Inflame My Heart (by p38-MAPK)

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Although many studies have explored the stimuli which promote hypertrophic growth or death in cardiac myocytes and the signaling pathways which they activate, the mechanisms by which these pathways promote the pathophysiological responses are still obscure. The mitogen-activated protein kinase (MAPK) cascades (in which MAPKs are phosphorylated and activated by upstream MAPK kinases [MKKs] which are, in turn, phosphorylated and activated by MKK kinases [MKKKs]) were identified in the early- to mid-1990s as potentially key regulatory pathways in cardiac myocyte pathophysiology. \(^1,2\) The principal MAPKs investigated in cardiac myocytes are the extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNKs), and p38-MAPKs. ERK1/2 are potently activated by hypertrophic stimuli, whereas JNKs and p38-MAPKs are potently activated by cellular stresses (eg, oxidative stress). However, there is cross-talk such that JNKs and p38-MAPKs are activated by hypertrophic stimuli and ERK1/2 are activated by cellular stresses, and the contribution of each pathway to the overall cardiac myocyte response is not entirely clear.

MAPKs phosphorylate a number of known transcription factors to alter their transactivating activities thus, presumably, influencing gene expression to elicit the cellular response.\(^3\) Nevertheless, the immediate consequences (ie, the transcription factors which are phosphorylated) and downstream consequences (ie, genes with altered expression) of MAPK signaling in the heart or specifically in cardiac myocytes are still largely unknown. To start to address this issue for the p38-MAPK pathway in the (rat) heart (Figure A), Tenhunen et al\(^4\) directly injected adenoviruses encoding wild-type (WT) p38-MAPK\(\alpha\) together with a mutated constitutively-activated (CA) upstream kinase (MKK3b) into the heart. The effects of stimulating p38-MAPK\(\alpha\) signaling on global gene expression in the left ventricle (assessed by microarray analysis), and cardiac function and pathology are reported. At 3 days, gene expression profiling identified a number of changes in gene expression, most notably in genes encoding proteins which regulate cell cycle progression or which are involved in inflammation. By 7 days, the hearts were noticeably fibrotic and contained many inflammatory cells. There was no evidence of cardiac myocyte hypertrophy. Initially, these results may perhaps seem surprising, given that p38-MAPK\(\alpha\) signaling in heart is usually considered in the context of cardiac myocyte hypertrophy or cytoprotection/apoptosis.\(^2,5\) However, in the historical perspective of p38-MAPK\(\alpha\) signaling in inflammation and allowing for the methodology used in the study, the responses are not necessarily entirely unexpected.

p38-MAPK\(\alpha\) and the Inflammatory Response

p38-MAPK\(\alpha\) was identified as the target of pyridinyl imidazole drugs (eg, SB203580 and SB202190) which suppress the production of proinflammatory cytokines in monocytes subjected to endotoxin challenge,\(^6\) and as a 38-kDa protein kinase which is phosphorylated and activated in response to endotoxin or other stresses.\(^7,8\) Other p38-MAPK isoforms were subsequently identified (\(\beta, \gamma, \delta\) isoforms)\(^9\) but, because pyridinyl imidazoles inhibit only the \(\alpha\)- and \(\beta\)-forms,\(^10\) these are the best studied. The best established substrate for p38-MAPK\(\alpha/\beta\) is a downstream kinase, MAPKAP2, which phosphorylates the small heat shock protein Hsp25/27.\(^11,12\) However, as discussed by Tenhunen et al,\(^4\) p38-MAPK\(\alpha/\beta\) and/or MAPKAP2 may also phosphorylate transcription factors.\(^11,12\) The importance of p38-MAPK\(\alpha\) in inflammation was particularly highlighted in a study of the in vivo effects of SB203580 on several animal models of inflammation.\(^13\) Indeed, the role of p38-MAPK\(\alpha\) in such responses is now so well-established that many companies are competing to produce selective p38-MAPK\(\alpha\) inhibitors for the treatment of inflammatory diseases.\(^11,12\)

It is important to note that the approach used by Tenhunen et al to investigate the effects of p38-MAPK\(\alpha\) is unlikely to assess the role of the kinase specifically in cardiac myocytes in relation to pathophysiological stresses which are relevant to the heart. Although the combination of WTP38-MAPK\(\alpha\) and CA-MKK3b gives selective activation of p38-MAPK (rather than JNKs or ERK1/2), it results in sustained, very potent activation of the pathway. This contrasts with the transient activation which is observed in response to, for example, ischemia or ischemia with reperfusion,\(^14\) raising the possibility that the response could be somewhat anomalous. In addition, expression of CA-MKK3b and WTP38-MAPK\(\alpha\) was regulated by a cytomegalovirus (CMV) immediate early promoter allowing expression in any cell type which may become infected rather than specific expression in cardiac myocytes (as could be obtained with the \(\alpha\)-myosin heavy chain promoter [see eg, Molkentin et al\(^15\)]) Apart from nonmyocytes in the heart (eg, fibroblasts, endothelial cells, resident macrophages), inflammatory cells may also become infected and express CA-MKK3b and p38-MAPK\(\alpha\) particularly because adenoviruses themselves can provoke an inflammatory response.\(^16\) Because of the potent proinflam-
Regulation of mRNA expression by the p38-MAPK pathway. p38-MAPKα/β are potently activated by cell stresses via their upstream kinases MKK3/6. They are also activated by GqPCR agonists. However, the events upstream of MKK3/6 for either GqPCR or cell stress signaling are not clear. p38-MAPKα/β phosphorylates and activates the downstream kinase MAPKAPK2, which phosphorylates the small heat shock protein Hsp25/27. In addition, p38-MAPKα/β may regulate mRNA expression by phosphorylating transcription factors to increase their transcriptional activities, and promote mRNA synthesis from gene promoters. Alternatively, p38-MAPKα/β (potentially via MAPKAPK2) can alter the phosphorylation status of mRNA binding proteins such as those which bind AU-rich elements in the 3′ untranslated regions of mRNAs (AU-rich element binding proteins, AREBP). This can increase the stability of ARE-containing mRNAs to upregulate mRNA expression. The p38-MAPKα/β pathway is particularly implicated in the inflammatory response as demonstrated in the heart by Tenhunen et al.4

Inflammatory effects of p38-MAPKα signaling, an unregulated inflammatory response is likely to ensue whichever cells express the genes. Overproduction of cytokines will cause recruitment and proliferation of inflammatory cells in addition to exerting effects on the resident cells in the heart. This could account for the increased expression of genes encoding cell cycle proteins and inflammatory mediators in hearts with activated p38-MAPKα, with the ensuing increase in fibrosis. Although Tenhunen et al control for the adenovirally-induced inflammation with adenoviruses encoding LacZ,4 these hearts would not necessarily be exposed to the escalating inflammatory response which is the likely consequence of simultaneously having an activated p38-MAPKα signal.

Signaling From p38-MAPKα to Gene Expression: Transcriptional Versus Posttranscriptional Regulation

The focus of the Tenhunen et al study4 is the potential effects of p38-MAPKα signaling on gene expression as a consequence of activation of specific transcription factors (Figure). In support of this, increases in DNA-binding activities for GATA-4, AP-1, NF-κB, and SRF transcription factors are shown for hearts 3 days after infection. Because direct injection of adenoviruses into the heart can result in maximal protein expression within ~4 hours,17 it cannot be ascertained whether the effects on gene expression are the result of p38-MAPKα (or MAPKAPK2) phosphorylating preexisting transcription factors to alter their activities, whether the signal is transmitted via alternative mediators to existing transcription factors, or whether the transcription factors are induced as a consequence of p38-MAPKα signaling. To complicate the issue further, gene expression profiling with microarrays examines the steady state levels of mRNAs and reflects the balance between synthesis and degradation. It is increasingly apparent that regulation of mRNA stability is an important factor in balancing mRNA levels in the cell. Thus, by comparing the profiles of steady-state poly(A) RNA expression with RNA generated from nuclear run-on assays in T cells, it is estimated that up to 50% of transcripts are regulated at the level of stability.18 Activation of p38-MAPKα/MAPKAPK2 is particularly implicated in stabilizing mRNAs containing AU-rich elements (AREs) in the 3′ untranslated regions (Figure).19,20 Many of these encode inflammatory mediators, and a number of mRNAs which are established to be regulated via p38-MAPKα/MAPKAPK2 acting on an ARE were detected in the Tenhunen study (eg, IL-6, cyclooxygenase 2, plasminogen activator urokinase, c-fos).4 Therefore, at least some of the effects of CA-MKK3b/p38-MAPKα on gene expression in the heart may reflect alterations in mRNA stability. At an extreme, it could even be argued (given the number of links from IL-6 to other genes) that the principal effect of p38-MAPKα signaling is to increase the stability of mRNAs for soluble mediators such as IL-6, which act in an autocrine/paracrine fashion to signal to gene transcription.

Significance of p38-MAPKα Signaling for Cardiac Myocytes

p38-MAPKs were first shown to be activated in hearts by ischemia/reperfusion in 1996.21 They are now known to be activated by a range of cellular stresses and receptor agonists in cardiac myocytes and perfused hearts ex vivo, and in hearts in vivo,2,5 but the isoform expression profile is still not fully characterized. As in other cells, the focus of attention has been p38-MAPKα/β (because of the availability of selective inhibitors for these isoforms), and early studies demonstrated that overexpression of activated components of the p38-MAPK pathway induce hypertrophic markers in cardiac myocytes.22 Further studies suggested that whereas p38-MAPKα is proapoptotic, p38-MAPKβ is hypertrophic.23 However, such overexpression studies only highlight potential signaling consequences and it should be borne in mind that endogenous proteins are probably subject to additional constraints. Although p38-MAPKs are implicated in cardiac myocyte hypertrophy, ischemic preconditioning, and apoptosis, the data are often conflicting. As discussed fully by Tenhunen et al,4 studies in transgenic animals with cardiac overexpression of activated or dominant-negative components of the p38-MAPK pathway have proved equally conflicting. For example, cardiосpecific overexpression of dominant-negative components of the p38-MAPKα pathway protects against ischemia/reperfusion injury,24 but overexpression of activated components of the pathway improve functional recovery.25 Another study simply suggests that activation of p38-MAPK is detrimental to the heart, causing cardiomyopathy.26 Possible differential effects of p38-MAPKα versus p38-MAPKβ (as suggested by Wang et al23)
could help to explain the apparently conflicting results but, while p38-MAPKα is clearly expressed in cardiac myocytes, the degree of expression of p38-MAPKβ has never been established.

What conclusions can be drawn from the Tenhunen et al study4 about the functional role of p38-MAPKα in cardiac myocytes? Presumably, p38-MAPKα was activated in at least some cardiac myocytes, so it is notable that there was no overt cardiac myocyte hypertrophy. However, the inflammatory response and fibrosis which were triggered may have served to suppress any hypertrophic effect. To determine a direct effect of p38-MAPKα signaling in cardiac myocytes, perhaps it may be useful to perform additional experiments with the expression of WT-p38-MAPKα and CA-MKK3β under the control of a cardiac myocyte-specific promoter. At a molecular level, p38-MAPKα/β signals to MAPKAPK2 in cardiac myocytes and perfused hearts, and promotes phosphorylation of Hsp25/27.27,28 but, other than this, very little is known of the functional effects of this pathway in the heart. In this context, although care must be taken with the interpretation, the study by Tenhunen et al4 has started to shed some light on a very confused area. It is perhaps reassuring that persistent activation of p38-MAPKα promotes inflammation in the heart, as it does in other systems, whether or not the effects are mediated directly through the cardiac myocytes. Even if, at an extreme, the study does not assess directly the effect of p38-MAPKα signaling in cardiac myocytes, the data are still highly relevant to cardiac pathologies resulting from myocardial infarction29 or in myocarditis30 in which there is a large inflammatory response. Understanding the changes in gene expression which are influenced by p38-MAPKα signaling may be useful in developing therapeutic strategies to modulate disease progression. The potential significance of many of the changes in gene expression is discussed by the authors, but it is notable that the increased expression of profibrotic genes and the increased fibrosis induced in the CA-MKK3β/p38-MAPKα may relate to the fibrosis which occurs subsequent to myocardial infarction. An additional point of interest is the large proportion of transcripts which are induced but which correspond to no known gene. This is perhaps surprising in the current era in which the human, mouse, and even rat genomes are apparently well-characterized (see www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomemapr for current status). It remains to be seen whether these “unknown” transcripts are physiologically relevant, whether they represent novel (as yet undiscovered) protein-coding genes, or whether they may represent non-protein-coding regulatory RNAs (e.g., antisense RNAs or microRNAs).

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References


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