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p38 δ mitogen-activated protein kinase regulates skin homeostasis and tumorigenesis

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Key words: p38 δ signaling, keratinocyte, multistage chemical skin carcinogenesis, knockout mice, epidermis

Abbreviations: MAPK, mitogen-activated protein kinase; JNK, c-Jun NH₂-terminal kinase; MK2, MAPK-activated protein kinase 2; DUSP, dual specificity phosphatase; LPS, lipopolysaccharide; TORC1, target of rapamycin complex 1; HNSCC, head and neck squamous cell carcinoma; CC, cholangiocarcinoma; PKC, protein kinase C; DMBA, 7,12-dimethylbenz(a)anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

The p38 δ mitogen-activated protein kinase (MAPK) is abundantly expressed in a wide array of tissues where it is likely to have specific functions. This review aims to highlight recent new insights into the biological roles of this relatively less studied p38 isoform. We focus on function of p38 δ in regulating of epidermal keratinocyte differentiation, apoptosis and skin tumor development.

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Introduction

The mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated protein kinases (ERKs), c-Jun NH₂-terminal kinases (JNKs) and p38, are evolutionary conserved families of serine/threonine kinases that control diverse cellular processes in response to a variety of extracellular stimuli (reviewed in refs. 1 and 2). MAPK activity is regulated by a three-module kinase cascade that include a MAPK, a MAPK kinase (MAP2K, MKK or MEK) and a MAPK kinase kinase (MAP3K, MAPKKK or MEKK).^{1,2} Activated MAPKs phosphorylate numerous downstream substrates, such as transcription factors, protein kinases, cytoskeletal proteins and other targets. The mammalian p38 MAPK family includes p38 α , p38 β , p38 γ and p38 δ isoforms, encoded by *MAPK14*, *MAPK11*, *MAPK12* and *MAPK13* genes, respectively (reviewed in refs. 3 and 4). The p38 family members are activated by environmental stresses, inflammatory cytokines and growth factors. p38 MAPKs regulate a plethora of cellular responses including inflammation, proliferation, differentiation, development, cell cycle control, apoptosis, migration, senescence and tumorigenesis.^{3,4} Dual specificity MAP2Ks MEK3 and MEK6 are the predominant immediate upstream activators of p38s that phosphorylate threonine-180 and tyrosine-182 residues within the activation loops of p38 isoforms. Additionally, MEK4- and MEK7-dependent, as

well as MEK-independent activation of p38 has been reported (reviewed in refs. 2–4). The MAP3Ks known to participate in p38 activation include MEKK1-4, ASK1, TAK1, TAOs, MLK3. Low molecular weight GTP-binding proteins of the Rho family, including Rac1 and Cdc42, and p21-activated kinases (PAKs) are among further upstream activators of p38 signaling.²⁻⁴ The physiological impact of the p38 MAPK activation is cell type- and stimulus-dependent, and the specific functions of the individual p38 isoforms under homeostatic and pathological conditions are not well defined. p38 isoforms are dissimilar in their pattern of expression, substrate specificities and sensitivity to pyridinyl imidazole inhibitors.^{3,4} p38 α and p38 β isoforms are ubiquitously expressed and are susceptible to inhibition by pyridinyl imidazoles, such as SB203580. p38 γ is highly expressed in skeletal muscle. p38 δ is abundant in many tissues, including skin, pancreas, testes, prostate gland, adrenal gland, small intestine, stomach, colon, kidney and lung.⁵⁻⁹ p38 γ and p38 δ isoforms are insensitive to the pyridinyl imidazole compounds. Protein kinases MK2 and MK3 exemplify substrates preferentially phosphorylated by p38 α and p38 β rather than p38 γ and p38 δ , while microtubule-associated protein Tau appears to be a better substrate for p38 γ and p38 δ than for p38 α and p38 β isoforms in transfected cells.^{3,4} These observations suggest highly specialized cellular functions for individual p38 isoforms. p38 α gene ablation in mice resulted in early embryonic lethality due to placental and vascular abnormalities.¹⁰⁻¹³ p38 β , p38 γ , p38 δ and double p38 γ /p38 δ knockout mice are viable and fertile, and exhibit no apparent health problems.^{14,15} Currently, p38 α , often referred to as p38, is by far the best characterized isoform among p38 family members. In contrast, considerably less attention has been focused on p38 δ . Here we intend to review the progress toward understanding the functions of the p38 δ signaling in physiological and pathophysiological settings, focusing, in particular, on its roles in epidermal keratinocytes and in epithelial carcinogenesis.

Properties of p38 δ

Regulation of p38 δ activity. p38 δ (also called SAPK4) was cloned and characterized as the fourth member of the p38 MAPK that is 61, 59 and 65% identical to p38 α , p38 β and p38 γ ,

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respectively.^{5-8,16} The human *p38δ/MAPK13* gene is located on chromosome 6 and encodes a protein of 365 amino acids.⁷ The expression of p38δ is regulated in different developmental stages in the mouse embryo, suggesting that p38δ is a developmentally regulated MAPK.⁷ Similar to the other p38 isoforms, p38δ is robustly activated by inflammatory cytokines (i.e., TNFα, IL-1β) and environmental stresses (such as UV radiation, hyperosmotic shock, oxidative stress, arsenite), and to a lesser degree by mitogenic stimuli. MAP2Ks reported to activate p38δ include MEK3, MEK6, MEK4 and MEK7.^{6,7,17} The activation loop (T-loop) of p38δ contributes to the specificity of activation by MEK3.¹⁸ MEK3 deficiency has been shown to abrogate the endogenous p38δ activation in response to TGFβ1 in murine glomerular mesangial cells, suggesting a critical role for MEK3 as an upstream activator of p38δ in this system.¹⁹ Of note, p38δ is activated in a glomerulonephritis disease model in rat, suggesting a possible involvement in the pathogenesis of this disease.⁶ The MLK3/MEK7/p38δ signaling module scaffolded by an adaptor protein islet brain-2 (IB2) has been described in cultured neuronal cells.²⁰ Future studies using MAP2K and MAP3K gene knockout and RNAi-mediated knockdown approaches will define the specific contributions of individual upstream kinases in regulation of p38δ activity in response to a given stimulus.

Intrinsically active mutants of all p38 isoforms have been recently developed and demonstrated to maintain substrate specificity and sensitivity to inhibitors of the parental native p38s.²¹ Active mutants of p38, including p38δ mutant generated by substituting phenylalanine-324 with serine (F324S), acquire strong autophosphorylation capabilities that may emulate a natural mechanism of p38 activation. Autophosphorylation activity results from conformational changes conferred by activating mutations that are located in the L16 loop region of each isoform. Notably, the L16 loop of p38δ is purported to undergo changes via regulatory mechanisms other than phosphorylation, for instance, through interaction with other proteins.²¹

A dual-specificity phosphatase MAPK phosphatase 1 (MKP-1, also known as DUSP1) has been implicated in downregulation of hypertonicity-induced activation of both p38δ and p38α in HEK293FT cell line.²² In contrast, MKP-1 fails to bind to and inactivate p38δ in NIH3T3 cell line, and, similar to MKP-5 (also known as DUSP10) and MKP-7 (also known as DUSP16), preferentially acts on p38α and p38β isoforms in this setting.²³ The involvement of protein serine/threonine phosphatases PP1 and PP2A in downregulation of endogenous p38δ activity is suggested by finding that PP1/PP2A inhibitor okadaic acid (OA) markedly increases p38δ activity in human epidermal keratinocytes.^{9,24} Interestingly, the endogenous p38δ is found in a monophosphorylated, tyrosine-182-phosphorylated, form in macrophages after LPS treatment.²⁵ Upon treatment with LPS and calyculin A, another inhibitor of PP1 and PP2A, p38δ activity is increased 25-fold versus LPS alone-treated cells, suggesting that threonine-180 may be efficiently dephosphorylated by the calyculin A-sensitive phosphatase(s) in this system.

p38δ substrates. Stathmin, a regulator of microtubule dynamics, has been identified as p38δ substrate both in vitro and in transfected cells, suggesting a potential role for p38δ in cytoskeletal

remodeling.²⁶ It is hypothesized that p38δ phosphorylates stathmin in times of stress, which would, in conjunction with phosphorylation by other kinases, enhance microtubule polymerization to increase cell survival under stress. A microtubule-associated protein Tau has been found to be a good substrate for p38δ and p38γ in transfected COS cells.^{27,28} Using RNAi approach, p38δ has been shown to be the major in vivo kinase that phosphorylates endogenous Tau at threonine-50 following osmotic shock of neuroblastoma cells.²⁹ Notably, phosphorylation of Tau at threonine-50 is suggested to be an early event after p38δ activation. This phosphorylation promotes microtubule assembly and may play a role in the adaptation of neurons to changes in osmolarity, while subsequent hyperphosphorylation of Tau at additional sites by p38δ and/or other kinases may negatively regulate the ability of Tau to interact with microtubules and destabilize the microtubule network.²⁹

p38δ phosphorylates and inactivates eukaryotic elongation factor 2 kinase (eEF2K), which causes dephosphorylation and activation of eEF2K substrate eEF2 in response to cellular stress induced by anisomycin, an agent that activates all stress-activated protein kinases, including p38δ.³⁰ Furthermore, the anisomycin-induced phosphorylation of eEF2K at serine-259 in HEK293 cells is prevented by overexpression of a catalytically inactive mutant of p38δ, and is unaffected by SB203580, U0126 or rapamycin, compounds that specifically inhibit p38α/p38β, MEK1 and TORC1, respectively. These data suggest that p38δ may mediate the inhibition of eEF2K and the resulting activation of eEF2, to stimulate the translation of mRNAs encoding proteins whose levels are increased by stress. Fibroblast growth factor homologous factors (FHF) are IB2-interacting proteins that purportedly function in facilitating the assembly of the MLK3/MEK7/p38δ complex scaffolded by IB2 in neurons.²⁰ FHF1 has been shown to serve as a p38δ substrate in transfected HEK293T cells treated with hydrogen peroxide.²⁰ A transcription factor c-myc is phosphorylated by p38δ and, to a lesser degree, by p38γ, in response to anisomycin-induced stress in transfected HEK293 cells, leading to c-myc degradation via a proteasome-dependent pathway.³¹ This regulation may have implications in hematopoietic cell development, inflammatory responses and cellular transformation. siRNA-mediated knockdown of p38δ enhances hypertonicity-induced transcriptional activation of the osmoprotective transcription factor TonEBP/OREBP, while overexpression of p38δ reduces it.²² Notably, p38α is shown to have an opposite effect on the activity of this transcription factor. The physiological implications of the differential effects of p38δ and p38α isoforms on TonEBP/OREBP are presently being investigated.²² PKD1 has been identified as a target of p38δ during regulation of insulin exocytosis.³² PKD1 has been found to associate with ectopically expressed activated mutant form of p38δ (p38δ^{F324S}) in HEK293T cells and in pancreatic β cell line. Phosphorylation of PKD1 by p38δ negatively regulates PKD1 activity. Moreover, PKD1 activity is enhanced in pancreatic β cells lacking p38δ, contributing to increased stimulated insulin secretion and enhanced glucose tolerance observed in p38δ-null mice.³² p38δ deficiency is shown to be protective against high fat diet-induced insulin resistance and oxidative stress-stimulated

pancreatic β cell failure. These findings suggest a pivotal role for p38 δ -PKD1 signaling module in regulation of pancreatic β cell function in diabetic conditions.³²

The Role of p38 δ in Regulation of Keratinocyte Function

The function of p38 δ in keratinocyte differentiation. Epidermis, the outermost layer of the skin, is a stratified squamous epithelium that possesses a remarkable regenerating capacity and provides a protective barrier against environmental assaults, dehydration and loss of nutrients. To accomplish this critical life-sustaining function, keratinocytes, the major cell type of the epidermis, undergo a tightly regulated terminal differentiation program, also known as cornification (reviewed in refs. 33–38). During this process, mitotically active keratinocytes of the innermost basal layer withdraw from the cell cycle, detach from the underlying basement membrane, and progressively differentiate to form spinous, granular and cornified epidermal layers, as they ascend toward the skin surface to ultimately develop into squames, dead flattened anucleated cornified cells.^{33,34} These terminally differentiated squamous cells are composed of keratin microfibrils surrounded by highly crosslinked insoluble proteinaceous structures referred to as cornified envelopes, which replace the plasma membranes of differentiating keratinocytes. Specialized lipids are extruded into the intercellular space between squames and become covalently attached to the proteins of the cornified envelope. Together, the cornified envelope and extracellular lipids are essential for effective physical and water barrier function in the skin.³⁵ Squamous cells are eventually sloughed off the skin surface and are replaced by differentiating cells from the layers below. In healthy epidermis, normal epidermal homeostasis is maintained by a precisely controlled balance of proliferation, differentiation and death.^{33,34,36,37} Malfunctions in control of this balance lead to a compromised epidermal barrier function and result in development of dermatological diseases, including skin cancer, psoriasis, disorders of keratinization and anomalous wound healing. The molecular mechanisms underlying epidermal growth and differentiation control remain incompletely understood. A number of signaling pathways implicated in regulation of keratinocyte differentiation and skin barrier function has been identified in recent years (reviewed in refs. 33, 34, 36 and 37), including MAPK/SAPK cascades.

We used involucrin gene as a model to study the signaling mechanisms that govern epidermal gene expression during keratinocyte differentiation.^{38–40} Involucrin is a component of the cornified envelope that is crosslinked to various other structural proteins during early stages in the envelope assembly, and provides a scaffold to which other envelope precursors are subsequently crosslinked.^{35,38,39} Involucrin is a marker of keratinocyte terminal differentiation that is expressed in the spinous and the granular, but not in the basal, epidermal layers. The investigation of involucrin gene regulation has revealed important mechanisms whereby intracellular signaling pathways and the downstream transcription factors regulate differentiation-dependent epidermal gene expression (reviewed in ref. 39), and, in particular, has

offered initial insights regarding the role of p38 δ in epidermis (reviewed in ref. 40).

A robust p38 δ expression is detected in developing mouse epidermis,⁷ as well as in neonatal and adult mouse and human epidermis,^{9,41} suggesting a physiological function for p38 δ in epidermal tissue. p38 δ is expressed in both proliferating and non-proliferating epidermal layers.^{9,41} Notably, p38 α and p38 δ are the predominant p38 MAPK isoforms expressed in normal keratinocytes, while p38 β is detected at lower levels, and p38 γ is virtually undetectable.^{42,43} We found that p38 δ is the major p38 isoform activated in cultured normal human epidermal keratinocytes by treatment with various differentiation-inducing agents, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA), calcium, OA and green tea polyphenol.^{9,44} Treatment of cultured keratinocytes with these and other differentiation-promoting agents is known to induce morphological and biochemical changes, which resemble those taking place during epidermal differentiation *in vivo*, such as growth arrest and expression of terminal differentiation markers, including involucrin expression. Cell culture and transgenic mouse studies show that the human involucrin (hINV) gene upstream regulatory region, containing functional activator protein 1 (AP1), CCAAT enhancer binding protein (C/EBP), and Sp1 transcription factor binding sites, is essential for differentiation-appropriate hINV gene expression both *in vitro* and *in vivo* (reviewed in ref. 39). We have shown that differentiating agent-mediated endogenous p38 δ activation correlates with increased levels of hINV promoter activity, and endogenous hINV mRNA and protein expression, as well as increased protein levels and enhanced binding of AP1 and C/EBP factors to the hINV promoter, in cultured human keratinocytes undergoing differentiation.^{9,44,45} These responses are maintained in the presence of SB203580, indicating the lack of involvement of the inhibitor-sensitive p38 α and/or p38 β isoforms. On the other hand, the differentiation-dependent increase in hINV promoter activity is inhibited by a dominant-negative mutant of p38 α , which is expected to block activation of all p38 isoforms as it binds to shared direct upstream p38 activators MEK3 and MEK6. Moreover, the endogenous hINV protein levels are increased in keratinocytes overexpressing p38 δ , and further increased in cells co-expressing both p38 δ and PKC η , a differentiation-promoting isoform that activates p38 δ signaling in keratinocytes.⁴⁵ Altogether, these data suggest a role for p38 δ isoform in activation of differentiation-dependent involucrin gene expression. However, the induction of involucrin expression by treatment with cholesterol-depleting agents involves p38 α ,⁴⁶ and constitutively active MEK6 or MEK7, when overexpressed in keratinocytes, increase involucrin via a mechanism that involves p38 α ,^{42,47} suggesting that the engagement of a specific p38 isoform may be stimulus-dependent. Interestingly, a genomic analysis has shown that *MAPK13* (a gene that encodes p38 δ protein) and involucrin are among genes commonly upregulated both in psoriasis, a benign inflammatory condition characterized by increased proliferation and differentiation, and in well-differentiated locally invasive cutaneous squamous cell carcinomas (SCC).⁴⁸ Moreover, many additional genes encoding proteins involved in epidermal differentiation are upregulated in both conditions, including

keratinocyte transglutaminase, desmoglein 3, S100 proteins and small proline-rich proteins. On the other hand, *MAPK14* (a gene that encodes p38 α protein) is detected to be significantly upregulated in SCC only.⁴⁸ Notably, invasion-associated matrix metalloproteinases (MMPs) 1 and 13 are also highly upregulated in SCC only.⁴⁸ In addition, MMP1 and MMP13 expression levels are reduced by up to 90% by SB203580 in head and neck SCC (HNSCC) cell lines, suggesting a key role for SB203580-sensitive p38 α in regulation of these enzymes (p38 β expression in HNSCC cells is minimal).⁴³ Of note, increased kinase activities of p38 α , p38 β and p38 δ isoforms are detected in lesional compared with nonlesional psoriatic skin.⁴⁹ Altogether, these data support the notion that p38 isoform-specific signaling may play distinct roles in regulation of epidermal homeostasis and may differentially contribute to the pathogenesis of various cutaneous pathologies, possibly via responding to different stimuli, as well as by targeting distinct sets of substrates.

p38 δ has been implicated in regulation of differentiation in other cell systems, including primary human erythroid cells,⁵⁰ intestinal epithelial cells⁵¹ and the myeloid lineage cells.²⁵ Thus, p38 δ is expressed and active only during the terminal phase of erythroid differentiation.⁵⁰ Additionally, p38 δ mRNA and protein expression are strikingly induced during blood monocyte differentiation into macrophages.²⁵ p38 δ is expressed exclusively in differentiated enterocytes, where it appears to be required for execution of enterocyte anoikis.⁵¹

The function of p38 δ in keratinocyte apoptosis. Our studies also show that p38 δ has a role in regulation of keratinocyte apoptosis.^{24,52} This dual role for p38 δ in regulation of both epidermal terminal differentiation and apoptosis may not be surprising, considering that both processes are known to share some common regulatory pathways.⁵³ Our studies show that OA, a potent inhibitor of serine/threonine protein phosphatases PP1 and PP2A, induces both differentiation- and apoptosis-associated responses in keratinocytes. On the one hand, OA increases the expression of involucrin, a marker of keratinocyte differentiation,⁹ while, on the other hand, it causes a disruption of mitochondrial membrane potential, and caspase-dependent apoptosis.²⁴ These responses occur simultaneously in cultured keratinocytes, and are associated with a selective, potent and sustained activation of p38 δ . What is more, p38 α and p38 β do not appear to contribute to any of these responses as they occur in an SB203580-independent manner.^{9,24} In addition, overexpression of p38 δ enhances OA-induced apoptosis.²⁴ These data indicate that p38 δ is a mediator of OA-stimulated apoptotic cell death in keratinocytes. Moreover, a co-expression of p38 δ together with either an activated form of its direct upstream activator MEK6, or its distal upstream activator PKC δ , causes apoptosis-like morphological changes in the absence of any additional external stimulus.⁵² These changes are similar to those elicited by OA exposure, and are associated with loss of the mitochondrial membrane potential, cytochrome *c* release, and killer caspase activation, the typical features of apoptotic cell death. This death response is specific for the combination of PKC δ and p38 δ , and does not occur when PKC δ is replaced with PKC α , or p38 δ with p38 α . In addition, treatment of cells with SB203580 does not inhibit this response,

further ruling out the role for p38 α and/or p38 β isoforms in this process.⁵²

PKC δ , a member of the novel PKC subfamily, has been implicated in regulating both the differentiation and the apoptosis processes in epidermal keratinocytes.^{24,45,54-59} We found that PKC δ potently activates both ectopically expressed and endogenous p38 δ in keratinocytes.⁴⁵ PKC η and PKC ϵ are additional members of the novel PKC subfamily that are expressed in keratinocytes.⁵⁹ Paradoxically, while PKC η is also a potent activator of p38 δ (a regulation associated with increased involucrin expression), coupling of PKC η and p38 δ fails to cause apoptosis in this cell culture model.^{45,52} This is consistent with a reported role for PKC η as a negative regulator of UVB-induced apoptosis in normal human keratinocytes.⁶⁰ In contrast, despite the fact that PKC ϵ has been implicated in promoting cell survival and tumorigenesis,⁶¹ PKC ϵ promotes apoptosis when coupled to p38 δ in our cell culture model.⁵² Additional studies are needed to elucidate the molecular basis underlying this diverse regulation.

Hydrogen peroxide (H₂O₂), a mediator of oxidative stress-induced cell death and a known inducer of keratinocyte apoptosis, produces similar apoptotic morphological changes, and promotes the endogenous PKC δ and p38 δ activation, when added exogenously to cultured keratinocytes.⁵² The function of p38 δ in promoting oxidative stress-mediated apoptosis in vivo has been substantiated by finding that p38 δ deficient mice exhibited 5-fold lower rates of pancreatic β cell apoptosis in response to treatment with apoptosis-inducing agent streptozotocin.³²

Interestingly, keratinocytes undergoing apoptosis in response to various stimuli, such as H₂O₂ or OA exposure, or co-expression of PKC δ /p38 δ , consistently display concurrent p38 δ activation and ERK1/2 inactivation.^{9,24,52} We found that reduction in ERK1/2 activity is essential for apoptosis, since forced ERK1/2 activation, by means of expressing constitutively active form of MAP3K Raf1, a specific upstream activator of ERK1/2, inhibits PKC δ /p38 δ -induced apoptosis.⁵² These data suggest that a balance between prosurvival ERK1/2 signaling and proapoptotic/prodifferentiation stress-activated protein kinase p38 δ signaling is a crucial determinant of cell fate decisions. We studied the mechanisms of coordinated and inverse regulation of p38 δ activation and ERK1/2 inactivation in keratinocytes. Our studies reveal two important aspects of this regulation: the formation of p38 δ -ERK1/2 complex that is constitutively present in stimulated and nonstimulated keratinocytes, and translocation of the complex from the cytoplasm into the nucleus upon stimulation with PKC δ .^{9,52} This PKC δ -stimulated nuclear co-localization of p38 δ -ERK1/2 complex is sustained up to 48 hours after initiation of treatment, and is associated with maintenance of p38 δ activity and inactivation of ERK1/2. The latter may be carried out by resident nuclear phosphatases that become activated in response to nuclear accumulation of ERK1/2, as previously reported.⁶² Moreover, the interaction of p38 δ and ERK1/2 may result in conformational changes in ERK1/2 that facilitate their interactions with phosphatases. Additionally, activated p38 δ may act to retain unphosphorylated ERK1/2 in the nucleus, thus preventing ERK1/2 re-localization into the cytoplasm where the upstream ERK1/2 activator, MEK1, resides. Activated p38 δ

may contribute to proapoptotic phenotype by stimulating apoptosis-related transcription changes the specific nature of which remains to be elucidated. Notably, a complex formation between p38 α splice variant, Mxi2 and ERK1/2 has been recently reported.^{63,64} Mxi2 constitutively binds to ERK1/2 and promotes stimulus-independent ERK1/2 nuclear import. Mxi2 promotes nuclear accumulation of both phosphorylated and unphosphorylated forms of ERK1/2. The biological rationale underlying the Mxi2 functions in nuclear shuttling of ERK1/2 remains to be established. Mxi2 overexpression in certain renal tumors is associated with ERK1/2 redistribution which may contribute to disease.⁶⁴ A physical interaction between p38 γ isoform and ERK1/2 in K-Ras-transformed intestinal epithelial cells has been recently reported.⁶⁵ It is suggested that unphosphorylated p38 γ may function to promote K-Ras transformation through an increased complex formation with ERK1/2 proteins. A direct association between p38 α and ERK1/2 has also been described that leads to ERK1/2 inhibition, purportedly via blocking MEK1-dependent ERK1/2 phosphorylation.⁶⁶ Overall, these findings, demonstrating the capacities of different p38 isoforms to physically interact with ERK1/2 kinases and to modulate ERK1/2 activity and/or subcellular distribution, highlight the potential functional significance of this regulatory mechanism in influencing cell fate decisions in response to stress.

The function of p38 δ in skin tumor development. Increased expression of p38 δ has been detected in human primary cutaneous SCCs,⁴⁸ and increased activation of p38 δ has been observed in HNSCC cells in culture as well as in HNSCC tumors in vivo.⁴³ In addition, a recent study reports increased p38 δ mRNA and protein levels in cholangiocarcinoma (CC), an aggressive liver malignancy, and in liver cancer cell lines.⁶⁷ p38 δ has been reported to regulate HNSCC and CC invasion.^{43,67} Taken together, these findings suggest a potential role for p38 δ in human malignancies.

Our recent study has suggested that p38 δ is required for the development of skin tumors in the multistage chemical carcinogenesis model in mouse skin.⁴¹ In this well characterized model (reviewed in refs. 68–72), during the initiation stage, a single topical treatment with a chemical mutagen 7,12-dimethylbenz(*a*) anthracene (DMBA) induces activating mutations in H-ras gene. The population of mutated “initiated” cells undergoes clonal expansion after repeated applications of a tumor promoting agent, such as TPA, leading to development of benign tumors, papillomas. Some of papillomas may spontaneously progress to invasive SCCs. The conversion of papillomas to SCCs is associated with progressive chromosomal abnormalities that occur independently of treatment with tumor promoting agents. As the multistage skin carcinogenesis model in mice recapitulates features of multistage carcinogenesis in humans, this model is a useful experimental tool to study the mechanisms of human epithelial carcinogenesis.⁶⁸⁻⁷²

Using the DMBA/TPA regimen, we found that p38 δ knockout mice¹⁵ displayed a significantly reduced susceptibility to the multistage chemical skin carcinogenesis.⁴¹ p38 δ deficiency resulted in a delay of tumor development and a significantly reduced numbers and size of tumors compared with wild-type

mice. Decreased skin carcinogenesis in p38 δ knockout mice correlated with reduced epidermal proliferation during tumor promotion with TPA. Interestingly, a major cancer promoting cascade, ERK1/2-AP1 signaling pathway, showed reduced activation in TPA-treated p38 δ -deficient epidermis. In this regard, it is noteworthy that deletion of ERK1 has been shown to inhibit DMBA/TPA-induced mouse skin carcinogenesis.⁷³ In addition, the epidermal-specific expression of dominant-negative AP1 transgene led to a marked resistance to chemical skin carcinogenesis.⁷⁴ Furthermore, a repressed ERK1/2-AP1 signaling correlated with reduced DMBA/TPA-stimulated skin tumor development in JNK2 knockout mice,⁷⁵ while increased ERK1/2-AP1 signaling was associated with enhanced susceptibility to chemical skin carcinogenesis in JNK1 knockout mice.⁷⁶ These findings suggest that a reduced proliferative response during tumor promotion may limit intensive tumor growth and thus account for a marked protection against chemical skin carcinogenesis commonly observed in these mouse models.

We also found that p38 δ -deficient mice exhibited markedly reduced levels of activated signal transducer and activator of transcription 3 (Stat3) oncogenic transcription factor in DMBA/TPA-induced papillomas, in comparison with tumors derived from their wild-type counterparts.⁴¹ This indicates that Stat3 is a downstream target of p38 δ -stimulated signaling in skin tumor development. Studies using skin-specific gain and loss of function Stat3 transgenic mouse models have shown that Stat3 has critical roles in both chemically and UVB-induced skin carcinogenesis.⁷⁷ Stat3 is required for the survival of keratinocyte stem cells during tumor initiation.⁷⁸ In addition, Stat3 drives epithelial proliferation necessary for clonal expansion of initiated cells.⁷⁸ Finally, Stat3 is known to play a role in malignant conversion by regulating a number of genes involved in angiogenesis and invasion.⁷⁹ Our data argue that p38 δ regulates Stat3 activation during epithelial carcinogenesis, suggesting that p38 δ promotes skin tumor development at least in part by regulating Stat3 signaling pathway. The molecular mechanisms whereby this regulation is executed remain to be elucidated.

p38 δ knockout mice also displayed a markedly reduced susceptibility to lung tumorigenesis induced by oncogenic mutation of *K-ras* gene, including significantly decreased number of tumors, average tumor volume and total tumor volume per lung, compared with their wild type counterparts.⁴¹ In contrast, p38 α deletion resulted in hyperproliferation of lung epithelium, and sensitized p38 α -deficient mice to *K-ras*-induced lung tumor development.⁸⁰ These data suggest that p38 δ and p38 α isoforms may have opposing functions in regulating oncogenic Ras-driven lung carcinogenesis. However, both p38 α and p38 δ genes are upregulated in human cutaneous SCCs,⁴⁸ and kinase activity of both isoforms is increased in HNSCCs.⁴³ Moreover, both p38 α and p38 δ are implicated in regulation of invasion and proliferation of HNSCC cells.⁴³ Therefore, although the function for p38 α as a tumor suppressor in vivo has been demonstrated by a plethora of studies using genetically engineered mouse models (reviewed in refs. 81–84), a notion that p38 α signaling may function to promote skin tumorigenesis needs to be explored in future investigations.

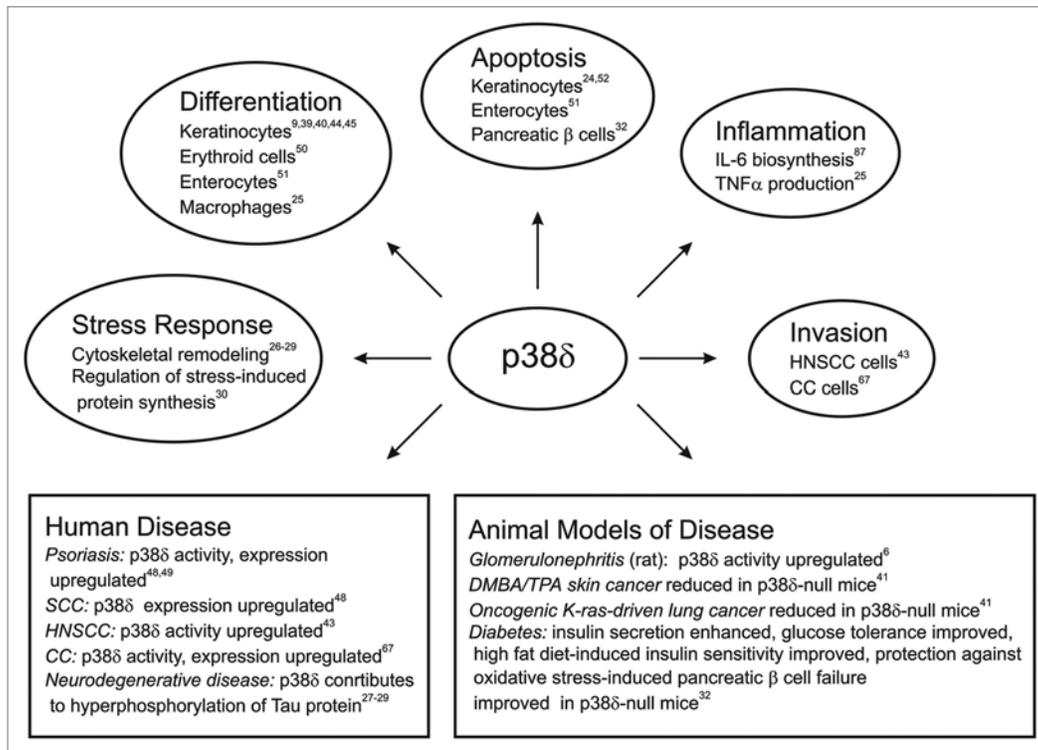


Figure 1. The involvement of p38 δ in regulation of biological processes and disease conditions.

A significant protection against epithelial carcinogenesis afforded by a germline p38 δ deletion in mice⁴¹ may potentially be mediated by specific and/or redundant contributions from both epithelial and non-epithelial cell types. Development of epithelial-derived tumors is known to be stimulated by inflammation, and inflammatory cells and mediators are present in the microenvironment of all tumors in experimental animal models and humans from the earliest stages of tumor development.^{84,85} In addition, activated oncogenic Ras signaling induces production of tumor-promoting inflammatory cytokines and chemokines, which contributes to the formation of tumor-promoting microenvironment.⁸⁵ Activation of p38 signaling is known to be important for the production of tumor-promoting inflammatory cytokines, such as TNF α , IL-1 β and IL-6 (reviewed in refs. 3, 4 and 84). p38 α and p38 δ are the predominant p38 isoforms expressed in immune (myeloid) cells.²⁵ Interestingly, studies using conditional knockout of p38 α in macrophages show that p38 α is essential for production of some cytokines (such as TNF α and IL-12), but not others (such as IL-6), in response to stimulation with LPS.⁸⁶ On the other hand, a recent report, using RNAi approach, suggests that p38 δ may have a role in regulation of IL-6 biosynthesis.⁸⁷ It is noteworthy that the mechanisms of constitutive Stat3 activation in many tumors include, among others, both autocrine and paracrine production of IL-6 leading to Stat3 phosphorylation.⁸⁸ It would be of interest to investigate whether p38 δ /IL-6/Stat3 signaling axis has a functional role in promoting epithelial carcinogenesis. Remarkably, analysis of the effect of p38 α ablation in myeloid and epidermal cells have revealed distinct cell type-specific functions of p38 α signaling in regulation

of inflammatory responses to various types of skin injury.⁸⁹ For example, epidermal p38 α was required for acute inflammatory responses to UVB-induced epidermal injury, whereas myeloid p38 α exerted anti-inflammatory regulation in acute UVB- and TPA-induced skin responses via activation of anti-inflammatory gene expression.⁸⁹ Future studies using conditional epithelial- and myeloid-cell-specific deletion of p38 δ will identify cell type-specific effects of p38 δ deficiency on epithelial inflammatory responses and carcinogenesis, and will provide insights into the underlying molecular processes.

Concluding Remarks

Recent studies using p38 δ deficient mice have begun to contribute to advancing our understanding of the *in vivo* roles of this enigmatic p38 isoform. Thus, p38 δ knockout mice have been demonstrated to exhibit improved glucose tolerance due to enhanced insulin secretion from pancreatic β cells.³² Moreover, these mice are protected against high fat diet-mediated insulin resistance and oxidative stress-induced β cell apoptosis. This study suggests that p38 δ is a potential therapeutic target for human diabetes.³² Our recent studies using p38 δ knockout mice have revealed essential *in vivo* role of p38 δ as a positive regulator of skin and lung tumorigenesis.⁴¹ Moreover, increased expression and/or activity of p38 δ are demonstrated in human malignancies, such as cutaneous SCC, HNSCC and liver malignancy CC.^{43,48,67} These findings suggest that targeting p38 δ is a promising strategy for cancer therapy. Much remains to be learned regarding the molecular mechanisms underlying p38 δ function(s) in regulation of epithelial tumorigenesis.

Genomic and proteomic approaches will assist in discovery of specific p38 δ -regulated genes and interacting protein partners. Epidermal-specific inducible p38 δ -deficient mice will need to be generated and characterized to define the role of p38 δ in each stage of multistage chemical carcinogenesis in mouse skin.⁹⁰ A potential contribution of non-epithelial cell types (i.e., cells of myeloid lineage) to reduced tumor development in p38 δ -null mice will need to be addressed by generation of mouse models featuring cell-type specific p38 δ deletion, and subjecting these mice to multistage chemical carcinogenesis regimen. Finally, it would be of interest to examine the impact of p38 δ deficiency on UVB-induced skin

carcinogenesis. **Figure 1** summarizes our current understanding of the involvement of p38 δ in regulation of various biological processes and disease conditions, as discussed in this review.

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