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Intracellular Signaling Pathways in Skin Inflammation and Cancer

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Claus Johansen

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Intracellular Signaling Pathways in Skin Inflammation and Cancer

Claus Johansen

Faculty of Health Sciences

University of Aarhus

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II. Johansen C, Kragballe K, Rasmussen M, Norman TD, Iversen L. Activator protein 1 DNA binding activity is decreased in lesional psoriatic skin compared with nonlesional psoriatic skin. British Journal of Dermatology 2004; 151: 600-607.

III. Johansen C, Flindt E, Kragballe K, Henningsen J, Westergaard M, Kristiansen K, Iversen L. Inverse Regulation of the Nuclear Factor-κB Binding to the p53 and Interleukin-8 κB Response Elements in Lesional Psoriatic Skin. Journal of Investigative Dermatology 2005; 124:1284-1292.

IV. Johansen C, Kragballe K, Henningsen J, Westergaard M, Kristiansen K, Iversen L. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. British Journal of Dermatology 2005; 152: 37-42.

V. Johansen C, Funding AT, Otkjaer K, Kragballe K, Jensen UB, Madsen M, Binderup L, Skak-Nielsen T, Fjording M, Iversen L. Protein Expression of TNF-α in Psoriatic Skin is Regulated at a Posttranscriptional Level by MAPK-Activated Protein Kinase 2. Journal of Immunology 2006; 176:1431-1438.

VI. Johansen C, Moeller K, Kragballe K, Iversen L. The Activity of Caspase-1 is Increased in Lesional Psoriatic Epidermis. Journal of Investigative Dermatology 2007; 127: 2857-2864.

VII. Johansen C, Vestergaard C, Kragballe K, Kollias G, Gaestel M, Iversen L. MK2 regulates the early stages of skin tumor promotion. Carcinogenesis, in press.

Arbejderne I-III har indgået som en del af ph.d.-afhandlingen "In vitro and in vivo regulation of the transcription factors AP-1 and NF-κB by 1α,25-Dihydroxyvitamin D3 and Calcipotriol in human skin" (Faculty of Health Sciences, University of Aarhus, 2003).

De øvrige arbejder IV-VII, eller deri indgående resultater, har ikke tidligere været indleveret med henblik på opnåelse af en akademisk grad.

LIST OF ABBREVIATIONS

AP-1	Activator protein 1
ARE	Adenylate/uridylate-rich elements
ATF	Activating transcription factor
AU	Adenylate/uridylate
BMK1	Big MAP kinase 1
CDC	Cell division cycle
CHK	Checkpoint kinase
cPLA2	Cytosolic phospholipase A2
CREB	CRE (cAMP-responsive element) binding protein
DC	Dendritic cell
DMF	Dimethylfumarate
DUSP	Dual-specificity protein phosphatase
eIF	Eukaryotic initiation factor
ERK	Extracellular signal regulated kinase
HMGN1	High-mobility group N1
hnRNP	Heterogeneous nuclear ribonucleoprotein
Hsp27	Heat shock protein 27
IκB	Inhibitor κB
IFN	Interferon
IKK	IκB kinase
IL	Interleukin
IP	Incontinentia pigmenti
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPK kinase kinase
Mdm2	Murine double minute 2
MKP	MAPK phosphatase
MNK	MAPK-integrating kinase
NEMO	NF-κB essential modulator
NF-κB	Nuclear factor κB
NIK	NF-κB inducing kinase
NLS	Nuclear localization signal
MMP	Metalloproteinase
PSF	PTB-associated splicing factor
PTB	Polypyrimidine tract-binding protein
RHD	Rel-homology domain
SAPK	Stress-activated protein kinase
SRF	Serum response factor
STAT	Signal transducers and activators of transcription
TCF	Ternary complex factor
TNF	Tumor necrosis factor
TPA	12-O-tetradecanoyl phorbol 13-acetate
TTP	Tristetraprolin
UV	Ultraviolet
VDR	Vitamin D receptor

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PREFACE

This thesis is a result of my work carried out from 2002 to 2009 at the Department of Dermatology, Aarhus University Hospital, Denmark. My studies would not have been possible without the help and support from all my colleagues, co-authors and supervisors.

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Aarhus, December 2009

Claus Johansen

INTRODUCTION

The skin has a crucial role as a barrier to the external environment and its associated microflora. Therefore, efficient functioning of the immune system in the skin is critical. Some of the most common inflammatory skin diseases are atopic dermatitis, allergic contact dermatitis and psoriasis. Recent discoveries have highlighted the importance of intracellular signaling pathways as key players in the pathogenesis of different inflammatory skin diseases, including psoriasis. However, the exact role by which intracellular signaling pathways are involved in the pathogenesis of psoriasis is still not fully understood. Therefore, a better understanding of these different signaling pathways may offer new therapeutic targets.

PSORIASIS

Psoriasis is a chronic inflammatory skin disease affecting approximately 2-3% of the population (1, 2). There are two main forms of psoriasis: psoriasis pustulosa and psoriasis vulgaris. The most common clinical form of psoriasis is psoriasis vulgaris, accounting for approximately 85-90% of the cases (2). It is characterized by sharply, demarcated, erythematous, scaling plaques which typically affect the elbows, knees, scalp and intergluteal cleft, but can be present all over the body (Fig. 1). Many different theories of the pathogenesis of psoriasis have been made over the last decades. Psoriasis was originally considered to be a primary keratinization disorder of the skin. However, more recent evidence indicated that the epidermal changes in psoriasis occur in response to cellular immune infiltrates in the skin, leading to the current general consensus that psoriasis is a T-cell-mediated inflammatory disease (3-5).

Histologically, psoriasis is characterized by marked thickening of the epidermis and elongation of the rete ridges, due to increased proliferation of the keratinocytes (Fig. 1). The differentiation of the keratinocytes is abnormal in psoriasis, which is reflected by the loss of the granular layer and presence of nuclei in the cornified layer (4). In addition, there are elongated blood vessels in the papillary dermal region owing to an increased angiogenesis, which also causes the visible redness of psoriatic skin lesions. Other distinctive histological characteristics of psoriatic skin lesions include the presence of neutrophils forming the Munroe micro abscesses in the stratum corneum, significant mononuclear infiltrates in the epidermis as well as marked infiltration of mononuclear leukocytes such as T cells and dendritic cells (DCs) into the dermis (6).

Psoriasis has a complex genetic basis and so far genome scans have identified at least 19 genetic susceptibility loci

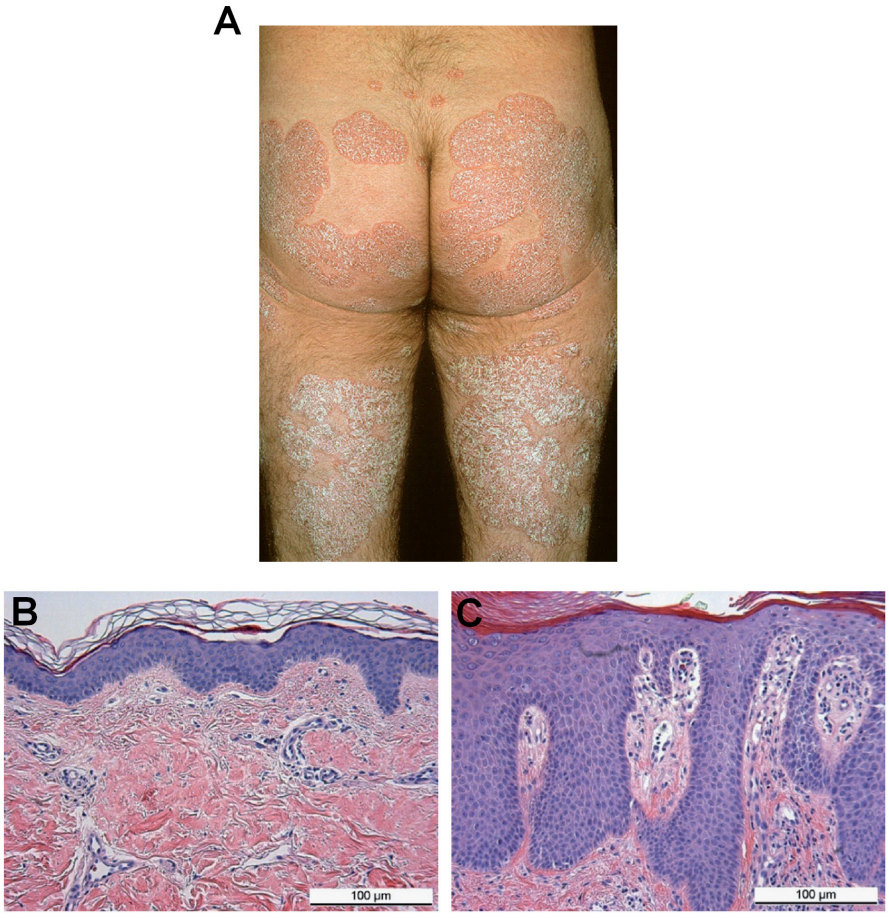

Fig. 1. A) Psoriasis vulgaris characterized by thickening, well demarked, erythematous plaques. B and C) Hematoxylin and eosin staining of (B) nonlesional and (C) lesional psoriatic skin. Scale bar = 100 µm.

(6, 7). So far, few of the genes within these loci have been identified and their association with psoriasis has not always been replicated in studies of other populations. The only locus which has consistently been identified in genetic screens of families with psoriasis is the MHC locus on the short arm of chromosome 6. This locus is known as psoriasis susceptibility 1 (PSORS1) (6). In addition to gene loci predisposing to psoriasis, molecular genetic studies have revealed a number of psoriasis related gene polymorphisms. These include promoter polymorphisms for TNF-α and IL-1β (8, 9), macrophage migration inhibitory factor (MIF) gene polymorphism (10), as well as gene polymorphisms in the IL-12/Il-23(p40), IL-23(p19) and IL-23R regions (11).

The pathogenesis of psoriasis is not fully understood at present, but since the early 1990s it has been considered as a T cell mediated disease where DCs ingest and process a putative exogenous or endogenous antigen, migrate to the regional lymph nodes where they activate T cells (12). Until recently, psoriasis has been considered mainly to be a TH1 driven autoimmune inflammatory skin disease defined by a cytokine pattern consisting of IFN-γ, TNF-α, IL-1, -2, -3, -6, -8, epidermal growth factor (EGF) and TGF-α (13, 14). However, recent findings have revealed a potential role for IL-23 and TH17 responses in the pathogenesis of psoriasis (15, 16). In support of this, an increased number of TH17 cells have been identified in the dermis and epidermis of psoriatic skin compared with normal skin (17). Moreover, increased expressions of IL-17A, IL-17C, IL-17F and IL-23 have been demonstrated in lesional psoriatic skin compared with nonlesional psoriatic skin (18-20). Thus, instead of being a TH1-mediated disease, psoriasis is now thought to be a mixed TH1 and TH17 inflammatory autoimmune disease.

Treatment of psoriasis

Vitamin D and different synthetic vitamin D analogues such as calcipotriol have been widely used in the treatment of psoriasis (21–23). These drugs were known to modulate epidermal growth and differentiation, suggesting their mode of action in psoriasis, but later a variety of effects on cells of the immune system were also demonstrated (23). The vitamin D analogues are mainly used in the treatment of mild to moderate psoriasis, whereas systemic treatments with cyclosporine, methotrexate and acitretin are used for moderate to severe psoriasis. However, the treatment of moderate to severe psoriasis has been challenged in recent years by the emergence of biological therapies. Especially, biological drugs targeting TNF-α such as etanercept, infliximab and adalimumab have proved to be very efficient in the treatment of psoriasis (12, 24, 25). The fact that psoriasis responds very well to anti-TNF-α therapy does not rule out T cells as important players in the pathogenesis of psoriasis. Also agents which target T cells, such as alefacept and efalizumab, have been shown to reduce the symptoms of psoriasis (26). However, the concept that T cells with a TH1 cytokine profile are key triggers of psoriasis has been challenged by an increased knowledge of the contribution to the pathogenesis of psoriasis made by other immune components such as keratinocytes, DCs and TH17 cells (4, 27). The latest addition to the biological drugs used in the treatment of moderate to severe psoriasis is ustekinumab, a human monoclonal antibody which binds the shared p40 subunit of IL-12 and IL-23 (28, 29). This therapeutic approach is conceptually new, because it targets mainly dendritic-cell-derived cytokines, in contrast to the broader targeting of anti-TNF-α therapies. Although the new biological therapies have marked effects on psoriasis they fail, as well, in some cases. For example disease responsiveness might lack, resistance in long-term treatment maybe induced and risks of opportunistic infections is present. Thus, there are still major needs for identification of new targets in the treatment of psoriasis. A new concept in the treatment of psoriasis could be to target intracellular signaling pathways by small molecules.

The role of intracellular signaling pathways as well as transcription factors in the pathogenesis of psoriasis is still not clearly defined. Transcription factors such as STAT1, STAT3, NF-κB and AP-1 are all dysregulated in psoriatic skin (II, III), (30, 31). However, the specific upstream activators or repressors of these transcription factors in psoriasis are still not fully known. Recently, several different intracellular signaling molecules have been suggested as targets for future therapies (32). Especially, the p38 MAPK signaling pathway has been demonstrated to play a significant role in inflammatory processes including psoriasis (IV-VI), (33, 34) and inhibitors of p38 MAPK and its downstream targets are considered as possible future treatments for psoriasis and other inflammatory diseases.

INTRACELLULAR SIGNALING

Communication between the cell membrane receptors and the nucleus allow cells to respond to extracellular danger signals. Rapid and adequate transduction of this information is critical for appropriate cell reactions and survival. Intracellular signal transduction pathways act as messengers of information from the extracellular environment to the genes inside the nucleus. Some of the most prominent among the known signal transduction pathways which control these events are the mitogen-activated protein kinase (MAPK) cascades.

The MAPK signaling pathway

The MAPK family members are serine/threonine protein kinases which play an essential role in signal transduction by modulating gene transcription in the nucleus in response to changes in the cellular environment (35). MAPKs control key cellular functions, including proliferation, differentiation, migration, apoptosis, cellular stress and inflammatory responses, and participate in a number of disease states including acute and chronic inflammation and cancer (36-40). In mammals, there are at least four distinctly regulated groups of MAPKs: the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2); c-Jun N-terminal kinases 1, 2 and 3 (JNK1, JNK2 and JNK3); p38 MAPKs (p38α, -β, -γ and -δ); and ERK5 (41). Each group of MAPKs can be stimulated by a separate protein kinase cascade which includes sequential activation of a specific MAPK kinase kinase (MAPKKK or MEKK) and a MAPK kinase (MAPKK, MKK or MEK), which in turn phosphorylates and activates their downstream MAPKs (35) (Fig. 2). In general, the activity of ERK1 and ERK2 is stimulated in response to growth factors and phorbol esters, whereas p38 MAPK and JNK are most potently activated by proinflammatory cytokines and environmental stresses such as osmotic shock and UV irradiation (42). That is why p38 MAPK and JNK proteins are also known as stress-activated protein kinases (SAPKs).

The numerous functions of the MAPKs are mediated through phosphorylation of several substrates, including transcription factors, phospholipases, and cytoskeletal proteins. MAPKs also phosphorylate, and thereby activate, several protein kinases termed MAPK-activated protein kinases (MKs), which represent an additional enzymatic and amplification step in the MAPK signaling cascade (Fig. 2). The MK family includes the MAPK-activated protein kinases (MKs), the mitogen- and stress-activated protein kinases (MSKs), the ribosomal S6 kinases (RSKs) and the MAPK-integrating kinases (MNKs), which perform overlapping and unique biological functions (43).

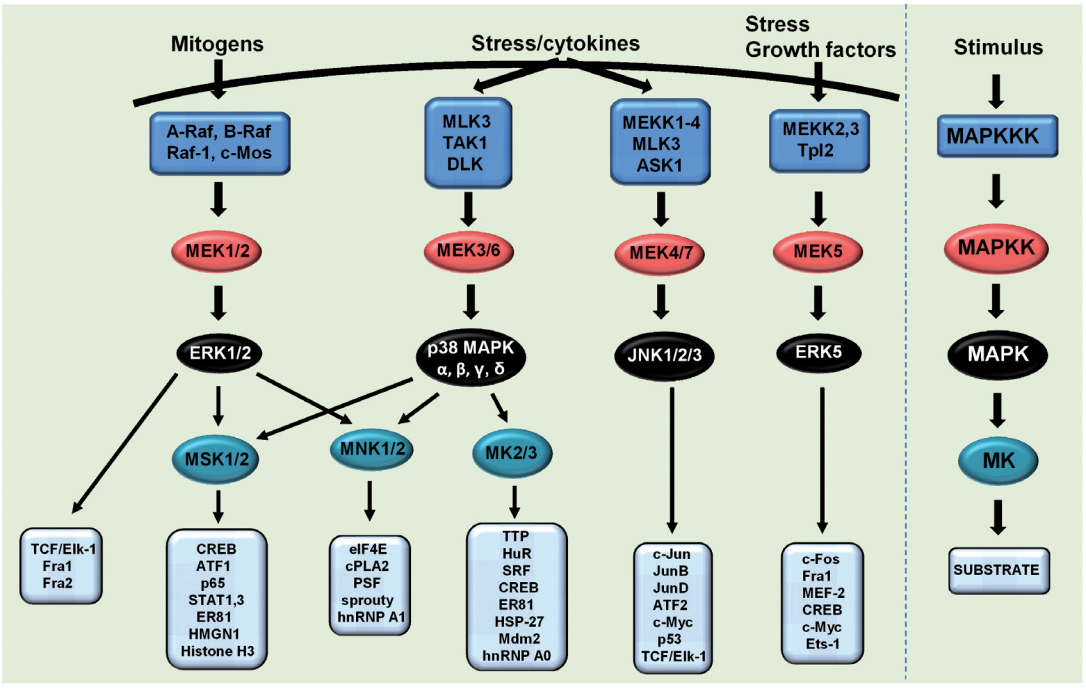

Fig. 2. A simplified schematic overview of the mitogen-activated protein kinase (MAPK) signaling cascade. Activation of the MAPK leads to activation of the MKs, which in turn leads to a regulation of the specific substrates.

p38 MAPK

Inflammatory stimuli, such as LPS, TNF and IL-1 are the major inducers of p38 MAPK. The fact that LPS induces p38 MAPK led to the first description of this molecule, because p38 MAPK was originally identified as an LPS-activated gene (44). p38 MAPK is activated by the upstream kinases MEK3 and MEK6 by dual phosphorylation at threonine 180 and tyrosine 182 (45). p38 MAPK has been demonstrated to be present both in the nucleus and cytoplasm of quiescent cells (43). However, upon cell stimulation, the cellular localization of p38 MAPK is not fully understood. Some evidence suggests that, following activation, p38 MAPK translocate from the cytoplasm to the nucleus (46), but other data indicate that activated p38 MAPK is also present in the cytoplasm of stimulated cells (47).

To date, four different p38 MAPK isoforms have been identified, and include p38α, -β, -γ and -δ. All isoforms are serine-threonine protein kinases which share the conserved phosphorylation motif Thr-Gly-Tyr (TGY) in their activation loop. The p38α is the best characterized isoform, and together with p38β it is ubiquitously expressed (48, 49). It has been suggested that p38α and, to a lesser extent, p38β play important roles in mediating keratinocyte responses to cellular stress (50). In support of this, recent results from our group using p38β/δ knockout mice in an acute contact dermatitis model, demonstrated that p38α is the most important isoform in the induction of skin inflammation (submitted data). Similar results were obtained in another study, using an acute and chronic inflammation model (51). p38α, but not p38β, was shown to be necessary for the development of acute and chronic inflammatory responses. Interestingly, recent results have suggested a dual function for p38α coordinating both inflammatory and anti-inflammatory responses (52). The p38δ has been shown to play an important role in inducing keratinocyte differentiation (50), and like all other p38 MAPK isoforms it responds to inflammatory and toxic environmental triggers (53). Together with p38α and –β, the phosphorylation/activation of p38δ was demonstrated to be increased in psoriatic skin (IV). p38γ is involved in myocyte differentiation, regulating proteins such as syntrophin which is a synapse-associated protein (54). It is predominantly expressed in skeletal muscle, whereas it is not expressed in human epidermis (IV) (55).

Activated p38 MAPK can upregulate cytokine production by several mechanisms, including direct phosphorylation of transcription factors (48) and through stabilization and increased translation of mRNAs containing 3´untranslated region adenylate/uridylate-rich elements (AREs) by phosphorylation of ARE binding proteins (56-58). IL-1β, IL-6, IL-8, TNF-α, IL-12/IL-23(p40), IL-23(p19), IL-18 and IL-20 are all inflammatory cytokines regulated through the p38 MAPK pathway (V, VI), (59–64). As for p38 MAPK, these cytokines have been demonstrated to be related to inflammatory diseases such as psoriasis and rheumatoid arthritis.

Recently, we and others demonstrated increased activity of p38 MAPK in lesional psoriatic skin (IV) (65) (Fig. 3). Therefore, p38 MAPK has been suggested to represent a possible target of therapeutic intervention in psoriasis and other inflammatory diseases. In animal studies, however, p38 MAPK inhibitors have demonstrated adverse effects affecting the liver and the heart (66, 67). It is therefore critical to describe and understand the p38 MAPK signaling pathway in inflammatory diseases like psoriasis in order to identify alternative and maybe even better targets for future anti-inflammatory therapies. Another encouraging approach is targeting downstream the p38 MAPK. This may avoid some of the adverse effects observed with p38 MAPK inhibitors, and kinases downstream p38 MAPK may be even better targets because they integrate signals from upstream pathways. For instance a recent publication demonstrated that p38α knockout cells have increased levels of activated JNK, suggesting that p38 MAPK antagonizes the JNK pathway (68). Targeting downstream p38 MAPK may therefore avoid compensatory increased activation of other signaling pathways. Recently, our group have demonstrated increased activities of the downstream kinases MSK1, MSK2 and MK2 in psoriatic skin, suggesting some of these to be possible targets for anti-inflammatory therapy (V) (33, 34). In addition, we have recently demonstrated increased activity of caspase-1 as well as increased levels of IL-18 in lesional psoriatic skin (VI). We showed that the secretion of IL-18 from cultured human keratinocytes was regulated by a p38 MAPK/caspase-1 dependent mechanism, making caspase-1 a potential target in the treatment of psoriasis (VI).

ERK1 and ERK2

ERK1 and ERK2 were the first MAPKs to be characterized. Two isoforms, ERK1 and ERK2 (also known as p44MAPK and p42MAPK, respectively) have been identified. ERK1 and ERK2 are predominantly activated by growth factors, serum and phorbol esters and, to a lesser extent, by cytokines

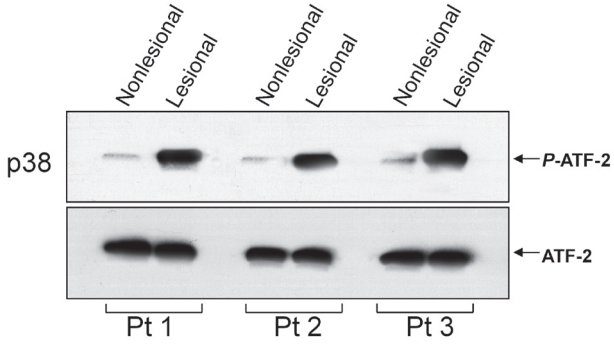

Fig. 3. The p38 mitogen-activated protein kinase (MAPK) kinase activity is increased in lesional psoriatic skin. Kinase activity was determined by an in vitro kinase assay using ATF-2 as a substrate.

and osmotic stress (69). ERK1 and ERK2 are distributed throughout quiescent cells, but upon stimulation, ERK1 and ERK2 accumulates in the nucleus (70, 71). An important role of ERK activation is the regulation or modulation of gene expression. Once activated, ERK1 and ERK2 can target numerous protein kinases and transcription factors, including MSK1/2, MNK1/2 and AP-1 (72, 73) (Fig. 2).

ERK1 and ERK2 have both been demonstrated in the epidermis as well as in normal human keratinocytes in vitro, and have been implicated as key regulators of keratinocyte differentiation (74). For instance, we demonstrated that vitamin D3-induced keratinocyte differentiation was mediated through an ERK1 and ERK2 dependent mechanism (I). Increased expression of ERK1 and ERK2 in the basal and lower suprabasal layer of lesional psoriatic skin has been demonstrated (75), as well as an increase in the phosphorylated/activated form of ERK1 and ERK2 (IV), (65, 76). Furthermore, in a previous study ERK activation was shown to play an important role in epidermal hyperproliferation and skin inflammation (77).

In addition, the MEK/ERK signaling pathway is involved in cell proliferation and survival, and for that reason, inhibitors of the MEK/ERK pathway are entering clinical trials as potential anticancer agents (78, 79). Several tumors are known to express constitutive levels of activated ERK1 and ERK2, and ERK activation is critical for a large number of Ras-induced cellular responses. Interestingly, Ras has been shown to be mutated in 30% of all human cancers, while B-Raf is mutated in 60% of malignant melanomas (80, 81).

JNK1-3

Three members of the JNK family have been identified in mammals, JNK1, JNK2 and JNK3 (also known as SAPKγ, SAPKα and SAPKβ, respectively) (82, 83). JNK was originally identified as the UV-induced factor responsible for phosphorylating and thereby activating the proto-oncogene transcription factor c-Jun (84). Similar to ERK1, ERK2 and p38 MAPK, JNK activation requires dual phosphorylation on tyrosine and threonine residues. The MAPKKs which catalyze this reaction are known as MEK4 and MEK7, which are themselves phosphorylated and thereby activated by several MAPKKKs, including MEKK1-4, MLK3 and ASK1 (Fig. 2). Once activated JNKs translocate to the nucleus where they exert their effects on numerous transcription factors such as c-Jun, JunB, JunD, ATF2, c-Myc, TCF/Elk-1 and p53 (39, 85-87). Activation of the JNK signaling pathway plays an important role in regulating apoptosis as well as tumorigenesis and inflammation. There are currently no known JNK-activated MKs.

In the skin, all three isoforms of the JNKs have been demonstrated in cultured human keratinocytes in vitro, and recent results have demonstrated that JNK1, but not JNK2 and JNK3, is involved in the UV-induced signal transduction in human epidermis (88). Furthermore, studies using JNK1-deficient mice have suggested that JNK1 is a crucial suppressor of skin tumor development (89). The fact that immunohistochemical analyses of normal human skin have revealed that phosphorylated JNK1 is expressed in the nuclei in the suprabasal-granular cell layer (90) and that vitamin D3-induced differentiation in human keratinocytes is mediated through a JNK1 dependent mechanism (I), indicates that JNK1 participates in the regulation of gene transcription in differentiating human keratinocytes.

ERK5

ERK5, also known as BMK1, is a recently identified MAPK which is being studied intensely (91, 92). Upon stimulation ERK5 is activated specifically by a member of the MEK family of kinases known as MEK5 (93) (Fig. 2). ERK5 is expressed in many human tissues, but is most abundant in heart and skeletal muscle (94). It has never been studied in human skin. Recently, however, it was demonstrated to be expressed in mouse keratinocytes (95). Moreover, previous studies have demonstrated ERK5 to be expressed in fibroblasts (96) and mast cells (97).

ERK5 activates a distinct set of transcription factors implicated in cell cycle regulation. Some of the transcription factors which are directly activated by ERK5 phosphorylation include c-Fos and Fra1 (98), two protein members of the AP-1 family. In addition, ERK5 plays a critical role in the regulation of c-Jun expression by controlling the activity of the transcription factor MEF-2 which binds to a MEF-2 response element within the c-Jun promoter (99). Other transcription factors regulated by ERK5 include CREB, Ets-1 and c-Myc, some of which are also shared by ERK1 and ERK2, reflecting a degree of redundancy among MAPK targets.

MK2

MAPK-activated protein kinase 2 (MK2) is a serine/threonine kinase regulated by direct phosphorylation by p38α (100). In its inactive form, MK2 is located in the nucleus. Upon activation by p38α, MK2 rapidly translocates from the nucleus to the cytoplasm and also cotransports p38α back to the cytoplasm (47, 101). Using a p38 MAPK inhibitor and a gene targeting approach in mice, it has been demonstrated that stress-induced MK2 activation is exclusively dependent on p38α (102, 103). MK2 is activated upon different stress conditions such as UV-irradiation, heat shock, LPS or IL-1 treatment and participate in diverse cellular processes such as cytokine production (V), (104, 105), endocytosis (106), reorganization of the cytoskeleton (107), cell migration (108), cell cycle control (109) and apoptosis (VII).

MK2 has been demonstrated to play a key role in inflammatory processes (110). MK2-deficient mice showed an increased resistance to LPS-induced endotoxic shock, due to a 90% reduction of the TNF-α production (104). In addition,

LPS stimulated cells derived from MK2-deficient mice have reduced levels of several inflammatory cytokines including IL-1β, IL-6, TNF-α and IFN-γ, indicating a pivotal role of MK2 in inflammatory responses (104). MK2 regulates the stability and translation of TNF-α and IL-6 mRNAs through a process which involves the AU-rich elements in the 3´non-coding region of these mRNAs (111). Tristetraprolin (TTP), a protein which controls the stability and translation of TNF-α mRNA (112), has recently been identified as a direct substrate for MK2 (113). Phosphorylation of TTP by MK2 increases its stability and binding to 14-3-3 proteins, and thereby stimulates TNF-α expression (57).

Recently, we demonstrated increased levels of the phosphorylated/activated form of MK2 in lesional psoriatic skin compared with nonlesional psoriatic skin (Fig. 4). In addition, we showed that the mRNA expression of TNF-α was unchanged between lesional and nonlesional psoriatic skin, whereas the protein level of TNF-α was increased in lesional compared with nonlesional psoriatic skin. These data, together with in vitro studies demonstrating decreased TNF-α protein levels after transfection of cultured human keratinocytes with MK2 siRNA, suggests that MK2 regulates TNF-α at a posttranscriptional level in psoriatic skin (V).

In another study, it was demonstrated that MK2-deficient mice show increased resistance to collagen-induced arthritis, a murine model of rheumatoid arthritis (114). In a model of experimental asthma, the endothelial permeability as well as expression of specific chemokines and adhesion molecules were reduced in the lung of allergen treated MK2-deficient mice, indicating that MK2 is critical for the development of lung inflammation (115). Finally, in a recent paper we demonstrated that MK2-deficient mice have reduced oxazolone-induced acute allergic contact dermatitis (116). The significant role of MK2 in psoriasis, collagen-induced arthritis, lung inflammation and acute allergic contact dermatitis disclose MK2 as a key player in the pathogenesis of these inflammatory diseases, and qualifies MK2 as a promising target for the development of small molecule inhibitors which can be used as anti-inflammatory therapeutics.

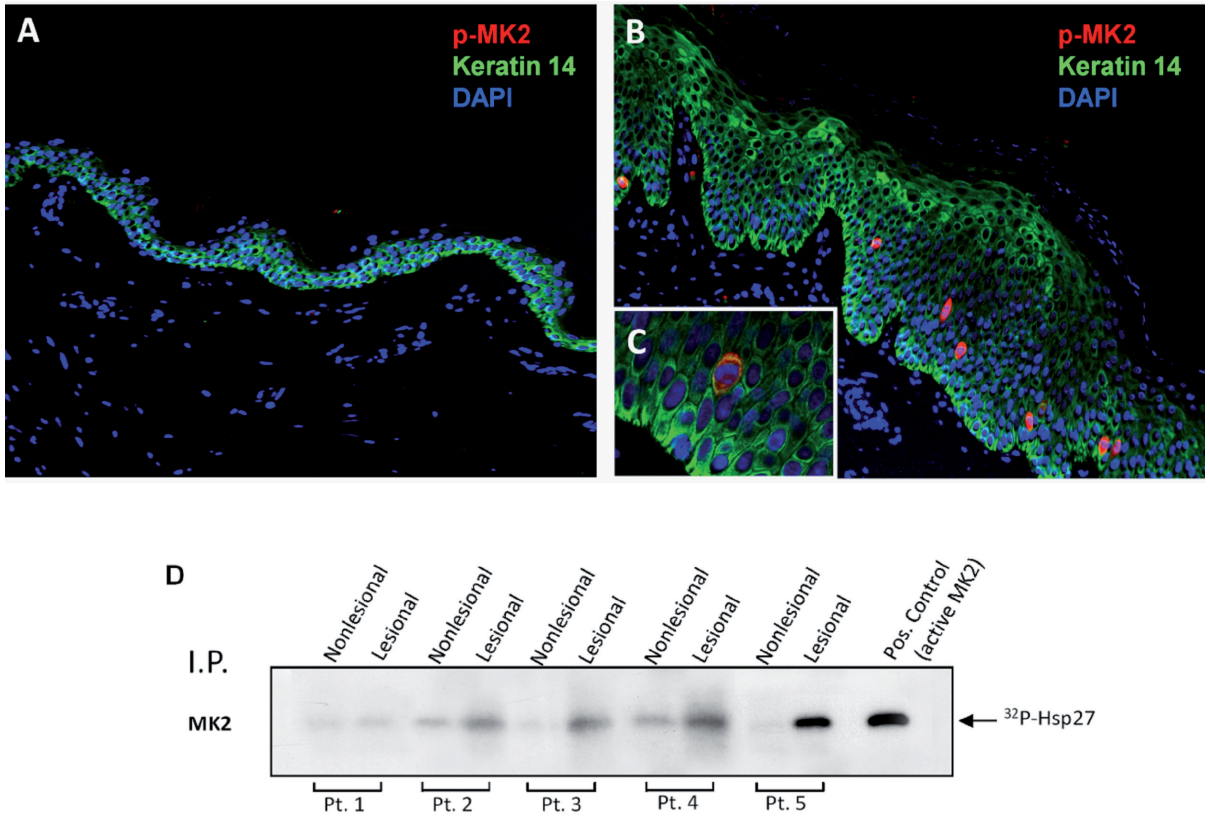

Fig. 4. Increased phosphorylation of MK2 in lesional psoriatic skin (B and C) compared with nonlesional psoriatic skin (A), as determined by immunofluorescence analysis. Nuclear staining was performed using DAPI (blue). Green demonstrates keratin 14 and red demonstrated phosphorylated MK2. Yellow indicates co-localization (B and C). (A and B) magnification x 10 and (C) magnification x 40. (D) Kinase activity was determined by an in vitro kinase activity assay using Hsp27 as a substrate.

Several substrates for MK2 have been identified, including Hsp27, TTP, hnRNP A0, HuR, CREB, SRF, ER81 and Mdm2 (113, 117-123) (Fig. 2). In a previous study, MK2 was demonstrated to directly phosphorylate CDC25B and CDC25C in UV-treated osteosarcoma cells. In the same study, it was shown that MK2 knockdown leads to loss of the G2/M checkpoint, and therefore MK2 was suggested to be a third member of the DNA-damage-checkpoint-kinase family which functions in parallel with CHK1 and CHK2 (109). The p53-interacting ubiquitin ligase Mdm2 was recently identified as a target of MK2. Mdm2 was demonstrated to be phosphorylated/activated by MK2 leading to increased degradation of p53, and MK2-deficeint mouse embryonic fibroblasts were found to have reduced Mdm2 phosphorylation and elevated p53 protein levels, suggesting that MK2 plays a role in moderating the extent and duration of the p53 response (123). MK2, therefore, seems to play a role in the regulation of both inflammation and apoptosis. In support of this, we recently demonstrated that MK2 regulates the early stages of skin tumor promotion. MK2-deficient mice developed significantly fewer skin tumors compared with both TNF-α-deficient mice and wild-type mice in a chemical carcinogenesis model (VII) (Fig. 5). MK2 was demonstrated to play a critical role during tumor initiation in regulating apoptosis in DNA-damaged cells through a Mdm2 mediated stabilization of p53 (VII).

Besides playing a critical role in the early stages of tumor promotion, MK2 was recently shown to be essential in mediating cell death. This was demonstrated in an experimental model resembling the late stage of tumor promotion using p53-deficient cells (124). Tumor cells lacking functional p53 survive DNA-damaging chemotherapeutic drugs through a p38 MAPK/MK2-dependent mechanism. In contrast, in p53-proficient cells, signaling through this pathway was dispensable for survival after DNA-damage. Therefore, the targeting of MK2 as a strategy to specifically eradicate p53-defective tumor cells with DNA-damaging chemotherapy was suggested (124).

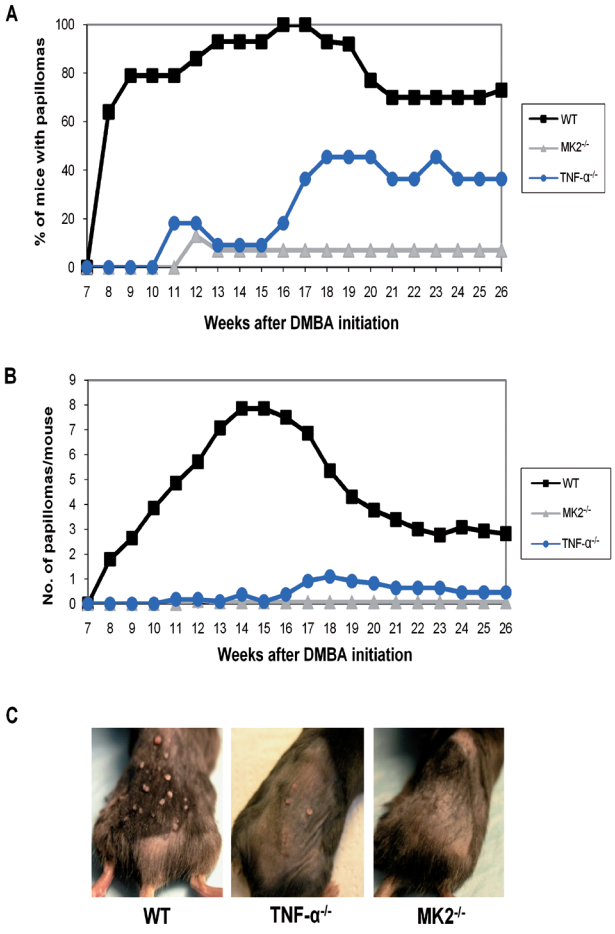

Fig. 5. Skin carcinogenesis in wild-type, TNF-α$^{-/-}$ and MK2$^{-/-}$ mice. (A-C) Wild-type mice (n=14), TNF-α$^{-/-}$ mice (*n*=11) and MK2$^{-/-}$ mice (*n*=15) were treated topically with one dose of 25 µg DMBA and then one week later with 4 µg TPA three times weekly for 15 weeks. (A) Percentage of papilloma-bearing mice in each group. (B) Average number of papillomas per mouse over time. (C) Representative picture of wild-type, TNF-α$^{-/-}$ and MK2$^{-/-}$ mice at the end of the experiment shown in (A) and (B).

MSK1 and MSK2

Mitogen- and stress-activated protein kinase (MSK) 1 and 2 are serine/threonine protein kinases which are activated by both the p38 MAPK and ERK1/2 signaling pathways (125, 126) (Fig. 2). Sequence comparisons between MSK1 (also called RLPK) and MSK2 (also called RSKB) revealed more than 75% identity between these two kinases (127). MSK1 and MSK2 are widely expressed in human tissues including the skin (33, 34, 127). MSKs are predominantly located in the nucleus of quiescent and activated cells, and a nuclear localization sequence has been found in the C-terminal region (128), indicating that MSKs preferentially phosphorylates nuclear substrates.

Because of the dual activation mode, MSKs can be activated by both mitogens such as serum and growth factors as well as stress stimuli such as inflammatory cytokines, UV-irradiation and hydrogen peroxide. Upon activation, MSK1 and MSK2 are capable of phosphorylating multiple transcription factors and nuclear proteins, thereby regulating the expression of a number of specific genes (Fig. 2). cAMP-response element binding protein (CREB) and activating transcription factor (ATF1) are two transcription factors phosphorylated by the MSKs (127, 129). Embryonic fibroblasts derived from MSK1/2 double knockout mice revealed a complete abolishment of CREB and ATF1 phosphorylation in response to anisomycin and UV-irradiation (130), supporting the role of MSK1 and MSK2 in CREB phosphorylation. Another target of the MSKs is the p65 subunit of the transcription factor NF-κB which has been demonstrated to be phosphorylated by MSK1 in response to TNF-α in fibroblasts (131). Other

transcription factors targeted by the MSKs include STAT1, STAT3 and ER81 (132-134). In a previous study, the phosphorylation of histone H3 and HMGN1 (also known as HMG-14) was demonstrated to be abolished in fibroblasts lacking MSK1 and MSK2, suggesting that the MSKs are the major kinases for histone H3 and HMGN1 in response to mitogenic and stress stimuli in fibroblasts (135).

MSK dysregulation has recently been linked to psoriasis. Significantly higher amounts of phosphorylated/activated MSK1 and MSK2 were demonstrated in lesional psoriatic skin compared with nonlesional psoriatic skin. Moreover, CREB and ATF1 phosphorylation levels were found to be elevated in psoriatic skin biopsies (33, 34). Furthermore, MSK1 was demonstrated to regulate the production of proinflammatory cytokines such as TNF-α, IL-6 and IL-8 (34). Interestingly, another study showed that dimethylfumarate (DMF), which is commonly used to treat psoriasis, is able to inhibit IL-1β-induced MSK1 and MSK2 phosphorylation in cultured normal human keratinocytes. Concomitantly, p65, CREB and ATF1 phosphorylation was also abolished (136). Together, these data suggest that MSK1 and MSK2 play a role in the pathogenesis of psoriasis.

On the contrary, we have also recently demonstrated that MSK1 and MSK2 act as negative regulators of toll-like-receptor (TLR) signaling. Using MSK1 and MSK2 double deficient macrophages, the MSKs were found to be required to limit TLR4-induced production of the inflammatory cytokines TNF-α, IL-6 and IL-12, through an induction of DUSP1 and IL-10 (137). In addition, mice double deficient in MSK1 and MSK2 were hypersensitive to LPS-induced endotoxic shock and showed prolonged inflammation in a model of irritative contact eczema (137). These data indicate that although ERK1/2 and p38 MAPK play an important role in inflammation, these kinases also activate negative feedback systems critical in the prevention of uncontrolled inflammation.

MNK1 and MNK2

The MAPK-interacting kinases (MNK) 1 and 2 form a subfamily of MKs which was discovered in 1997 (138). MNK1 and MNK2 show approximately 70% homology in regard to amino acids sequence, mostly differing within their C-terminal region (43). The MNKs are activated through both the p38 MAPK and the ERK1/2 signaling pathway, but not the JNK signaling pathway (139). Transcription of both the MNK1 and MNK2 gene generates two splice variants designated MNK1a, MNK1b, MNK2a and MNK2b (140–142). The MNK isoforms differ in their cellular localization, in that the MNK1b and MNK2b are primarily located in the nucleus of quiescent cells, whereas the MNK1a and MNK2a are mainly found in the cytoplasm, but with the capability of actively shuttling between the cytoplasm and the nucleus (43, 141, 143). The best characterized substrate for the MNKs is the eukaryotic initiation factor (eIF) 4E, a translation initiation factor which binds the 5´-cap structure found on mRNAs (139). Other substrates for the MNKs include cPLA2, hnRNP A1, PSF and sprouty (144-147) (Fig. 2).

An association between the activity of MNK1, as judged by phosphorylation of eIF4E, and the production of the inflammatory cytokines TNF-α, IL-1β and IL-6 has been demonstrated in different cell lines, including cultured normal human keratinocytes (145, 148–150). Moreover, the expression and phosphorylation levels of eIF4E were recently demonstrated to be augmented in lesional psoriatic epidermis (151), suggesting that MNK1 contributes to the expression of proinflammatory cytokines found in inflammatory skin diseases.

TRANSCRIPTION FACTORS

The ability to respond to extracellular signals is essential for the development, survival, and the potential of all living organisms to adapt to changing and adverse environmental conditions. A common response to extracellular signals involves changes in the rate of gene transcription. The most common way to regulate gene expression in response to extracellular signals is by modulating the activity of transcription factors. Transcription factors are proteins which bind to specific regulatory sequences in a target gene to increase (or sometimes decrease) gene transcription and subsequently protein synthesis. They are activated by numerous extracellular stimuli acting through surface receptors and several types of kinases, or directly by ligands such as corticosteroids and vitamins (152, 153).

Today numerous transcription factors are known. Two of these, which are known to play an essential role in the skin, are activator protein 1 (AP-1) and nuclear factor κB (NF-κB).

Activator protein 1 (AP-1)

Even though AP-1 was one of the first transcription factors to be identified (154), its biological relevance and physiological functions are still being unraveled. AP-1 regulates gene transcription in response to a variety of extracellular stimuli and has been demonstrated to be involved in a wide range of cellular processes, including cell proliferation, survival, death and differentiation. The AP-1 DNA binding site was originally described to be responsive to 12-O-tetradecanoylphorbol-13-acetate (TPA), and therefore the AP-1 DNA binding site in various promoter regions is also known as the TPA response element (TRE: 5'-TGA C TCA-3')(154, 155). It is now apparent that AP-1 is a dimer composed of proteins belonging to the Jun (c-Jun, JunB, JunD), Fos (c-Fos, FosB, Fra1 and Fra2), Maf (c-Maf, MafB, MafA, MafG/F/K, Nr1) and ATF (ATF2, Lrf1/ATF3, B-ATF, JDP1, JDP2) subfamilies (156, 157). However, this thesis will focus primarily on Jun and Fos.

Besides being able to form stable heterodimers with Fos proteins, Jun family proteins can also form homodimers. Unlike Jun, Fos proteins do not form homodimers, and because dimer formation is a prerequisite for DNA binding, Fos proteins do not bind to DNA by themselves (158). Jun and Fos proteins are regulated in response to extracellular stimuli such as growth factors, hormones, cytokines, bacterial and viral infections, and a variety of physical and chemical stresses (159). Serum and growth factors induce AP-1 through activation of ERK1/2 and JNKs. Once activated ERK1/2 and JNKs translocate to the nucleus to phosphorylate the ternary complex factor (TCF/Elk-1) which leads to an induction of c-Fos (87, 160, 161). Moreover, ERK1/2 also directly phosphorylates Fra1 and Fra2 in response to serum stimulation (162) (Fig. 2).

AP-1 in the skin

AP-1 has been suggested to be involved in important functions in the epidermis such as differentiation, wound repair and carcinogenesis (163, 164). The expression pattern of Jun and Fos proteins has been examined in both mouse and human epidermis (165, 166). In human epidermis c-Jun was found only in the granular layer whereas c-Fos was found both in the spinous and granular layer but not in the basal layer. In contrast, JunB and JunD were ubiquitously expressed in all epidermal layers. This distinct expression pattern of the AP-1 subunits in the epidermis suggests that AP-1 plays an essential role in skin physiology although this could not be confirmed in a knockout mouse model. Mice lacking c-Fos, FosB or JunD did not show any specific skin phenotype (167).

Several studies strongly suggest that AP-1 plays a pivotal role in the regulation of epidermal differentiation (168, 169). In cultured normal human keratinocytes we have demonstrated that the active form of vitamin D3, 1α,25(OH)2D3, increases the DNA binding activity of AP-1 resulting in an increased differentiation of the keratinocytes (170). In addition, we found that the 1α,25(OH)2D3-induced increase in the AP-1 DNA binding activity was mediated through a PI3-k/Ras/MEK/ERK1/2 and JNK1 dependent mechanism resulting in an increased expression of c-Fos, Fra1 and c-Jun (I). In a different study we demonstrated that the AP-1 DNA binding activity is impaired in lesional psoriatic skin compared with nonlesional psoriatic skin (II). Moreover, we found that the protein and mRNA expression of c-Fos, Fra1 and c-Jun was reduced in lesional compared with nonlesional psoriatic skin, whereas the protein and mRNA expression of JunB was elevated, demonstrating that a specific regulation of the various AP-1 proteins is taking place in lesional psoriatic skin. Furthermore, these data indicate a role of AP-1 in the pathogenesis of psoriasis which is further supported by the finding that topical treatment of psoriatic skin with calcipotriol (a vitamin D analogue) leads to a normalization of the AP-1 DNA binding activity and its subunits c-Fos, Fra1, c-Jun and JunB preceding the morphological normalization of the skin (II).

In contrast to our data and others demonstrating elevated levels of JunB in psoriatic skin (171), a study by Zenz et al. recently demonstrated reduced levels of JunB in lesional psoriatic skin (172). To further characterize Jun proteins in the skin, epidermis-specific single- and double-knockout mice for JunB and c-Jun were generated. Epidermis-specific knockout of JunB or c-Jun alone revealed no specific skin phenotype, whereas epidermis-specific JunB/c-Jun double knockout mice developed skin alterations resembling some of the observed hallmarks of psoriasis such as thickened epidermis with rete ridges, hyperkeratosis, parakeratosis and deregulated cytokine expression (172), suggesting that epidermis-specific altera-

tions are sufficient to initiate a psoriasis-like skin phenotype. Interestingly, these epidermis-specific knockout mice also developed arthritic lesions strongly reminiscent of psoriatic arthritis. Although these data indicate the involvement of AP-1 in the pathogenesis of psoriasis, the exact role of the particular composition of AP-1 dimers still remains to be determined.

AP-1 has also been described to play a role in skin carcinogenesis. This was initially demonstrated by the fact that constitutively elevated levels of AP-1 were present in malignant but not benign mouse tumors (173). Further studies using transgenic mice expressing dominant negative c-Jun have demonstrated a role for AP-1 in both UVB- and TPA-induced skin carcinogenesis (174-176). These data suggest that the specific signaling pathways which are inhibited by the expression of dominant negative c-Jun, are involved in both UVB- and chemical-induced skin carcinogenesis in mice.

Nuclear factor κB (NF-κB)

NF-κB is an eukaryotic transcription factor which is present in virtually all tissues and cell types investigated, including the skin. It is an inducible and ubiquitously expressed transcription factor regulating the transcription of specific genes involved in apoptosis, cell adhesion, inflammation, differentiation and proliferation (177-180). In mammals, the NF-κB/Rel family consists of five members: RelA (p65), RelB, c-Rel, NF-κB1 (p105/p50) and NF-κB2 (p100/p52). NF-κB1 and NF-κB2 are synthesized as large precursors, p105 and p100, which are posttranscriptionally processed to the DNA-binding subunits p50 and p52, respectively (180, 181). The NF-κB/Rel proteins carry a Rel-homology domain (RHD), which contains a nuclear localization sequence (NLS) and is involved in dimerization, sequence-specific DNA-binding and interaction with the inhibitory IκB proteins (182). The NF-κB/Rel proteins associate to form numerous

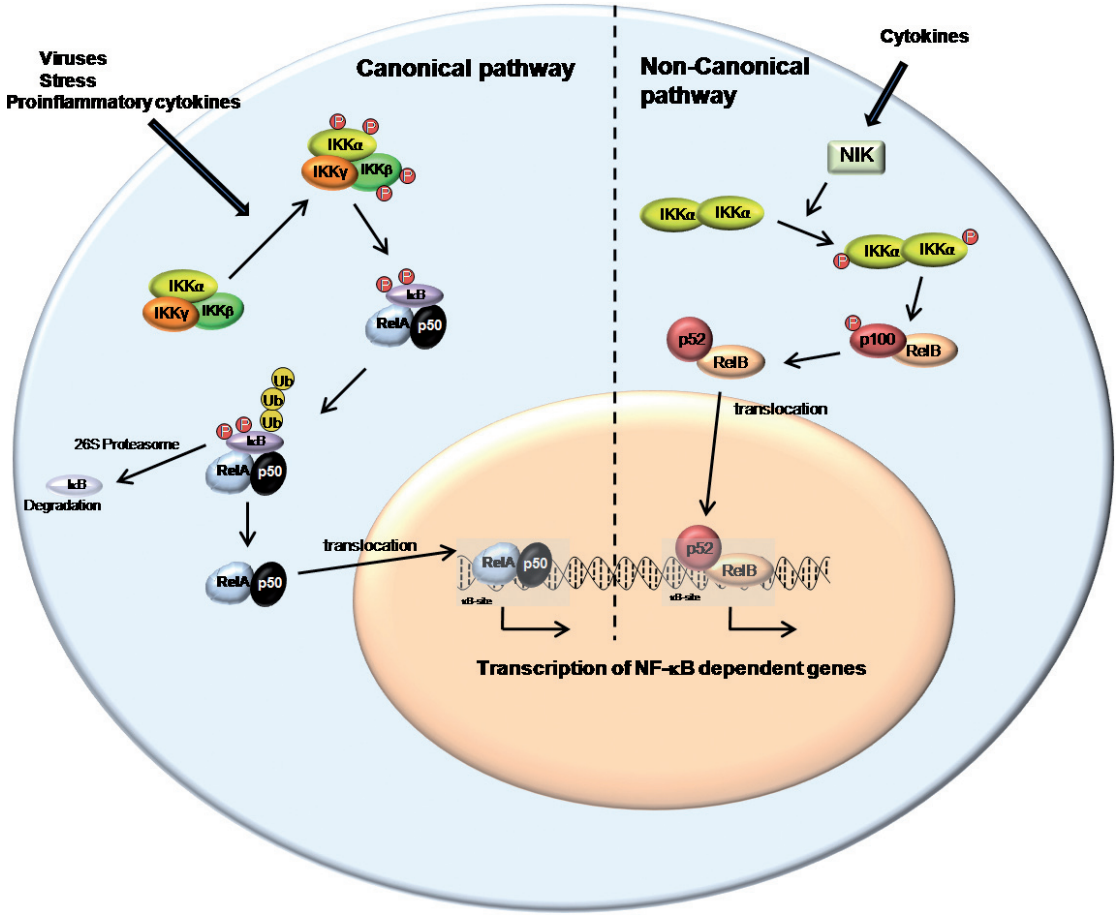

Fig. 6. NF-κB activation signaling pathway. The canonical pathway and the non-canonical pathway are included. The canonical pathway is triggered by viral infection and proinflammatory cytokines. This leads to an activation of IKK, which again leads to a phosphorylation of IκB. Upon phosphorylation, IκB is then ubiquitinylated and degraded. NF-κB now translocate to the nucleus where it regulate specific target genes. The non-canonical pathway is triggered by specific cytokines which cause activation of the p52/RelB complex by inducing processing of the p100 precursor protein.

homo- and heterodimers, the most studied being the p65/p50 heterodimer.

In resting cells NF-κB is generally retained in the cytoplasm as an inactive complex bound to the IκBs (180, 183, 184). The IκB family contains seven known members, IκBα, IκBβ, IκBγ, IκBε and Bcl-3 and the precursor Rel proteins p100 and p105 (180, 182). Stimulation of cells to a variety of agonists, such as IL-1β and TNF-α, results in phosphorylation/activation of a specific IκB kinase (IKK), which phosphorylates the IκBs and thereby tags them for polyubiquitination and subsequent degradation by the 26S proteasome (185) (Fig. 6). Degradation of IκB exposes the NLS of the NF-κB allowing it to translocate to the nucleus where it binds selectively to the consensus sequence G/(T)GGRNNYYC/(T)C, designated a κB site (N = any base), thereby regulating the transcription of more than 400 genes involved in inflammation, growth regulation, carcinogenesis and apoptosis (186). Interestingly, the gene encoding IκBα also contains a functional κB binding site in its promoter region and is transcriptionally activated by NF-κB (187). Thus the transcription of IκBα is induced in response to NF-κB activation, thereby constituting a negative feedback loop, which ensures that the activation of NF-κB is transient.

The IKK complex consists of the IKKα and IKKβ catalytic subunits (also referred to as IKK1 and IKK2, respectively) and the IKKγ regulatory subunit (also called NEMO (NF-κB essential modulator)) (188, 189). Even though IKKα and IKKβ are highly homologous proteins they are functionally different. Studies have demonstrated that activation of the IKK complex, resulting in IκB degradation upon inflammatory stimuli, is entirely dependent on IKKβ phosphorylation (190). Another study demonstrated that IKKα controls keratinocyte differentiation in a protein kinase activity- and NF-κB independent manner, which seems to involve a still unidentified protein (191).

Two NF-κB signaling pathways have been described. In the canonical signaling pathway, the activated IKK complex, predominantly acting through IKKβ in an IKKγ-dependent manner, catalyzes the phosphorylation of IκBs, polyubiquitination and subsequent degradation by the 26S proteasome (Fig. 6). The released NF-κB dimers (commonly RelA/p50) translocate to the nucleus, bind DNA at specific κB-sites and activate gene transcription (180, 192). Apart from this canonical pathway of NF-κB activation, there has been described a non-canonical pathway which specifically activates the p52/RelB heterodimers (Fig. 6). It involves the activation of IKKα homodimers by NF-κB inducing kinase (NIK). The activated IKKα homodimers then phosphorylate the p100 protein, which undergoes proteolytic processing to generate the p52 subunit of NF-κB. The p52/RelB heterodimer then translocates to the nucleus to regulate the transcription of specific genes (193, 194).

Table I. *Different mouse models with NF-κB alterations, displaying specific skin phenotypes.*

Ref	Mouse mutant	Skin phenotype
270	$ReIB^{-/-}$	Skin inflammation
195	$I\kappa B\alpha^{-/-}$	Severe dermatitis
271	$IKK\alpha^{-/-}$	Keratinocyte hyperproliferation and dysregulated epidermal differentiation
204	K14-Cre/$IKK\beta^{FL/FL}$	Severe inflammatory skin disease
203	$IKKy^{+/-}$	Skin inflammation in female mice similar to incontinentia pigmenti
196	$I\kappa B\alpha^{k5\Delta/K5\Delta}$	Thickned epidermis with acanthosis and hyperkeratosis
213	K5-IκBαDN	Hyperkeratosis, increased skin inflammation that progresses to squamous cell carcinoma
272	*$ReIA^{-/-}$	Epidermal hyperproliferation
273	K14-$p50^{-/-}$	Decreased epidermal thickness

*Epidermis specific ($ReIA^{-/-}$ embryo skin was grafted to immune-deficient mice)

NF-κB in the skin

NF-κB in the skin is crucial for morphogenesis and homeostasis. Dysregulations in its activity are linked to developmental skin defects, inflammatory skin diseases and skin cancer. Much of the experimental data in this area is derived from mouse models with genetic alterations in the NF-κB pathway (Table I). For example the constitutive activation of NF-κB in $I\kappa B\alpha^{-/-}$ mice leads to severe dermatitis (195, 196). The skin pathology of these mice includes epidermal hyperplasia, hyperkeratosis, acanthosis, loss of the granular layer and T cell infiltration. These pathological features are all hallmarks of psoriasis. Indeed the activity of NF-κB has been demonstrated to be dysregulated in psoriatic skin. Results from our group have demonstrated that there is an increased NF-κB DNA binding activity to the κB site in the IL-8 promoter region and a decreased NF-κB DNA binding activity to the κB site in the p53 promoter region in lesional psoriatic skin compared with nonlesional psoriatic skin, which is also reflected by an increase in IL-8 expression and decrease in p53 expression (III), (197, 198). These results demonstrate that NF-κB regulation is very complex and that there is a high degree of specificity of the genes transactivated by NF-κB in psoriasis. In addition to our study, other groups have also demonstrated disturbances in NF-κB in psoriatic arthritis (199) as well as psoriatic keratinocytes (200). A previous study demonstrated that dimethylfumarate (DMF) selectively prevents the nuclear entry of activated NF-κB (201). Later on a study from our group demonstrated that DMF specifically inhibits the activation of MSK1 and MSK2 and subsequently inhibits NF-κB-induced gene-transcription, and it was suggested that this may be the mechanism of action by which DMF mediates at least parts of its anti-psoriatic effects (136).

Although constitutive activation of NF-κB leads to inflammatory diseases of the skin, a block of NF-κB signaling also results in skin inflammation (Table 1). The inflammatory disease incontinentia pigmenti (IP) was the first familial syndrome linked to the NF-κB pathway, because patients with IP displayed nonfunctional mutations in the IKKγ gene (202). Female mice heterozygous for IKKγ develop a unique dermatopathy characterized by keratinocyte hyperproliferation, skin inflammation, hyperkeratosis and increased apoptosis, symptoms very similar to those of IP (203). Also the epidermis specific inhibition of NF-κB signaling in K14-Cre/IKKβ FL/FL leads to skin inflammation. K14-Cre/IKKβ FL/FL mice develop a severe inflammatory skin disease, which is caused by a TNF-mediated inflammatory response which develops in the skin shortly after birth (204). These results indicate that the significant function of IKKβ-mediated NF-κB activity in epidermal keratinocytes is to regulate mechanisms which maintain the immune homeostasis of the skin. Taken together, a constitutive activation as well as an inhibition of NF-κB activation results in inflammatory skin diseases, suggesting that skin homeostasis requires balanced NF-κB activity.

The use of specific NF-κB decoy oligonucleotides for blocking NF-κB activity has been developed and is considered to be a potential new class of antigene therapy (205). In mice, treatment either topically or systemically with NF-κB decoy oligonucleotides followed by exposure to UVB light has been shown to significantly reduce UV-induced cutaneous swelling, epidermal hyperplasia and secretion of proinflammatory cytokines such as IL-1β, TNF-α and IL-6 (206). In addition, prevention and regression of atopic dermatitis by ointment containing NF-κB decoy oligonucleotides have been demonstrated in chronic atopic dermatitis mice models (207, 208). Topical treatment of inflamed mice skin with NF-κB decoy oligonucleotides leads to decreased epidermal and dermal cell proliferation as well as downregulation of proinflammatory cytokines such as IL-1β, IFN-γ, IL-4, IL-13 and TNF-α (207, 208).

NF-κB has also been described to be involved in skin carcinogenesis (209, 210). Recent studies demonstrated that a reduction in IKKα expression promoted the development of skin papillomas and carcinomas in the DMBA/TPA chemical carcinogenesis model, and that mice overexpressing IKKα in the epidermis developed significantly fewer squamous cell carcinomas (SCC) compared with wild-type mice (211, 212). In addition, K5-IκBαDN transgenic mice have been reported to spontaneously develop squamous cell carcinomas. In this model, the inhibition of NF-κB resulted in massive inflammation, which was associated with hyperproliferation, prior to the development of SCCs (213). Taken together, these data suggest an involvement of the NF-κB signaling pathway in the development of skin carcinogenesis.

CANCER AND INFLAMMATION

Cancer is a hyperproliferative disorder originating from damaged DNA sequences which reroute crucial pathways regulating tissue homeostasis, cell survival and cell death (214). The carcinogenesis process which leads from a normal cell to cancer can be divided into three distinct phases: initiation, promotion and progression. The tumor initiation stage is a process in which chemical or physical carcinogens damage DNA of the cell, leading to the activation of oncogenes and the regulation of tumor-suppressor genes such as p53. During tumor promotion, the malignant cells are stimulated to grow, owing to increased cell proliferation and/or reduced cell death whereas tumor progression is the process in which the growing tumor becomes more aggressive (215-217). Over the years clinical and epidemiologic observations have suggested that the development of cancer is strongly associated with chronic inflammation (215, 218, 219). Now, it has been realized that the development of cancers from inflammation might be a process driven by inflammatory cells as well as a variety of mediators, including inflammatory cytokines and chemokines, which altogether establish an inflammatory microenvironment (215, 220). A proinflammatory cytokine known to be a key mediator in inflammation is TNF-α. Despite its name, TNF-α (tumor necrosis factor) is very important in the early stages of tumor promotion, regulating a number of cytokines, chemokines, adhesion proteins, matrix metalloproteinases (MMP's) and pro-angiogenic activities (221, 222). Previous studies have demonstrated that mice deficient in TNF-α are partially resistant to chemical induced skin carcinogenesis (VII) (223). In addition, the use of anti-TNF-α antibodies has been demonstrated to inhibit the development of skin tumors in a two stage chemical carcinogenesis mice model, suggesting that the early induction of TNF-α is critical for tumor promotion (224). Another proinflammatory cytokine which has been shown to be associated with cancer development is IL-6. In colon cancer patients, IL-6 serum levels are strongly elevated and positively correlated with disease status (225). Moreover, an in vitro study showed that IL-6 enhanced colony formation of human colon carcinoma cells in a dose-dependent manner, indicating its potential role in promoting cancer growth (226). Also in breast cancer IL-6 plays a key role because the presence of specific polymorphisms in the IL-6 promoter region and consequently high levels of IL-6 contributes to a worse breast cancer prognosis (227).

MK2 is a kinase known to play a key role in the regulation of both TNF-α and IL-6 (V), (111). Therefore, based on the above mentioned data, it is possible that MK2 plays an essential role in carcinogenesis. Indeed, we have recently demonstrated that MK2-deficient mice developed significantly fewer skin tumors compared with both TNF-α-deficient mice and wild-type mice using the two-stage chemical carcinogenesis model (VII) (Fig. 5). The TPA-induced inflammatory response was found to be reduced in both TNF-α-deficient mice and MK2-deficient mice, but most pronounced in TNF-α-deficient mice. In addition, we found that the p53 stabilization was increased in MK2-deficient mice resulting in increased numbers of DNA-damaged cells undergoing apoptosis in the epidermis. These data suggest that MK2 acts as a double-edged sword regulating the inflammatory response through the regulation of proinflammatory cytokines such as TNF-α and IL-6 as well as regulating apoptosis through the p53 signaling pathway (VII).

Several transcription factors have been demonstrated to play multiple roles in inflammation-linked tumor development. Recently, numerous studies have made great progress in delineating the role of NF-κB in linking inflammation and cancer (210, 228-230). For example different mouse models of carcinogenesis in which the classical NF-κB activation pathway has been blocked by genetic means have demonstrated a key role of NF-κB as a promoter of inflammation-linked cancers (231-233). Another transcription factor recognized as an important link between inflammation and cancer is STAT3 (234). Because STAT3 has been demonstrated to be constitutively activated in many types of cancer and the fact that IL-6 is a well-established inducer of STAT3 activation, it has been speculated that STAT3 plays a role in linking the inflammatory microenvironment and cancer development. In two recent studies, it was demonstrated that STAT3 is necessary for the growth of colitis-associated colorectal cancer in mice (235, 236), and a model was suggested whereby STAT3-dependent carcinogenesis is mediated by IL-6 signals from the tumor microenvironment. In support of this, STAT3 activation in human tumors is often observed at the invasive front of tumors adjacent to inflammatory cells (234). Finally, a previous study demonstrated that STAT3-deficient mice are completely resistant to chemical-induced skin carcinogenesis (237). Although these data demonstrate that the role of STAT3 in cancer development is significant, the exact function of STAT3 in inflammation-linked cancer development is still not fully elucidated.

DISCUSSION AND CONCLUSION

Intracellular signal transduction pathways as well as transcription factors are key regulators of cellular functions such as inflammatory processes, proliferation, differentiation, migration, apoptosis and cellular stress, and participate in a number of disease states including acute and chronic inflammation. A thorough understanding of these signaling pathways and transcription factors is, therefore, crucial in our search for new pharmaceuticals.

In this thesis, we first studied the DNA binding activity of AP-1 in cultured normal human keratinocytes and in psoriatic skin as well as the effect of 1α,25(OH)2D3 and calcipotriol on the AP-1 DNA binding activity (I, II). We found that the DNA binding and transactivating activity of AP-1 was increased by 1α,25(OH)2D3 in cultured human keratinocytes. We furthermore proposed a model of the signal transduction pathway induced by 1α,25(OH)2D3. In this model, 1α,25(OH)2D3 binds to the membrane receptor annexin II, which leads to an activation of the PI3-k/Ras/MEK/ERK1/2 and JNK1signaling pathway resulting in an increased expression of the AP-1 subunits c-Fos, Fra1 and c-Jun and subsequently an increased differentiation of the keratinocytes (I). Inconsistent data exist whether 1α,25(OH)2D3 mediates its rapid effects through a vitamin D membrane receptor or through the classical nuclear vitamin D receptor (VDR). Although, numerous studies have suggested a putative membrane receptor to be involved in the rapid actions of 1α,25(OH)2D3 (I), (238-242), a previous study demonstrated that 1α,25(OH)2D3-mediated rapid responses could not be observed in nuclear VDR knockout animals (243), indicating that the nuclear VDR is also involved in the rapid actions mediated by 1α,25(OH)2D3. This is further supported by a recent study demonstrating that rapid 1α,25(OH)2D3-induced activation of ERK1/2 and JNK in human osteosarcoma SaOS-2 cells is mediated by a VDR-dependent mechanism (244). Thus these data indicate that there might be a cross-talk between the rapid responses and the nuclear actions of 1α,25(OH)2D3.

The activity of AP-1 was also analyzed in psoriatic skin. We demonstrated that the AP-1 DNA binding activity was diminished in lesional psoriatic skin compared with nonlesional psoriatic skin (II). Moreover, the mRNA and protein expression of the c-Fos, Fra1 and c-Jun subunits was decreased in lesional psoriatic skin, whereas JunB was increased, demonstrating that a specific regulation of the various AP-1 subunits is taking place in lesional psoriatic skin. Although increased expression of JunB in psoriasis has been demonstrated by our group and others (II), (171, 245), a recent study reported that the expression of JunB was decreased in lesional psoriatic skin (172). Moreover, the authors demonstrated that epidermis-specific deletion of JunB and c-Jun in mice lead to skin alterations resembling lesions observed in patients with psoriasis (172). Even though these data indicate the involvement of JunB in the pathogenesis of psoriasis, additional experiments are necessary to establish the specific role of JunB in psoriasis and whether modulation of JunB expression is associated with the pathogenesis of psoriasis. Calcipotriol is a vitamin D analogue which has successfully been used in the treatment of psoriasis (22). We demonstrated that topical treatment of psoriatic skin with calcipotriol for four days increased the AP-1 DNA binding activity, and lead to a normalization of c-Fos, Fra1, c-Jun and JunB expression (II). Thus, it is possible that calcipotriol mediates some of its anti-psoriatic effects by restoring the imbalance in the AP-1 subunits and subsequently the AP-1 DNA binding activity, possibly by the same signaling pathway as demonstrated in vitro (I).

Another transcription factor known to be involved in cell adhesion, cell survival, differentiation, proliferation and inflammation is NF-κB (179). In this thesis we analyzed the NF-κB DNA binding activity in psoriatic skin using two different κB promoter sites. We demonstrated that the NF-κB DNA binding activity to the IL-8 κB promoter site was increased in lesional psoriatic skin compared with nonlesional psoriatic skin, whereas the NF-κB DNA binding activity to the p53 κB promoter site was decreased (III). In support of this we found that the IL-8 protein level was increased, whereas the p53 protein level was decreased in lesional compared with nonlesional psoriatic skin (III). These data suggest that NF-κB is able to differentiate between different genes and thereby activate or repress the expression of specific genes. Previous results have demonstrated that during the onset of inflammation, NF-κB activation in leukocytes was associated with proinflammatory gene expression, whereas during the resolution of inflammation NF-κB activation was associated with the expression of anti-inflammatory genes (246). Furthermore, during the onset of inflammation the dominating NF-κB complex was demonstrated to consist of c-Rel/p50, whereas there was a shift towards a predominance of p50/p50 at the resolution of inflammation (246). These results suggest that NF-κB regulate cellular functions in a highly subunit-specific manner. Moreover, it is possible that NF-κB differentiate between the κB promoter sites located on the different genes, by the formation of specific NF-κB complexes. Besides their prodifferentiating and anti-proliferative effects on the keratinocytes, vitamin D analogues are also known to modulate inflammation by the inhibition of IL-8 and stimulation of IL-10 expression (247-249). We demonstrated that topical treatment of psoriatic skin with calcipotriol for four days, decreased the NF-κB DNA binding to the IL-8 promoter κB site and increased the NF-κB DNA binding to the p53 promoter κB site (III), suggesting that there is an imbalance in the specific NF-κB complexes formed in psoriasis. In accordance with our data, previous studies using κB sites comprising

either the IL-6 or the IL-8 promoter κB site, respectively, have demonstrated that vitamin D inhibits NF-κB activity in both activated human lymphocytes (250) and activated human fibroblasts (251). Thus, it is possible that vitamin D mediates some immunomodulatory effects by restoring the delicate balance between the various NF-κB complexes.

The MAPK signal transduction pathways are among the best characterized intracellular signaling cascades and control a wide variety of cellular events, including cell differentiation, cell proliferation, apoptosis and processes involved in immune responses (252, 253). In this thesis, we analyzed the MAP kinases p38 MAPK, ERK1/2 and JNK1/2, as well as the p38 MAPK downstream target MK2 in psoriatic skin. We found that the kinase activity of the three p38 MAPK isoforms, p38α, -β and -δ were increased in lesional psoriatic skin compared with nonlesional psoriatic skin (IV). Moreover, we demonstrated an increased phosphorylation status of ERK1/2 in lesional compared with nonlesional psoriatic skin, whereas the phosphorylation status of JNK1/2 was unchanged between lesional and nonlesional psoriatic skin (IV). These results strongly suggest that p38 MAPK and ERK1/2 play a role in the pathogenesis of psoriasis. TNF-α is a proinflammatory cytokine known to be regulated at a posttranscriptional level through the p38 MAPK/MK2 signaling pathway (104, 254). In addition, the essential role of TNF-α in the pathogenesis of psoriasis is indisputable, which is also demonstrated by the successful treatment of the disease with TNF-α antagonists (255, 256). We demonstrated increased protein level of TNF-α in lesional compared with nonlesional psoriatic skin. Interestingly, we showed that the increased protein level of TNF-α was not paralleled by an increased mRNA expression of TNF-α, demonstrating that TNF-α is regulated at a post-transcriptional level in psoriatic skin (V). Because increased kinase activity of MK2 was demonstrated in lesional psoriatic skin (V) and the fact that in vitro studies conducted on cultured normal human keratinocytes showed that TNF-α is regulated through a p38 MAPK/MK2 dependent mechanism (V), it is possible that the increased activation of MK2 is responsible for the elevated and posttranscriptionally regulated TNF-α protein expression in psoriatic skin, making MK2 a potential future target in the treatment of psoriasis.

The p38 MAPK signaling pathway has been demonstrated to play a key role in numerous inflammatory processes. Studies have demonstrated how the p38 MAPK signaling pathway is involved in activation of caspase-1 and IL-18 (VI), (257-260). Caspase-1 belongs to the group of inflammatory caspases, and the predominant role of caspase-1 is considered to be the processing of pro-IL-1β and pro-IL-18, two cytokines believed to play an important role in the pathogenesis of inflammatory skin diseases, including psoriasis (261). The role of caspase-1 in the pathogenesis of psoriasis is still not fully elucidated. Recently, we demonstrated that the activity of caspase-1 was increased in lesional psoriatic skin compared with nonlesional psoriatic skin (VI). Moreover, using cultured normal human keratinocytes we showed that the secretion of pro-caspase-1 and active caspase-1 as well as activation of pro-IL-18 was mediated by a p38 MAPK/caspase-1 dependent mechanism, whereas secretion and new synthesis of pro-IL-18 was mediated by a p38 MAPK-dependent but caspase-1 independent mechanism (VI). The increased p38 MAPK and caspase-1 activity in lesional psoriatic skin, as well as the rapid secretion of both pro-caspase-1 and pro-IL-18 after p38 MAPK activation in cultured normal human keratinocytes, indicate that the extracellular environment in the epidermis may be metabolically active in inflammatory skin diseases such as psoriasis. The possible role of caspase-1 in the pathogenesis of psoriasis is further supported by results obtained by Yamanaka et al. They demonstrated that keratinocyte-specific caspase-1-transgenic mice spontaneously suffered from chronic dermatitis and skin ulcers (262). Analysis of biopsies taken in and around the skin ulcers revealed psoriasis-like changes, including parakeratosis. Moreover, the dermis of the ulcers was characterized by the infiltration of many mononuclear cells, and both the skin and sera of these mice showed elevated levels of mature IL-1β and IL-18 (262). In addition, a recent study in mice demonstrated that the expression of the caspase-cleaved form of Lyn (LynΔN) resulted in a chronic inflammatory condition resembling psoriasis (263).

The association between inflammation and cancer is well recognized, and studies have suggested that inflammation is involved in tumor initiation, promotion and progression (215, 216, 264, 265). Previously, TNF-α has been demonstrated to play an important role in tumor initiation. TNF-$\alpha^{-/-}$ mice subjected to the two stage chemical carcinogenesis model developed significantly fewer skin tumors compared with wild-type mice (223). In contrast, TNF-α had little influence on later stages of skin carcinogenesis, as tumors in wild-type and TNF-$\alpha^{-/-}$ mice had similar rates of malignant progression. In this thesis we used the two stage chemical carcinogenesis model to examine the role of MK2 in initiation of skin carcinogenesis. We demonstrated that MK2-deficient mice developed significantly fewer skin tumors compared with both wild-type and TNF-$\alpha^{-/-}$ mice (VII) (Fig. 5). The TPA-induced inflammatory response was found to be reduced in both, TNF-$\alpha^{-/-}$ mice and MK2$^{-/-}$ mice, but most pronounced in TNF-$\alpha^{-/-}$ mice, indicating that a reduced inflammatory response was not the only explanation for the inhibited tumorigenesis seen in the MK2$^{-/-}$ mice. Therefore, we next examined the role of MK2 in apoptosis and demonstrated increased numbers of apoptotic cells in the epidermis of MK2-deficient mice compared with wild-type and TNF-$\alpha^{-/-}$ mice. This increase in apoptosis was shown to be due to a MK2/Mdm2 regulated stabilization of p53, which was supported by in vitro experiments demonstrating that knockdown of p53 by siRNA significantly reduced the DMBA/TPA induced apoptosis in cultured MK2-deficient keratinocytes (VII). Together, these results demonstrate a dual role of MK2 in tumor initiation through a regulation of the inflammatory response and a stabilization of p53 leading to increased apoptosis of DNA-damaged cells. In a recent study, it was demonstrated that the p38δ isoform plays an essential

role in skin tumor development in mice. Mice lacking p38δ exhibited a marked resistance to DMBA/TPA induced skin tumors, with greatly reduced incidence, multiplicity, and size of tumors compared with wild-type mice (266). These data together with our results, demonstrate that the p38 MAPK signaling pathway not only plays a crucial role in inflammatory processes but also a significant role in skin tumor development, making the p38 MAPK signaling pathway an obvious therapeutic target for skin cancer.

MK2 not only plays an essential role in the early stages of tumor initiation. Recently, MK2 was demonstrated to mediate cell death, as shown in an experimental model resembling the late stage of tumor promotion (124). In this study, tumor cells lacking functional p53 were demonstrated to survive chemotherapeutic drugs through a p38 MAPK/MK2-dependent mechanism. In contrast signaling through this pathway was not necessary for survival after DNA-damaged in p53-functional cells. Together with our results, these data demonstrate that MK2 may act by different mechanisms to regulate the survival of cells in response to DNA-damage, depending on the distinct stage in the carcinogenesis process.

As demonstrated in this thesis signal transduction is a very complex process, where specific changes in the signaling pathways seem to play a key role in the pathogenesis of different skin diseases such as psoriasis and nonmelanoma skin cancer. Thus, targeting intracellular signaling pathways and transcription factors may be a novel and important preventive or therapeutic strategy not only against psoriasis and other inflammatory skin diseases, but also against skin cancers. Therefore, strategies focused on reducing the activity of for instance NF-κB, p38 MAPK or downstream targets of p38 MAPK such as MK2 by natural or synthetic inhibitors directed against these proteins, could become a novel and potent approach for the treatment of both inflammatory skin diseases and skin cancers. The most promising targets would be those which do not participate in complex feedback control loops and networks where inhibition of the transcription factor or kinase of interest would lead to the activation of other proinflammatory signaling pathways or simultaneously block beneficial anti-inflammatory pathways. Indeed a number of selective small-molecule kinase inhibitors have emerged over the last years as an important class of anti-cancer agents, and have demonstrated impressive clinical efficacy in several different diseases including breast and lung cancer (267, 268).The next decade will probably reveal a number of specific small-molecule inhibitors used in the treatment of inflammatory skin diseases as well as other inflammatory conditions.

FUTURE STUDIES

In this thesis we demonstrated that the MAPK signaling pathway played an essential role in the pathogenesis of the inflammatory skin disease psoriasis. Recently, new biological drugs targeting TNF-α and IL-12/IL-23(p40) have proved to be very efficient in the treatment of psoriasis. It would be interesting to examine the activities of the MAPKs and its downstream targets in psoriatic patients treated with these biological drugs. Especially, it would be interesting to examine the early time points after the start of treatment before any clinical and histological improvements are observed. Thereby, it might be assessed whether the MAPK signaling pathway is one the primary targets by which these drugs mediate their anti-psoriatic effects.

Until recently, psoriasis has been considered mainly to be a TH1 driven autoimmune inflammatory skin disease. However, recent findings have revealed an essential role for IL-23 and TH17 responses in the pathogenesis of psoriasis. In support of this, an increased number of TH17 cells have been identified in the dermis and epidermis of psoriatic skin compared with normal skin. Thus, studies analyzing the role of the MAPKs as well as AP-1 and NF-κB in the regulation of IL-23 and TH17 cell-derived cytokines such as IL-17A, IL-17F and IL-22 would be interesting, and could bring us a step closer in understanding the complicated role of TH17 cells in the pathogenesis of psoriasis.

Because the MAPK signaling pathway is activated through phosphorylation, dephosphorylation of the MAPKs mediated by phosphatases represents an important feedback control mechanism which regulates the MAPKs. A number of phosphatases are known, however, in mammalian cells, the dual-specificity protein phosphatases (DUSPs) are the primary phosphatases responsible for dephosphorylation/deactivation of the MAPKs (269). Therefore, these phosphatases are often referred to as MAP kinase phosphatases (MKPs). Because the MAPKs are involved in the pathogenesis of inflammatory diseases such as psoriasis, it would be of interest to analyze the expression level of these MKPs in skin biopsies taken from psoriatic patients. In addition, to further study the physiological function of MKP-1 in inflammatory skin conditions, it would be interesting to examine MKP-1 knockout mice subjected to experimental skin inflammation.

DANSK RESUMÉ

Celler responderer på udefra kommende stimuli ved at aktivere intracellulære signaltransduktions systemer, hvilket efterfølgende kan føre til en aktivering af specifikke transkriptionsfaktorer og derved en regulering af specifikke gener i cellekernen. Et af de bedst beskrevne signaltransduktions systemer er MAPK systemet. MAPK systemet repræsenterer en familie af protein kinaser, som gennem sekventiel fosforylering af specifikke kinaser regulerer en række forskellige cellulære processer herunder inflammation, celle-proliferation, differentiering og apoptose. Mindst fire forskellige, specifikt regulerede MAPK er beskrevet. Disse inkluderer ERK1/2, JNK1/2/3, ERK5 og p38 MAPK.

Psoriasis er en almindeligt forekommende, benign, hyperproliferativ, kronisk inflammatorisk sygdom karakteriseret ved velafgrænsede erytematøse plaques med flerlaget skældannelse. Behandlingen af psoriasis strækker sig fra topikal behandling af de milde former med vitamin D analoger som f.eks. calcipotriol til systemisk behandling af de svære former for psoriasis. Patogenesen ved psoriasis er endnu ikke fuldt ud afklaret, men flere undersøgelser tyder på, at ændringer i bestemte signaltransduktions systemer kan være en del af forklaringen på den inflammatoriske tilstand og ændrede vækstregulering, man finder ved psoriasis.

AP-1 er en transkriptionsfaktor der aktiveres gennem MAPK systemet. AP-1 er en dimer bestående af proteiner fra henholdsvis Jun (c-Jun, JunB, JunD) og Fos (c-Fos, FosB, Fra1, Fra2) familien.

Vi viser, hvorledes 1α,25(OH)2D3 (den aktive form af vitamin D) øger AP-1 DNA bindings aktiviteten gennem en PI3-k/Ras/MEK/ERK1/2 og JNK1 afhængig mekanisme, resulterende i en øget ekspression af c-Fos, Fra1 og c-Jun og en efterfølgende øget differentiering af humane keratinocytter dyrket i kultur. Disse data tyder på, at AP-1 spiller en væsentlig rolle ved keratinocyt-differentiering. I overensstemmelse med dette viser vi, at AP-1 DNA bindings aktiviteten er nedsat i involveret psoriasis hud sammenlignet med ikke involveret psoriasis hud. Ekspressionen af c-Fos, Fra1 og c-Jun er ligeledes nedsat, hvorimod ekspressionen af JunB er forhøjet i involveret sammenlignet med ikke involveret psoriasis hud. Interessant er det, at topikal behandling af involveret psoriasis hud med calcipotriol resulterer i en øget AP-1 DNA bindings aktivitet og en normalisering af c-Fos, Fra1, c-Jun og JunB ekspressionen. På baggrund af disse resultater er det således muligt, at calcipotriol medierer nogle af dets anti-psoriasis effekter ved at genoprette ubalancen i AP-1 DNA bindings aktiviteten gennem samme signalvej som påvist in vitro.

NF-κB er en transkriptionsfaktor der er vist, at spille en helt central rolle ved inflammatoriske processer. NF-κB er en dimer bestående af proteiner fra Rel familien herunder RelA (p65), RelB, c-Rel, p50 og p52. Vi beskriver, hvorledes NF-κB DNA bindings aktiviteten til IL-8 κB elementet er forhøjet i involveret sammenlignet med ikke involveret psoriasis hud, hvorimod NF-κB DNA bindings aktiviteten til p53 κB elementet er nedsat. Disse resultater er i overensstemmelse med tidligere studier, der har vist øget ekspression af IL-8, men nedsat ekspression af p53 i involveret sammenlignet med ikke involveret psoriasis hud. Ydermere viser vi, hvorledes topikal behandling af psoriasis med calcipotriol resulterer i en normalisering af NF-κB DNA bindings aktiviteten. Således synes NF-κB at udgøre et attraktivt terapeutisk mål ved inflammatoriske hudlidelser.

p38 MAPK spiller en væsentlig rolle for regulationen af cellers proliferation, differentiering, apoptose og respons ved inflammation. Ydermere er p38 MAPK vist, at regulere en række transkriptionsfaktorer herunder AP-1 og NF-κB. Vi beskriver, hvorledes aktiviteten af p38 MAPK er forhøjet i involveret psoriasis hud sammenlignet med ikke involveret psoriasis hud. MK2 er en kinase, der aktiveres af p38 MAPK via en direkte fosforylering, og som er vist at regulere ekspression af inflammatoriske cytokiner post-transkriptionelt gennem en stabilisering af mRNA for bl.a. TNF-α. MK2 var fokus for det næste studium, hvori vi beskriver, hvorledes aktiviteten af MK2 er øget i involveret sammenlignet med ikke involveret psoriasis hud. Interessant er det, at mRNA ekspressionen af TNF-α er uændret mellem involveret og ikke involveret psoriasis hud, hvorimod protein niveauet af TNF-α er forhøjet. Disse data sammenholdt med resultater fra samme studium, der viser hvorledes en specifik hæmning af MK2 nedregulerer TNF-α protein niveauet i humane keratinocytter in vitro, tyder på at MK2 regulerer ekspressionen af TNF-α post-transkriptionelt i psoriasis hud, hvilket gør MK2 til et attraktivt terapeutisk mål i behandlingen af psoriasis.

p38 MAPK er også involveret i regulationen af caspase-1. Caspase-1 hører til gruppen af inflammatoriske caspaser og er vist at spille en essentiel rolle i det innate immunrespons bl.a. ved en aktivering af de to inflammatoriske cytokiner IL-1β og IL-18. En bedre forståelse af de signalveje der ligger til grund for aktiveringen af caspase-1 ved stress-stimuli, vil derfor kunne bidrage til at identificere specifikke kinaser som angrebspunkter for fremtidig behandling af inflammatoriske sygdomme. Vi finder, at både proformerne og de aktive former af caspase-1 og IL-18 induceres post-transkriptionelt i humane keratinocytter in vitro, via en p38 MAPK afhængig mekanisme. Desuden beskriver vi, hvorledes niveauet af de aktive former af caspase-1 og IL-18 er forhøjet i involveret psoriasis hud sammenlignet med ikke involveret psoriasis hud.

Det er velkendt, at der er en sammenhæng mellem inflammatoriske faktorer og hudkræft. Således har tidligere studier vist, hvorledes TNF-α knockout mus ikke udvikler så mange DMBA/TPA-inducerede hudtumorer sammenlignet med vild-type musene. Ligeledes er det for nylig vist, hvorledes anti-TNF-α antistoffer kan hæmme udviklingen af DMAB/TPA-inducerede hudtumorer. Disse resultater understøtter, at inflammation og måske især TNF-α spiller en afgørende rolle for udviklingen af tumorer i huden. Vi undersøger MK2's rolle ved udviklingen af hudtumorer og beskriver, hvorledes MK2 knockout mus er resistente overfor DMBA/TPA-inducerede hudtumorer. Vi finder, at der er et hæmmet inflammatorisk respons i MK2 knockout musene, samt en øget stabilisering af p53 resulterende i et øget antal DNA-skadede celler i huden, der undergår apoptose. MK2 synes derfor at være et fremtidigt terapeutisk mål i behandlingen af hudkræft.

De beskrevne studier bidrager således til viden omkring de molekylære signaleringsmekanismer ved inflammatoriske hudsygdomme og hudkræft. Dette er af fundamental betydning for forståelsen af patogenesen for disse hudsygdomme, samt for muligheden for terapeutisk intervention.

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Contacts in the Societies for Private Practitioners

Denmark:

No information

Finland:

Jukka Juhela
Seponkatu 12
FI-29200 Harjavalta, Finland
E-mail: jukka.juhela@iki.fi

Marja Soini
Tiilimäki 5C
FI-00330 Helsinki, Finland
E-mail: marja.soini@pp1.inet.fi

Iceland:

No information

Norway:

No information

Sweden:

Dr Thor Bleeker
Hudmottagningen
Lidköping, Sweden
E-mail: thor bleeker@ptj.se

Contacts in the Societies for Young Dermatologists

Denmark:

Stine Lasthein Andersen
E-mail: formand@fyd.dk

Finland:

Sampsa Kauppi
University of Tampere
Tampere, Finland
E-mail: sakauppi@pp.tnnet.fi

Iceland:

No information

Norway:

Guttorm Guleng
Department of Dermatology
Rikshospitalet
NO-0027 Oslo, Norway
E-mail: gguleng@hotmail.com

Sweden:

Hanna Velin
Department of Dermatology
Gävle Hospital
SE-801 87 Gävle, Sweden
E-mail: hanna.velin@lg.se

Maria Brolin
Department of Dermatology
Södersjukhuset
SE-118 83 Stockholm, Sweden
E-mail: maria.brolin@sodersjukhuset.se

Contact the Editorial Office

Agneta Andersson, Managing Editor
Daniela Andersson, Editorial Assistant

Trädgårdsgatan 14
SE-753 09 Uppsala
Sweden

Phone: +46-18-580 50 91, +46-18-580 50 95
Fax: +46-18-580 50 90
E-mail: agneta@medicaljournals.se
daniela@medicaljournals.se

Web: http://forum.medicaljournals.se

Sender:
Forum for Nordic Dermato-Venereology
Trädgårdsgatan 14
SE-753 09 Uppsala
Sweden

B

SVERIGE
PORTO BETALT
PORT PAYÉ