MINIREVIEW

Activation and signaling of the p38 MAP kinase pathway

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ABSTRACT

The family members of the mitogen-activated protein (MAP) kinases mediate a wide variety of cellular behaviors in response to extracellular stimuli. One of the four main sub-groups, the p38 group of MAP kinases, serve as a nexus for signal transduction and play a vital role in numerous biological processes. In this review, we highlight the known characteristics and components of the p38 pathway along with the mechanism and consequences of p38 activation. We focus on the role of p38 as a signal transduction mediator and examine the evidence linking p38 to inflammation, cell cycle, cell death, development, cell differentiation, senescence and tumorigenesis in specific cell types. Upstream and downstream components of p38 are described and questions remaining to be answered are posed. Finally, we propose several directions for future research on p38.

Keywords: p38 MAP kinase, signaling pathway, nexus, inflammation, differentiation, senescence, tumorigenesis.

INTRODUCTION

Cellular behavior in response to extracellular stimuli is mediated through intracellular signaling pathways such as the mitogen-activated protein (MAP) kinase pathways [1]. MAP kinases are members of discrete signaling cascades and serve as focal points in response to a variety of extracellular stimuli. Four distinct subgroups within the MAP kinase family have been described: (1) extracellular signal-regulated kinases (ERKs), (2) c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), (3) ERK/big MAP kinase 1 (BMK1), and (4) the p38 group of protein kinases. The focus of this review will be to highlight the characteristics of the p38 kinases, components of this kinase cascade, activation of this pathway, and the biological consequences of its activation.

PROPERTIES OF p38 MAP KINASE MEMBERS

p38α (p38) was first isolated as a 38-kDa protein rapidly tyrosine phosphorylated in response to LPS stimulation [2,3]. p38 cDNA was also cloned as a molecule that binds puridinyl imidazole derivatives which are known to inhibit biosynthesis of inflammatory cytokines such as interleukin-1 (IL-1) and tumor-necrosis factor (TNF) in LPS stimulated monocytes [4]. To date, four splice variants of the p38 family have been identified: p38α, p38β [5], p38γ (ERK6, SAPK3) [6,7], and p38δ (SAPK4) [8,9]. Of these, p38 and p38β are ubiquitously expressed while p38γ and p38δ are differentially expressed depending on tissue type. All p38 kinases can be categorised by a Thr-Gly-Tyr (TGY) dual phosphorylation motif [10]. Sequence comparisons have revealed that each p38 isoform shares ~60% identity within the p38 group but only 40-45% to the other three MAP kinase family members.

REGULATION OF p38 SIGNALING PATHWAY

Extracellular stimuli

p38 activation has been observed in response to a variety of extracellular stimuli in different organisms and homologues of p38 have been identified and cloned in yeast (Hog1 & Spc/Sty1), worm (pmk-2), fly (p38a,b,c), and frog (p38) [1,11-13]. In yeast, the Hog1 & Spc/Sty1 pathways have been implicated in osmoregulation, responses to extracellular stress stimuli, and cell-cycle events [12-14]. Mammalian p38s show similar roles and activation has been shown to occur in response to extracellular stimuli such as UV light, heat, osmotic shock, inflammatory cytokines (TNF-α & IL-1), and growth factors (CSF-1) [1,3,15-21]. This plethora of activators conveys the complexity of the p38 pathway and this matter is further complicated by the observation that activation of p38α is not only dependent on stimulus, but on cell type as well. For example,

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insulin can stimulate p38 in 3T3-L1 adipocytes [22], but downregulates p38 activity in chick forebrain neuron cells [23]. Although the other three p38 group members have been cloned for quite some time now, little is known regarding their activation. Despite research that has shown that all four p38 group members display similar activation profiles [5,8,24,25], differences have been observed in the kinetics and level of activation of these isoforms. Furthermore, the activation of p38 isoforms can be specifically controlled through different regulators and coactivated by various combinations of upstream regulators [24,26].

Upstream kinases that activate p38

Like all MAP kinases, p38 kinases are activated by dual kinases termed the MAP kinase kinases (MKKs). However, despite conserved dual phosphorylation sites among p38 isoforms, selective activation by distinct MKKs has been observed. There are two main MAPKKs that are known to activate p38, MKK3 and MKK6. It is proposed that upstream kinases can differentially regulate p38 isoforms as evidenced by the inability of MKK3 to effectively activate p38β while MKK6 is a potent activator despite 80% homology between these two MKKs [27]. Also, it has been shown that MKK4, an upstream kinase of JNK, can aid in the activation of p38α and p38δ in specific cell types[8]. This data suggests then, that activation of p38 isoforms can be specifically controlled through different regulators and coactivated by various combinations of upstream regulators. Furthermore, substrate selectivity may be a reason why each MKK has a distinct function. In addition to the activation by upstream kinases, there is a MAPKK-independent mechanism of p38 MAPK activation involving TAB1 (transforming growth factor-β-activated protein kinase 1 (TAK1)-binding protein) [28]. The activation of p38 in this pathway is achieved by the autophosphorylation of p38α after interaction with TAB1. Although there is an indication that TAB1-dependent p38 phosphorylation occurs in LPS, TNF, and CpG treated B cell lines, a study using MKK3/6 knockout MEF cells showed that TNF-induced p38 activation is solely dependent on MKKs [29]. While the biological contexts of MKK-independent p38 activation still need further investigation, there is a couple of recent publications that support the role TAB-dependent p38 activation under physiological conditions [30-32]. This suggests that the activation mechanisms of p38 may vary in different cells under various physiological or pathological conditions.

Further upstream activators

The activation of p38 in response to the wide range of extracellular stimuli can be seen in part by the diverse range of MKK kinases (MAP3K) that participate in p38 activation. These include TAK1 [33], ASK1/MAPKKK5 [34], DLK/MUK/ZPK [35,36], and MEKK4 [35,37,38]. Overexpression of these MAP3Ks leads to activation of both p38 and JNK pathways which is possibly one reason why these two pathways are often co-activated. Specific activation of p38 or JNK has been observed, though, implying explicit activation of p38 at this level [39].

Also contributing to p38 activation upstream of MAPK kinases are low molecular weight GTP-binding proteins in the Rho family such as Rac1 and Cdc42 [40,41]. Rac1 can bind to MEKK1 or MLK1 while Cdc42 can only bind to MLK1 and both result in activation of p38 via MAP3Ks [35,42]. p21-activated kinases (PAKs) are yet another group of p38 activators. In vitro data has shown that PAK1, PAK2, and PAK3 are activated by binding to Cdc42 and Rac [41,43,44].

Downregulation of the p38 signaling pathway

Under physiological conditions, MAP kinase activation is often transient despite the unchanging level of MAP kinases throughout the course of stimulation.

Dephosphorylation, then, would seem to play a major role in the downregulation of MAP kinase activity. Many dual-specificity phosphatases have been identified that act upon various members of the MAP kinase pathway and are grouped as the MAP kinase phosphatase (MKP) family [45]. Several members can efficiently dephosphorylate p38α and p38β [46,47]; however, p38γ and p38δ are resistant to all known MKP family members. In addition, other types of phosphatases such as serine/threonine protein phosphotase type 2C (PP2C) has been shown to have a role in downregulating the MAP kinase HOG1 pathway as well as negatively regulating human MKK6 and MKK4 levels *in vitro* and *in vivo* [48-51]. Taken together, these results suggest a mechanism by which p38 isoforms are differentially regulated depending on phosphatase levels and specificity.

DOWNSTREAM SUBSTRATES OF p38 GROUP MAP KINASES

Protein kinase substrates of p38

The first p38α substrate identified was the MAP kinase-activated protein kinase 2 (MAPKAPK2 or MK2) [1, 15,52]. This substrate, along with its closely related family member MK3 (3pk), were both shown to activate various substrates including small heat shock protein 27 (HSP27) [53], lymphocyte-specific protein 1 (LSP1) [54], cAMP response element-binding protein (CREB) [55], transcription factor ATF1 [55], SRF [56], and tyrosine hydroxylase [57]. More recently, MK2 has been found to phosphorylate tristetraprolin (TTP), a protein that is known

to destabilize mRNA hinting at a role for p38 in mRNA stability [58]. MNK1 is another kinase substrate of p38 whose function is thought to reside in translational initiation due to the observation that MNK1 and MNK2 can phosphorylate eukaryotic initiation factor-4e (eIF-4E) [59,60]. p38 regulated/activated kinase (PRAK) is a p38α and/or p38β activated kinase that shares 20-30% sequence identity to MK2 and is thought to regulate heat shock protein 27 (HSP27) [61]. Mitogen- and stress-activated protein kinase-1 (MSK1) can be directly activated by p38 and ERK, and may mediate activation of CREB [62-64]. p38 is also thought to regulate S phase activation of histone 2B (H2B) promoter through OCA-S, a component of p38 [65].

Transcription factors activated by p38

Another group of substrates that are activated by p38 comprise transcription factors. Many transcription factors encompassing a broad range of action have been shown to be phosphorylated and subsequently activated by p38. Examples include activating transcription factor 1, 2 & 6 (ATF-1/2/6), SRF accessory protein (Sap1), CHOP (growth arrest and DNA damage inducible gene 153, or GADD153), p53, C/EBPβ, myocyte enhance factor 2C (MEF2C), MEF2A, MITF1, DDIT3, ELK1, NFAT, and high mobility group-box protein 1 (HBP1) [17,55,66-76] . An important *cis*-element, AP-1 appears to be influenced by p38 through several different mechanisms. ATF-2 is known to form heterodimers with Jun family transcription factors thereby directly associating with the AP-1 binding site [71]. Another possible mechanism comes from the observation that a component of AP-1 is c-fos. c-fos is known to be SRE dependent and SRE is able to bind Ternary Complex Factor (TCF). Ternary Complex Factor is comprised of Sap-1a, a protein that is phosphorylated by p38. Thus, p38 indirectly regulates AP-1 activity. ERK and JNK can also mediate another component of the TCF called Elk-1 [77]. It is thought then that there is coordinated participation of the three MAP kinases in regulation of c-fos expression. Recently, the HBP1 transcription factor has been identified as a substrate for p38. HBP1 has been linked to G1 cell cycle arrest and inhibition of p38 has been shown to decrease HBP1 protein levels [73].

Other types of substrates for p38

cPLA2, Na+/H+ exchanger isoform-1 (NHE-1), tau and keratin 8 have also been reported as substrates for p38α [78-81]. Furthermore, stathmin is another substrate for p38α [27]. Taken together, all the data suggest that the p38 pathway has a wide variety of functions.

GENES REGULATED BY THE p38 PATHWAY

Through the use of inactive and constitutively active mutants of MKK3 and 6 as well as the p38 inhibitor SB203580, numerous genes regulated by the p38 MAP kinase pathway have been identified. These genes encompass a wide range of families including cytokines, transcription factors and cell surface receptors. We have mentioned earlier that about half of p38 substrates identified so far are transcription factors. So, it is obvious that p38 has a role in regulating gene expression at the transcriptional level. Post-transcriptional regulation of inflammatory gene expression has also been linked with the p38 pathway [82, 83]. TNFα and IL-1β steady-state mRNA levels exhibited little or no change when protein synthesis was blocked with p38 inhibitors suggesting a role for p38 in the translation of these transcripts. MK2 knockout mice resulted in impairment of TNFα protein synthesis while TNFα mRNA steady-state levels remained unchanged [84,85]. Furthermore, a genomic deletion of a conserved AU rich element (ARE) in the TNFα 3' untranslated region (UTR) of mice caused overproduction of TNFα and a loss of sensitivity to p38 inhibitors. Taken together, this suggests p38 may act through MK2 to release TNFα mRNA from translational arrest imposed by the ARE [86].

BIOLOGICAL CONSEQUENCES OF p38 ACTIVATION

p38 and inflammation

A strong link has been established between the p38 pathway and inflammation. Rheumatoid arthritis, Alzheimer's disease and inflammatory bowel disease are all postulated to be regulated in part by the p38 pathway [87-89]. The activation of the p38 pathway plays essential roles in the production of proinflammatory cytokines (IL-1β, TNF-α and IL-6) [90]; induction of enzymes such as COX-2 which controls connective tissue remodeling in pathological conditions [91]; expression of intracellular enzymes such as iNOS, a regulator of oxidation [92,93]; induction of VCAM-1 and other adherent proteins along with other inflammatory related molecules [18]. In addition, a regulatory role for p38 in the proliferation and differentiation of immune system cells such as GM-CSF, EPO, CSF and CD-40 has been established [16,94].

p38 and apoptosis

Abundant evidence for p38 involvement in apoptosis exists to date and is based on concomitant activation of p38 and apoptosis induced by a variety of agents such as NGF withdrawal and Fas ligation [95-97]. Cysteine proteases (caspases) are central to the apoptotic pathway and are expressed as inactive zymogens [98,99]. Caspase inhibitors then can block p38 activation through Fas cross-linking, suggesting p38 functions downstream of caspase activation [97,100]. However, overexpression of domi-

nant active MKK6b can also induce caspase activity and cell death thus implying that p38 may function both upstream and downstream of caspases in apoptosis [101, 102]. It must be mentioned that the role of p38 in apoptosis is cell type and stimulus dependent. While p38 signaling has been shown to promote cell death in some cell lines, in different cell lines p38 has been shown to enhance survival, cell growth, and differentiation.

p38 in the cell cycle

The participation of p38α in cell growth has been observed in both yeast and mammals [103]. Overexpression of p38α in yeast led to significant slowing of proliferation while treatment in mammalian cells with p38α/β inhibitor SB203580 slowed proliferation as well. p38 has been implicated in G1 and G2/M phases of the cell cycle in several reports [73,104,105]. G1 arrest of NIH3T3 cells caused by microinjection of Cdc42 was found to be p38α-dependent [105]. Also, as mentioned earlier, a link between p38 and G1 cell cycle control has been proposed through the regulation of p38 substrates HBP1 and p21 [73]. HBP1 is thought to have a role in regulating G1 cell cycle progression through repression of cell cycle regulatory genes, similar in function to retinoblast protein (RB) while the p21 CDK inhibitor is established as a crucial factor in preventing G1 progression through blockage of CDK activity. p38 involvement in G2/M phase is seen through several examples as well. p38α is activated in mammalian cells upon M phase arrest by disruption of the spindle with nocodazole [104]. Furthermore, it has been shown that p38α and p38γ are required for UV-induced G2 cell cycle arrest [106].

p38 and cardiomyocyte hypertrophy

Since p38 is a stress-activated kinase, activation and function in cardiomyocyte hypertrophy has been studied. During progression of hypertrophy, both p38α and p38β levels were increased and constitutively active MKK3 and MKK6-elicited hypertrophic responses enhanced by sarcomeric organization and elevated atrial natriuretic factor expression. Also, reduced signaling of p38 in the heart promotes myocyte differentiation via a mechanism involving calcineurin-NFAT signaling [107].

p38 and development

Despite the non-viability of p38 knockout mice, evidence exists regarding the differential role of p38 in development. p38 has been linked to placental angiogenesis but not cardiovascular development in several studies. Furthermore, p38 has also been linked to erythropoietin expression suggesting a role in erythropoiesis [108-111]. PRAK has recently been implicated in cell development in murine implantation. PRAK mRNA, as well as p38 isoforms, were found to be expressed throughout blastocyst development [112].

p38 and cell differentiation

p38α and p38β have been implicated in cell differentiation for certain cell types. Differentiation of 3T3-L1 cells into adipocytes and PC12 cells into neurons requires p38α and/or β [113,114]. p38 was also found to be required and sufficient for SKT6 differentiation into haemoglobinised cells [115]. More recently, a cross-talk model has been proposed between the p38 pathway and phosphatidylinositol 3-kinase (PI3 kinase)/Akt in the orchestration of myoblast differentiation [116].

p38 in senescence and tumor suppression

p38 now seems to have a role in tumorigenesis and sensescence. There have been reports that activation of MKK6 and MKK3 led to a senescent phenotype dependent upon p38 MAPK activity. Also, p38 MAPK activity was shown responsible for senescence in response to telomere shortening, H_2O_2 exposure, and chronic RAS oncogene signaling [117-119]. A common feature of tumor cells is a loss of senescence and p38 may be linked to tumorigenesis in certain cells. It has been reported that p38 activation may be reduced in tumors and that loss of components of the p38 pathway such as MKK3 and MKK6 resulted in increased proliferation and likelihood of tumorigenic conversion regardless of the cell line or the tumor induction agent used in these studies [29].

DISCUSSSION

Although all research done on the p38 pathway cannot be reviewed here, certain conclusions can still be made regarding the operation of p38 as a signal transduction mediator. The p38 family (α,β,γ,δ) is activated by both stress and mitogenic stimuli in a cell dependent manner and certain isoforms can either directly or indirectly target proteins to control pre/post transcription. p38 MAPKs also have the ability to activate other kinases and consequently regulate numerous cellular responses. Because p38 signaling has been implicated in cellular responses including inflammation, cell cycle, cell death, development, cell differentiation, senescence, and tumorigenesis, emphasis must be placed on p38 function with respect to specific cell types.

Despite all that is known regarding p38 structure and function, many questions still remain. However, new evidence linking p38 to senescence, tumorigenesis and post transcriptional regulation has shed some more light on p38 function and regulation. The activity of p38α has been proven instrumental in cytokine gene expression. However,

the role of p38 becomes nebulous when the influence of p38 transcription factors on cytokine expression is considered. Transcription factors of p38 predicted to influence TNF transcription had little or no effect but new insights on post-transcriptional gene regulation by p38 have begun to elucidate possible mechanisms by which p38 regulates TNF gene expression.

Regulation of the p38 pathway is not an isolated cascade and many different upstream signals can lead to p38 activation. These signals may be p38 specific (MKK3/6), general MAPKKs (MKK4), or MAPKK independent signals (TAB1). Downstream signaling pathways of p38 are quite divergent and each component may interact with other cellular components, both upstream and downstream, to coordinate cellular processes such as feedback mechanisms. Furthermore, *in vivo* p38 is not an isolated event and exists in the presence of other MAP kinases and a plethora of other signaling pathways. The subcellular location of p38 activation may also play a critical role determining the resulting effect and may add yet another order of complexity to the investigation of p38 function. Future work would benefit from attention to the interaction between different pathways, the balance/regulation among signaling events, and subcellular location of p38 activation.

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