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Original Article

Proteomic analysis of the effect of iptakalim on human pulmonary arterial smooth muscle cell proliferation

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Aim: To investigate the anti-proliferative effect of iptakalim (Ipt), a newly selective K_{ATP} channel opener, in endothelin-1 (ET-1)-induced human pulmonary arterial smooth muscle cells (PASMCs) using proteomic analysis.
Methods: Human PASMCs were incubated with ET-1 (10^{-8} mol/L) and ET-1 (10^{-8} mol/L) plus iptaklim (10^{-5} mol/L) for 24 h. Analysis *via* 2-DE gel electrophoresis and MALDI-TOF-MS was employed to display the different protein profiles of whole-cell protein from cultures of control, ET-1 treatment alone, and treatment with ET-1 and iptaklim combined. Real time RT-PCR and Western blot analysis were used to confirm the proteomic analysis.
Results: When iptakalim inhibited the proliferative effect of ET-1 in human PASMCs by opening the K_{ATP} channels, the expression of different groups of cellular proteins was changed, including cytoskeleton-associated proteins, plasma membrane proteins and receptors, chaperone proteins, ion transport–associated proteins, and glycolytic and metabolism-associated proteins. We found that iptakalim could inhibit the proliferation of human PASMCs partly by affecting the expression of Hsp60, vimentin, nucleoporin P54 (NUP54) and Bcl-X_L by opening the K_{ATP} channel.
Conclusion: The data suggest that a wide range of signaling pathways may be involved in abolishing ET-1-induced proliferation of human PASMCs following iptakalim treatment.

Keywords: pulmonary arterial smooth muscle cells; K_{ATP} channel opener; iptakalim; proteomics; proliferation
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Introduction

Proliferation of pulmonary arterial smooth muscle cells (PASMCs) is a key feature during the development of hypoxia pulmonary hypertension (PH)[1, 2]. Endothelin-1 (ET-1) has been implicated in the development of the pulmonary hypertension through the remodeling of pulmonary vessels[3, 4]. In pulmonary hypertension, the amount of circulating ET-1 is increased, with the greatest increase seen in the small resistance arteries. The vascular remodeling associated with chronic hypoxia is likely mediated by the mitogenic effects of ET-1 on vascular smooth muscle cells (SMCs), resulting in their growth[5].

Dysfunction of the potassium channels in the plasma membrane plays a pivotal role in PASMC proliferation[6]. Changes in K^+ channel function may represent a universal mechanism by which Ca^{2+} signals are targeted toward the activation of gene expression and cell growth[7]. Furthermore, activation of K^+ channels can induce apoptosis in vascular SMCs[8]. Recently, K_{ATP} channels have received intense scrutiny because of their biophysical properties, regulation and pharmacology[9]. Vasoactive agonists such as ET-1 can alter the activity of K_{ATP} channels in vascular SMCs, which contributes to the regulation of arterial diameter[10]. This observation has prompted a widespread interest in K_{ATP} channels as potential targets to regulate proliferative vascular disorders in disease conditions such as PH [9].

Iptakalim (Ipt), a lipophilic para-amino compound with a low molecular weight, has been demonstrated *via* pharmacological, electrophysiological, and biochemical studies and a receptor binding test to be a new selective K_{ATP} channel opener[11, 12]. Our previous study revealed that iptakalim could alleviate pulmonary artery remodeling and had the

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potential to treat pulmonary arterial disorders in PH[13]. Moreover, iptakalim could inhibit the release and synthesis of ET-1 in cultured endothelial cells and suppress the proliferation of rabbit/human ET-1-induced PASMCs in a concentration-dependent manner *in vitro*, with 10^{-5} mol/L iptakalim abolishing the effect of 10^{-8} mol/L ET-1[14, 15]. However, the molecular mechanisms of its anti-proliferative effect remain poorly understood. In this study, a proteomic approach is used to investigate the anti-proliferative effect of iptakalim in ET-1-induced human PASMCs.

Materials and methods

Isolation and culture of human PASMCs

Approval for the use of human specimens in this study was obtained from the Jiangsu Province Hospital Ethical Review Board (the First Affiliated Hospital of Nanjing Medical University, Nanjing, China). Human PASMCs were cultured from stage 3–4 of pulmonary artery of a male lung cancer donor (specimens were obtained from Jiangsu Province Hospital). Briefly, the arteries were separated from their adventin and endothelium, minced into 1-mm squares with sterile scalpel blades and placed in a culture bottle containing Dulbecco's modified Eagle's medium (DMEM) with 20% FBS. The culture bottle was placed in a moist tissue culture incubator at 37 °C in an atmosphere of 5% CO_2. The purity of human PASMCs in the primary cultures was confirmed by positive staining with a smooth muscle α-actin antibody compared with a known positive control of SMCs as previously described[16].

Experimental design for 2-DE

Experiments were performed in the third to fifth passages of the cells. Human PASMCs were exposed to the normal (control) condition, the ET-1 (10^{-8} mol/L) condition, or the ET-1 (10^{-8} mol/L) + iptakalim (10^{-5} mol/L) condition in parallel culture. Human PASMCs were plated in 75 mL culture bottles and cultured for 72 h before the medium was changed to serum-free DMEM containing 100 units/mL (1%) penicillin/streptomycin. The human PASMCs were cultured in serum-free medium for 24 h prior to treatment with either ET-1 (10^{-8} mol/L) or ET-1 (10^{-8} mol/L)+iptakalim (10^{-5} mol/L). The control was left untreated for the same period of time. The control, ET-1-treated, and ET-1+iptakalim-treated cultures from one experiment were simultaneously submitted to all the steps of 2-DE, including protein extraction, isoelectric focusing, SDS-PAGE, silver staining and quantitative analysis, to minimize the variability between samples.

Sample preparation for 2-DE

The medium was removed from the culture bottles and the human PASMCs were detached from the culture bottle using trypsin digestion. The PASMCs were harvested and washed three times in ice cold 1×PBS before the addition of lysis buffer (500 μL) containing 7 mol/L urea, 2 mol/L thiourea, 4% (*w*/*v*) CHAPS, 1% (*w*/*v*) DTT, 1% (*v*/*v*) protease inhibitor cocktail, and 2% (*v*/*v*) IPG buffer (pH 3–10) on ice. Samples were sonicated (Five 1-s bursts) on ice and then centrifuged at 40 000×*g* for 60 min at 4°C. The supernatant was removed, assayed for protein concentration (using the Bradford method), and stored at -85°C for the next step in the analysis.

2-DE gel electrophoresis

Isoelectric focusing (IEF) was performed on an Ettan IPGphorII (Amersham Bioscience, Uppsala) with 24 cm immobilized pH gradient strips (pH 3–10; Amersham Bioscience, Uppsala). The IPG strips were rehydrated with samples containing 120 μg of protein and each IPG strip was focused simultaneously, as described previously[17]. After isoelectric focusing, the IPG strips were equilibrated at room temperature and then run in an Ettan Daltsix electrophoresis system (Amersham Bioscience, Uppsala), as described previously. Gels were silver stained according to published procedures[18].

Quantitative analysis of differential protein expression

Gels were scanned using an Attrix Scan 1010 plus (Microtek, Taiwan), and the resulting images were analyzed with the ImageMaster™ 2D platinum software (Amersham Bioscience, Uppsala) for spot detection quantification and comparative and statistical analyses. In brief, the amount of each protein spot was expressed as its volume, which was calculated as the volume above the spot border situated at 75% of the spot height (measured from the peak of the spot). To reflect the quantitative variations in the protein spot volumes, the spot volumes were normalized as a percentage of the total volume of all the spots present in a gel. The experiments were repeated three times with three independent samples, and the protein expression profiles were compared. We assessed the statistical significance between each 2-DE gel (control, ET-1-treated and ET-1+iptakalim-treated) using ImageMaster™ 2D Platinum Software. In brief, each protein spot from the control, ET-1-treated and ET-1+iptakalim-treated gels was manually matched and assigned a number. The protein spots that varied with the same trends of intensity between the 2-DE gels (control, ET-1-treated and ET-1

+iptakalim-treated) in three independent experiments were selected for protein identification.

Protein identification

In-gel Trypsin Digestion: protein spots were manually excised from the gels stained with silver (each piece approximately 1 mm square) using a tip, and digested with trypsin according to the previously described protocol[17].

Protein identification by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Lepzig): dried peptides were dissolved in 2 μL of 0.5% (*v*/*v*) TFA. The matrix material was dissolved until saturated in TA (CAN:0.1% TFA:acetone=3:6:1). The matrix and the analytic solution were mixed at a ratio of 1:1 and 1 μL of the mixture was deposited onto the stainless steel sample target. The solvent was allowed to evaporate at ambient temperature. MALDI-TOF analysis was performed using a Bruker Biflex IV MALDI-TOF-MS system coupled with an N2 laser (337 nm, 3-ns pulse length) in positive ion mode with an accelerating voltage of 19 kV. Peptide data were collected in the reflectron mode. External calibration for peptide analysis was performed with peptide calibration standards. Analyses of MS data were performed by searching against the SWISS-PROT database of the National Center for Biotechnology Information (NCBI) non-redundant database using the MASCOT search program (Matrixscience, London, UK; www.matrixscience.com) to identify proteins of interest. Searches were performed using peptide mass accuracy tolerances of 0.3 Da or 200 ppm for external calibration. Peptides occurring in the blank controls were excluded. One missed cleavage per peptide was allowed, and a molecular weight range of 10% of the relative molecular weight was allowed for the matching of peptide mass values. The criteria for positive identification of proteins were set as follows: (1) at least four matching peptide masses and (2) scores greater than 50 were considered significant (P<0.05).

Expression analysis *via* real time quantitative RT-PCR

Total cellular RNA from control, ET-1 (10^{-8} mol/L)-treated and ET-1 (10^{-8} mol/L)+iptakalim (10^{-5} mol/L)-treated human PASMCs was isolated using the TRIzol reagent (Invitrogen, Jefferson). Then, 2 μg of total RNA of each sample was reverse transcribed into cDNA using the Moloney murine leukemia virus reverse transcriptase (Invitrogen, Jefferson). To amplify the selected protein transcripts, the following gene primers were used:

Hsp60 5'-CAGATGCCCTTAATGCTACAAG-3' (forward)
5'-GCATCATAACCAACTTCTGAGG-3' (reverse)
Vimentin 5'-CAGATGCCCTTAATGCTACAAG-3' (forward)
5'-GCATCATAACCAACTTCTGAGG-3' (reverse)
NUP54 5'-CAAAGGAAGAGTGGTTATGCCATT-3' (forward)
5'-CGGCCCTTGAACTGAGTAGGT-3' (reverse)

Bcl-X_L was also amplified; the primers were 5'-AAGGAGATGCAGGTATTGGTGAGT-3' (forward) and 5'-TCTCGGCTGCTGCATTGTT-3' (reverse). GAPDH was used as control; the primers were 5'-GGAGCCAAACGGGTCATCATCTC-3' (forward) and 5'-GAGGGGCCATCCACAGTCTTCT-3' (reverse). The amplified fragment was about 200–250 bp. PCR conditions were as follows: 95 °C at 10 min for 1 cycle, 95 °C for 10 s and 60 °C for 1 min for 40 cycles in the 7300 System (Applied Biosystems Company, Foster City, CA).

Western blot analysis

Proteins (50 μg each) from whole-cell lysates were separated by SDS-PAGE in 12% SDS gels and then eletrotransferred onto nitrocellulose membranes (BIO-RAD, Hercules). Membranes were blocked overnight in blocking milk solution containing 5% (*w*/*v*) nonfat milk at 4 °C, followed by incubation with primary antibodies (anti-Hsp60, 1:1000; anti-vimentin, 1:100; anti-nucleoporin p54 (NUP54), 1:1000; anti-Bcl-X_L, 1:1000) overnight at 4 °C. The membranes were then rinsed with 1×PBS, applied in 3×15 min washes, followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:5000) at room temperature for 2 h. After a second set of the washes as described above, the reactive proteins were visualized using chemiluminescent substrates (Pierce, Rockford, IL). GAPDH was used as the control.

Statistical analysis

All values were expressed as mean±SEM of three independent experiments. The significance of the difference between the control and samples treated with the various drugs was determined by one-way ANOVA, followed by the *post-hoc* least significant difference (LSD) test. Differences were considered significant at P<0.05.

Results

The present results showed that human PASMCs exhibited the typical "hill and valley" growth morphology. The purity of the primary culture cells was about 99%, which was determined by immunocytochemistry for α-smooth muscle actin, as shown in Figure 1.

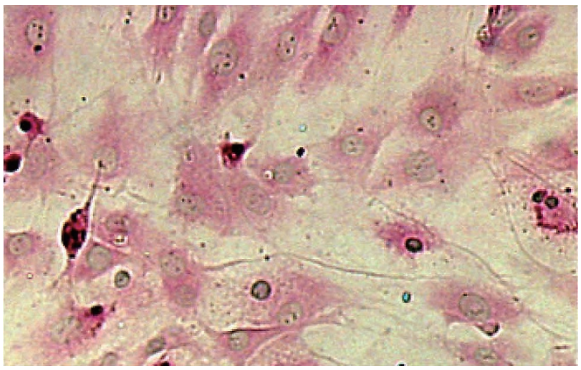

Figure 1. Immunocytochemical staining for α-smooth muscle actin in cells from human PASMC primary cultures (×100). Immunostaining showed that primary cultures were positive for α-smooth muscle actin.

The differential protein expression in human PASMCs was examined using 2-DE. The human PASMCs were treated with ET-1 (10^{-8} mol/L), ET-1 (10^{-8} mol/L)+iptakalim (10^{-5} mol/L), or left untreated. Figure 2 presents the results from one of three independent experiments. More than 3000 protein spots were separated in each gel image, with an apparent range of molecular masses from 11 to 170 kDa and a range of pH values from 3 to 10. Gels were analyzed with the ImageMaster™ 2D platinum software. Proteins that had at least a 5-fold difference in intensity among the gels were excised for identification by MALDI-TOF mass spectrometry using the MASCOT search. We positively identified 27 proteins whose expressions were induced or suppressed in ET-1-treated cells compared with the control group, and were suppressed or induced in ET-1+iptakalim-treated cells compared with the ET-1-treated group (Table 1). Different functional groups of protein were regulated by iptakalim in ET-1-induced human PASMCs, including cytoskeleton-associated proteins (actin, vimentin, lamin A/C, tubulin), plasma membrane protein and receptors (annexin A5, G protein subunit beta 1, nucleoporin p54, transmembrane channel-like protein 5), chaperone proteins (Hsp60, GRP 78, T-complex protein), an ion transport-associated protein (Lasp-1), and metabolism-associated proteins (purine nucleoside phosphorylase, PGAM1 protein, Pgk1, and others). One

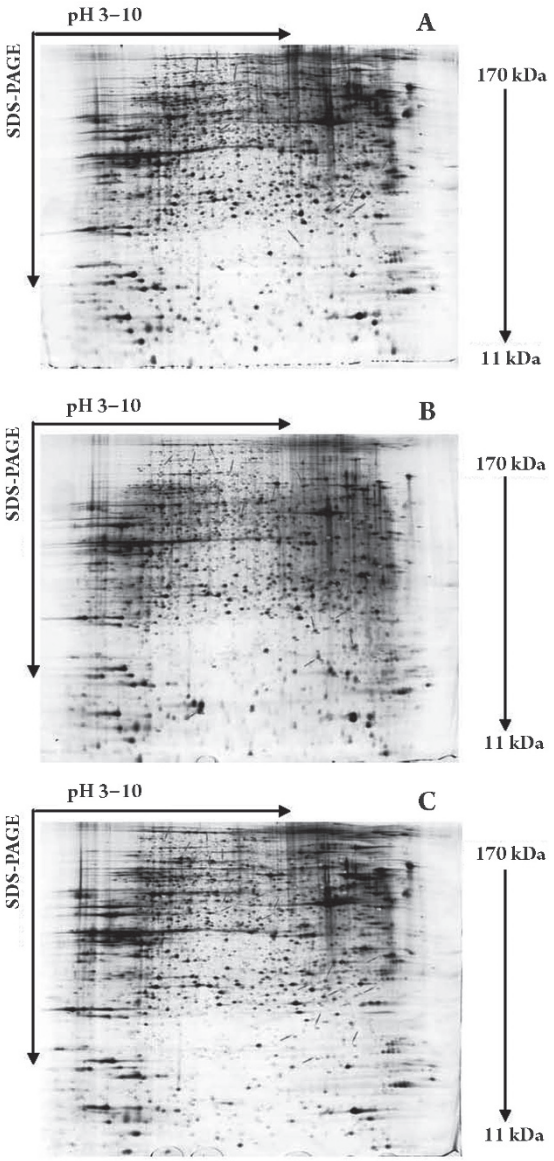

Figure 2. Two-dimensional gel electrophoresis maps of proteins in human PASMCs. Proteins (120 μg) from control (A), ET-1 (10^{-8} mol/L)-treated (B), and ET-1 (10^{-8} mol/L)+iptakalim (10^{-5} mol/L)-treated (C) human PASMCs were run on 2-DE gels and then stained with silver solution. The protein spots that were changed more than 5-fold by ET-1 and inhibited by iptakalim were marked with arrows.

interesting finding was that iptakalim could down-regulate the ET-1-induced expression level of the contractile protein actin in human PASMCs. We currently have no explanation for this phenomenon; the reasons for this change remain to be defined in future studies.

Among the 27 proteins, Hsp60, vimentin and NUP54 were selected for further analysis by real-time PCR and western blot analysis because their expressions were dramatically

Table 1. Twenty-seven differential proteins identified in this study.

Spot No	Accession No	Protein identified	Peptides matched	Mascot score	Molecular mass (Da)	PI	Functional class
Up-regulation in ET-1 treated group and down-regulation in ET-1+Ipt treated group							
1305	Q7Z3B4	Nucleoporin P54	17	93	55 515	6.53	signal transduction
757	P10809	60 kDa heat shock protein	35	230	61 187	5.7	chaperone
2433	P08758	Annexin A5	14	97	35 840	4.94	apoptosis
2478	Q5T8M8	Actin, alpha 1	9	81	32 370	5.26	cytoskeletal
1390	Q53GK6	Beta actin variant	26	198	42 038	5.29	cytoskeletal
2788	P00491	Purine nucleoside phosphorylase	25	193	32 325	6.45	metabolism
3164	Q6FHk8	PGAM1 protein	14	81	28 931	6.67	metabolism
2165	P11021	78 kDa glucose-regulated protein precursor	18	95	72 402	5.07	chaperone
2881	Q14847	LIM and SH3 domain protein 1 (LASP-1)	16	122	30 097	6.61	ion transport
1314	P08670	Vimentin	26	166	53 545	5.06	cytoskeletal
2326	P00558	Phosphoglycerate kinase 1	12	67	44 854	8.3	metabolism
2994	Q9H069	Leucine rich repeat containing 48	9	63	61 559	4.66	signal transduction
2461	P09651	Heterogeneous nuclear ribonucleoprotein A1	7	61	32 532	8.97	signal transduction
2281	P60709	ACTB actin, cytoplasmic 1	13	77	42 052	5.29	cytoskeletal
2828	Q6UXY8	Transmembrane channel-like protein 5	10	77	88 648	9.06	ion transport
1023	P68363	Tubulin alpha-ubiquitous chain	14	96	46 797	4.96	cytoskeletal
2041	P60709	ACTB actin, cytoplasmic 1	15	89	42 052	5.29	cytoskeletal
1293	P14618	PKM2 isoform M2 of pyruvate kinase isozymes M1/M2	20	114	58 470	7.96	metabolism
Down-regulation in ET-1 treated group and up-regulation in ET-1+Ipt treated group							
273	Q9UPY3	Endoribonuclease Dicer	17	62	220 227	5.45	signal transduction
655	Q14195	Dihydropyrimidinase-related protein 3	14	77	62 323	6.04	metabolism
799	Q16555	Dihydropyrimidinase-related protein 2	15	87	62 711	5.95	metabolism
547	P49368	T-complex protein 1 subunit gamma	25	136	61 066	6.1	chaperone
441	P43304	Glycerol-3-phosphate dehydrogenase, mitochondrial precursor	36	240	81 296	7.23	metabolism
950	P14618	Pyruvate kinase isozymes M1/M2	20	143	58 339	7.95	metabolism
783	P02545	Lamin-A/C	21	91	74 380	6.57	cytoskeletal
310	P02545	Lamin-A/C	21	91	74 380	6.57	cytoskeletal
1959	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta 1	10	54	38 020	5.6	signal transduction

changed on the 2-DE gels. The results of both real-time PCR and Western blot analysis confirmed the data from the 2-DE analysis. As shown in Figures 3 and 4, the mRNA levels and protein levels of Hsp60, vimentin, and NUP54 were up-regulated by ET-1, however, this up-regulation was abolished by iptakalim in human PASMCs. In light of the anti-apoptotic effect of Bcl-X_L and its possible interaction with Hsp60, the expression of Bcl-X_L was also examined. Interestingly, the expression of Bcl-X_L was increased in ET-1-treated cells and showed little change in ET-1+iptakalim-treated cells as compared to controls (Figures 3 and 5).

Discussion

It has been demonstrated that iptakalim could selectively open the K_{ATP} channels and increase the K_{ATP} current[11, 12, 15]. Our previous work has shown that iptakalim has an anti-proliferative effect on PASMCs and has potential for the treatment of pulmonary hypertension[13–15]. To further investigate the mechanism of iptakalim's anti-proliferative effect, we took a proteomic approach to examine the protein expression in human PASMCs. ET-1 was able to close the K_{ATP} channels and reduce the K_{ATP} current in a concentration-dependent manner in PASMCs[19]. It was selected as the agonist in our experimental model because it has been implicated in the development of pulmonary hypertension[3, 4].

One of the findings of this work was that the expression of Hsp60 in ET-1-induced human PASMCs was decreased by iptakalim. The chaperone molecular heat shock proteins are primarily anti-apoptotic in cells[20, 21]. Hsp60 can form a complex with Bax, Bak, and Bcl-X_L; when Hsp60 decreases, both Bax and Bak increase, while Bcl-2 decreases[22, 23]. It has

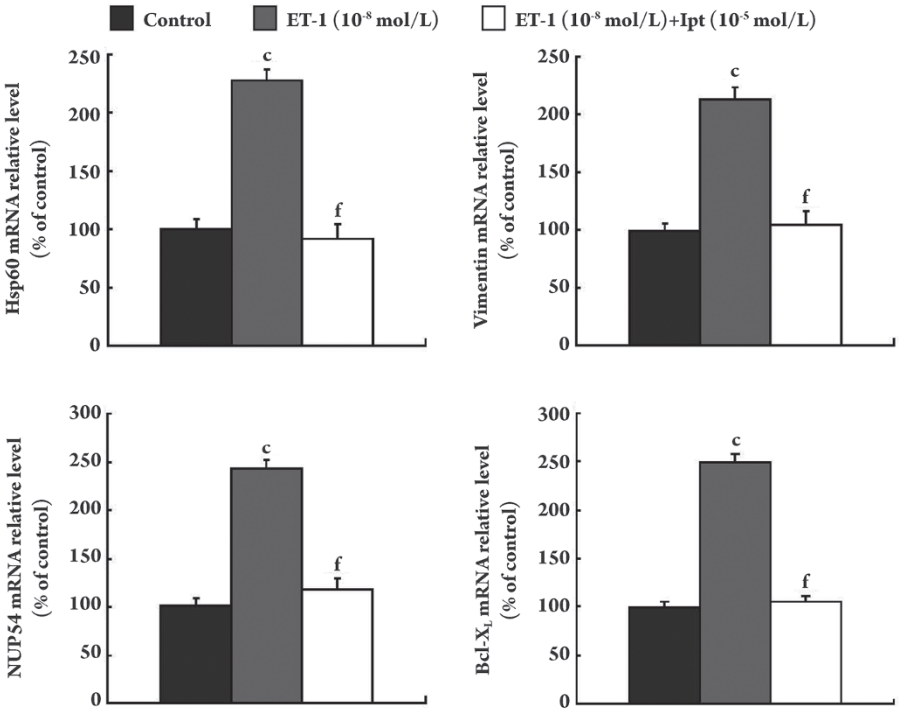

Figure 3. Real-time quantitative RT-PCR analysis of mRNA expression of Hsp60, vimentin, NUP54, and Bcl-X_L in human PASMCs. Human PASMCs were incubated with ET-1 (10^{-8} mol/L) or ET-1 (10^{-8} mol/L)+iptaklim (10^{-5} mol/L) or left untreated for 24 h. The mRNA was extracted and amplified by real-time quantitative RT-PCR. Values are represented as means±SEM from three independent experiments. The results showed that iptakalim could decrease the mRNA expression of Hsp60, vimentin, NUP54 and Bcl-XL induced by ET-1 in human PASMCs. n=3 experiments. Mean±SEM. ^cP<0.01 *vs* control. ^fP<0.01 *vs* ET-1 10^{-8} mol/L.

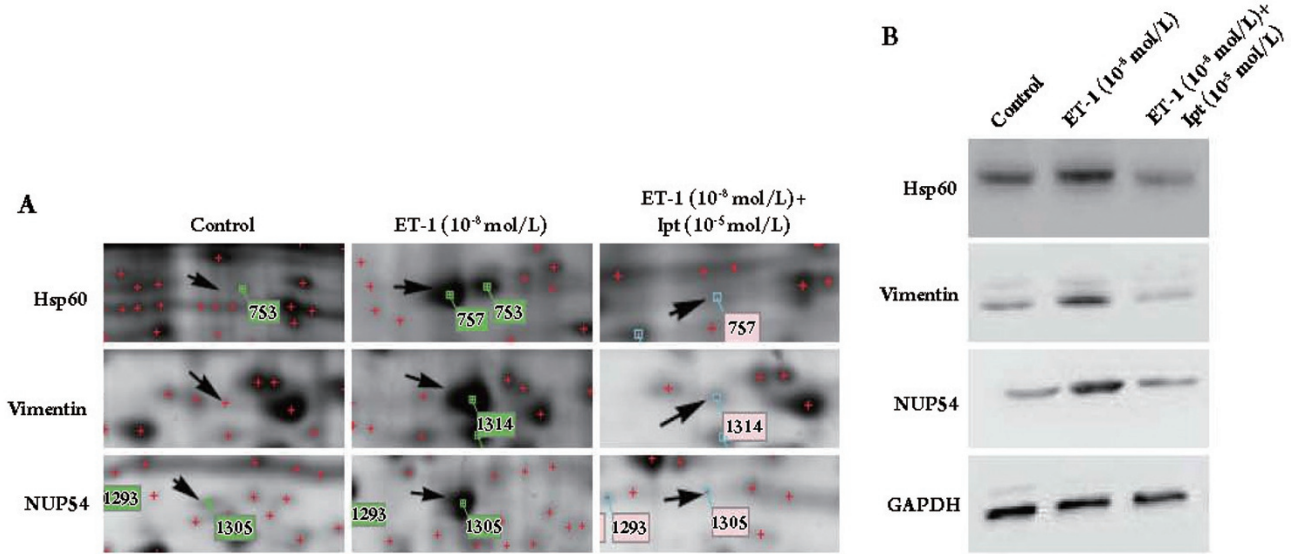

Figure 4. Protein expression of Hsp60, vimentin, and NUP54 in human PASMCs. Human PASMCs were incubated with ET-1 (10^{-8} mol/L) or ET-1 (10^{-5} mol/L)+iptakalim (10^{-5} mol/L) or left untreated for 24 h. Proteins (50 μg each) from whole-cell lysates were run on 2-DE gels (A) or separated by SDS-PAGE and immunoblotted with antibodies against Hsp60, vimentin, NUP54, or GAPDH (B). We confirmed that the Western blot and proteomic results were identical.

been reported that acute ablation of Hsp60 could activate caspase-dependent apoptosis by the disruption of an Hsp60-p53 complex, which results in p53 stabilization, increasing expression of pro-apoptotic Bax, and Bax-dependent apoptosis[22]. In contrast, overexpression of Hsp60 could lead to the increased expression of Bcl-X_L and Bcl-2 and decreased expression of Bax[23]. Therefore, the down-regulation of Hsp60 or interference with the binding of Hsp60 to Bax, Bak, or Bcl-X_L could potentially induce apoptosis[24, 25]. In this regard, iptakalim could decrease the ET-1-induced

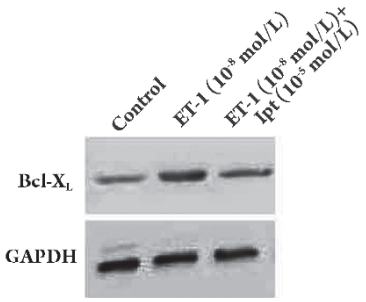

Figure 5. Protein expression of Bcl-X_L in human PASMCs. Human PASMCs were incubated with ET-1 (10^{-8} mol/L) or ET-1 (10^{-8} mol/L)+iptakalim (10^{-5} mol/L) or left untreated for 24 h. Proteins (50 μg each) from whole-cell lysates were separated by SDS-PAGE and immunoblotted with antibodies against Bcl-X_L or GAPDH.

Hsp60 expression in human PASMCs, suggesting that iptakalim might have a pro-apoptotic effect in ET-1-induced PASMCs.

Structural protein expression changes are usually involved in cell proliferation. In the present work, we also identified the protein vimentin, which has intermediate filaments (IFs) and may play an important role in cell integrity, mobility, and differentiation[26, 27], as well as in maintaining cellular function[28–30]. Various cellular processes, including signal transduction, cytoskeletal stability, and cell apoptosis, have been associated with alterations of IFs[29]. Vimentin contains several phosphorylation sites[31] and is a substrate for many protein kinases, such as p34 Cdc2 kinase[32], PKA, PKC[33], Ca^{2+}-calmodulin-dependent protein kinase II[34], p21-activated kinase[35] and MAPK-activated protein kinase-2[36]. The proteolytic degradation of vimentin is a component of the executive process of cell apoptosis[37]. However, whether the decreased expression of vimentin in ET-1-induced PASMCs results from proteolytic degradation has yet to be determined.

The nuclear pore complexes (NPCs), consisting of nucleoporins p62, p54, and p58/p45, may be the only known gateway between the cytoplasm and nucleus that allows for the exchange of macromolecules in eukaryotic cells and is involved in signal import and export[38, 39]. Functional endothelin receptors (ETAR and ETBR) are present on the plasma membrane and nuclei, and protein-protein interactions between the receptors and p62 subcomplex have been demonstrated[40]. Stimulation of the plasma membrane and nuclear ETRs by ET-1 can increase the $[Ca^{2+}]_{cyt}$ and nuclear cisterna or nucleoplasm[41]. Nucleoplasmic Ca^{2+} can regulate many key nuclear functions, including gene transcription, apoptosis, and gene repair[42, 43]. Moreover, the Ca^{2+} level can alter the conformational state of the nuclear pore complex and control the transport of molecules between the cytosol and nucleoplasm. It has been confirmed that a transient rise in $[Ca^{2+}]_{cyt}$ induced by ET-1 could be blocked by iptakalim in human PASMCs. Furthermore, the present study showed that iptakalim could abolish the effect of ET-1 on the induction of NUP54 expression in human PASMCs. The data suggest that iptakalim regulates signaling between the cytoplasm and nucleus, not only through the concentration of Ca^{2+} but also through the expression of NUP54 in ET-1-induced PASMCs.

Because of technical issues, there are limits to the ability of the 2-DE MS approach to analyze proteins of medium to low abundance. In this regard, we plan to investigate the mechanism of the anti-proliferative effect of iptakalim using the modified 2-DE method in association with another approach, such as microarray analysis.

Conclusion

The present work investigated the mechanism of the anti-proliferative effect of the newly selective K_{ATP} channel opener iptakalim in ET-1-induced human PASMCs. Our data suggested that iptakalim might achieve its anti-proliferative effect at least in part through the regulation of Hsp60, Bcl-X_L, vimentin and NUP54 expression and modulate a wide range of signaling pathways in ET-1-induced human PASMCs.

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Author contributions

Hong WANG, Chang-liang ZHU, Wei-ping XIE, and Ming-xia YANG designed the research; Ming-xia YANG, Zheng-xia LIU, Shi-jiang ZHANG, and Lei MA performed the research; Ming-xia YANG, Yu JING, and Shi-jiang ZHANG analyzed the data; and Ming-xia YANG, Zheng-xia LIU, and Shu ZHANG wrote and revised the paper.

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