

**ORIGINAL ARTICLE**

Iran J Allergy Asthma Immunol  
December 2014; 13(6):420-427.

## **Effects of Mitochondrial ATP-Sensitive Potassium Channels on the Proliferation and Secretion of Human Airway Smooth Muscle Cells**

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Received: 18 October 2013; Received in revised form: 16 December 2013; Accepted: 8 January 2014

### **ABSTRACT**

Bronchial asthma is the common chronic inflammatory disease and is characterized by chronic airway inflammation, airway remodeling, and airway hyperreactivity (AHR). Aim of this study was to investigate the effects of mitochondrial ATP-sensitive potassium channels (MitoKATP) on the proliferation and secretion of human airway smooth muscle cells (HASMCS). HASMCS were treated with the serum from asthmatic patients to establish HASMCS asthma model of passive sensitization. Rhodamine 123 (R-123) and 2,7-dichloro-dihydrofluorescein diacetate (DCFH-DA) fluorescence staining were used to detect mitochondrial membrane potential ( $\Delta\psi_m$ ) and the content of reactive oxygen species (ROS) in the cells, respectively.

The cell counting was used to detect cell proliferation, and RT-PCR was used to detect the expression of TGF- $\beta$ 1 mRNA.

In the normal + Diazoxide group, the fluorescence intensity of R-123, ROS content, cell proliferation and TGF- $\beta$ 1 expression were enhanced, compared with the normal control group ( $p < 0.05$ ). There were no significant differences between the normal + 5-hydroxydecanoate (5-HD) group and the normal control group. In the asthma model control group, the fluorescence intensity of R-123, ROS content, cell proliferation and TGF- $\beta$ 1 expression were enhanced, compared with normal control group, ( $p < 0.05$ ). The aforementioned indices were enhanced in the asthma model + Diazoxide group, when compared with the asthma model control group, whereas these indices were attenuated in the asthma model + 5-HD group, when compared with the asthma model control group ( $p < 0.05$ ).

In conclusion, asthma could activate MitoKATP channels in HASMCS, promote HASMC proliferation and TGF- $\beta$ 1 expression.

**Keywords:** Asthma; smooth muscle; Mitochondrial ATP-sensitive potassium channels (MitoKATP); Reactive oxygen species (ROS); Membrane potential

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## INTRODUCTION

Bronchial asthma is a common chronic inflammatory disease that seriously threatens human health and is characterized by chronic airway inflammation, airway reconstruction, and airway hyperreactivity (AHR). Chronic airway inflammation is the basis of asthma pathology. Previous studies presumed that airway smooth muscle cells (ASMCs) were merely passive target cells in the pathological process, which generated contractions and spasms through the stimulation of inflammatory mediators. However, recent studies<sup>1-3</sup> demonstrated that ASMCs not only have contractile function but also can generate phenotype transformation when stimulated by extracellular factors. This phenotype transformation, a change from the contraction to proliferation phenotype, enables ASMCs to synthesize and secrete multiple inflammatory factors and cytokines through complex mechanisms.

The ATP-sensitive K-channel is a critical channel that combines cellular energy metabolism and bioelectricity activity. These channels are generally distributed in many cells, such as nerve, heart, smooth muscle, skeletal muscle, and pancreatic  $\beta$  cells. ATP-sensitive K channels (KATP) can be divided into cell membrane KATP channels and mitochondrial inner membrane KATP channels (MitoKATP), according to their different locations.<sup>4</sup> In our previous studies, we found that asthma could induce the opening of MitoKATP (mitochondrial ATP-sensitive potassium channels) channels of ASMCs in rats, promote ASMC proliferation and phenotype transformation, and is involved in the airway reconstruction.<sup>5</sup> However, studies of HASMCs remain rare. Therefore, in this study, we aimed to explore the effects of MitoKATP on the proliferation and secretion of HASMCs to explore the role of MitoKATP in bronchial asthma and to find new approaches for the prevention and cure of asthma.

## MATERIALS AND METHODS

The study was approved by the Institutional Review Board of Huazhong University of Science and Technology.

### Reagents and Instruments

The following reagents were used in this study: diazoxide, 5-hydroxydecanoate (5-HD), methyl

thiazolyl tetrazolium (MTT), Rhodamine 123 (R-123), dimethyl sulfoxide (DMSO), propidium iodide (PI), and 2,7-dichloro-dihydrofluorescein diacetate (DCFH-DA). These reagents were purchased from Sigma (Sigma, USA). High-glucose DMEM (Dulbecco's minimum essential medium) culture medium and fetal bovine serum (FBS) were products of Hyclone (Hyclone, USA). Human smooth muscle cell  $\alpha$ -actin antibody and SPABC immunohistochemistry kit (Zhongshan Golden Bridge Biotech., China), cDNA reverse transcription kit and PCR-SuperMix were purchased from Toyobo (Toyobo, Japan); all PCR primers were synthesized by Shanghai Sangong (Sangong, China). Other reagents were analytical reagents from China.

### Sources of Human Serum

Asthma serum was obtained from 6 cases of acute stage asthma, consisting of 4 male and 2 female patients, who ranged in age from 20 to 45 years. The asthmatic patients were characterized by the following features: the diagnosis was in accordance with Prevention and Cure of Bronchial Asthma Manual;<sup>6,7</sup> patient history of hypersensitivity; the patients had no other respiratory system disease; patients had not used a glucocorticoid in the week prior to serum collection; and patient total serum IgE was  $>200$  IU/mL.

Control serum was obtained from 6 non-asthmatic patients, consisting of 3 male and 3 female patients, who ranged in age from 20 to 45 years. The non-asthmatic patients were characterized by the following features: patients had no history of hypersensitivity and asthma; patients had no other diseases of respiratory system; and patients had taken no drugs within a recent time period prior to the study.

### Culture and Identification of HASMC

HASMCs were cultured with the tissue adhesion method as described in a previous study.<sup>8</sup> Normal human bronchial tissues were obtained intraoperatively from 6 patients undergoing pulmonary lobectomy due to pulmonary diseases. These patients consisted of 4 male and 2 female patients, and the mean age was 40 years. Informed consent was given by the patients, who had no history of anaphylactic diseases, including asthma. The normal bronchial tissues of patients with pulmonary cancer were obtained from a location at least 5 cm from the tumor site. The pulmonary tissue was placed into cool D-Hanks solution, containing

penicillin and streptomycin at a dose of  $10^5$  U/L at  $4^\circ\text{C}$  in a germ-free condition. D-Hanks solution was used to carefully wash and separate the tracheal tissue, removing the affiliated connective tissue, blood vessels, and nerve fibers. Subsequently, the inner membrane was cut open and removed, and then the bronchi were sheared into slivers that measured  $1\text{ mm}\times 1\text{ mm}\times 1\text{ mm}$ . A small amount of fetal bovine serum (FBS) was added and mixed with the slivers in the culture flask, and then a small amount of high-glucose DMEM, containing 15% FBS, was placed into a 5%  $\text{CO}_2$  incubator at  $37^\circ\text{C}$ . After 10-16 h, a suitable amount of high-glucose DMEM, containing 15% FBS, was added to avoid the removal of tissue slivers. After this, high-glucose DMEM, containing 15% FBS, was used to change the solution once every 3 days. In 14-21 days, when the cell fusion had achieved 80%, pancreatin (0.125%) was used for the digestion and passage of cells. Human smooth muscle  $\alpha$ -actin was used to identify HASMCs by immunocytochemical stain.

#### Preparation of Asthma Model

The cultured HASMCs were used for passive sensitization for 24 h by DMEM culture medium containing 10% serum of asthma patients (total level of  $\text{IgE}>200$  U/ml) to establish the asthma model according to a previous study.<sup>9</sup> The serum of non-asthmatic patients was used for culturing for 24 h in the control group.

#### Grouping and Medicine Intervention

The high-glucose DMEM culture medium without FBS was used to culture cells to synchronize for 24 h in all groups. HASMCs were divided into 6 different groups: 1) Normal control group, which consisted of normal human ASMCs, with DMSO only added into the culture medium (the high-glucose DMEM, containing 15% FBS) at a final density of 0.1%; 2) Normal + diazoxide group, which consisted of normal ASMCs, with diazoxide (a selective opener of MitoKATP channels) dissolved in DMSO added into the culture medium at a final density of  $60\ \mu\text{mol/L}$ , with the final concentration of DMSO in the DMEM culture medium of 0.1%; 3) Normal + 5-HD group, which consisted of normal human ASMCs, with 5-HD (a selective blocker of MitoKATP channel) added into the culture medium at a final density of  $300\ \mu\text{mol/L}$  and DMSO added into the solution at a final density of 0.1%; 4) The asthmatic serum treated control group,

which consisted of the asthma model of HASMCs, with DMSO only added into the culture medium at a final density of 0.1%; 5) Asthma model + diazoxide group, which consisted of the asthma model of HASMCs, with diazoxide at a final density of  $60\ \mu\text{mol/L}$  and DMSO at a density of 0.1% added into the culture medium; and 6) Asthma model + 5-HD group, which consisted of the asthma model of HASMCs, with 5-HD at a density of  $300\ \mu\text{mol/L}$  and DMSO at a density of 0.1% added into the culture medium. The cells were cultured for 24 h after intervention.

#### Detection of HASMCs Mitochondrial Membrane Potential ( $\Delta\psi\text{m}$ )

The Rhodamine fluorescence technology was used.<sup>10</sup> The culture grouping and intervention for cells in the 6-well plates were performed as previously described. After intervention with the treatment factors, the cells in each group were washed by D-Hanks solution to avoid affecting the intensity of fluorescence, and the solution was incubated with R-123 at a final density of  $10\ \mu\text{g/mL}$  for 30 min at  $37^\circ\text{C}$  away from light. D-Hanks solution was used to wash out unabsorbed R-123. R-123 fluorescence was detected by laser confocal microscopy; the fluorescence was stimulated at 488 nm and detected at 530 nm. R-123 fluorescence showed a linear correlation with  $\Delta\psi\text{m}$ , and R-123 fluorescence was gradually enhanced with the depolarization of  $\Delta\psi\text{m}$ . The cells that were incubated by D-Hanks solution were set as controls, and the results were analyzed with the HPIAS-1000 image analyzer.

#### ROS Level Detected by DCFH-DA Fluorescent Staining

The culture grouping and intervention for cells in the 6-well plates were performed as previously described. After intervention with the treatment factors, the D-Hanks solution with DCFH-DA at a final density of  $20\ \mu\text{mol/L}$  was added, incubated for 30 min away from light at  $37^\circ\text{C}$ , and observed by fluorescence microscopy. DCFH-DA could enter into the cells for hydrolyzation and oxidation; DCFH-DA formed into DCF with high fluorescence and showed a direct ratio to the ROS level in the cells. Therefore, the mean fluorescence intensity of DCF in the cells could be used to reflect ROS levels directly. DCF emitted green fluorescence, and the results of fluorescence were analyzed with the HPIAS-1000 image analyzer.

### Cell Proliferation Detected by Cell Counting

Cells were seeded in 24-well plates (5,000 cells/well) and cultured overnight. Cells were then maintained in serum-free media for 24 h, followed by different treatments. At the end-point, cells were harvested and counted using a hemocytometer.

### The Transforming Growth Factor, Beta 1 (TGF- $\beta_1$ ) mRNA Level Detected by RT-PCR

The grouping and intervention methods were performed as previously described. Total RNA was extracted using Trizol with common methods after intervention with the treatment factors, cDNA was obtained by reverse transcription. The gene sequences of the load control  $\beta$ -actin primers were as follows: upstream 5GGAAATC-GTGCGTGACATTA3, and downstream 5GGACTCGTCATATCCTGCTTG3. The length of amplification products was 478 bp. The gene sequences of the TGF- $\beta_1$  primers were as follows: upstream 5TGTGACCGGAGTTGTGCG3, and downstream 5GGTCCTTGCGGAAGTCA. The length of the product was 323 bp. The renaturation temperatures in both PCRs were 60°C and 61°C, respectively. The PCR products were analyzed by 2% agarose gel electrophoresis;  $\beta$ -actin was set as positive control. The image was evaluated by a gel image analytical system and band-density scanning. Finally, the ratio of integral optical density between TGF $\beta$ -1 and  $\beta$ -actin ( $IOD_{TGF-\beta_1}/IOD_{\beta-actin}$ ) was used for semi-quantitative analysis.

### Statistical Analysis

Quantitative data are expressed as the mean  $\pm$  standard deviation. All of the analyses were conducted

using SPSS 13.0 (SPSS Inc., Chicago, IL). One-way ANOVA (analysis of variance) was used for comparisons among the means in multiple groups. The q test was used between the groups. Statistical significance was defined as  $p < 0.05$ .

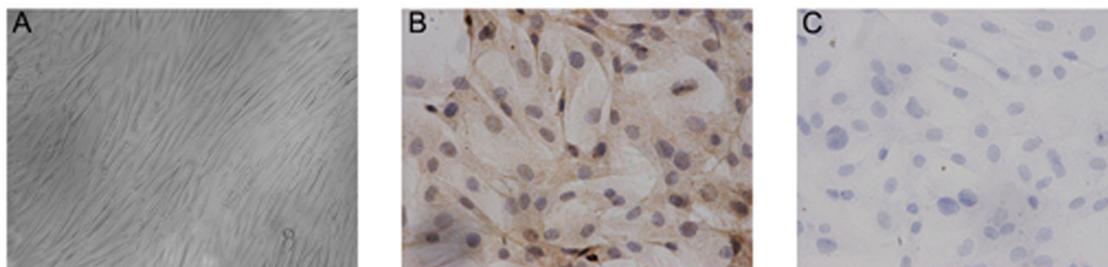
## RESULTS

### Characterization of HASMCs

HASMCs showed spindle cell type under phase contrast microscopy; the orbicular-ovate nucleus appeared in the center of the cells. The typical 'hill and valley' appearance of ASMCs was present in the cell growth. The cultured cells were positive for human  $\alpha$ -smooth muscle actin by immunocytochemistry. These results demonstrated that the cultured cells were smooth muscle cells (Figure 1).

### Variation of $\Delta\alpha$

The fluorescence intensity of R-123 in the normal + Diazoxide group was significantly enhanced compared with that in the normal control group ( $p < 0.05$ ). There was no significant difference in the fluorescence intensity of R-123 between the normal + 5-HD group and the normal control group. Nevertheless, both the asthma model control group and the asthma model + Diazoxide group could induce enhancement of R-123 fluorescence intensity in smooth muscle cells ( $p < 0.05$ ); the latter group showed more significant effects than the former group ( $p < 0.05$ ). The asthma model + 5-HD group could partially reverse the effects of asthma showing enhancement of R-123 fluorescence in smooth muscle cells ( $p < 0.05$ ) (Table 1, Figure 2).



**Figure 1.** Appearance and identification of HASMCs. **A:** Typical 'hill and valley' appearance of ASMCs under phase contrast microscopy. **B:** Cultured cells stained positive for  $\alpha$ -smooth muscle actin by immunohistochemical staining. **C:** Cells stained negative for  $\alpha$ -smooth muscle actin by immunocytochemical staining.

### Effects of Mitochondrial ATP-sensitive K<sup>+</sup> Channel on the ROS Level Reflected by DCF Fluorescence in HASMCs

ROS levels in the normal + Diazoxide group were significantly enhanced when compared with the normal control group ( $p<0.05$ ). ROS production in the normal +5-HD group was not markedly changed. ROS production was evidently increased in the asthma model control group compared with normal control group ( $p<0.05$ ). ROS levels were decreased in the asthma model + 5-HD group when compared with the asthma model control group ( $p<0.05$ ). However, ROS levels were significantly enhanced in the asthma model +diazoxide group compared with the asthma model control group ( $p<0.05$ ) (Table 1, Figure 3).

### Effects of MitoKATP Channels on HASMCs Proliferation

The cell number in the normal + Diazoxide group was significantly increased, compared with the normal

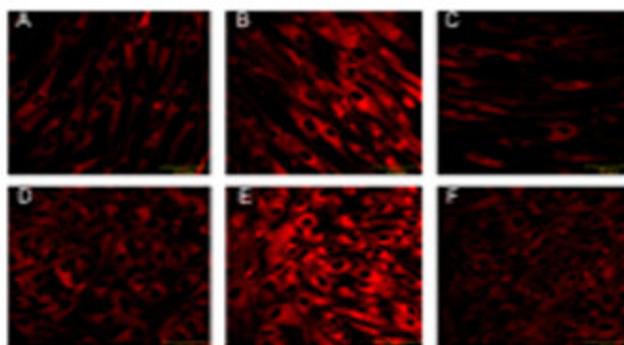
control group ( $p<0.05$ ). The cell number in the normal +5-HD group showed no significant change, whereas the cell number in the asthma model control group was increased ( $p<0.05$ ). Likewise, the cell number in the asthma model + diazoxide group was larger than that in the asthma model control group ( $p<0.05$ ). The cell number in the asthma model + 5-HD group was smaller than that in asthma model control group ( $p<0.05$ ) (Table 2).

### Effects of MitoKATP Channels on TGFβ<sub>1</sub> Expression in HASMCs

The expression of TGFβ<sub>1</sub> mRNA in the normal+Diazoxide group was enhanced compared with the normal control group ( $p<0.05$ ), whereas the expression showed no significant changes in the normal+5-HD group. The expression of TGFβ<sub>1</sub> mRNA in the asthma model control group was increased compared with the normal control group ( $p<0.05$ ). The expression of

**Table 1.** Effects of diazoxide, 5-HD and asthma on the mitochondrial membrane potential and ROS content in HASMCs. The mitochondrial membrane potential was detected using Rhodamine 123 (R-123) fluorescence. The level of reactive oxygen species (ROS) was detected by DCF fluorescence. Mean±SD. N=6. \* $P<0.05$  vs. Normal control group; # $P<0.05$  vs. Asthma model control group

Group	R-123 fluorescence	DCF fluorescence
Normal control	87.40±10.21	98.24±13.02
Normal+Diazoxide	152.34±21.77*	126.42±18.51*
Normal+5-HD	65.71±17.42	69.94±10.87
Asthma model control	195.26±27.65*	185.12±27.06*
Asthma model + diazoxide	237.03±17.92*#	228.91±13.62*#
Asthma model + 5-HD	103.79±26.81#	93.47±15.44#

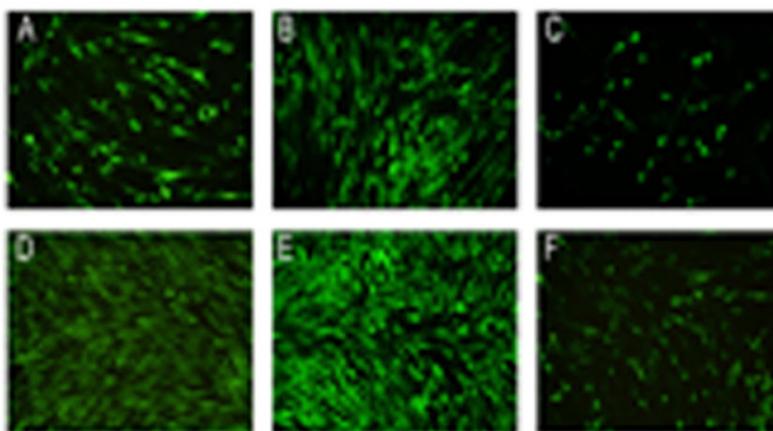


**Figure 2.** Effects of mitochondrial ATP-sensitive K<sup>+</sup> channel on mitochondrial membrane potential as reflected by R-123 fluorescence in HASMCs. A: Normal control group. B: Normal+Diazoxide group. C: Normal+5-HD group. D: Asthma model control group. E: Asthma model + diazoxide group. F: Asthma model + 5-HD group. Scale bar, 50 μm.

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**Table 2.** Effects of diazoxide, 5-HD and asthma on the expression of TGF- $\beta_1$  mRNA in HASMCs. Mean $\pm$ SD. N=6. \* $P$ <0.05 vs. Normal control group; # $P$ <0.05 vs. Asthma model control group

Group	Cell proliferation (cell counting) $\times 10^3$	TGF- $\beta_1$ mRNA (IOD <sub>TGF-<math>\beta_1</math></sub> /IOD $\beta$ -actin)
Normal control	8.25 $\pm$ 0.24	0.351 $\pm$ 0.017
Normal+Diazoxide	9.41 $\pm$ 0.01*	0.592 $\pm$ 0.047*
Normal+5-HD	7.31 $\pm$ 0.02	0.312 $\pm$ 0.053
Asthma model control	9.50 $\pm$ 0.03*	0.702 $\pm$ 0.076*
Asthma model + diazoxide	11.61 $\pm$ 0.03*#	0.998 $\pm$ 0.063*#
Asthma model + 5-HD	8.42 $\pm$ 0.02#	0.357 $\pm$ 0.068#



**Figure 3.** Effects of mitochondrial ATP-sensitive K<sup>+</sup> channel on ROS level as reflected by DCF fluorescence in HASMCs. A: Normal control group. B: Normal+Diazoxide group. C: Normal+5-HD group. D: Asthma model control group. E: Asthma model + diazoxide group. F: Asthma model + 5-HD group.



**Figure 4.** Effects of mitochondrial ATP-sensitive K<sup>+</sup> channel on the expression of TGF- $\beta_1$ mRNA. A: Normal control group. B: Normal+Diazoxide group. C: Normal+5-HD group. D: Asthma model control group. E: Asthma model+Diazoxide group. F: Asthma model+5-HD group.

TGF $\beta_1$  mRNA in the asthma model+diazoxide group was higher than that in the asthma model control group ( $p<0.05$ ). The expression of TGF $\beta_1$  mRNA in the asthma model+5-HD group was lower than that in the asthma model control group ( $p<0.05$ ) (Table 2, Figure 4).

## DISCUSSION

In recent years, many studies have indicated that the airway ion channel plays critical roles in the genesis and development of airway inflammation, airway hyperreactivity, and airway remodeling of asthma.<sup>11</sup> MitoKATP can be activated by diazoxide and inhibited by 5-hydroxyde.<sup>12,13</sup> In this study, diazoxide is a specific opener of MitoKATP, which could specifically open MitoKATP at a low concentration, while 5-HD is a selective blocker of MitoKATP; however, neither showed an effect on MitoKATP in the cell membrane.<sup>14</sup> MitoKATP plays a critical role in the maintenance of potassium balance in mitochondria, and the inhibition of mitochondrial volume changes, which is a critical factor that affects  $\Delta\psi_m$ . Further, MitoKATP is very sensitive to hypoxia. The cellular ATP and NDP contents regulate the activity of the MitoKATP channel.<sup>15</sup> When ATP is increased and NDP is decreased, MitoKATP channel activity, in this state of normoxia, is attenuated and closed. However, with the decrease of ATP and enhancement of NDP in hypoxia, the MitoKATP channel is activated and opens, which induces the depolarization of  $\Delta\psi_m$ . Currently, the effects of the MitoKATP channel in the proliferation and secretion of HASMCs in asthma remains uncertain.

As shown in our data in this study, we presumed that HASMCs in asthma might induce the reduction of ATP and enhancement of NDP in an anaerobic condition, which would induce the activation of MitoKATP channels, inflow of potassium ions, and partial depolarization of  $\Delta\psi_m$ . Meanwhile, there appeared to be a functional decoupling of the electron transport chain and oxidative phosphorylation; reactive oxygen species were generated and converted to hydrogen dioxide by superoxide dismutase in the mitochondria, which further enhanced ROS production and evoked HASMCs hyperplasia. The hyperplasia and proliferation of HASMCs might induce the transformation of HASMCs synthetic phenotype from a contraction type to a proliferation type and increase of synthesis of TGF- $\beta_1$ , which plays multiple roles in the

pathogenesis of asthma. TGF- $\beta_1$  could promote hyperplasia of HASMCs and goblet cells. Further, it can enhance the synthesis of collagen and fibronectin and facilitate their deposition out of the cells, which can induce trachea stenosis and irreversible changes of pulmonary function. Moreover, the expression of TGF- $\beta_1$  mRNA showed a positive correlation with basilar membrane thickness and asthma severity.<sup>16</sup> Plasmin is a critical serine stretch protein, which can split cellulose and is involved in airway repair and remodeling. TGF- $\beta_1$  can reduce the synthesis of plasmin of ASMs and fibroblasts, attenuate the spallation of cellulose, and induce airway fibrosis and remodeling.<sup>17-19</sup>

In conclusion, our results indicate that HASMCs in asthma under anaerobic conditions might induce the activation and opening of MitoK<sub>ATP</sub> channels, cause partial depolarization of  $\Delta\psi_m$ , enhance ROS levels, resulting in the remarkable proliferation of HASMCs, induce the transformation of HASMCs' synthetic phenotype from the contraction type to the proliferation type, and enhance synthetic TGF- $\beta_1$ . From our results, we can presume that the MitoK<sub>ATP</sub> channel plays critical roles in the pathogenesis of asthma. The relationship between the MitoK<sub>ATP</sub> channel and asthma might pave the way for the identification of pathological mechanisms of asthma airway remodeling and possible future asthma therapies.

## ACKNOWLEDGEMENTS

The following funds are gratefully acknowledged. The fund from Natural Science Foundation of China (No: 81070021;81300041), the fund from the Natural Science Foundation of the Anhui Higher Education Institutions of China(No.KJ2012Z184), the fund for the academic backbone of the excellent young and middle-age people of Anhui medical university (2013), the fund from the first affiliated hospital of Anhui medical university for Fostering Talents of the National Natural Science Foundation for young scholars of China(No. 2011KJ05).

## REFERENCES

1. McKay S, de Jongste JC, Saxena PR, Sharma HS. Angiotensin II induces hypertrophy of human airway smooth muscle cells: expression of transcription factors and transforming growth factor-beta1. *Am J Respir Cell*

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- Mol Biol 1998; 18(6):823-33.
2. Panettieri RA. Airway smooth muscle: immunomodulatory cells that modulate airway remodeling? *Respir Physiol Neurobiol* 2003; 137(2-3):277-93.
  3. Sukkar MB, Stanley AJ, Blake AE, Hodgkin PD, Johnson PR, Armour CL, et al. 'Proliferative' and 'synthetic' airway smooth muscle cells are overlapping populations. *Immunol Cell Biol* 2004; 82(5):471-8.
  4. Akrouh A, Halcomb SE, Nichols CG, Sala-Rabanal M. Molecular biology of K(ATP) channels and implications for health and disease. *IUBMB Life* 2009; 61(10):971-8.
  5. Zhao JP, Gao M, Ye YJ, Hu WH, Zhou ZG, Hu HL. Regulation of rat airway smooth muscle cell proliferation by mitochondrial ATP-sensitive K(+) channel in asthmatic rats. *Sheng Li Xue Bao* 2009; 61(1):65-71.
  6. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3). Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007; 120(5):S94-138.
  7. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; 31(1):143-78.
  8. Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am J Respir Crit Care Med*. 2001; 164(1):141-8.
  9. Johnson PR, Black JL, Carlin S, Ge Q, Underwood PA. The production of extracellular matrix proteins by human passively sensitized airway smooth-muscle cells in culture: the effect of beclomethasone. *Am J Respir Crit Care Med* 2000; 162(6):2145-51.
  10. Krick S, Platoshyn O, McDaniel SS, Rubin LJ, Yuan JX. Augmented K(+) currents and mitochondrial membrane depolarization in pulmonary artery myocyte apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2001; 281(4):L887-894.
  11. Csonka C, Kupai K, Bencsik P, Görbe A, Pálóczi J, Zvara A, et al. Cholesterol-enriched Diet Inhibits Cardioprotection by Atp-sensitivepotassium Channel Activators Cromakalim and Diazoxide. *Am J Physiol Heart Circ Physiol* 2013. [Epub ahead of print]
  12. Mironova GD, Negoda AE, Marinov BS, Paucek P, Costa AD, Grigoriev SM, et al. Functional distinctions between the mitochondrial ATP-dependent K+ channel (mitoKATP) and its inward rectifier subunit (mitoKIR). *J Biol Chem* 2004; 279(31):32562-8.
  13. Constant-Urban C, Charif M, Goffin E, Van Heugen JC, Elmoualij B, Chiap P, et al. Triphenylphosphonium salts of 1,2,4-benzothiadiazine 1,1-dioxides related to diazoxide targeting mitochondrial ATP-sensitive potassium channels. *Bioorg Med Chem Lett* 2013; 23(21): 5878-81.
  14. Ardehali H. Role of the mitochondrial ATP-sensitive K+ channels in cardioprotection. *Acta Biochim Pol* 2004; 51(2):379-90.
  15. Das B, Sarkar C. Cardiomyocyte mitochondrial KATP channels participate in the antiarrhythmic and antiinfarct effects of KATP activators during ischemia and reperfusion in an intact anesthetized rabbit model. *Pol J Pharmacol* 2003; 55(5):771-86.
  16. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997; 17(3):326-33.
  17. Hara K, Hasegawa T, Ooi H, Koya T, Tanabe Y, Tsukada H, et al. Inhibitory role of eosinophils on cell surface plasmin generation by bronchial epithelial cells: inhibitory effects of transforming growth factor beta. *Lung* 2001; 179(1):9-20.
  18. Yang ZC, Yi MJ, Ran N, Wang C, Fu P, Feng XY, et al. Transforming growth factor-β1 induces bronchial epithelial cells to mesenchymal transition by activating the Snail pathway and promotes airway remodeling in asthma. *Mol Med Rep* 2013; 8(6):1663-8.
  19. Firszt R, Francisco D, Church TD, Thomas JM, Ingram JL, Kraft M. Interleukin-13 induces collagen type-1 expression through matrix metalloproteinase-2 and transforming growth factor-β1 in airway fibroblasts in asthma. *Eur Respir J* 2013; 43(2):464-73.