Impairment of AMPK-α2 augments detrusor contractions in bladder ischemia

Jing-Hua Yang1, Wanting Niu2, Yedan Li2, Kazem M. Azadzoi3,4
1Department of Surgery, Boston University School of Medicine, Boston, MA, 2Research Section, VA Boston Healthcare System, Boston, MA, Departments of 3Urology and 4Pathology, VA Boston Healthcare System, Boston University School of Medicine, Boston, MA, USA

Purpose: Ischemia disrupts cellular energy homeostasis. Adenosine monophosphate-activated protein kinase alpha-2 (AMPK-α2) is a subunit of AMPK that senses cellular energy deprivation and signals metabolic stress. Our goal was to examine the expression levels and functional role of AMPK-α2 in bladder ischemia.

Materials and Methods: Iliac artery atherosclerosis and bladder ischemia were engendered in apolipoprotein E knockout rats by partial arterial endothelial denudation using a balloon catheter. After eight weeks, total and phosphorylated AMPK-α2 expression was analyzed by western blotting. Structural integrity of AMPK-α2 protein was assessed by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Functional role of AMPK-α2 was examined by treating animals with the AMPK activator 5-aminoimidazole-4-carboxamide-1-beta-D ribofuranoside (AICAR). Tissue contractility was measured in the organ bath and bladder nerve density was examined by immunostaining.

Results: Total AMPK-α2 expression increased in bladder ischemia, while phosphorylated AMPK-α2 was significantly downregulated. LC-MS/MS suggested post-translational modification of AMPK-α2 functional domains including phosphorylation sites, suggesting accumulation of catalytically inactive AMPK-α2 in bladder ischemia. Treatment of rats with AICAR diminished the force of overactive detrusor contractions and increased bladder capacity but did not have a significant effect on the frequency of bladder contractions. AICAR diminished contractile reactivity of ischemic tissues in the organ bath and prevented loss of nerve fibers in bladder ischemia.

Conclusions: Ischemia induces post-translational modification of AMPK-α2 protein. Impairment of AMPK-α2 may contribute to overactive detrusor contractions and loss of nerve fibers in bladder ischemia. AMPK activators may have therapeutic potential against detrusor overactivity and neurodegeneration in bladder conditions involving ischemia.

Keywords: Bladder; Ischemia; Metabolic stress; Overactive detrusor; Peripheral arterial disease

INTRODUCTION

Clinical and basic research suggest that impairment of bladder blood flow may contribute to detrusor overactivity and lower urinary tract symptoms (LUTS) [1-10]. Bladder dysfunction elicited by ischemia is associated with cellular stress responses [6-10]. Cells respond to ischemia by activation of defensive mechanisms to rebalance energy homeostasis. When exposed to ischemia, cells consume some of the energy to support functionality and preserve some energy to adjust
to the unforeseen energy deprivation consequences. Disruption of energy homeostasis instigates defensive responses via energy sensing molecules and survival signaling pathways to prevent cell damage and preserve cell function [11,12]. Energy sensors signal cellular energy deprivation and coordinate protective mechanisms to maintain homeostasis [11,12]. The relation between aberrant homeostasis elicited by ischemia and cell fate depends on the severity of nutrient deficiency, extent of hypoxia and the cell’s ability to handle the stress-eliciting elements [11,12]. Initial ramifications of disturbed energy homeostasis involve cellular stress and survival responses to signal cell danger [11,12]. When ischemia persists, protective mechanisms fail and noxious free radicals prevail. This provokes a cascade of detrimental cellular rejoiners leading to degeneration [11,12].

Adenosine monophosphate-activated protein kinase (AMPK), a key component of the energy-sensing system, senses cellular energy status and prevents cell stress by balancing energy homeostasis when cellular energy resources decline [13-15]. AMPK consists of 3 subunits, designated α, β, and γ [13-15]. The α-2 subunit with the catalytic kinase domain (AMPK-α2) is abundantly expressed in the bladder and is reactive to ischemia. The α-1 and β subunits are expressed at a much lower levels while the γ subunit is not expressed in the bladder and seems to be limited to the skeletal muscle [13-15]. Defective AMPK-α2 is incapable of sensing energy deficiency and fails to rebalance cellular energy status under the ischemic conditions [13-15]. Impairment of AMPK-α2 results in the integration of stress response molecules into downstream signaling pathways with critical impact on mitochondrial structure and function [16]. Mitochondrial biogenesis in response to cellular energy deprivation depends on functional AMPK [16].

Homeostatic mechanisms that sense energy deprivation and trigger cell survival signaling in bladder ischemia remain largely elusive. We hypothesized that ischemia may compromise homeostatic regulation of bladder function. Our goal was to define the expression levels and potential role of the cellular energy sensor AMPK-α2 in the ischemic bladder.

MATERIALS AND METHODS

1. The rat model of bladder ischemia

Animal care and experimental protocols were in accordance with the guidelines and approval of VA Boston Healthcare System Animal Care and Use Committee (approval number: 1586075-4). Apolipoprotein E knockout (ApoE−) rats (Envigo, Indianapolis, IN, USA) exhibiting spontaneous hypercholesterolemia were used to develop pelvic arterial atherosclerosis and bladder ischemia (n=6). ApoE− rat is a widely accepted model for studies of arterial atherosclerosis. To expedite plaque formation and arterial occlusive disease in the ApoE− rats, we performed balloon endothelial denudation of the iliac arteries using a 2F Fogarty arterial embolectomy catheter (Baxter Healthcare Corporation, Irvine, CA, USA), as previously described [4-6,8-10]. The sham control rats underwent similar surgical procedures without arterial endothelial denudation (n=6). After 8 weeks, animals were anesthetized with inhalation of 1% to 2% isoflurane mixed with oxygen. The bladder was exposed and bladder blood flow was measured with a laser Doppler needle probe connected to a blood flowmeter (Transonic Systems, Inc, Ithaca, NY, USA), as we have previously reported [4-6,8-10]. After this, bladder tissues were processed for analysis, as described below.

2. Western blotting of AMPK-α2

Frozen bladder tissues were homogenized and centrifuged then protein extracts were prepared and diluted to equal concentrations. Proteins separation was carried out using sodium dodecyl sulfate-PAGE then samples were transferred to polyvinylidene difluoride filter membranes (Millipore, Bedford, MA, USA). The membranes were incubated overnight with 2 µg/mL antibodies against either AMPK-α2 or phospho-AMPK-α2 (Millipore Sigma, Burlington, MA, USA) or β-actin (Cell Signaling, Danvers, MA, USA) then processed for incubation with the fluorescent-labelled secondary antibody. The fluorescent signals were scanned with Typhoon 8600 imager (GE Healthcare, Pittsburg, PA, USA) and protein levels were quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

3. Proteomic analysis of AMPK-α2

Frozen ischemic and control bladder tissues were processed for Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), as we have previously reported [17,18]. In brief, after centrifugation and homogenization, the supernatants were processed for fractionation using one-dimensional SDS-PAGE. After electrophoresis, the gel band proteins were digested, in-gel, with trypsin except reduction and alkylation steps. Post-translational modification of AMPK-α2 functional domains including phosphorylation/activation sites was analyzed using LC-MS/MS technology.

4. Treatment of rats with the AMPK activator

Six ApoE− rats undergoing arterial ballooning were treated with 0.5 mg of the AMPK activator 5-aminoimid
azole-4-carboxamide-1-beta-D ribofuranoside (AICAR; Cayman, Ann Arbor, MI, USA) subcutaneously in daily basis for four weeks. Another six ApoE<sup>−/−</sup> rats undergoing arterial ballooning were treated with placebo (distilled water) subcutaneously in daily basis for four weeks. The efficacy of AICAR treatment was assessed as described below.

5. Cystometric assessment of AICAR treatment

Animals were prepared for conscious cystometry as we have previously described [9,10]. In conscious animals, the bladder was emptied, and a syringe pump was used for intravesical infusion of saline at a rate of 200 µL min<sup>−1</sup>. Threshold and maximum intravesical pressure were recorded. To determine bladder capacity, the total amount of saline infused into the bladder was measured at the time when micturition commenced. Cystometric changes in bladder ischemia group treated with AICAR and bladder ischemia group treated with placebo were analyzed versus controls.

6. Assessment of AICAR treatment in the organ bath

Ischemic bladder tissues with intact mucosa were studied in the organ bath, as we have previously described [19,20]. In brief, tissues were submerged in organ baths containing physiologic solution, aerated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, and treated with either AICAR or placebo (distilled water). At isometric tension, contractile reactivity to electrical field stimulation (EFS, 10 volts, 0.8 msec and varying frequencies) was compared among ischemic tissues treated with AICAR, ischemic tissues treated with placebo, non-treated ischemic tissues, and control tissues. Contractile responses were expressed as tissue tension standardized by tissue cross-sectional area.

7. Histological assessment of AICAR treatment

Bladder tissues were processed for immunostaining, as we have previously reported [19,20]. In brief, tissue sections were incubated with 2 µg/mL anti-S-100 primary antibody for Schwann cells (Abcam, Cambridge, MA, USA) and 2 µg/mL anti-neurofilament primary antibody for myelinated nerve fibers (Fitzgerald, Acton, MA, USA). To determine nerve fiber density, the number of immunostained nerves was counted in 10 high power fields of each slide using a microscope at 400× magnification. Nerve fiber density in bladder ischemia group treated with AICAR was analyzed versus ischemia group treated with placebo and versus controls.

8. Statistical analysis

Data are expressed as mean±standard error of the mean. Data involving two group comparison was analyzed by Student t-test. Data involving greater than two groups was analyzed by analysis of variance (ANOVA) followed by post hoc comparisons using SigmaPlot statistical software (Systat, San Jose, CA, USA). Significant differences were determined at p<0.05 level.

RESULTS

1. AMPK-α2 expression in bladder ischemia

Bladder blood flow (mL/min/100 g tissue) in ApoE<sup>−/−</sup> rats undergoing arterial ballooning significantly decreased to 5.7±0.8 mL/min/100 g in comparison to 11.5±1.4 mL/min/100 g in sham controls (p=0.004), implying bladder ischemia. In
Impairment of AMPK-α2 in bladder ischemia

western blotting, total AMPK-α2 expression significantly increased in the ischemic bladder tissues versus sham controls (Fig. 1). However, expression levels of the phospho-AMPK-α2, the active form of AMPK-α2, was significantly downregulated in bladder ischemia (Fig. 1), suggesting a defect in the phosphorylation loop of AMPK-α2. These observations suggest accumulation of a defective form of AMPK-α2 that cannot be activated under the ischemic conditions.

2. Post-translational modification of AMPK-α2
LC-MS/MS revealed incongruity of the AMPK-α2 protein by means of post-translational modifications at multiple functional domains including phosphorylation sites (Fig. 2). The phosphorylation modification sites were identified near the C-terminal region of protein kinase A (PKA) domain at 54T+79.96 and 78S+79.96. Interestingly, other modifications were also observed in different functional domains of AMPK-α2, such as 168D+52.91 within the first CAP-ED motif, and 318K+315.15 within the second CAP-ED motif (Fig. 2). The data suggest that post-translational modifications may be one of the responsive mechanisms underlying lack of AMPK-α2 phosphorylation in bladder ischemia.

3. Effects of AICAR on bladder blood flow
Treatment with AICAR did not have a significant effect on bladder blood flow in animals with pelvic atherosclerosis. Although AICAR slightly increased the ischemic bladder blood flow (mL/min/100 g tissue) to 62±0.8 mL/min/100 g in comparison with 44±0.4 mL/min/100 g in bladder ischemia group treated with placebo, the increase was not statistically significant (p=0.167). Bladder blood flow in the ischemic groups treated with AICAR or placebo was significantly lower than 11.5±1.4 mL/min/100 g in the control group (p=0.001).

4. Effects of AICAR on detrusor contractions
Cystometrograms suggested that treatment with AICAR causes a significant decrease in both threshold and micturition pressure in comparison with bladder ischemia group treated with placebo (Fig. 3), suggesting that activation of AMPK diminishes the force of detrusor contractions in bladder ischemia (Fig. 4). AICAR caused a significant increase in bladder capacity versus ischemic group treated with placebo (Fig. 4). After AICAR treatment, capacity of the ischemic bladder was comparable to the control group. AICAR did not have a significant effect on the frequency of bladder contractions.

5. Effects of AICAR on tissue contractions in organ bath
Treatment of ischemic bladder tissues with AICAR caused a significant decrease in contractile responses to EFS, while treatment with placebo had no significant effect (Fig. 5). Contractile reactivity to 2, 4, 8, and 16 hertz EFS was significantly greater in the non-treated ischemic tissues and ischemic tissues treated with placebo versus control tissues (p=0.001, p=0.002, p=0.001, and p=0.012, respectively). Tissue treatment with AICAR decreased contractile reactivity of the ischemic tissues to EFS (Fig. 5). After treatment with AICAR, contractile reactivity of the ischemic tissues was comparable to the control tissues.

6. Effects of AICAR on nerve fiber density
We have previously reported that ischemia significantly diminished the bladder nerve fiber density [20, 21]. Our present study suggest that treatment with the AMPK activator AICAR may protect the bladder nerve fibers against ischemic injury. Nerve fiber density in the ischemia group treated with placebo was significantly less than the control group.
(Fig. 6). However, nerve fiber density in ischemia group treated with AICAR was significantly greater than the bladder ischemia group treated with placebo (Fig. 6). There was no significant difference between nerve fiber density of the ischemia group treated with AICAR and nerve fiber density of the control group.

**DISCUSSION**

Molecular mechanisms underlying cellular stress in bladder ischemia are not fully understood. Our present study suggests that bladder ischemia impairs the AMPK-α2, a key component of the cellular energy sensing system. Defective AMPK-α2 in bladder ischemia appears to involve post-translational modification of its protein. Beneficial effects of the AMPK activator AICAR provides further support that impairment of AMPK-α2 by ischemia may contribute to bladder dysfunction.

 Interruption of nutrient delivery to the cells initiates cellular responses via energy sensing system to maintain homeostasis and promote survival. Faulty energy sensing system exacerbates metabolic stress in ischemia and pro-
Impairment of AMPK-α2 in bladder ischemia

vokes deteriorating stress responses with profound impact on cellular structure and function. Our data implies significant upregulation of total AMPK-α2 in bladder ischemia, suggesting efficient cellular response to energy deficiency under the ischemic conditions. However, expression level of the phosphorylated and thus activated form of AMPK-α2 was significantly downregulated in bladder ischemia. These observations imply accumulation of a defective form of AMPK-α2 in the ischemic bladder that may not be capable of sensing cellular energy deprivation to promote homeostatic control of energy balance. Persistent disruption of cellular homeostasis due to dysfunctional AMPK-α2 may elicit damage to proteins, DNA, RNA and lipids and lead to structural and functional modifications in bladder ischemia.

Underlying mechanism of AMPK-α2 impairment in bladder ischemia appears to involve loss of AMPK-α2 structural integrity due to post-translational modifications of its protein. Under physiologic conditions, post-translational modifications can occur immediately after translation or in a later stage of a protein lifecycle to enable regulation of protein stability, localization, folding, and protein-protein interactions [22]. However, disease-associated post-translational modifications provoke adverse reactions by compromising functional properties of the affected protein [23]. In bladder ischemia, AMPK-α2 protein seems to undergo post-translational modifications at multiple functional domains including phosphorylation sites of protein kinase A2. Our western blotting and proteomic data together may suggest that protein upregulation do not necessarily epitomize functional significance. Upregulated proteins with post-translational modifications could be dysfunctional and may fundamentally differ from its upregulated form without post-translational modification. Proteins with post-translationally modified sites may display functional deficit and differential protein-
protein interaction properties [17,18,23].

Analysis of cardiac tissues from AMPK-α2 knockout mice (AMPK-α2⁻/⁻) has shown significant decrease in mitochondrial respiration rate regardless of carbohydrate or lipid being used as substrate [24]. This was shown to be due to modification of mitochondrial ultrastructure and functional deficit in mitochondrial complex-1 of the respiratory chain [24]. Cardiac muscle from the AMPK-α2⁻/⁻ mice exhibited a significant decrease in maximal oxidative capacity regardless of lipids, pyruvate, or glutamate+malate being used as substrate [24]. Mitochondrial stress in cardiac ischemia increased AMPK expression and activity, while the failure to upregulate AMPK was associated with poor outcome [25]. In addition, AMPK regulates endoplasmic reticulum (ER) homeostasis by suppression of sarcoendoplasmic reticulum calcium ATPase [26]. Inhibition of AMPK by pharmacological or genetic approaches provoked ER stress and reduced sarcoendoplasmic reticulum calcium ATPase activity [26]. These findings are consistent with mitochondrial and ER stress responses we reported in bladder ischemia [10,21]. Our previous studies showed upregulation of the mitochondrial stress proteins and decreased mitochondrial respiration rate in bladder ischemia [10]. Hypoxia provoked ER stress responses in cultured human and rat bladder smooth muscle cells [19,21]. Cumulatively, these observations allude to disruption of energy homeostasis by dysfunctional AMPK-α2 as a potential initiator of mitochondrial and ER stress responses.

To elucidate functional consequences of impaired AMPK-α2, we examined therapeutic role of the AMPK activator AICAR with the intention to invigorate AMPK-α2 activity in bladder ischemia. AICAR is an adenosine analogue that binds to

Fig. 5. Tissue organ bath studies showing increased contractile reactivity of the ischemic bladder tissues versus control tissues. Tissue treatment with placebo did not have a significant effect. Treatment with 5-aminoimidazole-4-carboxamide-1-beta-D ribofuranoside (AICAR) diminished contractile reactivity of the ischemic tissues to the control levels. *Represents significant differences versus control group (p<0.05).

Nerve fiber density

Control Ischemia-placebo

Ischemia-AICAR

Fig. 6. Immunohistochemical staining of bladder nerve fibers are shown at 400× magnification. Nerve fiber density in the ischemia group treated with placebo was significantly less than the control group (p=0.007), suggesting neurodegeneration in bladder ischemia. Nerve fiber density in bladder ischemia group treated with 5-aminoimidazole-4-carboxamide-1-beta-D ribofuranoside (AICAR) was significantly greater than the bladder ischemia group treated with placebo (p=0.027), suggesting protective role of AICAR against neural damage in ischemia. Nerve fiber density in ischemia group treated with AICAR was comparable to the control group (p=0.136). Scale bar=50 µm. Arrows point to the nerve fibers. *Represents significant difference versus control (p<0.05). †Represents significant difference versus ischemia-placebo (p<0.05).
AMPK and activates it via allosteric modification [27]. Beneficial effects of AICAR against ischemic injury have been documented in the kidney, heart and other organs [27]. In our study, subcutaneous administration of AICAR for four weeks diminished the force of overactive detrusor contractions and protected nerve fibers from ischemic injury in the rat model. Cystometrograms revealed significant decreases in threshold study, subcutaneous administration of AICAR for four weeks shown increased smooth muscle contractile activity in comparison with vascular tissues from wild-type control mice [28]. Studies of vascular tissues from AMPK-α2-/- mice have shown increased smooth muscle contractile activity in comparison with vascular tissues from wild-type control mice [28]. In addition, AMPK-α2-/- mice showed higher blood pressure, suggesting low arterial compliance [28]. Inhibition of AMPK in cultured smooth muscle cells augmented phosphorylation of myosin light chain (MLC) and myosin phosphatase targeting subunit one (MYPT1), while AICAR inhibited phosphorylation of MLC and MYPT1, suggesting that AMPK might improve tissue compliance by diminishing contractile activity of the smooth muscle cells [28].

Our immunostaining data suggest that activation of AMPK by AICAR may prevent nerve fiber degeneration in bladder ischemia. Preservation of nerve fiber density by AICAR may imply the involvement of AMPK in nerve fiber development, neuronal polarization, and neuronal reactivity to stimuli. We previously reported that bladder ischemia compromises structural integrity of the nerve fibers and leads to neurodegeneration [19-21]. Loss of nerve fibers in bladder ischemia was associated with metabolic stress signals such as depression of cell respiration and mitochondrial structural damage [20, 21]. It has been shown that AMPK plays a critical role in maintaining neuronal energy levels in the process of synaptic activation by mechanisms involving neural glycolysis and mitochondrial respiration [29]. Dysregulation of AMPK in neurodegenerative disorders was shown to impair neuronal plasticity by deteriorating metabolic response to synaptic activation [29]. It has been suggested that AMPK may act as a metabolic checkpoint by sensing energy homeostasis of the nerve fibers [29]. It is thought that AMPK maintains neural energy homeostasis by regulating mitochondrial respiration via mechanisms involving phosphorylation of A kinase anchor protein 1 [29]. These observations suggest that dysregulation of AMPK in metabolic stress conditions such as ischemia may compromise structural integrity of the nerve fibers and provoke neurodegeneration.

Neuroprotective effects of AICAR have been documented in a mouse model of retinopathy [30]. It was shown that activation of AMPK by AICAR increases the activity of cytochrome c oxidase by stimulation of AMPK and preservation of ATP synthesis [30]. It is suggested that stabilization of the ATP supply and restoration of cellular energy homeostasis may serve as a therapeutic approach to preserve neural integrity and prevent neurodegeneration. In addition, activation of AMPK by AICAR prevents oxidative neural injury by promoting antioxidant defense, thereby diminishing the production of oxygen free radicals and increasing mitochondrial quality control. Therefore, activation of AMPK by AICAR may be a viable therapeutic strategy to suppress the production of neurotoxic free radicals, promote antioxidant defense capability, and prevent the potential of neural oxidative injury in adverse conditions such as ischemia.

CONCLUSIONS

Total AMPK-α2 expression increased in bladder ischemia, while phosphorylated AMPK-α2 was significantly down-regulated. Downstream mechanisms impeding AMPK-α2 activation appeared to involve post-translational modifications of its functional domains including phosphorylation sites. Treatment of rats with the AMPK activator AICAR diminished the force of overactive detrusor contractions and increased capacity of the ischemic bladder. AICAR diminished contractile reactivity of the ischemic bladder tissues to EFS and protected the bladder nerve fibers from ischemic damage. Our data suggest that impairment of AMPK-α2 by ischemia may contribute to overactive bladder contractions. AMPK activators may have therapeutic potential against detrusor overactivity and neurodegeneration.

CONFLICTS OF INTEREST

The authors have nothing to disclose.
ACKNOWLEDGMENTS

This work was supported by Merit Review Award Number 101 BX004372 from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory R&D (BLRD) Service.

AUTHORS’ CONTRIBUTIONS

Research conception and design: Kazem M. Azadzoi. Data acquisition: Jing-Hua Yang. Statistical analysis: Wanting Niu and Yedan Li. Data analysis and interpretation: Kazem M. Azadzoi and Jing-Hua Yang. Drafting of the manuscript: Jing-Hua Yang. Critical revision of the manuscript: Kazem M. Azadzoi. Obtaining funding: Kazem M. Azadzoi. Administrative, technical, or material support: Wanting Niu and Yedan Li. Supervision: Kazem M. Azadzoi. Approval of the final manuscript: Kazem M. Azadzoi.

REFERENCES

1. Lin WY, Andersson KE, Lin CL, Kao CH, Wu HC. Association of lower urinary tract syndrome with peripheral arterial occlusive disease. PLoS One 2017;12:e0170288.
2. Gibbons EP, Colen J, Nelson JB, Benoit RM. Correlation between risk factors for vascular disease and the American Urological Association Symptom Score. BJU Int 2007;99:97-100.
3. Pinggera GM, Mitterberger M, Steiner E, Pallwein L, Frauscher F, Aigner F, et al. Association of lower urinary tract symptoms and chronic ischemia of the lower urinary tract in elderly women and men: assessment using colour Doppler ultrasonography. BJU Int 2008;102:470-4.
4. Thurmond P, Yang JH, Azadzoi KM. LUTS in pelvic ischemia: a new concept in voiding dysfunction. Am J Physiol Renal Physiol 2016;310:F738-43.
5. Andersson KE, Boedtkjer DB, Forman A. The link between vascular dysfunction, bladder ischemia, and aging bladder dysfunction. Ther Adv Urol 2017;9:11-27.
6. Azadzoi KM, Tarcan T, Kozlowski R, Krane RJ, Siroky MB. Overactivity and structural changes in the chronically ischemic bladder. J Urol 1999;162:1768-78.
7. Nomiya M, Yamaguchi O, Andersson KE, Sagawa K, Aikawa K, Shishido K, et al. The effect of atherosclerosis-induced chronic bladder ischemia on bladder function in the rat. Neurourol Urodyn 2012;31:195-200.
8. Azadzoi KM, Chen BG, Radisavljevic ZM, Siroky MB. Molecular reactions and ultrastructural damage in the chronically ischemic bladder. J Urol 2011;186:2115-22.
9. Zhao Z, Azad R, Yang JH, Siroky MB, Azadzoi KM. Progressive changes in detrusor function and micturition patterns with chronic bladder ischemia. Investig Clin Urol 2016;57:249-59.
10. Yang JH, Siroky MB, Yalla SV, Azadzoi KM. Mitochondrial stress and activation of PI3K and Akt survival pathway in bladder ischemia. Res Rep Urol 2017;9:93-100.
11. Kuznetsov AV, Janakiraman M, Margreiter R, Troppmair J. Regulating cell survival by controlling cellular energy production: novel functions for ancient signaling pathways? FEBS Lett 2004;577:1-4.
12. Rohas LM, St-Pierre J, Uldry M, Jäger S, Handschin C, Spiegelman BM. A fundamental system of cellular energy homeostasis regulated by PGC-1alpha. Proc Natl Acad Sci U S A 2007;104:7933-8.
13. Russell R 3rd. Stress signaling in the heart by AMP-activated protein kinase. Curr Hypertens Rep 2006;8:446-50.
14. Li J, McCullough LD. Effects of AMP-activated protein kinase in cerebral ischemia. J Cereb Blood Flow Metab 2010;30:480-92.
15. Cardaci S, Filomeni G, Ciriole MR. Redox implications of AMPK-mediated signal transduction beyond energetic clues. J Cell Sci 2012;125(Pt 9):2115-25.
16. Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, et al. AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc Natl Acad Sci U S A 2002;99:15983-7.
17. Su N, Choi HP, Wang F, Su H, Fei Z, Yang JH, et al. Quantitative proteomic analysis of differentially expressed proteins and downstream signaling pathways in chronic bladder ischemia. J Urol 2016;195:515-23.
18. Zhao Z, Azadzoi KM, Choi HP, Jing R, Lu X, Li C, et al. LC-MS/MS analysis unravels deep oxidation of manganese superoxide dismutase in kidney cancer. Int J Mol Sci 2017;18:319.
19. Azadzoi KM, Radisavljevic ZM, Siroky MB. Effects of ischemia on tachykinin-containing nerves and neurokinin receptors in the rabbit bladder. Urology 2008;71:979-83.
20. Azadzoi KM, Yalla SV, Siroky MB. Oxidative stress and neurodegeneration in the ischemic overactive bladder. J Urol 2007;178:710-5.
21. Azadzoi KM, Radisavljevic ZM, Golabek T, Yalla SV, Siroky MB. Oxidative modification of mitochondrial integrity and nerve fiber density in the ischemic overactive bladder. J Urol 2010;183:362-9.
22. Karlaftis V, Perera S, Monagle P, Ignjatovic V. Importance of post-translational modifications on the function of key haemostatic proteins. Blood Coagul Fibrinolysis 2016;27:1-4.
23. Smith LE, White MY. The role of post-translational modifications in acute and chronic cardiovascular disease. Proteomics Clin Appl 2014;8:506-21.
24. Athéa Y, Viollet B, Mateo P, Rousseau D, Novotova M, Garnier A, et al. AMP-activated protein kinase alpha2 deficiency affects cardiac cardiolipin homeostasis and mitochondrial function. Diabetes 2007;56:786-94.

25. Zhang P, Hu X, Xu X, Fassett J, Zhu G, Viollet B, et al. AMP-activated protein kinase-alpha2 deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction in mice. Hypertension 2008;52:918-24.

26. Dong Y, Zhang M, Liang B, Xie Z, Zhao Z, Asfa S, et al. Reduction of AMP-activated protein kinase alpha2 increases endoplasmic reticulum stress and atherosclerosis in vivo. Circulation 2010;121:792-803.

27. Lempiäinen J, Finckenberg P, Levijoki J, Mervaala E. AMPK activator AICAR ameliorates ischaemia reperfusion injury in the rat kidney. Br J Pharmacol 2012;166:1905-15.

28. Wang S, Liang B, Viollet B, Zou MH. Inhibition of the AMP-activated protein kinase-α2 accentuates agonist-induced vascular smooth muscle contraction and high blood pressure in mice. Hypertension 2011;57:1010-7.

29. Marinangeli C, Didier S, Ahmed T, Caillerez R, Domise M, Laloux C, et al. AMP-activated protein kinase is essential for the maintenance of energy levels during synaptic activation. iScience 2018;30:1-13.

30. Kawashima H, Ozawa Y, Toda E, Homma K, Osada H, Narimatsu T, et al. Neuroprotective and vision-protective effect of preserving ATP levels by AMPK activator. FASEB J 2020;34:5016-26.