SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Diabetic retinopathy special edition
• RESEARCH PAPER •

June 2015 Vol.58 No.6: 531–540
doi: 10.1007/s11427-015-4861-0

Apelin activates the expression of inflammatory cytokines in microglial BV2 cells via PI-3K/Akt and MEK/Erk pathways

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Received December 9, 2014; accepted March 2, 2015; published online May 6, 2015

This paper aims to observe the changes of the inflammatory cytokines in microglial BV2 cells stimulated by apelin, and investigate the mechanism of inflammatory cytokines secretion after apelin stimulation. Immunofluorescence and quantitative real-time PCR were performed to observe expression of TNF-α, IL-1β, IL-10, MIP-1α, and MCP-1 in BV2 cells. Western blot was used to investigate the expression of phosphorylation PI-3K/Akt and phosphorylation Erk signaling pathways in BV2 cells after stimulation by apelin. Furthermore, PI-3K/Akt inhibitor (LY294402) and Erk inhibitor (U0126) were used as antagonists to detect the secretion mechanisms of cytokines in BV2 cells stimulated by apelin. Exogenous recombinant apelin activated the expression of TNF-α, IL-1β, MCP-1 and MIP-1α in BV2 cells by the detection of fluorescence expression and mRNA. Apelin also unregulated the protein expression of p-PI-3K/Akt and p-Erk in BV2 cells induced by apelin. LY294002 and U0126 inhibited activation of p-PI-3K/Akt and p-Erk expression by Western blot and attenuated the expression of inflammation factors in BV2 cells by fluorescence staining. This study demonstrates that apelin is a potential activator of inflammation factors through the PI3K/Akt and Erk signaling pathway and is potential therapeutically relevant to inflammatory responses of microglia cells.

apelin, inflammation cytokines, BV2, PI-3K/Akt, MEK/Erk

Citation: Chen L, Tao Y, Jiang YR. Apelin activates the expression of inflammatory cytokines in microglial BV2 cells via PI-3K/Akt and MEK/Erk pathways. Sci China Life Sci, 2015, 58: 531−540, doi: 10.1007/s11427-015-4861-0

Apelin is a small molecular regulatory peptide, whose biological activity is mediated by its specific receptors named APJ, a G-protein-coupled receptor with seven transmembrane domains [1]. Apelin/APJ is expressed in multiple tissues, primarily in vascular endothelial cells, suggesting it plays an important role in angiogenesis [2,3]. During recent years, apelin has gained increasing attention in the function of angiogenesis, especially in retinal neovascularization. Kasai and others' studies [4,5] found that apelin is a crucial factor for retinal angiogenesis in prematurity of retinopathy (ROP) mice and apelin-deficiency can retard the retinal vascular development. In *in vivo* study, the sizes of the laser induced CNV lesions in apelin-KO or APJ-KO mice decreased significantly compared with those in the WT mice [6]. Our previous study also found plasma level of apelin was increased in proliferative diabetic retinopathy (PDR) patients [7]. However, the role of apelin for regulation of inflammation factors in *in vivo* and *in vitro* study is unclear. Han et al. [8] demonstrated that LPS, IL-6, or interferon-α (IFN-α) treatment can induce the increasing enteric apelin expression in rodents, suggesting a potential link between apelin and inflammation.

Microglial cells are blood-derived phagocytes, representing one major component of the innate immune system

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in the retina [9]. Studies have demonstrated that microglial cells are involved in phagocytosis, removal of apoptotic neuronal remnants and involved in remodeling [10]. Furthermore, when they are over-activated, microglial cells release soluble cytotoxins, such as tumor necrosis factor-alpha (TNF-α), interleukin1β (IL-1β), and IL-6 that can contribute to neuronal and vascular cell death and ultimately the progression of diabetic retinopathy (DR) [11]. In people with non-proliferative DR and proliferative DR, microglia largely accumulate around regions of vascular damage and around sites of neovascularisation [12]. These results indicate that activated microglia is a major cellular source of inflammatory and/or cytotoxic factors that cause retinal damage in DR. Therefore, microglial participation has been implicated in the pathogenesis of DR.

MAPKs are the most important signaling molecules with involvement in activated microglia and also appear to play key roles in inflammatory processes [13,14]. Previous studies have shown that activation of MAPKs has a significant effect on the regulation of cyclooxygenase (COX-2), inducible nitric oxide synthase (iNOS), and inflammatory cytokine gene expression in microglia [15]. Park et al. [16] also indicated that fucoidan treatment significantly attenuated expression of iNOS, COX-2, MCP-1, IL-1β and TNF-α in LPS-stimulated BV2 microglia through down-regulation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and AKT pathways.

However, the role and the mechanisms of apelin and its receptor signaling for expression of inflammatory factors in microglia BV2 cells remain unclear. Based on previous observations, the present study was designed to investigate whether apelin could effectively regulate the pro- and anti-inflammatory factors in BV2 cells and to study the mechanism of apelin on the expression of inflammatory cytokines in BV2 cells.

1 Materials and methods

1.1 Cell culture and treatment

BV2, a murine microglial cell line, which is a suitable model for *in vitro* study of microglia, was used in this study. BV2 cells were cultured at 37°C in 5% CO_2 in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 5 mmol L^{-1} glucose and 1% penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}) (Gibco, USA). Exogenous recombinant apelin-13 (100 ng mL^{-1}) (Sigma, USA) stimulated BV2 cells for 30 min, 1, 3 and 6 h. BV2 cells were pretreated with the indicated concentrations of PI-3K inhibitor LY294002 (10 μmol, Selleckchem, USA) and Erk inhibitor U0126 (10 μmol, Sigma, USA) 1 h before incubated with apelin-13 (100 ng mL^{-1}).

1.2 Immunofluorescence staining

Immunofluorescence analysis was performed using cell coverslip. Briefly, the cover slip was fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton-x 100 for 10 min, and blocked with 2% bovine serum albumin (BSA) at room temperature for 1 h, and then incubated with primary antibodies monocyte chemoattractant protein (MCP-1) (MABN712, 1:200, Millipore, Germany), macrophage inflammatory protein-1α (MIP-1α) (MAB66251, 1:100, RD Biosciences, USA), TNF-α (SC1069, 1:100, Sant Crus, USA), IL-1β (ab9722, 1:200, Abcam, USA), and IL-10 (ab33471, 1:300, Abcam, USA) overnight. Appropriate secondary antibodies were used by FITC-conjugation (goat anti mouse, 1:200, Invitrogen, USA; rabbit anti rat, 1:20, DAKO, Japan; chicken anti goat, 1:200, Invitrogen; chicken anti rabbit, 1:200, Invitrogen; rabbit anti rat, 1:20, DAKO; respectively). The coverslips were counterstained with Toto3 (1:500, Invitrogen, USA) for nucleus. Slides were examined by confocal microscopy (Leica TCS SP2, Germany) and images were analyzed by F'cell software.

1.3 Quantitative real time PCR

Total RNA was extracted from BV2 cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentrations of RNA were measured with Tecan RNA-nano plates (Tecan, Switzerland) and Icontrol software. 1 μg RNA was reverse-transcribed using QuantiTect Rev Transcription Kit (Qiagen, Germany). Then, cDNA was amplified by PCR. The PCR solution contained 2 μL specific primers (100 pmol L^{-1} each), 10 μL SYBR Green Master Mix (Eurogentech, Germany) and 2 μL cDNA with a final volume of 20 μL. PCR primers were as follows: MCP-1 forward: CAAGAAGGAATGGGTCCAGA, reverse: GCTGAAGACCTTAGGGCAGA; MIP-α forward: TATTTTGAAACCAGCAGCCTTT, reverse: ATTCTTGGACCCAGGTCTCTTT; IL-1β forward: TCTTCCTAAAGTATGGGCTGGA, reverse: GACTAAGGAGTCCCCTGGAGAT; β-actin forward: CACTGCAAACGGGGAAATGG, reverse: TGAGATGGACTGTCGGATGG. The reaction conditions for amplifying DNA were 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 58°C for 1 min, and 72°C for 1 min. The last step was 95°C for 1 min, 55°C for 30 s and 95°C for 30 s. The mRNA expression was normalized to the expression level of β-actin and was calculated using the following equation: fold change=$2^{-\Delta\Delta C_T}$. Each experiment was repeated at three times.

1.4 Western blot

Briefly, cells were harvested, washed once with ice-cold PBS and gently lysed for 30 min in 100 μL ice-cold 0.1% SDS lysis buffer including 3 μL mini cocktail (Roche, Ger-

many) and 2 μL orthovanadate. Protein concentrations determined using a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). Samples were analyzed on 10% SDS-PAGE gels, transferred to PVDF membranes (Millipore, Billerica, MA, USA), and processed for analysis using an enhanced chemiluminescence substrate (PerkinElmer, Inc., MA, USA). The primary antibodies were used at the following dilutions: anti-p-PI-3K (rabbit monoclonal, CST4228, 1:1,000), anti-PI-3K (mouse monoclonal, ab22653, 1:1,000); anti-p-Akt (rabbit monoclonal, CST4060, 1:1,000), anti-Akt (rabbit monoclonal, CST4691, 1:1,000); anti-p-Erk (mouse monoclonal, ab50011, 1:5,000), anti-Erk (Rabbit polyclonal, ab17942, 1:1,000), GAPDH (mouse monoclonal, ab9484, 1:5,000)

1.5 Statistics

The results were expressed as mean±SEM. Differences among groups were assessed using one-way analysis of variance (ANOVA) (GraphPad Prism 5.0), followed by Dunnett's test. A value of $P<0.05$ was considered as significantly different. We repeated all experiments at least three times, and representative experiments are shown.

2 Results

2.1 APJ is expressed in BV2 cells

Prior to exploring the effects of apelin in microglial BV2 cells, we performed immunofluorescence staining to examine the expression of APJ in BV2 cells. The results showed that expression of APJ staining was positive, which was localized in cytoplasm and cytomembrane in BV2 cells (Figure 1).

2.2 Apelin up-regulates the expression of inflammatory cytokines in BV2 cells

The expression of IL-1β, TNF-α, MCP-1, MIP-1α and IL-10 were investigated by immunofluorescence staining in microglial BV2 cells. After the microglial BV2 cells incubation with apelin, the result showed that IL-1β, TNF-α, MCP-1, MIP-1α were up-regulated, but IL-10 was down-regulated (Figure 2). Compared with the control group, the expression of MCP-1 was up-regulated by 188.3%±26.4%, MIP-1α by 88.1%±36.2%, IL-1β by 159.0%±38.9%, and TNF-α by 39.3%±5.5%, respectively. In contrast, the expression of IL-10 was decreased by 39.4%±15.1%.

2.3 Apelin increases the expression of mRNA of inflammatory cytokines in BV2 cells

Given the fact that inflammatory cytokines and chemokines play a significant role in the progression of inflammation, we then sought to determine whether apelin could regulate transcription of mRNA of these factors in BV2 cells. Microglial BV2 cells were cultured in 10% FBS RPMI 1640 medium in supplemented with 100 ng mL^{-1} apelin for various periods of times. Quantitative real-time PCR data revealed that compared with the basal level, mRNA expression began to be enhanced under stimulation of apelin (Figure 3). Among them, the mRNA expression of TNF-α began to rise at 1 h and reached a peak at 3 h by 13.84 folds ($P<0.01$). The mRNA expression of IL-1β was up-regulated by 10.38 folds at 1 h ($P<0.001$) and by 6.78 folds at 3 h ($P<0.01$). The mRNA expression of MCP-1 increased by 5.57 folds at 1 h and by 10.76 folds at 3 h ($P<0.01$). The mRNA expression of MIP-1α only increased by 3.75 folds at 3 h ($P<0.05$).

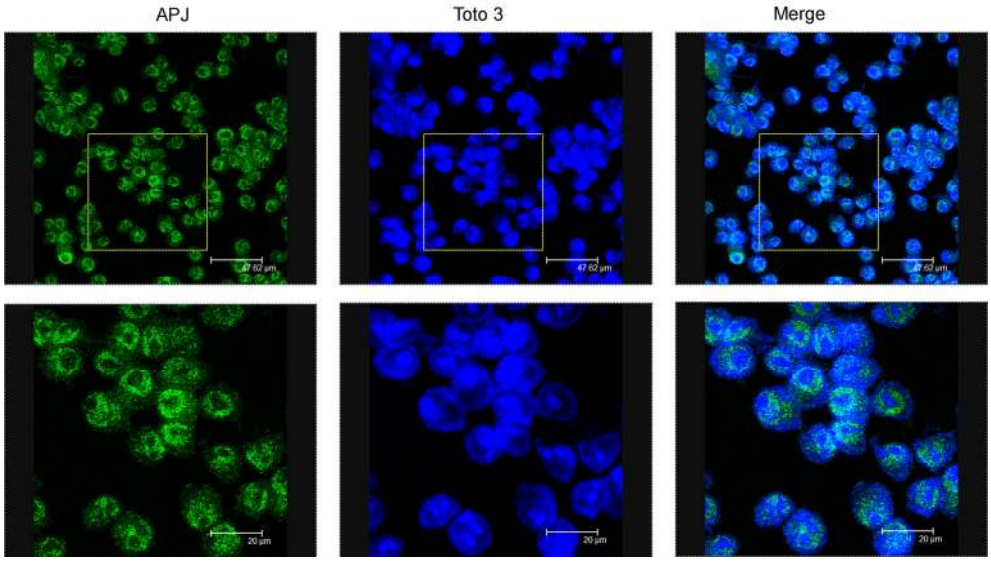

Figure 1 Localization of APJ in BV2 cells was visualized with fluorescence microscopy after immunofluorescence staining with Rabbit polyclone anti-APJ antibody (green). Cells were stained with Toto3 for visualization of nuclei (blue). The cellular localization of APJ was the cytoplasm.

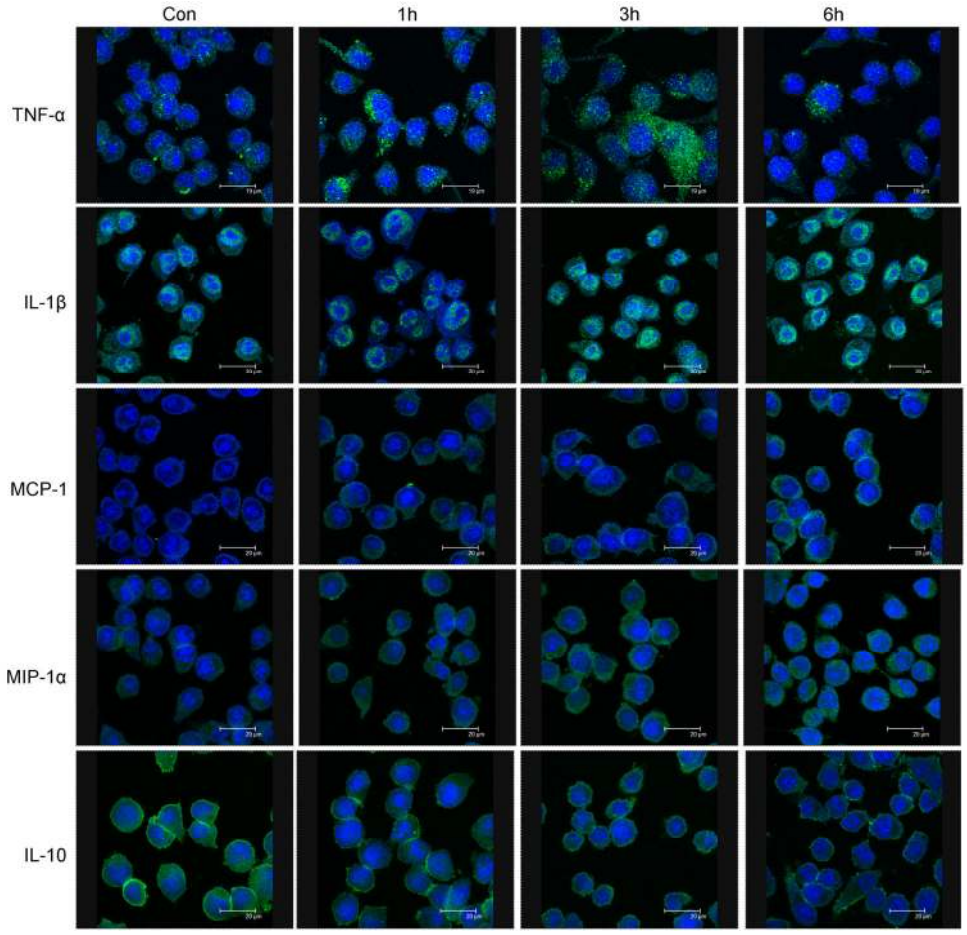

Figure 2 Immunofluorescent staining of inflammation cytokines in BV2 cells. Exogenous recombinant apelin-13 (100 ng mL^{-1}) stimulated BV2 cells for 1, 3 and 6 h. The expression of MCP-1, MIP-1α, IL-1β, and TNF-α were upregulated. The expression of IL-10 was decreased.

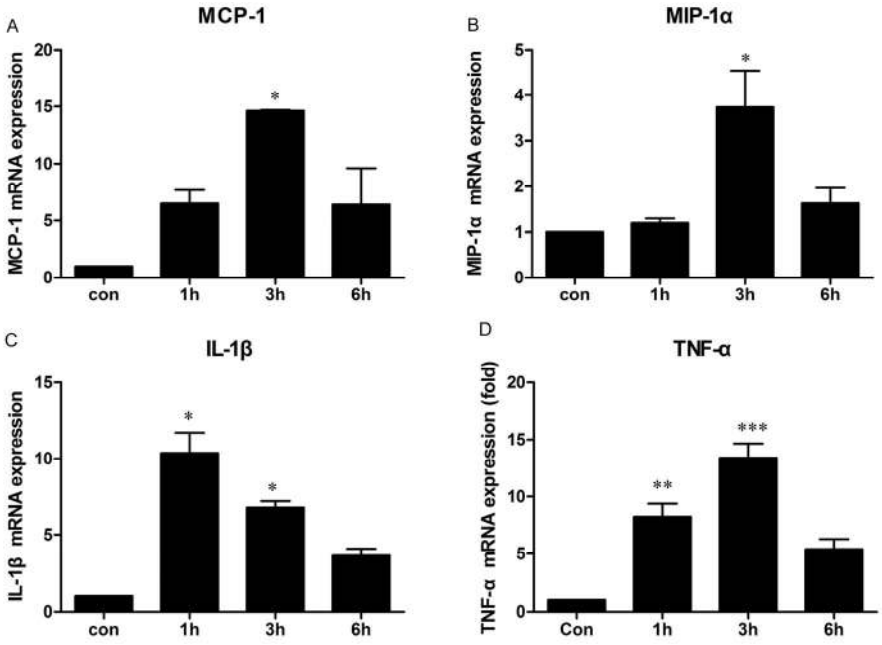

Figure 3 The mRNA expression of inflammation cytokines in BV2 cells by quantitative real time PCR. A, The mRNA expression of MCP-1 increased by 10.76 fold at 3 h (***P*<0.01 vs. con group). B, The mRNA expression of MIP-1α only increased by 3.75 fold at 3 h (**P*<0.05 vs. con group). C, IL-1β was up-regulated by 10.38 folds at 1 h (****P*<0.001 vs. con group), by 6.78 folds at 3 h (***P*<0.01 vs. con group). D, TNF-α reached a peak at 3 h by 13.84 folds (***P*<0.01 vs. con group). The data are expressed as mean±SEM. Results are representative of those obtained from three independent experiments.

2.4 apelin activates PI-3k/Akt and MEK/Erk signaling pathways in BV2 cells

The PI-3K/Akt and MAPKs are the most important signaling molecules involved in activated microglia and also appear to play key roles in inflammatory processes [14,17]. Therefore, using Western blot and immunofluorescence staining, we investigated the question of whether apelin activated PI-3K/Akt and Erk signaling pathways. We first examined the time curve of p-PI-3K, p-Akt and p-Erk in BV2 cells treated with apelin. BV2 cells were incubated with apelin (100 ng mL^{-1}) for 30 min, 1 h, 3 h, and 6 h. Western blot analysis results showed that apelin promoted expression of p-PI-3K, p-Akt and p-Erk in a time-dependent manner (Figures 4A, 4B, 5A, 5B, 6A and 6B).

Here, the influence of the PI-3K/Akt inhibitor LY294002 and Erk inhibitor U0126 were also examined. After pretreatment with 10 μmol L^{-1} LY294002 and 10 μmol L^{-1} U0126 for 1 h, respectively, BV2 cells were stimulated with apelin (100 ng mL^{-1}) for 6 h. Western blot analysis results revealed the expression of p-PI-3K, p-Akt and p-ERK1/2 were down-regulated significantly (Figures 4C, 4D, 5C, 5D, 6C and 6D). Immunofluorescence staining showed that apelin up-regulated the expression of p-Erk and U0126 did lower the expression of p-Erk in BV2 cells (Figure 6E).

2.5 Erk inhibitor U0126 suppresses expression of inflammatory cytokines in BV2 cells

We found that apelin treatment up-regulated the expression of inflammation factors in BV2 cells by immunofluorescence. In addition, apelin activated p-Erk signaling pathway through western blot analysis. We next examined the relationship between the apelin induced activation of the Erk signaling pathway and the change of inflammation factors after BV2 cells pretreated with Erk inhibitor 10 μmol L^{-1} U0126. Figure 7 shows the expression of inflammation factors was inhibited and translocation of the TNF-α and IL-1β was observed after using U0126 (Figure 7). The results showed that inhibition of inflammatory cytokines expression by U0126 in apelin-stimulated BV2 cells was associated with down-regulation of Erk phosphorylation. Erk signaling pathway participated in the activation of IL-1β, TNF-α, MCP-1 and MIP-1α in BV2 cells (Figure 7).

3 Discussion

In the present study, we investigated the relationship and its regulation mechanism between inflammatory cytokines and apelin in microglial BV2 cell. Our major findings were as follows: (i) Apelin enhanced the expression of inflammatory cytokines, TNF-α, IL-1β, MIP-1α, MCP-1 in BV2 cells through immunofluorescence staining and qRT-PCR methods; (ii) Apelin activated the PI-3K/Akt and MEK/Erk signaling pathways in BV2 cells; (iii) Erk signaling was involved in expression of inflammatory cytokines in BV2 cells induced by apelin.

Apelin is the endogenous ligand for the APJ receptor and

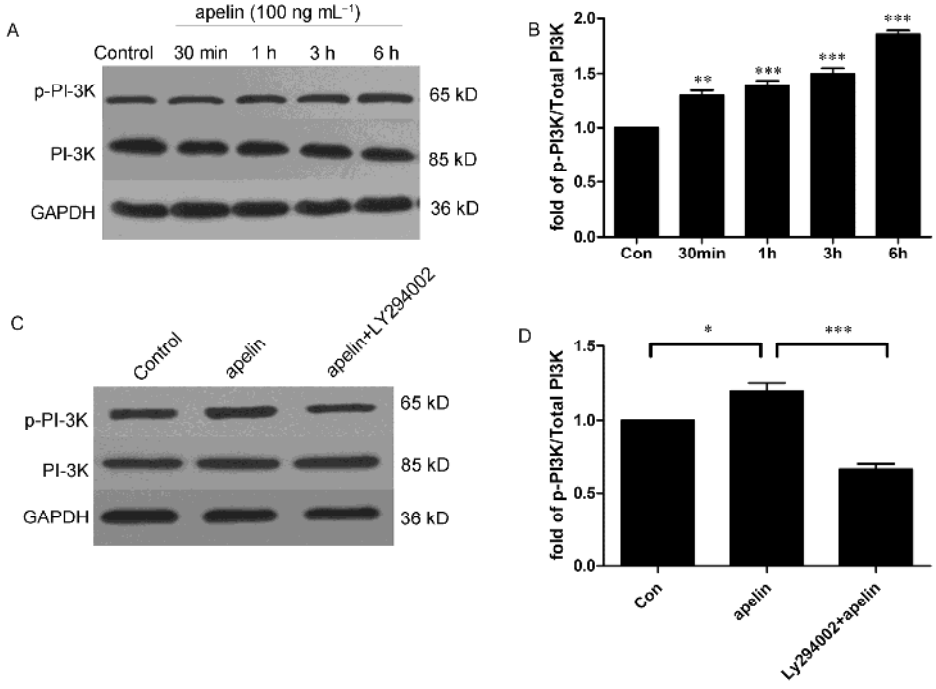

Figure 4 The expression of phosphor-PI-3K in BV2 cells stimulated by apelin. A and B, BV2 cells were pretreated with the indicated doses of apelin (100 ng mL^{-1}) for 30 min, 1 h, 3 h, and 6 h. C and D, Then BV2 cells were pretreated with the indicated concentrations of PI-3K/Akt inhibitor LY294002 (10 μmol) for 1 h. Total protein (30 μg) was subjected to 10% SDS-PAGE, followed by Western blotting using anti-p-PI-3K antibody. Proteins were visualized using an ECL detection system. GAPDH were used as internal controls. Results are representative of those obtained from three independent experiments.

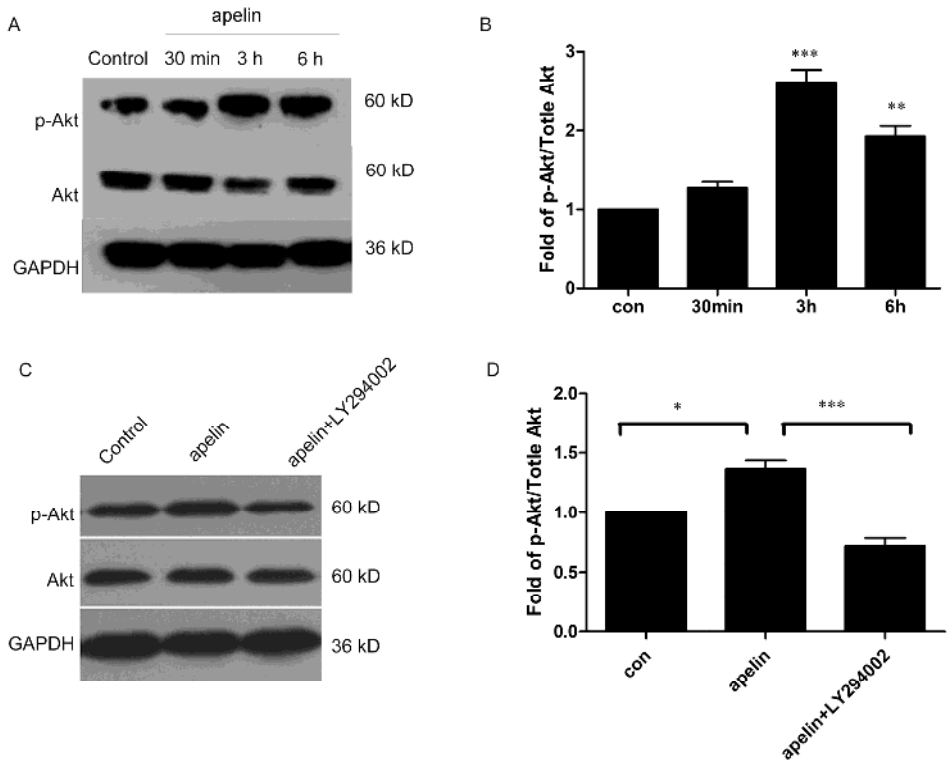

Figure 5 The expression of phosphor-Akt in BV2 cells stimulated by apelin. A and B, BV2 cells were pretreated with the indicated doses of apelin (100 ng mL^{-1}) for 30 min, 3 h, and 6 h. C and D, Then BV2 cells were pretreated with the indicated concentrations of PI-3K/Akt inhibitor LY294002 (10 μmol) for 1 h. Total protein (30 μg) was subjected to 10% SDS-PAGE, followed by Western blotting using anti-p-Akt antibody. Proteins were visualized using an ECL detection system. GAPDH were used as internal controls. Results are representative of those obtained from three independent experiments.

localization of apelin expression in tissues was observed in vascular endothelial cells (ECs), adipose tissue and epithelial cells, suggesting a role for apelin/APJ in angiogenesis and vascular formation [18,19]. Similarly, location of APJ is very important for Apelin/APJ signaling pathway to exert physiological function in various cells and tissues [20]. Previous study demonstrated that APJ expression was localized in the vascular and endocardial ECs and smooth muscle cells [21]. Our previous studies showed APJ was also expressed in pericyte and PRE (data not show). In the present study, through immunofluorescence staining, we first confirmed that APJ was positively expressed in BV2 cells. Our results showed that the expression of APJ is essential for the apelin/APJ system function in BV2 cells, which implies apelin might be involved in physiology and pathology of BV2 cells.

Many studies demonstrated the potential link between apelin and inflammation in different tissues *in vivo* and *in vitro* [8,22,23]. In the present study, we found the expression of IL-1β, TNF-α, MCP-1, and MIP-1α was increased in BV2 cells stimulated by apelin at different time. Especially, mRNA transcript of IL-1β reached the peak at 1 h firstly. IL-1β is known as a multifunctional inflammatory cytokine and the main trigger of the neuroinflammatory cascade [24]. Several observations support a role of IL-1β as a probable mediator of retinal damage in diabetes and IL-1β also is increased in the retina of experimental diabetes animals [11,25]. Therefore, we believe IL-1β plays an important role as trigger of the inflammatory cascade in DR.

Moreover, glial reactivity is a key component of neuroinflammation and retinal glial reactivity, which is a well documented early feature of both human and experimental diabetic retinopathy [26]. IL-1β can induce gliosis in *vivo* and in *vitro*. Stimulation of astrocytes with IL-1β leads to up-regulation or synthesis of several of the genes known to be regulated in reactive glia [27]. Our previous investigation demonstrated apelin stimulated Müller proliferation and migration, and induced gliosis of Müller in diabetic retinopathy [7,28,29]. In this study, our data directly demonstrate apelin may act as a direct regulator of increasing expression of IL-1β in microglial BV2 cells. Therefore, we presume in ischemia/hypoxia diseases, such as DR and ROP, up-regulation of apelin triggered releasing of IL-1β, which induced gliosis and aggravated progression of DR.

The activated native kidney cells and activated monocytes-macrophages can produce TNF-α. Moreover, TNF-α can increase the release of other cytokines, chemokines, growth factors, and acute phase proteins [30,31]. As a pleiotropic cytokine, TNF-α exerts multiple effects and it can contribute to the development of diabetic nephropathy through several mechanisms [30]. Similarly, studies demon-

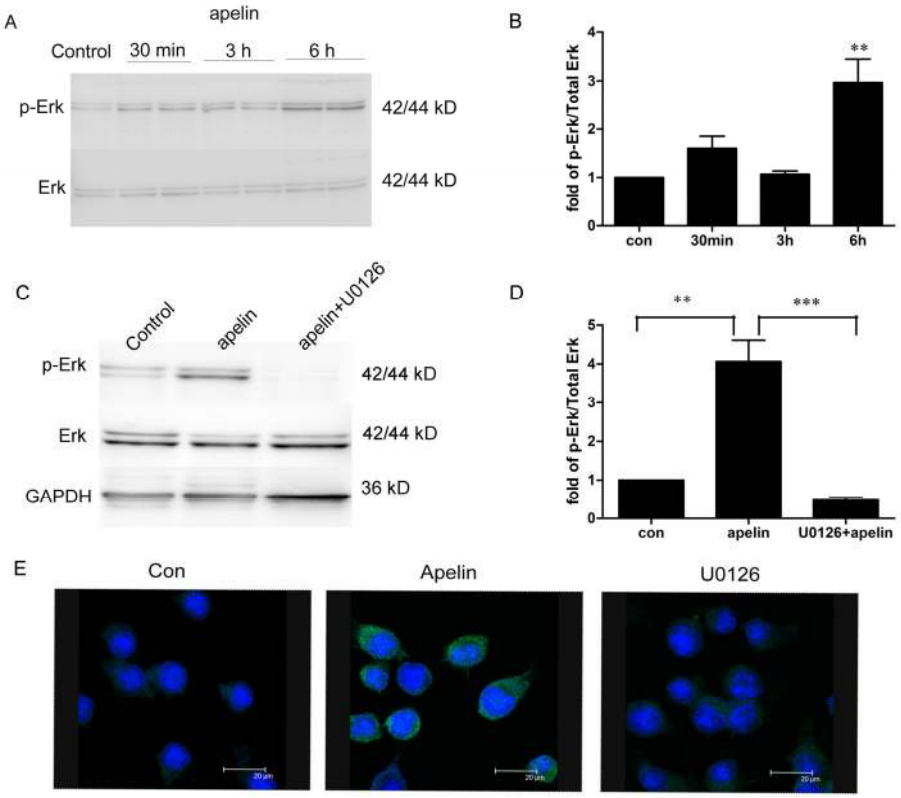

Figure 6 The expression of phosphor-Erk in BV2 cells stimulated by apelin. A and B, BV2 cells were pretreated with the indicated doses of apelin (100 ng mL^{-1}) for 30 min, 3 h, and 6 h. C and D, Then BV2 cells were pretreated with the indicated concentrations of Erk inhibitor U0126 (10 μmol) for 1 h. Total protein (30 μg) was subjected to 10% SDS-PAGE, followed by Western blotting using anti-p-Erk antibody. Proteins were visualized using an ECL detection system. GAPDH were used as internal controls. E, Immunofluorescent staining of p-Erk in BV2 cells stimulated apelin (100 ng mL^{-1}) and U0126 (10 μmol). Results are representative of those obtained from three independent experiments.

strated TNF-α genotypes were also significantly associated with PDR occurrence [32]. In progression of DR, the expression of TNF-α in retinal is associated with neuronal and endothelial cell death and inhibition of TNF-α is beneficial for the prevention of early DR [33]. *In vitro* experiment Daviaud has proved the expression of apelin was increased by endogenous TNF-α in adipocytes [34]. However, the relationship between apelin and TNF-α is unclear in microglia. Our study only demonstrated expression of TNF-α was upregulated in BV2 cells by apelin. Except for TNF-α, MIP-1α and MCP-1 have been implicated as potential modulators of the acute inflammatory response in the CNS [35]. They have potent chemoattractant capability and promote monocyte recruitment into an inflammatory or pathological site. Once they were activated near the site of pathology, the recruited cells can produce more inflammatory mediators, thus inducing inflammation response [10,36]. Similarly, MCP-1 and MIP-1α in vitreous fluid from PDR patients were significantly higher than that of from control groups [37]. IL-10, a pleiotropic TH_2 cytokine, has obvious suppressive effects on the production of pro-inflammatory cytokines by monocytes-macrophages and inhibits the expression of activating molecules on these cells and dendrite cells [38,39]. Kremlev demonstrated that IL-10 has protected LPS-induced microglial cell death in *in vivo* and in *in vitro* study [39].

Our study showed the expression of IL-1β, TNF-α, MCP-1 and MIP-1α were increased in BV2 cells after incubation with apelin. Moreover, the mRNA level of TNF-α, MCP-1 and MIP-1α reached the peak at 3 h in BV2 cells, which lagged the expression of IL-1β. These suggested IL-1β triggered the inflammation cascade and induced expression of other inflammatory cytokines in BV2 cells. At the same time, apelin is also a modulator of inflammatory cytokines, which up-regulated the expression of IL-1β, TNF-α, MCP-1 and MIP-1α. IL-10 is an anti-inflammation cytokine, and our results further showed IL-10 was decreased in BV2 cells induced by apelin. It was well known that the inflammatory cytokines secreted by over-activated microglia participated in the progression of DR and caused the damage of retina [40,41]. According to our results, we presumed apelin enhanced the expression of inflammatory cytokines secreted by microglia, which was involved in pathological damage of DR. Therefore, it is necessary to further investigate the expression of inflammatory factors induced by apelin in *in vivo* experiments.

Furthermore, we investigated the underlying mechanisms of up-regulation of inflammatory factors by apelin in BV2

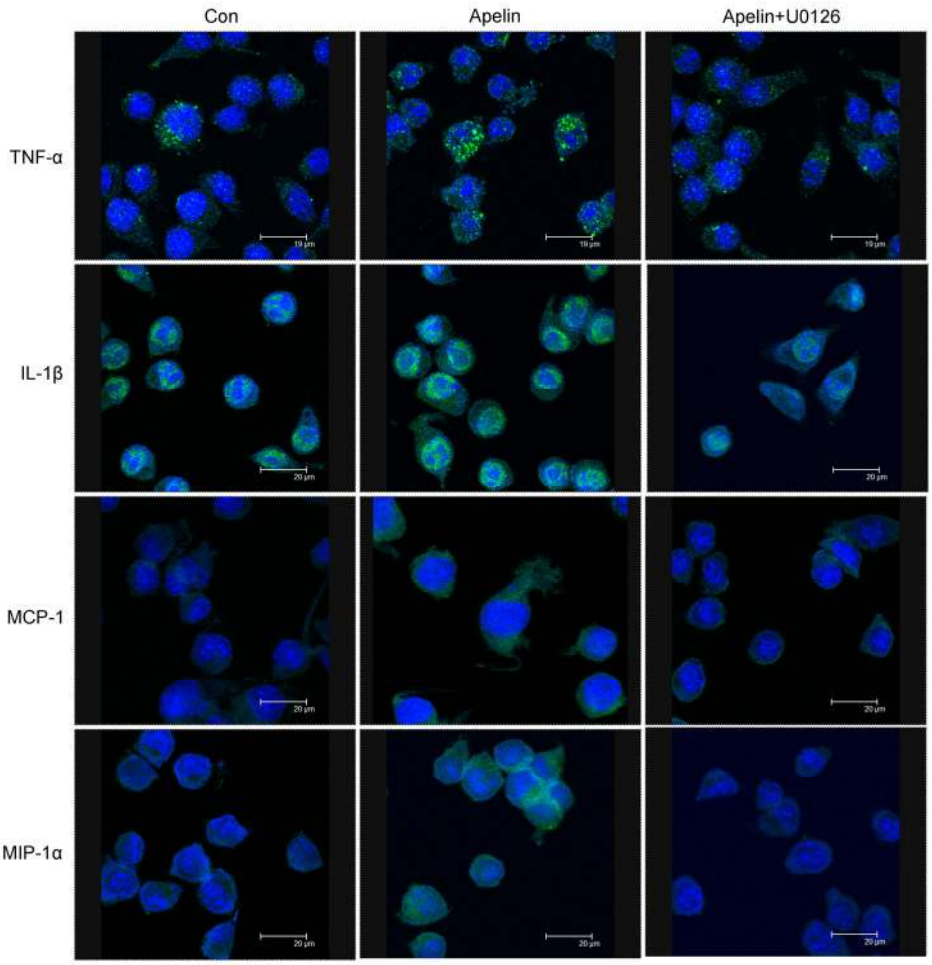

Figure 7 Immunofluorescent staining of pro-inflammation cytokines in BV2 cells after stimulated by U0126 (10 μmol). Exogenous recombinant apelin-13 (100 ng mL^{-1}) stimulated BV2 cells pretreated with U0126 (10 μmol) for 1 h. The expression of MCP-1, MIP-1α, IL-1β, and TNF-α were inhibited.

cells. Recently, involvement of various intracellular signaling pathways, such as PI-3K/Akt pathway and MAPKs/Erk, in inflammatory mediator induction of BV2 microglia has been reported [13,14]. Previous studies have shown that PI-3K has a significant role in cellular growth, adhesion, differentiation and the inflammatory response [42–44]. In addition, activation of MAPKs has significant effects on the regulation of COX-2, iNOS, and inflammatory cytokine gene expression in microglia [45]. To investigate potential mechanisms for apelin-activated inflammatory factors in BV2 cells, we evaluated the effect of apelin on the phosphorylated forms of PI-3K/Akt and MAPK/Erk pathways. The present results showed that apelin is a potent activator of PI-3K/Akt and MAPKs/Erk for expression of inflammation factors in BV2 microglia (Figure 8). Furthermore, our data obviously demonstrated that blockade of PI-3K/Akt and MAPKs/Erk pathways by PI-3K/Akt inhibitor LY294002 and Erk inhibitor U0126 significantly attenuated expression of phospho-PI-3K, phospho-Akt and phospho-Erk, respectively (Figure 8). Immunofluorescence staining results showed Erk inhibitor U0126 significantly decreased the expression of inflammatory factors, such as TNF-α, IL-1β, MIP-1 and MCP-1α. These results suggested that PI-3k/Akt and MAPK/Erk signaling pathway activation plays a key role in apelin-induced expression of inflammatory factors. Therefore, further studies are needed to evaluate the relation among downstream signaling pathway in the apelin-induced activation of inflammatory gene expression.

4 Conclusion

In summary, in the present study we presented novel data showing that apelin significantly increased the expression of inflammatory cytokines, such as TNF-α, IL-1β, MIP-1α, and MCP-1, and suppressed the expression of anti-inflammatory cytokines IL-10. Apelin mediated inflammatory cytokines secretion through activation of PI-3K/Akt and MAPKs/Erk pathways. In addition, the up-regulation of inflammatory responses was inhibited by LY294002 and U0126 in BV2 cells. These data suggest that apelin is an inflammatory mediator for inflammatory diseases, including

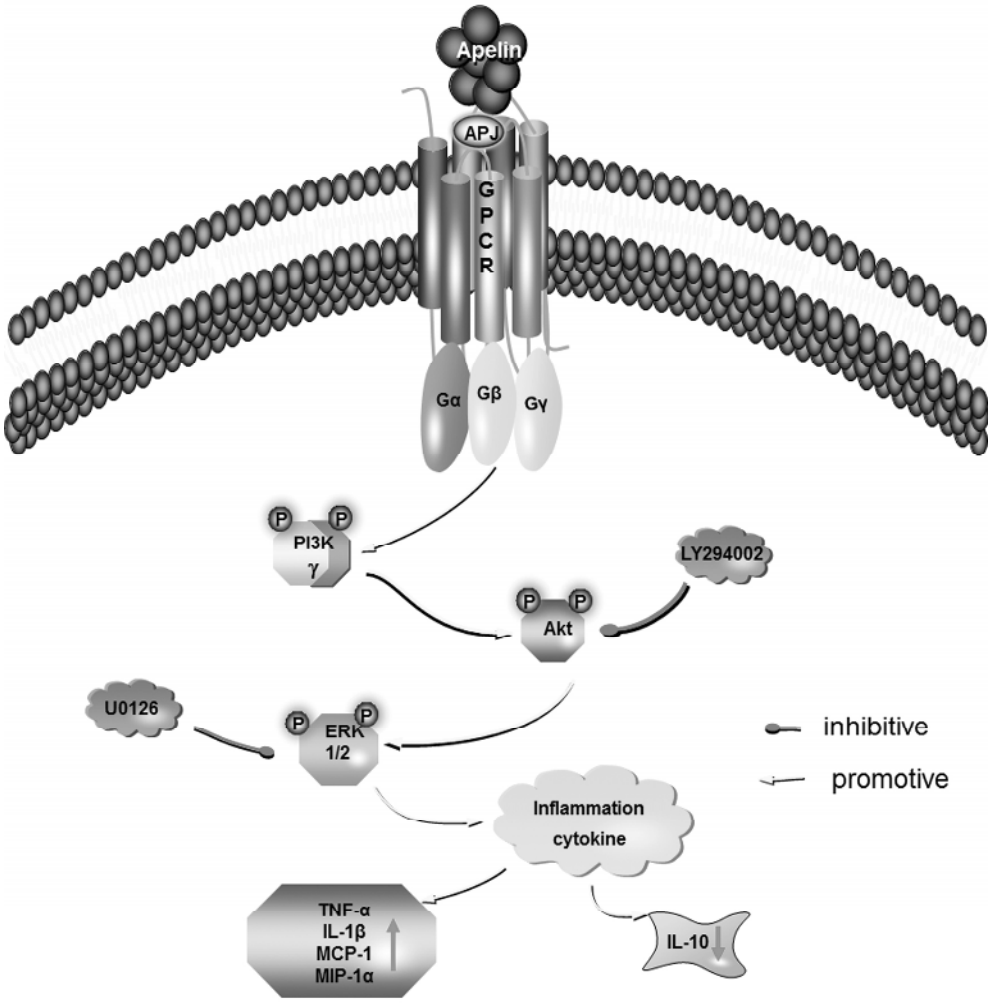

Figure 8 Schematic showing the putative apelin/APJ signalling mechanism that regulates inflammation factors. Apelin/APJ activates the PI-3K/Akt/Erk pathway. Then, active signaling pathway increases the expression of Il-1β, TNF-α, MCP-1 and MIP-1α and decreases IL-10 expression.

neurodegenerative disorders and DR. In that regard, the inhibition of apelin and a variety of inflammatory enzymes and cytokines in microglial cells would be an effective therapeutic approach against these diseases.

The authors declare that there is no conflict of interest regarding the publication of this paper.

This work was supported by European Foundation for the Study of Diabetes/Chinese Diabetes Society/Lilly grant (90561 and 94410). The authors thank Dr. Lin JiHong for technical assistance. We also sincerely acknowledge Prof. Hans-Peter Hammes for providing the BV2 cells and associated antibodies.

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