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Conditional expression of MAP kinase phosphatase-2 protects against genotoxic stress-induced apoptosis by binding and selective dephosphorylation of nuclear activated c-jun N-terminal kinase

Laurence Cadalbert*, Callum M. Sloss, Pamela Cameron, Robin Plevin

Strathclyde Institute for Biomedical Sciences, Department of Physiology and Pharmacology, University of Strathclyde, 27 Taylor Street, G4 0NR, Glasgow, Scotland, UK

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Abstract

MAP Kinase Phosphatase-2 (MKP-2) is a dual specific nuclear phosphatase which is selective for both ERK and JNK, MAP kinases implicated in the regulation of apoptosis in response to genotoxic stress. Here we report the conditional expression of MKP-2 in human embryonic kidney cells 293. We demonstrate that Flag-WT-MKP-2 is able to rescue cells from apoptotic commitment when subjected to UV-C or *cis*platin treatment. We establish that upon stimulation all three major MAP kinase families (ERK, JNK and p38 MAP kinases) are activated. However, MKP-2 is surprisingly only able to deactivate JNK in vivo. Furthermore, whilst pre-treatment of cells with either the JNK inhibitor SP600125, or the MEK-1 inhibitor PD98059, also reverses UV-C and *cis*platin-induced apoptosis, the anti-apoptotic effect of MKP-2 overexpression is not additive with SP600125 but is with PD098059, suggesting that MKP-2 is involved in specifically terminating JNK activity and not ERK. The inability of MKP-2 to dephosphorylate ERK in vivo is also not due to the inability of Flag-MKP-2 to bind both ERK and JNK; phosphorylated forms of each kinase are co-precipitated with both WT and CI-MKP-2. Immunofluorescence studies however demonstrate that ERK is exclusively cytosolic in origin and not translocated to the nucleus following UV-C and *cis*platin treatment whilst JNK is principally nuclear. These studies demonstrate the in vivo specificity of MKP-2 for JNK and not ERK and show that nuclear-targeted JNK is involved in genotoxic stress-induced apoptosis.

Keywords: MKP-2; UV; *Cis*platin; ERK; JNK; Apoptosis

1. Introduction

The mitogen-activated protein kinases are a family of kinases recognised to play many important cellular roles including cellular proliferation, apoptosis and inflammation. At least three major groupings are known, the extracellular signal-regulated kinases (ERKs) and isoforms of the stress activated protein kinases: JNK and p38 MAP kinase. Other recently identified isoforms include big MAP kinase (ERK5) and ERK7 (see for review, Ref. [1]). Individual members of each group play important roles in the regulation of cellular responses, dependent upon the kinetics of activity and the subcellular localisation of the kinase. For example sustained ERK activation is associated with cellular proliferation whilst transient ERK activation does not lead to further cellular response [2]. Similarly, sustained or transient JNK activity selectively discriminates between apoptosis or cell survival [3].

Abbreviations: BSA, bovine serum albumin; CI, catalytically inactive; *cis*platin, *cis*-Platinum (II) Diammine Dichloride; Dox, doxycyclin; DAPI, 4′,6′-diamidino-2-phenylindole hydrochloride; DUSP, dual specificity phosphatase; ECL, enhanced chemoluminescence; ERK, extracellular signal-regulated kinase; GST, glutathione S-transferase; HRP, horseradish peroxidase; JNK, c-Jun N-terminal kinase; MAP, mitogen-actived protein; MKP, MAP kinase phosphatase; PBS, phosphate buffer saline; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; Tet, tetracyclin; UV, ultra violet; WT, wild-type.

* Corresponding author. Tel.: +44 141 548 2964; fax: +44 141 552 2562.
E-mail address: Laurence.Cadalbert@strath.ac.uk (L. Cadalbert).

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The mitogen-activated protein kinases phosphatases (MKPs) regulate the activities of the MAP kinases through dual specific dephosphorylation at tyrosine and threonine residues and thus play important roles in limiting the amplitude and extent of cellular activation (see for review, Ref. [4]). Even though the MAP kinase phosphatases form a large family of proteins, each phosphatase has a unique function as suggested by the MKP-1 or MKP-5 gene knockout mouse [5,6], function which is determined by their selectivity for MAP kinase isoforms, a specific subcellular localisation, and mechanisms of regulation, either acutely through direct phosphorylation which enhances activity, e.g. MKP-1 [7], or through increased induction following growth factor stimulation of cells, e.g. MKP-1 [8], PAC-1 [9] and B23 [10].

MKP-2, an inducible nuclear phosphatase was one of the earliest MKPs to be identified [11–13]. However, relatively little attention has been devoted to its actions. Originally found to display selectivity for JNK and ERK over p38 MAP kinase in vitro [11–14], it was thought to play a role as a secondary mechanism for the termination of prolonged ERK activation as its induction is delayed relative to other nuclear MKPs such as MKP-1 [15,16]. Studies have shown induction of MKP-2 in response to growth factors [8,11], gonadotrophic hormones [17] and the oncogene v-jun [18], and is associated with the inactivation of ERK. However, because these interventions have the potential to induce the expression of other MKPs, the functional consequences of MKP-2 induction have not been clearly determined.

In this study we utilised for the first time a conditional expression system to specifically assess the role of MKP-2 in the termination of MAP kinase signals in relation to induction of cellular apoptosis. Two important cytotoxic stimuli have been selected in order to specifically determine which MAP kinase pathway was regulated by MKP-2. UV light which has been shown to initiate JNK-mediated apoptosis through disruption of mitochondrial function [19] and secondly, the chemotherapeutic agent, *cis*platin, which is believed to require ERK activation to mediate cell death in HeLa cells [20].

In our study we find that conditional overexpression MKP-2 reverses UV-C and *cis*platin-mediated apoptosis, suggesting that JNK signals generated from the nucleus are requisite for apoptosis in response to these agents. MKP-2 reduced UV-C and *cis*platin-stimulated JNK activity substantially. In addition, we found that whilst MKP-2 can bind to ERK and p38 neither of these two kinases can be regulated by MKP-2 in HEK 293 cells, since neither UV-C nor *cis*platin were able to mediate translocation of ERK to the nucleus. We further identified that JNK binding to MKP-2 is dependent on the phosphorylation status of JNK. These results thus question both the role of JNK in mitochondrial-mediated apoptosis in response to UV-C and the role of ERK in the apoptotic actions of *cis*platin and explain the discrepancy between MKP-2, JNK specificity and binding affinity.

2. Experimental procedures

2.1. Materials

Cells were stimulated using UV-C (254 nm) crosslinker (CL-1000 UVP, Anachem, Cambridge, UK) at 60 and 600 J/m^2 as stipulated. *cis*platin (*cis*-Platinum (II) Diammine Dichloride) was purchased from Sigma-Aldrich (Poole, UK) and was used at 50 μM.

2.2. Plasmid constructs

Human MKP-2 cDNA was cloned from Human Umbilical Vein Endothelial Cell cDNA library and catalytically inactive MKP-2 was obtained as previously described [21]. A Flag-Tag was added to the C-terminal end of both WT-MKP-2 and CI-MKP-2 by insertion into pCMV-Tag4 vector (Stratagene, Amsterdam Zuidoost, The Netherlands). p-CMV-Tag4-WT-MKP-2-Flag and p-CMV-Tag4-CI-MKP-2-Flag were further sequenced and co-transfected into HEK cells to check that Flag-Tag adjunction did not alter MKP-2 activity. WT- and CI-MKP-2-Flag sequences were further inserted into p-TRE2 vector (Clontech, Basingstoke, UK). p-TRE2-Flag was obtained by annealing the following complementary primers 5′-AAGCTTAAGCTTATGGATTACAAGGATGACGACGATAAGTAGGGCCCGGTACCTTAATCT-3′ and 5′-TCTAGATCTAGATTAAGGTACCGGGCCCTACTTATCGTCGTCATCCTTGTAATCCATAAG-3′ corresponding to the Flag-Tag sequence flanked by HindIII and XhoI restriction sites and containing a KOZAK sequence and a stop codon. The annealing product was digested by HindIII and XhoI restriction enzymes and inserted into p-TRE2 between the same restriction sites. p-TRE2 vector, p-TRE-Luc and p-TK-Hyg selection plasmid were purchased from Clontech, Basingstoke, UK.

2.3. Cell culture

HEK 293 (Human Embryonic Kidney cells) cells were grown in Minimum Essential Medium Eagle containing 10% heat inactivated Foetal Bovine Serum and supplemented with 2 mM L-Glutamine, 1 X Non-essential amino acids, 50 unit/ml Penicillin, 50 μg/ml Streptomycin, 0.375% (w/v) Sodium Bicarbonate (all from Invitrogen, Paisley, Scotland, UK). HEK cells tranfected with pTet-On, pTK-Hyg and pTRE2-flag, pTRE2-WT-MKP-2 or pTRE2-CI-MKP-2 were cultured under selective conditions in Minimum essential medium Eagle containing 10% heat-inactivated tetracycline-free Foetal Bovine Serum (Clontech, Basingstoke, UK) supplemented with 2 mM L-Glutamine, 1 X Non-essential amino acids, 0.375% Sodium Bicarbonate,