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p38^{MAPK}: stress responses from molecular mechanisms to therapeutics

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Abstract

The p38^{MAPK} protein kinases affect a variety of intracellular responses, with well-recognized roles in inflammation, cell-cycle regulation, cell death, development, differentiation, senescence and tumorigenesis. In this review, we examine the regulatory and effector components of this pathway, focusing on their emerging roles in biological processes involved in different pathologies. We summarize how this pathway has been exploited for the development of therapeutics and discuss the potential obstacles of targeting this promiscuous protein kinase pathway for the treatment of different diseases. Furthermore, we discuss how the p38^{MAPK} pathway might be best exploited for the development of more effective therapeutics with minimal side effects in a range of specific disease settings.

Introduction

p38 mitogen-activated protein kinases (p38^{MAPK}) are a class of evolutionarily conserved serine/threonine mitogen-activated protein kinases (MAPKs; see Glossary) that link extracellular signals to the intracellular machinery to regulate a plethora of cellular processes. Along with c-Jun N-terminal kinase (JNK), they are described as stress-activated protein kinases (SAPKs) because they are frequently activated by a wide range of environmental stresses and cytokines to induce inflammation, a key process in the host defence system. Excessive inflammation is a crucial factor in the pathogenesis of several different human diseases, making the MAPK pathway, and in particular p38^{MAPK}, potential targets for development of anti-inflammatory therapeutics [1]. However, more recent studies using specific inhibitors and knockout mice have demonstrated additional diverse roles of p38^{MAPK} in cellular processes including, but not exclusively, regulation of the cell cycle, induction of cell death, differentiation and senescence. This review focuses on the function and regulation of p38^{MAPK}, its role in the pathogenesis of several diseases and how this is currently – and could potentially be – exploited for the development of novel therapeutics against a range of chronic and acute pathologies.

Mammalian p38^{MAPK} pathway

p38^{MAPK} was discovered in a pharmacological screen for the identification of compounds that modulate the production of tumour necrosis factor alpha (TNF α) by lipopolysaccharide-stimulated human monocytic cells [2]. Since then, four isoforms of p38^{MAPK} (α , β , γ and δ) with >60% overall sequence homology and >90% identity within the kinase domains have been described in human tissues. Despite their high sequence homology these isoforms have notable differences in tissue expression, upstream activators and downstream effectors (Table 1), and differ in their sensitivity to chemical inhibitors. p38 α and p38 β are expressed in most tissues and are sensitive to pyridinyl imidazole inhibitors [3], whereas p38 γ and p38 δ have a more restricted pattern of expression and are insensitive to these inhibitors [4]. The various isoforms have been described in different compartments of the same cell, where they can have opposing effects on the same substrate, suggestive of dominant-negative regulatory pathways. However, the specific function of individual isoforms in physiological and pathological processes is not well defined [5,6]. In mice, genetic ablation of p38 α (*MAPK14*) results in embryonic lethality at embryonic day (E)10.5–11.5, a consequence of aberrant placental development and abnormal angiogenesis in the yolk sac and embryo. Disruption of single p38 β , p38 α , p38 δ genes, or double knockouts of p38 γ and p38 δ , results in viable fertile mice with no discernable phenotypic differences [7], ablation of one isotype having no apparent effect on the expression or activity of the other isotypes.

p38^{MAPK} can be phosphorylated by many extracellular stimuli through a classic MAPK kinase kinase (MAP3K)–MAP kinase kinase (MKK) pathway (Figure 1). p38^{MAPK} is inactive in the non-phosphorylated state, becoming rapidly activated by MKK-dependent dual phosphorylation on Thr-Gly-Tyr motifs, located within the regulatory loop between subdomains VII and VIII [4]. This phosphorylation induces a conformational change in the protein, enabling ATP and substrate to bind. The MKK required for phosphorylation of p38^{MAPK} is dependent upon cellular stimulus and cell type (Table 1). MKK3 and MKK6 typically phosphorylate p38^{MAPK} within a few minutes after exposure to the diverse activating stimuli. The duration of phosphorylation is crucial in regulating cell fate, sustained phosphorylation being frequently associated with induction of apoptosis [8,9]; by contrast, transient phosphorylation can be associated with growth-factor-induced survival [10]. Duration of signalling is controlled by phosphatases, including protein phosphatase 1, protein phosphatase 2A or MAPK phosphatases. These enzymes can be activated by phosphorylated p38^{MAPK}, creating a negative feedback loop that tightly regulates active p38^{MAPK} [11]. Crosstalk between different signalling pathways also influences the kinetics of p38^{MAPK} signalling and, consequently, its effect on cell fate [12,13].

Phosphorylated p38^{MAPK} can activate a wide range of substrates that include transcription factors, protein kinases, cytosolic and nuclear proteins (Figure 1). The downstream activities attributed to these phosphorylation events are frequently cell-type-specific and include inflammatory responses, cell differentiation, cell-cycle arrest, apoptosis, senescence, cytokine production and regulation of RNA splicing.

Pathological roles of p38^{MAPK}

p38^{MAPK} in inflammatory disease

Most studies of p38^{MAPK} have focussed on its functions in inflammatory cells and its prominence in cytokine signalling [14], consistent with roles in several chronic cytokine-dependent inflammatory diseases, including rheumatoid arthritis, Crohn's disease, psoriasis and asthma.

Rheumatoid arthritis—This is one of the most common autoimmune diseases and is characterized by thickening of the synovial membrane, with proliferation of macrophage-like and fibroblast-like synoviocytes (MLS and FLS, respectively) accompanied by extensive synovial infiltration of inflammatory cells, including B and T lymphocytes, macrophages and dendritic cells. All four p38^{MAPK} isoforms are expressed in the rheumatoid arthritis (RA) synovium [15], p38 α being the most highly expressed, particularly at the invasive edge of the pannus. Functional research is predominately focused on the biological properties of p38 α and p38 β , reflecting the specificity of current small molecule inhibitors of these protein kinases [16]. The TNF–p38^{MAPK} pathway is a key activator of the pathogenic events of RA, although only about one-third of RA sufferers respond well to anti-TNF therapy. Interestingly, up to a third of all TNF-induced genes in FLS are dependent on p38^{MAPK} signalling [17]. Not only does TNF contribute to destruction of bone and cartilage in RA [18–20], it also inhibits chondrocyte differentiation from FLS and neochondrogenesis [21]. p38^{MAPK} is implicated in the production of TNF, interleukin (IL)-1 β and IL-6 amongst other inflammatory cytokines in the pathogenesis of RA (Table 2). Both MKK3 and MKK6 have been shown to activate p38^{MAPK} in RA in humans [22], although MKK3 alone is largely responsible for p38^{MAPK} activation in the K/BxN passive transfer mouse model of arthritis [23]. Mice lacking MAPK-activated protein kinase (MAPKAP2, a major substrate for direct phosphorylation by p38^{MAPK}) are resistant to the collagen-induced arthritis [24], suggesting that an inhibitor of MAPKAP2 could be of therapeutic value in the treatment of inflammatory diseases such as RA.

Crohn's disease—This is a chronic, relapsing and remitting form of inflammatory bowel disease (IBD), which can involve the entire digestive tract. As in RA, cytokines are important in its pathogenesis, and several of them activate p38^{MAPK}; increased p38^{MAPK} kinase activity is frequently observed in the inflamed colonic mucosa of patients with Crohn's disease (CD) (Table 2). Expression and activity of p38^{MAPK} could be a useful predictive marker of patient response, as resistance to steroid treatment is associated with nuclear expression of p38^{MAPK} in the cells of the lamina propria [25]. Differential p38^{MAPK} pathway activation has also been reported in responders and non-responders to the chimeric TNF monoclonal antibody infliximab, with responders showing increased phosphorylation of activating transcription factor 2 (ATF2) and expression of heat-shock protein (HSP)-70 [26].

Psoriasis—This is a chronic, inflammatory skin disease, characterized by dry red silvery scales, affecting only the superficial layers of the skin. Biopsies of psoriatic lesions display increased levels of phosphorylated p38^{MAPK} in the cytoplasm and nucleus of epidermal cells [27]. The kinase activity of p38 α , p38 β and p38 δ isoforms is increased in these lesions but decreases to similar levels to those found in uninvolved skin as the psoriasis resolves [28]. This is suggestive of a role for p38^{MAPK} in the pathogenesis of this disease. Consistent with this hypothesis, fumaric acid esters, which are sometimes used in the treatment of psoriasis, effectively inhibit the activity of the p38^{MAPK} targets mitogen- and stress-activated protein kinase (MSK)1 and MSK2 [29]; MSK1 and MSK2 expression is frequently increased in psoriatic lesions, where they are thought to contribute to pro-inflammatory cytokine production [30].

Asthma—This is a chronic pulmonary disorder caused by inflammation of the airway leading to broncho-constriction, wheezing and coughing. It is characterized by infiltration of the lung tissue with inflammatory cells, largely caused by type 2 T-helper (Th2) cells, mast cells and eosinophils [31]. This is in contrast to RA, CD and psoriasis, in which the inflammatory response is predominantly effected through Th1 and Th17 T-helper cells [32]. p38^{MAPK} is an important regulator of the Th2 dependent cytokines, IL-4 and IL-13, which

produce monocyte chemoattractant protein (MCP)-1 in lung epithelial cells [33], and IL-9 in mast cells [34]. Furthermore, studies in the MAPKAP2 knockout mouse have shown MAPKAP2 to be an important downstream substrate for p38^{MAPK} in asthma [35], suggesting that this could be a useful target for development of novel asthma therapeutics. Although the majority of studies suggest that asthma is a Th2-dependent disease, there is accumulating evidence that p38^{MAPK} is also involved in Th1-cytokine responses in asthma [36,37]. p38^{MAPK} also has a role in the production of several cytokines, which are activated in allergic asthma (Table 2).

p38^{MAPK} in cardiovascular disease

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in the UK and encompasses several different conditions, including atherosclerosis, hypertension-induced cardiac hypertrophy, myocardial infarction (MI) and cerebral vascular disease. Activation of p38^{MAPK} has been implicated in both detrimental and protective processes in myocardium and the development of CVD. Although several inconsistencies remain to be resolved, some major principles have emerged [38,39]. First, enhanced p38^{MAPK} signalling promotes cardiomyocyte dysfunction, antagonising growth of individual cardiomyocytes and contributing to the development of injury during myocardial ischaemia. Inhibition of this activity is reported to have a cardio-protective effect [38]. Second, activation of p38 α occurs during remodelling of damaged cardiac tissue, for example after MI. Third, atherosclerotic lesions, which are the underlying cause of many forms of CVD, are characterized by lipid-laden macrophage foam cells that arise from p38^{MAPK}-dependent uptake of oxidised-low density lipoprotein (oxLDL) [40]. The mechanisms underlying these incongruent activities of p38^{MAPK} are not fully understood, although they could, in part, reflect important functional differences of p38^{MAPK} isoforms in different aetiologies of CVD [38].

p38^{MAPK} in cancer

The cancer phenotype is characterized by evasion of apoptosis, unlimited replicative potential, invasion and metastases, ability to initiate and sustain angiogenesis, avoidance of oncogene-induced replicative senescence and development of drug resistance [41]. MAPK signalling impacts in some way on all of these processes, with the p38^{MAPK} pathway being most frequently associated with a tumour-suppressor function. Most evidence for this role has come from studies using cell lines and mouse knockout models, where inactivation of the p38^{MAPK} pathway enhances cellular transformation [42,43].

The tumour-suppressive activity of p38^{MAPK} can largely be attributed to the inhibitory effects of the p38 α and p38 β isoforms on G0, G1/S and G2/M cell-cycle-checkpoint controls [44], to promote growth arrest and induction of apoptosis [42,45,46] or cellular senescence [47-49]. Collectively, these data suggest that inactivation of the p38^{MAPK} pathway will enhance cellular transformation by negatively regulating cell survival and proliferation. This hypothesis is supported by the increased tumorigenic potential of fibroblasts in nude mice in which MKK3, MKK6 or p38^{MAPK} have been disrupted [42,50,51], and the dependency of Ras-induced transformation of cells on suppression of p38^{MAPK} function [52,53].

Because p38^{MAPK} is predicted to have a tumour-suppressor function, one might anticipate that its activation would suppress development of the malignant phenotype. Indeed, forced expression of active p38^{MAPK} in rhabdomyosarcoma cells inhibits proliferation and induces terminal differentiation [54]. However, the ability of p38^{MAPK} to suppress tumour-forming capacity does not always correlate with decreased cell proliferation or induction of apoptosis [51], consistent with alternative anti-tumorigenic roles for p38^{MAPK} modulating cell migration and implantation [55,56]. Consistent with its anti-tumour function, induction of

apoptosis by several mechanistically distinct chemotherapeutics is effected in part through activation of p38^{MAPK} [57,58], suggesting that interference with regulators of the p38^{MAPK} pathway could provide novel generic strategies to enhance the efficacy of several conventional therapeutics. The efficacy of such a strategy will depend on whether cancer cells are more susceptible to p38^{MAPK}-mediated apoptosis than non-neoplastic cells. Encouragingly, p38^{MAPK} activity is reported to be decreased in some tumour types compared with that in normal tissues [59,60] and SCIO-469 (a small molecule inhibitor of p38 α) is currently in phase II studies of multiple myeloma [61]. However, more extensive investigations of p38^{MAPK}, its different isoforms and their specific functions in human tumours are needed to establish if this is a genuine tumour-suppressor pathway in human cancer.

p38^{MAPK} in neurodegenerative disease

Aberrant p38^{MAPK} signalling in neural cells contributes to the pathogenesis of many neurodegenerative diseases, including Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and Parkinson's disease (PD). p38 α and p38 β , expressed in the brain, are often activated in animal models of neurodegeneration, leading to altered physiological properties, activation of responsive genes and neurotoxicity [62,63]. Furthermore, p38^{MAPK} is frequently phosphorylated in association with tau deposits in several different tauopathies [64,65], consistent with a putative role in their development.

p38^{MAPK}-induced release of pro-inflammatory cytokines could contribute to the development of pathologies such as AD [66]. However, *in vitro* studies have shown that tau is a good substrate for p38 γ and p38 δ , tau phosphorylation resulting in a reduced capacity to promote microtubule assembly [67]. Because tau-dependent accumulation of neurofilaments is a major hallmark of tauopathies [68,69], these studies suggest that p38^{MAPK}-dependent regulation of tau hyperphosphorylation could contribute to development of some neurodegenerative diseases. Additional substrates of p38^{MAPK} that have been implicated in neurodegenerative diseases include MAPKAPK2 [66,70], c-Jun and ATF2 [63]. Taken together, these observations are consistent with the hypothesis that specific p38^{MAPK} isoforms have a role in the pathogenesis of neurodegenerative diseases, potentially making them attractive therapeutic targets. Although proof of principle experiments in preclinical models have shown that inhibitors of p38^{MAPK} can have neuroprotective effects, an evaluation of inhibitors that are able to bypass the blood-brain barrier is needed to evaluate this in human clinical trials. One potential agent is minocycline; this has a neuroprotective function in animal models of AD, PD, ALS, HD, MS and ischaemia [62,71] that could be attributed in part to inhibition of p38^{MAPK} signalling.

p38^{MAPK} pathways in hyperglycaemia and diabetes

Type 1 diabetes is an autoimmune disease affecting the insulin-producing pancreatic β cells, whereas in type 2 diabetes, the β cells progressively fail with time and have reduced sensitivity to insulin. In diabetes, one result of hyperglycaemia is the generation of reactive oxygen species (ROS), leading to increased oxidative stress and an imbalance of ROS and antioxidants, an important etiological factor in this disease [72]. Increased p38^{MAPK} signalling has been described in both forms of diabetes, and is associated with late complications such as ROS-mediated neuropathy [73] and nephropathy [74]. Consistent with these observations, studies in a hyper-insulinemic mouse model (db/db mice) have demonstrated that p38^{MAPK} signalling is required for progression of nephropathy [75]. Treatment of diabetic rats with the p38^{MAPK} inhibitor SB 239063 is reported to improve both motor and sensory nerve conduction velocity [73]) and elicits an anti-inflammatory

response in vascular smooth muscle of diabetic rats [76], suggesting that the p38^{MAPK} pathway could provide targets for the treatment and/or prevention of late complications in this disease.

p38^{MAPK} and pain

The physical causes of pain can be broadly grouped into nociceptive and neuropathic. Nociceptive (physiological) pain is usually time-limited (RA being a notable exception), and commonly arises as a result of a physical and/or inflammatory event. By contrast, neuropathic pain is frequently chronic and occurs in response to a malfunction or injury to the peripheral or central nervous system. Whereas nociceptive pain usually resolves when the causative tissue damage has been repaired, neuropathic pain is more difficult to manage and can be irreversible depending on the severity of the precipitating lesion. Increasing evidence supports the hypothesis that activation of p38^{MAPK} in spinal microglial cells has a key role in the pathogenesis of neuropathic pain [77-80]. Two isoforms of p38^{MAPK} have been reported in the spinal cord, p38 α in neurons and p38 β in microglia [81]. p38 α seems to have a role in central and p38 β in peripheral sensitization. Activation of p38 β in microglia has also been described in the spared nerve injury model [82] after ventral root lesion [83] and spinal cord injury [84], suggesting that p38 β is an isoform-specific target for the treatment of peripheral pain. Consistent with this hypothesis, spinal delivery of the inhibitor SB203580 attenuates pain in animal models, whereas equal doses of the inhibitor delivered systemically have no effect [85], indicating that these analgesic effects are mediated by local concentrations in the neural compartment. These findings suggest that spinal administration of inhibitors could be a therapeutic option for the treatment of peripheral and central nervous system pain. However, further development and evaluation of inhibitors of p38^{MAPK} capable of crossing the blood-brain barrier remains important. At least two proof of concept studies have reported successful use of p38^{MAPK} inhibitors in the management of peri- and post-operative dental pain; in a phase II study, ARRY-797 demonstrated analgesic benefit when administered either before or after dental surgery (<http://www.arraybiopharma.com>) and in a double-blind randomized study SCIO-469 had analgesic efficacy in post-surgical dental pain [86]. The regulation of current density in dorsal root ganglia by p38^{MAPK}-dependent activation of neurone-specific sodium channel Na_v1.8, provides further evidence for p38^{MAPK} as a therapeutic target for chronic pain [87].

Therapeutic opportunities and limitations

p38^{MAPK} has been a popular target for the design of anti-inflammatory drugs for well over a decade. RA has been the main clinical indication for such inhibitors, the rationale being that inhibition of the p38^{MAPK}-induced stress-response would prevent production of pro-inflammatory cytokines and, therefore, improve this inflammatory condition. More recently, a role for p38^{MAPK} in migration, senescence, apoptosis, proliferation and differentiation suggests that modulation of the p38^{MAPK} pathway could be of therapeutic benefit in a wider group of diseases.

Most inhibitors of p38^{MAPK} have been designed to fit the ATP-binding site (Figure 2) and are highly specific for p38^{MAPK} and its different isoforms [16]. Many have been valuable tools to unravel the complex and diverse biological and pathophysiological roles of the p38^{MAPK} family members. Several of the compounds have proven efficacy in preclinical models and good pharmacological properties. However, even though a number have entered clinical trials for several different indications, many trials have been stopped [88,89] (Table 3). Some studies have been stopped owing to a lack of inhibitor efficacy, although in most cases trials have been halted because of dose-limiting neurological, gastrointestinal and/or cardiovascular toxicities [90]. The main explanation for these toxicities is likely to be the

regulatory and feedback mechanisms in which $p38^{\text{MAPK}}$ participates, crosstalk between different intracellular pathways that interact, inhibit or regulate $p38^{\text{MAPK}}$ by phosphorylation or dephosphorylation limiting its clinical potential [91]. Inhibition of $p38^{\text{MAPK}}$ could, for example, stop feedback loops that suppress activity of upstream regulatory kinases such as TAK1 [92] and mixed lineage protein kinase (MLK2 or MLK3) [93], leading to activation of other pro-inflammatory pathways such as JNK, which in turn can compromise liver function [94]. Inhibition of $p38^{\text{MAPK}}$ might also suppress the beneficial anti-inflammatory effects of MSK1 or MSK2 and the proinflammatory activity of MAPKAPK2 or MAPKAPK3 [95,96]. Cell-specific differences in the function of regulatory kinases will also impact on their exploitation for therapeutic advantage. For example, in most cancers $p38^{\text{MAPK}}$ is associated with growth inhibition and/or pro-apoptotic activity; by contrast, extracellular signal-regulated kinase (ERK) is linked to proliferation; activation of the two pathways is inversely regulated. However, in melanoma both pathways are activated simultaneously and generate a positive feedback loop [56]. Consequently, whereas in most cancers one would anticipate that inhibition of ERK accompanied by activation of $p38^{\text{MAPK}}$ would be beneficial, in melanoma both pathways might have to be inhibited for optimal anticancer activity.

Inhibitors of the ATP-binding site have most frequently been designed to interact with Methionine 109 (Met109), to stabilize inhibitor interaction with $p38\alpha$ [97]. However, these ATP-mimicking inhibitors do not interact with the $p38\gamma$ and $p38\delta$ isoforms, because they lack the methionine residue at position 109 required to stabilize the interaction between the inhibitor and the ATP-binding site. Therefore, in clinical situations in which isoforms other than $p38\alpha$ have been implicated, many of the current inhibitors are likely to be ineffective. Allosteric inhibitors such as BIRB-796 and Kemia, which interact with residues in the kinase specificity region to induce a conformational change in $p38^{\text{MAPK}}$ (called DFG-out) to indirectly prevent ATP binding, should however inhibit all four $p38^{\text{MAPK}}$ isoforms [98]. Such inhibitors could be more effective at blocking $p38\beta$ -, $p38\gamma$ - and $p38\delta$ -dependent pathologies, although toxicity is likely to remain a challenge. Scepticism about the clinical value of inhibitors of $p38^{\text{MAPK}}$ might be tempered if therapeutics could be designed against individual $p38^{\text{MAPK}}$ isoforms, which have proven homologue-specific activity in specific disease states [99]. This might be achieved by targeting isoform-specific regions outside of the highly conserved ATP cleft or by direct inhibition of specific isoform activity using anti-sense or small interfering RNA (siRNA) technology, although low efficacy and therapeutic delivery are major challenges. However, inhibition of $p38^{\text{MAPK}}$ isoforms associated with specific pathologies could still result in unacceptable toxicity owing to crosstalk and feedback within and between regulatory pathways.

There is increasing evidence that developing drugs to upstream regulators or downstream targets of $p38^{\text{MAPK}}$ might be more attractive than directly targeting $p38^{\text{MAPK}}$ or its isoforms [88,89]. Such approaches might be less toxic if targeting specific regulators or substrates that do not interact in feedback and crosstalk loops. These are likely to be regulator- and cell-type-specific, exemplified by the MKK6-dependent suppression of cancer metastases [100] compared with the MKK6-dependent promotion of cell survival or MKK3-induced cell death in cultured cardiomyocytes [101]. However, some targets might be more generally attractive, for example the downstream $p38^{\text{MAPK}}$ target MAPKAPK2, which has been implicated in RA [24], asthma [35] and neurodegenerative disease [66,70].

For pathologies where local delivery of drugs is possible (e.g. ectopic treatment in psoriasis, injection into joints affected by RA, or intrathecal treatment for pain), some current inhibitors of $p38^{\text{MAPK}}$ could be of value. These might be particularly useful in those acute pathologies in which treatment is short (e.g. treatment of oral pain) [102,103]. However, for chronic diseases, such as neurodegenerative disorders, in which treatment could be required

for many years, compounds with a good pharmacological profile (including in some cases the ability to cross the blood–brain barrier) with improved safety profiles must be designed. How inhibitors of p38^{MAPK}, its regulators and substrates might interact and enhance the effectiveness of more traditional therapeutics in combination therapy must also be addressed.

Concluding remarks

Targeting the p38^{MAPK} pathway for therapeutic advantage might at first seem to be a generic strategy to treat a plethora of diseases, given the broad range of pathologies in which this pathway is implicated. However, the diversity of tissue- and pathology-specific functions of p38^{MAPK}, its different isoforms and their integration with other intracellular regulatory pathways creates many challenges to exploiting this pathway for therapeutic benefit.

Probably the biggest hurdle has been the high level of systemic toxicity observed in human clinical trials, toxicity being the main reason for the excessive failure rates for the many inhibitors of p38^{MAPK} that have entered clinical studies since the mid-1990 s. This toxicity most probably reflects the multiplicity of pathways and feedback loops in which p38^{MAPK} is implicated. However, some inhibitors of p38^{MAPK} have completed phase I and phase II trials, although direct proof of concept in the clinic has yet to be demonstrated. Nevertheless, the cell-specific activity of p38^{MAPK} is very precise and tightly regulated, which is one of the reasons why this pathway remains attractive for the design of novel therapeutics.

Inhibitors of p38^{MAPK} have been one of the most intensively studied classes of therapies for the treatment of inflammatory pathologies. Unfortunately, results from many of the clinical trials that have been performed to date are not publically available; this information is important to inform future developments in the field and prevent duplication of effort, which could slow progress. Increasingly, from the clinical trial information that is available and increased understanding of the role that p38^{MAPK} has in several different aetiologies, it seems that developing drugs to specific regulators or substrates of p38^{MAPK} could be a more attractive strategy to exploit this pathway for therapeutic benefit. This will be especially valuable if targets that are druggable, with specific functions that do not impact on other regulatory pathways and feedback loops, can be identified.

The greatest indication for clinical success targeting p38^{MAPK} could well be in the potential cytokine-independent functions of p38^{MAPK}, for example in the treatment of cancer, or where treatment can be delivered locally and over a short period of time for therapeutic benefit, for example in the treatment of dental pain. However, there is the possibility that targeting this pathway to treat a specific pathology could predispose a patient to developing further disorders. For example, agents designed to activate p38^{MAPK} and inhibit tumour growth and metastases might increase the risk of cancer patients developing inflammatory or neurodegenerative diseases. By contrast, inhibition of p38^{MAPK} to protect stroke victims from massive cerebral injury or to treat arthritis might result in an increased susceptibility to developing cancer. Because of these cell-type-specific activities, it will be important when evaluating new therapeutics exploiting the p38^{MAPK} pathway to focus on specific tissues in which dose-limiting toxicity might be anticipated.

A better understanding of the biology underlying the toxicity observed in trials using direct inhibition of p38^{MAPK} is crucial to inform the development of new classes of compounds that might exploit this pathway in the future. It is important to carefully dissect the roles of specific p38^{MAPK} isoforms in the physiology of normal cells and in the pathogenesis of disease, and how they control and are integrated with other regulatory pathways (Box 1). This knowledge should identify which p38^{MAPK} isoforms, substrates or regulators will be

the best targets in specific disease settings for the development of novel therapeutics with high efficacy but acceptable toxicity.

Box 1. Outstanding questions

- What further knowledge is required about the regulation and function of p38^{MAPK} isoforms in normal tissues and different pathologies?
- What are the results and biological explanations for toxicity and lack of efficacy in past trials?
- Can isoform-specific p38^{MAPK} inhibitors (especially γ and δ) be developed to address specific tissue expression and disease states?
- Will the array of pathways with which p38^{MAPK} interacts overcome targeted disruption of isoform-specific p38^{MAPK} functions, potentially preventing a specific desired clinical response?
- Will targeting regulators and/or substrates of p38^{MAPK} produce fewer side effects than targeting p38^{MAPK} directly?
- Can the new generation of p38^{MAPK} inhibitors benefit from advances in delivery (e.g. carriers or even stem cells)?
- Could inhibitors of p38^{MAPK} be exploited for clinical advantage in combination therapy?

Glossary

Angiogenesis	a physiological process involving the growth of new blood vessels from pre-existing vessels.
Apoptosis	a form of cell death involved in development, homeostatic regulation of cell types and destruction of cells that represent a threat to the body. Also known as programmed cell death.
Inhibitors of the ATP-binding site	small molecules that target residues within the ATP-binding site of protein kinases to inhibit kinase activity.
Cellular senescence	a process by which normal diploid cells enter an irreversible state owing to the loss of their ability to divide.
Differentiation	the process by which cells become progressively more specialized in cell type and function.
Knockout models	models in which usually one gene has been inactivated to study its biological effect and from which the probable function of that gene can be deduced.
Metastasis	the process by which tumours spread from the place of origin to distant locations in the body.
Mitogen-activated protein kinases (MAPKs)	a superfamily of serine/threonine protein kinases that phosphorylate substrates with a threonine-x-tyrosine motif, where x can be glutamate, proline or glycine.
RNA splicing	a process that removes introns and joins exons in a primary transcript. This is required to produce the correct protein during translation.

Tauopathy	a class of neurodegenerative diseases involving accumulation of the tau protein.
Tumour-suppressor function	protects a cell from the development of cancer. Mutations in genes encoding proteins with such activity result in loss or reduced function and can contribute to the development of cancer.

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Figure 1.

Schematic representation of the p38^{MAPK} signalling pathway. A variety of extracellular signals, such as cellular stresses and pro-inflammatory cytokines, can activate the p38^{MAPK} pathway. These lead to the initiation of a three-tiered MAPK phosphorylation cascade in which MAPKKKs phosphorylate the p38^{MAPK}-specific MAPKKs MKK3, MKK4 or MKK6. These subsequently phosphorylate four isoforms of p38^{MAPK} (α, β, δ and γ) and three alternatively spliced variants of p38α and p38β: Mxi2, Exip (both p38α variants, dark blue boxes) and p38β2 (p38β variant, pale blue boxes). Of note, only ASK1 can activate MKK4, which specifically activates p38α, and MKK6 preferentially phosphorylates p38β. It is not known which MKKs phosphorylate the splice variants (dashed line). p38^{MAPK} substrates can be isoform-specific or common to all isoforms. Substrates are coloured according to function: protein kinases (purple), transcription factors (orange) and cytosolic and nuclear proteins (green). Phosphorylated substrates go on to elicit varied biological responses that include inflammation, apoptosis, proliferation, cell-cycle regulation and differentiation. Dashed lines indicate potential links yet to be confirmed. Abbreviations: ASK1, Apoptosis signalling kinase 1; CHOP, CCAAT/enhancer-binding protein-homologous protein; GS, Glycogen synthase; MEKK, MEK kinase; MNK, MAPK interacting kinase; PRAK, p38-regulated/activated protein kinase; p75^{NTR}: p75 neurotrophin; STAT1, signal transducer and activator of transcription 1; TAK1, transforming growth factor activated kinase 1; TAO, thousand and one amino acid kinase.

Figure 2. p38^{MAPK} activation and inhibition. **(a)** p38^{MAPK} is activated in multiple steps. ATP is required for phosphorylation of the TGY motif on the activation loop. Once this has occurred, there is a conformational change in the kinase, exposing a binding site for a substrate. **(b)** Classical pyridinyl imidazole inhibitors (e.g. SB203580) are similar in shape to ATP molecules and compete for the ATP-binding site. Once the inhibitor is bound, ATP is blocked and phosphorylation of the TGY motif does not occur, leaving p38^{MAPK} inactive. **(c)** Newer p38^{MAPK} inhibitors (e.g. BIRB796) can act on distinct sites on the enzyme, which then cause a conformational change in the ATP-binding site, again blocking ATP and preventing activation.

Properties of p38^{MAPK} isoforms^a

Table 1

Isoform and splice variant	Pseudonyms	Chromosome location	Upstream MKK	Isoform-specific substrates	Expression	Function	Disease
α	SAPK2a, CSBP	6p21.3-p21.2	MKK3, MKK6 or MKK4	MAPKAPK-2, PRAK	Ubiquitous	Stress response differentiation, inflammation, cell cycle, myogenesis, proliferation	CVD, psoriasis, IPF, cancer, ND, pain, inflammatory disease
Mxi (α variant)		6p21.3-p21.2	Not MKK3 or MKK6	Max	Nucleus, distal portion of nephrons	Aids ERK nuclear entry	Not identified
Exip			Not MKK6	Unspecified		HeLa cell apoptosis	Not identified
β	SAPK2b, p38-2	22q13.33	MKK6	MAPKAPK-2, PRAK, c-Jun, GS	Ubiquitous	Stress response, inflammation, proliferation	Psoriasis, cancer, inflammatory disease, pain
$\beta 2$ (β variant)		22q13.33	MKK6	p75 ^{NTR}		Induces NF- κ B activity, resulting in decreased AP1 activation	Not identified
δ	SAPK4	6p21.31	MKK3 or MKK6	Transcription factors, statmin, eEF2K, Tau	Lung, pancreas, kidney, testis, epidermis	Development, differentiation, keratinocyte apoptosis proliferation	Cancer, psoriasis, tauopathies, RA
γ	SAPK3, ERK6	22q13.33	MKK3 or MKK6	Transcription factors, Tau, Rit	Skeletal muscle, heart, lung, thymus, testis	Regulates basal glucose uptake during exercise, proliferation	Tauopathies, IPF

^aAbbreviations: CSBP, cytokine-suppressive anti-inflammatory drugs binding protein; Erk CVD, cardiovascular disease, eEF2K, elongation factor 2 kinase; GS, glycogen synthase; IPF, idiopathic pulmonary fibrosis; ND, neurodegeneration; NF- κ B: nuclear factor-kappa B; PRAK, p38-regulated/activated protein kinase; p75^{NTR}, p75 neurotrophin; RA, rheumatoid arthritis.

Table 2

Inflammatory disease pathways in which p38MAPK has been implicated^{a,b}

Disease	Cell type	Stimulus	Product	Refs
Rheumatoid arthritis	FLS	MDP	IL-6, IL-8	[104]
	FLS	Adiponectin	IL-6	[105,106]
	FLS	SAA	IL-6	[107]
	FLS	IL-17	IL-23p19	[108]
	FLS	IL-1 β	IL-6	[22]
	FLS	IL-18	SDF- α	[109]
Psoriasis	MLS	CD40 ligation	TNF, IL-1 β	[110]
	Neutrophil	TNF	Mac-1	[111]
	Synovial cell line	Spontaneous	IL-6, IL-8, MMP-13	[112]
	Osteoblast cell line	IL-1 β	COX-2, PGE2	[113]
	Keratinocyte	IL-1 β	TNF	[114]
	Keratinocyte	IL-1 β	IL-20	[115]
	Keratinocyte	Corticotrophin releasing hormone	IL-18	[116]
	Keratinocyte	IFN- β with IL-18	CXCL9/10/11	[117]
	Neutrophil	Antimicrobial agents: psoriasin, cathelicidin	IL-8, ROS	[118,119]
	Dendritic cell	HSP70, CD40 ligand	TNF	[120]
Crohn's Disease	Colon epithelium	<i>E. coli</i>	IL-8	[121]
	Macrophage	TLR ligands	IL-6, TNF, MCP-1	[122]
	Plasma cell	Norepinephrine	IgE	[123]
Asthma	ASMC	IL-17	TNF, IL-8	[124]
	ASMC	IL-17 with IL-1 β	CXCL8	[125]
	ASMC	IL-17	Eotaxin	[126]
	Bronchial epithelium	IL-31	CCL2	[127]
	Lung epithelium	IL-4, IL-13	MCP-1	[33]
	Lung macrophages	LPS	TNF, L-6, GM-CSF	[128]

^a Abbreviations: ASMC, airway smooth muscle cell; CD, cluster designation; COX-2, cyclo-oxygenase-2; GM-CSF, granulocyte monocyte-colony stimulating factor; HSP70, heat shock protein 70; IFN- γ , interferon- γ ; IgE, immunoglobulin E; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MDP, muramyl dipeptide; MMP13, matrix metalloproteinase 13; PGE2, prostaglandin E2; SAA, serum amyloid A; SDF α , stromal derived factor- α ; TLR, toll-like receptor.

^b Inhibitors used: SB203580 in Refs [33, 105-109, 111, 113, 116-121, 124-127]; DN-MKK3/6 in Ref. [22]; SB202190 in Refs [110, 114, 115]; R-130823 in Ref. [112]; and PCG in Ref. [128]. SB203580 and SB202190 inhibits p38^{MAPK}_α and p38^{MAPK}_β; R-130823 inhibits p38^{MAPK}_α.

Table 3

Summary of p38MAPK inhibitors that have reached clinical trials^{a,b}

Inhibitor ^c	Company	Disease indication	Phase	Adverse effects and comments
AMG-548	Amgen	COPD ^d , RA ^d	I	Elevated liver enzymes.
ARRY-797	Array Biopharma	AS ^e , Dental pain, RA	I/II	Aching, conjunctival and ocular hyperaemia, dizziness, diarrhoea, headache, nausea.
ARRY-614	Array Biopharma	MDS ^e	I	
AZD-6703	Astra Zeneca	RA ^d	I	
AVE-9940	Sanofi-Aventis	RA ^d	I	
BIRB796 (Doramapimod)	Boehringer Ingelheim	Crohn's disease ^d , psoriasis ^d , RA ^d	II/III	Elevated liver enzymes, no efficacy in RA, some histological response in Psoriasis.
BMS-582949	Bristol-Myers Squibb	Atherosclerosis ^e , psoriasis, RA ^e	II	
GSK-681323 (SB-681323)	GlaxoSmithKline	COPD, neuropathic pain, patients with coronary heart disease undergoing percutaneous coronary interventions, RA ^e	II	Active RA trial is using [¹⁴ C]SB-681323 preparation; trials with non-radioactive SB-681323 completed.
GSK-856553 (losmapimod)	GlaxoSmithKline	Atherosclerosis ^e , COPD ^e , CVD, depression	II	
GSK-610677	GlaxoSmithKline	COPD	I	Inhaled preparation. Dose escalation trial in healthy volunteers completed.
KC706	Kemia	RA ^c , metabolic disorders ^d , CVD ^d , pemphigus vulgaris ^d	II	
PH-797804	Pfizer	COPD ^e , Neuropathic pain associated with post-herpetic neuralgia, RA	II	
SC80036	Pfizer	RA ^d		
PS-540446	Pharmacopoeia	Psoriasis, RA	I	
RO4402257 (Pamapimod)	Roche	RA ^d	II	No efficacy. Elevated liver enzymes, GI disorders, infection, RA flare, skin disorders.
RO3201195	Roche	RA ^d	I	
RJW-67657	Johnson and Johnson	Inflammatory disease ^d	I	
SCIO-469	Scios Inc, Johnson and Johnson	Dental pain, MDS, multiple myeloma ^e , RA ^d	II	Cutaneous lesions, dizziness, light headedness, liver enzyme abnormalities. Some evidence for efficacy in dental pain.
SCIO-323	Scios Inc, Johnson and Johnson	Cerebral ischemia ^d , diabetes ^d , MDS ^d , RA ^d	I	Cutaneous lesions

Inhibitor ^c	Company	Disease indication	Phase	Adverse effects and comments
TAK-715	Takeda	RA ^d	II	
VX-745	Vertex	RA ^d	II	GI and liver toxicity, neurological problems in dogs
VX-702	Vertex	Acute coronary disease ^d , RA ^d	II	Infection, skin disorders, renal impairment

^aThe information was compiled from reviews cited in the text, www.clinicaltrials.gov and from company websites. Some data could be incomplete, reflecting the paucity of data from some trials.

^bAbbreviations: AS, ankylosing spondylitis; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; GI, gastrointestinal; MDS, myelodysplastic syndromes; RA, rheumatoid arthritis.

^cInhibitor targets: AMG-548, ARRY-797, ARRY-614, AZD-6703, AVE-9940, KC706, RO4402257, RO3201195, SCIO-469, SCIO-323, VX-745 and VX-702 used p38 α ; BIRB796 used p38 α , β , γ and δ , and the specific isoform information is unknown for BMS-582949, GSK-681323, GSK-856553, GSK-610677, PH-797804, SC80036, PS-540446, RJW-67657 and TAK-715.

^dDiscontinued.

^eClinical trial currently active or recruiting patients.