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Omentin-1 Suppressed Inflammation by NLRP3 through AMPK Function

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KEYWORDS Acute Cerebral Infarction. AMP-Activated Protein Kinase. Cerebral Vessels. NOD-Like Receptor Family Pyrin Domain Containing 3. Omentin-1

ABSTRACT This experiment investigated the biological roles of Omentin-1 in cerebral vessels of acute cerebral ischemia (ACI). The expressions of Omentin-1 in mouse model or patients with ACI were down-regulated. Moreover, Omentin-1 was negatively correlated with NIHSS score or serum IL-1β expression in patients undergoing intravenous thrombolysis for ACI. Omentin-1 was positively correlated with Barthel index in patients undergoing intravenous thrombolysis for ACI. In the mouse model or the in-vitro model, Omentin-1 mitigated inflammation factors. Additionally, in the in-vitro model of ACI, overexpression of Omentin-1 induced AMPK/ NLRP3 signaling pathway. Down-regulation of Omentin-1 also inhibited AMPK/ NLRP3 signaling pathway. Taken together, Omentin-1 suppressed ACI in the ACI model through inhibiting inflammation, which might serve as a potential treatment strategies of cardiovascular and cerebrovascular diseases in ACI.

INTRODUCTION

At present, stroke has gradually become the primary cause of human death and disability in China and the world, in which ischemic stroke accounts for a large proportion (Carpio et al. 2021). Studies have shown that ischemic stroke accounts for 77.8 percent of the stroke incidence population in China, and its risk is about twice that of hemorrhagic stroke (Ferro et al. 2021; Kopeva et al. 2021).

In recent years, the incidence rate of cerebrovascular disease has gradually increased, and it has become one of the major diseases endangering human health (Miller et al. 2021). About 75 percent of the surviving patients with cerebrovascular diseases are accompanied by dysfunction, which brings great pain to the patients (Marchel et al. 2021). There are many manifestations of cerebral ischemia, such as focal and diffuse cerebral ischemia, permanent and acute cerebral ischemia and so on (Marchel et al. 2021). Among them, the sequelae of cognitive impairment, dementia and even vegetative state caused by neuronal injury caused by insufficient cerebral blood perfusion in patients with acute cerebral ischemia seriously affect the living standards of patients (Sun et al. 2018b). Therefore, whether it can promote neurogenesis and add new neurons to better restore the brain function of these patients has always been the focus of neurological research (Sun et al. 2018b).

Cerebral infarction is a disorder of blood supply in local brain tissue, which leads to ischemic and hypoxic necrosis of brain tissue (Nuñez et al. 2021). Cerebral infarction is a central nervous system disease that seriously affects human life and quality of life (Nevulis et al. 2018; Steven et al. 2019). Because of its high incidence rate, disability and mortality, it has brought a heavy economic and social burden to our country (Nevulis et al. 2018; Steven et al. 2019). Inflammatory response is an important mechanism for the occurrence and development of pathological process of ischemic brain injury (Haybar et al. 2019; Rane et al. 2020). NLRP3 inflammasome promotes the release of IL-1β and IL-18, and induce cell death (Zhang et al. 2021b). It plays an important role in exacerbating the inflammatory response of ischemic brain injury after cerebral infarction. Studying the relevant mechanism of regulating NLRP3 inflammasome can provide one new tendency for the prevention and treatment of cerebral infarction (Sun et al. 2018b; Pu et al. 2022).

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Most cerebrovascular diseases are associated with ischemia and hypoxia (Sun et al. 2018a). On the one hand, the oxygen consumption of brain tissue accounts for about 20 percent of the total body (Wang et al. 2019). On the other side, brain tissue is poorly tolerant to hypoxia. Short-term hypoxia can cause neuronal death (Pineda-Ramírez et al. 2020). Hypoxic stress activates the AMPK pathway. In addition to energy metabolism, activated AMPK is also involved in cell transcriptional regulation and division cycle, thereby inhibiting cell proliferation (Jiang et al. 2018; Pu et al. 2020).

In recent years, Omentin-1 is a newly discovered fat factor (Li et al. 2020; Liu et al. 2020). Most of the current research reports are based on the specific expression of adipokines in omental tissue or adipocytes, focusing on revealing the possible specific role of adipokines themselves. The decrease of Omentin-1 is related to the development and occurrence of varieties of clinical diseases (cardiovascular disease, obesity, diabetes, tumor, polycystic ovary syndrome and sleep apnea) (Askin et al. 2020; Li et al. 2020; Liu et al. 2020). The level of omentin-1 is significantly reduced in blood patients with acute coronary syndrome and acute myocardial infarction (Askin et al. 2020; Chai et al. 2020).

Objective of the Study

This paper investigated the biological roles of Omentin-1 in cerebral vessels of cerebral ischemia.

MATERIAL AND METHODS

Experimental Clinical Medicine

This study of the experimental clinical medicine was approved by the Ethics Committee of The PLA Naval Anqing Hospital. The serum samples of 12 patients with ACI and 12 normal volunteer were collected from The PLA Naval Anqing Hospital. All subjects signed written informed consent.

Animal Experiments and 2,3,5 Triphenyltetrazolium Chloride (TTC) Analysis

Male C57BL/6 mice were housed at 22 ± 2.0 °C and 50 percents ±5% humidity with a 12-h:12-h light/dark cycle and fed food and water ad libitum. All animal procedures were approved by the Ethics Committee of The PLA Naval Anqing Hospital. Mice were induced MCAO as literature (Sui 2019).

The randomized animal division results in 2 groups in total, MCAO-induced mice group (Model, n = 8), sham control group (sham, n = 6). Next, the randomized animal division results in 2 groups in total, MCAO-induced mice group (Model, n = 8), Omentin-1 group (sham, n = 7), mice were administered with Omentin-1 recombinant protein (100 ng/mice) for 24 h.

Brain tissues were extracted after sacrifice, and then olfactory bulb, cerebellum and brain stem were removed. Brain tissues was cut into coronal slices, and slices were put into 2 percents TTC phosphate buffer solution (Sigma-Aldrich, St. Louis, MO, USA) for 20 min at 37^0C.

Cell Culture

The murine BV2 microglial cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 percent foetal bovine serum (FBS, Gibco BRL, Grand Island, NY, USA) at 37°C and 5 percent CO2. Plasmids or simimics were transfected into BV2 using Lipofectamine 2000. After 48 h of transfection, cells were induced with 0.1 mg/ml LPS for 4 h, and then induced with 2 mM of ATP for 0.5 h.

Quantitative PCR

Total RNA was extracted from serum, brain tissues or cell samples according to instructions of the RNA extraction kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Quantitative PCR were performed using Light Cycler® 480 SYBR Mix (Roche, Germany) using LightCycler® 480 real time PCR system.

Western Blot Analysis

Total protein lysates from brain tissue (100 mg) or cell samples were solubilized in SDS-PAGE sample buffer using Radio-Immunoprecipitation Assay (RIPA) and PMSF reagent, separated on a 10 percent SDS-polyacrylamide gel, and transferred electrophoretically onto polyvi-

nylidenedifluoride (PVDF) membranes. The membranes were blocked with non-fat-milk (5%) for 2 h at room temperature and incubated with anti- p-AMPK, anti-AMPK, anti-Omentin-1, anti-NLRP3 and anti-β-actin antibody at 4°C. The membranes were incubated with horseradish peroxide-coupled sheep anti-mouse secondary antibody at room temperature for 2 h. The bound antibodies were detected using enhanced chemiluminescence (ECL) with β-actin used as a control.

ELISA Kits

Serum and cell samples were collected and used to measure these inflammatory factors (IL-1β, IL-6, IL-10, INF-γ and TNF-α) according to the instructions of the ELISA kit (Beyotime Institute of Biotechnology, China).

Immunofluorescence

Cells were fixed with 4% paraformaldehyde and then permeabilized with 0.1% Triton X-100 in PBS. Cells were blocked with 5 percent BSA for 30 min at 37°C and treated with primary antibodies at 4°C overnight: anti- p-AMPK and anti-Omentin-1. Cells were incubated with Cy3-conjugated goat anti-rabbit (1:200) or goat anti-mouse IgG (1:200) DyLight 488 conjugated secondary antibodies for 2 h at 37°C. Cells were stained with DAPI and viewed by fluorescent confocal microscopy (Nikon, Tokyo, Japan).

Statistical Analysis

$P<0.05$ was considered statistically significant using SPSS 22.0 software (SPSS, Chicago, IL, USA). All data are presented as mean ± SEM. ANOVA followed by Tukey's post hoc analysis was used for comparisons between multiple groups or Student's t test (two groups).

RESULTS

Omentin-1 Expression Levels in Patients with ACI

This work first examined cerebral vessels in patients with ACI by MRI, MRV and CTA assays.

First, MRI, MRV and CTA assays were conducted to examine cerebral vessels in patients with ACI (Fig. 1A). According to the results of this study, the serum Omentin-1 mRNA expression was down-regulated in patients with ACI (1.131±0.496 vs 0.363±0.246, all $P<0.05$, Fig. 1B). In patients receiving intravenous thrombolysis for ACI, the serum Omentin-1 mRNA expression was negatively correlated with NIHSS score or serum IL-1β level (all $P<0.05$, Fig. 1C-1D). However, the serum Omentin-1 mRNA expression was positively correlated with Barthel index in patients undergoing intravenous thrombolysis for ACI (all $P<0.05$, Fig. 1E). Further, the diagnostic value of Omentin-1 level was assessed by plotting the receiver operating characteristic (ROC) curve (AUC = 0.9861, $P<0.05$, Fig. 1F). As a result, compared with sham group, mice with ACI showed lower expression levels of Omentin-1 mRNA and protein in brain tissue (1.083±0.413 vs 0.373±0.168, 0.940±0.044 vs 0.333±0.064, $P<0.05$, Fig. 1G-1H). In general, Omentin-1 was lowly expressed in ACI and correlated with disease progression.

Omentin-1 Reduced Inflammation In the In-Vitro Model

This work further explored the potential function of Omentin-1 in inflammation. In the in-vitro model, Omentin-1 mRNA expression was induced by Omentin-1 plasmid and suppressed by si-omentin-1 mimics (1.107±0.500 vs 10.073± 3.848, 1.142±0.601 vs 0.645±0.254 vs 0.389±0.207 vs 0.203±0.082, $P<0.05$, Fig. 2A). Overexpression of omentin-1 did not regulate IL-1β levels in LPS-induced BV2 cells, but it reduced IL-1β levels in LPS+ATP-induced BV2 cells (75±24.49 vs 73.333±33.99 vs 95±36.28590176 vs 103.33±15.45 vs 586.67±61.28 vs 285±59.30, $P<0.05$, Fig. 2B). Meanwhile, si-omentin-1 did not regulate IL-1β levels in LPS-induced BV2 cells. In LPS+ATP-induced BV2 cells, down-regulation of omentin-1 promoted IL-1β levels (95±10.8 vs 105±8.16 vs 100±14.72 vs 98.33±28.67441756 vs 526.67±76.63 vs 1143.33±140.79, all $P<0.05$, Fig. 2B). Conversely, overexpression of omentin-1 reduced the levels of IL-6, IL-10, INF-γ and TNF-α in LPS+ATP-induced BV2 cells (100±12.27 vs 33.5±12.57, 545±57.15 vs 81.67±28.67, 266.67±45.89 vs 120±26.77, 201±40.10 vs 73±17.20, Fig. 2C-2F). Furthermore, down-regulation of omentin-1 promoted the levels of IL-6, IL-10, INF-γ and TNF-α in LPS+ATP-induced BV2 cells

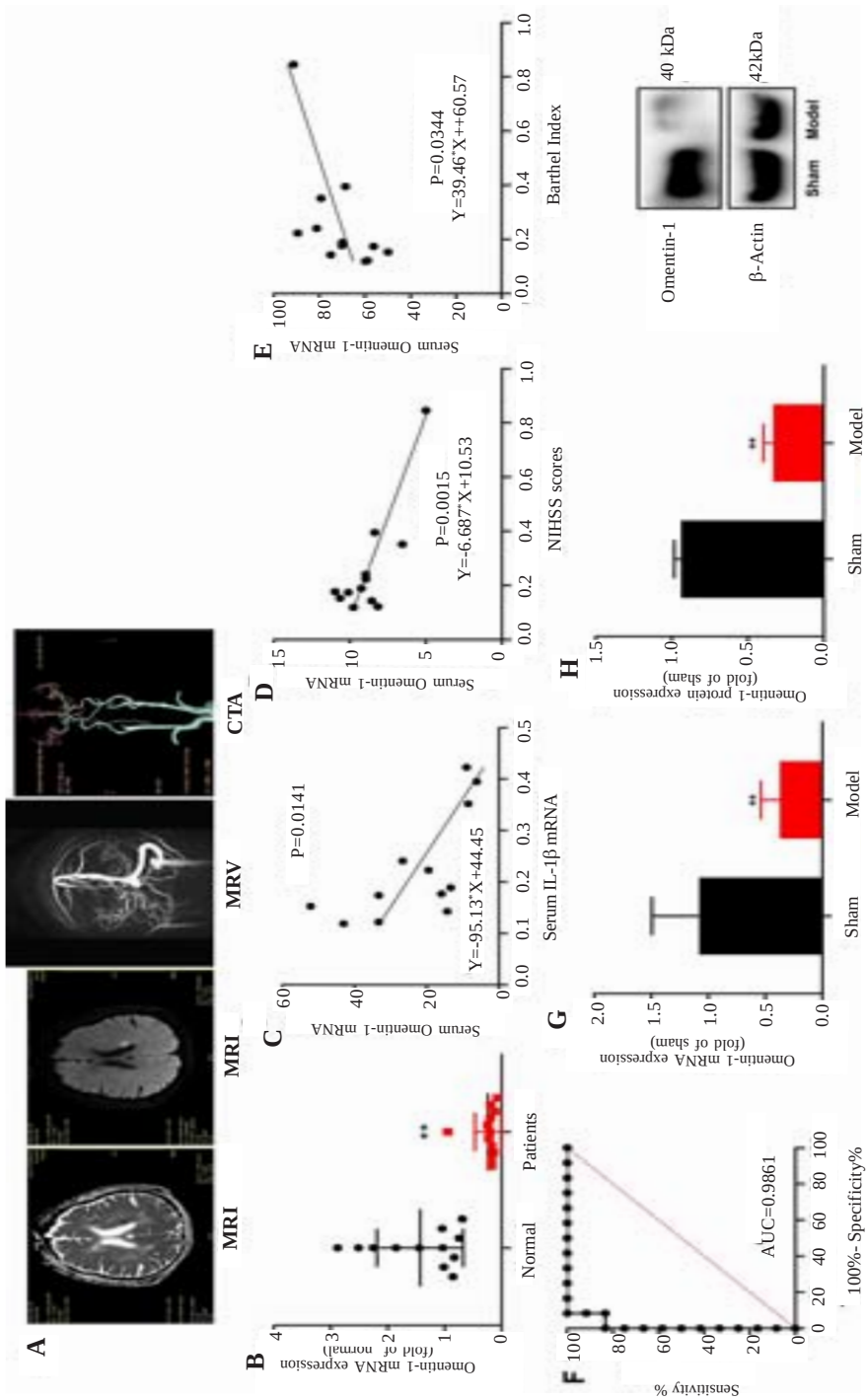

Fig. 1. Omentin-1 Expression Levels in Patients with ACI
Patients using MRI, MRV and CTA assay (A), Omentin-1 mRNA expression (B), serum mRNA of Omentin-1 was correlation with serum IL-1β levels (C), serum mRNA of Omentin-1 was correlation with NIHSS score (D), serum mRNA of Omentin-1 was correlation with Barthel index (E), ROC (F) in patients with ACI;
Omentin-1 mRNA (G) and protein expression (H) in mice with ACI
****p<0.01 compared with normal volunteers group or sham control group**
***Source:* Author**

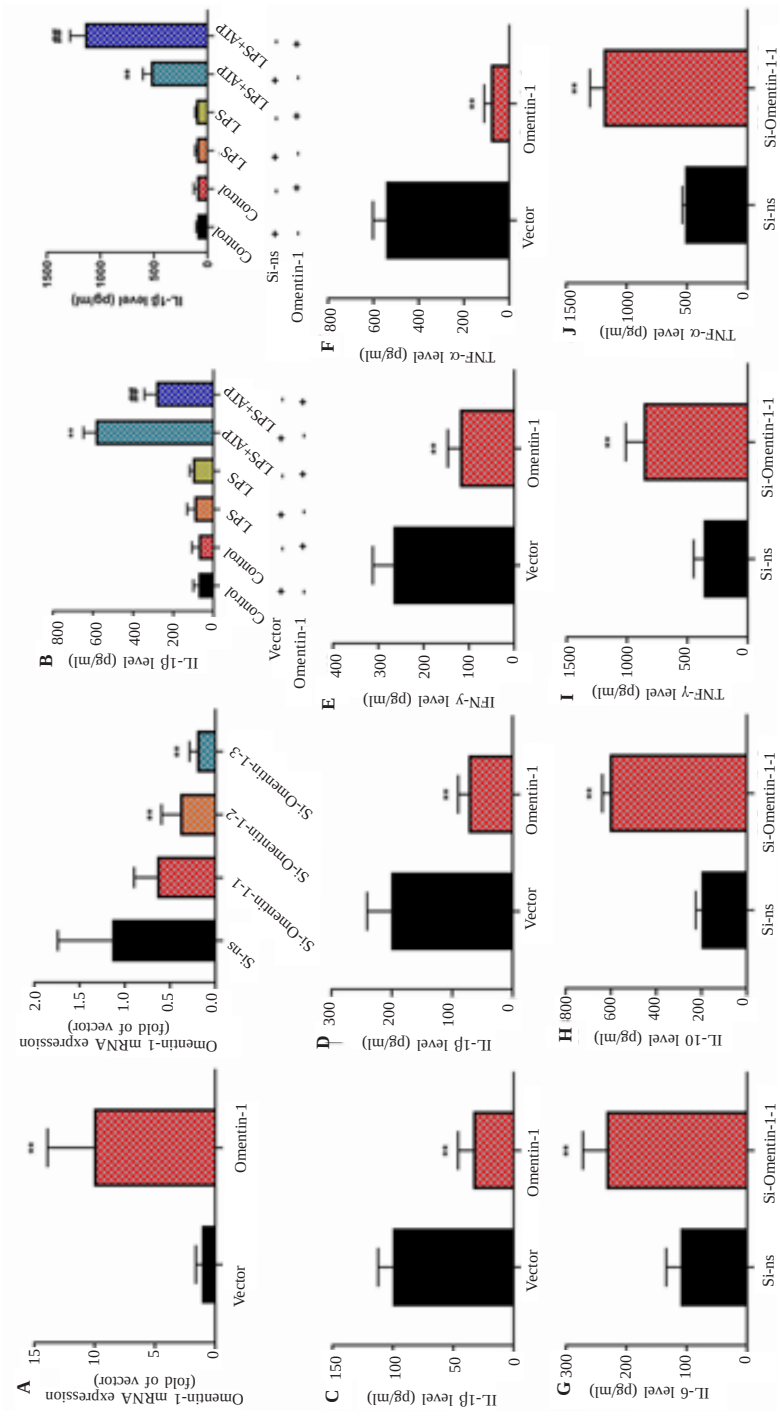

Fig. 2. Omentin-1 reduced inflammation in vitro model
Omentin-1 mRNA expression (A),
IL-1β levels in vitro model (B),
IL-6 (C), IL-10 (D), INF-γ (E) and TNF-α (F) levels in vitro model by over-expression of Omentin-1 group;
IL-6 (G), IL-1β (H), INF-γ (I) and TNF-α (J) levels in vitro model by down-regulation of Omentin-1 group
****p<0.01 compared with control negative group or si-negative group**
***Source:* Author**

(110±23.95 vs 233±39.20, 515±22.73 vs 1190±114.96, 365±83.37 vs 863.33±147.44, 200±25.14 vs 606± 32.03, Fig. 2G-2J). On the whole, these data suggest that Omentin-1 reduces inflammation in vitro, while knockdown of circCUL3 promotes inflammation in vitro.

Omentin-1 Presented ACI in Mouse Model

Thereafter, this study determined the effects of Omentin-1 on ACI in the mouse model. It was found that Omentin-1 virus reduced cerebral infarction, infarction scope (3.11±0.40 vs 1.93±0.31, P<0.05) and neurological impairment scores (35.54±5.86 vs 9.85±1.41, P<0.05), and inhibited the levels of IL-6, IL-10, INF-γ and TNF-α (123.5±18.99 vs 22±8.15, 356.67±168.64 vs 81.67±10.27, 151.67±33.25 68.33±31.18, 191±23.79 vs 50±11.05, all P <0.05, Fig. 3).

Moreover, microarray experiments were performed to explore the mechanism of Omentin-1 in regulating gene expression levels in the ACI model (Fig. 4A). According to the results of signaling pathway, Volcano map and KEGG terms, AMPK and NLRP3 might be the important targets for Foxp1 in the inflammation of ACI (Fig. 4B-4D). The results of this study indicate that Omentin-1 virus reduced the serum IL-1β levels in mice with ACI (713.33±140.55 vs 173.33±38.59, P<0.05, Fig.4E). In LPS+ATP-induced BV2 cells, further experiments suggested that overexpression of Omentin-1 suppressed the NLRP3 mRNA expression, while down-regulation of Omentin-1 had an opposite effect (1.068±0.369 vs 1.600±0.585 vs 1.607±0.318 vs 1.172±0.559 vs 11.173±3.113 vs 5.169±1.010, 1.086±0.455 vs 0.976±0.454 vs 0.714±0.339 vs 1.251±1.088 vs 6.929±3.827 vs 37.889±8.265, all P<0.05, Fig. 4F-4G). Furthermore, it was discovered that Omentin-1 virus suppressed the expression of NLRP3 (1.382±0.353 vs 0.382±0.109, P<0.05) protein and induced that of p-AMPK protein in mice with ACI (1.187±0.179 vs 2.143±0.141, P<0.05, Fig. 4G-4H). In general, the above data suggest that Omentin-1 presents ACI in the mouse model.

Omentin-1 Suppressed NLRP3 Inflammasome Via AMPK

Subsequently, this work verified the mechanism of Omentin-1 in acute inflammation in the ACI model. NLRP3 mRNA expression was suppressed in the in-vitro model of ACI in Omentin-1 overexpression group (1.036±0.270 vs 0.740± 0.482 vs 0.363±0.127 vs 0.251±0.005, P<0.05, Fig. 5A), but was induced in the si-Omentin-1 group (1.252±0.651 vs 2.196±1.680 vs 5.148±1.765 vs 11.188±3.028, P<0.05, Fig. 5B). As demonstrated by immunofluorescence analysis, Omentin-1 induced the expression of Omentin-1 and p-AMPK in the in-vitro model (Fig. 5C). To be more specific, Omentin-1 induced the expression of Omentin-1 and p-AMPK proteins, and suppressed that of NLRP3 protein in the in-vitro model (1.210±0.209 vs 3.753±0.551, 1.143±0.102 vs 2.679±0.332, 0.937±0.045 vs 0.304±0.149, all P<0.05, Fig. 5D-5F). By contrast, down-regulation of Omentin-1 suppressed the expression of Omentin-1 and p-AMPK proteins, and induced that of NLRP3 protein in the in-vitro model (1.067±0.054 vs 0.417±0.101, 1.243± 0.202 vs 0.283±0.126, 1.053±0.133 vs 2.724±0.364, all P<0.05, Fig. 5G-5I). In conclusion, these new discoveries show that Omentin-1 targets AMPK/NLRP3 in the ACI model.

AMPK Affected the Effects of Omentin-1 On the In-Vitro Model

Next, this work investigated whether the AMPK-NLRP3 regulatory axis affected the roles of Omentin-1 in acute inflammation of ACI.

To this end, the AMPK agonist Buformin (7 mmol/kg) was applied to reduce the serum levels of IL-1β (70±15.41 vs 865±92.13 vs 522.5±65.48 vs 202.5±24.49 vs 660±132.90, P<0.05) and NLRP3 mRNA expression (1.049±0.29 vs 60.128±0.060, P<0.05). In comparison, the AMPK inhibitor Dorsomorphin dihydrochloride (10 mg/kg) raised the serum IL-1β levels and the NLRP3 mRNA expression (1.045±0.282 vs 7.578±2.811, P<0.05) in mice with ACI by Omentin-1 (Fig. 6A-6C). In mice with ACI, the AMPK agonist Buformin (7 mmol/kg) suppressed the expression of NLRP3 protein (1.181±0.136 vs 0.446±0.085, P<0.05), and induced that of p-AMPK protein (1.073±0.116 vs 2.930± 0.202, P<0.05) in mice with ACI by Omentin-1 (Fig. 6D-6E). Furthermore, AMPK inhibitor inhibited the expression of p-AMPK protein (0.928±0.092 vs 0.288±0.046, P<0.05) and promoted that of NLRP3 protein (1.212±0.297 vs 2.599±0.345, P<0.05) in mice with ACI by Omentin-1 (Fig. 6F-6G). In conclusion, these findings imply that Omentin-1 suppresses inflammation by NLRP3 through regulating AMPK activity.

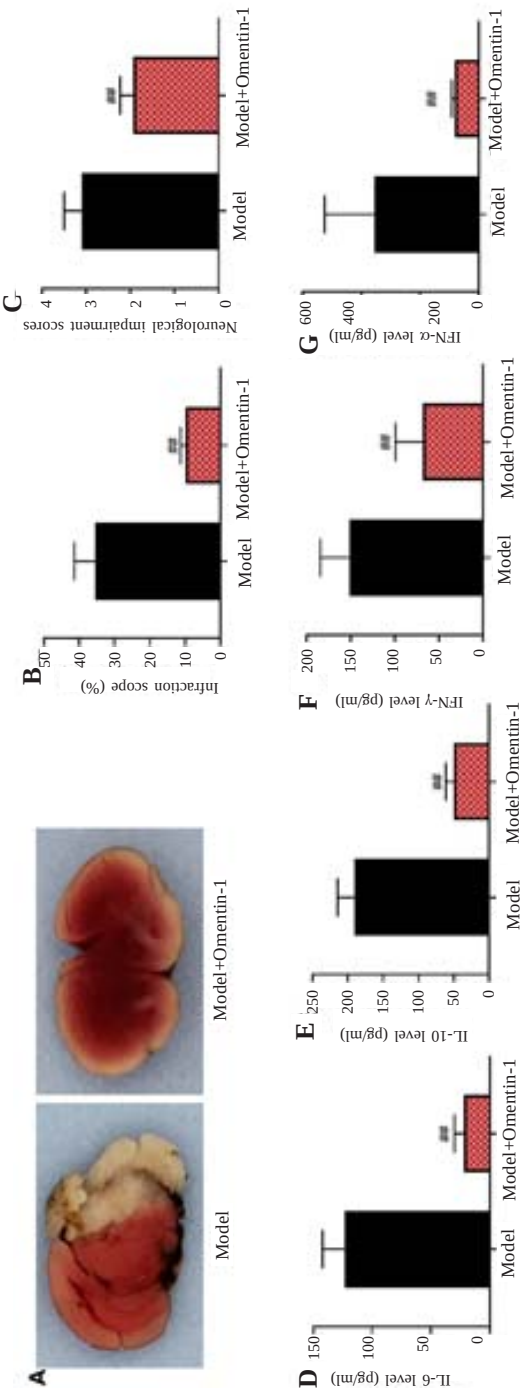

Fig. 3. Omentin-1 presented ACI in mice model
Brain tissue by TTC staining (A), infarction scope (B) and neurological impairment scores (C), IL-6, IL-10, INF-γ and TNF-α levels (D, E, F and G)
Model, mice model of acute cerebral infarction; Model+ Omentin-1, mice model of acute cerebral infarction with Omentin-1
****p<0.01 compared with mice model of ACI group**
***Source:* Author**

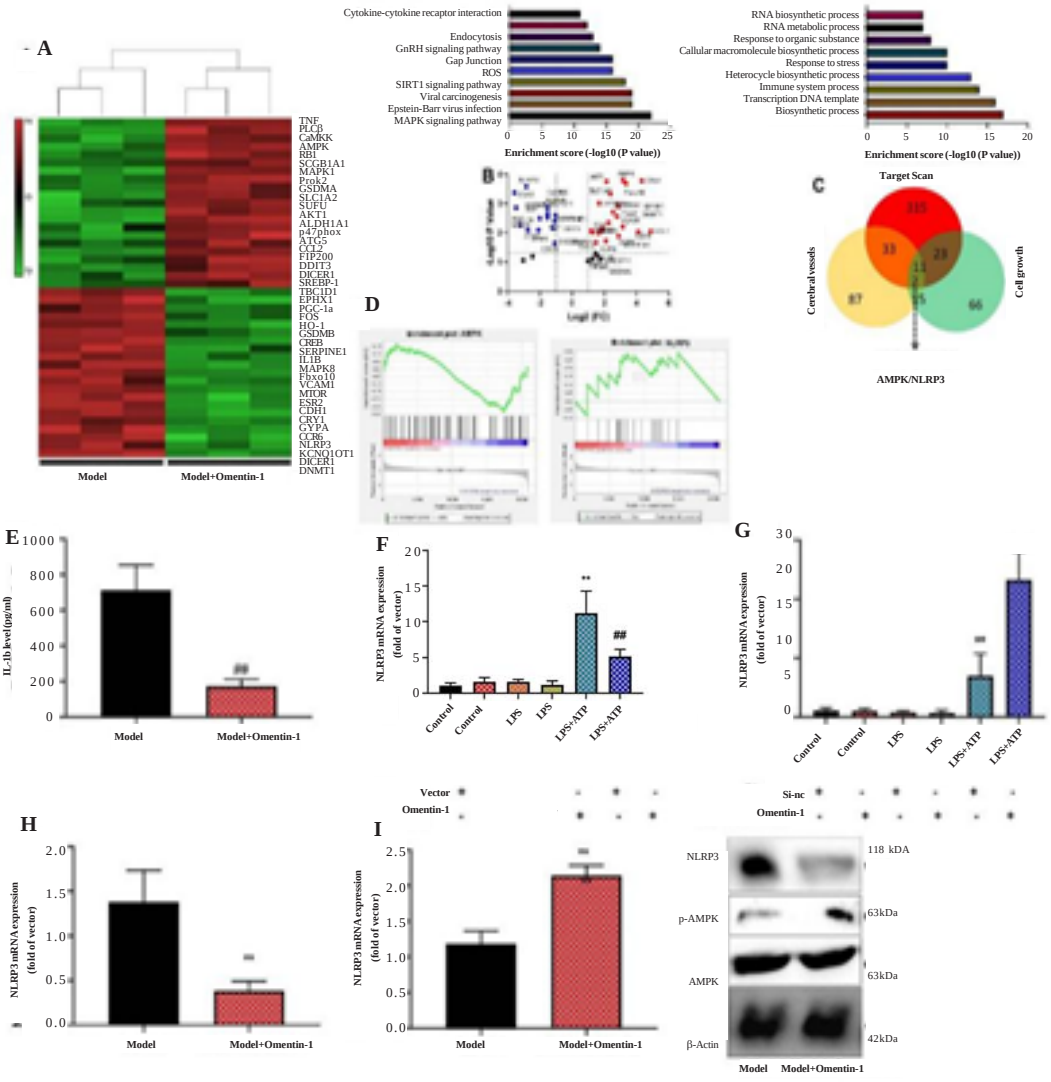

Fig. 4. Omentin-1 suppressed NLRP3 inflammasome by AMPK function
Serum IL-1β levels (A) in mice with ACI,
NLRP3 mRNA expression levels (B and C) in vitro model,
NLRP3 (D) and p-AMPK (E) protein expression in mice with ACI,
Model, mice model of ACI; Model+ Omentin-1, mice model of ACI with Omentin-1
****p<0.01 compared with mice model of ACI group**
****p<0.01 compared with control negative group or si-negative group**
Source: **Author**

DISCUSSION

CVD is one of the diseases with high mortality and disability rate. Among the pathological mechanisms of ischemic stroke, neuroinflammation is considered to be one of the most important pathophysiological mechanisms. Reducing neuroinflammation is expected to become an-

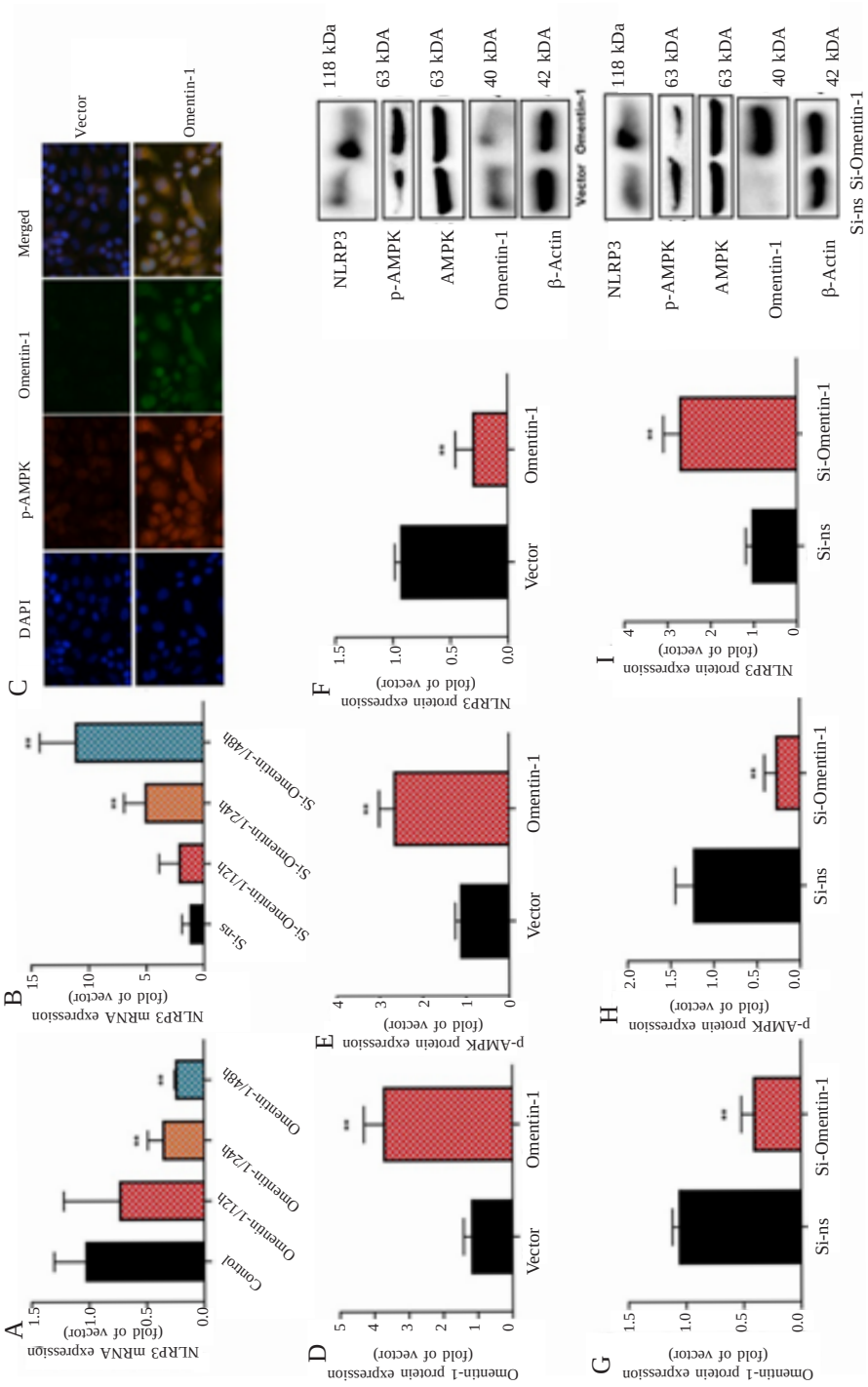

Fig. 5. Omentin-1 suppressed NLRP3 inflammasome by AMPK function in vitro model
NLRP3 mRNA expression levels (A and B), Omentin-1 and p-AMPK expression (Immunofluorescence, C), Omentin-1/p-AMPK/NLRP3 protein expression (D, E and F) in vitro model by over-expression of Omentin-1;
Omentin-1/p-AMPK/NLRP3 protein expression (G, H and I) in vitro model by down-regulation of Omentin-1
"p<0.01 compared with control negative group or si-negative group
***Source:* Author**

other important treatment strategy after thrombolytic therapy.

For the modern population, common CVDs include cerebrovascular disease, coronary heart disease, and pulmonary heart disease (Sinning et al. 2021). CVD has become the first or second leading cause of death in most countries of the world, which poses a serious threat to human life (Wang et al. 2021). It has become the first and second cause of death in most countries in the world (Sprenger et al. 2021).

In this analysis, omentin-1 mRNA expression levels of patients with ACI were reduced (1.131±0.496 vs 0.363±0.246). Omentin-1 mRAN and protein expression were reduced in mice with acute cerebral infarction (1.083±0.413 vs 0.373±0.168, 0.940±0.044 vs 0.333±0.064). Omentin-1 reduced cerebral infarction, infarction scope (3.11±0.40 vs 1.93±0.31, P<0.05) and neurological impairment scores (35.54±5.86 vs 9.85±1.41, P<0.05), and inhibited IL-6, IL-10, INF-γ and TNF-α levels (123.5±18.99 vs 22±8.15, 356.67±168.64 vs 81.67±10.27, 151.67±33.25 68.33±31.18, 191± 23.79 vs 50±11.05).

Xu et al. displayed that the serum levels of omentin-1 was attenuated in patients with ischemic stroke (Xu et al. 2020). The researchers suggest that omentin-1 may precisely control cerebrovascular diseases in acute cerebral infarction.

Inflammatory response plays a double-edged sword role in the pathological process of ACI. The persistent inflammatory response enhances the secondary injury of neurons after ischemic stroke. On the other hand, it plays a potential role in nerve repair. Therefore, regulating the inflammatory response in the brain after ischemic stroke is considered to be one of the effective strategies for the treatment of the disease. Many kinds of cells in the brain participate in the occurrence and development of neuroinflammation after cerebral ischemia. Among them, microglia, as the primary immune cells in the brain, play a very important role in resisting brain injury.

When inflammatory substances enter the brain parenchyma via BBB, microglia that accumulate around damaged blood vessels are over-activated and then phagocytose the damaged vascular endothelial cells, thereby further destroying BBB (Carrizales-Sepúlveda et al. 2018; Hansen 2018). Inflammatory mediators released by microglia, such as IL-1β, IL-6 and TNF-α, further aggravate local ROS-induced oxidative stress (Golia et al. 2014).

Omentin-1 plasmid increased Omentin-1 mRNA expression levels, and si-omentin-1 mimics reduced Omentin-1 mRNA expression levels in vitro model (1.107±0.500 vs 10.073±3.848, 1.142±0.601 vs 0.645± 0.254 vs 0.389±0.207 vs 0.203±0.082). In LPS-induced BV2 cells, omentin-1 not regulated IL-1β levels, however, omentin-1 reduced IL-1β levels in LPS+ATP-induced BV2 cells (75±24.49 vs 73.333±33.99 vs 95±36.28590176 vs 103.33±15.45 vs 586.67±61.28 vs 285±59.30). In LPS-induced BV2 cells, si-omentin-1 not regulated IL-1γb levels, and omentin-1 promoted IL-1β levels in LPS+ATP-induced BV2 cells (95±10.8 vs 105±8.16 vs 100±14.72 vs 98.33±28.67441756 vs 526.67± 76.63 vs 1143.33± 140.79). Over-expression of omentin-1 reduced IL-6, IL-10, INF-γ and TNF-α levels in LPS+ATP-induced BV2 cells (100±12.27 vs 33.5±12.57, 545±57.15 vs 81.67±28.67, 266.67±45.89 vs 120±26.77, 201±40.10 vs 73±17.20). Down-regulation of omentin-1 promoted IL-6, IL-10, INF-γ and TNF-α levels in LPS+ ATP-induced BV2 cells (110±23.95 vs 233±39.20, 515±22.73 vs 1190±114.96, 365±83.37 vs 863.33± 147.44, 200±25.14 vs 606±32.03).

Additionally, Rao et al. suggest that omentin-1 prevented inflammatory bone diseases (Rao et al. 2018). Therefore, the researchers speculate that Omentin-1 also suppressed inflammation to prevent cardiovascular and cerebrovascular diseases in acute cerebral infarction.

Existing reports show that NLRP3 inflammasome plays an important role in the pathophysiological development of ACI in various organs and is the main component mediating inflammatory response (Nevulis et al. 2018; Nuñez et al. 2021). NLRP3, as the core component of NLRP3 inflammasome, is the key regulatory protein in NLRP3 inflammasome promoting inflammatory response (Zhang et al. 2021a). At rest, the content of NLRP3 protein in tissue cells is very low, and most of them are in a stable state of ubiquitination inactivity. When NLRP3 increases sharply, it can effectively activate inflammasome (Nevulis et al. 2018; Nuñez et al. 2021).

Next, these results of this experiment indicated that over-expression of Omentin-1 induced Omentin-1 and p-AMPK protein expression levels, and suppressed NLRP3 protein expression level in vitro model (1.210±0.209 vs 3.753±0.551, 1.143±0.102 vs 2.679±0.332, 0.937±0.045 vs 0.304± 0.149).

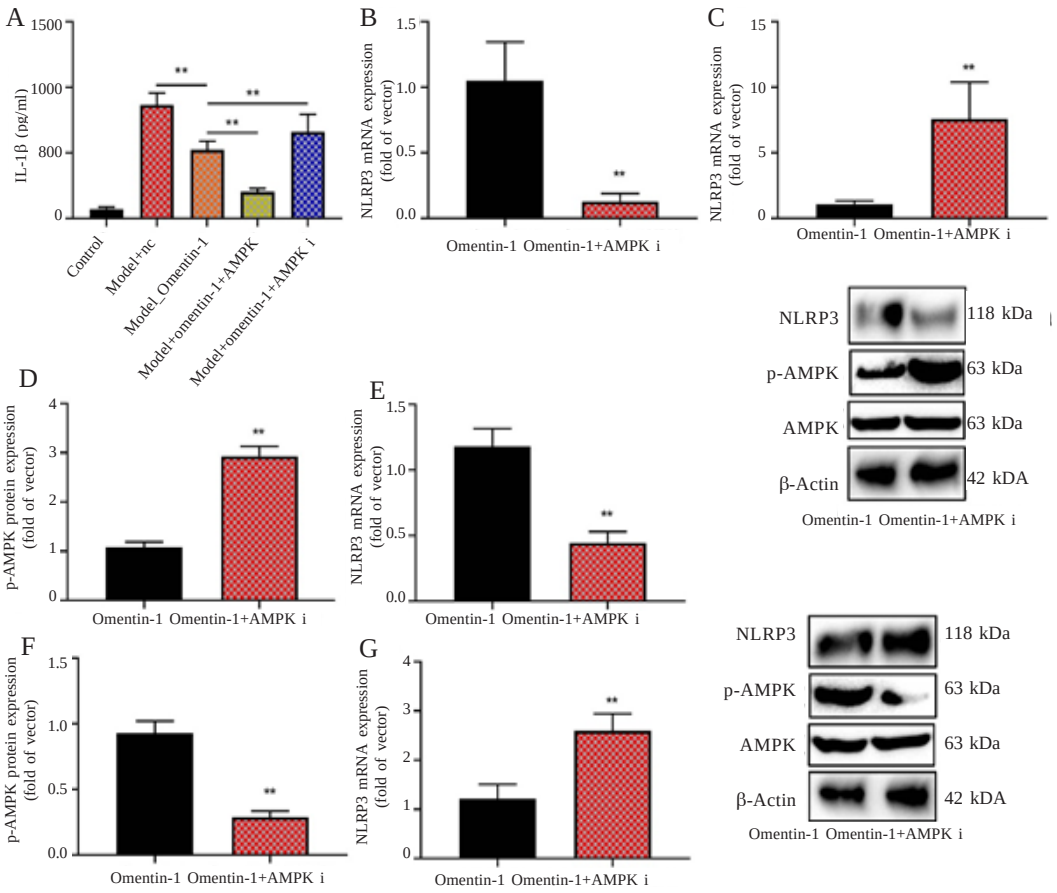

Fig. 6. AMPK affected the effects of Omentin-1 in vitro model
Serum IL-1β levels (A), NLRP3 mRNA expression (B and C), p-AMPK and NLRP3 protein expression in brain tissue by Omentin-1 and AMPK agonist (D and E), p-AMPK and NLRP3 protein expression in brain tissue by Omentin-1 and AMPK inhibitor (F and G)
Omentin-1, mice model of ACI with Omentin-1; AMPK, AMPK agonist; AMPK i, AMPK inhibitor
****p<0.01 compared with mice model of ACI with Omentin-1group**
Source: **Author**

Zhou et al. suggested that omentin-1 restrains TXNIP/NLRP3 signaling pathway to reduce adipose tissue inflammation (Zhou et al. 2020). This may, in part, NLRP3 is one anti-inflammatory target for Omentin-1 on the prevention of cardiovascular and cerebrovascular diseases in acute cerebral infarction.

AMPK induces the activation of NLRP3 inflammatory complex by reducing intracellular K+ concentrations and mediating mitochondrial damage to promote the release of ROS-producing cytoplasm (Zhang et al. 2020). Antioxidants with the potential to induce AMPK activation can inhibit the activation of NLRP3 complex. Endogenous antioxidant biosynthesis regulated by AMPK may be a protective target for inhibiting aging mitochondrial ROS (Dong et al. 2020).

The researchers used AMPK agonist (7 mmol/kg of Buformin) to reduce the serum IL-1β levels (70±15.41 vs 865±92.13 vs 522.5±65.48 vs 202.5±24.49 vs 660±132.90) and NLRP3 mRNA expression levels (1.049±0.29 vs 60.128±0.060, P<0.05), AMPK inhibitor (10 mg/kg of Dorsomorphin dihydrochloride) increased the serum IL-1β levels

and NLRP3 mRNA expression levels (1.045±0.282 vs 7.578±2.811) in mice with ACI by Omentin-1. In mice with ACI, AMPK agonist (7 mmol/kg of Buformin) suppressed NLRP3 protein expression (1.181±0.136 vs 0.446±0.085), and induced p-AMPK protein expression (1.073± 0.116 vs 2.930±0.202) in mice with acute cerebral infarction by Omentin-1 (Fig. 6D-6E). AMPK inhibitor suppressed p-AMPK protein expression (0.928±0.092 vs 0.288±0.046) and induced NLRP3 protein expression (1.212±0.297 vs 2.599±0.345, $P<0.05$) in mice with acute cerebral infarction by Omentin-1.

The researchers also observed that AMPK affected the effects of Omentin-1 in vitro model. Liu et al. indicated that omentin-1 induced AMPK/PPARδ pathway to reduce vascular endothelial dysfunction by high glucose (Liu et al. 2020). Thus, it is possible that the effects of Omentin-1/ AMPK axis may be the prevention of cardiovascular and cerebrovascular diseases in acute cerebral infarction.

CONCLUSION

Omentin-1 mRNA expression levels in patients or mice model of ACI were reduced. The mRNA of Omentin-1 was negative correlation with NIHSS score or serum IL-1β levels in patients with intravenous thrombolysis of ACI. The mRNA of Omentin-1 was positive correlation with Barthel index in patients with intravenous thrombolysis of ACI. Omentin-1 induced AMPK/ NLRP3 signaling pathway in model of ACI.

RECOMMENDATIONS

Therefore, the researchers speculate that Omentin-1 may be clinical significance for cardiovascular and cerebrovascular diseases, a potential treatment strategies of cardiovascular and cerebrovascular diseases in acute cerebral infarction.

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