Importance of PLC-Dependent PI3K/AKT and AMPK Signaling in RANTES/CCR5 Mediated Macrophage Chemotaxis

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Abstract

Regulated upon activation, normal T cell expressed, and secreted (RANTES), also known as chemokine ligand 5 (CCL5), has been reported to facilitate macrophage migration, which plays a crucial role in tissue inflammation. The aim of this study is to investigate the characteristics and underlying mechanism of RANTES on macrophage chemotaxis under physiological and pathological conditions. The study was conducted on macrophage RAW264.7 cell and bone marrow-derived macrophages (BMDM) isolated from CCL receptor 5 (CCR5) knockout mice. The macrophage migration and glucose uptake was assessed in time and dose dependent manners. Moreover, reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis were used to characterize mRNA and protein level related to the underlying mechanism. The present result showed that the maraviroc, a selective CCR5 inhibitor, dose-dependently suppressed RANTES-induced rapid increases in glucose uptake and cell migration in RAW264.7 cells. Similar effects were observed in the BMDM isolated from CCR5 knockout mice compared with wild type control. RANTES treatment promptly enhanced membrane glucose transporter 1 (GLUT1) expression, glucose uptake as well as phosphorylation of AKT on Thr308, Ser473 within min and has prolonged effect on phosphorylation of AMPK on Thr172, which were abrogated by maraviroc, CCR5 siRNA or phospholipase C (PLC) inhibitor in RAW264.7 cells. Inhibition of PI3K and AMPK by LY294002 and Compound C significantly suppress RANTES-stimulated macrophage glucose uptake and migration, respectively. RANTES has biphasic effect on activating PLC signaling including prompt action on PI3K/AKT phosphorylation and prolong action
Introduction

Macrophage infiltration plays a critical role in tissue inflammation. While human body is attacked by pathogens or in the development of obesity-associated adipose tissue inflammation, it could induce the immune responses and lead to chemokine secretion in inflammatory tissue. Chemokines attract macrophage to inflammatory site by binding the G-couple protein receptor and activating downstream signaling (20). It has been demonstrated that several chemokines are important in macrophage chemotaxis. For instance, chemokine MCP-1^-/- (17) and CCR2^-/- (34, 37) mice showed lowering macrophage infiltration in adipose tissue. However, the time and dose dependent effects of chemokines on macrophage chemotaxis remain elusive, which is important to determine the pathophysiological role of chemokines in the regulation of body inflammatory response.

Regulated upon activation, normal T cell expressed, and secreted (RANTES), also known as chemokine ligand 5 (CCL5), is a pro-inflammatory chemokine. By binding its receptors CCR1, CCR3 and CCR5, it could activate many downstream signaling like phosphoinositide 3-kinase (PI3K), RhoA, phospholipase C (PLC), AKT and AMP-activated protein kinase (AMPK) to regulate cell reactions such as T cell migration, tumor growth and cell apoptosis (3, 5, 6, 14, 33, 36). Accumulating evidence has also implicated that the imperial role of RANTES for macrophage chemotaxis. For example, the concentration of RANTES has a positive correlation with CD11b positive cells in adipose tissue of human. Moreover, RANTES has been demonstrated to increase macrophage infiltration into human adipose tissue (18, 24, 38). On the other hand, RANTES recruits circulating immune cells and augments acute inflammatory responses in many clinical diseases such as atherosclerosis, stroke, myocardial and acute kidney ischemia reperfusion injury (IRI). RANTES has been demonstrated to play a dominant role in infiltrating inflammatory cells and concomitantly promotes the release of other inflammatory cytokines in the site of post-ischemia inflammatory injury (2, 23, 29, 31, 35, 40).

However, it remains unclear that the cellular and molecular mechanisms under the chemotaxis effect of RANTES on immune cell infiltration under acute and chronic tissue IRI.

Key Words: CCR5, macrophage chemotaxis, phospholipase C, RANTES
and promptly increase AKT and AMPK phosphorylation time-dependently via activating PLC-mediated pathway, which leads to increase the GLUT1-mediated glucose uptake to facilitate macrophage migration in the absence of activating inflammatory signaling. Our finding provides compelling evidence to clarify the pathophysiological role of RANTES on macrophage recruitment and tissue inflammation.

**Materials and Methods**

**Animal**

C57BL/6J wild type and C57BL/6J background CCR5 knockout (CCR5−/−) mice were obtained from Jackson Lab. The mice were housed in an animal center certified by Association of Assessment and Accreditation of Laboratory Animal Care. All animals were handled according to the guidelines and manual of the animal care and use committee of this institute.

**Bone Marrow-Derived macrophages (BMDM)**

Primary BMDM of wild type and CCR5−/− mice were obtained from the following protocol (11). Briefly, mouse was euthanized by overdose anesthesia and removed limb and leg muscles to obtain femurs and tibias. The 25G needle filled with cold Dulbecco’s phosphate-buffered saline (PBS) was inserted into bone marrow cavity of femur and tibia and injected to pour out monocytes. The suspension was collected, centrifuged and removed supernatant. The suspension was added to DMEM/F12 supplement with M-CSF (Peprotech) for 7 days.

**Cell Culture and Reagent**

Murine RAW264.7 cells were purchased from Food Industry Research and Development Institute (FIRDI) in Taiwan and cultured in DMEM supplement with 10% FBS and 1% penicillin/streptomycin (GIBCO) at 37°C in a humidified incubator with 5% CO2. Recombinant mouse RANTES was purchased from R&D Systems. Maraviroc, U 73122 and 2-deoxy glucose were purchased from Tocris, 3H-2-Deoxy-D-glucose was purchased from Perkin Elmer Life Sciences. Inhibitors LY294002, a PI3K inhibitor, and Compound C, an AMPK inhibitor, were purchased from Sigma. Antibodies for phospho-AKT (Thr308, Ser473), phospho-AMPK (Thr172), AKT and AMPK were purchased from Cell Signaling Technology. Antibody for GLUT1 was purchased from GeneTex. Antibodies for mouse CCR1, CCR3, CCR5 and beta-actin were purchased from Novus Biologicals. Protein Extraction kit was purchased from Thermo Scientific Inc.

**siRNA Transfection**

An anti-CCR5 siGenome smartpool siRNA was purchased from Dharmacon. For transfection, RAW264.7 cells were seeded in an antibiotic-free medium well for 24 h before the experiment. Cells were transfected with siRNA (25 nM) and dharmaFECT transfection reagent in serum-free medium according to the manufacturer’s instructions. Cells were incubated at 37°C in 5% CO2 for 48 h and thus CCR5 mRNA expression was measured by real-time polymerase chain reaction (RT-PCR).

**Western Blot**

Cells were incubated with RANTES in different time courses (5, 10, 30, 60, and 240 min) and concentration (0.1, 1, 10 and 100 nM) and lysed with lysis buffer (50 mM Tris-base). Protein concentration was determined using Bio-Rad protein assay (Bio-Rad). 35 μg of proteins were denatured, and dissolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane, and blocked with 5% milk in Tris buffered saline with Tween 20 (TBST) for 1 h at room temperature. After wash for 3 times with TBST, proteins were probed with primary antibodies in TBST for overnight at 4°C. Next day, proteins were probed with secondary antibodies (1:10000) in TBST for 1 h at room temperature. After adding ECL, proteins were detected by using UVP BioSpectrum 500 Imaging System. A CCR5 or PLC inhibitor was added 1 h before administration of RANTES.

**Migration Assay**

Macrophage migration assay was conducted with 24-well Transwell chambers with 8-μm pores (Corning). In brief, macrophages were seeded in the upper chamber. Different concentrations of RANTES were added to the lower wells, and the chambers were incubated for 4 h at 37°C. Then the cells were fixed in 4% paraformaldehyde, and migrated macrophages were stained with crystal blue in PBS for 20 min. Cells in the bottom of upper chamber were counted with microscope. Each chamber was counted in triplicate in each experiment and each experiment was repeated three times.

**Glucose Uptake Assay**

1 × 10⁶ RAW264.7 cells and BMDM were cultured in 12 well dishes, respectively. In the beginning, the cells were washed with PBS and then replaced medium with 1 ml Krebs-Ringer-Phosphate-HEPES
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(KRPH) for 2 h. $^3$H-2-Deoxy-D-glucose (1 $\mu$Ci/ml) was added in the presence of RANTES for 10 min. Cells were washed with ice-cold PBS, and added cold 0.2 M NaOH to solubilize cells. Radioactivity was measured by using a liquid scintillation counter. A CCR5 or PLC inhibitor was added before the administration of RANTES and $^3$H-2-Deoxy-D-glucose.

Statistical Analysis

Statistical analysis was performed according to the repeated-measure one-way analysis of variance followed by Bonferroni's test. A level of $P < 0.05$ was considered to have a significant difference between the mean values. The values are given as means ± standard error of the mean (SEM).

**Results**

**Time and Dose Dependent Effects of RANTES on Macrophage Glucose Uptake**

After the addition of 10 $\mu$M RANTES, the glucose uptake of RAW264.7 cells was significantly increased, and the maximal uptake was shown after RANTES administration for 10 min and lasted for at least 240 min (Fig. 1A). Different concentrations of RANTES also enhanced glucose uptake in RAW264.7 cells and reached maximal uptake in 10 nM (Fig. 1B). Accordingly, the experiment conducted with BMDM showed the similar trend as those in RAW264.7 cells (Fig. 1, C and D), suggesting that the increase of RANTES level under pathological condition could promptly increase macrophage glucose uptake within min.

While co-treated with 2-deoxy glucose (2-DG), the effect of RANTES on macrophage migration is significantly decreased in a dose-dependent manner (Fig. 1E). Furthermore, the RANTES-stimulated migration was abrogated in BMDM after treating 2-DG 100 mM (Fig. 1F).

**CCR5 Receptor is Crucial in RANTES-Induced Macrophage Migration and Associated Glucose Uptake**

In migration assay as shown in Fig. 2A, inhibition of CCR5 with maraviroc could reduce the ability of migration in a dose-dependent manner and this phenomenon was also shown in those pretreated with CCR5 siRNA. Accordingly, the RANTES-induced migration ability of CCR5$^{-/-}$ BMDM also decreased compared to that in wild type BMDM (Fig. 2B), suggesting that RANTES could stimulate macrophage migration through CCR5 receptor.

Consistently, after treating maraviroc (a selective CCR5 antagonist), the glucose uptake of RAW264.7 cells was significantly reduced in a dose-dependent manner (Fig. 2C). Accordingly, knockdown of CCR5 gene expression by using siRNA method also significantly reduced macrophage glucose uptake (Fig. 2C). Furthermore, we compared glucose uptake of BMDM isolated from wild-type and CCR5$^{-/-}$ mice. Our result showed that glucose uptake of CCR5$^{-/-}$ BMDM was significantly lower than that in wild-type BMDM under treatment with 0.1, 1 and 10 nM RANTES (Fig. 2D), indicating that CCR5 is crucially involved in RANTES-stimulated macrophage glucose uptake, which is essential for chemotactic response of macrophages.

**Dose and Time Dependent Effects of RANTES on PI3K/AKT and AMPK-Mediated Signaling**

Whether RANTES could enhance AKT or AMPK phosphorylation in RAW264.7 cells was examined. As shown in Fig. 3A to 3D, RANTES increased AKT phosphorylation on Thr308 and Ser473 and AMPK phosphorylation on Thr172 in different time-dependent manners. The AKT phosphorylation reached peak after adding RANTES 10 nM for 10 min, which is similar to the pattern of RANTES-stimulated glucose uptake (Fig. 2). Nevertheless, AMPK phosphorylation gradually increased by time after adding RANTES 10 nM for 60 min. On the other hand, RANTES dose-dependently increased AKT (Thr308, Ser473) and AMPK (Thr172) phosphorylation before reached the peak level (Fig. 3, F-H). The maximum augmentation was exhibited in those with RANTES 10 nM, which was also identical to that of RANTES-stimulated glucose uptake (Fig. 2).

In addition, LY294002 (PI3K inhibitor) and Compound C (AMPK inhibitor) were used to evaluate the casual relationship of PI3K/AKT and AMPK pathways in RANTES-induced macrophage migration. Our data showed that the macrophage migration and associated glucose uptake induced by RANTES 10 nM significantly decreased after the administration of LY294002 and Compound C compared to those of control group (Fig. 4, A and B). These data suggested that RANTES could facilitate macrophage migration by activating PI3K/AKT and AMPK-mediated signaling in different time-dependent patterns.

**CCR5 Receptor Activation is Crucial for RANTES-Induced PI3K/AKT and AMPK-Mediated Signaling**

The effect of RANTES on AKT and AMPK phosphorylation of AKT Thr308, Ser473, and AMPK Thr172 significantly reduced after treating maraviroc 10 mM, indicating that CCR5 receptor plays an important link between RANTES and acti-
Fig. 1. RANTES facilitate macrophage migration through increased its glucose uptake. (A-B) Time and concentration-dependent effect of glucose uptake treated with RANTES in RAW264.7 cells. (C-D) Time and concentration-dependent effect of RANTES-treated glucose uptake in BMDM obtained from C57BL/6J mice. (E) The concentration-dependent effect of 2-DG in RANTES-induced migration in RAW264.7 cells. (F) The effect of 2-DG in RANTES-induced migration in BMDM obtained from C57BL/6J mice. Values are expressed as means ± SEM. *P < 0.05 vs. control, †P < 0.05 vs. RANTES-treated cell.
Fig. 2. CCR5 receptor is crucial in RANTES-induced macrophage glucose uptake and migration.  (A) The effect of maraviroc and CCR5 siRNA on the glucose uptake in RAW264.7 cells.  (B) The comparison of glucose uptake between wild-type and CCR5-/- mice treated with RANTES 10 nM.  (C) The effect of maraviroc and CCR5 siRNA on migration and CCR5 gene expression after administration of CCR5 siRNA in RAW264.7 cells.  (D) CCR5-/- mice migration ability compared to wild-type mice treated with RANTES 10 nM. Values are expressed as means ± SEM.  *P < 0.05 vs. control, †P < 0.05 vs. RANTES-treated only group.
Fig. 3. RANTES enhance AKT Ser473, Thr308 and AMPK Thr172 phosphorylation in RAW264.7 cells. (A-D) immunoblotting on AKT Ser473, Thr308 and AMPK Thr172 phosphorylation in time-dependent manner treated with RANTES 10 nM. (E-H) immunoblotting on AKT Ser473, Thr308 and AMPK Thr172 phosphorylation in concentration-dependent manner treated with RANTES in 10 min. Values are expressed as means ± SEM. *P < 0.05 vs. control.
**Fig. 4.** Inhibition of PI3K and AMPK reduce RANTES-induced macrophage glucose uptake and migration in RAW264.7 cells. (A) The effect of LY and CC on the RANTES-stimulated glucose uptake. (B) The effect of LY and CC on the RANTES-stimulated migration. LY: LY294002, CC: Compound C. Values are expressed as means ± SEM. *P < 0.05 vs. control. †P < 0.05 vs. RANTES-treated only group.

**Fig. 5.** CCR5 inhibition decrease AKT Ser473, Thr308 and AMPK Thr172 phosphorylation. (A-D) immunoblotting on RANTES-stimulated AKT Ser473, Thr308 and AMPK Thr172 phosphorylation treated with selective CCR5 antagonist maraviroc in RAW264.7 cells. Values are expressed as means ± SEM. *P < 0.05 vs. control. †P < 0.05 vs. RANTES-treated only group.
vation of PI3K/AKT and AMPK pathways as shown in Fig. 5A to 5D.

**Stimulatory Effect of RANTES on Membrane Protein Expression of GLUT1 in RAW264.7 Cells**

The main glucose transporter of macrophage is GLUT1 protein. Previous report showed that LPS could increase the expression of GLUT1 to enhance macrophage glucose uptake (25). Our results demonstrated that RANTES significantly enhanced membrane protein expression of GLUT1 in a time-course manner and reached peak after 10 min (Fig. 6, A and B). Additionally, RANTES-stimulated GLUT1 membrane protein expression was significantly increased in dose-dependent manner, with a maximum in 10 nM (Fig. 6, C and D), consistent with RANTES-mediated glucose uptake, suggesting that RANTES could enhance GLUT1 expression to increase macrophage glucose uptake.

**Induced PI3K/AKT and AMPK-Mediated Signaling and Macrophage Migration**

PLC is an important regulator enzyme in CCR5 downstream signaling pathway. Previous report showed that RANTES/CCR5 could activate PLC to increase matrix metallopeptidase 9 (MMP-9) expression to promote migration of cancer cell (22). The present result showed that the augmentation of RANTES-induced macrophage migration and associated glucose uptake significantly diminished after treating PLC inhibitor U 73122 (Fig. 7, A and B). In addition, the increases in the phosphorylation of AKT Thr308, Ser473 and AMPK Thr308 were all reversed after adding U 73122 (Fig. 7, C-E). Our observation suggested that PLC activation play a critical upstream regulator in RANTES-stimulated AKT and AMPK phosphorylation and subsequent macrophage migration.

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Fig. 7. Inhibition of phospholipase C (PLC) reduced macrophage glucose uptake and migration. (A) The effect of U 73122 on the glucose uptake in RAW264.7 cells treated with RANTES 10 nM. (B) The effect of U 73122 on the glucose uptake in RAW264.7 cells treated with RANTES 10 nM. (C-E) Immunoblotting on RANTES-stimulated AKT Ser473, Thr308 and AMPK Thr172 phosphorylation treated with U 73122 in RAW264.7 cell. U 73122: PLC inhibitor. Values are expressed as means ± SEM. *P < 0.05 vs. control. +P < 0.05 vs. RANTES-treated only group.

**Pathways Were not Involved in Acute Effect of RANTES-Stimulated Glucose Uptake and Migration**

Macrophage glucose uptake could be enhanced under inflammatory condition. To examine whether
the RANTES could activate the inflammatory signaling pathway to increase glucose uptake, we measured the activation of NF-κB and ERK, which were two important inflammatory pathways in macrophages. Our data showed that RANTES 10 nM did not significantly enhance phosphorylation of NF-κB and ERK in a time-dependent manner (Fig. 8, A-C), suggesting that RANTES-stimulated macrophage migration and associated glucose uptake are not mediated by activating inflammatory signaling pathways in current experimental condition.

Discussion

Macrophage infiltration plays a critical role in tissue inflammation. This study provides new insight to elucidate the characteristics and possible mechanism underlying the dose- and time-dependent effect of RANTES on macrophage chemotaxis. The present result demonstrated that RANTES could promptly initiate the effect on macrophage chemotaxis without activating inflammatory signaling pathway and further clarify the underlying mechanism that RANTES through CCR5 receptor to activate PLC and downstream PI3K/AKT and AMPK signaling pathways, which result in augmentation of GLUT1-mediated glucose uptake and macrophage migration. In particular, the PLC-dependent PI3K/AKT signaling is crucial for the prompt effect of RANTES/CCR5 signaling on macrophage chemotaxis. The PLC-dependent AMPK signaling is progressively increased in time and dose dependent manner, which might play an important role in RANTES-induced macrophage migration under inflammatory condition. It is the first study to demonstrate the functional effect of RANTES on macrophage migration under physiological and pathological conditions.

In the present result, RANTES could promptly initiate its chemotaxic effect on macrophages indicated by rapid increase in macrophage glucose uptake with the maximum at 10 min and also lasting at least 240 min, indicating that elevated tissue RANTES level is crucially involved in the regulation of macrophage migration during the states of acute
and chronic inflammation. In addition, the *in vitro* experiment showed that the given physiological levels of RANTES could significantly increase macrophage glucose uptake indicated its action on chemotaxis and maximal chemotactic effect of RANTES on macrophages took place at 10 nM. Compared to the RANTES concentration in mice measured by enzyme-linked immunosorbent assay (ELISA) in our lab (data not shown), 10 nM is higher than physiological concentration (about 0.5-1 nM) in mice, indicating that the pathological elevation of tissue RANTES level such as in the state of obesity or inflammatory diseases is important for the recruitment of macrophage into the inflamed tissues. On the other hand the chemotaxis effect of RANTES on cell migration was different in different immune cells and cell lines. For instance, RANTES could increase about 40-50% cell migration in RAW264.7. RANTES could increase 120% cell migration in isolated BMDM with the same dose. Nevertheless, these results showed the similar trend about the promoting effect of RANTES on macrophage chemotaxis.

Previous investigation demonstrates that obesity-induced macrophage infiltration in adipose tissue was significantly reduced in high fat-fed CCR5 knockout mice compared to that in wild-type mice (20). However, the direct casual relationship between RANTES receptor CCR5 and macrophage chemotaxis remains unknown. Our data further suggest that RANTES could rapidly increase macrophage glucose uptake to facilitate macrophage migration through activating CCR5 receptor-mediated signaling.

On the other hand, the present result further demonstrated that RANTES could promptly stimulate Thr308 and Ser473 phosphorylation on AKT and blockade of signaling by PI3K/AKT significantly reduced macrophage glucose uptake and migration, indicating the importance of the PI3K/AKT-mediated pathway in this rapid response of RANTES-stimulated macrophage chemotaxis. In addition, AMPK also plays an important role in the regulation of energy metabolism. When energy is deficient, AMPK will be phosphorylated and activate downstream signaling such as inhibition of cholesterol synthesis and stimulation of glucose uptake. Previous investigation has reported the deterioration of insulin resistance was deteriorated in AMPK deficient mice with high fat diet feeding, indicating the importance of AMPK in the regulation of glucose metabolism. The present result demonstrated that RANTES would enhance AMPK phosphorylation at Thr172 and reached the plaque at 60 min after treatment. The different time effects of RANTES-stimulating PI3K/AKT and AMPK pathways might account for the acute and time delayed effects of RANTES-mediated glucose uptake and migration under physiological and pathological conditions. The detail mechanism is still needed to be further investigated. In addition, our result also demonstrated that CCR5 inhibition could significantly suppress AKT and AMPK phosphorylation, especially Thr308 phosphorylation site of AKT, indicating that the important role of CCR5 receptor in this RANTES-mediated activation of PI3K/AKT and AMPK pathways.

GLUT1 maintains basal glucose metabolism in many tissues. Previous investigation has demonstrated that LPS could enhance macrophage glucose uptake by increasing GLUT1 expression (11). GLUT1 has also been reported to play a crucial role in RANTES-induced glucose uptake in T-cell (5), indicating the importance of GLUT1 in immune cell glucose uptake. In the present result, we demonstrated that RANTES-associated GLUT1 translocation has similar response pattern as RANTES-induced glucose uptake in time and dose-dependent manners, indicating that RANTES could stimulate macrophage glucose uptake by enhancing membrane GLUT1 protein expression.

Compared with previous report (5) about RANTES effect on activated human peripheral blood-derived T cell chemotaxis, our observation suggested that macrophages are more sensitive to the change of RANTES levels than T cells, implicating that the RANTES might involve in the regulation of macrophage migration not only in the pathological condition but also in the physiological condition. Accordingly, the mechanisms underlying their chemotaxic action are different. Based on the time and dose dependent results of RANTES on macrophage chemotaxis, the activation of PI3K/AKT signaling seems to account for the rapid physiological action of RANTES and the activation of AMPK signaling account for the pathological effect of RANTES on macrophage. However, the discrepancy of these two studies such as species, cells, treatment and protocol design, experimental model and observed parameters could affect the data interpretation and the comparison. It is needed to be further investigated.

PLC is an important effector for transferring CCR5 receptor-mediated signaling. Previous article has shown that PLC pathway is crucially involved in insulin-dependent glucose uptake (16). On the other hand, PLC-induced calcium variation has been reported to affect calcium/calmodulin-dependent protein kinase kinase (CaMKK), which is an important upstream regulator of AMPK (1, 9, 15, 39). Our observation further suggests that PLC is an upstream regulator of RANTES-induced phosphorylation of AKT and AMPK, which subsequently affect macrophage glucose uptake and chemotaxis.

Inflammation has been reported to affect
glucose metabolism. Previous study showed that RANTES would enhance NF-κb and ERK phosphorylation in cancer cell (14, 36). In the given concentration and observed time course of our experiment, the phosphorylation of NF-κb and ERK fail to change in the dose- and time-dependent manners. It is suggested that RANTES-mediated glucose uptake was not indirectly through activating the cellular inflammatory response during the current experimental condition. Collectively, we not only characterize time course and dose-dependent relationship of RANTES-directed macrophage migration but also clarify the possible inflammatory independent mechanism.

**Conclusion**

In summary, we demonstrate RANTES/CCR5 signaling has biphasic effect on macrophage chemotaxis through increasing glucose uptake by activating PLC-mediated PI3K/AKT and AMPK signaling in different time-dependent patterns. This article provides new insight to clarify the regulatory role of RANTES on macrophage migration under physiological and pathological conditions.

**Acknowledgments**

This work was funded by the National Science Council of the R.O.C. under NSC 102-2320-B-016-009-MY3 and the Tri-Service General Hospital under TSGH-C104-007-S01. This work also supported by the Armed Forces Taichung General Hospital, Taichung, Taiwan under 104B04 and Chi-Mei Medical Center, Tainan, Taiwan under CMNDMC10303.

**Conflict of Interests**

The authors declare that there are no conflicts of interests.

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