[Frontiers in Bioscience 13, 3786-3792, May 1, 2008]

Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1

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1. ABSTRACT

Apelin is the endogenous ligand of the G protein-coupled receptor, APJ. Vascular smooth muscle cells express both apelin and APJ, which are important regulatory factors in the cardiovascular and nervous systems. Importantly, APJ is also involved in the pathogenesis if HIV-1 infection. We investigated whether vascular smooth muscle cell proliferation was regulated through an apelin-pERK1/2-cyclin D1 signal transduction pathway. Apelin-13 significantly stimulated vascular smooth muscle cell proliferation and increased cell cycle progression. Apelin-13 a decreased the proportion of cell in the G0/G1 phase while increasing the number of cells in S phase. Apelin-13 also increased the levels of cyclin D1, cyclin E and pERK1/2. Treatment of cells with the MEK inhibitor PD98059 attenuated the apelin-3-induced pERK1/2 activation. Similarly, treatment with PD98059 partially diminished the apelin-13-induced expression of cyclin D1 and vascular smooth muscle cell proliferation. Taken together, these data established that apelin-13 stimulates vascular smooth muscle cell proliferation by promoting the G1-S phase transition, and that this effect is mediated in part by an apelin-pERK1/2-cyclin D1 signal cascade.

2. INTRODUCTION

Apelin is the endogenous ligand for APJ, a G protein-coupled receptor (GPCR) that is structurally related to the angiotensin II type 1 receptor (1). The apelin and APJ proteins are found in various tissues, including the heart, blood vessels, brain and gastrointestinal tract. The apelin/APJ system has multiple biological activities. For example, apelin has been shown to play an important role in cardiovascular homeostasis (2). Furthermore, apelin is a potent, endogenous, positive inotropic peptide and can increase cardiac contraction disproportionately in the failing myocardium (3-5). Apelin also modulates abnormal aortic vascular reactivity in response to both angiotensin II and acetylcholine by augmenting eNOS and phosphorylation of Akt in diabetic mice. The apelin-APJ system therefore may be an important regulator of vascular function in diabetes (6).

Functionally, apelin can stimulate cell proliferation and is an angiogenic factor for retinal endothelial cells (7) It can also promote thymidine incorporation into the DNA of CHO cells expressing the APJ receptor in human umbilical endothelial cells (HUVECs), and is a new mitogenic peptide for endothelial cells (7, 8). More recently, apelin

was reported to stimulate gastric cell proliferation and growth of human inotropic osteoblasts (9, 10) and the introduction of apelin into breast cancer cells induced tumor growth and neo-angiogenesis in xenografts (11).

The wide range of mitogenic effects of apelin in a variety of cell types, and the convincing evidence of vascular apelin and APJ expression, prompted us to speculate that apelin may have mitogenic effects on Vascular smooth muscle cells. Furthermore, the molecular mechanism by which apelin/APJ stimulates cell proliferation is unclear. The aims of this study were to assess the *in vitro* effects of apelin-13 on rat vascular smooth muscle cells and to determine the signaling pathways induced.

3. MATERIAL AND METHODS

3.1. Cell culture

Cell cultures of vascular smooth muscle cells (vascular smooth muscle cells) from the thoracic aortas of 7-8-week-old male Sprague-Dawley rats were prepared by an explant method and cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS)(12) in a 5% CO_2 humidified-atmosphere incubator until they displayed a typical "hill and valley" morphology. Immunohistochemical staining with a monoclonal antibody against α-actin confirmed that there were no co-cultured fibroblasts. Only vascular smooth muscle cells from passages 5 to 8 were used. The cells were grown to 70-80% confluence and then rendered quiescent by incubation with DMEM containing 0.1% FBS for 24 h. On the day of the experiment, the cells were washed twice with serum-free medium and then incubated with a synthetic apelin-13 peptide. This synthetic apelin-13 peptide (pGlu-Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Met-Pro-Phe) was purchased from Phoenix Biotech (Phoenix, AZ), and rehydrated as a stock solution in distilled water before use.

3.2. Proliferation

The extent of vascular smooth muscle cell proliferation was measured by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were plated in 96-well plates at a density of 5×10^4 cells/well, synchronized by incubation in DMEM containing 0.1% FBS for 24 h and then incubated with apelin-13 (0.001-10 μM) for 24 h. Cells treated with 10% FBS were used as control cells. Following the designated incubation interval, 20 μl of MTT was added to each well of the 96-well plates and incubated for 4 h. After removal of the medium from each well, 150 μl of DMSO was added and incubated for 15 min at 37°C. Finally, optical density (OD) readings were taken at 570 nm.

3.3. Flow cytometry

Flow cytometry analyses were performed on treated cells. After 24 h of serum deprivation, cells were incubated with apelin-13 (0.001-1 μmol/L) for 24 h, and then collected by trypsinization. Single-cell vascular smooth muscle cell suspensions were washed three times with cold PBS (pH 7.2) and fixed in cold 70% (v/v) ethanol overnight at 4°C. The cells were resuspended in PBS and were incubated in RNase (1 mg/ml) at 37°C for 30 min followed by staining with propidium iodide (1 μg/μl) for 30 min. The percentages of cells with G1, S or G2+M DNA contents were measured using an Epics Altra Flow Cytometer (Beckman Coulter Inc., Fullerton, CA). The results obtained from the flow cytometry enabled us to calculate the proliferation index (PI) according to the following equation: $PI = (S+G_2M)/(G_0G_1+S+G_2M)$. Change in the sub-G1 population of cells was used as a measure of apoptosis.

3.4. Western blotting

Immunoblotting was performed on vascular smooth muscle cells. After treatment, Vascular smooth muscle cells were washed twice with ice-cold PBS and lysed in a HEPES buffer (20 mM, pH 7.4) containing 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 2 mM sodium orthovanadate, 1 mM PMSF, 2 mg/ml aprotinin, 20 mM leupeptin and 1% Triton X-100 for 10 min on ice. After clarification of the cell lysates by centrifugation at 13,000 *g* for 15 min, the supernatants were collected and their protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Hyclone Pierce, Rockford, Il). Aliquots containing 30 μg of protein were electrophoresed in 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in Tris-buffered saline (10 mM Tris-HCl pH 7.6, 0.15 M NaCl) containing 5% milk and 0.1% Tween 20 for 1 h at room temperature. The proteins were analyzed with the following primary antibodies: anti-pERK1/2 (Santa Cruz Biotechnology Co., Santa Cruz, CA); anti-cyclin D1 and anti-cyclin E (Boster Co., Wuhan, China). The membranes were incubated with a primary antibody diluted in blocking solution for 2 h, and then incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin G as the secondary antibody. Peroxidase activity was detected by enhanced chemiluminescence and analyzed by densitometry using a densitometer and an imager. Data were expressed as the mean (±SD) and analyzed using the SPSS 10.0 for Windows software package. Statistical significance was determined by ANOVA, and values of $p<0.05$ were considered as statistically significant.

4. RESULTS

4.1. Apelin-induces proliferation of cultured vascular smooth muscle cells

To examine the influence of apelin on vascular smooth muscle cell proliferation, cells were cultured with synthetic apelin-13 at varying concentrations (0.001, 0.01, 0.1, 1 and 10 μM) for 24 h. Compared to control cells, apelin-13-treatment increased proliferation in a dose-dependent manner. The maximal response to apelin-13 was observed at a concentration of 1 μM (Figure 1). These results indicate that apelin-13 can stimulate vascular smooth muscle cell proliferation

Table 1. Cell cycle progression of vascular smooth muscle cells

Treatment (24 Hrs)	G0-G1 (%)	S (%)	G2-M (%)	Apoptosis (%)	PI[1]
FBS (0.1%)	93.5 +/- 0.46	3.4 +/- 0.15	2.8 +/- 0.46	1.7 +/- 0.78	0.06+/-0.01
Apelin (0.001μM)	93.1 +/- 0.26	3.7 +/- 0.21	3.2 +/- 0.26	3.7 +/- 0.78	0.07+/-0.01
Apelin (0.01μM)	91.5 +/- 1.76	4.8 +/- 1.25	3.8 +/- 1.27	5.1 +/- 0.53	0.09+/-0.02
Apelin (0.1μM)	88.6 +/- 1.01*	6.5 +/- 0.70	4.8 +/- 1.76	2.7 +/- 2.27	0.14+/-0.05
Apelin (1.0 μM)	81.5 +/- 1.50*	12.5 +/- 5.32	6.1 +/- 3.96	2.5 +/- 1.47	0.18+/-0.02*
FBS (10%)	74.7 +/- 0.50*	17.3 +/- 1.15	8.0 +/- 1.59	2.2 +/- 1.92	0.25+/-0.01*

Cell cycle progression was analyzed by flow cytometry. The data represent the mean ± SD (n=3). p<0.05 vs. 0.1% FBS control *, Proliferation Index [1].

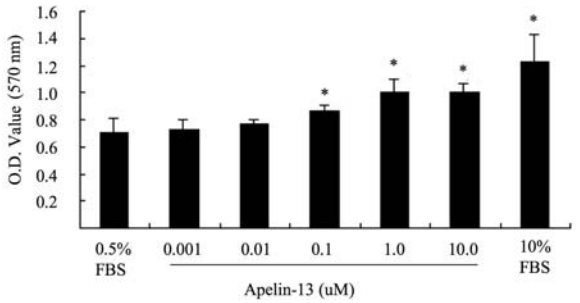

Figure 1. Effects of apelin-13 on rat vascular smooth muscle cell proliferation. Vascular smooth muscle cells were growth-arrested by incubation in DMEM containing 0.1% FBS for 24 h, followed by treatment with apelin-13 (0.001-1 μmol) for 24 h and analysis by the MTT assay. The data represent the mean ± SD (n=4-6). *p<0.05 vs. 0.1% FBS.

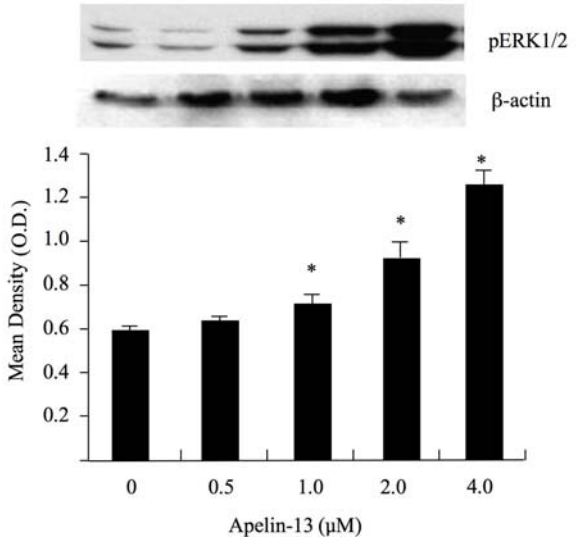

Figure 2. Effects of apelin-13 on the phosphorylation of pERK1/2. Vascular smooth muscle cells were cultured in DMEM containing 0.1% FBS for 24 h, and then incubated with apelin-13 at the indicated concentrations for 30 min. Western blots were probed with an anti-pERK1/2 antibody. The data represent the mean ± SD (n=4-6). *p<0.05 vs. 0 μM apelin-13.

4.1.1. Effects of apelin-13 treatment on the cell cycle in cultured vascular smooth muscle cells

Using flow cytometry, we further examined the effect of apelin on cell cycle distribution. After 24 h of apelin treatment, there was a significant decrease in the percentage of cells in G0/G1-phase, which was accompanied by an increase in the percentage of cells in S-phase (Table 1). The percentage of cells in G0/G1-phase after treatment with 1 μM apelin versus untreated cells was 81.5±1.50% and 93.5±0.46%, respectively (p<0.05). The percentage of cells in S-phase treated with 1 μM apelin compared with untreated cells was 12.5±5.32% and 3.4±0.15%, respectively (p<0.05). Furthermore, the proliferation index of the treated cells gradually increased in a concentration-dependent manner. These data demonstrate that apelin promotes quiescent vascular smooth muscle cell progression from the G0/G1-phase of the cell cycle into S-phase. No appreciable changes in cell apoptosis were observed between the different groups during the 24-hour observation period (Table 1).

4.2. Effects of apelin-13 on ERK1/2 phosphorylation and cell cycle regulatory proteins in cultured vascular smooth muscle cells

Next, the relationship between apelin-13 and ERK1/2 was examined. Treatment of cells with apelin-13 (0.5, 1, 2 and 4 μM) induced pERK (activation of ERK1/2 by phosphorylation) in a dose-dependent manner (Figure 2). The protein levels of cyclin D1 and cyclin E were also increased (Figure 3). A time course of cyclin D1 induction was performed and maximal induction of cyclin D1 protein levels by apelin-13 occurred at 12 hours, as expected (Figure 4).

4. 2. 1. Effect of PD98059 on apelin-13 induced pERK1/2 activation and cyclin D1 expression

To suppress p44/p42 MAPK activation after apelin-13 stimulation, 2'-Amino-3'-methoxyflavone (PD98059), a mitogen-activated protein kinase (MAPK) inhibitor was used. Growth arrested vascular smooth muscle cells were exposed to 10 μmol/L of PD98059 dissolved in DMSO (<0.1%) for 30 minutes followed by the addition of 2μM apelin-13. As shown in Figure 5, MAPK inhibition reduced the levels of apelin-induced pERK1/2 in vascular smooth muscle cells, and PD98059 also attenuated the expression of cyclin D1.

4. 3. Effect of PD98059 on cell proliferation induced by apelin-13

Next, vascular smooth muscle cells were treated with PD98059 or vehicle and cell proliferation was assessed by MTT assay at 24 hours after PD98059 application. The results demonstrated that the cell proliferation induced by apelin-13 was decreased as a result of treatment with PD98059 (Figure 6). These observations suggest that the ERK1/2–cyclin D1 signaling pathway may play an important role in the mediation of apelin-induced proliferation in vascular smooth muscle cells.

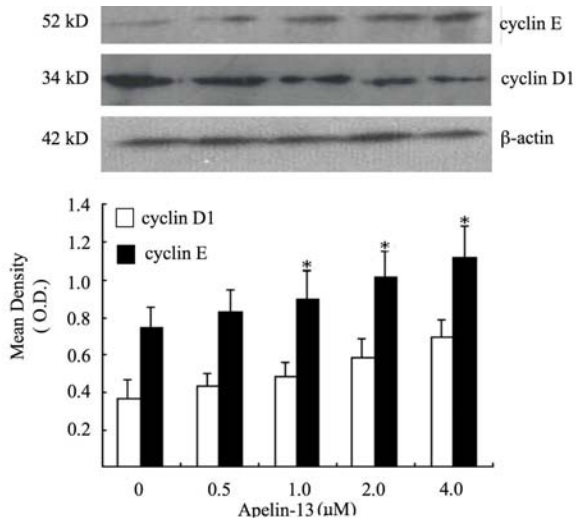

Figure 3. Effects of apelin-13 in vascular smooth muscle cells on the expression of cyclin D1. Vascular smooth muscle cells were serum-starved for 24 h, and then stimulated by apelin-13 for 12 h. Following cell lysis, the lysates were separated by SDS-PAGE and immunoblotted. The western blots were probed with anti-cyclin D1 and anti-cyclin E antibodies. The data represent the mean ± SD (n=3). *p<0.05 vs. 0 μM apelin-13.

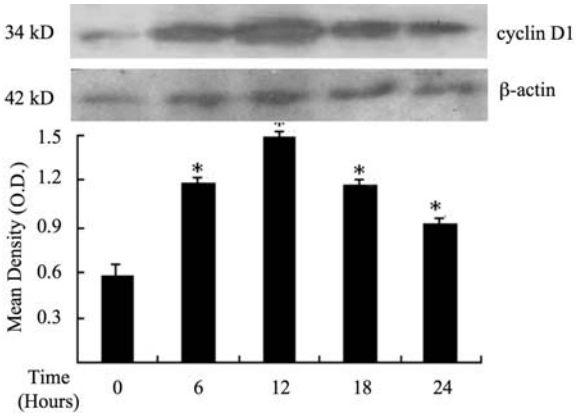

Figure 4. Time course of the effects of apelin-13 on cyclin D1 expression in vascular smooth muscle cells. The cells show a clear cyclic variation. The data represent the mean ± SD (n=3). *p<0.05 vs. 0.1% FBS.

5. DISCUSSION

5.1. General considerations and role in infection

The apelin receptor, APJ belongs to a family of 7- transmembrane domain receptors and is coupled to inhibitory G-proteins. Apelin, is first synthesized as a 77 amino acid pre-pro-peptide which is then cleaved into different size fragments which in turn activate APJ (reviewed in (13)), and the biologically-active apelin peptides serve many functions in both health and in disease (14). The Apelin/APJ pathway functions as a modulator of biological activity in the neuroendocrine, cardiovascular and immune system and apelin has recently been demonstrated to have positive inotropic and vasodilator effects and has been therefore viewed as a possible drug target.

Interestingly, apelin and APJ has been extensively examined with respect to the pathogenesis of HIV-1 infection. The CD4 receptor of T-cell is the major surface protein that determines HIV infection, however other receptors have also been implicated in facilitating HIV entry. For example, two 7-transmembrane chemokines GPCRs, receptors, CCR5 and CXCR4 fuse with the gp120 envelope protein of the HIV. This multimeric protein complex is regarded as an important co-receptor in enabling HIV infection.

APJ is one of a several alternative HIV co-receptors that enhance the entry of HIV-1 and simian immunodeficiency in cultured human cells. In addition, there is also strong evidence strongly suggesting that APJ may function as a co-receptor *in vivo* (15). A structural feature of GPCRs that enables these receptors to function as HIV co-receptors appears to be the tyrosine-rich region in the N-terminus of the receptor as this region is common to CCR5, CXCR4 and APJ. With respect to apelin's role in potentially regulating HIV entry, apelin peptides have been demonstrated to protect cells against HIV infection (16). Interestingly, incubation of cultured cells that are CD4 negative (i.e. lack the major surface receptor involved in HIV uptake) but are APJ positive with soluble CD4 failed to block HIV infection (17), indicating that CD4 is not necessary for infection, and fails to act as a dominant inhibitor of co-receptor-mediated HIV infection. Conversely, Zhou *et al.* (18) has reported that a small molecule antagonist of CXCR4, ALX40-4C, was successful in inhibiting the utilization of APJ as well as CXCR4 by HIV.

5.2. Discussion of experimental data

The major goal of these studies was to investigate the possible role of apelin-13 in vascular smooth muscle cell proliferation. The work presented here demonstrates that vascular smooth muscle cell exposure to apelin-13 increased vascular smooth muscle cell proliferation and increased levels of both activated ERK and cyclin D1. The *cyclin D1* gene is a target of many proliferative signals (19) and apelin has mitogenic effects on a wide variety of cell types. Apelin is capable of stimulating the growth of retinal endothelial cells, the human umbilical endothelial cells (HUVEC), gastric cell proliferation and the growth of human osteoblasts. Our data demonstrated that apelin induces vascular smooth muscle cell proliferation and that apelin induced vascular smooth muscle cell proliferation by promoting cell cycle progression. The restriction point in the G1/S phase of the mammalian cell cycle is a widely accepted control element regulating cell division and flow cytometry analyses revealed that the G1 phase fraction in the apelin-13 treated Vascular smooth muscle cells was lower and the S phase fraction was higher than the control group, demonstrating that apelin-13 induces cell cycle progression through promoting a G_0/G_1 phase to S phase transition.

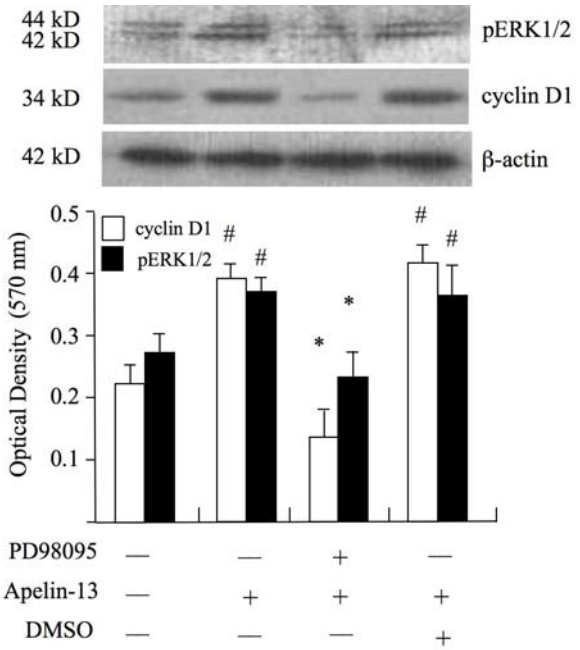

Figure 5. Effects of PD98059, a MEK inhibitor, on apelin-13-induced cyclin D1 and pERK1/2 activities in vascular smooth muscle cells. After serum-starvation for 24 h, vascular smooth muscle cells were incubated with or without PD98059 for 30 min, and then incubated with apelin-13 (2 μmol/L) for 30 min. Apelin+DMSO treatment shows no significant differences from apelin treatment. The data in the bottom panel represent the mean ± SD (n=3). *p<0.05 vs. apelin-13+PD98059; #P<0.05 vs. 0.1% FBS.

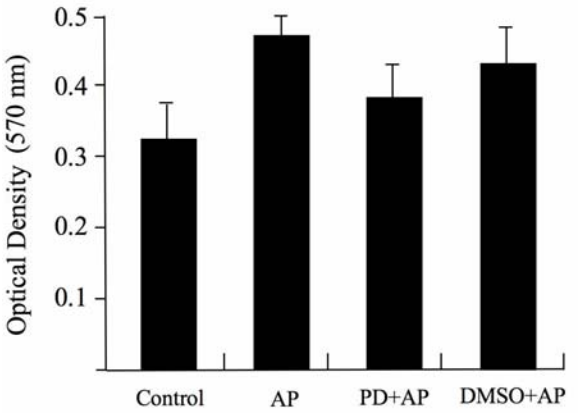

Figure 6. Effects of PD98059 on the cell proliferation induced by apelin-13. Cells at passages 5-8 were plated in 96-well plates. After synchronization for 24 h, the cells were incubated with or without PD98059+DMSO for 60 min, and then incubated with apelin-13 (2 μM) for 24 h. The data represent the mean ± SD (n=4).

The p44/42 ERK MAP kinase pathway is an important signal transduction pathway previously implicated in G_1-S progression and critical in controlling cell proliferation (20, 21). ERK also contributes to the synthesis of cyclins, and the cell cycle G1/S transition requires cyclin D-dependent kinase (CDK4 and CKD6) complexes and cyclin E/CKD2 activity, which act to phosphorylate target proteins such as Retinoblastoma (Rb), thereby promoting entry into the DNA synthetic phase of the cell cycle (22, 23). For example, in the kidney, angiotensin II induction of the angiotensin type I receptor (which is structurally related to the apelin receptor, APJ) induced cell cycle proliferation and cyclin D1 gene activation proliferation through ERK (24). Our results showed an up-regulation of ERK1/2, cyclin D1 and cyclin E by apelin. We also observed that pretreatment of vascular smooth muscle cells with PD98059 resulted in suppression of both apelin-induced ERK1/2 activity and cyclin D1 expression, and we showed that the stimulatory effect of apelin on cell proliferation was attenuated by treatment with PD98059. Since apelin and APJ are expressed in the kidney, it is interesting to speculate that apelin/APJ may play important roles in HIV-induced renal damage. Interestingly, infection of podocytes with HIV resulted in cell cycle progression, increased cyclin D1 protein levels and cyclin D1-dependent Rb phosphorylation, thereby contributing to HIV-induced nephropathy, while infection with *nef* deficient HIV failed to induce cyclin D1 (25). Conversely. molecular analysis of the effect of HIV on T cells differentiation and proliferation showed that,CD4 expression, cell proliferation and cyclin D1 were repressed through Nef (26). These data establish that, despite the fact that HIV/Nef regulation of cellular activity appears to be tissue specific, cyclin D1 remains a critically important target gene.

The present study clearly demonstrates that apelin-13 can induce smooth muscle cells proliferation by accelerating cells through the G1 phase to S phase of the cell cycle. Furthermore, our data also show for the first time that an apelin-pERK1/2-cyclin D1 signal cascade may be involved. It must be noted that apelin's effect of stimulation may involve other pathways as well. For example, apelin stimulated proliferation of MC3T3-E1 cells which was mediated via JNK and PI3-K/Akt (27). In addition, apelin can enhance human osteoblast proliferation, with the PI3K kinase/Akt pathway involved in the proliferative response (10). Whether apelin-13-induced vascular smooth muscle cell proliferation involves the PI3K/Akt pathway, and whether cyclin D1 is a target, requires further investigation.

In summary, apelin is capable of promoting vascular smooth muscle cell proliferation and is a mitogenic agent for vascular smooth muscle cells. As the mitogenic effect of apelin may be associated with angiogenesis and since vascular smooth muscle cell vascular smooth muscle cell proliferation plays a critical role in the pathogenesis of atherosclerosis, it is reasonable to consider the apelin-APJ-cyclin D1 pathway as a potential therapeutic target.

6. ACKNOWLEDGEMENTS

Drs Feng Li and Lanfang Li contributed equally to this work. This work was supported by grant-30371645 from China National Natural Scientific Foundation, the

Hunan Provincial Outstanding Younger Foundation of P. R. China (03B036) and NIH Grant AI052739 (HBT). We thank Professor Kai Li for helpful discussion and the valuable help in preparing the manuscript.

7. REFERENCES

1. O'Dowd, B. F., M. Heiber, A. Chan, H. H. Heng, L. C. Tsui, J. L. Kennedy, X. Shi, A. Petronis, S. R. George & T. Nguyen: A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene*, 136, 355-60 (1993)

2. Kleinz, M. J., J. N. Skepper & A. P. Davenport: Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul Pept*, 126, 233-40 (2005)
Szokodi, I., P. Tavi, G. Foldes, S. Voutilainen-Myllyla, M. Ilves, H. Tokola, S. Pikkarainen, J. Piuhola, J. Rysa, M. Toth & H. Ruskoaho: Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res*, 91, 434-40 (2002)

3. Ashley, E. A., J. Powers, M. Chen, R. Kundu, T. Finsterbach, A. Caffarelli, A. Deng, J. Eichhorn, R. Mahajan, R. Agrawal, J. Greve, R. Robbins, A. J. Patterson, D. Bernstein & T. Quertermous: The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading *in vivo*. *Cardiovasc Res*, 65, 73-82 (2005)

4. Dai, T., G. Ramirez-Correa & W. D. Gao: Apelin increases contractility in failing cardiac muscle. *Eur J Pharmacol*, 553, 222-8 (2006)

5. Zhong, J. C., X. Y. Yu, Y. Huang, L. M. Yung, C. W. Lau & S. G. Lin: Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice. *Cardiovasc Res*, 74, 388-95 (2007)

6. Kasai, A., N. Shintani, M. Oda, M. Kakuda, H. Hashimoto, T. Matsuda, S. Hinuma & A. Baba: Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochem Biophys Res Commun*, 325, 395-400 (2004)

7. Masri, B., H. Lahlou, H. Mazarguil, B. Knibiehler & Y. Audigier: Apelin (65-77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochem Biophys Res Commun*, 290, 539-45 (2002)

8. Wang, G., Y. Anini, W. Wei, X. Qi, O. C. AM, T. Mochizuki, H. Q. Wang, M. R. Hellmich, E. W. Englander & G. H. Greeley, Jr.: Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. *Endocrinology*, 145, 1342-8 (2004)

9. Xie, H., S. Y. Tang, R. R. Cui, J. Huang, X. H. Ren, L. Q. Yuan, Y. Lu, M. Yang, H. D. Zhou, X. P. Wu, X. H. Luo & E. Y. Liao: Apelin and its receptor are expressed in human osteoblasts. *Regul Pept*, 134, 118-25 (2006)

10. Sorli, S. C., S. Le Gonidec, B. Knibiehler & Y. Audigier: Apelin is a potent activator of tumour neoangiogenesis. *Oncogene* (2007)
11. Chamley-Campbell, J., G. R. Campbell & R. Ross: The smooth muscle cell in culture. *Physiol Rev*, 59, 1-61 (1979)

12. Beltowski, J.: Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? *Med Sci Monit*, 12, RA112-9 (2006)

13. Kleinz, M. J. & A. P. Davenport: Emerging roles of apelin in biology and medicine. *Pharmacol Ther*, 107, 198-211 (2005)

14. Edinger, A. L., T. L. Hoffman, M. Sharron, B. Lee, Y. Yi, W. Choe, D. L. Kolson, B. Mitrovic, Y. Zhou, D. Faulds, R. G. Collman, J. Hesselgesser, R. Horuk & R. W. Doms: An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J Virol*, 72, 7934-40 (1998)

15. Zou, M. X., H. Y. Liu, Y. Haraguchi, Y. Soda, K. Tatemoto & H. Hoshino: Apelin peptides block the entry of human immunodeficiency virus (HIV). *FEBS Lett*, 473, 15-8 (2000)

16. Puffer, B. A., M. Sharron, C. M. Coughlan, F. Baribaud, C. M. McManus, B. Lee, J. David, K. Price, R. Horuk, M. Tsang & R. W. Doms: Expression and coreceptor function of APJ for primate immunodeficiency viruses. *Virology*, 276, 435-44 (2000)

17. Zhou, N., X. Zhang, X. Fan, E. Argyris, J. Fang, E. Acheampong, G. C. DuBois & R. J. Pomerantz: The N-terminal domain of APJ, a CNS-based coreceptor for HIV-1, is essential for its receptor function and coreceptor activity. *Virology*, 317, 84-94 (2003)

18. Albanese, C., J. Johnson, G. Watanabe, N. Eklund, D. Vu, A. Arnold & R. G. Pestell: Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. *J Biol Chem*, 270, 23589-97 (1995)

19. Zhang, W. & H. T. Liu: MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res*, 12, 9-18 (2002)

20. Rescan, C., A. Coutant, H. Talarmin, N. Theret, D. Glaise, C. Guguen-Guillouzo & G. Baffet: Mechanism in the sequential control of cell morphology and S phase entry by epidermal growth factor involves distinct MEK/ERK activations. *Mol Biol Cell*, 12, 725-38 (2001)

21. Weinberg, R. A.: The retinoblastoma protein and cell cycle control. *Cell*, 81, 323-30 (1995)

22. Tamamori-Adachi, M., H. Ito, P. Sumrejkanchanakij, S. Adachi, M. Hiroe, M. Shimizu, J. Kawauchi, M. Sunamori, F. Marumo, S. Kitajima & M. A. Ikeda: Critical role of

cyclin D1 nuclear import in cardiomyocyte proliferation. *Circ Res*, 92, e12-9 (2003)

23. Watanabe, G., R. J. Lee, C. Albanese, W. E. Rainey, D. Batlle & R. G. Pestell: Angiotensin II activation of cyclin D1-dependent kinase activity. *J Biol Chem*, 271, 22570-7 (1996)

24. Nelson, P. J., M. Sunamoto, M. Husain & I. H. Gelman: HIV-1 expression induces cyclin D1 expression and pRb phosphorylation in infected podocytes: cell-cycle mechanisms contributing to the proliferative phenotype in HIV-associated nephropathy. *BMC Microbiol*, 2, 26 (2002)

25. Ndolo, T., N. K. Dhillon, H. Nguyen, M. Guadalupe, M. Mudryj & S. Dandekar: Simian immunodeficiency virus Nef protein delays the progression of CD4+ T cells through G1/S phase of the cell cycle. *J Virol*, 76, 3587-95 (2002)

26. Tang, S. Y., H. Xie, L. Q. Yuan, X. H. Luo, J. Huang, R. R. Cui, H. D. Zhou, X. P. Wu & E. Y. Liao: Apelin stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3-E1 via JNK and PI3-K/Akt signaling pathways. *Peptides*, 28, 708-18 (2007)

Abbreviations: ERK: extracellular regulated kinase, MAP kinase: mitogen activated protein kinase. JNK: Jun N-terminal kinase, CDK: cyclin-dependent kinase, Rb: retinoblastoma protein, PI3 Kinase: phosphatidylinositol-3 kinase

Key Words: Apelin, APJ, cyclin D1, cell cycle, Vascular Smooth Muscle

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