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Ste20-like kinase, SLK, activates the heat shock factor 1 – Hsp70 pathway

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ABSTRACT

Expression and activation of SLK increases during renal ischemia-reperfusion injury. When highly expressed, SLK signals via c-Jun N-terminal kinase and p38 to induce apoptosis, and it exacerbates apoptosis induced by ischemia-reperfusion injury. Overexpression of SLK in glomerular epithelial cells (GECs)/podocytes in vivo induces injury and proteinuria. In response to various stresses, cells enhance expression of chaperones or heat shock proteins (e.g. Hsp70), which are involved in the folding and maturation of newly synthesized proteins, and can refold denatured or misfolded proteins. We address the interaction of SLK with the heat shock factor 1 (HSF1)-Hsp70 pathway. Increased expression of SLK in GECs (following transfection) induced HSF1 transcriptional activity. Moreover, HSF1 transcriptional activity was increased by in vitro ischemia-reperfusion injury (chemical anoxia/recovery) and heat shock, and in both instances was amplified further by SLK overexpression. HSF1 binds to promoters of target genes, such as Hsp70 and induces their transcription. By analogy to HSF1, SLK stimulated Hsp70 expression. Hsp70 was also enhanced by anoxia/recovery and was further amplified by SLK overexpression. Induction of HSF1 and Hsp70 was dependent on the kinase activity of SLK, and was mediated via polo-like kinase-1. Transfection of constitutively active HSF1 enhanced Hsp70 expression and inhibited SLK-induced apoptosis. Conversely, the proapoptotic action of SLK was augmented by HSF1 shRNA, or the Hsp70 inhibitor, pifithrin-μ. In conclusion, increased expression/activity of SLK activates the HSF1-Hsp70 pathway. Hsp70 attenuates the primary proapoptotic effect of SLK. Modulation of chaperone expression may potentially be harnessed as cytoprotective therapy in renal cell injury.

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1. Introduction

The serine/threonine Ste20-like protein kinase, SLK, belongs to the group five germinal center kinase family [1–3]. Regulation, signaling, and physiological roles of germinal center kinases are incompletely understood [1,2]. Some germinal center kinases can be activated by stresses, e.g. heat shock, arsenite, staurosporine, ischemic injury or ATP depletion [4,5]. Certain members of the germinal center kinase family, including SLK, are mitogen-activated protein kinase kinase kinase kinases (MAP4Ks), and SLK can activate c-Jun N-terminal kinase (JNK) and p38 signaling cascades [6,7].

SLK is a large protein consisting of ~1235 amino acids. There is a N-terminal catalytic/kinase domain (amino acids 1–338), and coiled-coil structures in the C-terminal region (amino acids 826–929 and 942–1038) [3,8–10]. The N-terminal domain is also a site of regulation of kinase activity. SLK activation is associated with phosphorylation of T183 and S189, and mutation of these two residues to alanine greatly reduces SLK activity [10,11]. Homodimerization of the catalytic domain is key to enhancement of kinase activity, and this process appears to be mediated by the C-terminal coiled-coil regions [8,10,11].

SLK is ubiquitously expressed in mammalian cells, and its activity remains constant during most of the cell cycle, slightly increasing in mitosis. Overexpression of SLK induced apoptosis in various cell lines [3,6,12–14]. SLK co-localizes with α-tubulin, especially during metaphase re-assembly of the mitotic spindle, demonstrating a distinctive role for SLK in cell cycle progression, such that inhibition of SLK results in cell death [15]. SLK can also regulate cytoskeletal remodeling and cell motility. Activation of SLK via focal adhesion kinase increased actin stress fiber disassembly and destabilized F-actin, while inhibition disrupted focal adhesion turnover, adhesion, spreading and motility [3,16–18]. Regulation of SLK activity appears to be complex and may occur at the level of SLK mRNA or interaction of the SLK protein with binding partners [10,19]. So far, no mutations in SLK have been linked to human disease, but based on reports of SLK knockout mice, important mutations in SLK may confer embryonic lethality [3,20].

In the adult kidney, SLK is expressed in tubular and glomerular epithelial cells [12], and expression of SLK is enriched in the glomerulus, compared with other kidney compartments [21]. SLK expression and activity were increased during kidney development and in renal

Abbreviations: GEC, glomerular epithelial cell; GFP, green fluorescent protein; HA, hemaglutinin antigen epitope tag; HSF1, heat shock factor 1; ΔHSF1, constitutively active HSF1; Hsp, heat shock protein; JNK, c-Jun N-terminal kinase; PLK, polo-like kinase.

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ischemia-reperfusion injury, a pathophysiological event that mimics certain cellular processes of kidney development [12]. In cultured glomerular and renal tubular cells, ischemia-reperfusion activates SLK leading to activation of p38 kinase, which is associated with an increase in apoptosis [7]. Overexpression of SLK in podocytes in vivo resulted in podocyte injury [22]. Moreover, SLK was among several proapoptotic genes up-regulated in the lung after renal ischemia-reperfusion injury, suggesting a role for SLK in mediating the adverse distant organ effects of acute kidney injury [23].

Many cellular proteins undergo folding in the cytosol or endoplasmic reticulum. Molecular chaperones play a key role in folding newly synthesized proteins or refolding denatured proteins [24–26]. In response to various types of stress/injury, cells can increase the expression of chaperones or "heat shock proteins" (Hsps). Hsp70 and Hsp90 are involved in the folding and maturation of newly synthesized proteins in the cytosol, and Hsp70, coordinated with other molecular chaperones, can also refold denatured or misfolded proteins [24,27–31]. Hsp70 can also block steps in apoptotic pathways (e.g. caspase and Bax activation), and negatively regulate proapoptotic kinases [27,28,31]. A role for Hsp70 in restricting renal ischemia-reperfusion injury has been demonstrated [31,32]. Hsp70 expression in response to stress occurs mainly at the transcriptional level and depends on heat shock transcription factor 1 (HSF1) [25,33]. Under resting conditions, HSF1 is found in the cytosol as an inactive monomer bound to certain chaperones. Following stress, HSF1 trimerizes, translocates to the nucleus, binds to heat shock elements to initiate transcription of Hsps. Phosphorylation of HSF1 on specific serine residues further enhances its transcriptional activity. Therapeutic targeting of HSF1 with small molecules has received considerable attention [25,30,33,34]. In the present study, we assessed whether SLK can interact with the HSF1-Hsp70 pathway. We demonstrate that increased expression/activity of SLK activates HSF1 transcriptional activity and enhances expression of Hsp70 pathway. In turn, HSF1-Hsp70 attenuate the primary proapoptotic effect of SLK.

2. Materials and methods

2.1. Materials

Tissue culture media and Lipofectamine 2000 were from Invitrogen-Life Technologies (Burlington, ON) and Wisent (Saint-Jean-Baptiste, QC). Electrophoresis reagents were from Bio-Rad Laboratories (Mississauga, ON), and GE Healthcare (Baie d'Urfé, QC). Antimycin A, pifithrin-μ (2-phenylethynesulfonamide), and rabbit anti-actin antibody were purchased from Sigma-Aldrich Canada (Mississauga, ON). AP20187 was from Clontech Laboratories (Mountain View, CA). Mouse anti-hemaglutinin antigen epitope tag (HA) and mouse anti-green fluorescent protein (GFP) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-HA antibody was from Zymed Laboratories (South San Francisco, CA). Mouse anti-Hsp70 (inducible Hsp70) was from Enzo Life Sciences (Farmingdale, NY). Rabbit anti-RRXpS/T (anti-phospho-protein kinase A substrate) antibody, which reacts with SLK phospho-S189 [11] was from Cell Signaling Technology (Danvers, MA). Plasmids encoding full-length HA-SLK, HA-Fv-SLK 1–373 (wild type), HA-Fv-SLK 1–373 T183A/S189A, and myc-SLK K63R were described previously [6–8,11,12]. Dominant negative JNK (FLAG-JNKapf) and p38 (FLAG-p38apf) cDNAs were described previously [6]. Plasmid pGL-HSP70B (HSF1 luciferase reporter), which contains a 1.44 kb HSP70B gene promoter fragment upstream of firefly luciferase [35] was kindly provided by Dr. Michael Sherman (Boston University). pSIREN-HSF1 and pSIREN-Hsp72, which encode HSF1 and Hsp70 shRNAs were also provided by Dr. Sherman, and were validated previously [36,37]. pcDNA-ΔHSF1, which encodes a constitutively active truncation mutant of HSF1 (ΔHSF1; BH-S mutant in ref. [38]) was kindly provided by Dr. Jason Young (McGill University). Plasmids encoding EGFP-polo-like kinase 1 (PLK1; wild type), kinase dead PLK1 (D176N), and constitutively active PLK1 (T210D) were kindly provided by Dr. Michael B. Yaffe (Massachusetts Institute of Technology) [39].

2.2. Cell culture and transfection

Experiments were carried out in rat GECs and COS-1 monkey kidney cells. Rat GECs were characterized previously [40], and were cultured in K1 medium (DMEM, Ham F-12, with a 5% NuSerum and hormone mixture) [12]. COS-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum [7,8]. Cells were seeded into plates 24 h prior to transfection. Cells were transiently transfected using Lipofectamine 2000 according to the manufacturer's instructions, 24 h after plating.

To produce HSF1 and Hsp70 shRNAs, lentivirus stocks were prepared in 293 T cells. Briefly, 293 T cells were cultured in DMEM-10% fetal bovine serum and cells were transfected with plasmids using Lipofectamine 2000. Medium was changed after 24 h. Viruses were collected 48–72 h after the start of transfections. Medium containing the HSF1 shRNA and Hsp70 shRNA lentiviruses was added to GECs 24 h after plating in the presence of polybrene (8 μg/ml), and cells were incubated for 48 h.

2.3. Immunoblotting

Cells in 35 mm wells were rinsed and lysed with buffer ("lysis buffer"), containing 1% Triton X-100, 125 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EGTA, 2 mM Na_3VO_4, 5 mM $Na_4P_3O_7$, 25 mM NaF, 20 μM leupeptin, 10 μM pepstatin, 50 μM bestatin, 15 μM E64, 0.8 μM aprotinin, 1 mM 4-(2-aminoethyl)benzenesulfonylfluoride. The lysates were then centrifuged at 14,000 *g* for 10 min. After addition of Laemmli buffer, proteins were loaded onto gels with equal amounts of protein per lane. Proteins were separated by SDS-PAGE and were then electrophoretically transferred to a nitrocellulose membrane. Membranes were blocked with 5% BSA and incubated with primary antibody followed by horseradish peroxidase-conjugated secondary antibody. Membranes were developed with ECL. Density of specific bands was measured using National Institutes of Health Image J software. Preliminary studies demonstrated that there was a linear relationship between densitometric measurements and the amounts of protein loaded onto gels [7,8,11,12].

2.4. Quantification of cell death

Cells were stained using Hoechst H33342 dye (1 μg/ml) for 10 min at 37 °C without fixation. After washing with PBS, cells were stained with propidium iodide (5 μg/ml). Images at high magnification were acquired using a Zeiss AxioObserver fluorescence microscope with visual output connected to an AxioCam digital camera, and the cells were then counted manually. In every experiment, 4 random fields of cells were photographed and all of the cells in these fields were then counted. Each field contained ~200 cells; therefore each experimental measurement is based on ~800 cells. Cells in which nuclei showed chromosomal condensation and/or fragmentation, but which had an intact plasma membrane impermeable to propidium iodide, were counted as apoptotic. However, in cell culture propidium iodide-positive cells are typically "late apoptotic," as apoptotic cells are not phagocytosed and may proceed to necrosis. These cells were included in apoptotic cell counts. Hoechst H33342-stained cells with normal appearing nuclei and without propidium iodide staining were designated as normal [11]. Representative photographs have been published previously [11].

2.5. Dual luciferase reporter assay

Cells were plated in 9 mm wells and co-transfected after 24 h with the cDNA of interest, pRL-TK (renilla luciferase), and pGL-HSP70B (HSF1 firefly luciferase reporter) [6,11]. pRL-TK serves as an internal control that quantifies transfection efficiency, whereas firefly luciferase serves as the principal reporter. Cell lysates were assayed using the

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