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

API, 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 adipoin- sular 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
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 aggravate 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 protein participates 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. Furthermore, 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+/+ APOE2/2 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
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 angiogenesis. 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

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Figure 2 Schematic diagram of intracellular signal transduction pathways and effects on the vascular smooth muscle cells proliferation in apelin/APJ system
improves cardiac repair post-MI by a mechanism involving the upregulation of SDF-1α/CXCR-4 and homing of vascular progenitor cells. Augmented 11βHSD1<sup>−/-</sup> 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 inhibits 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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