adenovirus (AdV-GFP) at a multiplicity of infection of 100 in basic medium. After 2 hours at 37°C, the adenovirus was washed off, and fresh growth medium was added. Cells were further cultivated for 24 hours before addition of 20 ng/ml TNFα and harvested 24 and 48 hours later for Western blot analysis and real-time PCR.

Western blot analysis:

Skin shave biopsies from 14 patients with psoriasis were obtained after informed consent. Shave biopsies were floated on ammonium thyocyanate (Sigma) in PBS for 30 minutes at 37°C. Dermis and epidermis were separated with watch-maker's forceps and epidermal sheets were extensively washed in PBS (Gibco). Western blot analysis was performed as described previously (Mildner et al., 1999) with minor modifications. KC and epidermal sheets were lysed in SDS-PAGE loading buffer. After sonication and centrifugation proteins were size fractionated by SDS-PAGE through an 8 to 18% gradient gel (Amersham Pharmacia Biotech, Uppsala, Sweden) and transferred to nitrocellulose membranes (BioRad,

Hercules, CA, USA). Immunodetection was performed with either a mouse monoclonal anti-IKK2 antibody (Alexis, Lausen, Swizerland) or a rabbit anti-matriptase-1 antiserum (dilution 1:3000), followed by a HRP-conjugated goat anti-mouse IgG antiserum (dilution 1:10000, Amersham) or a goat anti-rabbit IgG antiserum (dilution 1:10000; Pierce, New York, NY, USA), respectively. Reaction products were detected by chemiluminescence with the ChemiGlow reagent (Biozyme Laboratories Limited, South Wales, U.K.) according to the manufacturer's instructions.

RESULTS

Matriptase-1 expression co-localizes with filaggrin expression

To determine matriptase-1 localization in the epidermis and its expression during KC-differentiation, we generated organotypic skin cultures and analyzed them from day 1 to day 6 after initialization of the culture. As shown in Fig.1 matriptase-1 expression was detectable in the granular layer of the epidermis as early as 5 days after initializing the differentiation process by culturing the organotypic skin model exposed to air. Similar expression kinetics and localization were detected for filaggrin, a specific marker protein for the granular layer of the epidermis (Fig. 1). Both antigens were not detectable in organotypic skin samples at day 1 to day 4. Like in the fully differentiated organotypic skin, an identical expression pattern was observed in normal human skin samples (Fig.1).

Matriptase-1 expression and activity is strongly reduced in psoriatic skin lesions.

Immunofluorescence staining of normal skin and psoriatic skin lesions revealed that in contrast to normal skin, which shows strong matriptase-1 expression in the granular layers of the epidermis (Fig. 2a), matriptase-1 expression was virtually absent from psoriatic skin lesions (Fig. 1b-f), especially underneath parakeratotic plaques. The absence of matriptase-1 expression was observed in all of 10 psoriasis samples tested. Like in normal skin, strong matriptase-1 expression was also found in uninvolved epithelium adjacent to psoriatic plaques (Fig. 2b). Although the adjacent skin still showed acanthosis there was no parakerathosis visible (Fig. 2b), suggesting an important role of matriptase-1 in the late cornification process. To further substantiate our finding, we performed Western blot analysis of involved and uninvolved skin samples of 14 different donors (Fig. 3a). A clear reduction of matriptase-1 protein in samples of psoriatic lesions was observed in 11 donors. Two donors showed no regulation and in one donor an induction of matriptase-1 expression was observed. To further investigate whether reduced matriptase-1 expression in psoriatic lesions also results in a reduced activity of the enzyme, we prepared lysates from epidermis of lesional skin and compared them to the activity of epidermal lysates of adjacent uninvolved skin. By incubation with an amino-methyl coumarin (AMC)-labeled peptide substrate (Boc-Gln-Ala-Arg-AMC), we found a strong reduction of matriptase-1 activity in lysates from epidermis of psoriatic plaques compared to adjacent skin in 3 out of 3 donors (Fig. 3b). The specificity of the substrate was tested by analyzing lysates of organotypic skin cultures generated from normal KC or KC transfected with either scrambles siRNA or two different matriptase-1 specific siRNAs (Mildner, BBRC). As shown in Fig. S1 matriptase-1 mRNA was

reduced by up to 85% in both specific siRNAs (Fig. S1a). The reduction of matriptase-1 expression strongly correlated with a significant reduction in the matriptase-1 activity assay (Fig. S1b)

TNFα strongly down-regulates matriptase-1 expression in keratinocytes in monolayer culture and in organotypic skin cultures.

Since TNFα is a key cytokine in the pathogenesis of psoriasis, we investigated if TNFα shows a direct influence on the expression of matriptase-1 *in vitro*. As shown in Fig. 4, stimulation of 70 % confluent KC in monolayer culture with TNFα dramatically reduced matriptase-1 expression on mRNA (Fig. 4a) and protein levels (Fig. 4b). Treatment of organotypic skin cultures with TNFα led to disturbed KC-differentiation as evidenced by a loss of the stratum granulosum and a defective stratum corneum with retention of nuclei. As in monolayer cultures, this was also accompanied with a complete loss of matriptase-1 expression (Fig. 4c).

TNFα down-regulates matriptase-1 expression in keratinocytes *via* the NFκB-signalling pathway.

Since database search revealed two NFκB-consensus binding sites in the matriptase-1 promoter region, we investigated the involvement of the NFκB-signaling pathway on the down-regulation of matriptase-1. KC in monolayer cultures were therefore transduced with adenoviral constructs either expressing green fluorescent protein (AdV-GFP) or mutant IKK2 (AdV-dnIKK2). Twenty four hours after addition of the recombinant adenovirus nearly all cells were infected, as determined by the portion of GFP-expressing KC (Fig. 5 a,b). Transduction with AdV-GFP did not affect matriptase-1 or IKK2 expression (data not shown). At this time point TNFα was added to the culture, and cells were analyzed by Western blot analysis forty-eight hours later. Transduction with AdV-dnIKK2 resulted in high expression of the mutant form of IKK2 (Fig. 5c, upper panel) in both TNFα treated (Fig. 5c, upper right panel) and untreated KC (Fig. 5c, upper left panel). Over-expression of dominant negative IKK2 by itself showed no effect on matriptase-1 expression in unstimulated KC (Fig. 5c, lower left panel). When TNFα was added to KC after transduction with either AdV-dnIKK2 or AdV-GFP, down-regulation of matriptase-1 was only observed AdV-GFP transduced but not the AdV-dnIKK2 transduced KC (Fig. 5c lower right panel) demonstrating that the IKK2/NFκB pathway was responsible for negative regulation of matriptase-1.

DISCUSSION

It has recently been established that the serine protease matriptase-1 plays an important role in the development of a regular epidermal barrier (List et al., 2002), and that loss of matriptase-1 expression and function {Basel-Vanagaite et al., 2007) results in an altered stratum corneum. In the present study, we show that matriptase-1 expression in the human skin is restricted to the granular layer of the epidermis and co-localizes with filaggrin expression. Its expression is strongly reduced in psoriatic skin lesions and we demonstrate that TNFα, a key cytokine in psoriasis, negatively regulates matriptase-1 expression in human primary KC *in vitro* and that this effect is mediated *via* the NFκB-signaling pathway.

The loss of matriptase-1 function appears to be the central event in the pathogenesis of autosomal recessive ichthyosis with hypotrychosis (List et al., 2003; Basel-Vanagaite et al., 2007), a disorder which is characterized by thickened, grayish, scaly skin and slow growing curly, sparse and fragile hair. At the biochemical level a defect in filaggrin-processing and impaired function of the stratum corneum is found in these patients (List et al., 2002; List et al., 2003; Basel-Vanagaite et al., 2007) and can be reproduced in a ST14 hypomorphic mouse model expressing vastly reduced levels of matriptase-1. We have observed altered stratum corneum development with thickened corneocyte layer and retention of nuclei in an organotypic skin model using siRNA technology to knock down matriptase-1 (Mildner et al., 2006). The virtual absence of matriptase-1 in lesional psoriatic skin, was striking. Although various epidermal genes have been reported to show altered expression in psoriatic epidermis in the past (Tschachler et al., 2007), the absence of this enzyme might signify more than a mere bystander effect. The fact that the reduction of matriptase-1 expression in individuals with mutations of the ST14 gene leads to a pronounced defect in epidermal barrier formation (List et al., 2003; Basel-Vanagaite et al., 2007) suggests rather strongly that the absence of matriptase-1 directly contributes to the disturbed epidermal morphology of psoriatic lesions. In contrast to autosomal recessive ichthyosis with hypotrychosis where a mutation of the gene coding for matriptase-1 is causative, the central pathogenic event in psoriasis is thought to be the dysregulation of inflammatory cytokines, in particular a strong up-regulation of TNFα production (de Kerkhof et al., 2007; Lowes et al., 2007). The important role of TNFα is further stressed by the therapeutic efficacy of antibodies targeting TNFα or soluble TNFα receptor (Gottlieb et al., 2005; Gottlieb et al., 2007). In our study the addition of TNFα to cultures of KC in monolayers and to an organotypic skin model led to a reduction of matriptase-1 mRNA and protein expression. This finding strongly supports the idea that over-expression of TNFα in psoriasis might directly be involved in down-regulating matriptase-1.

TNFα acts on several cellular signaling pathways (Aggarwal et al., 2003), including the activation of NFκB (Barnes et al., 1997), AP-1 (Karin et al., 1997), c-jun N-terminal kinase (Minden et al., 1994), Raf-1 (Belka et al., 1995) and subgroups of the mitogen-activated protein kinase (MAPK) (Raingeaud et al., 1995). The activation of NFκB requires the IKK-multiprotein complex which contains IKK1, IKK2 and NEMO (Israel et al., 2000). Targeted deletion of IKK1 leads to a defect of KC differentiation in experimental animals despite functional NFκB-activation by exogenous cytokines (Takeda et al., 1999; Li et al., 1999; Hu et al., 1999). By contrast deletion of IKK2 renders NFκB unresponsive to inflammatory cytokines including TNFα (Li et al., 1999; Tanaka et al., 1999; Li et al., 1999). Our finding that transduction of KC with a dominant negative mutant of IKK2 reverses the effect of TNFα on matriptase-1 expression shows that activation of the NFκB *via* IKK2 is indeed the signaling pathway involved. As to the mechanism(s) which lead to the inhibition of matriptase-1 expression by TNFα / NFκB we have currently no explanation to offer. Although inhibitory effects mediated by NFκB activation have been reported previously (Todorov et al., 2002; Liu et al., 2003) the pathways involved have not yet been clarified. Whether or not specific inhibition of this pathway might represent another potential target for the therapy of psoriasis remains to be determined. Targeting IKK2 as a potential and therapeutic approach has been recently suggested for the treatment of inflammation induced bone loss (Ruocco et al., 2005; Ruocco et al., 2005; Jimi et al., 2004). However extensive pharmacological evaluations of such an approach are still missing.

Taken together our finding that matriptase-1 expression is strongly reduced in psoriatic lesions suggests that the absence of this serine protease might contribute to the impaired barrier formation in psoriasis. Whether dysregulation of matriptase-1 expression is seen in other inflammatory skin diseases with disturbed barrier formation such as atopic dermatitis is currently under investigation. The finding that the down-regulation of matriptase-1 by TNFα occurs *via* IKK2/NFκB supports the importance of this signaling pathway in the pathogenesis of psoriasis and suggests that drugs which are able to inhibit NFκB activation *via* IKK2 might represent an interesting alternative or supplement to existing therapeutics.

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FIGURE LEGEND

Fig.1: Matriptase-1 expression co-localizes with filaggrin in the granular layer of the epidermis:

Expression of matriptase-1 and filaggrin in normal human skin as well as in organotypic skin cultures on day 4, 5 and 6 after initializing the differentiation process were analyzed.

Fig. 2: Matriptase-1 expression is lost in psoriatic skin lesions.
(a) Immunofluorescence staining of normal human skin shows that matriptase-1 expression is restricted to the granular layer of the epidermis. In contrast to normal epidermis, matriptase-1 expression is completely lost in the epidermis of psoriatic skin lesions **(b-f)**. Whereas strong matriptase-1 expression can be detected in adjacent skin, it was immediately lost beneath the parakeratotic psoriatic plaques **(b)**.

Fig. 3: Matriptase-1 activity is strongly reduced in epidermis of psoriatic skin.
Epidermis of normal skin and psoriatic skin lesions from three different patients were analyzed for matriptase-1 activity. Activity assays reveal a strong down-regulation of matriptase-1 activity in all 3 donors, as measured by use of the specific substrate Boc-Gln-Ala-Arg-AMC. Data are means +/- SD. Mean represents statistic analysis of all three donors; *$p<0.05$ *vs.* control; unpaired Student's *t* test.

Fig. 4: TNFα down-regulates matriptase-1 expression in KC and impairs normal KC-differentiation in an organotypic skin model. Matriptase-1 mRNA **(a)** and protein expression **(b)** in KC were analyzed after treatment with TNFα (20 ng/ml) after 24 and 48 hours, respectively. Both matriptase-1 mRNA **(a)** and protein expression **(b)** are strongly down-regulated after addition of TNFα. Data are means +/- SD of one representative experiment each done in triplicates. *$p<0.05$ *vs.* control; unpaired Student's *t* test. After 6 days of culture, organotypic skin cultures, either untreated **(c,f)** or treated with TNFα (5ng/ml **(d,g)** or 25ng/ml **(e,h)**) were analyzed by matriptase-1 immunofluorescence **(c-e)** and H&E-staining **(f-h)**. Treatment with TNFα leads to loss of matriptase-1 expression **(c-e)** accompanied with hyper- and parakeratosis (arrows) and loss of keratohyaline granules **(f-h)**. Down-regulation of matriptase-1 expression by TNFα was confirmed by Western-blot analysis **(i)**. TNFα induced matriptase-1 down-regulation was accompanied with strongly reduced filaggrin expression.

Fig. 5: Matriptase-1 expression is regulated by TNFα *via* the NFκB-pathway.
Preconfluent KC were infected with adenoviral constructs either expressing green fluorescence protein (AdV-GFP) or dnIKK2 (AdV-dnIKK2). **(a,b)** Twenty-four hours after addition of the recombinant adenovirus, expression of GFP was determined by fluorescence microscopy. **(c)** Forty-eight hours after addition of TNFα (20 ng/ml) cells were lysed, and the expression of IKK2 (upper panel) and matriptase-1 (lower panel) was determined by Western blot analysis. One representative experiment of two is shown.

Fig. 1

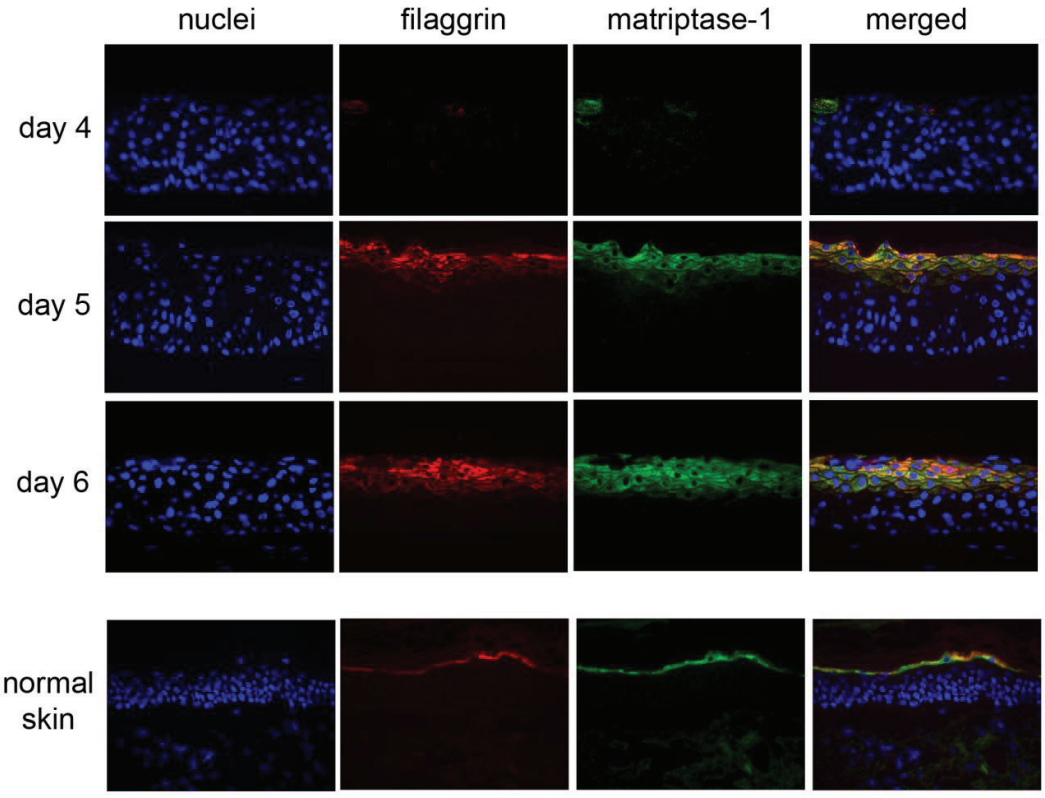

nuclei
filaggrin
matriptase-1
merged
day 4
day 5
day 6
normal skin

Fig. 2

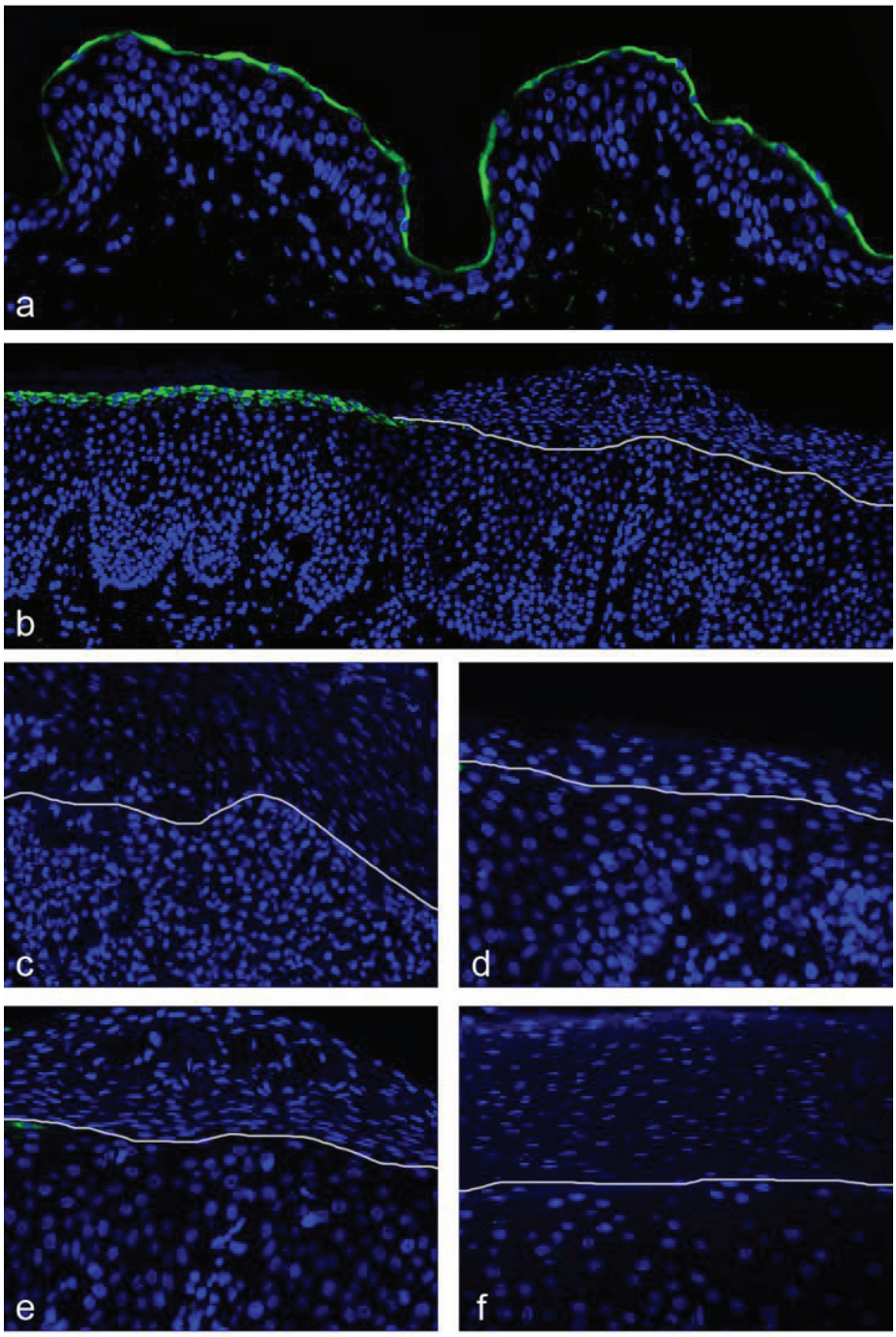
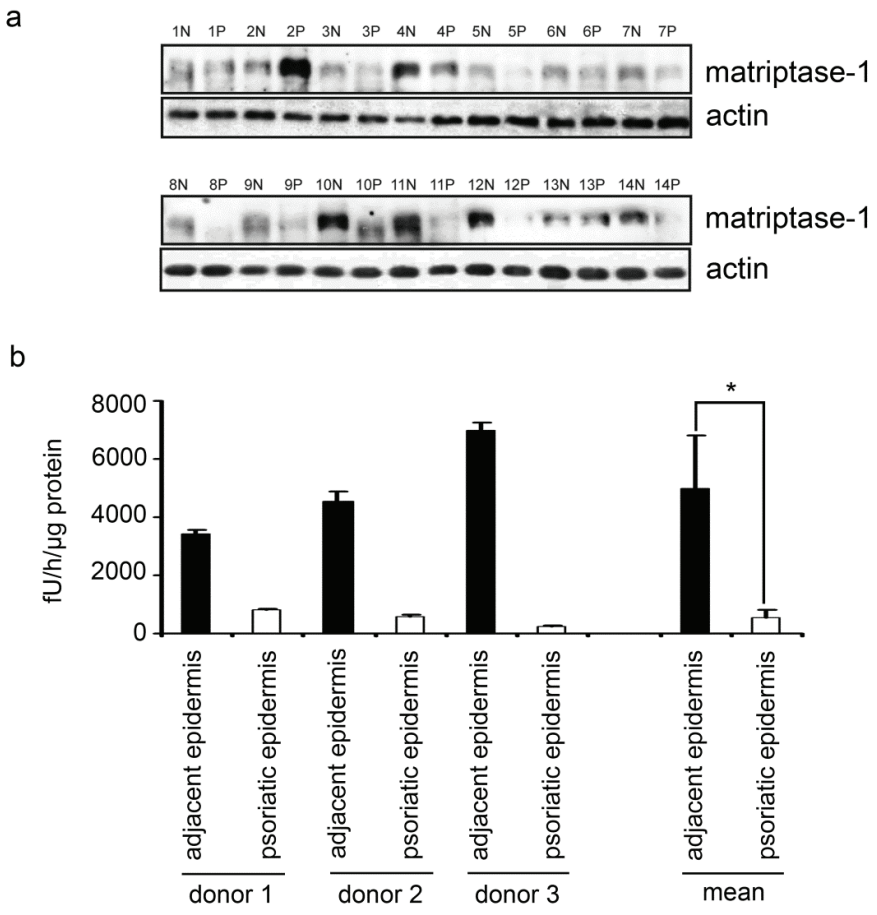


Fig. 3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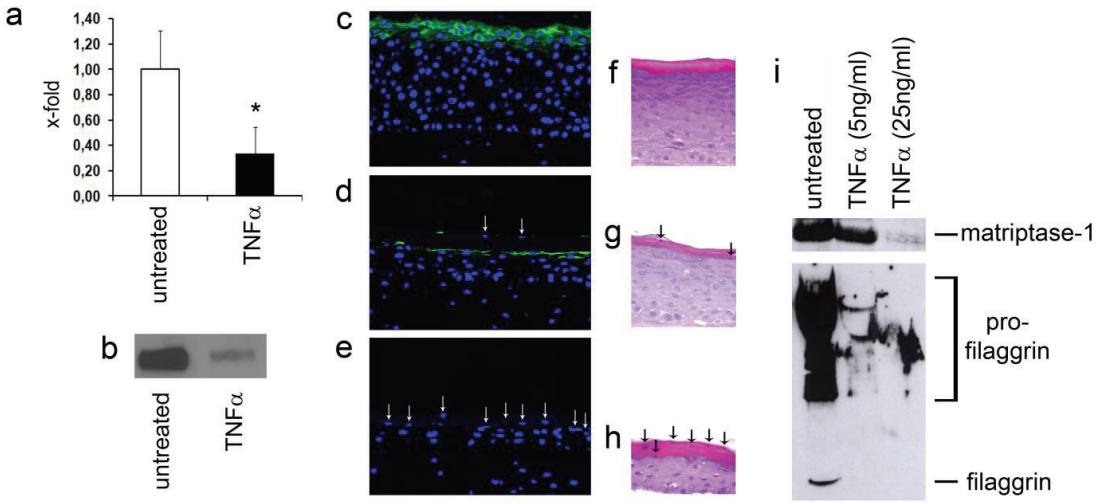
matriptase-1
actin
8N 8P 9N 9P 10N 10P 11N 11P 12N 12P 13N 13P 14N 14P
matriptase-1
actin
b
fU/h/µg protein
8000
6000
4000
2000
0
*
adjacent epidermis
psoriatic epidermis
adjacent epidermis
psoriatic epidermis
adjacent epidermis
psoriatic epidermis
adjacent epidermis
psoriatic epidermis
donor 1
donor 2
donor 3
mean

Fig. 4


a
x-fold
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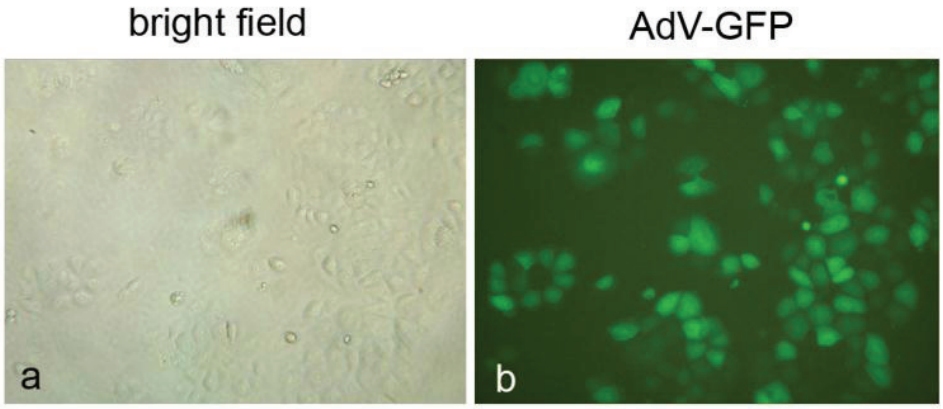
1,20
1,00
0,80
0,60
0,40
0,20
0,00
*
untreated
TNFα
b
untreated
TNFα
c
d
e
f
g
h
i
untreated
TNFα (5ng/ml)
TNFα (25ng/ml)
—matriptase-1
pro-
filaggrin
— filaggrin

Fig. 5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AdV-GFP
a
b

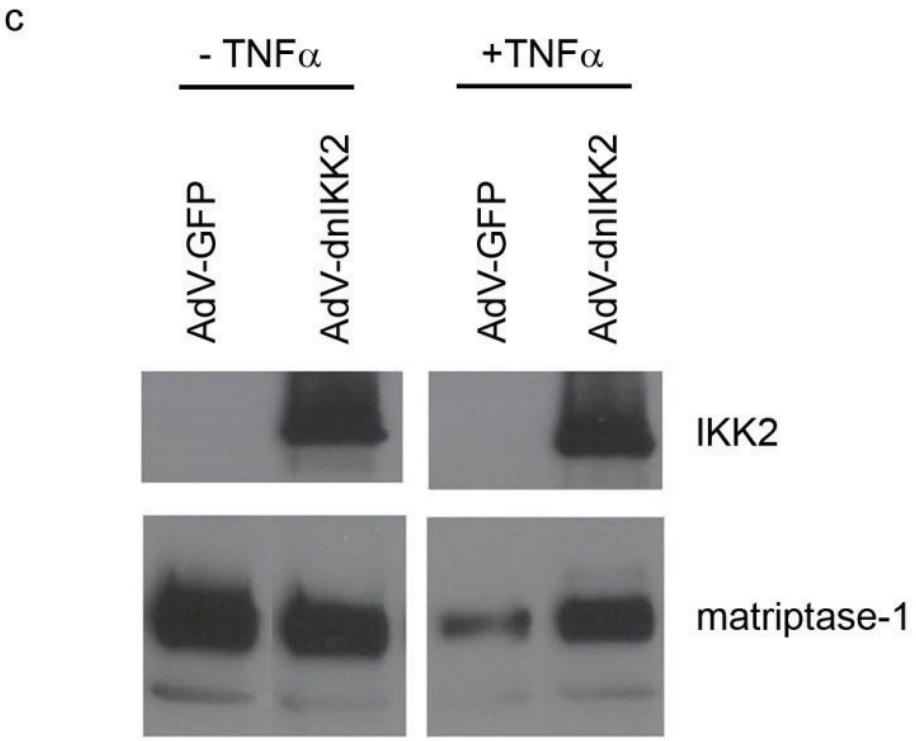
c
- TNFα
+TNFα
AdV-GFP
AdV-dnIKK2
AdV-GFP
AdV-dnIKK2
IKK2
matriptase-1

Figure S1

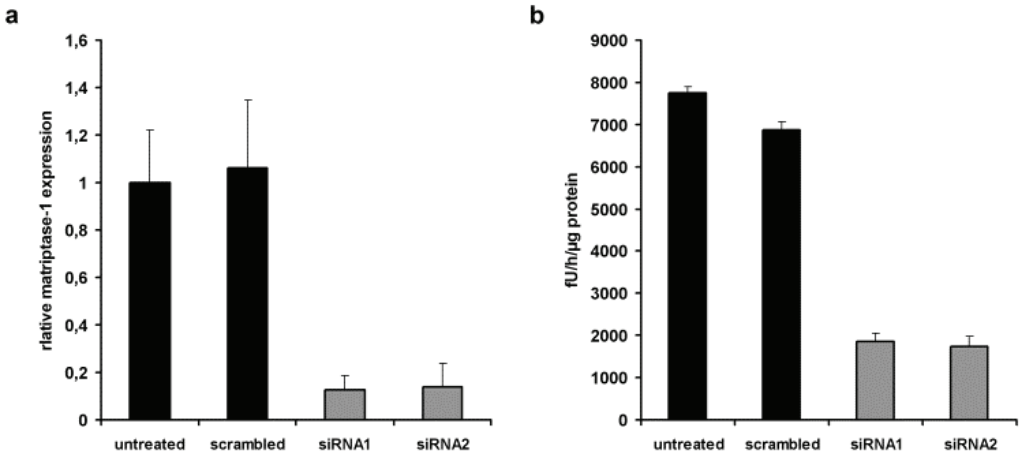
a
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
rlative matriptase-1 expression
untreated
scrambled
siRNA1
siRNA2
b
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
fU/h/µg protein
untreated
scrambled
siRNA1
siRNA2

Figure S2


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NFkB
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SP1
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