The Expanding Role of p38 Mitogen-Activated Protein Kinase in Programmed Host Cell Death

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ABSTRACT: The p38 mitogen-activated protein kinase (MAPK) is involved in a multitude of essential cellular processes. The kinase is activated in response to environmental stresses, including bacterial infections and inflammation, to regulate the immune response of the host. However, recent studies have demonstrated that pathogens can manipulate p38 MAPK signaling for their own benefit to either prevent or induce host cell apoptosis. In addition, there is evidence demonstrating that p38 MAPK is a potent trigger of pathogen-induced necrosis driven by mitochondrial membrane disruption. Given the large number of p38 MAPK inhibitors that have been tested in clinical trials, these findings provide an opportunity to repurpose these drugs for improved control of infectious diseases.

KEYWORDS: apoptosis, necrosis, mitochondria, p38 MAPK, *Mycobacterium tuberculosis*

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Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases involved in a variety of cellular processes, including differentiation, proliferation, and cell survival. Following activation by extracellular stimuli, MAPKs are responsible for transducing signals from the cell membrane to the nucleus. Three subgroups of conventional MAPK have been described: the c-Jun NH2-terminal kinase (JNK), the extracellular signal-regulated kinase (ERK), and the p38 MAPK. While ERK is mainly activated in response to growth stimuli, JNK and p38 MAPK are activated in response to environmental stresses, such as inflammation, DNA damage, UV irradiation, and osmotic as well as oxidative stress. MAPKs are activated by dual phosphorylation of tyrosine and threonine residues, catalyzed by dual specificity kinases, called MAPK kinases (MAP2K). The activation can be reversed by MAPK phosphatases (MKP).[1] In this commentary, we will focus on p38 MAPK and summarize recent findings on p38 MAPK mediated programmed host cell death.

There are four isoforms of p38 MAPK (p38α, p38β, p38γ, and p38δ), which vary in their substrate specificities and their expression patterns, resulting in different sensitivities to p38 MAPK inhibitors.[2] p38 MAPK has a variety of targets, including protein kinases, phosphatases, cell-cycle regulators, and transcription factors. The α and β isoforms are able to autophosphorylate. Otherwise, p38 MAPK activation follows the signaling module of MAP3K (such as apoptosis signal-regulating kinase 1 [ASK-1] and MEKK1-4) phosphorylating MAP2K (MKK3 and 6), which in turn phosphorylate and activate p38 MAPK.[3] In bacterial infections, p38 MAPK activation is initiated either by secreted factors or components of the bacterial cell wall. Alternatively, release of proinflammatory cytokines like interleukin (IL) 1β or tumor necrosis factor (TNF) α from infected host cells can trigger activation. Therefore, p38 MAPK plays an important role in managing the immune response of the host and is often targeted by pathogens to promote virulence and ensure pathogen survival.[4]

Apart from its prominent and well-recognized role in the cellular stress response and inflammation, p38 MAPK has also been linked to execution of regulated cell death. Initially, several studies identified p38 MAPK as a key mediator of apoptosis,[2] an immunologically silent form of cell death that occurs regularly during development to maintain homeostasis in tissues. There are two major apoptotic pathways, namely the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. Both pathways lead to the activation of the effector cysteine proteases (caspases) 3, 6, and 7 and to the degradation of apoptotic bodies by phagocytes.[5] The extrinsic pathway can be activated by extracellular stimuli, such as lipopolysaccharide (LPS), a proinflammatory component of the outer envelope of gram-negative bacteria. LPS is mainly recognized by toll-like receptors (TLR), which recruit caspase 8 to complete apoptosis by the death-inducing signaling complex (DISC).[6] Most bacterial infections also induce an increased production of TNF-α by immune cells, which is recognized by the TNF receptor superfamily (death receptors), resulting in capsase 8 activation and apoptosis. The intrinsic pathway is mainly initiated by DNA damage and leads to mitochondrial outer-membrane permeabilization (MOMP), which triggers the release of cytochrome *c* (CytoC). CytoC

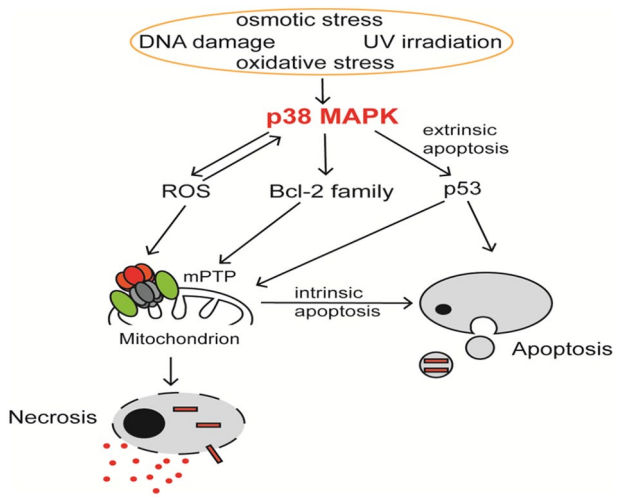

Figure 1. Schematic representation of the role of p38 mitogen-activated protein kinase (MAPK) in host cell death. Activation of p38 MAPK in response to environmental stress triggers host cell death either by apoptosis or by necrosis. p38 MAPK initiates caspase activation via the extrinsic and intrinsic pathway to execute apoptosis. In addition, p38 MAPK induces programmed necrosis by opening of the mitochondrial permeability transition pore (mPTP). Bcl-2 indicates B-cell lymphoma 2; ROS, reactive oxygen species.

binds to the apoptotic protease activating factor 1 (APAF1) to activate the initiator caspase 9, which cleaves and activates the effector caspases.[7] Intrinsic apoptosis often requires the activation of p38 MAPK by MKK3 or MKK6. Subsequently, p38 MAPK phosphorylates and activates proteins of the B-cell lymphoma 2 (Bcl-2) family. The Bcl-2 family consists of anti-apoptotic proteins, such as Bcl-2, Bcl-xL and myeloid cell leukemia-1 (Mcl-1), and pro-apoptotic proteins, like the Bcl-2 associated X protein (Bax) and Bim, which are essential for the regulation of mitochondrial integrity and function. p38 MAPK can either phosphorylate Bim at serine 65 to induce apoptosis or the tumor protein p53, a transcription factor, at serine 46, to initiate the pro-apoptotic transactivating function of p53.[8,9] Upon stress p53 ubiquitylation is suppressed, leading to p53 stabilization and accumulation in the nucleus. p53 induces the expression of death receptors and can activate members of the Bcl-2 family to promote apoptosis (Figure 1). Therefore, p53 induces the expression of pro-apoptotic Bcl-2 proteins and simultaneously represses anti-apoptotic proteins, resulting in permeabilization of the outer mitochondrial membrane (OMM).[3] Overexpression of Bcl-2 or Bcl-xL has been shown to reduce stress-mediated accumulation of p53 at the mitochondria and thereby prevent apoptosis.[8]

Many pathogens modulate p38 MAPK signaling pathways to either prevent or induce apoptosis for their own benefit.[4] One example is the bacterium *Bacillus anthracis* which releases a protease-containing toxin that degrades components of the MAPK signaling cascade and thereby suppresses MAPK activation and apoptosis.[4] *Salmonella enterica* interferes with the phosphorylation of MAPK utilizing acetylases to prevent MAP2K activation or by modifying threonine residues in the activation motif of p38α to irreversibly inactivate p38 MAPK, resulting in the inhibition of cell death.[4] In neutrophils, infection with *Coxiella burnetii* leads to p38 MAPK-dependent upregulation and stabilization of the anti-apoptotic protein Mcl-1 resulting in increased cell survival.[10]

Most pathogens inhibit apoptosis in early stages of infection, but promote cell death in later stages to initiate dissemination of disease. *Mycobacterium tuberculosis* (*Mtb*) is another clinically relevant bacterium that modulates host cell death as a part of its pathogenic mechanisms.

Aguilo et al[11] described that *Mtb* increases the levels of calcium (Ca^{2+}) and reactive oxygen species (ROS) inside the host cell to induce endoplasmic reticulum (ER)-stress-associated pathways. These events are regulated by ASK1 and p38 MAPK, resulting in the activation of apoptosis. Although in recent years, it has been suggested that only attenuated *Mtb* strains lacking important virulence factors induce apoptosis, while fully virulent *Mtb* evades apoptosis by the induction of necrosis.[12]

We and others have recently shown that virulent *Mtb* induces programmed necrosis by opening of the mitochondrial permeability transition pore (mPTP) triggering loss of mitochondrial integrity and function.[12,13] In healthy cells, the mPTP preserves the mitochondrial membrane potential ($\Delta\Psi m$) across the inner mitochondrial membrane (IMM). Under certain circumstances, opening of the mPTP results in massive influx of ions which disrupts the $\Delta\Psi m$ and decreases the oxidative phosphorylation and adenosine triphosphate (ATP) production resulting in necrotic cell death. We were now able to show that *Mtb* initiates necrosis via mPTP opening in a p38 MAPK-dependent way.[13] p38 MAPK induces the dissociation of the enzyme hexokinase II (HK II), an OMM bound antagonist of ROS-mediated mPTP opening. siRNA-mediated downregulation of p38 MAPK as well as chemical p38 MAPK inhibition using the pan-p38 MAPK inhibitor doramapimod (BIRB 796) potently prevents host cell death and preserves intracellular ATP levels in a variety of cell lines and primary human macrophages.[13] Key mediators of *Mtb*-driven hyperinflammation and tissue damage are neutrophil granulocytes.[14] Interestingly, chemical p38 MAPK inhibition was also highly cytoprotective for infected primary human neutrophils (Figure 2) which, by repurposing of clinically tested p38 MAPK inhibitors, may provide a therapeutic approach against tuberculosis.

It is unlikely that p38 MAPK directly interacts with hexokinase II (HKII) or other OMM bound mitochondrial proteins. Thus, the link between p38 MAPK and mPTP opening still needs to be investigated. One potential candidate is once more p53, which is activated downstream of p38 MAPK. Upon activation, ubiquitinated p53 migrates to mitochondria and possibly interacts with cyclophilin D (CypD) to promote mPTP opening.[8] In addition, p53 mediates the transcription of other proteins involved in mPTP regulation, such as HK II.[15] Therefore, p53 inhibition may be another possible target to prevent host cell death in infectious diseases such as tuberculosis. Recently, we could demonstrate that chemical inhibition of p53 using pifithrin α is nearly as cytoprotective as p38 MAPK

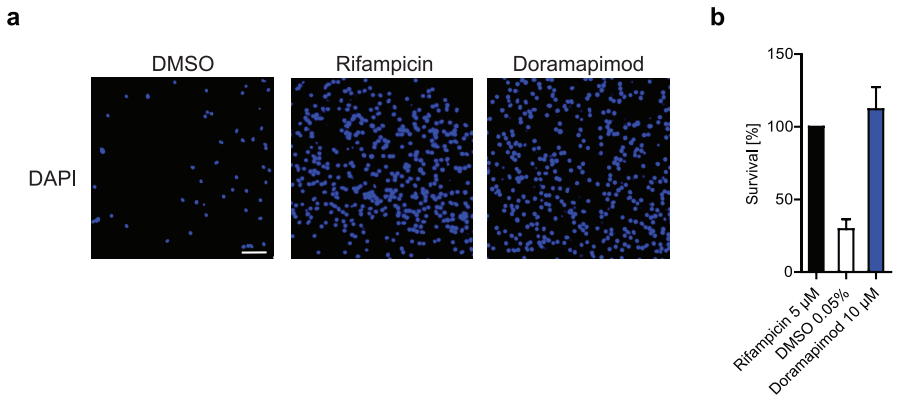

Figure 2. Cytoprotective effect of the p38 MAPK inhibitor doramapimod in *Mycobacterium tuberculosis* (*Mtb*) infected primary human neutrophils. (A) Representative fluorescent microscopy images of *Mtb*-infected primary human neutrophils (MOI 1) treated with doramapimod (10 μM), rifampicin (5 μM), or DMSO (0.05%). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; scale bar: 100 μm). Images are representative of two individual experiments with multiple replicates. (B) Doramapimod (10 μM)-treated neutrophils were infected with the wild-type *Mtb* strain Erdman (MOI 1) and surviving cells were stained with DAPI to determine the number of living cells 24 h post infection. Data from two experiments with multiple replicates are shown in (B). Results are expressed as the mean ± SEM.

inhibition in *Mtb*-infected MRC-5 lung fibroblasts and J774.2 macrophages.[13]

Alternatively, ROS could be a possible link between p38 MAPK signaling and mPTP opening. Mitochondrial ROS may induce DNA damage and protein oxidation, which in turn activates the redox-sensitive kinase p38 MAPK to promote host cell death. Furthermore, ROS can inhibit MKP to allow persistant p38 MAPK phosphorylation, which results in hyperinflammation and tissue damage.[16]

The precise mechanism and components involved in opening of the mPTP are still debated. Future studies will help to investigate the signaling pathways involved in mPTP-mediated cell death and can lead to new therapeutic strategies. One candidate might be p38 MAPK which could be targeted by host-directed therapy to assist and improve antibiotic therapy and thereby decrease bacterial spread, hyperinflammation, tissue damage, and relapse of the disease.[13]

Author Contributions

JG and JR wrote the article. JG performed experiments.

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