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Original Article

Modulation of connexin 43 expression by histone acetylation dependent mechanisms in human bladder smooth muscle cells

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Abstract: We investigated the role of a histone acetylation dependent mechanism in the transcriptional repression of connexin43 (Cx43) in cultured human bladder smooth muscle cells (HBSMCs). Expression of Cx43 mRNA was assessed by RT-PCR and qPCR after treatment of HBSMCs with either the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA) or the histone acetyltransferase (HAT) inhibitor, anacardic acid (AA). Modulation of histone acetylation and recruitment of the transcription factors AP-1 and Sp1 at the Cx43 promoter region in response to TSA or anacardic acid was analyzed by chromatin immunoprecipitation (ChIP) assay. While the treatment with TSA promoted the expression of both the Cx43 mRNA and protein in HBSMCs, the expression of both the Cx43 mRNA and protein were suppressed in the presence of anacardic acid, compared to the levels in the untreated cells. ChIP assays confirmed that TSA-induced transcriptional up-regulation of Cx43 in HBSMCs was associated with increases in the accumulation of acetylated histones H3 and H4 accompanied with the enrichment of accessible AP-1 and Sp1 in the critical promoter region of the Cx43 gene. On the contrary, ChIP assay after treatment with anacardic acid showed that repression of Cx43 in HBSMCs was associated with decreased acetylation levels of histone H3 and H4 accompanied with subsequent reduction in the binding of AP-1 and Sp1. Our finding suggested that TSA-mediated induction and anacardic acid-mediated reduction of Cx43 expression in HBSMCs might be associated with the histone acetylation dependent mechanism linked to the transcription factors AP-1 and Sp1 in the Cx43 promoter.

Keywords: Connexin43, histone modification, anacardic acid, detrusor muscle

Introduction

The main function of the bladder is a balanced storage and timely emptying of urine. The capability to efficiently hold urine stand on the basis of the ability to comply with accumulation of urine produced continuously while keeping a low pressure. If the bladder does not act as a reservoir and the detrusor pressure at storage phase rises sharply, the elevated intravesical pressure adversely affect the kidney [1]. There is growing evidence that the pathogenesis of detrusor over-activity is related to increased electrical signaling of smooth muscle cells that causes an involuntary intravesical pressure rises [2, 3]. Intercellular gap-junctional channels are shown in various kinds of mammalian tissues which accelerate electrical and chemical communications between neighboring cells [4]. Gap-junctional channels are assembled by two hemichannels, specifically connexons which are individually given by either of the adjacent cells [5]. Connexons are composed of hexameric assembly of membrane-spanning proteins, called as connexin (Cx), which are affiliated to a family of at least 20 members in mammals [6]. Gap-junctional Cx43 was constitutively expressed in human bladder [7]. Cx43 expression in the normal bladder smooth muscle is generally low, but Cx43 expression increase after partial bladder outlet obstruction in rats and in patients with an overactive bladder (OAB) [8, 9]. Smooth muscle cells of the bladder continuously meet with mechanical

stimuli such as hydrostatic pressure and stretching force during the storage and emptying cycles. Appropriate mechanical stimuli are beneficial to the developmental process of a urinary bladder [10]. Cell to cell communication through gap-junctional channels may contribute to the regulation of variable cellular functions, including cellular growth, migration, differentiation, electric coupling and hormone secretion [11, 12]. Cx43 is a gap-junction protein, which seems to be the well-demonstrated connexin in human bladder smooth muscle cells (HBSMCs) [7]. The number of gap junctions and the expression of connexin were identified to have increased secondary to partial bladder outlet obstruction [13]. But, the manifestation of Cx43 expression decreases under sustained hydrostatic pressures above the physiologic level in the longer term (>72 h) in HBSMCs [14]. In human study, low levels of Cx43 expression were observed in normal bladder, but a significant increase in the expression of Cx43 was identified in women with urge symptoms [15]. Therefore, expression of Cx43 increases in the bladder smooth muscle cells of patients with overactive bladder. More recently the contributions of epigenetic and post-transcriptional mechanisms like DNA methylation, histone modification and microRNAs have been identified in regulation of connexin expression [16]. The involvement of histone modifications, and histone acetylation in particular, in the control of connexin expression has been demonstrated by works with histone deacetylase (HDAC) inhibitor [16]. In the current study, we investigated the role of a histone acetylation dependent mechanism in the transcriptional repression of Cx43 in cultured HBSMCs.

Materials and methods

Cell culture and reagents

The human bladder smooth muscle cell line was obtained from ScienCell Research Laboratories (Carlsbad, CA, USA), and was cultured in Smooth Muscle Cell Medium (ScienCell Research Laboratories). Complete growth media for culturing HBSMCs consisted of 10% fetal bovine serum (FBS), smooth muscle cell growth supplement, and penicillin/streptomycin solution. All the cells were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO_2. HBSMCs were treated with either trichostatin A (TSA; Sigma-Aldrich, St. Louis, MO, USA) for 24 h or anacardic acid (Sigma-Aldrich) for 48 h.

Reverse transcription PCR

Total RNA was isolated from the cell lines using the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Next, cDNA was synthesized from 1 μg of total RNA using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA). The sequences of primer used for RT-PCR amplification of Cx43 cDNA are 5'-CAATCACTTGGCGTGACTTC-3' (forward) and 5'-GTTTGGGCAACCTTGAGTTC-3' (reverse). The cycling conditions were as follows: initial denaturation at 94°C for 3 min; 27-29 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 40 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. All amplifications were performed using AccuPower PCR PreMix (Bioneer, Daejeon, Korea). The human ACTB gene (encoding β-actin) was amplified as an endogenous control.

Quantitative real-time PCR

Real-time cDNA synthesis was performed by conducting a two-step RT-PCR with 1 μg total RNA as the template, using the QuantiTect Reverse Transcription Kit (Qiagen). Next, 1 μl diluted cDNA was used as the template for PCR with the Rotor-Gene SYBR Green PCR Kit (Qiagen), according to the manufacturer's instructions. Amplification and quantitative analysis were performed using the Rotor-Gene Q 5plex HRM system (Qiagen). The cycling conditions consisted of 5 min at 95°C followed by 40 rounds of 5 s at 95°C and 10 s at 60°C.

Immunoblotting

Total protein extracts from HBSMCs were prepared in a lysis buffer of 1.5% SDS, 62.5 mM Tris-HCl (pH 6.8), 5 mM EDTA, 1% 2-mercaptoethanol, 1 mg/mL of antipain, 1 mg/ml of chymostatin, and 1 mg/mL of leupeptin (all from Sigma-Aldrich). The constituent proteins were separated using 12% SDS-PAGE and then transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked for 1 h in Tris-buffered saline-Tween [TBST; 0.2 M NaCl, 0.1% Tween-20, and 10 mM Tris (pH 7.4)] containing 5%

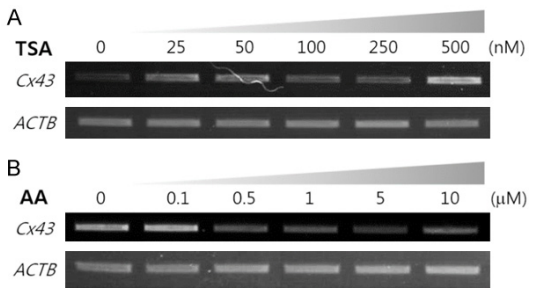

Figure 1. Connexin43 (Cx43) transcription modulated by trichostatin A (TSA) and anacardic acid (AA) in human bladder smooth muscle cells (HBSMCs). A. TSA led to the induction of Cx43 mRNA in a dose-dependent manner in HBSMCs. B. AA gave rise to down-regulation of Cx43 transcription in a dose-dependent manner.

non-fat dry milk. The blocked membranes were then incubated with rabbit anti-connexin43 (1:1,000; Abcam, Cambridge, UK) and mouse monoclonal anti-ACTB (1:2,000; Santa Cruz Biotechnology Inc. Dallas, TX, USA) antibodies in TBST. After incubation, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000; Cell signaling, Beverly, MA, USA) in TBST for 1 h at room temperature. After each step, the membranes were washed several times with TBST, and bound antibodies were detected using an enhanced chemiluminescence detection system (Amersham Biosciences, Amersham, UK) according to the manufacturer's instructions.

Chromatin immunoprecipitation

ChIP analysis was carried out using an EZ ChIP kit (Millipore, Billerica, MA, USA) following the manufacturer's protocol. Briefly, HBSMCs (4×10^5 cells) were plated in a 100-mm culture dish the day before treatment with TSA or anacardic acid. After 48 h of incubation, formaldehyde-treated cells were resuspended in SDS lysis buffer, and the cell lysates were sheared by sonication. The chromatin fragments were immunoprecipitated overnight with antibodies against AcH3, AcH4, AP-1 and Sp1 (all antibodies were purchased from Millipore). Precipitated chromatin was then washed, reverse-cross-linked, and digested with RNase A and proteinase K. The purified DNA was analyzed by quantitative PCR and the quantitative analysis was run in the Rotor-Gene Q 5plex HRM system (Qiagen). The sequences (encompassing nucleotides -256 to -96 bp upstream of the TSS) of the PCR primers used were as follows: forward (5'-ATACACCTTTTTCCCCCATTATCCC-3') and reverse (5'-GATCAGGATAAAGTTGTAGTTTAA ATTTGC-3'). Thermal cycling was performed using the default conditions of the Rotor-Gene Q Series Software (Qiagen), which consisted of 5 min at 95°C followed by 45 rounds of 5 s at 95°C and 10 s at 58°C.

Statistical analysis

Statistical analyses were conducted using IBM SPSS for Windows, Version 21.0 (IBM Inc., Armonk, NY, USA). Data were presented as the mean ± standard error of the mean and analyzed using the Student's *t*-test. A *P*-value of < 0.05 was considered statistically significant.

Results

Histone acetylation-mediated regulation of Cx43 expression in HBSMCs

We investigated whether Cx43 expression in human detrusor smooth muscle cells was affected by histone acetylation-mediated regulation. As shown in **Figure 1A**, the inhibition of histone deacetylation by increasing doses of TSA led to the induction of Cx43 mRNA in HBSMCs. In contrast, the exposure to histone acetyltransferase (HAT) inhibitor, anacardic acid gave rise to down-regulation of Cx43 transcription in a dose-dependent manner (**Figure 1B**). We next demonstrated whether contrasting effects on Cx43 expression were triggered by TSA and anacardic acid in HBSMCs. While the treatment with TSA promoted the expression of both the Cx43 mRNA (**Figure 2A**) and protein (**Figure 2B**) in HBSMCs, the expression of both the Cx43 mRNA and protein were suppressed in the presence of anacardic acid, compared to the levels in the untreated cells (**Figure 2**). These results suggested that the different histone acetylation patterns might modulate the up- or down-regulation of the Cx43 expression levels in HBSMCs.

Changes in the histone acetylation and transcription factors AP-1/Sp1 of the Cx43 promoter in HBSMCs

To determine the TSA- and anacardic acid-responsive regions important for Cx43 regulation, we examined the histone acetylation changes in the Cx43 promoter encompassing

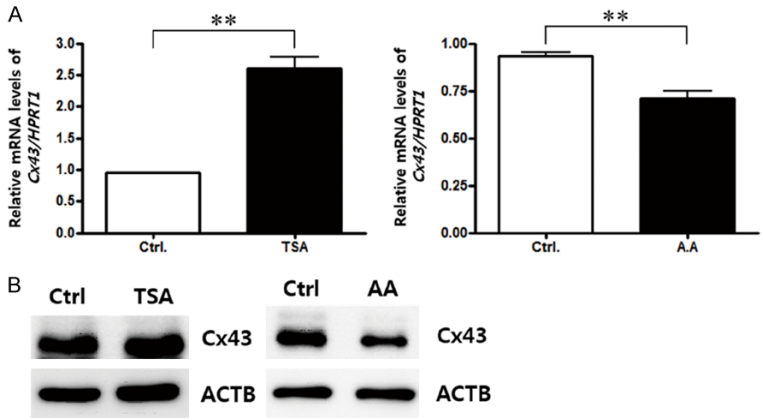

Figure 2. Contrasting effects on Cx43 expression triggered by TSA or AA in HBSMCs. A. Treatment with TSA promoted the expression of Cx43 mRNA and treatment of AA suppressed the expression of Cx43 mRNA. B. Treatment with TSA promoted the expression of Cx43 protein and treatment of AA suppressed the expression of Cx43 protein. **P < 0.01.

binding elements for transcription factors AP-1 and Sp1 (**Figure 3A**). ChIP assays confirmed that TSA-induced transcriptional up-regulation of Cx43 in HBSMCs was associated with increases in the accumulation of acetylated histones H3 and H4 in the critical promoter region of the Cx43 gene (**Figure 3B**). We also found that the hyperacetylation of histones was accompanied with the enrichment of accessible AP-1 and Sp1 to the corresponding sequence element in the Cx43 promoter, compared to the untreated control cells (**Figure 3B**). On the contrary, ChIP assay after treatment with anacardic acid showed that repression of Cx43 in HBSMCs was associated with decreased acetylation levels of histone H3 and H4 in the same sequence element in the Cx43 promoter (**Figure 3B**). Moreover, we also determined that subsequent reduction in the binding of AP-1 and Sp1 to the Cx43 promoter region, which was marked by significant hypoacetylation of histone H3 and H4, compared to the untreated control cells (**Figure 3B**). Therefore, our findings suggested that a promoter region of Cx43 containing conserved sequence elements for AP-1 and Sp1 might be responsive to both TSA and anacardic acid modulating the histone acetylation status in the opposite manners.

Discussion

In a variety of studies, the involvement of histone modifications in connexin expression has been identified by means of control agents that regulate these specific epigenetic processes. Chromatin structure is changeable and histone acetylation and deacetylation are essential parts of gene regulation. These reactions are typically carried out by enzymes with HAT or HDAC activity. Acetylation is the process where an acetyl functional group is transferred from one molecule to another. Deacetylation is simply the reverse reaction where an acetyl group is removed from a molecule. Histone acetylation usually promotes transcriptional activation through chromatin decondensation. On the other hand, the opposite reaction suppresses gene expression [17]. In this study, we investigated whether Cx43 expression in HBSMCs was affected by histone acetylation-mediated mechanism. In HBSMCs, inhibition of histone deacetylation by increasing doses of TSA gave rise to induction of Cx43 mRNA in a dose-dependent manner. In contrast, exposure to anacardic acid, a HAT inhibitor brought about down-regulation of Cx43 transcription in a dose-dependent manner. Anacardic acid is a mixture of 2-hydroxy-6-alkylbenzoic acid homologs and is usually found in Anacardiaceae family [18, 19]. It has been generally known as a non-specific histone acetyltransferase inhibitor of p300 [20]. Anacardic acid, a natural plant extracts, is considered to be a promising anti-tumor drug with acceptable toxicity and high anti-tumor spectrum. We looked into whether contrasting effects on Cx43 expression were triggered by TSA and anacardic acid in HBSMCs. While the treatment with TSA promoted the expression of both Cx43 mRNA and protein in HBSMCs, the expression of both Cx43 mRNA and protein were suppressed in the presence of anacardic acid, compared to the levels in the untreated cells. These results suggested that the differential histone acetylation patterns might be associated with the up- or down-regulation of the Cx43 expression levels in HBSMCs. Inhibitors of HDAC enzymes have been verified to increase connexin production [21-31], usually related to trigger of gap junction opening [21,

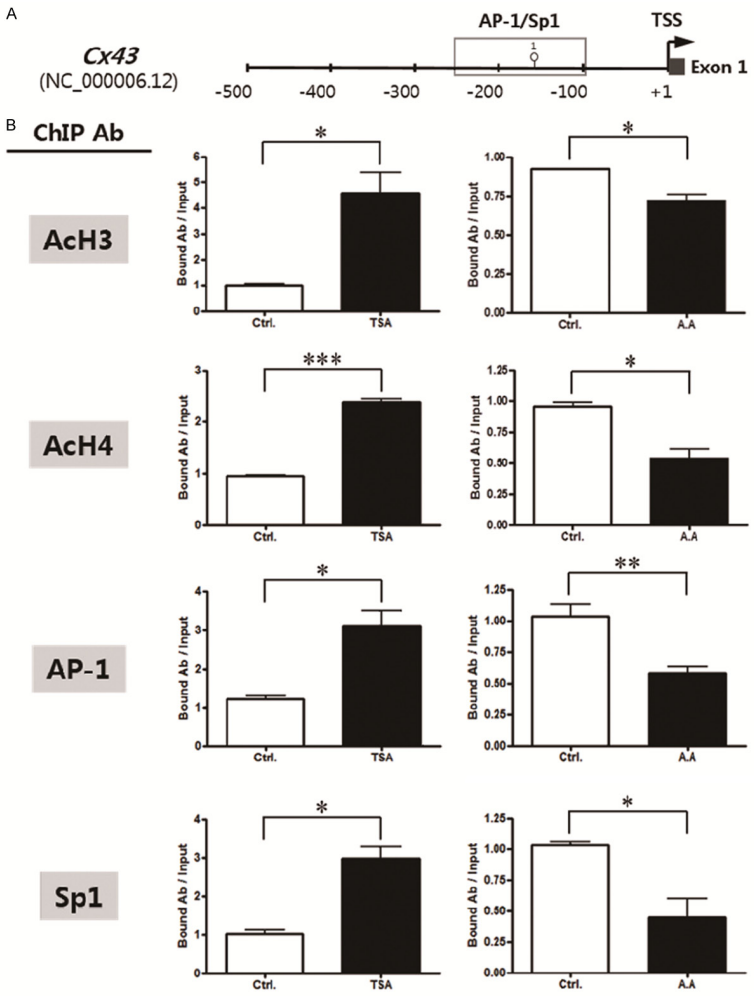

Figure 3. Chromatin immunoprecipitation analysis of acetylated histone proteins (i.e., AcH3 and AcH4) and transcription factors AP-1 and Sp1 in the Cx43 promoter in HBSMCs after the treatment with either TSA or AA. A. Cx43 promoter encompassing binding elements for transcription factors AP-1 and Sp1. B. Treatment of TSA led to increased level of AcH3 and AcH4 and transcription factors AP-1 and Sp and treatment of AA led to decreased level of AcH3 and AcH4 and transcription factors AP-1 and Sp. *P < 0.05, **P < 0.01 and ***P < 0.001.

22, 24-27, 32-35] in diverse experimental settings. The effects of HDAC inhibitors depend on the character of the connexin species and epigenetic modulator as well as the cellular condition. Consequently, HDAC inhibitors have a direct impact on the transcription of the connexin gene. Hernandez et al. [21] reported that the TSA, a HDAC inhibitor, leads to the expression of Cx43 in human prostate cancer cells. They demonstrated that the integrity of both Sp1 and a run of AP-1 in a regulatory region between -234 and -287 from the transcription start site of the basal Cx43 promoter is essential for TSA-induced Cx43 promoter activity, showing that the Sp1/AP-1 responsive elements act in a synergy with the coactivator p300/CREB-binding protein in TSA-mediated Cx43 transcription. This is accompanied by hyperacetylation of histones H4 surrounding the AP-1 and Sp1-responsive gene elements. In this study, in order to determine TSA- and anacardic acid-responsive regions for the regulation of Cx43 in HBSMCs, we examined the histone acetylation changes in the Cx43 promoter encompassing the binding elements for transcription factors AP-1 and Sp1. ChIP assays confirmed that the TSA-induced transcriptional up-regulation of Cx43 in HBSMCs was associated with increases in the accumulation of acetylated histones H3 and H4 in the critical promoter region of the Cx43 gene. We also found that the hyperacetylation of histones was accompanied with the enrichment of accessible AP-1 and Sp1 to the corresponding sequence element in the Cx43 promoter, compared to the untreated control cells. On the contrary, ChIP assay after the treatment with anacardic acid showed that the repression of Cx43 in HBSMCs was associated with the decreased acetylation levels of histone H3 and H4 in the same sequence element in the Cx43 promoter. Moreover, we also determined that the subsequent reduction in the binding of AP-1 and Sp1 to the Cx43 promoter region, which was marked by significant hypoacetylation of histone H3 and H4, compared to the untreated control cells. Therefore, our findings suggested that a promoter region of Cx43 containing conserved sequence elements for AP-1 and Sp1 might be responsive to both TSA and anacardic acid modulating the histone acetylation status in the opposite manners. Expression of Cx43 increases in the detrusor smooth muscle cells of patients with overactive bladder [9]. More recently the contributions of epigenetic and

post-transcriptional mechanisms like DNA methylation, histone modification and microRNAs have been identified in regulation of connexin expression. Our data suggested that different histone acetylation patterns different histone acetylation patterns different histone acetylation patterns histone acetylation patterns might modulate the up- or down-regulation of the Cx43 expression levels in the detrusor smooth muscle of OAB patients. Synthetically, epigenetic aberrations may play crucial roles in development and progression of OAB by a histone acetylation dependent mechanism in the transcriptional expression of Cx43.

Conclusions

Our finding suggested that TSA-mediated induction and anacardic acid-mediated reduction of Cx43 expression in HBSMCs might be associated with the histone acetylation dependent mechanism linked to the transcription factors AP-1 and Sp1 in the Cx43 promoter. Also, epigenetic aberrations may play crucial roles in the development and the progression of OAB.

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Disclosure of conflict of interest

None.

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References

[1] Lee SD, Misseri R, Akbal C, Jung C, Rink RC and Kaefer M. Muscarinic receptor expression increases following exposure to intravesical pressures of < or = 40 cm-H2O: a possible mechanism for pressure-induced cell proliferation. World J Urol 2008; 26: 387-393.

[2] Brading AF. A myogenic basis for the overactive bladder. Urology 1997; 50: 57-67; discussion 68-73.

[3] Fry CH and Wu C. The cellular basis of bladder instability. Br J Urol 1998; 81: 1-8.

[4] Han JH, Kim KA, Shah A, Park BH, Park WH and Kim SH. Regulation of renin release by connexin 43 in As 4.1 cell line. Peptides 2010; 31: 899-902.

[5] White TW and Bruzzone R. Multiple connexin proteins in single intercellular channels: connexin compatibility and functional consequences. J Bioenerg Biomembr 1996; 28: 339-350.

[6] Beyer EC, Paul DL and Goodenough DA. Connexin family of gap junction proteins. J Membr Biol 1990; 116: 187-194.

[7] Neuhaus J, Weimann A, Stolzenburg JU, Wolburg H, Horn LC and Dorschner W. Smooth muscle cells from human urinary bladder express connexin 43 in vivo and in vitro. World J Urol 2002; 20: 250-254.

[8] Haferkamp A, Mundhenk J, Bastian PJ, Reitz A, Dorsam J, Pannek J, Schumacher S, Schurch B, Buttner R and Muller SC. Increased expression of connexin 43 in the overactive neurogenic detrusor. Eur Urol 2004; 46: 799-805.

[9] Christ GJ, Day NS, Day M, Zhao W, Persson K, Pandita RK and Andersson KE. Increased connexin43-mediated intercellular communication in a rat model of bladder overactivity in vivo. Am J Physiol Regul Integr Comp Physiol 2003; 284: R1241-1248.

[10] Haberstroh KM, Kaefer M, Retik AB, Freeman MR and Bizios R. The effects of sustained hydrostatic pressure on select bladder smooth muscle cell functions. J Urol 1999; 162: 2114-2118.

[11] Michon L, Nlend Nlend R, Bavamian S, Bischoff L, Boucard N, Caille D, Cancela J, Charollais A, Charpantier E, Klee P, Peyrou M, Populaire C, Zulianello L and Meda P. Involvement of gap junctional communication in secretion. Biochim Biophys Acta 2005; 1719: 82-101.

[12] Haefliger JA, Castillo E, Waeber G, Bergonzelli GE, Aubert JF, Sutter E, Nicod P, Waeber B and Meda P. Hypertension increases connexin43 in a tissue-specific manner. Circulation 1997; 95: 1007-1014.

[13] Li L, Jiang C, Hao P, Li W, Song C and Song B. Changes of gap junctional cell-cell communication in overactive detrusor in rats. Am J Physiol Cell Physiol 2007; 293: C1627-1635.

[14] Wang Y, Wang K, Li H, Chen L, Xu F and Wu T. Effects of different sustained hydrostatic pressures on connexin 43 in human bladder smooth muscle cells. Urol Int 2013; 90: 75-82.

[15] Neuhaus J, Pfeiffer F, Wolburg H, Horn LC and Dorschner W. Alterations in connexin expression in the bladder of patients with urge symptoms. BJU Int 2005; 96: 670-676.

[16] Vinken M, De Rop E, Decrock E, De Vuyst E, Leybaert L, Vanhaecke T and Rogiers V. Epigenetic regulation of gap junctional intercellular communication: more than a way to keep cells quiet? Biochim Biophys Acta 2009; 1795: 53-61.

[17] Olzscha H, Sheikh S and La Thangue NB. Deacetylation of chromatin and gene expres-

sion regulation: a new target for epigenetic therapy. Crit Rev Oncog 2015; 20: 1-17.
[18] Sowmyalakshmi S, Nur-E-Alam M, Akbarsha MA, Thirugnanam S, Rohr J, Chendil D. Investigation on Semecarpus Lehyam--a Siddha medicine for breast cancer. Planta 2005; 220: 910-918.
[19] Rea AI, Schmidt JM, Setzer WN, Sibanda S, Taylor C and Gwebu ET. Cytotoxic activity of Ozoroa insignis from Zimbabwe. Fitoterapia 2003; 74: 732-735.
[20] Devipriya B and Kumaradhas P. Probing the effect of intermolecular interaction and understanding the electrostatic moments of anacardic acid in the active site of p300 enzyme via DFT and charge density analysis. J Mol Graph Model 2012; 34: 57-66.
[21] Hernandez M, Shao Q, Yang XJ, Luh SP, Kandouz M, Batist G, Laird DW and Alaoui-Jamali MA. A histone deacetylation-dependent mechanism for transcriptional repression of the gap junction gene cx43 in prostate cancer cells. Prostate 2006; 66: 1151-1161.
[22] Ogawa T, Hayashi T, Kyoizumi S, Ito T, Trosko JE and Yorioka N. Up-regulation of gap junctional intercellular communication by hexamethylene bisacetamide in cultured human peritoneal mesothelial cells. Lab Invest 1999; 79: 1511-1520.
[23] Dovzhanskiy DI, Hartwig W, Lazar NG, Schmidt A, Felix K, Straub BK, Hackert T, Krysko DV and Werner J. Growth inhibition of pancreatic cancer by experimental treatment with 4-phenylbutyrate is associated with increased expression of Connexin 43. Oncol Res 2012; 20: 103-111.
[24] Ammerpohl O, Thormeyer D, Khan Z, Appelskog IB, Gojkovic Z, Almqvist PM and Ekstrom TJ. HDACi phenylbutyrate increases bystander killing of HSV-tk transfected glioma cells. Biochem Biophys Res Commun 2004; 324: 8-14.
[25] Asklund T, Appelskog IB, Ammerpohl O, Ekstrom TJ and Almqvist PM. Histone deacetylase inhibitor 4-phenylbutyrate modulates glial fibrillary acidic protein and connexin 43 expression, and enhances gap-junction communication, in human glioblastoma cells. Eur J Cancer 2004; 40: 1073-1081.
[26] Khan Z, Akhtar M, Asklund T, Juliusson B, Almqvist PM and Ekstrom TJ. HDAC inhibition amplifies gap junction communication in neural progenitors: potential for cell-mediated enzyme prodrug therapy. Exp Cell Res 2007; 313: 2958-2967.
[27] Ogawa T, Hayashi T, Tokunou M, Nakachi K, Trosko JE, Chang CC and Yorioka N. Suberoylanilide hydroxamic acid enhances gap junctional intercellular communication via acetylation of histone containing connexin 43 gene locus. Cancer Res 2005; 65: 9771-9778.
[28] Forster T, Rausch V, Zhang Y, Isayev O, Heilmann K, Schoensiegel F, Liu L, Nessling M, Richter K, Labsch S, Nwaeburu CC, Mattern J, Gladkich J, Giese N, Werner J, Schemmer P, Gross W, Gebhard MM, Gerhauser C, Schaefer M and Herr I. Sulforaphane counteracts aggressiveness of pancreatic cancer driven by dysregulated Cx43-mediated gap junctional intercellular communication. Oncotarget 2014; 5: 1621-1634.
[29] Kim J, Hwangbo C, Hu X, Kang Y, Papangeli I, Mehrotra D, Park H, Ju H, McLean DL, Comhair SA, Erzurum SC and Chun HJ. Restoration of impaired endothelial myocyte enhancer factor 2 function rescues pulmonary arterial hypertension. Circulation 2015; 131: 190-199.
[30] Colussi C, Rosati J, Straino S, Spallotta F, Berni R, Stilli D, Rossi S, Musso E, Macchi E, Mai A, Sbardella G, Castellano S, Chimenti C, Frustaci A, Nebbioso A, Altucci L, Capogrossi MC and Gaetano C. Nepsilon-lysine acetylation determines dissociation from GAP junctions and lateralization of connexin 43 in normal and dystrophic heart. Proc Natl Acad Sci U S A 2011; 108: 2795-2800.
[31] Liu F, Levin MD, Petrenko NB, Lu MM, Wang T, Yuan LJ, Stout AL, Epstein JA and Patel VV. Histone-deacetylase inhibition reverses atrial arrhythmia inducibility and fibrosis in cardiac hypertrophy independent of angiotensin. J Mol Cell Cardiol 2008; 45: 715-723.
[32] Kaufman J, Gordon C, Bergamaschi R, Wang HZ, Cohen IS, Valiunas V and Brink PR. The effects of the histone deacetylase inhibitor 4-phenylbutyrate on gap junction conductance and permeability. Front Pharmacol 2013; 4: 111.
[33] Vinken M, Henkens T, Vanhaecke T, Papeleu P, Geerts A, Van Rossen E, Chipman JK, Meda P and Rogiers V. Trichostatin a enhances gap junctional intercellular communication in primary cultures of adult rat hepatocytes. Toxicol Sci 2006; 91: 484-492.
[34] Ammerpohl O, Trauzold A, Schniewind B, Griep U, Pilarsky C, Grutzmann R, Saeger HD, Jansen O, Sipos B, Kloppel G and Kalthoff H. Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells. Br J Cancer 2007; 96: 73-81.
[35] Vinken M, Henkens T, Snykers S, Lukaszuk A, Tourwe D, Rogiers V and Vanhaecke T. The novel histone deacetylase inhibitor 4-Me2N-BAVAH differentially affects cell junctions between primary hepatocytes. Toxicology 2007; 236: 92-102.