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ATP Depletion-induced Actin Rearrangement Reduces Cell Adhesion *via* p38 MAPK-HSP27 Signaling in Renal Proximal Tubule Cells

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Key Words

Actin cytoskeleton • ATP depletion • Cell adhesion • Tubular epithelial cells • p38MAPK • HSP27

Abstract

Ischemia causes desquamation of proximal tubular epithelial cells leading to acute renal failure. However, the molecular mechanisms underlying the detachment of proximal tubule cells remain unknown. In this study, we reported that ATP depletion resulted in actin polymerization, a shift of filamentous actin from web-like structure to fragmented parallel stress fibers, followed by a reduction of cellular adhesion ability. The pre-treatment with Jasplakinolide, an actin stabilizer, prevented ATP depletion-induced actin polymerization and reduction of cell adhesion, indicating that the cytoskeleton reorganization decreased the cellular adhesion ability. Furthermore, the ATP depletion markedly increased the levels of p38MAPK and HSP27 phosphorylation with enhanced translocation of phosphorylated HSP27 from cytoskeleton to cytoplasm. The inhibition of p38MAPK by SB203580 blocked the ATP depletion to induce HSP27 phosphorylation and actin polymerization. These findings suggest that ischemia remodels filamentous actin leading to desquamation of proximal tubular epithelial cells through p38 MAPK-HSP27 signaling.

Introduction

Ischemia is well known to be a major cause of acute renal failure [1]. Renal proximal tubule cells are the most susceptible to ischemia [2, 3]. Renal ischemia results in the alteration of cell polarity and impairment of tight junction structure, which causes the detachment of tubular epithelial cells to obstruct tubular lumen leading to the acute renal failure [4]. However, the cellular and molecular mechanisms underlying ischemia-induced detachment of proximal tubular epithelial cells are poorly understood.

Actin cytoskeleton plays important roles in the structure and function of proximal tubular epithelial cells

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through modulating cell-cell and cell-extracellular matrix (ECM) adhesions [5, 6]. The actin cytoskeleton anchors plasma membrane proteins such as integrin in the cell adhesion complex to increase the ability of cell adhesion [7]. Previous studies reported that ischemia severely disrupted the actin cytoskeleton in kidney epithelial cells, resulting in degeneration of microvillar F-actin core and plasma membrane, loss of surface membrane polarity, and breakage of tight junction structures [8]. In addition, either ischemia or ATP-depletion impairs the attachment of cell-cell and cell-substratum [9, 10]. However, direct evidence regarding to ischemia-impaired actin cytoskeleton to reduce cellular adhesion ability is absent to date.

p38MAPK is a member of the mitogen-activated protein kinase (MAPK) family, which also includes extracellular signal-regulated kinase, big MAP kinase1 and c-Jun NH_2-terminal kinase [11]. A growing body of studies indicate that p38MAPK can be activated by some stress stimuli such as renal ischemia [12]. In a study of myocardial ischemia, p38MAPK pathway activation is associated with mitochondria damage in cardiomyocytes [13]. The activation of p38MAPK during small intestinal ischemia-reperfusion evokes detachment of epithelial cells from the tip of villi [14]. Inhibition of p38MAPK by FR167653 is also reported to prevent ischemia and reperfusion-induced renal injury [15]. In addition, the activation of the small heat shock protein 27(HSP27), as an F-actin cap-binding protein, inhibits actin polymerization [16]. White et al provided evidence that ischemia induced the phosphorylation of HSP27 [17]. The phosphorylation of HSP27 is associated with endothelial barrier dysfunction in lung endothelial cells *in vivo* [18]. A recent study showed that ischemia activated HSP27 signaling in a p38MAPK-dependent manner, leading to apoptosis and necrosis [19]. Thus, it is interesting to explore whether the p38MAPK and HSP27 signaling pathway is involved in the ischemia-impaired actin cytoskeleton in proximal tubular epithelial cells.

Although ischemia is a complex process, many of its consequences result from, and can be recapitulated by, depletion of the normal intracellular concentration of ATP. In the present study, we examined the effects of ATP depletion on actin cytoskeleton and cell adhesion in renal proximal tubule cells. Using pharmacologic and western blot analysis, we further explored the involvement of p38MAPK and HSP27 signals in ATP-depletion induced alteration of actin cytoskeleton and cell adhesion.

Materials and Methods

Cell culture

The rat proximal tubule cell line, NRK52E cell, was maintained in Dulbecco's MEM medium(high glucose) supplemented with 10% (v/v) fetal bovine serum, 100 unit penicillin/ml,100μg/ml streptomycin and cultured at 37°C in a humidified atmosphere of 5% CO_2. The cells were grown on coverslips for fluorescent studies and on plastic dishes for protein extraction. Cells were made quiescent by serum starvation overnight followed by drug treatment.

Reagents and antibodies

Antimycin was purchased from Sigma, USA. Trypsin and Dimethyl Sulfoxide (DMSO) were obtained from Amresco, USA. Dulbecco's MEM medium (high glucose) was the product of Gibco, France. Fetal bovine serum was purchased from Hyclone, USA. Enhanced Chemiluminescence (ECL) reagent kit was purchased from Pierce, USA. Matrigel was product of BD Biosciences, USA. SB203580 was obtained from Promega, USA. Jasplakinolide was obtained from Calbiochem, USA. FITC-labeled phalloidin was purchased from Molecular Probes, USA. All the primary antibodies such as rabbit anti-p38MAPK antibody, rabbit anti-phospho p38MAPK(Thr180/Tyr182) antibody, rabbit anti-HSP27 antibody and mouse anti-actin antibody were purchased from Chemicon, USA. Rabbit anti-phospho-HSP27(Ser 82) antibody was purchased from Upstate, USA. HRP-conjugated secondary antibody raised in goats was obtained from Santa Cruz, USA.

Preparation of ATP depletion cellular model

ATP depletion was induced using depleted medium and the electron transport chain inhibitor Antimycin A as previously described [20]. After washing three times with PBS, confluent NRK52E cells were incubated in depleted medium (Dulbecco's MEM medium without glucose, pyruvate, or amino acids) containing 0.1 μM antimycin A for 5, 15, and 30 min, and then media containing Antimycin A were removed and replaced with normal depleted medium for 30 min and 60 min. Untreated cells were incubated in the same media as treated cells except that no Antimycin A was added. Total ATP measurements were made on cell extracts using an ATP bioluminescent assay kit. Measurements were made on a TD 20/20 luminometer. Actual concentrations of ATP were calculated by generating a standard curve using known amounts of ATP.

Cell adhesion assay

The 96-well plates were coated with *Matrigel* at 37 °C for 1h and then blocked with 1% BSA in PBS. The cells were detached from dishes and plated in the wells (2×10^5cells/well) and allowed to attach for 1h at 37 °C . The media were changed to control media or ATP depletion buffer for indicated times. For drug studies, 20μM SB203580 or 0.1μM Jasplakinolide was added to the medium for 1h before ATP depletion. The cells were washed twice with PBS and fixed with 3.7% paraformaldehyde in PBS for 30 min. The number of

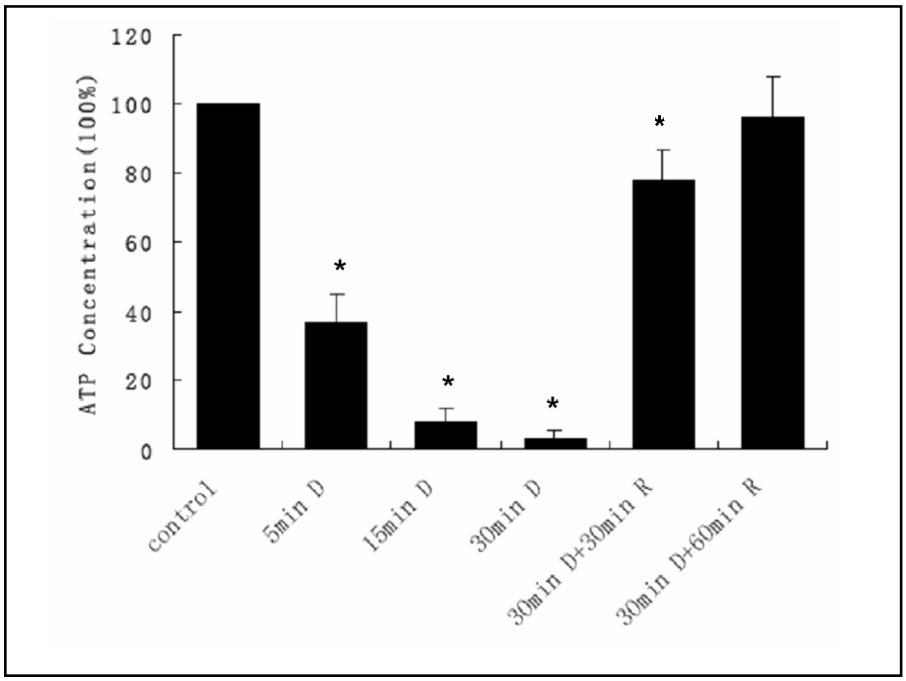

Fig. 1. The cellular ATP levels following treatment of antimycin A in NRK-52E cells. ATP levels were measured by luminometer using the luciferin-luciferase assay. Cells were treated with ATP depletion buffer (containing 0.1μM antimycin A) for the indicated times (5, 15, and 30min) inducing depletion and replaced with normal medium for 30 or 60min (30min D+30min R, 30min D+60min R). Values are means ± SD; *n*=6. *VS control, p<0.05.

attached cells was obtained with an ocular micrometer and at least 10 fields/filters were counted. To compare multiple experiments, adhesion of control cells was designated as 100%, and all other values were normalized to the control value. All of the experiments were independently performed in triplicate.

Actin cytoskeleton staining and immunofluorescence

Cells were fixed in 3.7% paraformaldehyde in PBS for 30 min, permeabilized in 0.1% Triton X-100 and blocked in PBS containing 2% BSA for 1 h at room temperature. F-actin was stained with FITC-labeled phalloidin (0.1 μg/ml) for 1 h at room temperature. After being washed with 0.05% Tween20-PBS, the samples were mounted on a chamber. The images were photographed using an Olympus DP70 charge-coupled device (CCD) camera coupled to an Olympus BX51 microscope. For the quantitation of F-actin fluorescence, cells from several fields were analyzed using the NIH Image program. The area around each cell was delineated, and the mean fluorescence intensity was measured in pixels.

Total cell extraction

Subconfluent cells were washed twice with PBS, then lysed with ice-cold RIPA lysis buffer (50mM Tris, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM EDTA, 1 mM PMSF, 1% cocktail) pH7.4. The lysates were then clarified by centrifugation at 12,000 g for 20 min at 4 °C .

Extraction of the triton X-100-soluble and -insoluble fractions

The shift between detergent-soluble and detergent-insoluble fractions of the actin cytoskeleton-associated protein HSP27 was assessed. The treated subconfluent cells were washed twice with PBS, then lysed with ice-cold Triton lysis buffer (0.5% Triton X-100, 300mM Sucrose, 5mM Tris-Cl, 2mM EDTA, 1% Cocktail, 1mM Na_3VO_4, 0.5mM PMSF) pH7.4. After 20min of centrifugation at 12,000 g, the supernatant was saved as Triton X-100 soluble fractions. The pellet was then suspended in sample buffer referred to as Triton X-100 insoluble fractions.

SDS-PAGE and western blot analysis

Total protein content was determined using the BCA assay and equal amount of samples was run on a 10% sodium dodecyl sulphate polyarcylamide gel electrophoresis (SDS-PAGE). The samples were then transferred onto polyvinylidene difluoride (PVDF) membrane with a Bio-Rad transfer unit at 20 V for 1 h at room temperature. Membranes were blocked in blocking buffer for 60 min followed by incubation with primary antibody and then incubated with the corresponding HRP-conjugated secondary antibody. The immunoblotted proteins were visualized with ECL reagents. Immunoblotting for β-actin was used as protein loading control.

Statistical analysis

Statistical analysis was carried out using SPSS software. Student's *t* test was used to analyze the differences between two groups. When comparisons between multiple groups were carried out, one-way ANOVA followed by SNK tests were employed. Statistical significance was considered at $p<0.05$.

Results

Influence of ATP depletion on actin cytoskeleton

NRK52E cells were treated with antimycinA (inhibitor of electron transport chain) to produce a cell model of chemical anoxia. The amount of ATP was

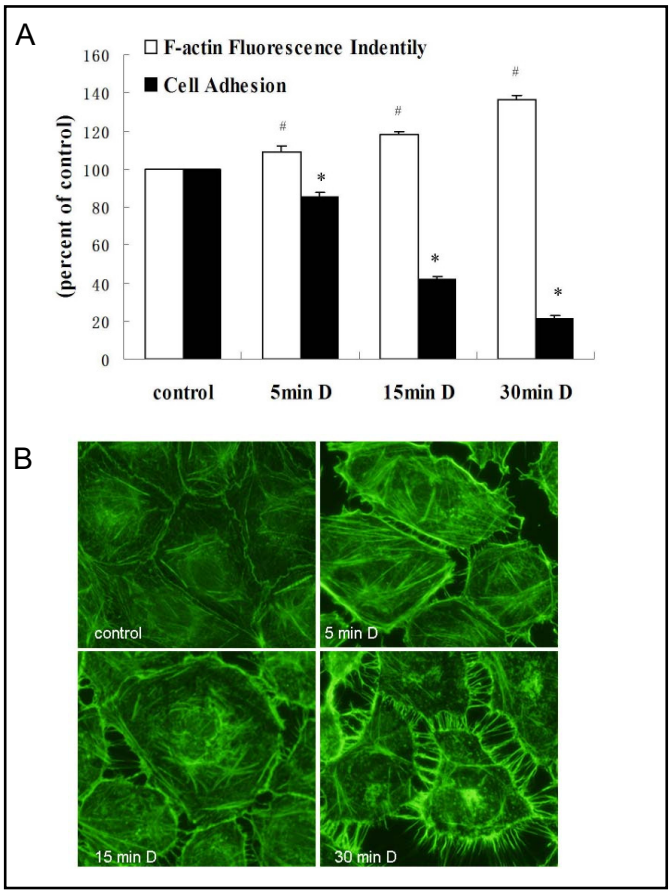

Fig. 2. Effects of ATP depletion on cell adhesion, F-actin content and cell morphology in NRK52E cells. (A) Effects of ATP depletion on cell adhesion and F-actin content. Cells were treated with ATP depletion buffer for 0, 5, 15 and 30min, and then washed with PBS. Attached cells were enumerated using ocular micrometer and at least 10 fields/filter were counted. To assess the changes in filamentous actin, FITC fluorescence was quantified in micrographs from experiment. * VS control, # VS control, $p<0.05$. (B) Effects of ATP depletion on stress fibers and cell morphology. Cells were treated with ATP depletion buffer for 0, 5, 15 and 30min (control, 5 min D, 10 min D, 30 min D). The cells were fixed with 3.7% paraformaldehyde in PBS for 30 min. Phalloidin-staining actin filaments were observed by fluorescence microscope. Magnification, ×400.

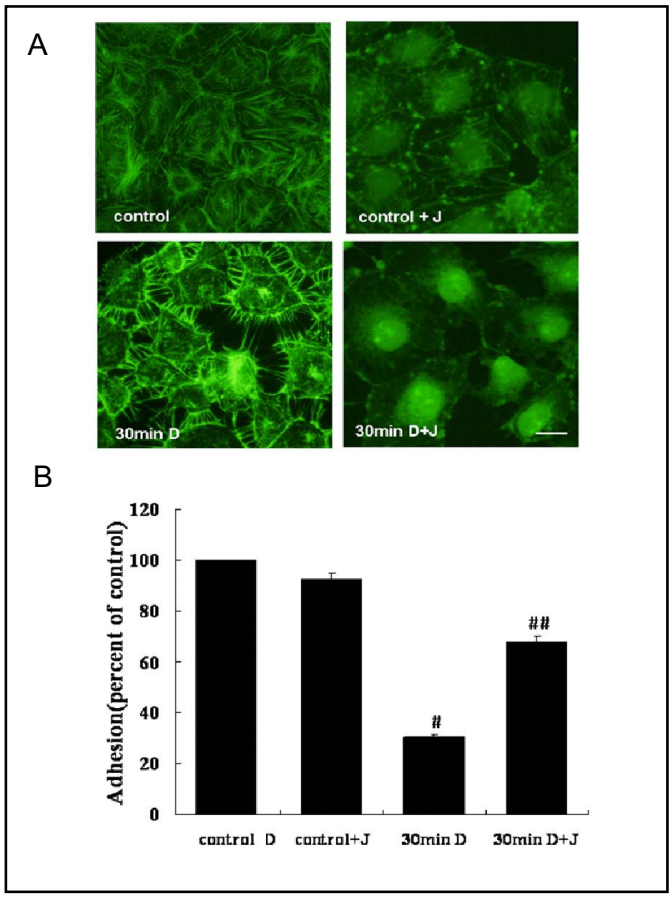

Fig. 3. Effects of Jasplakinolide on the actin cytoskeleton and cell adhesion. NRK52E cells were treated with vehicle (control), 0.1μM Jasplakinolide (control+J), ATP depletion buffer for 30 min (30min D) or the combination of ATP depletion buffer with 0.1μM Jasplakinolide for 30 min (30min D+J). Jasplakinolide was added for 1h before the treatment of ATP depletion buffer. (A) Effects of Jasplakinolide on the actin cytoskeleton and cell morphology. Cells were fixed and then stained with FITC-phalloidin to label filamentous actin. The actin cytoskeleton was visualized by fluorescence microscopy. (B) Effect of Jasplakinolide on cell adhesion. Cells were fixed with 3.7% paraformaldehyde in PBS for 30 min. Attached cells were enumerated using ocular micrometer and at least 10 fields/filter were counted. #VS control, $p<0.05$, ##VS 30 min D, $p<0.05$.

measured by luciferin-luciferase assay at 5, 15 and 30 min after antimycinA addition. As shown in Fig. 1, the amount of ATP rapidly declined within 5 min after antimycinA-addition (36.59 ±8.10 % of control value, $P<0.05$), and then further reduced at 15 min (7.95±4.21% of control value, $P<0.05$) and 30 min after antimycinA-addition (3.21±1.98% of control value, $P<0.05$). It should be noted that the amount of ATP could recover to the basal level following 60 min washout of antimycinA (96.16 ±12.31% of control value, $P>0.05$), indicating a reversible ATP depletion induced by antimycinA.

The experiment was designed to examine whether the ATP depletion influences actin cytoskeleton in NRK52E cells. The results of phalloidin staining showed that mean fluorescence intensity of phalloidin-labeled actin filaments following the antimycinA-addition was rapidly increased within 5 min (108.82±3.4% of control

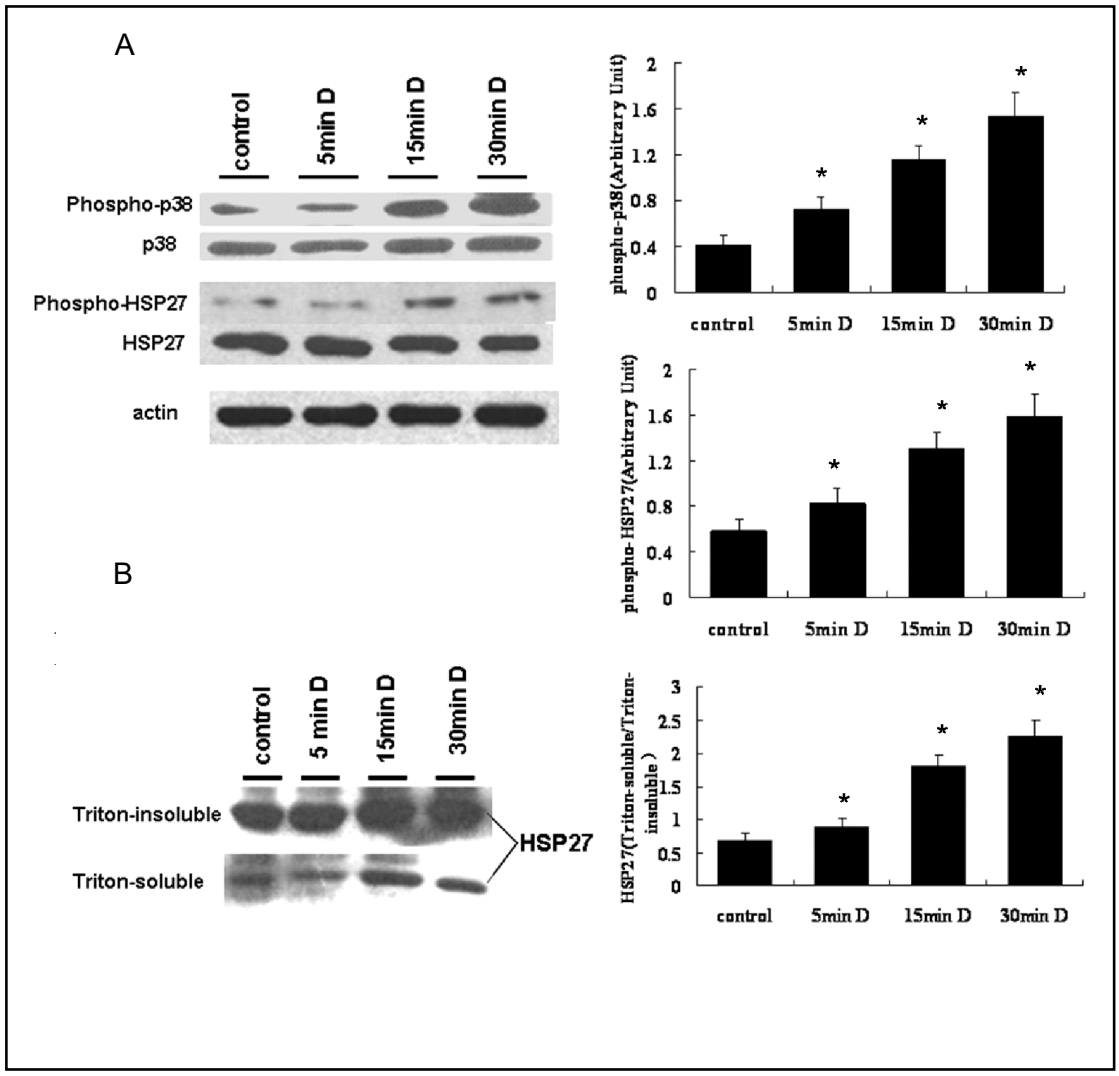

Fig. 4. Effects of ATP depletion on activation of p38 MAPK and HSP27 in NRK52E cells. Cells were treated with ATP depletion buffer for 0, 5, 15 and 30min. (A) phospho-p38 and phospho-HSP27 were detected by western blotting. The same blot was stripped off and re-probed with anti-p38 and anti-HSP27 antibodies respectively to detect the levels of p38 and HSP27. β -Actin staining was performed to ensure equal loading. (B) Redistribution of HSP27 during ATP depletion. Equal amount of Triton X-100-soluble and -insoluble fractions was detected by western blotting. Each bar graph represents means ± SD of three experiments. *VS control, p<0.05.

value, P<0.05; Fig. 2A), and then was further augmented after 15 min (118.26±1.50% of control value, P<0.05) and 30 min (136.27±2.58% of control value, P<0.05). In addition, we observed that the fragment of actin filaments increased at 5 min after addition of antimycinA, and further aggravated by prolonged exposure duration ATP depletion (Fig. 2B).

Furthermore, the ATP depletion induced actin filaments to largely accumulate in the cortical region underlying the lateral borders and the roughness of cellular outline in comparison to that treated with vehicle (Fig. 2B). Different from control, filopodia were obviously formed at lateral borders of cell-cell junctions, actin filaments were fragmented and web-like structure of actin cytoskeleton was disrupted in cytoplasm after ATP depletion. The augmentation of actin polymerization and disruption of parallel stress fibers induced by the ATP depletion indicated an actin rearrangement.

Influence of ATP depletion on cell adhesion ability

Previous study reported that remodeling actin cytoskeleton can influence the ability of cell adhesion [21]. It is possible that the actin rearrangement induced by ATP-depletion affects the adhesion of proximal tubule epithelial cells. We observed that after 5 min exposure to Antimycin A, the number of cells decreased approximately 20% compared to control level after adhesion assay (85.30±2.64%, P<0.05; Fig. 2A). Following the prolongation of antimycinA-treatment period, the number of cells after adhesion assay further decreased (15 min: 41.77±1.57% of control, P<0.05; 30 min: 20.97±1.94% of control, P<0.05).

Jasplakinolide, binding to both ends of actin filaments, causes stabilization of actin. Treatment with Jasplakinolide alone resulted in the appearance of large aggregates of actin filaments in cytoplasm. By the pre-incubation of 0.1 μM Jasplakinolide for 1 h, we observed that the

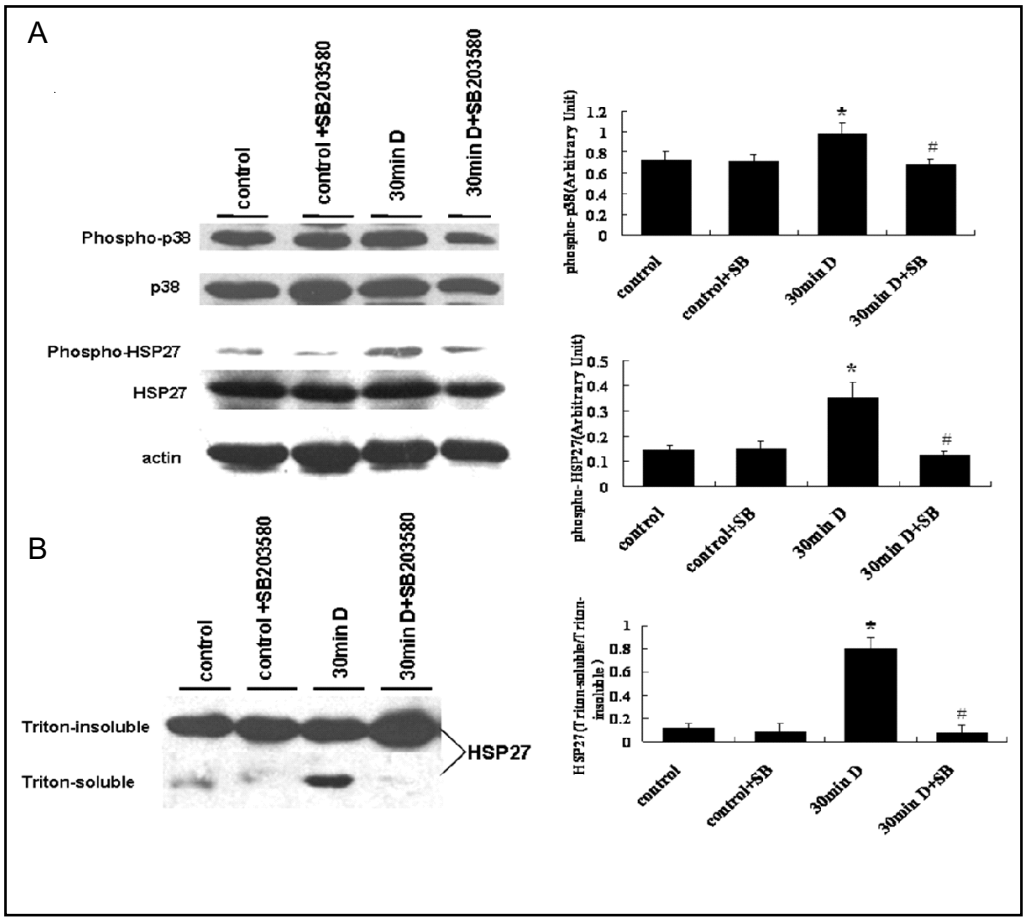

Fig. 5. Phosphorylation and redistribution of HSP27 are dependent on the activation of p38 MAPK during ATP depletion. Subconfluent NRK52E cells were preincubated for 1h with 20μM SB203580 or vehicle and then treated with or without Antimycin A for 30 minutes. (A) phospho-p38 and phosphor-HSP27 were detected by western blotting. The same blot was stripped off and respectively re-probed with anti-p38 and anti-HSP27 antibodies to detect the levels of p38 and HSP27. β-actin staining was performed to ensure equal loading. (B) Redistribution of HSP27 during ATP depletion. Equal amount of Triton X-100-soluble and -insoluble fractions was detected by western blotting. Each bar graph represents means ± SD of three experiments. *VS control, $p<0.05$, #VS 30 min D, $p<0.05$.

treatment of antimycinA failed to disrupt the structure of actin filaments (Fig. 3A). Similarly, the pre-treatment with Jasplakinolide completely prevented the reduction of cell adhesion ability induced by antimycinA, whereas it had no effect on the ability of cell adhesion of the cells treated with vehicle (Fig. 3B).

Influence of ATP depletion on activation of p38MAPK and HSP27

The influence of ATP-depletion on the time-course of p38 MAPK and HSP27 activation was further investigated, since the signals have been well known to play a critical role in remodeling actin cytoskeleton [22, 23]. The analysis of immunoblot showed that the antimycinA treatment largely increased the level of p38MAPK phosphorylation (control: 0.40±0.09; 5 min: 0.73±0.11, $P<0.05$; 15 min: 1.16±0.12, $P<0.05$; 30 min: 1.54±0.20, $P<0.05$), whereas the total protein amounts of p38MAPK were not altered (Fig. 4A).

Expectedly, the antimycinA-treatment triggered a significant increase in the levels of HSP27 phosphorylation (phospho-HSP27) (control: 0.58±0.11; 5 min: 0.82±0.13, $P<0.05$; 15 min: 1.26±0.12, $P<0.05$; 30 min: 1.59±0.20, $P<0.05$), whereas the total protein amounts of HSP27 showed no difference between control and ATP-depletion groups (Fig. 4A). In addition, western blot analysis revealed that the antimycinA-treatment not only enhanced the translocation of HSP27 from cytoskeletal fraction to cytosolic fraction but also significantly increased the ratio of soluble/insoluble HSP27 fraction (increased approximately 3.2-fold compared with that of control)(Fig. 4B). Furthermore, the pre-treatment with 20μM SB203580, a specific inhibitor of p38MAPK, blocked the antimycinA-treatment to increase the level of phospho-HSP27 and the solubility of HSP27 (Fig. 5).

Role of p38MAPK-HSP27 activation in ATP depletion-induced actin rearrangement

Finally, we investigated whether the p38MAPK-HSP27 signaling pathway is involved in ATP depletion-induced actin rearrangement. As shown in Fig. 6A, the inhibition of p38MAPK by SB203580 before the antimycinA-treatment prevented an increase in fluorescence intensity of phalloidin-labeled actin filaments. Furthermore, the addition of SB203580 partially prevented antimycinA-induced collapse of actin filaments, and

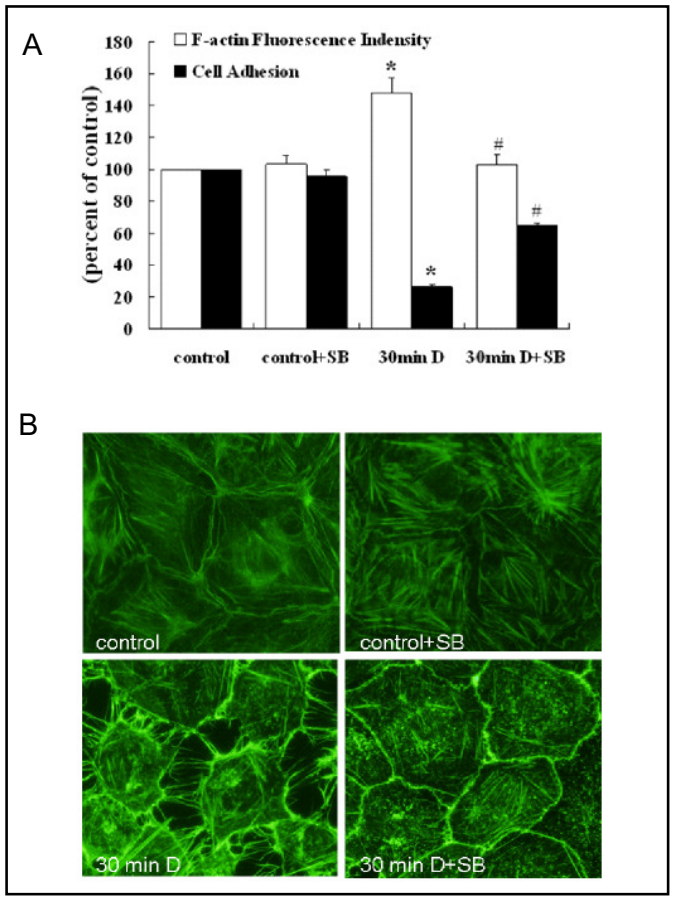

Fig. 6. Effects of p38 MAPK on cell adhesion, F-actin content and cell morphology during ATP depletion in NRK52E cells. (A) Effects of SB203580 on cell adhesion and F-actin content during ATP depletion. NRK52E cells were preincubated for 60 minutes with 20μM SB203580 or vehicle and then treated with or without Antimycin A for 30 minutes and then washed with PBS. Attached cells were enumerated using ocular micrometer and at least 10 fields/filter were counted. To assess the changes in filamentous actin, FITC fluorescence was quantified in micrographs from experiment. *VS control, $p<0.05$, #VS 30 min D, $p<0.05$. (B) Effects of SB203580 on stress fibers and cell morphology during ATP depletion. Cells were preincubated for 60 minutes with 20μM SB203580 or vehicle and then treated with or without Antimycin A for 30 minutes, then the cells were fixed with 3.7% paraformaldehyde in PBS for 30 min. Fluorescence microscopy micrographs show NRK52E cells stained with FITC- phalloidin to label filamentous actin. Magnification, ×400.

blocked the filopodia formation at lateral borders of cell-cell junctions (Fig. 6B). As above mentioned, after 30 min of antimycinA-treatment, the number of cells during adhesion assay decreased to approximately 20% compared to control level. Expectedly, SB203580 pretreatment partially rebound the number of cells (66.08±2.24% compared to control) in adhesion assay (Fig. 6A).

Discussion

The present study demonstrates that ATP-depletion results in actin rearrangement through p38MAPK-HSP27 signaling pathway, which leads to deficits in cell adhesion. This conclusion is deduced mainly from following observations: *(1)* the ATP-depletion caused actin polymerization with reduction of cell adhesion ability, *(2)* the stabilization of actin cytoskeleton prevented the ATP-depletion induced reduction of cell adhesion ability, *(3)* the ATP-depletion activated p38MAPK-HSP27 signaling pathway with increased translocation of HSP27 from actin cytoskeleton into cytoplasm, *(4)* the blockade of p38MAPK prevented the ATP depletion-induced actin rearrangement and rescued the impairment of cell adhesion.

ATP depletion-induced rearrangement of actin cytoskeleton reduces cell-adhesion ability

A primary observation in the present study is that ATP depletion for 5 min can induce an increase in actin polymerization and parallel stress fibers, indicating a rearrangement of actin cytoskeleton. A previous study reported that a remarkable rearrangement of actin cytoskeleton in renal cells occurs immediately after the ischemic insult [24]. The actin cytoskeleton is a highly dynamic structure consisting of spatial and temporal polymerization and depolymerization of preexisting filaments. The subcellular distribution of filamentous actin (F-actin), particularly the balance between F-actin and monomeric G-actin, plays an important role in regulating the function of actin cytoskeleton. In renal proximal tubule cells, the microvillar F-actin core and its overlying plasma membrane show signs of degeneration following 5 min of ischemia [25].

A previous study has demonstrated that the rearrangement of actin cytoskeleton corresponds with the loss of cell adhesion [21]. Similarly, we observed that the actin rearrangement induced by the ATP-depletion was associated with the loss of cell adhesion. Importantly, preventing the rearrangement of preexisting filaments by stabilizing actin cytoskeleton rescued the loss of cell adhesion after the ATP depletion, further indicating that the ischemia-induced rearrangement of actin cytoskeleton was responsible for the detachment of proximal tubule cells. The change in ratio of actin polymerization and depolymerization might affect the cell adhesion ability.

For example, cytochalasin D blocks elongation of actin filaments by binding to the barbed end. After cytochalasin D treatment, squamous lung cancer cells, Calu-1, decrease cell adhesion ability which may be due to depolymerization of actin filaments [26]. Meanwhile, the increased actin polymerization increases cell-cell adhesion ability in primary keratinocytes [27]. In prostate epithelial cell line, NPTX, actin filaments are aggregated in response to TGF-β1, which is associated with increased cell-substrate adhesion [28]. Ischemia results in the disorganization and polymerization in F-actin and that is an important aspect of cellular injury [29, 30]. Normally, long actin filaments emerge at the focal adhesion site contacting the extracellular matrix. We observed that actin filaments were fragmented and basal actin network was disrupted after ATP depletion compared to the control, although the F-actin was significantly increased. These results indicate that the disruption of the basal actin network during ATP depletion causes the loss of cell-ECM interaction.

ATP-depletion induces actin rearrangement through p38MAPK-HSP27 signaling pathway

Ischemia and hypoxia-stimulated p38MAPK activation has been reported in various cell types such as myocardial cell [31], neuroblastoma cell [32], pulmonary artery fibroblast [33]. Kim et al. [34] showed that ATP-depletion produced by ceramide increases p38MAPK activity in human glioma cells. Our results revealed that ATP-depletion not only elevated the level of p38MAPK phosphorylation but also increased HSP27 activity in renal proximal tubule cells. In addition, Sakamoto et al. [35] reported that ischemic preconditioning induces HSP27 redistribution from cytosol to sarcomere in rat heart. However, we observed that the ATP-depletion enhanced the translocation of activated HSP27 from cytoskeleton (insoluble fraction) to cytoplasm (soluble fraction), which is sensitive to p38MAPK inhibitor. Interestingly, blocking the p38MAPK-HSP27 signaling pathway significantly prevented the ATP depletion-induced actin rearrangement. Thus, a question we should address may be how the activation of HSP27 following the ATP-depletion causes the rearrangement of actin cytoskeleton. HSP27 is supposed to act as an actin-capping protein, which inhibits actin polymerization when bound to the plus end of the actin filament [36]. This process is abolished when the HSP27 is phosphorylated and released from the actin microfilament. In addition, the activation of p38MAPK in response to chemical stressors induces the actin reorganization, which may be associated with HSP27 phosphorylation [37, 38]. Therefore, ATP-depletion causing actin rearrangement may be mediated by activation of p38MAPK-HSP27 signals.

It is not clear how ATP-depletion mediates p38MAPK activation in renal proximal tubule cells. Previous studies demonstrated that hypoxia enhanced Rho GTPases activation, and p38MAPK activation by Rho GTPases has been reported in a variety of cell types [39]. Rho GTPases are GTP-binding proteins that regulate many essential cellular processes, including regulation of actin cytoskeleton organization. In renal cells, for example, hypoxia increases expression and activation of Cdc42, Rac, and RhoA, which are main isoforms of Rho GTPases [39]. In fibroblast cells, there is good evidence for a link from Rac and Cdc42, via PAK, to p38MAPK activation, a pathway that is essential for actin cytoskeleton rearrangement [40]. We assume that the increased Rho GTPases activity leading to p38MAPK activation possibly relate to the destruction of actin cytoskeleton during ATP depletion. The role of Rho GTPases in the control of p38MAPK activation and actin cytoskeleton remodeling remains to be determined.

In summary, this study provides novel information regarding ATP-depletion induced p38MAPK-HSP27 signaling, and the actin cytoskeleton rearrangement mediated by this signaling that might take part in cell detachment. In particular, these findings are of potential pathophysiological importance for understanding the overall involvement of the actin cytoskeleton remodeling in cell detachment during ischemia. Further studies will be necessary to determine whether cytoskeletal changes described in this study are toxic or protective to cells during acute renal failure.

Abbreviations

ECM (extracellular matrix); MAPK (mitogen-activated protein kinase); HSP27 (heat shock protein 27); SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoreses); NRK52E (normal rat kidney epithelial cells); PVDF (polyvinylidene difluoride).

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