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# **Therapeutic Regulation of Cardiac Fibroblast Function: Targeting Stress-Activated Protein Kinase Pathways**

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## **Summary**

Cardiac fibroblasts (CF) are key players in the myocardial remodeling process and respond to myocardial damage or dysfunction by adopting a myofibroblast phenotype and undergoing increased proliferation, migration, secretion of bioactive molecules and turnover of the extracellular matrix. Many of the key cellular responses of the heart to injury or stress are mediated via specific signaling cascades involving the stress-activated protein kinases (SAPKs). The SAPKs comprise the p38 MAPK and c-Jun N-terminal kinase (JNK) families, both of which have been implicated in promoting myocardial damage and adverse cardiac remodeling. This review focuses on SAPK signaling cascades in the heart, with particular emphasis on their modulatory effects on CF function, inflammation and fibrosis. It also describes current and future therapeutic strategies for inhibiting SAPKs in the myocardium. Understanding the role of SAPK signaling at the cellular level holds potential for developing novel therapies to ameliorate cardiac dysfunction in man.

## **Keywords**

Cardiac fibroblast - stress-activated protein kinase - p38 - JNK - myocardial remodeling - therapeutic agents

## **Introduction**

Adverse structural remodeling of the myocardium is the major contributor to morbidity and mortality associated with multiple cardiovascular diseases including hypertension, myocardial infarction, cardiomyopathy and heart failure. Both the major resident cell types of the heart, namely cardiomyocytes and cardiac fibroblasts (CF), play important yet divergent roles in the myocardial remodeling process. Cardiomyocytes are responsible for providing the contractile force of the heart and undergo hypertrophy in an attempt to compensate for cardiac dysfunction. In contrast, CF undergo cell proliferation and remodel the extracellular matrix (ECM) by altering the balance of ECM synthesis and degradation, in order to facilitate adaptation to altered myocardial function. Many such responses of myocytes and fibroblasts in the heart are regulated by the mitogen-activated protein kinase (MAPK) intracellular signaling cascades. In particular the stress-activated protein kinases (SAPKs), comprising the p38 MAPK and c-Jun N-terminal kinase (JNK) families, are activated by the ischemic and inflammatory stresses associated with myocardial injury and have been implicated in promoting myocardial damage and remodeling.

This review describes the key roles played by the p38 MAPK and JNK families in the heart, with particular emphasis on their modulatory effects on CF function with respect to fibrosis and inflammation. By identifying and understanding the function of myocardial SAPK pathways in a cell type-specific context, it is hoped that therapeutic strategies can be designed to reduce adverse myocardial remodeling and improve clinical outcomes in man.

## **Myocardial Fibrosis and Inflammation**

Cardiac fibrosis, the disproportionate deposition of collagen-based ECM within the cardiac interstitium, is a common feature of ischaemic, dilated and hypertrophic cardiomyopathies and results in increased myocardial stiffness, ventricular dysfunction and arrhythmia,

ultimately leading to heart failure [1,2]. Cardiac fibrosis can be broadly classified as ‘reparative’ or ‘reactive’ depending on the initiating stimulus and the pattern of collagen deposition [1,2].

Reparative fibrosis describes the response of the heart to acute injury, for example scar formation after myocardial infarction (MI). Post-MI remodeling is characterized by a number of distinct phases including cardiomyocyte injury and death, acute inflammatory response, formation of granulation tissue, degradation of ECM, neovascularization, deposition of new ECM to form a scar, and ultimately resolution of the inflammatory response [3,4]. Pro-inflammatory and profibrotic cytokines and chemokines play integral roles in orchestrating reparative fibrosis of the infarcted myocardium by temporally altering the balance between ECM deposition (e.g. type I and III collagen synthesis) and degradation (increased matrix metalloproteinase (MMP) activity and/or decreased activity of their native inhibitors, TIMPs) [5].

Reactive fibrosis involves chronic deposition of collagen-based ECM in the absence of acute injury, for example in response to hypertension-induced pressure overload or in non-infarcted regions of the post-MI heart. Reactive fibrosis represents an adaptive compensatory response aimed to increase the contractile force of the heart and is mediated by elevated levels of profibrotic hormones and growth factors and activation of mechanosensitive receptors [6,7]. Together, these changes alter the balance of ECM turnover in favor of collagen deposition.

Dissecting the cellular and molecular mechanisms underlying cardiac fibrosis and inflammation is imperative for designing therapeutic strategies for prevention and treatment of pathologies associated with adverse myocardial remodeling. Fibroblasts are the major producers of ECM proteins in the heart and CF are highly responsive to proinflammatory stimuli. As such, the CF represents an important cellular target for anti-fibrotic strategies.

## **Cardiac Fibroblasts**

Whilst cardiomyocytes occupy most of the cellular volume of the heart, the smaller CF are actually the most prevalent resident cell type in the adult human and rat heart, accounting for up to two-thirds of total cells [8,9]. Other non-myocyte cells permanently resident in the myocardium include vascular endothelial and smooth muscle cells, neuronal cells, mast cells and progenitor cells, which together account for an estimated 10% of resident cardiac cells [8,9]. Interestingly, a FACS-based analysis of the relative proportion of different cell types within adult murine heart revealed that CF are less prevalent (one-quarter of total cells) than in rat heart [9]. Irrespective of the absolute proportion of fibroblasts in the heart, this cell type has historically been under-represented in the scientific literature in comparison to cardiomyocytes. Although widely recognized as regulating turnover of ECM, it is becoming increasingly apparent that CF also have wide-reaching functions that are fundamental to the overall development, physiology and pathophysiology of the heart [10-13]. CF are in close contact with myocytes and vascular cells within the myocardium and may regulate cardiac electrophysiology through heterotypic cell-cell coupling via gap junctions [14,15]. CF also secrete a wide range of bioactive molecules including growth factors, cytokines and vasoactive peptides that can modulate local myocardial cell function through autocrine/paracrine actions [10]. Following damage to the heart (e.g. after MI or with pressure overload), CF undergo a series of functional changes that drive structural remodeling of the myocardium (see Box 1) [10]. These events initially serve to compensate for lack of cardiac function, but ultimately may lead to fibrosis and development of heart failure. In response to altered mechanical strain or specific humoral factors such as transforming growth factor- $\beta$  (TGF- $\beta$ ) or angiotensin II (Ang II), CF undergo phenotypic modulation to become myofibroblasts, differentiated cells that exhibit increased migratory,

proliferative and secretory properties, and express contractile proteins such as  $\alpha$ -smooth muscle actin ( $\alpha$ SMA) [12]. Recent evidence suggests that in addition to derivation from resident CF, a proportion of myofibroblasts in the remodeling heart originate from hematopoietic bone marrow-derived fibrocytes, monocytes or endothelial cells [10-13]. In response to proinflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and IL-6, myofibroblasts undergo increased proliferation and migration, and actively remodel the cardiac interstitium by increasing secretion of MMPs, increasing collagen turnover and secreting an array of growth factors and cytokines [10] (Box 1). The multifactorial role of CF in the myocardial remodeling process therefore makes them an attractive therapeutic target, distinct from the cardiomyocyte.

### **Mitogen-Activated Protein Kinase (MAPK) Signaling Pathways**

MAPK signaling pathways are highly conserved three-tier phosphorylation/activation cascades that play fundamental roles in regulating every aspect of cell behavior from development, growth, survival, metabolism and movement to cell death. There are four classical MAPK signaling cascades, incorporating extracellular signal-regulated kinases (ERK1/2), p38 MAPKs, JNKs and ERK5 [16-18]. Additional atypical MAPKs (ERK3/4, ERK7, NLK) have also been identified [19], but these have not been well studied in the heart and will not be reviewed here. The canonical MAPK signaling cascade (Fig. 1) involves a series of specific phosphorylation events in which a MAP kinase kinase kinase (MAPKKK) phosphorylates and activates a MAP kinase kinase (MAPKK), which in turn phosphorylates and activates a MAPK. The dual phosphorylation of MAPK occurs on specific threonine and tyrosine residues within a conserved Thr-X-Tyr (TXY) motif, where X is Glu (ERK-1/2, ERK5), Gly (p38) or Pro (JNK). MAPK pathways exhibit a high degree of specificity, but also display crosstalk, synergism and redundancy [16-18]. Together, these signaling cascades

operate across species to regulate numerous aspects of cellular function through phosphorylation of a plethora of downstream substrates situated within the nucleus, cytosol, cytoskeleton, mitochondria and plasma membrane of the cell (Fig. 1).

The ERK-1/2 (p44/42-MAPK) pathway is activated predominantly by mitogenic stimuli (e.g. growth factors and G-protein coupled receptor ligands), and to a lesser degree by stress stimuli (e.g. proinflammatory cytokines), and is important for promoting cell proliferation, motility, differentiation and survival. The p38 and JNK pathways are activated primarily by environmental stresses and inflammatory cytokines, and as such are collectively known as stress-activated protein kinases (SAPKs). SAPKs can also be activated by mitogenic stimuli, albeit to a smaller extent. SAPK activation is often coupled to apoptotic and inflammatory responses. The more recently described ERK5, also known as big MAPK (BMK), is activated by both growth and stress stimuli and is generally coupled to increased proliferation and cell survival. The roles of ERK1/2 and ERK5 will not be discussed further, but the reader is referred to some excellent recent reviews on the roles of these MAPKs in the heart [16-18].

### **Stress-Activated Protein Kinases (SAPKs)**

#### **p38 MAPK**

The p38 MAPK pathway is activated primarily by physical and chemical stresses and proinflammatory cytokines [16,17,20]. There are four known subtypes of p38 MAPK; p38 $\alpha$  (also termed SAPK2a/MAPK14), p38 $\beta$  (SAPK2b/p38-2/MAPK11), and the more distantly related p38 $\gamma$  (SAPK3/ MAPK12/ERK6) and p38 $\delta$  (SAPK4/MAPK13); each of which is encoded by a separate gene. Several alternatively spliced forms of p38 $\alpha$  have been described (e.g. CSBP1, Mxi2, Exip) and p38 $\beta$  also exists as two splice variants (p38 $\beta$  and p38 $\beta$ 2).

P38 $\alpha$  and  $\beta$  share high sequence homology and can be inhibited by pyridinyl imidazole



compounds (e.g. SB203580), whereas the  $\gamma$  and  $\delta$  subtypes are less homologous and resistant to inhibition by SB203580 and other similar inhibitors [21]. Although p38 $\alpha$  appears to be ubiquitously expressed, expression of p38 $\beta$ ,  $\gamma$  and  $\delta$  is restricted to particular tissues and cell types [22,23]. The canonical p38 MAPK signaling pathway (Fig. 2) involves phosphorylation of p38 on threonine and tyrosine residues in the TGY activation motif by the dual-specificity kinases MKK3 and MKK6 (and possibly MKK4). These MAPKKs are activated by upstream MAPKKKs including MEKKs 1-4, MLK3, TAK1 and ASK1. The downstream substrates of the p38 family include transcription factors (e.g. ATF2, ELK1, CREB, MEF2, p53 etc) as well as additional kinases such as MAPK-activated protein kinases (MAPKAPK2/3), MAP kinase interacting kinases (MNK1/2) and mitogen and stress-activated protein kinases (MSK1/2) [16,17,20].

Although the four p38 MAPK subtypes possess structural homology at the protein level and share a number of upstream activators and downstream substrates, several differences in their regulation and functional coupling are apparent. For example, MKK3 and MKK6 can differentially activate individual p38 subtypes depending on the initiating stimulus [24,25]. Furthermore, non-canonical activation of p38 $\alpha$  can be induced through a unique mechanism involving the TAB1 scaffold protein which promotes autophosphorylation of p38 $\alpha$ , but not the other p38 subtypes [26]. These levels of specificity are likely due to subtle differences in protein structure, and may be influenced by binding of p38 subtypes and MKKs to scaffolding proteins of the JNK-interacting protein (JIP) family (e.g. JIP-2, JIP-4, OSM) [27]. However, the role of JIPs is less well defined for p38 than it is for JNK (see below). The duration and amplitude of p38 signaling is regulated by negative feedback involving dephosphorylation of the conserved Thr and Tyr residues by serine/threonine phosphatases (e.g. PP2A, PP2C), tyrosine phosphatases (e.g. HePTP, STEP) and dual-specificity phosphatases (e.g. MKPs 1, 2, 4, 5, 7) [16,28].

## **JNK**

Members of the JNK family of SAPKs are activated primarily by stress stimuli and proinflammatory cytokines [16,17,29,30] (Fig. 3). JNKs are so-called because one of their earliest described actions was that of phosphorylating the N-terminal transactivation domain of c-Jun, a component of the AP-1 transcription factor. JNKs have subsequently been found to phosphorylate several additional transcription factors including JunD, ATF2, ELK1, p53, SMAD4, NFAT4 and HSF1 [17]. There are at least 10 human JNK isoforms derived from differential splicing of 3 genes (Jnk1, Jnk2 and Jnk3). JNK1 (SAPK $\gamma$ /1c) and JNK2 (SAPK $\alpha$ /1a) are ubiquitously expressed, whereas JNK3 (SAPK $\beta$ /1b) expression is localized mainly to the central nervous system. All three JNKs exist as both short (46-48 kDa) and long (54-57 kDa) forms with distinct C-termini due to differential splicing [31]. JNKs are activated by dual phosphorylation in the TPY activation motif by the upstream dual-specificity kinases MKK4 and MKK7, which themselves are activated by several MAPKKKs including MEKKs, MLKs, ASK1 and TAK1 (Fig. 3). JNKs are dephosphorylated and inactivated by a range of protein phosphatases including serine/threonine and tyrosine phosphatases, as well as the dual-specificity phosphatases MKP-1, 2, 5 and 7 [28]. The sequential components of the JNK pathway are physically associated through binding to particular scaffolding proteins (e.g. JIPs 1-4) that confer signal specificity and compartmentalization [27] (Fig. 3). Nuclear translocation of JNK enables transcription factor phosphorylation and altered expression of an array of genes, several of which play key roles in apoptosis [29]. In addition to these genomic effects, JNKs are also important for inducing cell death by inhibiting anti-apoptotic proteins (e.g. Bcl-2), stimulating pro-apoptotic proteins (e.g. Bad, Bim) and promoting mitochondrial cytochrome c release [29].

## **SAPK Signaling in the Heart**

SAPK signaling pathways play important roles in regulating many aspects of cardiac function. In addition to roles in normal physiology and development, SAPK pathways are activated in response to a range of stimuli associated with myocardial dysfunction including oxidative stress, inflammatory cytokines and altered mechanical force [16,18,32]. The vast majority of stimuli that activate the p38 pathway in the heart also activate the JNK pathway, albeit with differences in the magnitude and time course of activation. However, there is also limited evidence of differential activation of the p38 and JNK pathways in response to the same stimulus in myocardial tissue and cells. A particularly good example is the signaling response to myocardial ischemia. In perfused rat heart, ischemia activates p38 MAPK but does not activate JNK; subsequent reperfusion activates both p38 and JNK pathways [33]. Similarly, exposure of cultured cardiac myocytes or myoblasts to simulated ischemia in vitro activates the p38 pathway without stimulating the JNK pathway, whereas reperfusion activates both pathways [34,35]. Interestingly, reoxygenation-induced activation of the JNK pathway in hypoxic cardiomyocytes in vitro was not observed in parallel cultures of reoxygenated CF [36], suggesting cell-type specific signaling occurs in response to ischemia/reperfusion injury in the heart. Indeed, cell-specific ERK and p38 MAPK signaling has been observed in vivo following experimental myocardial infarction [37].

The roles of individual p38 subtypes ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) and JNK isoforms (JNK1, 2, 3) in translating stress signals into changes in cardiac function are discussed below.

### **p38 MAPK subtypes**

The most highly expressed p38 MAPK subtype in the adult human heart is p38 $\alpha$ , with lower levels of p38 $\gamma$  and p38 $\delta$ , and no detectable p38 $\beta$  mRNA or protein [38]. Similarly, adult mouse and rat hearts express mostly p38 $\alpha$ , with p38 $\beta$  protein expression occurring at low or

undetectable levels [39-44]. P38 $\gamma$  protein has been detected in adult cardiac tissue from a number of species [22,38,45], but p38 $\delta$  has relatively low expression in the heart [38,45].

### **p38 $\alpha$**

P38 $\alpha$  (often referred to simply as p38) is by far the most well studied isoform and has been investigated in the heart using a range of approaches. In contrast to the other p38 subtypes, p38 $\alpha$  knockout mice die in utero due to placental abnormalities [46]. Therefore in vivo studies on myocardial p38 $\alpha$  function have relied on pharmacological p38 inhibitors [21], p38 $\alpha$ <sup>+/-</sup> heterozygous animals [40], dominant negative (DN) p38 mutants [47] or modulation of upstream/downstream components of the p38 signaling pathway [47]. More recently, application of conditional Cre-lox technology to delete the p38 $\alpha$  gene in particular cell types has overcome the lethality of the systemic knockout and provided a useful tool for investigating the function of p38 $\alpha$  at the level of the cardiomyocyte in in vivo murine models [43,48].

Since the role of p38 $\alpha$  in the heart has been extensively reviewed in recent years [16,18,49-51], only a summary will be provided here. Much of the evidence for the importance of p38 $\alpha$  has been acquired through the use of pharmacological inhibitors of p38 $\alpha/\beta$  (e.g. SB203580). P38 $\alpha$  is generally viewed as a critical mediator of cardiac dysfunction, making it an attractive target for therapeutic intervention [21,50,52]. P38 $\alpha$  exacerbates ischemia/reperfusion injury and promotes cardiomyocyte apoptosis, myofilament modulation, proinflammatory gene induction and ECM remodeling, all of which can contribute to adverse myocardial remodeling and development of heart failure [16,18,49-51]. Conversely, p38 $\alpha$  activation underlies some of the protective effects conferred by ischemic preconditioning [53]. Nevertheless, strategies aimed at inhibiting p38 $\alpha$  in the heart are being

developed as therapies for treating cardiac dysfunction [50,52].

### **p38 $\beta$**

Despite being expressed at relatively low levels in the myocardium, p38 $\beta$  is purported to have important functional effects in the heart. However, it should be noted that most of the evidence for this has been compiled from approaches involving ectopic expression of wild-type or mutant p38 $\beta$  in cultured neonatal cardiomyocytes, rather than studying the endogenous protein. Several such studies have indicated that p38 $\alpha$  and p38 $\beta$  have opposing roles in cardiomyocytes. For example, simulated ischemia activates p38 $\alpha$ , but reduces p38 $\beta$  activity [54], and while overexpression of p38 $\alpha$  promotes apoptosis of cardiomyocytes, p38 $\beta$  overexpression increases myocyte hypertrophy and survival [55,56]. In transgenic mice with systemic overexpression of DN-p38 $\alpha$  or DN-p38 $\beta$ , pressure overload-induced hypertrophy was similar to that in wild type animals, but mutant mice exhibited reduced cardiac fibrosis [57]. An elegant chemical-genetic study using p38 $\beta$  knockout mice and mice expressing SB203580-resistant forms of p38 $\alpha$  and p38 $\beta$  recently determined that p38 $\alpha$  rather than p38 $\beta$  was important for ischemic preconditioning [53]. Thus, there remains scant information on the role of endogenous p38 $\beta$  in the heart and given its low expression in the myocardium the importance of this subtype remains questionable.

### **p38 $\gamma$ and p38 $\delta$**

P38 $\gamma$  (SAPK3) is expressed in human, mouse, rat, dog and pig heart [22,38], and its mRNA and protein levels in mouse heart are similar to those of p38 $\alpha$  [45]. In contrast to the cytoplasmic and nuclear localization of p38 $\alpha$  and p38 $\beta$ , p38 $\gamma$  was found to be localized exclusively in the cytoplasm in cultured neonatal rat cardiomyocytes [22]. However, nuclear

p38 $\gamma$  has been observed in cardiomyocytes in vivo following pressure overload [45]. P38 $\gamma$  activity is increased in the infarcted and remote myocardium following experimental MI in mice [37]. However, despite the clear evidence of p38 $\gamma$  expression and activation in the heart, its functional role in the myocardium remains undefined. Further studies with p38 $\gamma$  knockout mice are clearly warranted to determine the precise role of this prevalent cardiac p38 subtype.

The role of p38 $\delta$  in the heart is less clear still. Analysis of p38 subtype expression in mouse heart revealed that p38 $\delta$  is lowly expressed at the mRNA and protein level (similar to p38 $\beta$ ) [45], although our own studies in human CF from multiple patients suggest somewhat higher expression than p38 $\beta$  [58]. We recently showed that the p38 $\alpha/\beta/\gamma/\delta$  inhibitor BIRB-0796 significantly reduced IL-1-induced MMP-1 and MMP-10 expression in human CF; effects not observed with the classical p38 $\alpha/\beta$  inhibitor SB203580 [59]. These data implicate p38 $\gamma$  and/or p38 $\delta$  in modulating expression of MMP-1 and MMP-10 in these cells, although further studies are needed to confirm this.

### **JNK subtypes**

Western blot analysis has identified JNK1 and JNK2 as the major subtypes expressed in mouse heart, with no apparent expression of JNK3 [60]. Although neonatal rat cardiomyocytes have been reported to express all three JNK subtypes [61], the evidence for JNK3 is unclear and may rather reflect splice variants of JNK1 and 2.

The role of the JNK family in cardiac remodeling has been studied in vivo and in vitro using both gain-of-function models (e.g. overexpression of constitutively active mutants of JNKs or their upstream activators) and loss-of function models (e.g. knockout of JNK isoforms, or overexpression of DN mutants of JNKs or their upstream activators). These investigations have implicated the various JNK isoforms, especially JNK1, in the induction of cardiac hypertrophy, myocyte cell death and myocardial remodeling [16]. However, the role

of JNK is complex and many studies have reported conflicting results [16]. Some of these discrepancies may be explained by the functional redundancy that is evident between JNK isoforms. For example, knockout mice deficient in either JNK1, JNK2 or JNK3 all developed pressure overload-induced hypertrophy similar to wild-type animals [62], whereas deletion of upstream activators of these JNK isoforms, MKK4 [63] or MKK7 [64], reduced cardiac hypertrophy due to inhibition of JNK signaling. Recent evidence also suggests that JNK1 can have a dual function, conferring both beneficial and detrimental effects depending on the metabolic state of the heart [65]. Thus, JNK1 is proposed to act as a conditional survival kinase that protects the heart against brief ischemic episodes, but not against prolonged ischemia.

### **Therapeutic Targeting of SAPK Pathways in the Heart**

Evidence gathered from genetic manipulation of SAPKs indicates that the p38 and JNK pathways represent viable therapeutic targets for ameliorating myocardial dysfunction. Several pharmacological SAPK inhibitors, particularly those targeting p38 MAPK, have been evaluated in animal models of cardiovascular disease and in clinical trials (Table 1 and 2).

#### **p38 MAPK**

The most widely studied p38 inhibitors are the pyridinyl imidazole compounds, such as the archetypal SB203580 and the structurally similar SB202190 (Table 1). This class of molecules inhibit p38 $\alpha$  and p38 $\beta$  by binding to the active site in an ATP-competitive manner, but they do not inhibit the  $\gamma$  or  $\delta$  subtypes due to fundamental differences in the protein sequence of the active site [21]. SB203580 and SB202190 are relatively specific for p38 $\alpha/\beta$ , but can also effectively inhibit several other kinases including glycogen synthase kinase (GSK)-3 $\beta$ , casein kinase 1 $\delta$  and cyclin G-associated kinase [66]. A number of second and

third generation p38 $\alpha$ / $\beta$  inhibitors have been designed that are orally active, including SB239063, SB681323 (Dilmapimod), RWJ-67657, GW85655 (Losmapimod) and SCIO-469 (Talmapimod) (Table 1). Several inhibitors that preferentially target p38 $\alpha$  over p38 $\beta$  have also been developed including SD-282, VX-702 and RO4402257 (Pamapimod) (Table 1). None of the above inhibitors affect p38 $\gamma$  or p38 $\delta$  activity. In contrast, the diaryl urea compound BIRB-0796 is structurally and functionally distinct from the other p38 inhibitors and can inhibit all four p38 subtypes in a non-competitive manner in vivo and in vitro, albeit with slight selectivity for the  $\alpha$  and  $\beta$  subtypes [67]. BIRB-0796 does not inhibit any of the other kinases inhibited by pyridinyl imidazoles, but it is a potent inhibitor of JNK2 (but not JNK1 or JNK3) [66].

There is good evidence that pharmacological inhibitors of p38 can improve contractile function, reduce ischemic injury and ameliorate fibrosis in animal models of cardiac dysfunction [16,21,50,52]. Given their potent anti-inflammatory effects, most clinical trials of p38 inhibitors to date have assessed their impact on inflammatory disorders such as rheumatoid arthritis and Crohn's disease [68]. Early trials did not progress beyond phase 2 due to adverse side effects. More recently, however, trials of lower doses of p38 inhibitors have been better tolerated, although they have failed to provide much benefit when compared with alternative anti-inflammatory drugs [68]. Nevertheless, a number of trials are underway to investigate the effects of p38 inhibition on cardiovascular disease and its complications, including angioplasty (VX-702, SB681323[69]), atherosclerosis (GW85655) and non-ST elevation MI (GW85655) [50].

## **JNK**

In contrast to the diverse range of studies with p38 inhibitors, there are relatively few studies using pharmacological JNK inhibitors to modulate cardiac remodeling. The main JNK



inhibitors investigated to date are SP600125, AS601245 and XG-102 (Table 2), all of which are pan-specific i.e. inhibit JNK1, 2 and 3 with similar efficacies [30,70]. SP600125 was one of the first small molecule ATP-competitive JNK inhibitors to be developed and is the most widely studied. However, this compound is actually relatively non-selective for JNK, inhibiting at least 15 other kinases with similar or greater potency [66]. SP600125 had detrimental effects on myocardial remodeling in a hamster cardiomyopathy model, contrary to the effects of a p38 inhibitor [71]. However, SP600125 had beneficial effects on cardiac function and fibrosis in a recent study employing a murine model of cardiomyopathy [72]. AS601245 is an ATP-competitive small molecule JNK inhibitor that appears to be slightly more selective than SP600125 [66]. AS601245 reduced infarct size and cardiomyocyte cell death following ischemia-reperfusion injury in rats [73]. XG-102 (also known as D-JNKI-1) is a novel cell-permeable peptide based on the JNK-binding sequence of JIP-1 [74]. XG-102 appears to be more selective than the non-peptide inhibitors, and has been shown to be protective against myocardial ischemia-reperfusion injury in rats [75]. Thus, there is limited pre-clinical evidence that JNK inhibitors may be beneficial in reducing adverse myocardial remodeling, but none of these molecules have yet undergone clinical evaluation. The development of a new generation of more selective JNK inhibitors [30] may make this goal achievable in the coming years.

### **SAPK Roles in Cardiac Fibroblasts**

Animal models have proved important for determining the effects of inhibiting SAPK pathways on global myocardial remodeling. However, the complexity of the interrelationship between myocytes and fibroblasts and other cell types within the heart makes precise interpretation of pharmacological inhibitor experiments difficult, as effects on one cell type can manifest themselves in another cell type through direct cell-cell contact or

autocrine/paracrine signaling. Given the cell-specific nature of signaling pathways, it is not surprising (but often overlooked) that MAPK and SAPK signaling differs between myocytes and CF in vivo. For example, in a murine model of MI, p38 phosphorylation was increased in both myocytes and non-myocytes in the infarct and border zone and in the myocytes of the remote non-ischemic area [37]. In contrast, ERK phosphorylation was localized to non-myocytes in areas of fibrosis in both infarct and remote regions of the myocardium. This heterogeneity of cellular signaling responses is rarely considered and may offer avenues for selective therapeutic modulation.

Components of SAPK pathways have been manipulated selectively in cardiomyocytes in vivo by exploiting the  $\alpha$ MHC promoter to drive cardiomyocyte-specific expression or deletion of specific proteins [63,64,76]. Such models are cardiomyocyte-specific, not “cardiac-specific” as they are sometimes misleadingly referred to in the literature; an important distinction when considering the role of the CF. In stark contrast to the situation with cardiomyocytes, SAPK-targeting strategies aimed specifically at the fibroblasts of the heart have not yet been reported. In vitro cell culture models have therefore proved invaluable in delineating the function of distinct SAPK signaling pathways in regulating CF function, as discussed below.

### **p38 MAPK**

The role of p38 MAPK has been studied in vitro predominantly through the use of pharmacological inhibitors of p38 $\alpha/\beta$  (SB203580, SB202190, RWJ-67657) and p38 $\alpha/\beta/\gamma/\delta$  (BIRB-0796), as well as through the use of molecular biological techniques such as overexpression of DN-p38 $\alpha$  mutants. Such studies (summarized in Table 3) have provided evidence that p38 MAPK subtypes, particularly p38 $\alpha$ , are involved in regulating many of the key functions of CF in the myocardial remodeling process (Box 1). For example, p38

regulates expression of a range of proteins involved in inflammation, including cytokines and chemokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ , MCP-1, SDF-1, COX-2) [77-85] and intercellular adhesion molecules (ICAM-1 and VCAM-1) [86].

The evidence for p38 MAPK regulating IL-6 expression is particularly well documented and has been observed in CF of human, rat and mouse origin in response to numerous stimuli [77-82]. In human CF, we demonstrated that both TNF $\alpha$  and IL-1 could stimulate the p38 MAPK pathway and induce IL-6 mRNA and protein secretion that was attenuated by SB203580 [77,78]. Ang II-induced IL-6 mRNA expression in neonatal rat CF was mediated via a p38-dependent mechanism culminating in activation of the CREB transcription factor and IL-6 gene transcription [79]. A similar p38- and CREB-dependent mechanism appears to underlie  $\beta$ 2-adrenergic receptor-induced IL-6 expression in neonatal mouse CF [81]. In adult mouse CF, both an agonist of AMP kinase (AICAR) [82] and adenosine [80] could stimulate IL-6 secretion in a SB203580-sensitive manner, with the results of the latter study being confirmed by overexpression of DN-p38 [80]. Interestingly, a gene profiling study highlighted IL-6 as a central molecule upregulated in the adult rat heart following adenoviral gene transfer of constitutively active MKK3 and wild-type p38 $\alpha$  [87]. This is likely to be important in the remodeling heart, as IL-6 stimulates left ventricular hypertrophy and fibrosis in the absence of an inflammatory response or effects on blood pressure [88].

The p38 pathway has been implicated in both degradation and synthesis of ECM components. For example, cytokine-induced expression of ECM-degrading MMPs 1, 3, 9 and 10 occurs via p38-dependent pathways [59,89,90]. In human CF, we obtained evidence for a differential role of individual p38 subtypes in this context. While IL-1-induced MMP-3 was inhibited by either a p38 $\alpha/\beta$  inhibitor (SB203580) or a p38 $\alpha/\beta/\gamma/\delta$  inhibitor (BIRB-0796), IL-1-induced MMP-1 and MMP-10 expression was sensitive only to inhibition by BIRB-0796

[59]. As human CF express the  $\alpha$ ,  $\gamma$  and  $\delta$  subtypes of p38 at the mRNA and protein level, but relatively little p38 $\beta$  (NA Turner, unpublished observations and [58]), this suggests roles for p38 $\alpha$  in stimulating MMP-3 expression, and for p38 $\gamma$  or p38 $\delta$  in stimulating MMP-1 and MMP-10 expression. However, it should be noted that BIRB-0796 is also a potent inhibitor of JNK2 [66]. Conflicting reports exist for the role of the p38 pathway in mediating IL-1-induced MMP-3 and MMP-9 expression in rat and human CF [59,90]. In adult rat CF, IL-1-induced protein expression of MMP-3 was p38-independent and that of MMP-9 was p38-dependent [90]. However, in adult human CF we reported that IL-1-induced MMP-3 mRNA expression was p38-dependent and MMP-9 mRNA expression was p38-independent [59], suggesting important inter-species differences may exist.

ADAMTS1 (a disintegrin and metalloproteinase domain with thrombospondin motifs 1) is a secreted protease that is a potent inhibitor of angiogenesis [91]. We determined that both SB203580 and BIRB-0796 inhibit ADAMTS1 mRNA expression in human CF in the absence or presence of IL-1 stimulation [59]. These data imply that p38 MAPK inhibition could promote neovascularization in vivo.

Profibrotic effects of the p38 pathway in CF have also been reported. For example, p38 inhibition with SB203580 or RWJ-67657 reduced type I collagen gene expression in rat CF [92-94]. Moreover, oncostatin M-induced expression of TIMP-1 (a negative regulator of MMP activity) was sensitive to p38 inhibition in human CF [95]. Additionally, mechanical stretch induced angiotensinogen gene expression in neonatal rat CF via RhoA-induced activation of p38 $\alpha$  [96,97], likely contributing to Ang II-mediated pro-fibrotic changes in the heart. The involvement of p38 in both degradation and synthesis of ECM by CF makes the net effect of p38 inhibition on ECM remodeling complex. However, the majority view is that p38 inhibition reduces cardiac fibrosis in vivo [16,57,92,98]. Whether these effects are due to direct modulation of ECM turnover by CF rather than downstream consequences of improved

cardiomyocyte function is not clear. In support of the latter scenario, overexpression of DN-p38 $\alpha$  specifically in cardiomyocytes reduced fibrosis in a diabetic cardiomyopathy model without directly affecting p38 activity in CF [99].

Besides effects on gene expression, p38 also regulates several cellular properties of CF that are important to the myocardial remodeling process. For example, TGF $\beta$ -induced differentiation of CF to myofibroblasts is inhibited by the p38 $\alpha/\beta$  inhibitor RWJ-67657 both in vivo and in vitro [92,100]. IL-1-induced migration of neonatal rat CF can be attenuated by p38 inhibition, although JNK and ERK appear to play more pronounced roles [101]. In a recent study, the adipokine visfatin was shown to increase DNA synthesis (a marker of cell proliferation) in rat CF via a p38-dependent, JNK-independent mechanism [94]. Finally, SB203580 protected neonatal rat CF against ischemia-induced cell death, suggesting that p38 is important for mediating the detrimental effects of ischemia on these cells [102].

Taken together, the results of these in vitro studies on CF (Table 3) suggest that p38 MAPK regulates production of inflammatory mediators, ECM turnover and cell proliferation, migration and myofibroblast differentiation, all of which are important in the myocardial remodeling process. As such, the p38 MAPK pathway in CF represents a potential therapeutic target for reducing adverse myocardial remodeling.

### **JNK**

The role of the JNK pathway in CF is much less well studied than the p38 pathway. Almost all of our current knowledge on the role of JNK in CF has been acquired from the use of a single pharmacological JNK inhibitor, SP600125 (Table 4). As highlighted earlier, in addition to JNK this agent also inhibits at least 15 other kinases with similar or greater potency [66], and hence an element of caution must be applied when interpreting results obtained solely with this inhibitor.

In rat CF, SP600125 reduced ET-1-induced  $\alpha$ SMA expression and collagen synthesis, both markers of myofibroblast differentiation [103]. SP600125 also inhibited CF migration induced by IL-1 family cytokines (IL-1 or IL-18) [101,104], and cell proliferation induced by the chemokine CXCL16 [105]. Additionally, SP600125 inhibited IL-1-induced protein expression of MMP-3 in adult rat CF [90]. Although MMP-9 expression was not sensitive to SP600125 in that study [90], another group reported that IL-1-induced MMP-9 expression was mediated by a pathway involving specific protein kinase C subtypes and JNK in adult rat CF [106]. Matricellular proteins are non-structural components of the ECM that regulate cell-matrix interactions and cellular function [107]. The ability of CF to synthesize two such matricellular proteins (periostin and osteopontin) has also been shown to be inhibited by SP600125, and a role for JNK signaling downstream of ROS generation suggested [108,109]. Plasminogen activator inhibitor-1 (PAI-1), which is associated with increased risk of cardiovascular disease, can be secreted by both myocytes and fibroblasts in the heart [110]. Adenovirus-mediated gene transfer of DN kinase mutants in neonatal rat CF revealed that Ang II or ET-1-induced PAI-1 expression occurred via a JNK-dependent (p38-independent) mechanism [110].

The JNK pathway can also negatively regulate expression of some genes in CF. For example, SP600125 or overexpression of DN mutants of JNK1 or JNK2 significantly increased basal and mechanical stretch-induced angiotensinogen expression in neonatal rat CF; opposite to the effects of p38 MAPK inhibition [96,97]. Moreover, in our own studies using adult human CF, SP600125 increased basal and IL-1-induced IL-6 mRNA expression; again converse to the effects of p38 inhibition [77].

Taken together, the above studies suggest a role for JNK signaling pathways in regulation of CF proliferation, migration, differentiation and ECM regulation (Table 4). Although the evidence is not as strong as it is for p38 MAPK, the JNK pathway in CF may

also represent a viable therapeutic target for reducing adverse myocardial remodeling.

### **Future Perspective**

The role of MAPK signaling pathways and their coupling to cellular function are clearly different in cardiomyocytes and non-myocytes and hence prevention of adverse myocardial remodeling may require cell type-specific manipulation of MAPK activation. As the importance of the CF is increasingly recognized, strategies for selectively targeting this cell type in the myocardium will begin to emerge. The three major approaches that offer potential in this respect are pharmacological, genetic and cell-based therapies [111].

Pharmacological approaches can offer cell type-specificity depending on the target in question. Several classes of widely prescribed drugs for patients with cardiovascular disease (e.g. statins, beta-blockers, ACE inhibitors) have beneficial effects on myocardial remodeling that appear to be due in part to modulation of CF function [10]. With respect to SAPK signaling, *in vitro* studies have shown defined roles for SAPK pathways in modulating diverse cellular functions of import to the remodeling process. However, the net effect of pharmacological inhibition *in vivo* depends upon the role of SAPK pathways in all cell types and therefore is far more complex. One avenue that may be of value is in exploiting the differential isoform expression of specific JNKs and p38 MAPKs in different cell types. By understanding the cell-specific expression patterns of SAPK isoforms, it may be possible to design therapies targeted at particular isoforms which would then favor a particular cell type in the heart. The question remains as to whether global inhibition of all JNKs or p38 MAPKs is more beneficial than isoform-specific inhibitors. In the case of p38, most pharmacological studies to date have utilized  $\alpha/\beta$ -selective inhibitors, but the potentially opposing roles of the  $\alpha$  and  $\beta$  subtypes (e.g. in cardiomyocyte hypertrophy and apoptosis [55]) cannot be ignored. Moreover, inhibition of p38 $\gamma$  or p38 $\delta$  in the heart has not been well explored, although agents

such as BIRB-0796 can inhibit these subtypes in addition to p38- $\alpha/\beta$ . Isoform-selective JNK inhibitors are also being developed [30,70].

Another aspect that deserves consideration is whether SAPK signaling differs in CF compared with fibroblasts from other organs. This is important because SAPK-targeting therapies that are ineffective in one clinical setting may be more effective in another. Cardiac fibroblasts arise from the primitive heart tube and are developmentally distinct from fibroblasts from other tissues [112]. A systematic review of *in vitro* studies on skin, lung, synovial, liver and heart fibroblasts highlighted several important differences in their response to proinflammatory cytokines [113]. It is also likely that diversity in SAPK signalling exists between different fibroblast populations as a result of differential SAPK subtype expression. For example, synovial fibroblasts in tissue from rheumatoid arthritis patients expressed p38 $\beta$  and p38 $\gamma$ , but did not express p38 $\alpha$  even though this was observed in macrophages [114]. This is in marked contrast to human CF, in which p38 $\alpha$  is the most abundant isoform [58].

In comparison to the cardiomyocyte, the CF has been relatively unexplored as a target for gene therapy. Whilst there has been much progress in studying the function of specific proteins in cardiomyocytes through use of  $\alpha$ MHC gene promoter-driven strategies, the lack of a CF-specific enhancer/promoter has clearly held back *in vivo* research at the level of the CF. Fibroblasts from other tissues (e.g. skin and lung) have been targeted using fibroblast-specific enhancers (e.g. Col1a2 promoter [115]), but this technology has not yet been applied to the heart. Fibroblasts and cardiomyocytes have been differentially targeted in the myocardium using a specific promoter region of the mouse periostin (Postn) gene [116]. Periostin is a matricellular protein whose expression is restricted to the non-myocyte lineage of the neonatal heart and is not expressed by cardiomyocytes [117], although it is expressed in other connective tissues within the body. The GATA4 transcription factor is expressed by



cardiomyocytes and CF, but interestingly does not appear to be expressed by fibroblasts from other organs [118]. Whether elements within the GATA4 gene could be exploited to facilitate gene therapies aimed specifically at the CF has yet to be investigated. Thus, identification of a CF-specific promoter / transcriptional enhancer is an important goal if the full potential of selectively modulating CF function in vivo is to be realized [111]. This may then open up new avenues for assessing the role of SAPK pathways in CF and their role in myocardial remodeling.

Cell-based therapies involve the introduction of exogenous cells (e.g. cardiomyocytes, stem cells or fibroblasts) into the heart to ameliorate cardiac dysfunction. There is a small body of evidence that transplantation of wild-type or genetically-modified CF into the heart can offer therapeutic benefit in reducing adverse cardiac remodeling [111]. For example, injection of CF over-expressing the GATA4 transcription factor [119], erythropoietin [120] or VEGF [121] has been used to attempt to ameliorate myocardial remodeling in rat models of MI. Although the erythropoietin approach did not offer long-term benefit [120], the GATA4-expressing CF improved cardiac function and reduced adverse myocardial remodeling [119]. Moreover, transplantation of VEGF-expressing CF into infarcted areas of the heart one day after MI promoted neovascularization and attenuated cardiac dysfunction measured 4 weeks later [121]. This latter study offers particular therapeutic potential as the favorable effects of CF transplantation were apparent after induction of MI. By adopting a similar strategy for activating or inhibiting selective SAPK pathway components in exogenous CF, and then transplanting them into the heart, we may be able to gain a more detailed understanding of the role of SAPK signaling specifically in CF in vivo.

### **Concluding Remarks**

In summary, SAPK signaling pathways play important roles in regulating cardiac

development and physiology, but are also key proponents of cardiac dysfunction. Whilst the p38 and JNK pathways have been well explored in cardiomyocytes, increasing evidence supports a role for SAPK signaling in many aspects of CF function that are central to the myocardial remodeling process. This knowledge provides an opportunity to design focused interventional therapies targeted specifically at CF. In this respect, pathway-specific targeting holds potential for modulating fibroblast functions that are perceived as detrimental, whilst sparing other favorable effects of this important myocardial cell type.

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## **Figure Legends**

### **Fig.1. Canonical MAPK signaling cascades.**

MAP kinase kinase kinases (MAPKKKs) phosphorylate MAP kinase kinases (MAPKKs) on specific serine/threonine residues. This results in MAPKK activation and subsequent dual phosphorylation of MAPKs on specific threonine and tyrosine residues within the conserved Thr-X-Tyr activation motif. MAPKs regulate multiple facets of cellular function by phosphorylating downstream substrates localized in the nucleus (e.g. transcription factors), cytosol, cytoskeleton, mitochondria and plasma membrane. Abbreviations: ASK: apoptosis signal regulating kinase; ERK: extracellular signal regulated kinase; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; MEK: MAPK/ERK kinase; MEKK: MAPK/ERK kinase kinase; MKK: MAP kinase kinase; MLK: mixed lineage kinase; TAK: transforming growth factor- $\beta$  activated kinase.

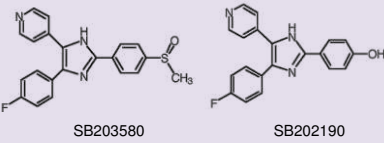
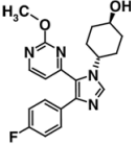
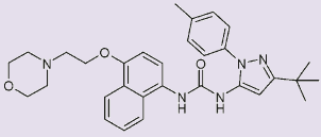
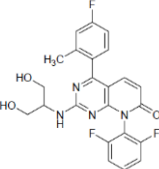
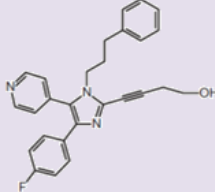
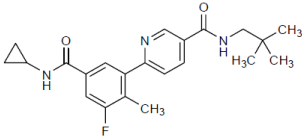
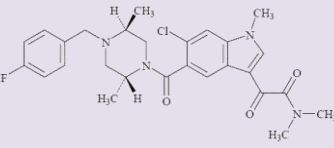
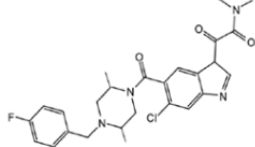
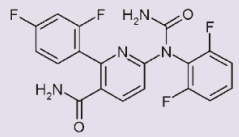
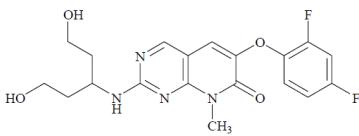
### **Fig. 2. The p38 MAPK signaling pathway.**

Abbreviations: ASK: apoptosis signal regulating kinase; ATF: activating transcription factor; C/EBP: CCAAT/enhancer binding protein; CHOP: C/EBP-homologous protein; CREB: cyclic AMP response element binding protein; DLK: delta like homolog; ELK: Ets-like gene; HBP: high-mobility group box protein; MAPK: mitogen-activated protein kinase; MAPKAPK: mitogen-activated protein kinase-activated protein kinase; MEF: myocyte enhancer factor; MEKK: MAPK/ERK kinase kinase; MKK: mitogen-activated protein kinase kinase; MAPKK: mitogen-activated protein kinase kinase; MAPKKK: mitogen-activated protein kinase kinase kinase; MLK: mixed lineage kinase; NFAT: nuclear factor of activated T-cells; NHE: Na<sup>+</sup>/H<sup>+</sup> exchanger; MNK: mitogen-activated protein kinase interacting kinase; MSK: mitogen and stress-activated protein kinase; PLA<sub>2</sub>: phospholipase A2; PRAK: p38-regulated/activated protein kinase; SAP1: serum response factor accessory protein;

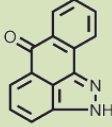
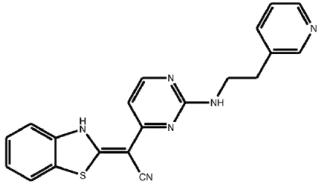
TAK: transforming growth factor- $\beta$  activated kinase; TAO: thousand and one amino acid protein kinase; TPL2: tumor progression locus 2; USF: upstream transcription factor; ZAK: leucine zipper and sterile- $\alpha$  motif kinase.

**Fig. 3. The JNK signaling pathway.**

Abbreviations: ASK: apoptosis signal regulating kinase; ATF: activating transcription factor; ELK: Ets-like gene; GR: glucocorticoid receptor; HSF: heat shock factor; IRS: insulin receptor substrate; JIP: JNK-interacting protein; JNK: c-Jun N-terminal kinase; MAP2/1B: microtubule associated protein 2 and 1B; MAPK: mitogen-activated protein kinase; MAPKK: mitogen-activated protein kinase kinase; MAPKKK: mitogen-activated protein kinase kinase kinase; MEKK: MAPK/ERK kinase kinase; MKK: mitogen-activated protein kinase kinase; MLK: mixed lineage kinase; NFAT: nuclear factor of activated T-cells; PPAR: peroxisome proliferator-activated receptor; RXR: retinoid receptor; SMAD: SMA and MAD homolog; TAK: transforming growth factor- $\beta$  activated kinase.

Table 1. Properties of common p38 inhibitors			
Inhibitor	Primary Target	Notes	Chemical Structure
SB203580 SB202190	p38 $\alpha/\beta$	First generation inhibitors. Pyridinyl imidazole compounds. Also inhibit other kinases including GSK3 and CK.	 SB203580                      SB202190
SB239063	p38 $\alpha/\beta$	Second generation pyridinyl imidazole inhibitor. Orally active.	
BIRB-0796 (Doramapimod)	p38 $\alpha/\beta$ > p38 $\gamma/\delta$	Second generation diaryl urea inhibitor. Orally active. Slightly selective for p38 $\alpha/\beta$ over $\gamma/\delta$ . Also potently inhibits JNK2.	
SB681323 (Dilmapiomod)	p38 $\alpha/\beta$	Orally active.	
RWJ-67657	p38 $\alpha/\beta$	Potent. Orally active.	
GW85655 (Losmapimod)	p38 $\alpha/\beta$	Orally active.	
SCIO-469 (Talmapiomod)	p38 $\alpha/\beta$	Orally active third generation inhibitor.	
SD-282	p38 $\alpha$	Orally active. Selective for p38 $\alpha$ over p38 $\beta$ .	
VX-702	p38 $\alpha$	Orally active third generation inhibitor. Highly selective.	
RO4402257 (Pamapiomod)	p38 $\alpha$	Orally active third generation inhibitor. Selective for p38 $\alpha$ over p38 $\beta$ . Also inhibits JNK2 and JNK3.	

**Table 2. Properties of common JNK inhibitors**

Inhibitor	Primary Target	Notes	Chemical Structure
SP600125	JNKs 1-3	Very low specificity. Inhibits >15 other kinases with similar or greater potency.	
AS601245	JNKs 1-3	Low specificity. Inhibits GSK3 with much greater potency.	
XG-102 (D-JNKI-1)	JNKs 1-3	Cell permeable peptide inhibitor of JNK/JIP interaction. Also inhibits MKK4 and MKK7.	Asp-Gln-Ser-Arg-Pro-Val-Gln-Pro-Phe-Leu-Asn-Leu-Thr-Thr-Pro-Arg-Lys-Pro-Arg-Pro-Pro-Arg-Arg-Arg-Gln-Arg-Arg-Lys-Lys-Gly

**Table 3. Effect of p38 MAPK inhibition on cardiac fibroblast function *in vitro***

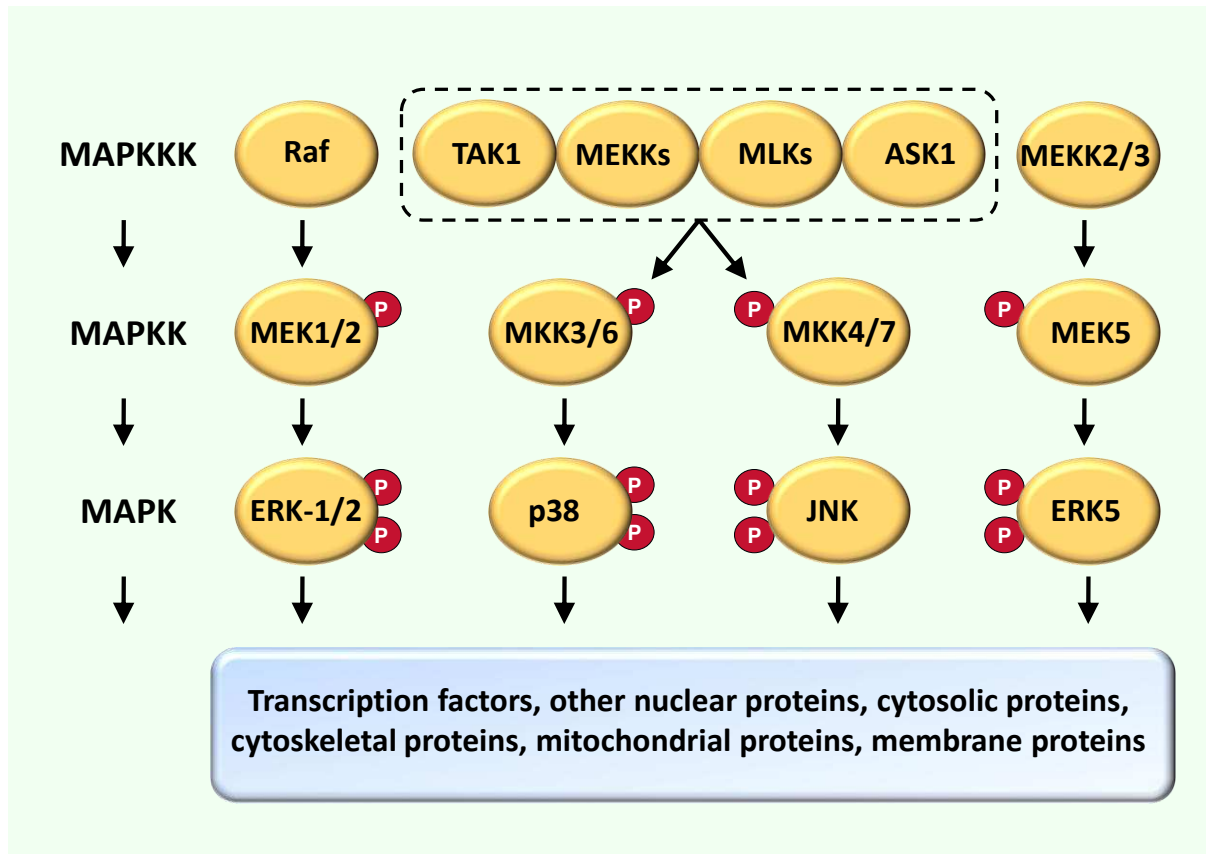
<b>Target</b>	<b>Stimulus</b>	<b>Inhibitor</b>	<b>CF Source</b>	<b>Reference</b>
<b>Inflammation</b>				
↑ IL-1 $\alpha$ , IL-1 $\beta$	TNF $\alpha$	SB203580	Human	[78]
↑ IL-6	IL-1, TNF $\alpha$ , Ang II, Adenosine, AMP kinase, $\beta$ -AR stimulation	SB203580, DN-p38 $\alpha$	Human, Rat, Mouse	[77-82]
↑ TNF $\alpha$	IL-1	SB203580	Human	[77]
↑ MCP-1	Ang II	DN-p38 $\alpha$	Rat	[83]
↑ SDF-1	Oncostatin M	SB202190	Human	[84]
↑ COX-2	Ang II	SB203580	Rat	[85]
↑ ICAM-1, VCAM-1	IL-1	SB203580	Rat	[86]
<b>ECM turnover</b>				
↑ MMP-1	IL-1, IL-17	SB203580, BIRB-0796	Human	[59,89]
↑ MMP-3	IL-1	SB203580, BIRB-0796	Human	[59]
↑ MMP-9	IL-1	SB202190	Rat	[90]
↑ MMP-10	IL-1	BIRB-0796	Human	[59]
↑ TIMP-1	Oncostatin M	SB202190	Human	[95]
↑ Type I collagen	TGF- $\beta$ , AGEs, visfatin	RWJ-67657, SB203580	Rat	[92-94]
<b>Cell function</b>				
↑ Myofibroblast	TGF- $\beta$	RWJ-67657	Rat	[92]
↑ Cell proliferation	Visfatin	SB203580	Rat	[94]
↑ Cell migration	IL-1	SB202190	Rat	[101]
↓ Cell survival	Ischaemia	SB203580	Rat	[102]
<b>Other</b>				
↑ Angiotensinogen	Mechanical stretch	SB202190, SB203580, DN-p38 $\alpha$	Rat	[96,97]
↓ ADAMTS1	IL-1	SB203580, BIRB-0796	Human	[59]
Abbreviations: ADAMTS: a disintegrin and metalloproteinase domain with thrombospondin motifs; AGEs: advanced glycation end products; Ang II: angiotensin II; $\beta$ -AR: beta-adrenergic receptor; DN: dominant negative; ICAM: intercellular cell adhesion molecule; IL: interleukin; MCP: monocyte chemoattractant protein; MMP: matrix metalloproteinase; SDF: stromal-derived factor; TIMP: tissue inhibitor of metalloproteinase; TGF: transforming growth factor; TNF: tumor necrosis factor; VCAM: vascular cell adhesion molecule.				

**Table 4. Effect of JNK inhibition on cardiac fibroblast function *in vitro***

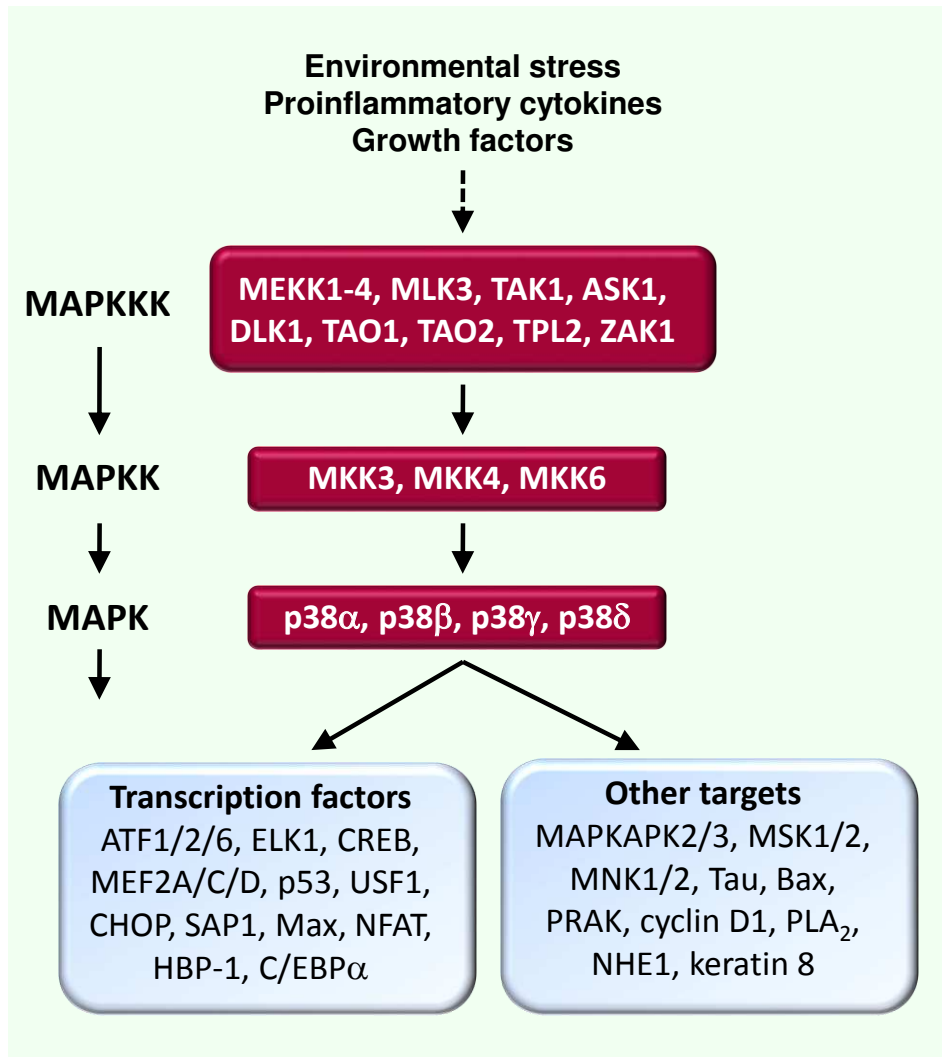
<b>Target</b>	<b>Stimulus</b>	<b>Inhibitor</b>	<b>CF Source</b>	<b>Reference</b>
<b>ECM turnover</b>				
↑ MMP-3	IL-1	SP600125	Rat	[90]
↑ MMP-9	IL-1	SP600125	Rat	[106]
<b>Cell function</b>				
↑ Myofibroblast	ET-1	SP600125	Rat	[103]
↑ Cell migration	IL-1	SP600125	Rat	[101]
↑ Cell migration	IL-18	SP600125	Rat	[104]
↑ Cell proliferation	CXCL16	SP600125	Rat	[105]
<b>Other</b>				
↑ Periostin	High glucose	SP600125	Rat	[108]
↑ Osteopontin	Ang II + IL-1/TNF $\alpha$	SP600125	Rat	[109]
↑ PAI-1	Ang II, ET-1	DN-JNK	Rat	[110]
Abbreviations: <i>Ang II</i> : angiotensin II; <i>CXCL</i> : CXC ligand; <i>DN</i> : dominant negative; <i>ET</i> : endothelin; <i>IL</i> : interleukin; <i>MMP</i> : matrix metalloproteinase; <i>PAI</i> : plasminogen activator inhibitor; <i>TNF</i> : tumor necrosis factor.				



Fig. 1. MAPK signaling cascades



**Fig. 2. The p38 signaling pathway**



**Fig. 3. The JNK signaling pathway**

