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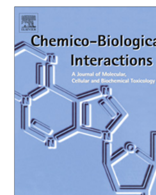

Modulation of albumin-induced endoplasmic reticulum stress in renal proximal tubule cells by upregulation of mapk phosphatase-1

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A B S T R A C T

High amounts of albumin in urine cause tubulointerstitial damage that leads to a rapid deterioration of the renal function. Albumin exerts its injurious effects on renal cells through a process named endoplasmic reticulum (ER) stress due to the accumulation of unfolded proteins in the ER lumen. In addition, albumin promotes phosphorylation and consequent activation of MAPKs such as ERK1/2. Since ERK1/2 activation promoted by albumin is a transient event, the aims of the present work were to identify the phosphatase involved in their dephosphorylation in albumin-exposed cells and to analyze the putative regulation of this phosphatase by albumin. We also sought to determine the role played by the phospho/dephosphorylation of ERK1/2 in the cellular response to albumin-induced ER stress. MAP kinase phosphatase-1, MKP-1, is a nuclear enzyme involved in rapid MAPK dephosphorylation. Here we present evidence supporting the notion that this phosphatase is responsible for ERK1/2 dephosphorylation after albumin exposure in OK cells. Moreover, we demonstrate that exposure of OK cells to albumin transiently increases MKP-1 protein levels. The increase was evident after 15 min of exposure, peaked at 1 h (6-fold) and declined thereafter. In cells overexpressing flag-MKP-1, albumin caused the accumulation of this chimera, promoting MKP-1 stabilization by a posttranslational mechanism. Albumin also promoted a transient increase in MKP-1 mRNA levels (3-fold at 1 h) through the activation of gene transcription. In addition, we also show that albumin increased mRNA levels of GRP78, a key marker of ER stress, through an ERK-dependent pathway. In line with this finding, our studies demonstrate that flag-MKP-1 overexpression blunted albumin-induced GRP78 upregulation. Thus, our work demonstrates that albumin overload not only triggers MAPK activation but also tightly upregulates MKP-1 expression, which might modulate ER stress response to albumin overload.

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1. Introduction

Whenever excreted into urine in high amounts, albumin is responsible for tubulointerstitial damage [1], causing a rapid deterioration of the renal function that often leads to severe kidney disease [2,3]. Albumin, which is the most abundant component of plasma proteins, exerts its injurious effects on renal cells through a process named endoplasmic reticulum (ER) stress due to the accumulation of unfolded and/or misfolded proteins in the ER lumen [4]. ER stress produces the activation of stress-response signaling pathways which allow cells to respond to perturbations and recover homeostasis [5]. These pathways are collectively named unfolded protein response (UPR). One major pro-survival mechanism prompted by the UPR is mediated by chaperone glucose-regulated protein 78 (GRP78). GRP78 plays an essential role in counteracting the apoptotic potential of ER stress by multiple mechanisms, such as binding to the unfolded proteins to alleviate ER stress conditions [6]. Accordingly, it has been established that albumin overload induces the expression of GRP78 in mouse proximal tubular cells [7].

Abbreviations: ER, endoplasmic reticulum; UPR, unfolded protein response; GRP78, glucose-regulated protein 78; ERKs, extracellular signal-regulated kinases; JNKs, c-Jun NH2-terminal protein kinases; SAPKs, stress-activated protein kinases; MAPKKs, MAPK kinases; MKP, MAPK phosphatase; BSA, Free fatty acid albumin; Act D, actinomycin D; CHX, cycloheximide; PD98059, 2-amino-3-methoxyphenyl)4H-1-benzopyran-4-one; SP600125, Anthra[1-9-cd]pyrazol-6(2H); OK, Opossum kidney.

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It is documented that albumin interacts with renal tubular cells through an unspecific receptor involved in its internalization [8] and promotes ER stress. It has been reported that the albumin reabsorbed by proximal tubular cells triggers a signal transduction pathway that includes ROS generation and the subsequent activation of the tyrosine kinase Src and the serine/threonine kinase mTOR (the mammalian target of rapamycin), and that these events play a central role in ER stress promoted by albumin [9].

This albumin-induced ER stress could involve MAPK family members. Indeed, in several systems the induction of ER stress is linked to the activation of MAPKs [10–12]. Moreover, albumin overload is known to promote the activation of MAPKs in renal tubular cells [13,14]. MAPKs play a crucial role in signal transduction pathways related to cell growth, differentiation and apoptosis and are classified in three subgroups: extracellular signal-regulated kinases (ERKs), c-Jun NH2-terminal protein kinases (JNKs) or stress-activated protein kinases (SAPKs), and p38 MAPKs [15]. MAPKs are phosphorylated in threonine and tyrosine residues by specific kinases named MAPK kinases (MAPKKs), such as MEK1 and MEK2 [15]. After dual phosphorylation, MAPKs acquire maximal activity and translocate to the nucleus, where they phosphorylate transcription factors driving the expression of MAPK-dependent genes. While JNK and p38 activation promote signaling pathways related to apoptosis [16], the activation of the MEK/ERK pathway is a common cause for cell resistance to death [15]. It has been demonstrated that the inhibition of the MEK/ERK pathway sensitizes cells to ER stress-induced apoptosis, and that this is associated with the downregulation of GRP78 expression and blockage of its induction by the UPR in colorectal cancer cells [17].

Because MAPK activity depends on phosphorylation processes, the magnitude and duration of this activity are linked to phosphatases capable of promoting MAPK dephosphorylation. The MAPK phosphatase (MKP) family comprises dual specificity enzymes that promote MAPK inactivation [18]. MKP-1 is a short-lived nuclear enzyme – product of an early gene – which is rapidly induced by a wide range of stimuli [19,20]. Due to its cellular localization, MKP-1 is responsible for nuclear MAPK inactivation. Consequently, this phosphatase controls the activity and/or expression of MAPK-dependent transcription factors and, ultimately, gene transcription [20]. MKP-1 expression is tightly regulated by a broad array of stimuli like hormones [21–25], drugs [26] and physical stress like heat shock [27] and UV radiation [28]. Moreover, this regulation is exerted through several mechanisms including the increase in gene transcription, as was documented in adrenocortical cells [22,29] and in Leydig cells [21] under stimulation with the corresponding trophic hormone. Other mechanisms include the stabilization of MKP-1 mRNA and the increase in its translational activity [30]. In addition, the regulation of MKP-1 by post-translational modifications has been described in several systems. In particular, different works have described MKP-1 stabilization mediated by ERK1/2 phosphorylation [21,31], although enhanced MKP-1 degradation after ERK-mediated phosphorylation has also been reported [32].

Although albumin overload has been shown to promote ERK1/2 and JNK1/2 activation in renal tubular cells [13,14] and MAPK activity has been linked to ER stress-induced apoptosis in a colorectal model of cancer cells exposed to albumin [17], information about MAPK regulation of albumin-induced ER stress is scarce. Proximal tubule cells are among the first to be exposed to high protein concentrations. This tubular segment is responsible for tubular reabsortion mechanisms but it is also sensitive to the cytotoxic effects of albumin. A tight regulatory mechanism is therefore crucial for an appropriate handling of filtered proteins. The fact that activation of MAPKs by albumin is transient [13,14] points to the involvement of a dephosphorylation event operating in the cellular response to the noxious effects of albumin. The dephosphorylation of ERK after albumin exposure could therefore modulate the response to ER stress and, consequently, the aim of the present work was to identify the phosphatase involved in MAPK dephosphorylation in albumin-exposed cells and to determine whether this phosphatase is regulated by albumin overload. Our results demonstrate that albumin rapidly induces MKP-1, which promotes ERK1/2 dephosphorylation and also modulates the expression of GRP78 and ER stress-induced MAPK-regulated gene expression in OK cells.

2. Materials and methods

2.1. Reagents

Bovine serum albumin (BSA) (Cat # A8806, fatty acid free and low endotoxin lyophilized powder), LPS (lipopolysaccharides), okadaic acid, sodium orthovanadate, actinomycin D (Act D), cycloheximide (CHX), PD98059 (PD) and SP600125 (SP) were obtained from Sigma (St. Louis, MO, USA). Cell culture supplies were obtained from Life Technologies Inc. BRL (Carlsbad, CA, USA). Plasticware was purchased from Corning-Costar (Corning, NY, USA). Specific polyclonal antibody against MKP-1 was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies against phospho-ERK1/2 (pERK1/2) and total ERK1/2 (ERK1/2) were from New England Biolabs Inc. (Beverly, MA, USA). Electrophoresis supplies, polyvinylidene difluoride membrane and secondary antibody (horseradish peroxidase-conjugated goat antibody) were from Bio-Rad Laboratories Inc. (Hercules, CA, USA). The Bio-Lumina kit for enhanced chemiluminescence was provided by Kalium Technology (Bernal, Buenos Aires, Argentina). All other chemicals were commercial products of the highest grade available.

2.2. Cell culture

OK is a cell line derived from Opposum (*Didelphis virginiana)* kidney proximal tubular cells. OK cells were maintained in low glucose Dulbeccós modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated bovine fetal serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin and maintained in a humidified atmosphere containing 5% CO_2 as already described [33]. Cells were arrested early in the G1 phase of the cell cycle by transferring the cultures in the logarithmic phase of growth to serum-free medium for 24 h. This medium was then replaced with fresh medium, where cells were maintained during 2 h for stabilization to be later incubated with or without BSA as stated in the legend of the corresponding figures. When indicated, different reagents were preincubated for the following times, before BSA addition: 5 μg/ml Act D or 2 μg/ml CHX, 30 min; 50 μM PD, 40 min; 20 μM SP, 1 h; 100 nM okadaic acid (OA) plus 10 μM pervanadate (PV), 15 min. PV was prepared from orthovanadate as previously described [34]. After treatments, total RNA or cell lysates were obtained.

2.3. Plasmids and transfections

The FLAG-tagged MKP-1 expression vector (pFLAG-MKP-1) was generated from pRc/CMV-MKP-1 expression vector, as already described [21]. This vector contains murine MKP-1 cDNA cloned in *Eco*RI/*Xba*I sites of p3xFLAG-CMV-7.1. OK cells were seeded the day before transfection, grown up to 80% confluence, then transfected during 16 h using Lipofectamine 2000 reagent in Opti-MEM medium according to the manufacturer's instructions and finally

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