Role of p38 MAP Kinase Signal Transduction in Solid Tumors

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Abstract

Mitogen-activated protein kinases (MAPKs) mediate a wide variety of cellular behaviors in response to extracellular stimuli. One of the main subgroups, the p38 MAP kinases, has been implicated in a wide range of complex biologic processes, such as cell proliferation, cell differentiation, cell death, cell migration, and invasion. Dysregulation of p38 MAPK levels in patients are associated with advanced stages and short survival in cancer patients (e.g., prostate, breast, bladder, liver, and lung cancer). p38 MAPK plays a dual role as a regulator of cell death, and it can either mediate cell survival or cell death depending not only on the type of stimulus but also in a cell type specific manner. In addition to modulating cell survival, an essential role of p38 MAPK in modulation of cell migration and invasion offers a distinct opportunity to target this pathway with respect to tumor metastasis. The specific function of p38 MAPK appears to depend not only on the cell type but also on the stimuli and/or the isoform that is activated. p38 MAPK signaling pathway is activated in response to diverse stimuli and mediates its function by components downstream of p38. Extrapolation of the knowledge gained from laboratory findings is essential to address the clinical significance of p38 MAPK signaling pathways. The goal of this review is to provide an overview on recent progress made in defining the functions of p38 MAPK pathways with respect to solid tumor biology and generate testable hypothesis with respect to the role of p38 MAPK as an attractive target for intervention of solid tumors.

Keywords: mitogen-activated protein kinase (MAPK), p38 signaling pathway, solid tumors

Background

Mitogen-activated protein kinase (MAPK) signal transduction pathways are evolutionarily conserved among eukaryotes and have been implicated to play key roles in a number of biological processes, including cell growth, differentiation, apoptosis, inflammation, and responses to environmental stresses.[1,2] They are typically organized in 3-tiered architecture consisting of a MAPK, a MAPK activator (MAPK kinase), and a MAPKK activator (MAPKK kinase). The MAPK pathways can be regulated at multiple levels as well as via multiple mechanisms, of which the regulation of mitogen-activated protein kinase kinase kinase (MAPKKK/MAP3K) has been proved to be the most challenging due to the great diversity and versatility between different modules at this level. The complex array of growth factors and other ligands that can initiate intracellular cell signaling requires a very high level of coordination among the different proteins involved. The cascade events can thus amplify the incoming signal and generate a threshold subject to multiple activation cascades. The transduction of exogenous signals is achieved through sequential phosphorylation and activation of the components in the individual cascade leading to the activation of certain transcription factors and other kinases.[3,4] The specificity is less clearly defined for elements upstream of the MAPKK modular level because the MAPKKK are highly promiscuous and can interact with and activate a number of downstream components. When stimulated, MAPKs phosphorylate their specific substrates at serine and/or threonine residues; however, substrate selectivity is often conferred by specific interaction motifs located on physiological substrates, including phospholipases, transcription factors, and cytoskeletal proteins, which can either positively or negatively regulate the substrate or ultimately regulate the entire signaling pathway.[5-7] Whereas many kinases gain their activation via autophosphorylation, yeast MAPK Hog1 is usually phosphorylated by specific kinases MAPKK Pbs2, via an autophosphorylation activity that is induced by osmotic pressure,[8] suggesting a key aspect to understand how adaptation to external stimuli is accomplished.

Signaling by MAPKs

There are 4 major MAP kinase (MAPK) pathways: the extracellular-signal regulated kinase (ERK, also known as p42/44

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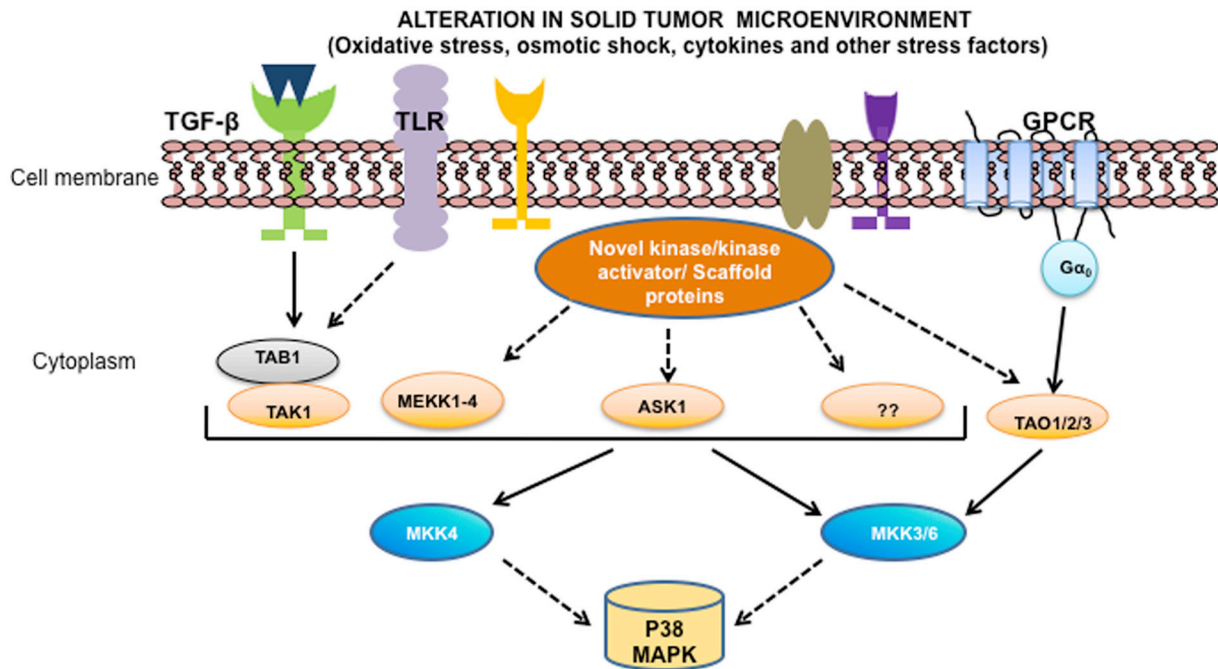(Email: hkoul@lsuhsc.edu).

Figure 1. Schematic representation of the activation of p38 MAPK. Numerous physical and chemical stresses, including oxidative stress and osmotic shock, cytokines, and growth factors such as TGF-β (transforming growth factor-β) activate p38 MAPKs through complex kinase cascades signaling pathways. The mechanism of p38 MAPK activation is mediated by dual phosphorylation (at Thr-Pro-Tyr motif) by MAPK kinase 4 (MKK4) and MKK3/6. Upstream of the MKKs are their MAPKKKs, which include MEKKs 1-4, a member of the mixed-lineage kinases (MLK3) and ASK-1. In turn, these are activated by cellular intermediates, like GTPases, kinase activator, or receptor adapter proteins, among others, which transmit the stimulus to the kinase cascades. TGF-β also activates Smad-independent signaling pathways, including TAK1, which in turn triggers the activation of downstream p38 MAPK signaling cascades. GPCR (G protein-coupled receptor) stimulation results in the activation of heterotrimeric G protein that transmits the signal to TAOs (thousand-and-one amino acid), which then activate p38 MAPK cascades. Once activated, the different p38MAPKs either phosphorylate cytoplasmic targets or translocate into the nucleus leading to the regulation of transcription factors involved in cellular responses.

MAP kinase), c-*jun* N-terminal kinase (JNK, also known as stress activated protein kinase-1 [SAPK1]), Big MAP kinase (BMK, also known as ERK5), and p38 MAPK (also known as SAPK2/RK). In general, ERK1 and ERK2 are key transducers of proliferation signals and are often activated by mitogens. BMK is activated by various stimuli such as oxidative stress and growth factors. In contrast, SAPKs/JNKs and p38 are poorly activated by mitogens but strongly activated in cells in response to stress signals, growth factors, and inflammatory cytokines.[9,10] The transfer of signal between a MAPK and its immediate upstream kinase (MAPKK) is highly specific; with p42/p44 (ERK) MAP kinases being phosphorylated exclusively by MAP/ERK kinase (MEK) 1 and 2; p38 MAP kinase is selectively activated by MAP kinase kinases (MKK) 3 and 6, while JNK is activated by MKK7 and MKK4. In most conditions though, MKK4 can sometimes activate p38 MAP kinase when overexpressed.[11] MKK6 can phosphorylate the 4 p38 MAPK family members, while MKK3 phosphorylates p38α, p38γ, and p38δ but not p38β. Both will also phosphorylate JNK isoforms.[12]

Several MAP3Ks have been shown to trigger p38 MAPK activation (Figure 1); they include ASK1 (apoptosis signal-regulating kinase 1), DLK1 (dual-leucine-zipper-bearing kinase 1), TAO (thousand-and-one amino acid) 1 and 2, TPL2 (tumor progression loci 2), MLK3 (mixed lineage kinase 3), MEKK3 (MEK kinase 3) and MEKK4, and ZAK1 (leucine zipper and sterile-α motif kinase 1).[12] The diversity of MAP3Ks and their regulatory mechanisms may provide the ability to respond to a wide range of stimuli and to integrate p38 MAPK activation with other signaling pathways. It should be noted that some MAP3Ks that trigger p38 MAPK activation can also activate the JNK pathway.[12] The mechanism of p38 MAPK activation is mediated by dual phosphorylation at the Thr-Gly-Tyr motif and is generally activated by environmental stresses, including heat, osmotic and oxidative stresses, as well as inflammatory cytokines and transforming growth factor-β (TGF-β) signaling.[13] Recent reports suggest that GPCR (G protein-coupled receptor) can also regulate cellular growth and differentiation by controlling p38 MAPK activity. GPCR stimulation results in the activation of heterotrimeric G protein that transmits the signal to TAOs, which then activate p38 MAPK cascades.[14,15] The upstream kinases acting on p38 include MKKs 4, and 3/6 has preferential effects on different p38 isoforms,[16] which are in turn activated by MEKKs, MLKs, and ASK1. GTPases are responsible for the transmission of stress stimuli to the MAPKKKs of this pathway, including the Racs, Rhos, and Cdc42s. Recently, a major concept change for MAPK regulation was developed for p38α. The p38α enzyme is activated by TAK1-TAB1 kinase enzyme system,[17] but
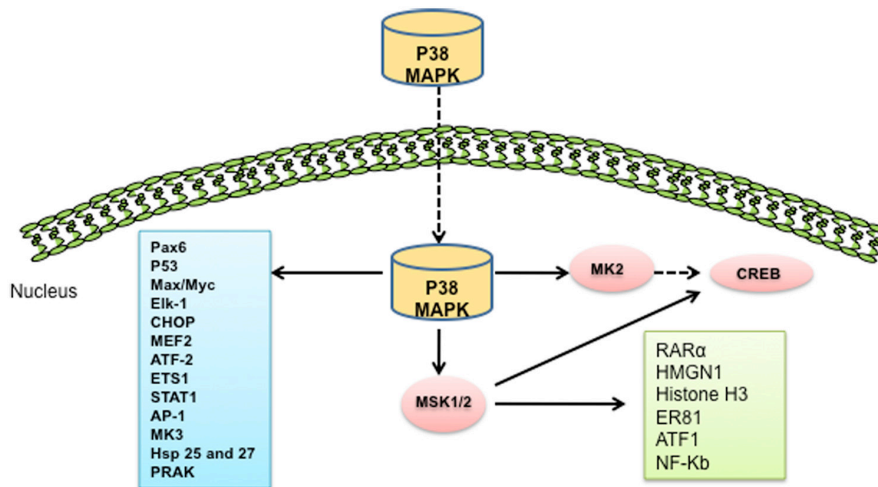
Figure 2. Schematic diagram of the downstream targets of p38 MAPK. Activated p38 MAPK regulates downstream targets including several kinases and transcription factors. These kinases include PRAK (p38-related/activated protein kinase), MSK1/2 (mitogen- and stress-activated protein kinase-1/2), and MK2 (MAPK-activated protein kinase 2). Some important transcription factors phosphorylated by p38 MAPK include tumor suppressor protein p53, CHOP (C/EBP-homologous protein), STAT1 (signal transducers and activators of transcription-1), CREB (cAMP response element-binding protein), NF-κB, Max/Myc complexes, and so on. Other substrates of the p38 signaling pathway include Pax6, Elk-1, MEF2, ATF-2, Hsp 25 and 27, ETS1, MK3, RARα, HMGN1, Histone H3, ER81, Activator Protein 1 (AP-1), and ATF1 for regulation of gene expression, affecting cell motility, transcription, and chromatin remodeling.

TAB1 is not a MKK and TAB1 appears to be an adaptor or scaffolding protein and has no known catalytic activity. This is the first demonstration that another mechanism exists for the regulation of MAPKs in addition to the MKKK-MKK-MAPK regulatory module. This important observation indicates that other adaptor proteins should be scrutinized for potential roles in regulating MAPK activity. Transforming growth factor-β (TGF-β) activated kinase 1 (TAK1) belongs to the MAPKKK family. This serine/threonine kinase is a key intermediate in inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1)[18,19] as well as TGF-β[20] mediated signaling pathways. In the absence of TGF-β stimulation, TAK1 associates with TGF-β receptor through complex formation with TAB2 and TRAF6. On TGF-β stimulation, autopolyubiquitination of TRAF6 leads to polyubiquitination (Ub) of TAK1, by which TAK1 is released from the receptor complexes. The released TAK1 interacts with TAB1, which in turn induces autophosphorylation of TAK1 for activation.[21-23] Activated TAK1 has the capacity to stimulate its downstream MAPK and NF-κB-inducing kinase-IκB kinase cascades.[24] The former activates *c-jun* N-terminal kinase (JNK) and p38 MAPK while the latter activates NF-κB.[18,25,26]

p38 MAPK modulates cellular functions by activation of a number of downstream targets (Figure 2). The different p38 isoforms defined to date (p38α, p38β, p38γ, p38δ) vary, based on their substrate specificity. The α and β isoforms of p38 are responsible for the activation of heat shock proteins (hsps) 25, 27, and the MAPK-activated protein (MAPKAP)-2 or MK2. The γ and δ isoforms of p38 activate ATF2.[27] Other transcription factors affected by the p38 family include STAT1, Max/Myc complexes, MEF-2, Elk-1, and CREB through the activation of MSK1/2. Other substrates of the p38 signaling pathway include Pax6, ETS1, PRAK, MK3, RARα, HMGN1, Histone H3, ER8, Activator Protein 1 (AP-1), ATF1,

NF-κB, and CHOP for regulation of gene expression, affecting cell motility, transcription, and chromatin remodeling.[28-31]

p38 MAPK Signaling

The p38 MAPK family consists of 4 isoforms—p38-MAPKα, p38-MAPKβ, p38-MAPKγ, and p38-MAPKδ—each encoded by a separate gene with differences in substrate specificity and tissue distribution (Table 1).[32] The most common form, p38α, was first isolated as a 38-kDa protein rapidly tyrosine phosphorylated in response to lipopolysaccharide (LPS) stimulation. Of these, p38α and p38β are ubiquitously expressed while p38γ and p38δ are differentially expressed depending on tissue type.[33] The p38 MAPK pathways are activated in response to various environmental and cellular stresses, inflammation, and other signals.[34] The 4 vertebrate isoforms of p38 are characterized by the presence of the conserved Thr-Gly-Tyr (TGY) phosphorylation motif in their activation loop.[35] Sequence comparisons have shown that each p38 isoform shares ~60% identity within the p38 group but only 40% to 45% to the other 3 MAP kinase family members. This motif is phosphorylated by MEK3 and MEK6, which themselves are activated by various MAPKKKs that are induced by physical and chemical stresses, such as oxidative stress, hypoxia, X-ray and UV irradiation, and cytokines.[36] In some instances, p38 can also be activated by MEK4, a kinase that is better known as an activator of JNK. Once activated, p38 proteins can translocate from the cytosol to the nucleus where they phosphorylate serine/threonine residues of their many substrates. In addition to its role in stress responses, recent studies have suggested a role for p38 MAPK in mediating pathways leading to cell apoptosis and growth inhibitory signals.[37] However, other studies with malignant cells have shown that activation of p38-MAPK signaling pathways may produce just the opposite effect: anti-apoptotic and proliferative effects and various

Table 1. Phenotypes of p38 Mitogen-Activated Protein Kinase Knockout Mice.

MAPK	Distribution by Immune or Inflammatory Cells[a]	Phenotype	Disease and Cancer Model	References
p38α	All cells	Placental defect, death by E12.5	Not applicable	260, 261
p38β	T cells	No phenotype	Not determined	249
p38γ	—	No phenotype	Not determined	262
p38δ	Tcells, macrophages/monocytes and neutrophils	Impaired insulin secretion and survival of pancreatic β-cells	Diabetes	263
			Skin cancer (DMBA and TPA) and lung cancer (KrasG12V) induced	264

[a]Tissue distribution as described in Hale *et al.*[236] p38γ is not expressed by immune or inflammatory cells.

Table 2. Role of p38 MAPK in Human Cancers.

Human Cancer	MAPK Implication	References
Liver cancer	p38α activity downregulated	47
Prostate, breast, bladder, lung, thyroid, head and neck squamous cell carcinomas	p38α activity upregulated	40, 41, 43, 46, 48, 49, 254, 265-267

cell survival pathways.[38] These studies suggest that tumor cell growth might be regulated by coordination between cell proliferation and apoptosis. The inability of malignant cells to respond to p38 MAPK signaling toward apoptosis could possibly be due to alterations in regulators of apoptosis that help in cell survival.[39] The emerging views on how these pathways might affect human cancers, as well as evidence from human genetic studies, are discussed below and are summarized in Table 2.

p38 MAPK Pathways in Human Cancer

Several studies have suggested that p38 MAPK plays an important role in prostate cancer,[40-42] breast cancer,[43,44] bladder cancer,[45,46] liver cancer,[47] lung cancer,[48] transformed follicular lymphoma,[49,50] and leukemia.[51] Therefore, the relevance of the p38 MAPK pathway in the context of tumor formation has yet to be investigated. The activation of p38 MAPKs has been reported to contribute to the epithelial-mesenchymal transition (EMT) of cells in the primary tumor, to the acquisition of invasion and migrating capabilities, and to the extravasation of migrating tumor cells.[52,53] In contrast, p38 MAPK inhibition has been correlated with the resistance to anoikis, which allows circulating cancer cells to survive.[54] Tumor cell dormancy has been associated with high p38 MAPK activity in combination with low activity of the ERK1/2 pathway. Analysis of the phenotype of mice disrupted in both the MEK3 and MEK6 genes and the p38α gene has led to the suggestion that p38 can function as a tumor suppressor. The transforming potential of oncogenes is increased in fibroblasts from these animals as well as their tumorigenic potential in nude mice.[55-58] Suppression of p38 function also plays a critical role in Ras-induced transformation.[59,60] The tumor suppressive effects of p38 are involved in both the activation of p53 and in p53-induced apoptosis and acts as negative regulator of cell cycle progression.[57,61-63] p38 is also activated by oncogenic stresses and plays an important role in Ras-induced senescence in mouse embryo fibroblasts.[64] Overall, it suggests that decreasing p38 activity plays an important role in cancer.

The significance of p38 MAPKs in controlling cellular responses to the environment and in regulating gene expression, cell growth, and apoptosis has made them a priority for research related to many human diseases.[65] Therefore, p38 MAPK signaling pathways appear to be a distinguished molecular feature as molecular targets for drug development, and inhibitors of MAPKs will undoubtedly be one of the next groups of drugs developed for the treatment of different solid tumors of the prostate, breast, bladder, liver, and lungs.

p38 MAP Kinase and the Prostate

Prostate cancer is the second-leading cause of cancer deaths in men, after lung and bronchial cancer, according to the National Cancer Institute. Diseases of the prostate can vary from benign enlargement (benign prostatic hyperplasia [BPH]) causing discomfort to prostatic intraepithelial neoplasia and prostate carcinoma, which are a tremendous source of morbidity and mortality in aging males.[66] Most of the prostate cancer deaths are due to the emergence of an androgen-resistant phenotype of prostate cancer either by certain co-activators or through mitogen-activated cell signaling activities,[67,68] which lead to the overexpression of anti-apoptotic genes and survival of the cancer cells. Unfortunately, treatment options for these androgen-resistant prostate cancer patients are few and generally ineffective. These facts underline the need to develop new therapies that will improve outlook for hormone-independent prostate cancer.

The effects of various manipulations, both genetic and chemical, including

Table 3. p38 MAP Kinase Activating Agents in Prostatic Cells.

Agent	Cell Line	Apoptosis	References
FGF-1	DU145	?	74
FGF-2	DU145	?	74
Keratinocyte growth factor (FGF-7)	DU145	?	74
IL-6	LNCaP	?	72
TGF-β	PC3	+	94
Adenosine triphosphate	1E8, 2B4	?	89
Neu differentiation factor (NDF)/Heregulin	LNCaP	?	76
Vitamin D	LNCaP	?	268
Selenite	DU145	?	269
Arsenic trioxide	DU145, PC3	–	270
PEITC	PC3	–	271
Tyrphostin AGE82	LNCaP	+	272
FTY720	DU145	–	91
Phorbol esters	LNCaP	+	83
Sodium butyrate	DU145	+	273
IL-1	Patient samples	–	80
TNF-α	LNCaP	–	274

growth factors, chemical modifiers, and androgens on prostatic cells, have been described so far. Despite these studies, the structure and function of the MAP kinase pathways in prostate are far from being clearly understood. In normal human prostate tissue, p38 MAP kinase protein is present in the basal cells and epithelial cells of the prostate gland, but Magi-Galluzzi *et al.* show it to be absent in the epithelial layer.[69] While likely present, it is not normally activated in epithelial tissue samples that have been studied, but has stronger activity in the prostatic stroma similar to ERK. Epithelial p38 MAP kinase can become active in situations of neoplasia and benign hypertrophy of the prostate gland.[70] In a study using TRAMP mice, strong epithelial p38 MAP kinase activation was shown to be present in intraepithelial neoplasia and well-differentiated tumors but was probably lost in poorly differentiated tumors.[71] Similar to JNK, p38 MAP kinase is a kinase primarily activated by external stresses, some of which are listed in Table 3. Many growth and endocrine factors are able to activate p38 MAP kinase, including FGF-1 and -2, keratinocyte-derived growth factor (KGF), IL-6, heparin binding epidermal growth factor (HB-EGF), ATP, vitamin D, and Neu differentiation factor

(NDF).[72-76] Other factors that provoke stress response (phorbol-12-myristate-13-acetate [PMA], carbachol, osmotic shock, UV light, and anisomycin)[77] are also known to cause p38 activation. Most of the data regarding the effect of these inducers were obtained in studies using cell lines (mostly DU145 and LNCaP) but the PC3 cells are conspicuously absent from these studies. AT3 cells have been used to show drug resistance in prostate cancer due to the inhibition of p38 together with the activation of ERK and JNK by Id-1.[78] Response of p38 MAP kinase to various hormones can range in time from several minutes to several hours, which suggests indirect inducible activation of p38 MAPK.

Protein Signaling

Intracellular signaling prior to the p38 MAP kinase module appears to be complex and incompletely described. Many of the factors that have not been shown to act in other MAPK pathways like IL-1 and TNF-α have been shown to activate the p38 pathway.[79,80] Activation of p38 via IL-1 is through MEK-6 and has been attributed to cell proliferation. Activation of JNK via TNF-α is mediated by MEK-4 and can cause apoptosis in prostate carcinoma,[79] but the pathway can be diverted to cell proliferation via the

activation of p38 by MEK-4. Protein kinase C is able to activate p38 MAP kinase, although perhaps indirectly.[10] The JAK/STAT family of proteins also appears to be able to activate p38 MAP kinase while PAR4 loss causes impairment of p38 MAP kinase phosphorylation.[81]

Results of p38 Activation

Metastatic cancer of the prostate occurs through the concerted proliferation, migration, adhesion, and differentiation of normal prostate cells. These activities are brought about by many intrinsic growth factors like epidermal-derived growth factor (EGF), transforming growth factor (TGF)-α, and insulin-like growth factor (IGF).[82] The cell surface receptors for these factors, on binding their ligands, induce intrinsic kinase activities. The kinases subsequently initiate signal transduction cascades including Ras/Raf/MAPK, phosphoinositol 3-kinase (PI3K)-Akt, signal transducers and activators of transcription (STAT), and protein kinase C pathways. After activation, the cytosolic proteins translocate to the nucleus and initiate gene expression through the activation of transcription factors. Continuous cell proliferation requires the activity of p38 in most of the cancers studied. This role is carried out by the activation of downstream targets like ATF-2 and Elk-1. Activating transcription factor (ATF-2) has been reported in the control of cell cycle progression.[83] Enhancement of cell proliferation by ATF-2 has been reported in some neoplastic cells such as skin adenocarcinoma[84] and melanoma.[85] Experimental studies in rat ventral prostate has shown that reduction in p-Elk-1 is associated with an enhancement in apoptosis,[86] while in the human prostate cell lines DU145 and PC-3, its presence has been correlated with cell proliferation.[87] Recent evidence provided by Ricote *et al.*[80] shows that overexpression of α-PAK, MEK-6, p38, p-Elk-1, and p-ATF-2 occurs in benign prostate hyperplasia and more intensely in prostate cancer patients, enhancing cell proliferation and survival.

In BPH proliferation can be counteracted in part by TNF-α/JNK/AP-1 mediated apoptosis, but in tumors from patients and LNCaP cells, this pathway is diverted to p38 and proliferation.[88]

Chen *et al.*[89] demonstrated that ATP mediated P2Y (metabotropic GPCR family) receptor induced prostate cancer cell invasion is regulated through the involvement of p38. The p38 MAP kinase is well known to activate the transcription factor NF-Kappa B[90] and may also have some role in activating caspases.[91] p38 is also able to increase the expression of certain proteins including chromium induced HIF-1α expression, upregulation of the U-PA promoter in PC3 cells, and PSA induced by IL-6 in LNCaP cells.[78,92,93] A decrease in the activation of actin stress fibers in DU145 cells was observed when p38 was inhibited,[74,94] probably by growth factors. Kim *et al.*[75] demonstrated neuroendocrine differentiation in LNCaP cells via a p38 MAP kinase dependent mechanism. Some authors have postulated that the determination of the ratio of p-ERK to p-p38 MAP kinase might be able to predict the *in vivo* behavior of cancer, including the prostate.[95] Skjoth and Issinger[96] suggest that the presence of an active p38 may be responsible for the high sensitivity to apoptosis induction leading to cell death, p38 MAP kinase is not a requisite for cellular apoptosis, and clearly the balance of p38 MAP kinase activation versus other signals probably determines cellular outcome in which the PI3K/Akt pathway has an apparent role. Previous studies demonstrated that inhibition of p38 MAP kinase pathway resulted in inhibition of the DNA synthesis, growth, and proliferation of PC3 cells.[97] Additional studies further evaluate the function of p38 MAP kinase signaling pathway in other prostate cancer cell lines. In addition, it has also been demonstrated that prostate cancer cell invasion is mediated through the p38 MAPK pathway; this leads to the phosphorylation of heat shock protein 27, which in turn regulates MMP-2 activation and cell invasion.[98] The activation of MMP-2 and the invasiveness have been shown to be mediated through p38 MAPK, but not ERK, signaling in human melanoma cells.[99] Studies from our laboratory have demonstrated a critical role for p38 MAP kinase activation in response to hypoxia/reoxygenation and emergence of castrate resistant phenotype in prostate cancer.[41] We have observed that p38 MAPK associates AR in immunoprecipitation assays and that inhibition of p38 MAPK results in decrease in AR levels and activity in prostate cancer cells in culture (S. Koul *et al.*, unpublished observations). Given the critical role of AR signaling in prostate cancer, p38 MAPK may thus offer an attractive alternate target in management of prostate cancer.

p38 MAP Kinase and the Breast

Breast cancer is the second most common cancer in women in the United States, after skin cancer, according to the Mayo Clinic. Breast cancer is a heterogeneous disease with several subtypes that have been identified through analysis of gene expression patterns.[100] Metastatic breast tumors represent the final lethal stage of the disease, with cancer cells from the primary mammary gland site having spread to a secondary site (e.g., bone, lung, or liver). The cells from the initial tumor that is able to invade the surrounding tissue by a degrading basement membrane disperse through the lymph and vascular systems to produce metastasis. They usually undergo EMT, which provides epithelial cell plasticity and gain a proliferative and migratory/invasive phenotype with an ability to degrade and migrate through ECM.[101] A range of growth factors and cytokines (e.g., human growth factor, FGF, and TGF-β) have been found to trigger EMT.[102-106] But in particular, TGF-β signals through transmembrane receptor kinases activate p38 through both Smad-dependent and Smad-independent mechanisms and the MAPK4 pathway.[107,108] TGF-β inhibits growth of normal epithelial cells but induces proliferation and EMT in immortalized, phenotypically normal MCF10A cells and in cells from advanced carcinomas.[109] Previous studies have shown that p38 MAPK is strongly activated by a mutant form of H-Ras in human breast epithelial cells.[110,111] Not only the small G-proteins but the large G-proteins such as GPCR and regulators of G-protein signaling (RGS) proteins are also involved in the p38 MAPK signaling pathway.[112,113]

The role of MAPKs and their endogenous negative regulators, mitogen-activated protein kinase phosphatases (MKPs) in chemotherapy resistance is well established. MKPs dephosphorylate MAPK are part of the dual-specificity family of phosphatases. MAPKs have been shown to be involved in resistance to tamoxifen, and MKPs have been linked to resistance to treatment with doxorubicin, mechlorethamine, paclitaxel, proteasome inhibitors, and oxidative-stress-induced cell death in breast cancer.[114] Growing evidence suggests that modulating MKP-1 activity could be a viable option to make breast cancer chemotherapy more effective.[114] Inhibition of p38 signaling by overexpression of MKP-1 also increased resistance to H_2O_2-induced death in MCF7 breast cancer cells, with a correlation between MKP-1 induction and the disappearance of phosphorylated MAPKs, suggesting that MKP-1 might play a physiologic role in the inactivation of oxidative-damage-induced MAPK activities.[115]

Protein Signaling

p38 MAPK exerts its biological effects by activating several transcription factors that are involved in various cellular functions such as apoptosis, cell growth, and differentiation. On activation of p38 MAPK, transcription factors present in the cytoplasm or nucleus become phosphorylated and activated, leading to expression of target genes resulting in a biological response. These transcription factors include an activating transcription factor (ATF)-1, ATF-2, GADD153, myocyte enhancer factor (MEF) 2C, CREB, C/EBP homologous protein

(CHOP), p53, and NF-κB.[31,116-119] ATF-2, a substrate of p38 MAPK, is a possible partner of *c-jun* in the activator protein (AP)-1 complex. A recent study has demonstrated that ATF-2 mediates MMP-2 transcriptional activation induced by p38 MAPK in MCF10A, human breast epithelial cells.[120] p38 MAPK can modulate transcriptional upregulation of TNF-α expression and regulate NF-κB-dependent gene expression in LPS-stimulated macrophages.[121,122] Genes that are regulated by p38 MAPK have been revealed by using dominant negative mutants of the upstream activators of p38 MAPK, MKK3, and MKK6, as well as pharmacological inhibitors of p38 MAPK. Expression of many cytokines, transcription factors, cell surface receptors, and enzymes including IL-1, IL-6, IL-8, TNF-α, iNOS, COX-2, MMP-1, MMP-2, MMP-9, MMP-13, *c-jun*, c-fos, junB, and LDL receptor have been demonstrated to be regulated by the p38 MAPK pathway.[120] p38α regulates the induction of the pro-inflammatory mediator cyclo-oxygenase 2 (COX2), which could potentially contribute to breast cancer progression.[123] Further studies identifying p38 MAPK-regulated genes would be of great importance to understand the p38 MAPK signaling pathway and application of its inhibitors for therapeutic use.

Results of p38 Activation

Activated p38 phosphorylates and activates many transcription factors (including activating transcription factor-2, Max, myocyte enhancer factor-2, Mac, p5,3 and Stat1),[124,125] which are involved in regulating existing invasive behavior in cultured cells: phospho-p38, which is elevated in cultured invasive breast cancer cells. Constitutive p38 activity induces the overproduction of the pro-invasive uPA,[126] which might have an important role in invasion and metastasis of breast cancer.[127] Also, treatment of the invasive BT549 breast cancer cells with a p38 MAPK inhibitor diminished both uPA and uPAR expression and inhibited the ability of these cells to invade matrigel.[126,128] Furthermore, activated Src overexpression was shown to activate

p38 during TGF-β-induced breast cancer cell proliferation and invasion.[129] Studies on mammary cells indicate that WAVE-3 (a regulator of cytoskeletal dynamics and cell motility) regulates breast cancer progression, invasion, and metastasis through the p38 pathway and MMP production. Knockdown of WAVE-3 using small interfering RNA in triple negative MDA-MB-231 breast cancer cells decreases p38 activity but not AKT, ERK1/2, or JNK.[130] Elevated phospho-p38 levels have been associated with high expression of EGFR and ErbB2 as well in tamoxifen-resistant xenografts.[131] p38 would not be seen as driving apoptosis in this context; it acts to support nuclear functions of the estrogen receptor.[132] Interestingly, a relationship between phospho-p38 level and lymph node metastasis was identified in human breast cancer samples.[131]

Recent studies indicated that the p38 MAPK pathway regulates the expression of the MMP family,[120,133,134] which is significantly involved in tumor invasion and metastasis formation in various cell systems.[135] Activation of the p38 MAPK pathway plays a crucial role in the invasive phenotype of transformed squamous epithelial cells.[127,136] The p38 MAPK pathway stimulated MMP-9 expression/secretion and *in vitro* invasion of human squamous carcinoma cell lines.[137] The activation of p38 MAPK is critical for induction of the expression of invasion-associated MMPs such as MMP-13, MMP-1, and MMP-9 in transformed keratinocytes.[138,139] p38 MAPK is activated in invasive H-Ras MCF10A human breast epithelial cells but not in noninvasive N-Ras MCF10A cells.[110] It has been shown that H-Ras-specific activation of the Rac/MKK-6/p38 MAPK signaling pathway upregulated MMP-2, leading to invasion and migration of MCF10A cells.[134] The p38 MAPK pathway activates ATF-2, which then results in transcriptional activation of MMP-2 through binding to the functional AP-1 site.[120] Phosphorylation of heat shock protein 27 by p38 MAPK has been shown to induce the migration of MDA-MB-231 breast cancer cells on a laminin-5 coated

dish.[140] It was also suggested that p38 MAPK might be critical in heregulin-b1-mediated MMP-9 induction in breast cancer cells.[141] p38 signaling was found to be required for resveratrol-induced apoptosis in triple negative MDA-MB-231 breast cancer cells,[62,142] whereas p38 was not required for vitamin E succinate–induced apoptosis of MDA-MB-435 breast cancer cells.[143] Recently, it has been demonstrated that breast carcinoma cells in effusions have markedly different expressions of key molecules that are involved in the biology of breast tumors, as well as in cancer invasion and metastasis in general, including tyrosine kinase receptors, hormone receptors, matrix metalloproteinases, Ets transcription factors, and angiogenic molecules.[144,145] Expression of several of these molecules was found to correlate with more aggressive clinical behaviour.[145,146] Carcinoma cells in effusions showed significant upregulation of p-p38, which would make sense biologically in a microenvironment lacking access to vessels and therefore considerably deprived of oxygen and nutrients, as well as to the often beneficial cross-talk with peritumoral myofibroblasts in solid tumors. The fact that the upregulation of phopho-p38 does not trigger apoptosis provides further proof to the adaptive capabilities of these cells in suboptimal growth conditions. One target that is known is mitogen-activated protein kinase activated protein kinase 2 (MAP-KAP-K2), which phosphorylates RNA binding effectors, such as tristetraprolin, heterogeneous ribonuclear protein A0, and poly (A) binding protein. These molecules are involved in reducing the stability of MKP-1, vascular endothelial growth factor, and MMP mRNA.[147] p38 phosphorylation prevents the effectors from carrying out their function, thereby increasing the stability of their target mRNAs.[147]

p38 MAP Kinase and the Bladder

Bladder cancer, the fourth most common malignancy in men, the ninth in women

in the United States, and the tenth frequent cancers worldwide, is characterized by frequent recurrence and poor clinical outcome when tumors from noninvasive flat and papillary urothelial neoplasia progress to muscle invasive or metastatic disease.[148] More than 90% of bladder cancers are transitional cell carcinoma (TCC), which is characterized by a multifocal origin and an immunogenic cancer. Although transurethral resection (TUR) of tumor combined with intravesical BCG immunotherapy has become the standard treatment for this type of bladder cancer, the tumor recurrence rate is still high, and most of which will progress to a higher stage or grade.[149,150]

In our previous studies we found that p38 MAPK is activated during the log phase growth of bladder cancer cells and activation of MAPK is a frequent event in tumor progression and metastasis.[45] As metastatic disease is the principal cause of death in cancer patients, a greater understanding of tumor invasion and metastasis is essential and should lead to the identification of new therapeutic targets. There is increasing evidence to indicate that several MMPs in tumor invasion not only have a direct role in tumor invasion by degrading extracellular matrix protein, but as a consequence they also have an important role in maintaining the tumor microenvironment and thus promoting tumor growth. Our previous findings also suggest that p38 MAPK modulates MMP-2 and MMP-9 expression, stability, and activity as well as invasion of bladder cancer cells.[46] Recently, it was demonstrated that exposure to LPS caused a remarkable increase in the level of phosphorylated p38 in T24 cells and p38 played a key role in LPS induced IL-6 syntheses.[151-153] However, these studies focused on the signaling pathway of regulating IL-6 syntheses in normal cells including airway smooth muscle cells, biliary epithelial cells, and some immune cells.[154,155] Very little is known about the mechanisms by which IL-6 is regulated in bladder cancer cells. Interleukin-6 is a pleiotropic cytokine with both pro- and anti-inflammatory roles on immune and nonimmune cells.[156] Recent studies suggested that elevated levels of IL-6 in the serum and urine of patients suffering from bladder cancer correlated with tumor size and grade.[157] Therefore, tumors associated IL-6 were reported to function as an autocrine growth factor for bladder cancer.[158] Previous studies also found that the activation of TLR4 by LPS increased the secretion of IL-6 in a dose- and time-dependent manner and was regulated positively by activation of p38. Horwood *et al.* demonstrated that LPS-induced expression of IL-6 in human monocytes/macrophages was significantly less sensitive to p38 MAPK inhibition than TNF; it might be explained by this kind of double-modulation mode.[159] Moreover, recent findings demonstrate that activation of PI3K/Akt pathway neutralizes the IL-6 induction effect of p38 activation in bladder cancer cells and may contribute to an improved understanding of the overall regulatory process of IL-6 expression through TLR4 signaling. It has also been shown that p38 is essential for the downregulation of COX-2 expression by sulforaphane (SFN), a dietary isothiocyanate in human bladder T24 cells. SFN suppressed both COX-2 mRNA and protein levels by inhibiting NF-κB DNA-binding to the COX-2 promoter. It is known that COX-2 plays a role in cell proliferation, metastasis, and angiogenesis, and thus recent findings suggest that targeting COX-2 through p38 MAPK is a novel mechanism for cancer chemoprevention by SFN in human bladder cancer. Therefore, SFN activated p38 MAPK, and then inhibited NF-κB binding to the COX-2 promoter as well as the downregulation of COX-2 expression, suggesting that activators of p38 MAPK may be beneficial in the down-regulation of COX-2 and, in combination with SFN, may provide potential therapeutic strategies for bladder cancer.

Protein Signaling

Several studies have identified that p38 MAPK signal transduction pathways are involved in the regulation of MMP-9 expression in several bladder cancer cell lines.[46,160-162] A recent study identified potential cisacting elements of the p38 MAP kinase signal pathway–mediated control of the MMP-9 gene in HT1376 bladder cancer cells in response to TNF-α. Although multiple signaling pathways were previously shown to be involved in MMP-9 expression in different cell types,[141,163] a recent study reported that TNF-α upregulates MMP-9 via the simultaneous activation of different pathways in bladder cancer cells. This finding has broadened the understanding of the regulation of this important molecule, which is involved in urinary bladder tumor invasion and metastasis. The transcription factors, NF-κB, AP-1, and Sp-1, were the focus of this investigation into how p38 MAP kinase mediates MMP-9 expression, as they are necessary for MMP-9 secretion. The transcription factors that are involved in p38 MAP kinase-mediated control of the MMP-9 gene in HT1376 cells in the presence of TNF-α were identified. Therefore, it clearly shows that TNF-α regulates MMP-9 expression through NF-κB, AP-1, and Sp-1 *cis*-elements of the gene promoter, and p38 MAP kinase mediates TNF-α-induced MMP-9 expression by coordinating the regulation of the binding activity of the transcription factors, NF-κB, AP-1, and Sp-1, in HT1376 cells.[107]

Results of p38 Activation

It has been demonstrated that p38 MAPK plays an important role in the expression of both ARE-dependent enzymes and COX-2 manipulated by sulforaphane (SFN). p38 activation positively modulates the induction of GSTA1-1 and TR-1 by SFN and enhances the effect of SFN on downregulation of COX-2. In accordance with these findings, positive regulation of p38 MAPK was also reported in other ARE-dependent enzymes, such as HO-1, γ-GCS.[164,165] However, only a few studies have reported the converse, that p38 MAPK negatively controls ARE-dependent enzymes. Keum *et al.*[166] found that p38 MAPK overexpression suppressed

SFN-induced heme oxigenase-1 (HO-1) expression in human hepatoma HepG2 cells. The underlying mechanism remains to be clarified and the specificities in stimuli and cell types should also be considered. In nonstimulated cells, Nrf2 is sequestered in the cytoplasm by its inhibitor Keap1. On activation mediated by p38 MAPK following exposure to SFN, Nrf2 translocates into the nucleus and binds to ARE sites in the promoter regions of many detoxification and antioxidant genes, leading to coordinated upregulation of downstream targets that enhance cellular detoxification and antioxidative potency.[167] Previous studies have revealed that intracellularly, hypericin-PDT-induced photodynamic therapy (PDT) cell death is mediated by endoplasmic reticulum (ER) Ca^{2+} store emptying and executed through caspase-dependent or caspase-independent pathways.[168] However, hypericin-PDT also activates rescuing responses, chiefly governed by the activation of p38 MAPK.[169-171] The pleiotropic p38 MAPK appears to contribute to carcinogenesis by regulating growth factor expression,[43,49,50] inflammatory,[40] and invasion-associated genes, including members of the MMP family.[44,46,136,139] Previous results confirm the crucial role of p38 MAPK in modulating immune and inflammatory responses by upregulation. For example, COX-2, IL8, and CSF2 additionally assign novel important molecular functions to this pathway in PDT signaling by inducing genes including AKR1C1, AKR1C3, GCLC, NQO1, as well as HMOX1. These cytoprotective genes are induced by oxidants and electrophilic carcinogens through activation of the redox-dependent Keap1/Nrf2 pathway.[172,173] Accordingly, it was observed that the activation of p38 MAPK signaling pathways by PDT contributes to HO-1 protein induction and survival through a mechanism involving nuclear accumulation of Nrf2.[174]

Furthermore, a previous study reveals that MMP-13, a member of the collagenase subfamily of MMPs, is a p38 MAPK target. MMP-13 is expressed at the invading edges of urinary bladder transitional cell carcinoma (TCC) *in vivo* and is regulated *in vitro* in T24 cells stimulated by TNF-α through p38 MAPK.[175] MMP-13 upregulation in bladder cancer cells after PDT is remarkably p38 MAPK dependent, suggesting that p38 MAPK inhibition could be required to halt the invasiveness of cancer cells escaping photokilling. Thus, the role of p38 MAPK-MMP-13 cascade in the modulation of tumor growth, as well as the immune response *in vivo*, deserves to be further addressed.[176] Interestingly, the marked activation of angiogenesis in urinary bladder *lamina propria* with chronic radiation cystitis was associated with a dramatic increase of p38 and p65 cytoplasmic and nuclear expression in endothelial cells. These findings indicate a critical role for the p38 MAPK cascade and the NF-kB p65 subunit in endothelial cell activation as an important component in the pathogenesis of Chernobyl cystitis.[177] T24 cells have an activating *ras* mutation,[178] and previously it has been shown that T24 cells have very low *NF1* tumor-suppressor gene expression levels.[179] As the *NF1* gene has been suggested to have inhibitory effects on *ras*, high *ras* activation in T24 cells could lead to activation of MAPK pathways, for example, p38 when compared to RT4 cells.[175]

p38 MAP Kinase and the Liver

Liver cancer is one of the most common forms of cancer around the world, but is uncommon in the United States, according to the Mayo Clinic. However, its rates in America are rising. MAPKs are essential components of intracellular signal transduction and are activated in response to various extracellular stimuli, including growth factors, cytokines, and environmental stress. Proinflammatory cytokines such as TNF-α and IL-1 are elevated in liver cell injury induced by hepatitis B and C virus infection.[180,181] Accordingly, these cytokines may activate the p38 MAPK pathway in chronic liver diseases. Of 4 distinct MAPK groups, p38 MAPKs are phosphorylated on Tyr and Thr by a stress-induced signal transduction pathway and activated by proinflammatory cytokines and environmental stress.[10,182] It has been demonstrated that activation of p38 and concurrent inhibition of ERK are critical for induction of apoptosis in rat PC-12 pheochromocytoma cells and other cell lines.[183-188] Recent studies indicate the enzymatic activity of p38 MAPK requires dual phosphorylation of threonine and tyrosine residues by members of the MAPK kinases (e.g., MKK6 and MKK3). MKK6 is identified as a common activator of entire isoforms, whereas MKK3 activates some isoforms of p38 MAPK.[189] Because resistance to apoptosis is a molecular event underlying unrestricted cell growth other than acceleration of cell cycle, a recent study focuses on determining how p38 MAPK and MKK6 activations participate in the apoptosis of human hepatocellular carcinoma (HCC).

Protein Signaling

Recent studies indicate apoptosis is induced by genotoxic stresses, such as UV, X-ray, H_2O_2, heat,[190,191] and cell surface receptor Fas.[192,193] Consistently, blockade of JNK activation promotes cell survival and stimulates p38 MAPK mediated by MKK3/6 and MKK4/7 in human hepatoma cell lines.[47] In addition, it is established that p38 MAPK cascade is involved in the apoptotic pathway, mutant MKK6-induced p38 MAPK activation inhibited cell growth, and induced apoptosis in the 2 hepatoma cell lines, HepG2 and HuH7.[47] Alterations in the apoptosis pathways might lead to unrestricted malignant cell growth. Therefore, there has been recent interest in the interactions between the MAPK pathways and apoptosis because caspases participate in one of the major death process pathways.[194] These observations indicate that activation of p38 MAPK enhances caspase 3 activities, accompanied by apoptosis of hepatoma cell lines.

Results of p38 Activation

Recent findings suggest that p38 MAPK modulates the caspase cascade downstream of Bid cleavage and upstream of cytochrome c release in human hepatoma cell lines. Recently, activation of MAPK superfamily in human HCC tissue specimens was evaluated. p38 MAPK and MKK6 activities in human HCCs were significantly lower than in adjacent uninvolved liver tissue specimens,[47] whereas ERK1/2 activity was significantly higher in malignant lesions from the same tissue specimens. There is a significant positive correlation between p38 MAPK and MKK6 activities in human HCC tissue specimens,[47] which has been clarified by transfecting mutant MKK6 in the modulation of caspase cascade through p38 MAPK activation. The proapoptotic protein Bid, a member of the *bcl*-2 family, is a substrate for caspase-8.[194] Cleavage of Bid is mediated by p38 MAPK during apoptosis in human leukemic cells.[195] Since caspase-8 activation and Bid cleavage in MKK6-transfected hepatoma cells was not observed, caspase-8 and Bid may not be involved downstream of MKK6-p38 MAPK in hepatoma cells. On the other hand, mitochondria are influenced by proapoptotic signal transduction through the p38 MAPK pathway. ASK1, a MAPK kinase kinase that activates p38 MAPK and JNK/SAPK via MKK3/6 and MKK4/7, induces cell death-mediated cytochrome c release from the mitochondria and caspase activation.[196]

The attenuation in the p38 MAPK and MKK6 activities in human HCC tissue specimens may account for the resistance to apoptosis, leading to unrestricted cell growth of human HCCs.[197] The p38 MAPK inhibitor completely blocked p38 MAPK activity and markedly suppressed cell death induced by TNF-α treatment.[198] It was established that TNF-α receptor levels were reduced in HCCs.[199] Therefore, reduced TNF-α apoptotic signals might account, in part, for the downregulation of p38 MAPK cascade in human HCCs. Conversely, there are reports of MAPK regulation

through dephosphorylation by specific protein phosphatases[200,201] and inhibition of p38 MAPK activation through protein phosphatase 2C alpha.[201] The functional alternation of protein phosphatase would serve as another mechanism for the downregulation of the p38 MAPK cascade. Some anticancer drugs induce apoptosis in human cancer cells in association with the p38 MAPK activation.[202,203] Recent studies suggest that p38 MAPK activation induces apoptosis in hepatoma cells. p38 MAPK activation by anticancer drugs or other chemical agents may be a potent approach to targeted therapy for human HCCs. Overall, *in vitro* findings in hepatoma cell lines and the *in vivo* observations of human HCCs suggest that diminished p38 MAPK and MKK6 activities may account, in part, for the resistance to apoptosis, leading to unrestricted cell growth of human HCCs. In support of this notion, p38 activity has been shown to be reduced in hepatocellular carcinomas in comparison to adjacent normal tissue, with tumor size inversely related to p38 activity.[47]

p38 MAP Kinase and the Lung

Lung and bronchial cancer is the top killer cancer in the United States. p38 MAPK is a ubiquitous and highly conserved, proline-directed serine/threonine protein kinase considered important in many processes that are critical to the inflammatory response and tissue remodeling[204] that are hallmarks of pulmonary diseases such as chronic obstructive pulmonary disease (COPD).[205] Although a role for p38 kinase inhibitors in the treatment of pulmonary disease has only recently been postulated,[205,206] it is well known that inflammatory cytokines and chemokines are capable of regulating or supporting chronic airway inflammation.[207] Several reports support the association of p38 kinase activation with a plethora of pulmonary events: LPS and TNF-α-induced intercellular adhesion molecule-1 expression on pulmonary microvascular endothelial cells,[208]

MMP-9 activation,[138] hypoxia-induced stimulation of pulmonary arterial cells,[209] hyperosmolarity-induced IL-8 expression in bronchial epithelial cells,[210] and enhanced eosinophil trafficking and survival.[211,212]

Protein Signaling

Although there is a scantiness of reports specifically addressing the role of p38 kinase in pulmonary disease, it is known that inflammatory cytokines play an important role in airways inflammation. Thus, cytokines such as TNF-α, IFN-γ, IL-4, IL-5, IL-6, and chemokines such as IL-8, regulated on activation normal T-cell expressed and secreted (RANTES), and eotaxin have all been shown to be capable of regulating or supporting chronic airway inflammation,[207] suggesting that p38 MAPK inhibition would have an impact in models that reflect chronic lung diseases.[207] There is also evidence for an involvement of p38 kinase in allergic mechanisms. For example, human monocyte IL-4-induced release of soluble cellular differentiation antigen 23 was dramatically inhibited by the p38 kinase inhibitor SB 203580,[213] suggesting IgE synthesis also might be regulated by p38 kinase activity. In addition, aggregated IgA- or IgG-stimulated eosinophil degranulation has been shown to involve the MAP kinases and phosphatidylinositol 3-kinase.[214]

Results of p38 Activation

TNF-α and IL-1β have been implicated as mediators of LPS-induced airway inflammation[215] by stimulating the release of chemotactic factors from alveolar macrophages and airway epithelial cells and by upregulating the expression of leukocyte and endothelial adhesion molecules.[216] Furthermore, instillation of recombinant TNF-α into the tracheobronchial tree induces chemokine release in bronchoalveolar lining fluid[217] and upregulation of intercellular adhesion molecule-1 (ICAM-1) on pulmonary vascular endothelium.[218] Several therapeutic strategies have been employed to control the release of pro-inflammatory

cytokines such as IL-1 and TNF-α. Recently, attention has focused on the bicyclic pyridinyl imidazole class of compounds such as SB 203580 and SB 202190, which are potent inhibitors of TNF-α and IL-1β release.[219-222] The intracellular target of these compounds is the p38 mitogen-activated protein kinase,[221] a key component in cytokine and stress-induced signal transduction pathways.[223] Analysis of the inhibitory mechanisms of these compounds indicated that the site of action was primarily at the translational level.[222,224]

Several lines of evidence suggest the involvement of TNF-α as a mediator of LPS-induced airway inflammation[215,225-229] mediated through the 55-kDa type 1 TNF receptor (TNFR1). It has been demonstrated that TNFR1-deficient mice exposed to LPS aerosol showed a persistent reduction in neutrophil recruitment to the air spaces of the lungs, in comparison with wild-type animals, that was associated with depressed chemokine levels in BAL fluid.[230] Studies in monocytes[172] and alveolar macrophages[231] have shown that the p38 MAPK pathway is critical for LPS-induced cytokine release. Specific inhibition of the p38 kinase pathway by SB 203580 resulted in reduced cytokine release secondary to a defect in translation. In alveolar macrophages, the regulatory effect of p38 kinase on LPS-induced TNF-α and IL-6 gene expression was mediated partly through changes in gene transcription.[231] The potential reasons for this differential effect are unclear but may be related to the differential expression and the sensitivity of the different p38 kinase isoforms or cell types. Indeed, 4 genes encode the known members of the p38 family, p38α,[221] p38β,[27,232] p38γ,[233] and p38δ.[234,235] Hale *et al.* have shown that the expression of p38 family members is not ubiquitous but is controlled during cell differentiation and in a lineage-specific fashion.[236] Differential expression was most striking in monocytes that strongly expressed p38α, but did not express p38β or p38γ. As blood monocytes differentiated into macrophages, a striking induction of p38δ

mRNA and protein occurred so that p38δ protein became at least as abundant as p38α.[236] In neutrophils, only p38α and p38δ were detected.[237] Although p38γ mRNA was present in endothelial cells, p38γ protein was not detected in any cell type.[236] This is consistent with other reports that p38γ is a muscle-specific protein.[233] Furthermore, in terms of regulation it has been shown that p38α is preferentially activated by MKK-3 in PC-12 cells, whereas p38α is predominantly activated by MKK-6 in monocytes cells, suggesting that p38 is activated by different MKKs in a cell type–dependent manner.[238] Unlike p38α, p38δ was activated by MKK-3, -4, -6, and -7 in an approximately equal manner in 293 T cells, suggesting that the regulation of p38δ may be distinct from p38α. However, it is still not clear whether the regulation of p38δ depends on cell type.[238] A role for p38δ in inflammation was suggested recently by Jiang *et al.*,[235] who reported the activation of renal p38δ during glomerulonephritis in rats. The widely used pyridinyl imidazole inhibitors of p38, SB 203580 and SB 202190, are nearly equipotent against p38α and p38β, but do not inhibit p38γ or p38δ.[27,234,235,239,240] Thus, if p38γ or p38δ are the predominant isoforms involved in the release of TNF-α in BAL fluid following LPS inhalational challenge, this could explain the apparent ineffectiveness of SB 203580. However, the development of inhibitors with greater selectivity for the different isoforms of p38 kinase will undoubtedly allow a more precise definition of the relative contribution of p38 kinases to airway inflammation and airway inflammatory diseases. Alternatively, it may indicate that the release of TNF-α may not be solely controlled via the p38 kinase signal transduction pathway and could be due to the activation of other signaling pathways. In fact, both p38 and ERK are involved in LPS-induced TNF-α production from macrophages *in vitro*.[241] In conclusion, p38 MAPK appears to be involved in the release of IL-1β and the associated sustained phase of neutrophilia following aerosolized LPS. This may

suggest a role for p38 inhibitors in the treatment of airway inflammatory diseases (e.g., septic shock, COPD, and acute respiratory distress syndrome) associated with neutrophilia of the airways.

Functional Role of p38 MAPK

Based on the above discussion, it is quite evident that p38 MAPK plays a critical role in several aspects of cancer cell biology: cell survival,[45] cell death,[115,174] cell differentiation/dedifferentiation,[10] apoptosis,[242] check-point control,[243] overcoming dependence on addictive oncogenic pathways,[244] drug resistance,[78] actin cytoskeletal remodulation,[30] cell migration,[110] invasion,[41] and metastasis.[45,46] In addition, although not discussed in detail here, p38 MAPK as a key regulator of inflammation and immune modulation with respect to tumor progression cannot be undermined. The most common isoform, p38α, has a key role in the production of many cytokines, such as TNF-α, IL-1, and IL-6, which have pro-inflammatory, prosurvival, and angiogenic effects[35] and also regulate cytokine expression by modulating transcription factors, such as NF-κB,[245] or at the post-transcriptional level, by regulating mRNA stability and protein translation, which is mostly mediated by the downstream kinase MK2.[246] Specific deletion of different forms of p38 in genetically engineered mouse models has provided *in vivo* evidence for the importance of this pathway in cytokine production and inflammatory responses (Table 1),[247,248] whereas p38β does not seem to be required for acute or chronic inflammatory responses.[249,250] p38α may also directly affect tumor invasion and angiogenesis independently of its role in inflammation by inducing the expression of the matrix metalloproteinases MMP1, MMP3, and MMP13, which regulate matrix remodeling and degradation by metastatic cancer cells, as well as vascular endothelial growth factor A (vEGFA), a potent inducer of tumor survival and angiogenesis.[251] In addition, it is also reported that p38α can activate hypoxia

inducible factor 1 (HIF1), involved in hypoxia-driven expression of angiogenic factors, at least partly through the stabilization of its α-subunit.[252] p38γ has been associated with Ras-induced invasion[253] and p38δ regulates squamous cell carcinoma invasion.[254] By contrast, the p38 MAPK activator MKK6 has been reported to suppress metastasis[255] or to have no effect,[11] depending on the experimental model used. In addition to facilitating metastasis, p38α also regulates cancer.[110,256,257] MK2 has an important role in cell migration downstream of p38α by remodeling the actin cytoskeleton. This cytoskeletal remodeling may be mediated by the phosphorylation of heat shock 27 kDa protein (HsP27), inducing its release from F-actin caps.[258]

Conclusions and Future Directions

Cells in solid tumors in general live in a hostile environment. They have to live in periods of deprived oxygen, altered pH, and deprived nutrition and avoiding consequences of additional barrage of the host responses, inflammation, and immune surveillance. Thus, it is imperative for their survival that these cells are able to tightly monitor the changes to their environment and adequately respond to such changes.[259] Our discussion as listed above, suggests that mammalian p38 MAPK plays varying roles in normal conditions, but the role of this signaling pathway in solid tumor biology (cell survival, invasion and progression, and inflammatory response) may be critical determinants of tumor cell survival and metastasis, although the mechanisms involved seem to be more diverse and complex than previously anticipated. In addition to other roles, the p38 MAPK signaling pathway may be essential for mere survival of cancer cells in general especially during nutrient deficiency and hypoxia reoxygenation periods. There have been a large number of previous studies examining the presence of different isoforms of p38 MAPK in different types of tumors. Although several of these studies have suggested prognostic significance for different isoforms of p38 MAPK, few studies have described actual usefulness of the individual targets in different types of solid tumors. Moreover, none of the signal transduction pathways exist as individual entities, as such understanding of the interplay between p38 MAPK family members and the crosstalk with other signaling pathways in a context-specific response under normal physiological and pathological conditions is necessary for better understanding the role of this signaling pathway. Systems biology approaches and high-throughput genomic and proteomic technologies may provide valuable insights into these questions. Moreover, the use of genetically modified mice to modulate the activity of the p38 MAPK signaling pathway in a time- and tissue-specific manner as well as to express at physiological levels p38 MAPK proteins with particular mutations will be very useful to elucidate *in vivo* functions to test the effectiveness of p38 MAPK-inhibiting drugs. Irrespective of our understanding of the specific mechanisms, mere preponderance of evidence for the critical role of p38 MAP kinase in various aspects of tumor progression (hypoxic survival, reoxygenation injury, inflammation and immune response modulation, cell migration and invasion, to name a few aspects) would imply that this stress kinase pathway might offer potential therapeutic targets for intervention in solid tumors. Additional studies are warranted to evaluate the differential requirement of the p38 MAP kinase pathway for survival and metastasis of cancer cells, to fill the gaps that exist in our current understanding of the functional cosequences of p38 MAP kinase inhibition with respect to solid tumor biology.

Declaration of Conflicting Interests

Funding

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