Tech Science Press

Oct-1 Mediates ACTH-Induced Proliferation of Vascular Smooth Muscle Cells

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Abstract: Adrenocorticotrophic hormone (ACTH), a 39-amino acid peptide hormone, has been reported in the appreciation of the proliferation of vascular smooth muscle cells (VSMCs), however, the mechanism in molecular scale supporting the appreciation remains to be elucidated. In this study, we observed that the protein expression levels of ACTH at 24 h after exposure to 15% cyclic stretch were significantly higher than that after 5% cyclic stretch. When VSMCs were treated with 1000 nM ACTH directly, Oct-1 and lamin B1 expression were both up-regulated associating with each other, and the presence of Oct-1 was found shuttling between the cytosol and nucleus. When we silenced Oct-1 expression with RNA interference, the proliferation of VSMCs decreases significantly, which also validates a dominant contribution of Oct-1 in ACTH-induced VSMC proliferation. We further screened the target molecules of Oct-1 related to the proliferation with ingenuity pathway analysis (IPA), and found that superoxide dismutase 1 (SOD1) was significantly induced by ACTH stimulation yet suppressed by Oct-1 interference. All these findings in the present study highlight a new molecular mechanism that ACTH up-regulates Oct-1 expression and activates the protein expression of downstream target SOD1, finally induces the VSMC proliferation. The present work proved Octamer transcription factor-1 (Oct-1) as a key transcription factor in the mechanical regulation of VSMC proliferation, which in turn, provide a new target for the treatment of hypertension.

Keywords: Oct-1; proliferation; ACTH; VSMCs; cyclic strech; lamin B1

1 Introduction

Hypertension is one of the most serious cardiovascular diseases. During the progression of hypertension, the mechanical strains on the arterial wall increase abnormally, which induces the remodeling of vascular vessels through the mechanotransduction of proliferation, apoptosis, and migration of vessel cells, and the variations of extracellular matrix components as well [1-4]. As we know, the vascular smooth muscle cells (VSMCs) in arterial wall bear most of the cyclic mechanical stress [5] among all vascular cells, and aberrant proliferation of VSMCs was often observed under hypertension [6].

The adrenocorticotrophic hormone (ACTH), a 39-amino acid peptide hormone synthesized and secreted primarily by the pituitary gland [7] when the precursor peptide proopiomelanocortin (POMC) is processed by prohormone convertase 1/3 (PC1/3) [8], whose main function is to stimulate glucocorticoid production in the adrenal glands. It also modulates both diurnal secretion of glucocorticoids and acute

release of glucocorticoids as part of stress response [9]. Since glucocorticoids are critical for survival, which affect the transcription of up to 20% of the expressed human genome [10], it is reasonable that many studies have reported that ACTH plays an important role in the transcription of peripheral tissues. For example, ACTH in leukocytes affects the anti-inflammatory properties [11]. It also promotes chondrogenic nodule formation and induces transient elevations in intracellular calcium levels in rat bone marrow cell cultures via melanocortin receptor type 2 (MC2R) signaling [12]. Furthermore, ACTH could regulate blood pressure by promoting the synthesis of aldosterone under stress conditions [13]. Our previous study also suggested that ACTH could be synthesized by VSMCs and be involved in abnormal cell proliferation when subjected to high cyclic strain [14]. Usually, specific kinds of transcription factors are required to regulate the expression of specific protein molecules when tissues or cells are stimulated by hormones, growth factors, or others. However, the transcription factors in VSMC proliferation due to ACTH stimulation still remains unknown.

Octamer Transcription factor-1 (Oct-1) belongs to the family of Pit-Oct-Unc (POU) proteins whose members contain highly conserved DNA-binding domain POU. Proteins of the POU family are generally involved in transcriptional regulation and cellular differentiation, and are often found in proliferating eukaryotic cells. Thereafter, Oct-1 transcription factor is intensively literatured in the transcriptional regulation of a variety of genes related to cell cycle, development, and hormonal signals [15,16]. In recent years, suppression of AMPK phosphorylation has been reported to promote Oct-1-directed miR-451a levels and osteoblast differentiation, and an miR-451/AMPK feedback loop has been shown to allow glioblastoma multiforme (GBM) cells to adapt to metabolic stress through direct activation of miR-451 transcription by Oct-1 [17,18]. In addition, activation of the LOX-1 receptor, by either intravenous infusion of oxLDL or an HCD, induces a switch in endothelial signal transduction from the protective Oct-1/SIRT1 pathway to prothrombotic ERK1/2 pathway with the suppression of Oct-1 expression and lack of SIRT1 activation, thus increases tissue factor activity and enhances thrombus formation and vascular occlusion *in vivo* [19]. The *POU2F1* gene encoding the Oct-1 protein can be activated at very early stages of embryonic development, and gets involved in the regulation of many genes such as "housekeeping" genes including snRNA and histone H2B genes, and those specific for the immune, endocrine, and nervous systems [20].

As previous studies have showed that ACTH induces proliferative effects in VSMCs [14], and Oct-1 is involved in the proliferation of HeLa and MCF-7 cells and in hormonal signals [21]. In the present study, we assumed Octamer transcription factor-1 (Oct-1) as a key transcription factor in the mechanical regulation of VSMC proliferation. The rest of the paper investigated whether Oct-1 is involved in VSMC proliferation, and how it mediates ACTH-induced VSMC proliferation with certain molecular mechanisms.

2 Materials and Methods

2.1 Cell culture and Cytokine Stimulation

Animal care and experimental protocols strictly abided by the Animal Management Rules of China (Documentation 55, 2001, Ministry of Health, China), and the study was approved by the Animal Research Committee of Shanghai Jiao Tong University. Primary rat aortic VSMCs were obtained by the explant technique [22], cultured in Dulbecco's modified Eagle medium (Gibco, Carlsbad, CA) supplemented with 10% Newborn calf serum, incubated at 37°C in a humidified 5% CO_2 environment [23], and characterized by the VSMC marker SMA (DAKO, Glostrup, Denmark). VSMCs at passages 4-7 were used. ACTH concentration was 1000 nM.

2.2 Cyclic Strain Application

VSMCs were plated on gelatin-coated flexible silicone-bottom plates (Flexcell International, Burlington, NC) at a density of 2×10^5 cells per well. After 24 h, the culture medium was replaced with serum-free medium for another 24 h to synchronize the cells. Cells in the serum-free medium were exposed to cyclic strain, provided by the FX-5000T Strain Unit (Flexcell International, Burlington, NC), with an elongation magnitude of either 5% or 15%, at a constant frequency of 1.25 Hz.

2.3 Western Blotting

Proteins were electrophoretically separated by 10% SDS-PAGE and transferred to nitrocellulose membrane (Hybond, Amersham, Piscataway, NJ), which was then blocked with 5% non-fat dry milk in TBST buffer, and incubated overnight at 4°C with primary antibodies against Oct-1 (1:1000, Abcam, Cambridge, UK), lamin B1(1:1000, Proteintech, Chicago), lamin A/C (1:1000, Abcam, Cambridge, UK), and GAPDH (1:1000, Proteintech, Rosemont, IL). Membranes were washed thrice with TBST buffer, incubated for 1.5 h with a secondary antibody at room temperature, and finally washed thrice again with TBST. Immunoreactive proteins were detected using BCIP/NBT phosphatase substrate system according to the instruction of the manufacturer (KPL, Milford, MA), scanned with an image scanner (Amersham, Piscataway, NJ), and quantified with Quantity One software (Bio-Rad, Hercules, CA).

2.4 Cell Proliferation Assay

VSMCs were seeded in triplicates at 1×10^4 cells per well in 100μl complete medium in 96-well plates. Cells were cultured overnight and then cultured in serum-starved medium for 24 h. Thereafter, appropriate concentration (1000 nM) of ACTH (Zhong tai Peptide Biochemical, Hangzhou, China) was added and left for another 24 h. The BrdU labeling reagent was added to the culture medium 8 h before detection (1:1000). For BrdU ELISA, VSMC proliferation was analyzed using a colorimetric BrdU kit (Roche, Basel, Switzerland).

2.5 RNA Interference

The mRNA sequence of rat Oct-1 (NM_001100639.1) was acquired from NCBI GenBank. Several small interfering RNAs (siRNAs) targeting rat Oct-1 were designed and synthesized by a company named Gene Pharma Biological, China; the sequences are as follows. For all interference experiments, we used siRNA-Oct-1-Rat-1063.

Table 1: The siRNA sequences of Oct-1

Oct-1 siRNA	siRNA sequences, 5′ to 3′
Oct-1-Rat-485	Fwd: GCA ACA CUC CGC CAG CCA ATT Rev: UUG GCU GGC GGA GUG UUG CTT
Oct-1-Rat-588	Fwd: GCA CAG GAU CUU CAA CAA UTT Rev: AUU GUU GAA GAU CCU GUG CTT
Oct-1-Rat-1063	Fwd: CCU UGA ACC UCA GCU UUA ATT Rev: UUA AAG CUG AGG UUC AAG GTT
NC	Fwd: UUC UCC GAA CGU GUC ACG UTT Rev: ACG UGA CAC GUU CGG AGA ATT

VSMCs were seeded on a six-well plate at a density of 1.5×10^5 cells per well and incubated overnight. RNA interference was studied according to the standard protocol [22]. Interference efficiency was measured after 24-48 h.

2.6 Immunoprecipitation (IP)

Cells were lysed using RIPA Lysis Buffer in presence of complete protease inhibitor cocktail (Sangon Biotech, Shanghai, China). Lysates were pre-cleared twice using protein G PLUS-Agarose (Sangon Biotech, Shanghai, China) and immunoprecipitated overnight at 4°C using anti-lamin B1 (Proteintech, Rosemont, IL). IP complexes were captured using protein G PLUS-Agarose and eluted using 1 × Loading Buffer. The eluted immunoprecipitate was used in SDS-PAGE. Antibodies used for IP, in the current study, were anti-lamin B1 (Proteintech, Rosemont, IL) and anti-Oct-1 (Abcam, Cambridge, UK).

2.7 Radioimmunoassay (RIA) for ACTH Concentration

VSMCs were seeded on flexible silicone-bottom plates (Flexcell International, Burlington, NC) at a density of 2×10^5 cells per well and cultured overnight in an incubator. Once the cells were completely adhered, the culture medium was changed to serum-free medium for 24 h for synchronization. After the cells were exposed to cyclic strain for 24 h, the culture media from VSMCs were collected, and ACTH concentrations were measured using ACTH RIA kit (HY-098, Beijing DORUN International Technology Co., Ltd).

2.8 Immunofluorescence Staining

VSMCs were cultured on coverslips placed in cell cultured plates. Following different treatments, cells were fixed with 4% paraformaldehyde for 20 min, followed by permeabilization with 0.2% Triton X-100 in PBS for 10 min. The slides were blocked with PBS containing 10% horse serum for 1 h at 25°C, and incubated overnight with primary antibodies at 4°C. Appropriate secondary antibodies were added for incubation over 1 h at 37°C. The nuclei were stained with DAPI; images were captured using confocal microscopy (Leica TCS SP8).

2.9 Statistical Analysis

All data are presented as mean ± SD. Statistical comparisons were made with the Student's *t*-test for paired data, and one-way or two-way ANOVA was used to compare the difference between two groups, followed by Fisher's exact-test for multiple comparisons. Values with $P < 0.05$ were considered statistically significant. All analyses were performed with GraphPad Prism Software (Version 7.0).

3 Results

3.1 Cyclic Strain Up-Regulates ACTH Secretion in the Medium

VSMCs were exposed to different magnitudes of cyclic stretch for 12 h and 24 h, and the expressions of ACTH in culture supernatants were measured (Fig. 1(A)). Compared to 5% cyclic stretch (as normal and physiological stretch), 15% cyclic stretch (as high and pathological stretch) slightly repressed ACTH in the culture supernatants after 12 h, but the difference was not significant. When VSMCs were exposed to different cyclic stretches for 24 h, the expressions of ACTH in the culture supernatants of cells exposed under 15% cyclic stretch were significantly increased compared to that exposed under 5% cyclic stretch (Fig. 1(B)). These results indicate that high cyclic stretch applied to VSMCs up-regulates ACTH secretion.

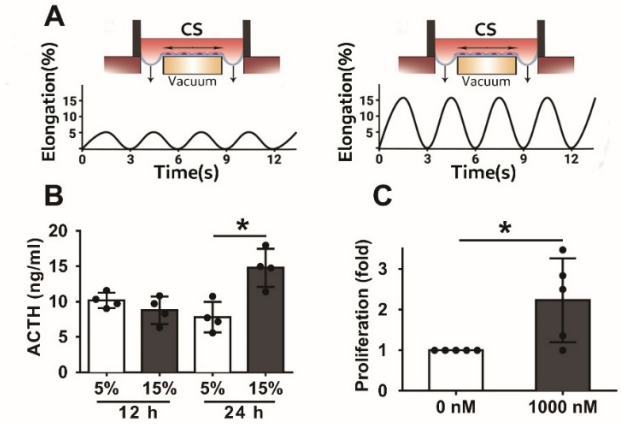

Figure 1: Cyclic strain regulated ACTH expression in culture fluids and ACTH promoted VSMC proliferation. (A) Pattern diagram of strain loading. (B) Protein levels of ACTH in culture fluids after cyclin strain loading for 12 h and 24 h. (C) Proliferation of VSMCs stimulated by 1000 nM ACTH. VSMCs were either untreated or treated with ACTH for 24 h, and their proliferation was assessed using BrdU. The results are represented as the means ± SD of five independent experiments, $*P < 0.05$, $n = 5$

3.2 ACTH Induces Proliferative Effects with Enhanced Oct-1 Expression in VSMCs

Using certain concentrations of ACTH to stimulate VSMCs, our data showed significant increase of VSMC proliferation compared to that in control cells (Fig. 1(C)). To determine whether ACTH directly induces Oct-1 expression in VSMCs, cells were stimulated with ACTH (1000 nM), and cell lysates were analyzed for Oct-1 protein expression. After 6 h, 12 h, and 24 h of ACTH stimulation, the up-regulation of Oct-1 protein expression appears (Fig. 2(A)). In addition, ACTH treatment significantly increased lamin B1 protein expression in the 24 h group (Fig. 2(B)) and had little effect on the expression of lamin A/C (Fig. 2(C)) in VSMCs. These results suggest that ACTH is capable to stimulate VSMC proliferation and induce the up-regulation of both Oct-1 and lamin B1.

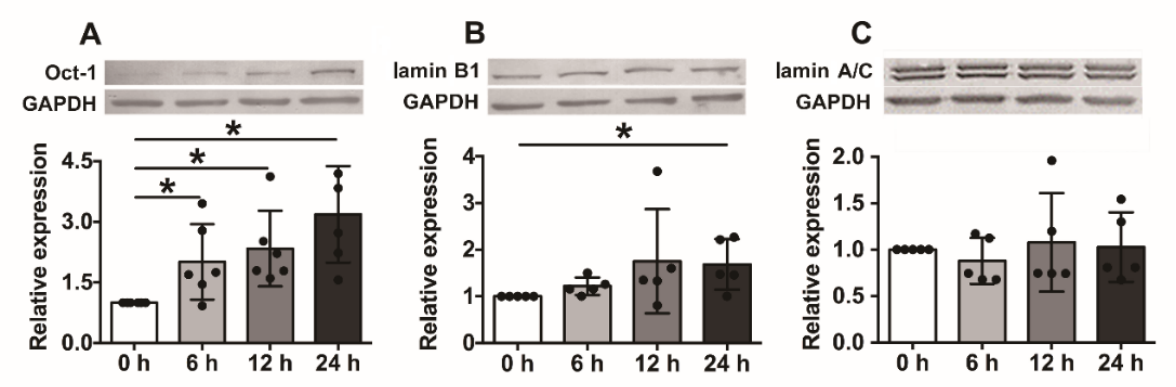

Figure 2: ACTH enhanced Oct-1 and lamin B1 expression in VSMCs. Total protein extracts of VSMCs were either untreated or treated with ACTH (1000 nM) for 0 h, 6 h, 12 h, and 24 h. Protein levels of Oct-1 (A), lamin B1 (B), and lamin A/C (C) were subjected to immunoblot analysis by western blotting in both control and ACTH-treated cells. GAPDH served as the loading control. Data are presented as mean ± SD of six independent experiments, $*P < 0.05$, $n = 6$

3.3 ACTH Could Induce Oct-1 Shuttling Between Cytosol and Nucleus

To examine the ACTH-treatment induced proliferation, subcellular distribution of Oct-1after ACTH stimulation was studied. Oct-1 was well distributed in all VSMCs at 0 h. Using immunofluorescence staining, we found that with the treatment of ACTH, Oct-1 expression was induced, which concentrated mainly in the nucleus at 6 h compared to that in the control (0 h) (Fig. 3). However, similar distributions were not observed at 12 h and 24 h (Fig. 3). This indicates that ACTH could affect the subcellular distribution of Oct-1, and Oct-1 could shuttle between the cytosol and nucleus during VSMC proliferation.

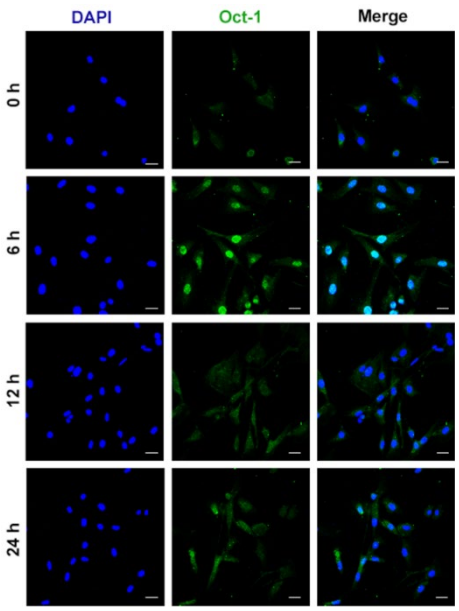

Figure 3: ACTH could induce Oct-1 shuttling between cytosol and nucleus. ACTH (1000 nM) stimulated VSMCs for 6 h, 12 h, and 24 h; the cells were then immunostained with anti-Oct-1 antibodies (green) while nuclei were stained with DAPI (blue), and localization of Oct-1 analyzed by confocal microscopy. Bars = 25 μm

3.4 Oct-1 Mediates the VSMC Proliferation Induced by ACTH

As shown in Fig. 2(A), ACTH significantly induced Oct-1 expression in VSMCs in a time-dependent manner. To further investigate the role of Oct-1 on the VSMC proliferation, gene silencing of Oct-1 by siRNA Oct-1 was employed. Oct-1 protein expression was evaluated by western blot analysis, after 24 h of siRNA transfection to verify whether Oct-1 protein expression was effectively silenced (Fig. 4(A)). Knockdown studies were performed to further confirm the contribution of Oct-1. Results suggested that Oct-1 does not affect lamin B1 expression in VSMCs (Fig. 4(B)), although VSMC proliferation was significantly decreased (Fig. 4(C)). Therefore, the present study showed that ACTH induced proliferation response, which was alleviated by Oct-1 deficiency (Fig. 4(D)). These findings comprehensively suggest that Oct-1 is necessary for ACTH-mediated VSMC proliferation.

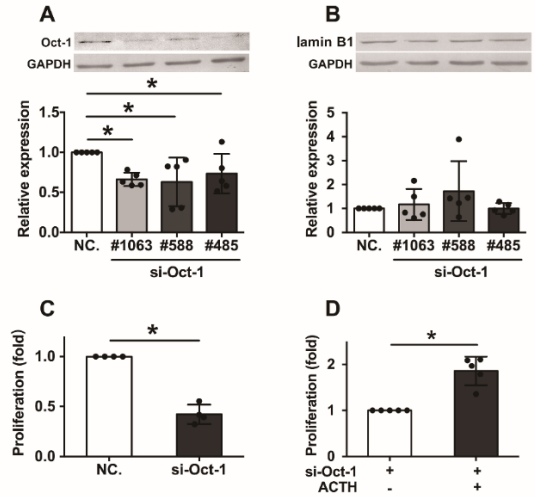

Figure 4: Oct-1 mediated VSMC proliferation induced by ACTH. (A, B) VSMCs were transfected with Oct-1 siRNA for 24 h and protein expression of Oct-1 and lamin B1 was examined by western blotting. (C)

Proliferation of VSMCs was reduced when Oct-1 expression was reduced; proliferation was assessed using BrdU in VSMCs transfected with control siRNA or siRNA directed against Oct-1 for 24 h. (D) VSMCs were transfected with Oct-1 siRNA, and then either left untreated or treated with 1000 nM ACTH for 24 h. Cell proliferation was evaluated by BrdU. The sequence of siRNA used siRNA-Oct-1-Rat-1063 in (C, D). The results are represented as the means ± SD of five independent experiments, $*P < 0.05$, $n = 5$

3.5 Oct-1 Associates with Lamin B1 in VSMCs

To investigate the correlation between Oct-1 and lamin B1, interaction between them was analyzed after ACTH stimulation.

To address this issue, we immunoprecipitated lamin B1 using an anti-lamin B1 antibody and assessed the levels of co-immunoprecipitated Oct-1 using western blotting. Expression of lamin B1 and Oct-1 could not be detected in the negative control (Fig. 5(A)). However, after 6 h, 12 h, and 24 h of ACTH stimulation (1000 nM), Oct-1 was pulled down with lamin B1 using co-immunoprecipitation (Fig. 5(B)). The Co-IP experiments showed an interaction of Oct-1-lamin B1 during the ACTH-induced proliferation.

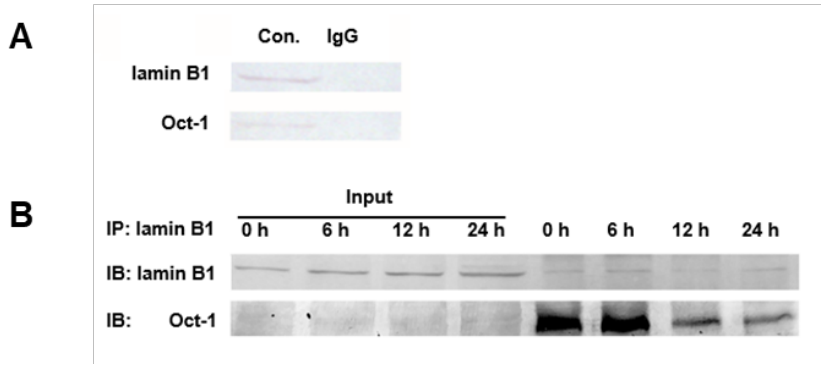

Figure 5: Oct-1 association with lamin B1 in VSMCs. (A) Same amount of IgG was used as a negative control in co-immunoprecipitation. The negative control used lamin B1 antibody, and IP samples were analyzed using anti-lamin B1 and anti-Oct-1 antibodies. The IgG group did not use any antibody and IP samples were also analyzed using anti-lamin B1 and anti-Oct-1 antibodies. (B) After VSMCs were treated with 1000 nM for 6 h, 12 h, and 24 h, control and ACTH-treated VSMCs cells were subjected to immune-precipitation using an anti-lamin B1 antibody. IP samples were then analyzed using anti-lamin B1 and anti-Oct-1 antibodies. One of three similar experiments is presented here

3.6 Correlation of the Downstream Targets of Oct-1 with Cell Proliferation in Cardiovascular Diseases, as Revealed by Ingenuity Pathway Analysis

Ingenuity Pathway Analysis (IPA) was used to analyze the downstream targets of Oct-1 that are associated with cell proliferation in cardiovascular diseases. According to the data obtained, probably more than 20 downstream targets of Oct-1 are closely related to cell proliferation in cardiovascular diseases, which include ICAM1, ATM, SOD1, and IL6 (Fig. 6(A)). After 6 h, 12 h, and 24 h of ACTH stimulation, an up-regulation of SOD1 protein expression at 24 h was observed (Fig. 6(B)). In addition, Oct-1 siRNA transfection significantly reduced SOD1 levels (Fig. 6(C)). The present work demonstrates that ACTH directly up-regulates the protein expression level of Oct-1, thereby regulating the downstream target gene SOD1.

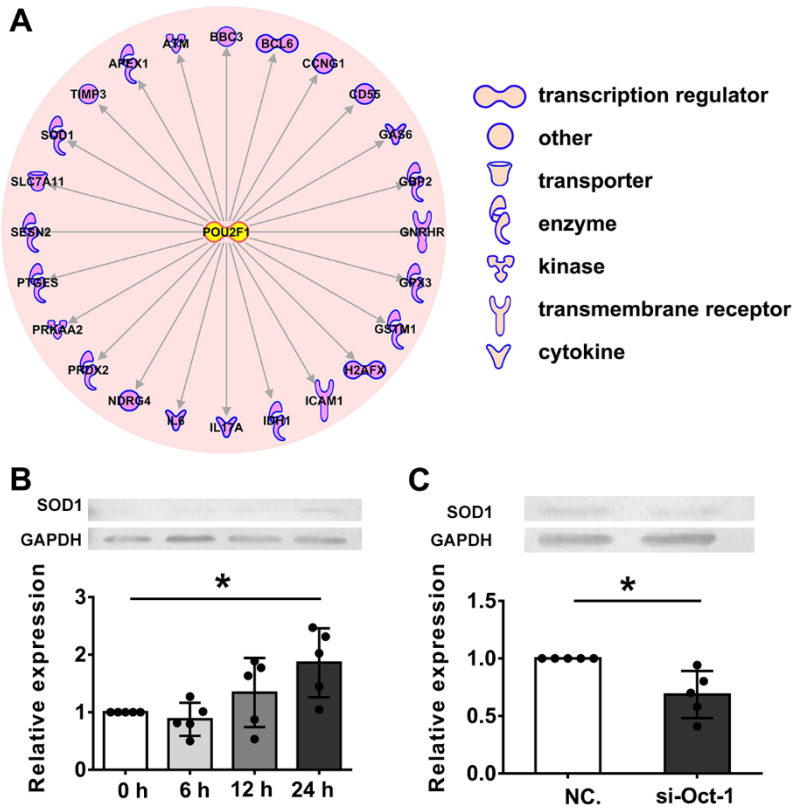

Figure 6: Correlation of the downstream targets of Oct-1 with cell proliferation in cardiovascular diseases, as revealed by IPA. (A) IPA analyzed the downstream targets of Oct-1 that were associated with cell proliferation in cardiovascular diseases. (B) VSMCs were treated with ACTH (1000 nM) for 0 h, 6 h, 12 h, and 24 h. Protein levels of SOD1 were checked with western blotting. (C) VSMCs were transfected with Oct-1 siRNA for 24 h, protein level of SOD1 was analyzed by western blotting, and the sequence of siRNA used was siRNA-Oct-1-Rat-1063 in this experiment, $*P < 0.05$, $n = 5$

4 Discussion

Cyclic stretch is an important inducer of VSMC proliferation, which is crucial in vascular remodeling during hypertension [6]. However, molecular mechanism underlying the vascular remodeling in hypertension has not been fully elucidated. Neuropeptides are endogenous active substances which present in the nerve tissues and participate in the function of the nervous system [24]. Studies on neuropeptides are mainly focused on the nervous system and endocrine system. In recent years, the role of neuropeptides in other local tissues, acting as a special kind of information material, has attracted the attention of researchers. In the humoral regulation, neuropeptides are transported to the target organ through the blood circulation system, and then regulate the function of target organ via specific receptor. For example, the spectrum of ghrelins function has been expanded to include Cardiovascular actions and modulation of proliferation of neoplastic cells, as well as of the immune system [25].

ACTH, a small neuropeptide molecule, is synthesized and secreted by basal cells of the anterior pituitary [26]. Studies have shown that ACTH upregulates intracellular caveolin-1 expression, inhibits G-protein signaling (RGS2 and RGS5) by activating surface-related receptors of ECs and VSMCs [2], and promotes apoptosis by the activation of caspase 3 in ECs [3]. After activation of the ACTH receptor, mononuclear macrophages could adhere and accumulate in the circulatory system, which induces EC dysfunction [4, 27]. In addition to the expression of ACTH receptor, ECs also synthesize and secrete ACTH which interactively participates in EC regulation and angiogenesis by regulating the activity of endogenous

nitric oxide synthase (eNOS) [27]. Interestingly, ACTH has been reported to be able to regulate VSMC proliferation via the ERK/STAT3 pathway [14]. In addition, STAT3 regulated the transcription and expression of Oct-1 by directly targeting its promoter and hence regulating cell proliferation in esophageal carcinogenesis [28]. In this study, ACTH stimulation significantly increases the proliferation of VSMCs *in vitro*, which is consistent with previous reports, thereby suggests that neuropeptides are directly involved in the regulation of function of vascular wall cells. Our previous study had shown that different amplitudes of cyclic strain can regulate the production of neuropeptide Y (NPY) in VSMCs and ultimately affect the proliferation and migration of VSMCs [29]. All these data comprehensively suggest that neuropeptides may play an important role in the regulation of vascular cell function.

To study the molecular mechanism underlying ACTH-induced proliferation of VSMCs, we focused on the transcription factor Oct-1. Oct-1 is a transcription factor that plays crucial roles in modulating cell proliferation, migration, and apoptosis [30]. Previous studies had suggested that Oct-1 is overexpressed in pituitary and breast tumors [31], and Oct-1 expression also correlates with the proliferation of prostate cancer cells [32]. In addition, Oct-1 has been shown in the promotion of HeLa and MCF-7 cell proliferation via a mechanism involving the activation of histone H2B transcription [33]. The present study found that ACTH stimulation can promote the proliferation of VSMCs, which agrees with previous reports those suggested the ACTH/ERK/STAT3 pathway in promoting the proliferation of VSMCs [14]. Our study also demonstrated the increasing expression of Oct-1 due to ACTH. To investigate the roles of Oct-1 in VSMCs proliferation, RNAi was used to downregulate the expression of Oct-1. Inhibition of Oct-1 led to significant decrease in VSMC proliferation which suggests that Oct-1 is necessary for inducing VSMC proliferation. Furthermore, there exists a novel signaling cascade Pak1/MEK/ERK/Oct-1, for both insulin and curcumin, in exerting glucose-lowering effects via the promotion of hepatic ChREBP production [34]. All these results indicated that ACTH might activate STAT3 or ERK to regulate the expression of Oct-1 and promote proliferation of VSMC. These results collectively indicate the central role played by Oct-1 in cell proliferation.

Furthermore, after ACTH treatment, the expression of lamin B1 was found to be remarkably increased. To discover the interactions between lamin B1 and Oct-1, we adopted co-immunoprecipitation. The nuclear lamina is involved in anchoring chromatin to the nuclear envelope during DNA replication and repair, and in the control of gene expression [35,36]. As reported in previous work, lamin B1 contributes to the regulation of gene expression by tethering to specific chromosomes [37] and the transcription factor Oct-1. In addition, phosphorylation of lamin B1 by JNK could result in Oct-1 release from NE as well as the downstream effects that Oct-1 has on GADD45A expression [38]. Previously, Oct-1 was observed to undergo DNA-PK-dependent phosphorylation in response to DSBs, and was considered to make an important contribution to the survival ability of cells against damage [39].

Our immunofluorescence results indicate that Oct-1 could shuttle between the cytosol and nucleus after being stimulated by ACTH. When VSMCs were treated with ACTH, the expression of Oct-1 was significantly increased and it shuttled into the nucleus. We propose that lamin B1 may likely get phosphorylated at T575 [38], which results in partly release of Oct-1 from lamin B1, and the accumulation of Oct-1 in the nucleus in response to ACTH stimulation. After that the increased Oct-1 could bind to its target gene sequence, subsequently up-regulating the expression of downstream target genes such as SOD1. After completion of its effects, Oct-1 may shuttle back from nucleus into cytosol and be degraded therein [40].

It has reported that 15% cyclic stretch could induce more proliferation of VSMCs compared with 5% cyclic stretch [6]. In addition, our previous study had also proved that cyclic stretch upregulates the protein expressions of ACTH and its receptor MC2R in VSMCs, which promoted the proliferation of VSMCs [14]. Our present study focuses more on clearing the function and molecular mechanism of VSMCs with exogenous ACTH rather than discovering the total comprehensive pathways through Oct-1. The molecular mechanisms under mechanical conditions are very complicated. Several experiments in our follow-up series work about this topic revealed that the protein expressions of Oct-1 and lamin B1 in VSMCs sometime conjugated and significantly increased under 15% cyclic stretch compared with 5% cyclic stretch which suggested mechano-responsive correlations between the Oct-1 and lamin B1. Yet, it is unclear whether the molecular mechanisms

of Oct-1 increased in VSMC induced by high cyclic stretch and exogenous ACTH are consistent. The role of Oct-1 in different levels of cyclic stretch needs to clarify in our following researches.

5 Conclusions

In summary, ACTH stimulation was seen to significantly promote the expression of Oct-1 and lamin B1, and it may activate the expression of related target gene SOD1. As a result, the proliferation of VSMCs is significantly appreciated (Fig. 7).

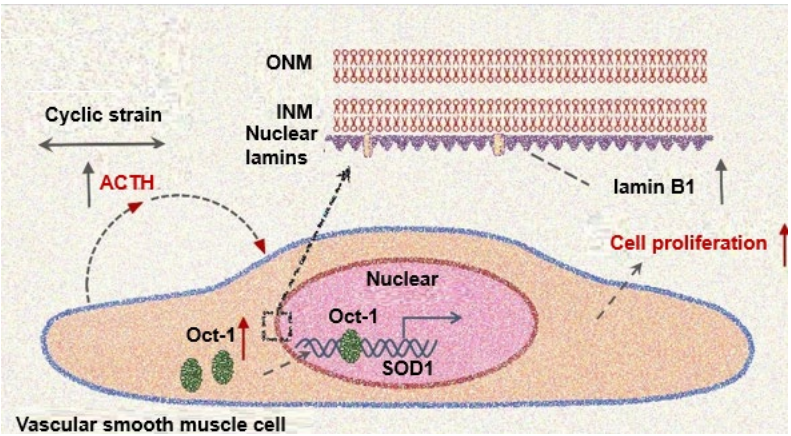

Figure 7: Proposed working model of ACTH in modulating Oct-1 expression and cell proliferation in VSMCs. ACTH stimulation significantly up-regulates the protein expression of Oct-1 and lamin B1. Simultaneously, ACTH treatment promotes more Oct-1 accumulation in the nucleus, which increases the binding of Oct-1 to DNA-binding sequences, thereby regulating the downstream signaling cascade involved in cell survival

Acknowledgement: This study was supported by grants from the National Natural Science Foundation of China (Grant Nos. 11372190 and 11232010). Authors appreciate many instructive discussions from Prof. Yingxin Qi for this study.

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