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Review

Apelin and APJ, a novel critical factor and therapeutic target for atherosclerosis

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Apelin is a bioactive peptide discovered recently that has been proved to be an endogenous ligand of the APJ receptor. Apelin and APJ are widely distributed in the central nervous system and peripheral tissues. Researches have confirmed that apelin/APJ involved in a wide range of physiological and pathological functions in the cardiovascular system. Investigations indicated that apelin is a novel critical factor in the development of atherosclerosis (AS). In this review, we discuss the roles of apelin in the vascular smooth muscle cell proliferation, monocytes-endothelial cell adhesion, and angiogenesis that potentially reveals a new cellular mechanism of AS. Considering these roles, apelin and APJ may be novel therapeutic targets of AS.

Keywords apelin; APJ; atherosclerosis; vascular smooth cell; endothelial cell

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Introduction

APJ, a new G protein-coupled receptor with typical seven transmembranes, was found in 1993 by homology cloning [1], which was once considered to be an orphan receptor until its endogenous ligand apelin peptide was isolated from bovine stomach [2]. Researches showed that apelin/APJ are widely distributed in the central nervous system and peripheral tissues and involved in a wide range of physiological and pathological functions including dilatation of arteries, systolic effect, regulation of fluid homeostasis, the adipoinsular axis, cell proliferation, angiogenesis and so on. Apelin isoforms have at least four bioactive forms, including apelin-12, -13, -17, and -36. Apelin was identified as a novel adipokine and became popular in cardiovascular disease in the past decades. Only 32 articles were found in PubMed database using apelin and atherosclerosis (AS) as mesh medical subject heading terms as of December 18, 2012. Although there are some different results, the existing articles tend to support that the apelin/APJ system may be a risky factor in AS. In this review, we discuss the *in vitro* and *in vivo* results of apelin/APJ related to AS and give the reasonable suggestion for future research. The study of APJ in AS will probably open new routes for AS prevention and therapy.

Expression and Location of Apelin/APJ in Atherosclerosis Patients

There have been some reports of apelin/APJ levels in AS patients with somewhat controversial results. Rittig *et al*. [3] found that serum apelin-36 levels were not associated with early AS using apelin-36 as an early risk indicator in young subjects prone to AS. However, plasma apelin levels were lower in patients with elevated low-density lipoprotein-cholesterol (LDL-C) compared with healthy controls [4]. In addition, apelin-12 was negatively correlated with ox-LDL in the pregnancy group [5]. The decrease of LDL-cholesterol through statin treatment was accompanied with the increase of plasma apelin in dyslipidemia patients [6]. But Kadoglou *et al*. [7] found that apelin increase and LDL-C reduction were independently associated with the atorvastatin-induced GSM (gray-scale median) increase. They inferred that the atorvastatin-induced modification of apelin and LDL-C may be beneficial to carotid plaque stability. Although apelin-12 decreased in some dangerous state associated with AS, this did not totally reflect AS itself as well as other apelin subtypes. Indeed, apelin-13, an important active form, was up-regulated in human atherosclerotic coronary artery and this additional peptide was localized to the plaque, co-localizing with markers for macrophages and smooth muscle cells [8]. Moreover, in calcified aortic valve disease patients, the gene expression of apelin and the APJ receptor were shown to be significantly up-regulated in stenotic valves [9]. Which factors cause the overexpression as well as the role of apelin in AS is worth being further studied. The contradictory results may be caused by the sample, the method and the pleiotropic effects of apelin/APJ. The apelin expression and substantial role of this peptide in the AS needs more experimental research to be confirmed.

Effect of Apelin on Atherosclerosis

Apelin, a novel adipokine, *via* inhibition of food intake, may promote weight loss resulting in a beneficial effect of antiatherogenic. It is too complicated to define beneficial or harmful role of apelin/APJ on AS based on the current studies. In end-stage heart failure rats, the down-regulation of apelin/APJ expression was significantly increased by angiotensin (Ang)II receptor blocker. Angiotensin had already been thought to be an AS inducer. It suggested that APJ may possess anti-atherogenic effect. And the simultaneously increased phosphorylation of Akt (also known as protein kinase B, PKB) and endothelial nitric oxide synthase (eNOS) indicated that APJ may inhibit AS formation through Akt and eNOS pathways [10]. Chun *et al*. [11] found that apelin decreased AS formation by blocking AngII actions in mouse. But Hashimoto *et al*. [12] found that apelin/APJ system is the mediator of oxidative stress-linked AS in vascular tissue. Apelin can promote AS, but it can also inhibit AS in AngII-knockout mice, which is puzzling and interesting. The interaction between apelin and AngII in AS deserves further study. No matter what is the effect of apelin on AS, it is now clear that apelin is an important factor for AS. The mechanism of apelin action in the occurrence and development of AS needs to be further explored by more experimental researches, especially *in vitro* experiments.

Apelin Is Involved in Lipid Metabolism and Insulin Resistance

Apelin is the most recently identified adipocytokines that may associate with improved lipid metabolism. Apelin decreases free fatty acid releasing, which attributes to its dual inhibition on adipogenesis and lipolysis. Apelin suppresses adipogenesis through adenosine monophosphate protein kinase (MAPK) kinase/extracellular-signal-regulated kinase (ERK)-dependent pathways. And by preventing lipid droplet fragmentation, apelin inhibits basal lipolysis through AMP-activated protein kinase-dependent enhancement of perilipin expression and hormone-stimulated acute lipolysis through decreasing perilipin phosphorylation [13]. Further researches confirmed that sports [14] and high-fat sucrose diet [15] increase the expression of apelin. These results indicate that the apelin may possess a lipid-lowering effect. High-fat sucrose diet increased *apelin* gene expression and decreased cyclin-dependent kinase inhibitor 1A and fatty acid synthase (Fasn) expression [15]. Interestingly, circulating apelin is increased in children with type 1 diabetes mellitus, but it is not a significant increase between the apelin and body mass index, glucose, lipids and adiponectin, or insulin sensitivity [16]. In patients with type 2 diabetes mellitus (T2MD), both rosiglitazone and metformin significantly improved glycemic profile and apelin levels. Insulin resistance (IR) was significantly attenuated in both groups. The aforementioned changes of apelin were independently associated with HOMA-IR (homeostasis model assessment-insulin resistance) [17]. However, the levels of apelin in obese T2DM patients are closely related to IR. The increased levels of apelin may be a result of compensatory response to IR, and also may be the causative factor of IR. The levels of apelin correlate closely with oxidative stress and inflammation [18]. Apelin plays its role in IR or lipid metabolism that may not act directly on insulin or insulin receptor, but by promoting lipid use in muscle through mitochondrial biogenesis and matching between fatty acid oxidation and the tricarboxylic acid cycle. Apelin could contribute to insulin sensitivity improvement [19]. Another adipokine resistin (similar to apelin) on IR is controversial on inflammatory processes and endothelial dysfunction [20]. The brief discussion of apelin effects on IR is not significant. Apelin expression was increased during adipogenesis, and augmented by blocking renin–angiotensin system (RAS) [21]. RAS blockers also have beneficial effects that prevented excessive lipid accumulation and the generation of reactive oxygen species (ROS) in different adipocytes. It is indicated that angiotensin can inhibit the expression of apelin. Whether the beneficial effects resulted from inhibiting RAS were related to apelin or not still needs to be explored by more observations. Perhaps the action of apelin on lipid metabolism in AS will become an important research topic in the future.

Apelin Induces Adhesion of Monocytes-endothelial Cells by Inducing the Expression of Adhesion Molecules and Chemokines

Atherosclerotic plaques formation is the most important part in atherosclerotic disease. Li and coworkers [22] found that apelin-13 induced adhesion of monocytes (MCs) to human umbilical vein endothelial cells (HUVECs) by inducing the expression of vascular cell adhesion molecule 1 (VCAM-1) at first. The subsequent investigations revealed that apelin-13 promoted PI3K phosphorylation in concentration-dependent and time-dependent manners in HUVECs. And PI3K inhibitor LY294002 considerably inhibited the MC adhesion to HUVECs and the expression of VCAM-1 induced by apelin-13. Lu *et al*. [23] reported similar results that the expression of intercellular adhesion molecule-1, VCAM-1, and monocyte chemoattractant protein-1 (MCP-1) in HUVECs is significantly increased when treated with apelin. Our previous studies also showed that apelin-13 induced the expression of 14-3-3 in concentration- and time-dependent manners [24]. Furthermore, the potent

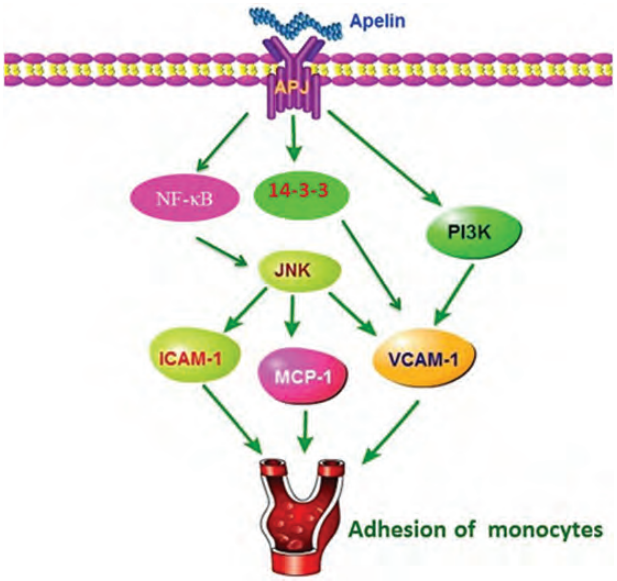

Figure 1 Apelin induces adhesion of monocytes by inducing the expression of adhesion molecules and chemokines

14-3-3 inhibitor difopein significantly reduced the expression of 14-3-3 and VCAM-1 in apelin-13-stimulated HUVECs, and extremely inhibited the effect of apelin-13 on induction of MCs adhesion to HUVECs [24]. The apelin/APJ system in endothelial cells is involved in the expression of adhesion molecules and chemokines, which is essential for the initiation of endothelial inflammation-related AS. Therefore, apelin/APJ and the cell signaling pathways activated by this system in endothelial cells may be potential targets for therapy of AS (**Fig. 1**).

Apelin Promotes VSMC Proliferation

Atherosclerotic plaques stimulate proliferation of vascular smooth muscle cells (VSMCs) that aggravated vascular stenosis. Li *et al*. [25] confirmed that apelin-13 stimulated VSMC proliferation in a concentration-dependent manner by promoting the cells from G0/G1 phase to S phase for the first time. By *in vitro* analysis, it was found that the expression of cyclin D1 and activation of pERK1/2 induced by apelin-13 can be inhibited by ERK1/2 inhibitor PD98059, which leads to an inhibition of VSMC proliferation stimulated by apelin-13 [26]. Our lab summarized that apelin-13 could stimulate the proliferation of VSMCs and the effect was potentially mediated by apelin/APJ-pERK1/2-cyclin D1 signal cascades. In 2010, we showed that apelin-13 promoted the phosphorylation of PI3K in dose- and time-dependent manners. LY294002 significantly decreased the expression of phospho-PI3K, phospho-Akt, phospho-ERK1/2, and cyclin D1 induced by apelin-13. The Akt inhibitor 1701-1 significantly diminished the expression of phospho-Akt, phospho-ERK1/2, and cyclin D1 stimulated by apelin-13 [27]. Subsequent researches found that 14-3-3 participated in the above signal pathway of cell proliferation regulation because 14-3-3 inhibitor difopein inhibited the VSMC proliferation induced by apelin. It is believed that 14-3-3 protein combines with Raf-1 that can stabilize rather than activate Raf-1. Our results showed that apelin promoted 14-3-3 expression and its combination with Raf-1. Apelin can also induce the phosphorylation of Raf-1 that is inhibited by 14-3-3 inhibitor difopein [28]. This indicated that 14-3-3 is involved in the activity of Raf-1. However, the effect of Raf-1 in the form of monomer or in complexes with 14-3-3 also requires to be clarified by more evidence. Futhermore, we also found that difopein decrease the phosphorylation of ERK and expression of cyclin D1. In addition, apelin-13 promotes the proliferation of VSMCs by down-regulating caveolin-1 expression. Caveolae may likewise participate in the above mentioned pathways, because apelin-13 induce the dissociation of PI3K and ERK1/2 with caveolin-1 in VSMCs [29]. Although these phenomena are observed in normal rat VSMCs, we believe that apelin maintains its effects in human AS. Hashimoto *et al*. [12] found that vascular superoxide radicals and nicotinamide-adenine dinucleotide phosphate oxidase subunits were decreased in $APJ^{-/-}ApoE^{-/-}$ mice compared with $APJ^{+/+}ApoE^{-/-}$ mice fed a standard normal diet. In VSMCs, apelin induced nicotinamide-adenine dinucleotide phosphate oxidase subunit expression. Apelin also induced VSMC proliferation, which was inhibited by superoxide dismutase or diphenylene iodonium. The apelin/APJ system is a mediator of oxidative stress in vascular tissue, thus we propose that APJ may be a critical factor in AS under high-cholesterol dietary conditions. Our results further reveal the precise cellular mechanisms responsible for VSMC proliferation induced by apelin-13 [30]. Apelin-13 treatment increased the expression of NADPH oxidase 4 in a dose-dependent manner. Down-regulation of NADPH oxidase 4 using siRNA prevented apelin-13-induced ROS generation, phosphorylation of ERK, and VSMC proliferation. APJ deficiency or inhibition may be preventative against oxidative stress-related AS. Although the role as well as the mechanism of apelin on VSMC proliferation has been found *in vitro*, it still lacks straightforward clinical data. The *in vivo* experiments are necessary to further clarify the effect of apelin on VSMC proliferation (**Fig. 2**).

Apelin, An Important Angiogenesis Factor

For vessel intimal lack of oxygen due to atherosclerotic plaque formation caused compensatory angiogenesis, abundant macrophages and T lymphocytes can permeate into lesions *via* the neovascular. Activation of macrophages can secrete matrix metalloproteinase that undermines the stability of atherosclerotic plaque and accelerates

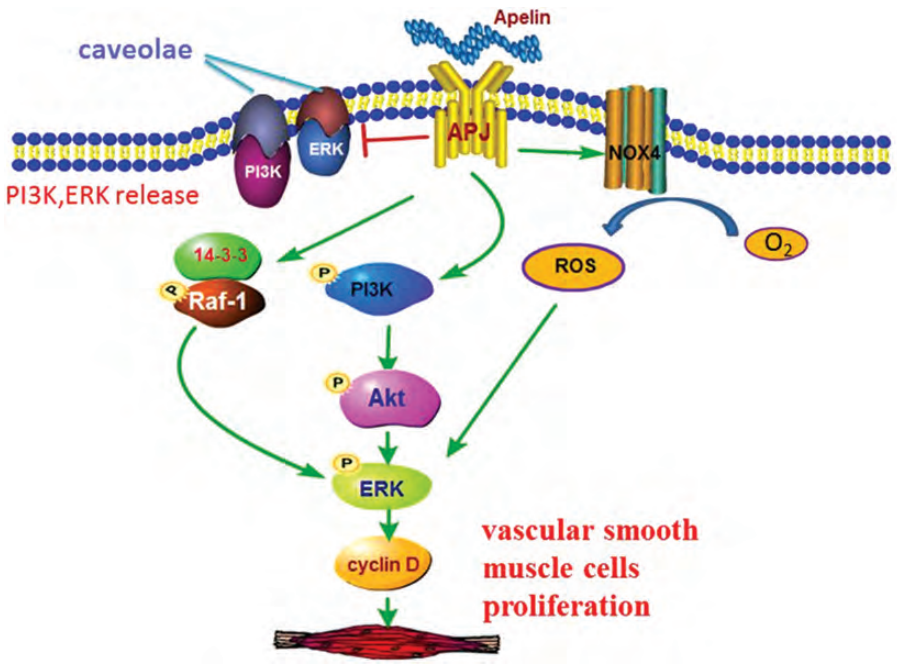

Figure 2 Schematic diagram of intracellular signal transduction pathways and effects on the vascular smooth muscle cells proliferation in apelin/APJ system

atherosclerotic plaque rupture. Neovascularization in the atherosclerotic plaque may be a core event and play a critical role in AS. Studies showed that apelin promotes angiogenesis, lymphangiogenesis and tumor growth, indicating that apelin is an angiogenic factor.

Apelin promotes angiogenesis

Either endogenous or exogenous apelin shows a role in promoting angiogenes. Kunduzova *et al*. [31] confirmed that apelin/APJ signaling pathways play a critical role in the development of the functional vascular network in adipose tissue. Epididymal white adipose tissue (EWAT) transplantation was performed as a model of adipose tissue angiogenesis. Transplantation leads to the increased apelin mRNA levels 2 and 5 days after transplantation associated with tissue hypoxia, as evidenced by hydroxyprobe staining on tissue sections. Graft revascularization evolved in parallel, as the first functional vessels in EWAT grafts were observed in 2 days after transplantation and a strong angiogenic response was apparent in 14 days. Prophylactic treatment with apelin improved alveolarization and angiogenesis, increased lung cGMP levels in neonatal rats with hyperoxia-induced lung injury. In the injury-recovery model, apelin also improved angiogenesis [32]. Injection of apelin in the vitreous induces the sprouting and the proliferation of endothelial cells from the retinal vascular network [33]. Increasing apelin levels by gene transfer to non-small cell lung cancer cells significantly stimulated tumor growth and microvessel densities and perimeters *in vivo* [34].

Inhibition of apelin/APJ causes angiogenesis decrease

Retinal angiogenesis in the oxygen-induced retinopathy model was rarely observed in apelin-deficient mice. In addition, clinical study showed that vitreous concentrations of apelin were significantly higher in the proliferative diabetic retinopathy group than in the control group [35]. It suggested that apelin plays an important role in proliferative diabetic retinopathy, which involves in an angiogenesis mechanism. Knockout mice and zebrafish morpholino knockdown of apelin showed delayed angiogenesis [36]. In addition, the application of apelin or APJ inhibitors can inhibit angiogenesis. Apelin and its receptor APJ was overexpressed in the splanchnic vasculature of portal hypertensive rats. APJ inhibitor F13A effectively decreased, by 52%, splanchnic neovascularization and expression of proangiogenic factors, vascular endothelial growth factor (VEGF), platelet-derived growth factor, and angiopoietin-2 in portal hypertensive rats. F13A also reduced, by 35%, the formation of portosystemic collateral vessels [37]. Rats with cirrhosis treated with the apelin receptor antagonist showed diminished hepatic fibrosis and vessel density [38].

The mechanism of apelin in angiogenesis

Although the role of apelin in angiogenesis was determined, the mechanism of apelin promoting angiogenesis is incompletely understood. Li *et al.* [39] found that treatment with apelin-13 promoted myocardial angiogenesis and attenuated cardiac fibrosis and hypertrophy together with a significant improvement of cardiac function at 14 days postmyocardial infarction (post-MI). Apelin-13 increases angiogenesis and

improves cardiac repair post-MI by a mechanism involving the upregulation of SDF-1α/CXCR-4 and homing of vascular progenitor cells. Augmented $11\beta HSD1^{-/-}$ adipose tissue angiogenesis is associated with enhanced peroxisome proliferator-activated receptor gamma-inducible expression of the potent angiogenic factors, VEGF-A and apelin [40]. Kasai *et al.* [41] confirmed that apelin is a prerequisite factor for hypoxia-induced retinal angiogenesis. Preventing the new blood vessel forming can inhibit tumor growth or spread. The angiogenesis inhibitor bevacizumab has been applied in clinic for cancer therapy. Inhibition of angiogenesis is an excellent way for the treatment of cancer, angiogenesis inhibition may represent a new and effective strategy for the treatment of AS. Inhibitor of APJ, new anti-angiogenesis drug may be more effective to reverse AS.

Apelin and Inflammation

Adipocytokines secreted by adipose tissue play an important role in inflammation that is considered to be a crucial step in the pathogenesis of AS. Apelin is a peptide with relevant functions in inflammation. However, the correlation of apelin and inflammation is still not fully understood in AS. In experimental models without AS, apelin seems to be an anti-inflammatory cytokine. It suggested that apelin may possess an anti-AS effect. In end-stage heart failure, Pyr-AP13 effectively suppressed the expression of inflammation factors such as tumor necrosis factor-alpha and interleukin (IL)-1β protein [42]. Apelin treatment was associated with significantly reduced aortic macrophage colony-stimulating factor expression and decreased MCP-1, macrophage inflammatory protein-1α, IL-6, and tumor necrosis factor (TNF)-α mRNA levels in the elastase model of human abdominal aortic aneurysm disease mice [43]. Endogenous apelin is required for the suppression of inflammation-induced vascular hyperpermeability. Apelin inhibited the down-modulation of vascular endothelial-cadherin by VEGF, resulting in suppression of hyperpermeability [44]. In non-circulation system diseases, apelin can also suppress the generation of inflammation. Prophylactic treatment with apelin improved alveolarization and angiogenesis, increased lung cGMP levels, and reduced inflammation, arteriolar wall thickness, and right ventricular hypertrophy in neonatal rats with hyperoxia-induced lung injury [32]. However, apelin can promote inflammation in patients. Apelin is significantly correlated with *in vivo* TNF-α and MAP in patients with the metabolic syndrome after a diet-induced weight loss [45]. Notably, a recent study found that there was no correlation among apelin-12 or -36 and inflammatory or oxidative markers in hemodialysis patients [46], suggesting a possible association between apelin and inflammation. But further study is needed to understand the role of other apelin subtype such as apelin-13. In the human body, the effect of apelin on inflammation may be more complex compared with *in vitro* activation. If the new finding was convinced, apelin might play pro-inflammatory role in human AS.

Concluding Remarks

Apelin has been known as a novel adipokine that has a close relation with AS confirmed by increasing evidence. In atherosclerotic diseases including hypertension, diabetes and stroke, apelin has also been proved to play an important pathological role. However, studies barely showed the surface of the research on the relationship between apelin and AS. There are still many questions to be answered. The role of apelin in AS in some literatures mentioned above is still not convinced yet. Is the high expression of apelin in AS patients the pathogen or the consequence? Apelin levels in serum are not associated with early AS, which suggested that overexpression of apelin is the result rather than cause of AS. However, some results show that apelin is negatively correlated with ox-LDL. We predict that apelin may not be a determinant but an important predisposing factor in AS. Sustained preeminent expression of apelin promotes the occurrence of AS rather than a compensatory mechanism. Apelin secretion is decreased when the deterioration secretory function of endothelial cell is compromised. Apelin may be a candidate for the treatment of AS. Considering the role of promoting MCs adhesion, VSMC proliferation, and angiogenesis, apelin/APJ may be more effective as a therapeutic target. So, more experiments directly in AS model instead of atherosclerotic dangerous situations or hallmarks are essential to reveal the substantial role as well as the pathogenesis of apelin in AS. The role of APJ in AS should be studied separately too. Because Pitkin *et al*. [8] found that apelin receptor density was significantly reduced in left ventricle from patients with dilated cardiomyopathy or ischemic heart disease, but apelin peptide levels remained unchanged. APJ might not be always 'loyal' to apelin that also existed in AS. Furthermore, the based potent small molecule APJ functional agonist had been synthesized [47], which may be used as a compound to synthesize apelin/APJ system related anti-AS drug in the future.

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References

1 O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL and Shi X, *et al*. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. Gene 1993, 136: 355–360.

2 Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX and Kawamata Y, *et al*. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998, 251: 471–476.

3 Rittig K, Hildebrandt U, Thamer C, Staiger H, Peter A, Stefan N and Fritsche A, *et al*. Apelin serum levels are not associated with early atherosclerosis or fat distribution in young subjects with increased risk for type 2 diabetes. Exp Clin Endocrinol Diabetes 2011, 119: 358–361.

4 Tasci I, Dogru T, Naharci I, Erdem G, Yilmaz MI, Sonmez A and Bingol N, *et al*. Plasma apelin is lower in patients with elevated LDL-cholesterol. Exp Clin Endocrinol Diabetes2007, 115: 428–432.

5 Kourtis A, Gkiomisi A, Mouzaki M, Makedou K, Anastasilakis AD, Toulis KA and Gerou S, *et al*. Apelin levels in normal pregnancy. Clin Endocrinol 2011, 75: 367–371.

6 Tasci I, Erdem G, Ozgur G, Tapan S, Dogru T, Genc H and Acikel C, *et al*. LDL-cholesterol lowering increases plasma apelin in isolated hypercholesterolemia. Atherosclerosis 2009, 204: 222–228.

7 Kadoglou NP, Sailer N, Moumtzouoglou A, Kapelouzou A, Gerasimidis T, Kostakis A and Liapis CD. Adipokines: a novel link between adiposity and carotid plaque vulnerability. Eur J Clin Invest 2012, 42: 1278–1286.

8 Pitkin SL, Maguire JJ, Kuc RE and Davenport AP. Modulation of the apelin/APJ system in heart failure and atherosclerosis in man. Br J Pharmacol 2010, 160: 1785–1795.

9 Peltonen T, Napankangas J, Vuolteenaho O, Ohtonen P, Soini Y, Juvonen T and Satta J, *et al*. Apelin and its receptor APJ in human aortic valve stenosis. J Heart Valve Dis 2009, 18: 644–652.

10 Fukushima H, Kobayashi N, Takeshima H, Koguchi W and Ishimitsu T. Effects of olmesartan on Apelin/APJ and Akt/endothelial nitric oxide synthase pathway in Dahl rats with end-stage heart failure. J Cardiovasc Pharmacol 2010, 55: 83–88.

11 Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R and Zheng L, *et al*. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. J Clin Invest 2008, 118: 3343–3354.

12 Hashimoto T, Kihara M, Imai N, Yoshida S, Shimoyamada H, Yasuzaki H and Ishida J, *et al*. Requirement of apelin-apelin receptor system for oxidative stress-linked atherosclerosis. Am J Pathol 2007, 171: 1705–1712.

13 Than A, Cheng Y, Foh LC, Leow MK, Lim SC, Chuah YJ and Kang Y, *et al*. Apelin inhibits adipogenesis and lipolysis through distinct molecular pathways. Mol Cell Endocrinol 2012, 362: 227–241.

14 Kadoglou NP, Vrabas IS, Kapelouzou A, Lampropoulos S, Sailer N, Kostakis A and Liapis CD, *et al*. The impact of aerobic exercise training on novel adipokines, apelin and ghrelin, in patients with type 2 diabetes. Med Sci Monit 2012, 18: CR290–CR295.

15 Paternain L, de la Garza AL, Batlle MA, Milagro FI, Martinez JA and Campion J. Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner. Stress 2013, 16: 220–232.

16 Meral C, Tascilar E, Karademir F, Tanju IA, Cekmez F, Ipcioglu OM and Ercin CN, *et al*. Elevated plasma levels of apelin in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2010, 23: 497–502.

17 Kadoglou NP, Tsanikidis H, Kapelouzou A, Vrabas I, Vitta I, Karayannacos PE and Liapis CD, *et al*. Effects of rosiglitazone and metformin treatment on apelin, visfatin, and ghrelin levels in patients with type 2 diabetes mellitus. Metabolism 2010, 59: 373–379.

18 Yu S, Zhang Y, Li MZ, Xu H, Wang Q, Song J and Lin P, *et al*. Chemerin and apelin are positively correlated with inflammation in obese type 2 diabetic patients. Chin Med J 2012, 125: 3440–3444.

19 Attane C, Foussal C, Le Gonidec S, Benani A, Daviaud D, Wanecq E and Guzman-Ruiz R, *et al*. Apelin treatment increases complete fatty acid oxidation, mitochondrial oxidative capacity, and biogenesis in muscle of insulin-resistant mice. Diabetes 2012, 61: 310–320.

20 Karbowska A, Boratynska M and Klinger M. Resistin: a pathogenic factor or a biomarker of metabolic disorders and inflammation? Postepy Hig Med Dosw (Online) 2009, 63: 485–491.

21 Hung WW, Hsieh TJ, Lin T, Chou PC, Hsiao PJ, Lin KD and Shin SJ. Blockade of the renin-angiotensin system ameliorates apelin production in 3T3-L1 adipocytes. Cardiovasc Drugs Ther 2011, 25: 3–12.

22 Mao X, Su T, Zhang X, Li F, Qin X, Li X and Liao D. Apelin-13 promote monocytes adhesion to HUVECs via PI3K signaling. Prog Biochem Biophys 2011, 38: 1162–1170.

23 Lu Y, Zhu X, Liang GX, Cui RR, Liu Y, Wu SS and Liang QH, *et al*. Apelin-APJ induces ICAM-1, VCAM-1 and MCP-1 expression via NF-kappaB/JNK signal pathway in human umbilical vein endothelial cells. Amino Acids 2012; 43: 2125–2236.

24 Li X, Zhang X, Li F, Chen L, Li L, Qin X and Gao J, *et al*. 14-3-3 mediates apelin-13-induced enhancement of adhesion of monocytes to human umbilical vein endothelial cells. Acta Biochim Biophys Sin 2010, 42: 403–409.

25 Li F, Li LF, Qin XP, Pan WN, Feng F, Chen F and Zhu BY, *et al*. The study of vascular smooth muscle cells proliferation stimulated by apelin-13. Chin Pharmacol Bull 2007, 23: 949–953.

26 Li F, Li L, Qin X, Pan W, Feng F, Chen F and Zhu B, *et al*. Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1. Front Biosci 2008, 13: 3786–3792.

27 Liu C, Su T, Li F, Li L, Qin X, Pan W and Feng F, *et al*. PI3K/Akt signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by apelin-13. Acta Biochim Biophys Sini 2010, 42: 396–402.

28 Pan W, Li F, Mao X, Qin X, Deng Y, Feng F and Chen F, *et al*. 14-3-3 protein is involved in ERK1/2 signaling transduction pathway of rat vascular smooth muscle cells proliferation induced by apelin-13. Prog Biochem Biophys 2011, 38: 1153–1161.

29 Mao X, Li L, Gao J, Yang L, Liu W, Qin X and Chen L. Apelin-13 promotes proliferation of vascular smooth muscle cells by down regulating caveolae expression. J Clin Rehabil Tissue Eng Res 2011, 15: 8603–8608.

30 Li L, Li F and Li F. NOX4-derived reactive oxygen species drive apelin-13-induced vascular smooth muscle cell proliferation via the ERK pathway. Int J Pept Res Ther 2011, 17: 307–315.

31 Kunduzova O, Alet N, Delesque-Touchard N, Millet L, Castan-Laurell I, Muller C and Dray C, *et al*. Apelin/APJ signaling system: a potential link between adipose tissue and endothelial angiogenic processes. FASEB J 2008, 22: 4146–4153.

32 Visser YP, Walther FJ, Laghmani el H, Laarse A and Wagenaar GT. Apelin attenuates hyperoxic lung and heart injury in neonatal rats. Am J Respir Crit Care Med 2010, 182: 1239–1250.

33 Masri B, van den Berghe L, Sorli C, Knibiehler B and Audigier Y. Apelin signalisation and vascular physiopathology. J Soc Biol 2009, 203: 171–179.

34 Berta J, Kenessey I, Dobos J, Tovari J, Klepetko W, Jan Ankersmit H and Hegedus B, *et al*. Apelin expression in human non-small cell lung cancer: role in angiogenesis and prognosis. J Thorac Oncol 2010, 5: 1120–1129.

35 Kasai A. Pathological role of apelin in angiogenic eye disease. Yakugaku Zasshi 2011, 131: 1201–1206.

36 del Toro R, Prahst C, Mathivet T, Siegfried G, Kaminker JS, Larrivee B and Breant C, *et al*. Identification and functional analysis of endothelial tip cell-enriched genes. Blood 2010, 116: 4025–4033.

37 Tiani C, Garcia-Pras E, Mejias M, de Gottardi A, Berzigotti A, Bosch J and Fernandez M. Apelin signaling modulates splanchnic angiogenesis and

portosystemic collateral vessel formation in rats with portal hypertension. J hepatol 2009, 50: 296–305.

38 Principe A, Melgar-Lesmes P, Fernandez-Varo G, del Arbol LR, Ros J, Morales-Ruiz M and Bernardi M, *et al*. The hepatic apelin system: a new therapeutic target for liver disease. Hepatol 2008, 48: 1193–1201.

39 Li L, Zeng H and Chen JX. Apelin-13 increases myocardial progenitor cells and improves repair postmyocardial infarction. Am J Physiol Heart Circ Physiol 2012, 303: H605–H618.

40 Michailidou Z, Turban S, Miller E, Zou X, Schrader J, Ratcliffe PJ and Hadoke PW, *et al*. Increased angiogenesis protects against adipose hypoxia and fibrosis in metabolic disease-resistant 11beta-hydroxysteroid dehydrogenase type 1 (HSD1)-deficient mice. J Biol Chem 2012, 287: 4188–4197.

41 Kasai A, Ishimaru Y, Kinjo T, Satooka T, Matsumoto N, Yoshioka Y and Yamamuro A, *et al*. Apelin is a crucial factor for hypoxia-induced retinal angiogenesis. Arterioscler Thromb Vasc Biol 2010, 30: 2182–2187.

42 Koguchi W, Kobayashi N, Takeshima H, Ishikawa M, Sugiyama F and Ishimitsu T. Cardioprotective effect of apelin-13 on cardiac performance and remodeling in end-stage heart failure. Circulation 2012, 76: 137–144.

43 Leeper NJ, Tedesco MM, Kojima Y, Schultz GM, Kundu RK, Ashley EA and Tsao PS, *et al*. Apelin prevents aortic aneurysm formation by inhibiting macrophage inflammation. Am J Physiol Heart Circ Physiol 2009, 296: H1329–H1335.

44 Kidoya H, Naito H and Takakura N. Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. Blood 2010, 115: 3166–3174.

45 Heinonen MV, Laaksonen DE, Karhu T, Karhunen L, Laitinen T, Kainulainen S and Rissanen A, *et al*. Effect of diet-induced weight loss on plasma apelin and cytokine levels in individuals with the metabolic syndrome. Nutr Metab Cardiovasc Dis 2009, 19: 626–633.

46 Leal VO, Lobo JC, Stockler-Pinto MB, Farage NE, Calixto A, Geloneze B and Mafra D. Apelin: a peptide involved in cardiovascular risk in hemodialysis patients? Ren Fail 2012, 34: 577–581.

47 Khan P, Maloney PR, Hedrick M, Gosalia P, Milewski M, Li L and Roth GP, *et al*. Functional agonists of the apelin (APJ) receptor. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD), 2010.