Asthma and Airway Remodelling:

Targeting Mitogen-activated Protein Kinases as Future Therapeutics

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Asthma is a chronic disorder of the airways affecting millions of people worldwide. Airways are remodelled, or thickened, resulting in airway obstruction and a decline in lung function. Airway remodelling is considered to be a consequence of long-term inflammation. As the current drugs for treating airway remodelling have side effects, we urgently need to target the inflammatory pathways that control the development of the remodelled phenotype in the airway. A wealth of studies has implicated the mitogen-activated protein kinase (MAPK) family of phosphoproteins as critical signalling molecules that drive pro-inflammatory pathways. Thus, inhibition of MAPKs has emerged as an attractive strategy for reversing inflammation and remodelling in asthma. This chapter will focus on targeting MAPKs as future therapeutics. We will briefly outline the use of small molecule MAPK inhibitors, and then explore the potential of harnessing the power of an endogenous MAPK deactivator – MAPK phosphatase 1 (MKP-1) – in inhibiting MAPK-mediated pro-remodelling

functions. Our recent studies demonstrate that MKP-1 deactivates MAPK signalling in airway smooth muscle cells; a pivotal airway cell in asthma and airway remodelling. Thus, this chapter will focus on the role of MAPKs in the development of the pro-remodelling phenotype in asthma and highlight the promise of novel anti-inflammatory strategies designed to reverse the development of the airway remodelling phenotype by regulating the anti-inflammatory protein – MKP-1.

What is asthma?

If you have asthma, you experience episodes of wheezing, chest tightness and shortness of breath in response to a variety of "triggers". This occurs because the airways in your lungs narrow, making it more difficult for air to get through. Over 2.2 million Australians have asthma. This includes 1 in 4 children, 1 in 7 teenagers and 1 in 10 adults (Source: National Asthma Campaign). Because "when you can't breathe, nothing else matters" (American Lung Association), asthma is an important and debilitating disease that we need to understand more about so that we can beat it!

What causes asthma?

Asthma is a complex disease with both genetic and environmental causes. Why do we get asthma? The causes are many and varied. Allergy may play a big role. According to the National Asthma Campaign, 8 in 10 Australians with asthma have positive allergy test results. Allergy occurs when your immune system reacts to substances (known as allergens) in the environment that do not bother most people. These allergens can be found in house dust mites, pets, pollen, moulds and foods and can "trigger" asthma. You may be born with a genetic tendency to develop allergic diseases (called "atopy"); or allergy may develop throughout life. There are still a large number of unanswered questions about the development of allergic disease but currently much intense research is being performed all around the world to understand more about the links between allergic disease and asthma.

What is the cellular basis of asthma?

Asthma is characterized by inflammation and airway hyper-responsiveness. An acute asthma attack can be brought on by exposure to triggers. Exposure to triggers induces airway inflammation characterized by mast cell degranulation and an influx of lymphocytes and eosinophils. These cells secrete various agents capable of perpetuating inflammation and provoking airway smooth muscle contraction (bronchospasm). Accordingly, the majority of therapeutic agents used for asthma seek to minimize the development or consequences of airway inflammation (corticosteroids) or directly promote airway smooth muscle relaxation (β_2-agonists).

What is airway remodelling?

Asthma is a treatable health condition and we have a number of effective drug treatments to tackle acute asthmatic attacks. With good asthma management, asthmatics can lead normal, active lives. But, there are still a number of unanswered questions. We now know that the airways of asthmatics can become "thickened" or remodelled over time. This occurs when the inflammation that is part of an acute asthmatic attack is not treated or controlled effectively. The consequence of uncontrolled asthma is that permanent changes in the airways can occur and unfortunately, these cannot be completely reversed with current treatments. As development of remodelled airways is correlated with deterioration of lung function, we urgently require therapies that reduce and reverse structural changes in remodelled airways. Although corticosteroids can inhibit some aspects of remodelling, side effects exist and thus, corticosteroid-sparing strategies to prevent airway remodelling require further investigation.

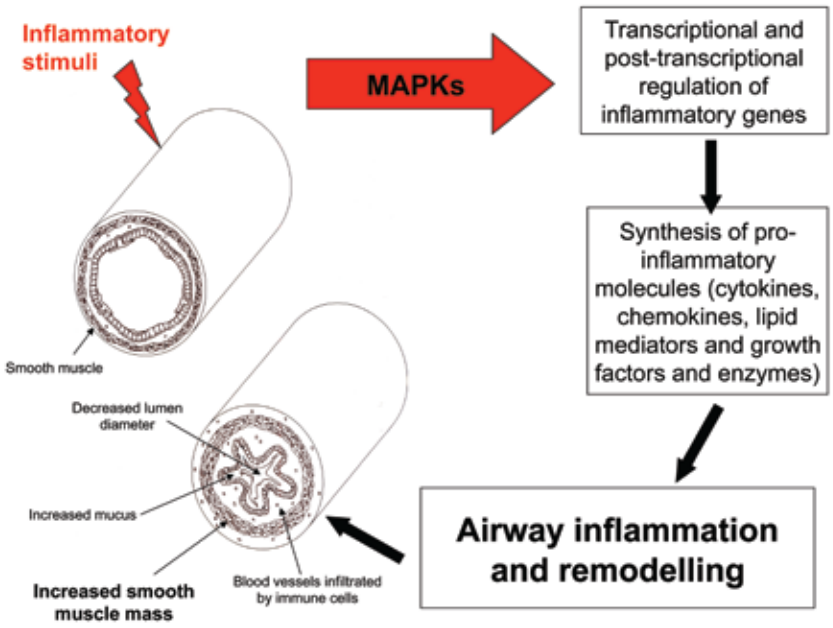
Airway smooth muscle plays an integral role in acute asthma and airway remodelling
In the Respiratory Research Group at the University of Sydney we have focussed on the role of the airway smooth muscle in asthma and airway remodelling. Airway smooth muscle is no longer viewed simply as a bystander

structural cell passively maintaining the airway wall integrity and responsible for bronchoconstriction; it has now emerged that airway smooth muscle plays a critical active role in the pathogenesis of asthma and airway remodelling. When an acute asthma attack is triggered, airway smooth muscle is bathed in a wide variety of inflammatory mediators released by mast cells upon degranulation and by-products of gene expression from lymphocytes and eosinophils. The airway smooth muscle then undergoes a number of important cellular reactions in response to these stimuli. Cells undergo calcium–mediated contraction and undergo bronchoconstriction that is typical of an asthmatic attack. Additionally, a number of important pro-remodelling genes are activated in airway smooth muscle cells. The protein products of this gene expression result in increased cell growth and increased production of pro-fibrotic proteins. It is this increase in airway smooth muscle mass (due to both increased airway smooth muscle cells numbers and also an increase in the amount of fibrotic proteins in and around the airway smooth muscle cells) that is considered to contribute strongly to the "thickening" of the airway wall. Because increased airway wall thickening results in reduced airway calibre (4), airway remodelling is a significant contributor to the exaggerated airway narrowing observed in asthmatics. It is generally considered that airway remodelling is a consequence of long-term inflammation, and although numerous cell types contribute to airway remodelling, the increase in airway smooth muscle mass has the largest impact on airway narrowing in asthma (5). Thus, airway smooth muscle plays an integral role in acute asthma and chronic airway remodelling and understanding the underlying signalling pathways will allow us to design future drug strategies for reversing inflammation and remodelling in asthma.

MAPKs - signalling pathways that drive development of airway remodelling

Our recent work has highlighted the importance of the mitogen-activated protein kinase (MAPK) super family of signalling molecules in the pro-remodelling functions of airway smooth muscle cells. There are three members of the MAPK superfamily: (1) p38 MAPK; (2) c-Jun N-terminal kinase (JNK); (3) extracellular signal-regulated kinase (ERK, also known as p44/p42 or ERK1/ERK2). These enzymes are involved in the intracellular signal transduction pathways mediated by a variety of stimuli (including cytokines, chemokines, growth factors, neurotransmitters and environmental stresses, such as allergens, respiratory viruses and airborne pollutants). To become activated by these various signals, the MAPKs pass through ATP-dependent phosphorylation cascades that consist of three protein kinases connected in series. Activation of MAPKs requires dual phosphorylation at the threonine (Thr) and tyrosine (Tyr) residues of the Thr-X-Tyr sites in the activation loop (where X is glutamic acid in the case of ERKs, proline in JNKs, and glycine in p38 MAPK). Many important substrates for MAPKs are transcription factors and after activation the MAPKs migrate to the nucleus of the cell and there they phosphorylate diverse transcription factors on specific sites and therefore control gene expression. In this way, MAPKs work at the key positions of many intracellular signalling pathways and regulate various cellular processes, from gene expression of pro-fibrotic proteins and inflammatory cytokines, to regulation of proliferative pathways by increasing levels of cell cycle proteins to induce cell growth. A wealth of studies has implicated the MAPK family of phosphoproteins as critical signalling molecules that drive pro-inflammatory pathways. Thus, inhibition of MAPKs has emerged as an attractive strategy for reversing inflammation and remodelling in asthma. Thus, MAPKs have emerged as critical pathways that drive development of airway remodelling to significantly contribute to asthma pathophysiology (6, 7)

The Respiratory Research Group at the University of Sydney has been at the forefront of discovery of the MAPK pathways responsible for critical pro-remodelling functions in airway smooth muscle cells, such as: increased synthetic function, production of cytokines (8-10) and pro-fibrotic proteins (11-13); and increased cell growth ((13-16)). We now wish



to use our knowledge to aid the future design of drugs that help tackle the development of airway remodelling in asthma.

Mitogen-activated protein kinases (MAPKs) as future therapeutic targets

As recent research underscores the importance of the MAPK signalling pathways in key airway smooth muscle functions that underlie the development of the remodelled phenotype, these pathways may be targeted as therapeutic strategies in the future. As the current drugs for treating inflammation (corticosteroids) have side effects, we urgently need to target the inflammatory pathways that control the development of the remodelling phenotype in the airway, as this knowledge may yield corticosteroid-sparing strategies in the future. In this chapter we focus on two approaches: firstly, use of small molecule inhibitors to inhibit MAPK pathways; and secondly, harnessing the power of endogenous MAPK deactivators, such as MAPK phosphatase 1 (MKP-1).

MAPK inhibitors

Recent research has begun to elucidate signalling pathways responsible for key airway smooth muscle functions that underlie the development of the remodelled phenotype (reviewed in (17)). Of the MAPK super family, most research in the airway smooth muscle cell arena has focused on the role of the p38 MAPK- and ERK-mediated pathways. Although an oversimplification, p38 MAPK pathways are considered responsible for synthetic function and the secretion of cytokines, while ERK-mediated pathways dominate in airway smooth muscle proliferation (reviewed by (18, 19)). JNK has only been investigated somewhat more recently and has been shown to regulate cytokine gene expression and protein secretion (20). Thus, as the importance of the MAPK pathways in airway smooth muscle cell biology and inflammatory airway diseases such as asthma and airway remodelling are emerging, these pathways may be targeted as therapeutic strategies in the future.

p38 MAPK: p38 MAPK is activated by an ATP-dependent dual phosphorylation at Thr180 and Tyr182 residues. Many laboratories around the world have designed and synthesized small molecules that block the ATP binding site of

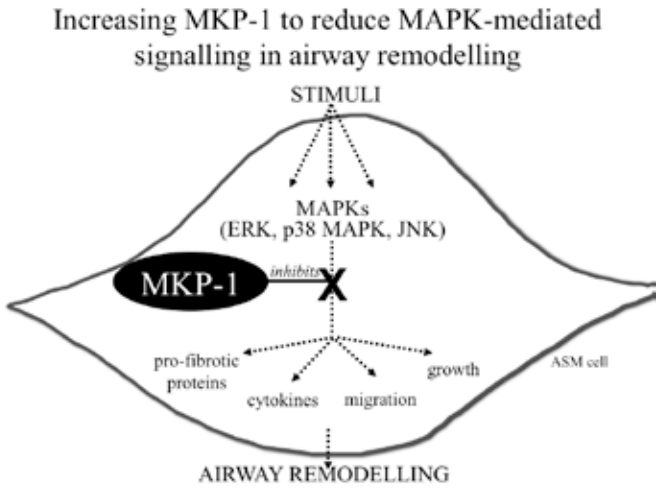
p38 MAPK, yielding a large number of potential p38 MAPK inhibitors (reviewed in (21, 22)). The prototypical p38 MAPK inhibitor is SB203580, one of the first generation p38 inhibitors and widely used as pharmacological tool to study p38 MAPK pathways. We (9, 10, 23) and others (24, 25) have used SB203580 in vitro to highlight the key role played by p38 MAPK in secretion of pro-inflammatory cytokines from airway smooth muscle cells (9, 10). The importance of p38 MAPK inhibition as a pharmacotherapeutic approach in asthma has been further underscored by demonstration of reduced pulmonary inflammation and airway hyperreactivity in a mouse model of asthma (26). In vivo, the use of SB203580 as an anti-inflammatory drug has been obstructed by its liver toxicity, as the pyridinyl imidazoles were found to inhibit hepatic cytochrome P450 enzymes (27). Promising anti-inflammatory actions have been observed in an in vivo model with the p38 MAPK inhibitors SD-282 (28) and further investigations into non-toxic p38 MAPK inhibitors are currently under intense investigation worldwide.

ERK: Dual threonine and tyrosine phosphorylations activate both ERKs, at Thr202/Tyr204 for ERK1 and Thr185/Tyr187 for ERK2. MAPK pathways are activated by dual phosphorylation by respective upstream activators. For ERK, the upstream kinase is mitogen-activated protein kinase, known as MEK. There are a number of MEK inhibitors such as U0126 and PD98059. These have been used extensively in vitro in airway smooth muscle cells to delineate pro-proliferative pathways. We (15, 29) and others (30, 31) have demonstrated that ERK-dependent pathways control growth.

JNK: The JNK phosphorylation sites are Thr183 and Tyr185. Relatively less is known about the role of JNK in airway smooth muscle pro-remodelling functions in vitro, although airway smooth muscle hyperplasia and inflammatory cytokine release in mice chronically exposed to allergens is inhibited by the administration of SP600125, a JNK inhibitor (32).

MKP-1: what is it?

Cellular function is profoundly affected by both strength and duration of MAPK activation, which must be strictly controlled to modulate functional outcome. This crucial negative feedback control is achieved by the balanced interplay between MAPK activation by diverse environmental and chemical stimuli and the negative feedback mechanism mediated by protein phosphatases such as MAPK phosphatases (MKPs) (33, 34). Several MKPs have been classified (34) and in general they all regulate MAPK activity in a negative feedback mechanism by dephosphorylating the threonine and the tyrosine residues in the activation loop "Thr-X-Tyr-motif" of these signalling enzymes. Since these phosphatases dephosphorylate both



the threonine and the tyrosine residues they are also called dual-specificity phosphatases (DUSPs). Although all MKPs have highly conserved structural elements they vary in subcellular localization, substrate specificity, tissue distribution and also in their inducibility by various stimuli. The characteristic structural elements of MKPs are within all MKPs homologue dual-specificity phosphatase (DUSP) domain in the C-terminal half which leads to the dual dephosphorylation on the threonine and tyrosine residues of MAPKs, and a MAPK-binding domain situated in the N-terminal half. This domain is very important for the quality of the interaction between MKPs and MAPKs and therefore plays a crucial role in the regulation of the enzymatic specificity (35, 36).

MKP-1, the prototypical member of the MKP family, is an immediate early gene located in the nuclear compartment and that means that its transcription is induced instantly after exposure to diverse stimuli such as growth factors, heat shock and stress. Many of these stimuli are the same as those that also activate MAPKs (36-39). MKP-1 is the first-discovered MKP and controls the MAPK signalling pathway by inactivating these enzymes through dephosphorylation (40-42). MAPKs are activated by pro-inflammatory mediators and diverse stress stimuli and therefore play a very important role in the innate and inflammatory immune response (43). The inhibitory effect of MKP-1 on MAPK activation and therefore the modulation of pro-inflammatory processes indicate the importance of MKP-1 in this cellular pathway. Therefore the attenuation of MAPK signalling by MKP-1 could be a promising target to reduce the inflammatory responses mediated by MAPK activation.

Current anti-asthma therapies and MKP-1: what do we know so far?

Inhaled corticosteroids are first-line anti-inflammatory therapy in asthma. However, there is increasing evidence that the combination of an anti-inflammatory corticosteroid with long-acting bronchodilator β_2-agonists results in superior therapeutic benefit, when compared with each component administered alone (reviewed in (44)). "Understanding the molecular basis of this fundamental clinical observation is a Holy Grail of current respiratory diseases research as it could permit the rational exploitation of this effect with the development of new 'optimized' corticosteroid/β_2-agonists combination therapies" (44). It is this question that we have begun to address. We have focused our investigations on the endogenous MAPK deactivator – MKP-1 – as MKP-1 acts as a critical negative regulator of the myriad pro-inflammatory pathways activated by MAPKs. We (10) and others (45) have recently discovered that the anti-inflammatory action of corticosteroids in ASM cells occurs via upregulation of MKP-1. Moreover, the corticosteroid-inducible gene MKP-1 is enhanced by long-acting β_2-agonists (46), and the enhanced expression of MKP-1 may explain the beneficial effects of β_2-agonists/corticosteroid combination therapies in the repression of inflammatory gene expression in asthma (44). Thus, further investigations into the molecular basis of MKP-1 regulation are urgently required as this new knowledge may lead to elucidation of "optimized" corticosteroid-sparing therapies.

How is MKP-1 regulated?

MKP-1 is a 367 amino acid protein expressed by an immediate-early gene (37). Stimuli which activate MAPKs also induce MKP-1 protein that then acts back on MAPKs as an important negative feedback controller to limit the strength and duration of MAPK-mediated cellular signalling (reviewed in (47)). However, the increase in MKP-1 protein levels is transient, as MKP-1 protein (expressed as a result of increased transcription and/or mRNA stability) then undergoes rapid degradation by the proteasomal machinery. Investigations into how MKP-1 is regulated will allow us to fully explore the potential of harnessing the power of an endogenous MAPK deactivator – MKP-1 – to inhibit MAPK-mediated pro-remodelling functions.

It is known that the activity of MKP-1 can be regulated at different levels. There are several approaches to regulate the expression of MKP-1 not only on the transcriptional level but also

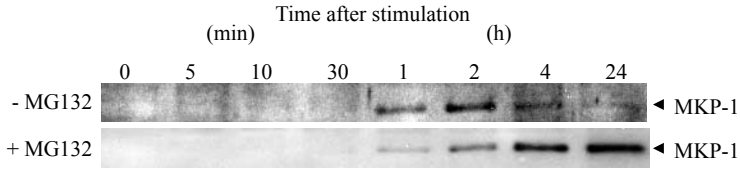


Figure C. MKP-1 is proteasomally degraded in airway smooth muscle cells. Airway smooth muscle cells were pretreated with and without the proteasome inhibitor MG132 (10 μM), before stimulation with 10 ng/ml platelet derived growth factor-BB. Western blotting for MKP-1 was performed.

on the post-transcriptional and on the post-translational level. Shortly after exposure to the diverse stimuli (such as stress and growth factors) the transcription of MKP-1 is induced and mRNA levels are highly increased. The mechanism by which the transcriptional induction is mediated is currently not completely understood but since MKP-1 can be induced by various stimuli the transcriptional induction and regulation seems to be a promising target for the modulation of the inflammatory response (42). The post-transcriptional regulation of MKP-1 stability is another mechanism to alter the enzymatic activity of this phosphatase. It has been shown that the MKP-1 stability can be increased through phosphorylation by ERK1/2 and that leads to an accumulation of MKP-1 in the cell and therefore may enhance the activity of MKP-1 (42, 48). To what extent the enhanced expression of MKP-1 has positive or maybe also negative outcomes is not completely known. The modulation of the interactions between MKP-1 and its substrates is a subject of the post-translational regulation of MKP-1 activity. The substrate specificity depends on diverse structural elements of MKP-1 and the modulation of this interaction could also be a mechanism to enhance the phosphatase activity and hence the inactivation of the MAPK pathway and therefore the efficacy of the anti-inflammatory reaction in the immune response. If it is possible to inhibit or attenuate the proteosomal degradation of MKP-1, that would provide another promising opportunity to regulate the MKP-1 activity. We are currently working on achieving a greater understanding of the multiple levels of regulation of MKP-1 regulation so that we can increase MKP-1 and reduce MAPK-mediated signalling.

Our current work: how is MKP-1 degraded?

Thus, because MKP-1 serves a crucial negative feedback role in regulating pro-remodelling signal transduction, discovering mechanisms to regulate the protein level or enzymatic activity of this endogenous MAPK-deactivator may be exploited as a novel anti-inflammatory strategy in asthma and airway remodelling. Thus, in order to achieve our aim of increasing MKP-1 to reduce MAPK-mediated signalling, we need a greater understanding of the three levels of MKP-1 regulation: (i) transcriptional; (ii) post-transcriptional; and (iii) translational. We are currently examining how MKP-1 is regulated at the translational level, that is, how MKP-1 is degraded by the proteasome – the garbage bin of the cell. As mentioned earlier, stimuli which activate MAPKs also induce MKP-1 protein. However, the increase in MKP-1 protein levels is transient, as MKP-1 then undergoes rapid degradation by the proteasomal machinery. If we can understand how and why MKP-1 is degraded, we could design molecules that could specifically block MKP-1 degradation and thus allow MKP-1 protein levels to remain high. We are currently a long way from our goal but our preliminary evidence obtained in airway smooth muscle cells shows that stimulation increases MKP-1 protein levels (peaking at ~ 2 hours) but the protein degrades over time. We have used a non-specific proteasome inhibitor – MG132 – and confirmed that we can inhibit proteasomal degradation and keep

MKP-1 levels high. We now need to investigate whether we can do this just for MKP-1 protein.

Future directions and significance

Asthma is a widespread chronic health problem. Airway remodelling underlies asthma, but unfortunately, the pharmacotherapy currently available for combating remodelling has had limited success. We need to understand how to control airway remodelling to be able to improve treatments for asthma. With our work we will increase our knowledge of the mechanistic basis of asthma focusing on the role of the MAPK pro-remodelling signalling pathways. In this chapter we have highlighted the use of small molecule MAPK inhibitors in vitro and in vivo and explored the potential of harnessing the power of an endogenous MAPK deactivator – MKP-1 – in inhibiting MAPK-mediated pro-remodelling functions. It is our hope that the elucidation of novel molecular strategies and drug candidates targeting the pro-remodelling MAPK signalling pathway will serve as a future pharmacotherapeutic approach to treating and preventing airway remodelling.

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