Biochemical and Biophysical Research Communications 445 (2014) 645–650

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Clusterin stimulates the chemotactic migration of macrophages through a pertussis toxin sensitive G-protein-coupled receptor and $G_{\beta\gamma}$-dependent pathways

Byeong-Ho Kang [a,1], Young-Jun Shim [a,1], Yoo-Keung Tae [a], Jin-A Song [a], Byong-Kwan Choi [b], In-Sun Park [c], Bon-Hong Min [a,*]

[a] Department of Pharmacology, College of Medicine, Korea University, Seoul, Republic of Korea
[b] Department of Internal Medicine, College of Medicine, Dongguk University Ilsan Hospital, Republic of Korea
[c] Department of Anatomy, College of Medicine, Inha University, Republic of Korea

ARTICLE INFO

Article history:
Received 12 February 2014
Available online 22 February 2014

Keywords:
Clusterin
Chemotaxis
Macrophage
Pertussis toxin
G-protein-coupled receptor

ABSTRACT

Clusterin induces the expression of various chemotactic cytokines including tumor necrosis factor-α (TNF-α) in macrophages and is involved in the cell migration. According to the results of this study, clusterin induced the directional migration (chemotaxis) of macrophages based on a checkerboard analysis. The chemotactic activity of clusterin was prevented by pretreatment with pertussis toxin (PTX), indicating that the $G_{\alpha i/o}$-protein coupled receptor (GPCR) was involved in the chemotactic response of clusterin. Clusterin-stimulated chemotaxis was abrogated in a dose-dependent manner by pretreatment with gallein (a $G_{\beta\gamma}$ inhibitor), indicating the involvement of $G_{\beta\gamma}$ released from the GPCR. In addition, inhibitors of phospholipase C (PLC, U73122) and phosphoinositide 3-kinase (PI3K, LY294002), the key targets of $G_{\beta\gamma}$ binding and activation, suppressed chemotactic migration by clusterin. The phosphorylation of Akt induced by clusterin was blocked by pretreatment with gallein or LY294002 but not with U73122, indicating that $G_{\beta\gamma}$ released from the PTX-sensitive G_i protein complex activated PLC and PI3K/Akt signaling pathways separately. The activation of cellular MAP kinases was essential in that their inhibitors blocked clusterin-induced chemotaxis, and $G_{\beta\gamma}$ was required for the activation of MAP kinases because gallein reduced their phosphorylations induced by clusterin. In addition, the inflammation-induced migration of macrophages was greatly reduced in clusterin-deficient mice based on a thioglycollate-induced peritonitis model system. These results suggest that clusterin stimulates the chemotactic migration of macrophages through a PTX-sensitive GPCR and $G_{\beta\gamma}$-dependent pathways and describe a novel role of clusterin as a chemoattractant of monocytes/macrophages, suggesting that clusterin may serve as a molecular bridge between inflammation and its remodeling of related tissue by recruiting immune cells.

1. Introduction

Clusterin is a secreted multifunctional glycoprotein expressed ubiquitously in a wide variety of tissues [1,2]. This disulfide-linked glycoprotein has been implicated in diverse age-related diseases such as neurodegenerative diseases, atherosclerosis, and tumor progression [3,4]. In addition, clusterin is dramatically increased in the involuting mammary gland after weaning [5], in the regressing ventral prostate [6], and in ventricular myocytes during myosin-induced myocarditis [7], presumably as a protective mechanism from tissue damage. Most of the aforementioned pathological conditions in which clusterin has been implicated are characterized by increased oxidative stress and chronic inflammation. The intriguing multifunctions of clusterin have been found to depend mainly on the existence of secretory glycosylated and intracellular nonglycosylated forms and their cellular location [8]. The secretory glycosylated form of clusterin has been extensively studied as a major protein, and it is generally accepted to play an important role in the cytoprotection of cells from oxidative injury through a small heat shock protein-like stress-induced chaperone activity [9]. However, no study has examined clusterin's functions except for its extracellular chaperone activity.

Monocytes/macrophages are predominant immune cells that infiltrate areas of inflammation, and macrophages are generally be-

* Corresponding author. Address: Department of Pharmacology, College of Medicine, Korea University, 126-1 Anam-Dong 5Ga, Seongbuk-Gu, Seoul 136-705, Republic of Korea. Fax: +82 2 927 0824.
E-mail address: bhmin@korea.ac.kr (B.-H. Min).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.02.071
0006-291X/

lieved to play important roles in the elimination of invading pathogens, tissue remodeling, and metastasis by secreting various growth factors, proteases, and cytokines [10,11]. Resident macrophages in tissues are derived mainly from circulating blood monocytes, and their migration is known to be mediated by a number of chemotactic cytokines [12]. The upregulation of clusterin in tissues with inflammatory processes has led to the hypothesis that clusterin functions as an immune modulator for the recruitment and activation of macrophages in tissues when the degradation of the extracellular matrix (ECM), the secretion of cytokines, and cell migration occur actively. Indeed, clusterin has been demonstrated to induce and activate matrix metalloproteinase-9 expression in macrophages [13]. In addition, clusterin has been shown to upregulate the expressions of chemotactic cytokines including tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1β (MIP-1β) in Raw264.7 cells [14], which are all known chemoattractants of macrophages [15,16]. Further, clusterin-induced TNF-α secretion has been found to play a critical role in the migration of Raw264.7 macrophages [14], although the involvement of other chemokines in clusterin-enhanced macrophage migration cannot be ruled out. These findings indicate a need to investigate whether the observed migration by clusterin is based on chemotactic activity by the gradient of clusterin itself or the enhanced movement in all directions by clusterin-induced cell activation and to determine which signaling pathways are involved.

2. Materials and methods

2.1. Reagents and antibodies

The PLC inhibitor U73122 or its inactive analog U73343 and pertussis toxin (PTX) obtained from TOCRIS Bioscience (Ellisville, MS) were used. PD98059, SB202190, SP600125, and LY294002 were from Calbiochem (San Diego, CA), and antibodies against ERK1/2, p38, JNK, Akt total proteins or the corresponding phospho-specific antibodies were purchased from Cell Signaling Technology (Beverly, MA).

2.2. Cell culture

Murine macrophage cell lines Raw 264.7 and J774A.1, human peripheral blood monocytes, and mouse peritoneal macrophages were grown in an appropriate medium as described earlier [13].

2.3. Purification of clusterin

Clusterin was purified as previously described [13].

2.4. Thioglycollate-induced peritonitis

Thioglycollate-elicited peritoneal macrophages were obtained from specific pathogen-free male C57BL/6 mice (Orient Bio, Korea) as previously described [13].

2.5. Transwell filter assay

Cell migration was assessed as previously described [14]. Cell numbers were assessed by counting the number of migrated cells in five random microscopic fields per well.

2.6. Checkerboard analysis

A checkerboard analysis was conducted in a 24-well transwell plate by using polycarbonate filters with 8-μm pores (Becton Dickinson, CA). J774A.1 cell suspension (4×10^5 cells/ml) in serum-free RPMI1640 was added to the upper chamber. Clusterin (1–25 μg/ml) was added to the bottom chamber, to the upper chamber containing the cell suspension, and to both chambers to set positive, negative, and zero gradients, respectively. After 4 h incubation at 37 °C in a humidified incubator containing 5% CO_2, migrated cells attaching to the lower surface of the filter were fixed with 4% paraformaldehyde for 5 min and stained with 0.1% crystal violet for 15 min. For each sample, cells in three randomly picked fields under were counted.

2.7. Western blot analysis

Western blot assays were conducted under standard conditions as described previously [13], using appropriate antibodies (pERK1/2, p38, JNK, and Akt).

2.8. Statistical analysis

All values in the figures represent the mean ± S.D. Data were analyzed through the one-way analysis of variance (ANOVA), followed by a post hoc Dunnett's *t*-test for multiple comparisons. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Clusterin induced the chemotactic migration of monocytes/macrophages in vitro

Previous research has found that clusterin upregulates the expression of various chemokines such as TNF-α and can induce the migration of Raw264.7 macrophages. In addition, clusterin-induced TNF-α has been found to sufficiently stimulate the migration of macrophages [14]. Based on these findings, this study examines whether the observed migration by clusterin is based on chemotaxis from the chemoattractant activity of clusterin or from clusterin-induced cell activation resulting in enhanced movements in all directions. Clusterin's ability to induce the migration of monocytes/macrophages other than the Raw264.7 macrophage was examined first. When cells were treated with clusterin, the cell migration of human primary monocytes, mouse primary macrophages, the macrophage cell line J774A.1 as well as Raw264.7 was observed (Fig. 1A). In addition, J774A.1 cells migrated to the lower side of the filter at 4 h after clusterin stimulation, whereas Raw264.7 cells and primary monocytes and macrophages, at 12 and 24 h after stimulation, respectively. For this reason, a checkerboard analysis was conducted using J774A.1 macrophages by placing different concentrations of clusterin in lower and/or upper compartments of the chemotaxis chamber. As shown in Fig. 1B, J774A.1 cells migrated only when higher concentrations of clusterin were present in lower compartments. There was no increased cell migration to the bottom side of the filter when higher concentrations of clusterin were placed in upper wells. These results indicate that clusterin induced the directional chemotactic migration of monocytes/macrophages, not chemokinesis.

3.2. A G-protein coupled receptor sensitive to pertussis toxin and $G_{\beta\gamma}$-dependent pathways were involved in clusterin-induced chemotaxis in J774A.1 macrophages

Many chemoattractants activate GPCRs expressed on the surface of migrating cells. In particular, the G_i family of α subunits has been implicated in coupling chemokine and chemoattractant receptors to leukocyte functions including chemotaxis [17]. PTX, which inhibits G_i signaling, blocks chemotaxis and many other re-

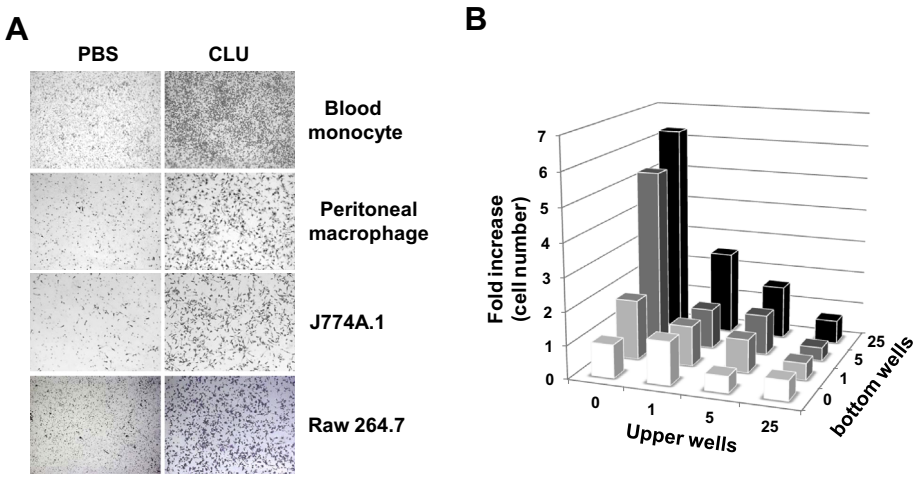

Fig. 1. Clusterin induced the chemotaxis of monocytes/macrophages. (A) Human blood monocyte, mouse peritoneal macrophage, and mouse macrophage cell lines (J774A.1 and Raw264.7) were treated with clusterin (3 μg/ml). Cells (4×10^5 cells/ml) were seeded in upper wells, and clusterin was added to lower wells to induce cell migration for 24 h (human blood monocytes and mouse peritoneal macrophages), 4 h (J774A.1), and 12 h (Raw264.7). (B) A checkerboard analysis of clusterin-induced macrophage migration. J774A.1 macrophages were treated with various concentrations of clusterin (0–25 μg/ml) into upper and/or lower wells of transwell filters for 4 h. The degree of migration was assessed by counting the number of migrated cells on the underside of the filter. Data are expressed as the mean fold induction of unstimulated cells from experiments performed in triplicate.

sponses to ligands that act on chemokine receptors [17]. Accordingly, PTX's ability to inhibit the clusterin-mediated chemotaxis of J774A.1 cells was investigated. Cells were preincubated with PTX (0–1 μg/ml) for 30 min and then exposed to clusterin in the lower chamber, and cells migrating to the lower side of the filter for 4 h were counted. The migration of J774A.1 cells by clusterin was inhibited by the pretreatment of the cells with PTX in a dose-dependent manner (Fig. 2A), which indicates that a $G_{\alpha i}$ protein-coupled receptor was involved in the clusterin-mediated chemotactic migration. GPCRs transmitted extracellular signals through heterotrimeric G-proteins composed of G_α and $G_{\beta\gamma}$ subunits. Although both the $G_{\alpha i}$ and $G_{\beta\gamma}$ subunits can activate downstream effectors, $G_{\alpha i}$ itself is not required for chemotaxis mediated by $G_{\alpha i}$-coupled receptors [18]. Instead, the release of $G_{\beta\gamma}$ proteins on heterotrimeric G_i-protein complex activation generally believed to be involved in many of GPCR functions, including chemotaxis [18–21]. To test the involvement of $G_{\beta\gamma}$ released from a PTX-sensitive GPCR in clusterin-induced chemotaxis, cells were pretreated with galleon (0–30 μM), which is a small molecule that binds to G-protein βγ subunits and inhibits interactions with downstream targets required for chemotaxis [21]. As shown in Fig. 2B, cell migration induced by clusterin was blocked in a dose-dependent manner by gallein pretreatment. PTX (1 μg/ml) or gallein (30 μM) almost inhibited clusterin-induced chemotaxis (Fig. 2C), but was not cytotoxic to cells after short-term treatment in terms of cell viability after drug treatment. Taken together, these results indicate that clusterin induced the chemotactic migration of macrophages through a PTX-sensitive GPCR and $G_{\beta\gamma}$-dependent pathways.

3.3. Inhibitors of PI3K (LY294002) and PLC (U73122) attenuated the chemotactic migration of J774A.1 cells induced by clusterin

Because the free $G_{\beta\gamma}$ subunit of the G_i-protein complex has been shown to activate diverse effectors such as PI3K [22] and PLC [23], the role of key targets of $G_{\beta\gamma}$ in the clusterin-stimulated chemotaxis of J774A.1 cells was examined. Cells were preincubated with LY294002 or U73122 for 30 min and then exposed to clusterin in the lower chamber. Here those cells migrating to the lower side of the filter for 4 h were counted. Clusterin increased cell migration relative to the PBS-treated control, and clusterin-stimulated chemotaxis was abrogated in a dose-dependent manner by pretreatment with LY294002 (0–20 μM) or wortmannin (Fig. 3A and C). Similarly, clusterin-induced cell migration was suppressed by a U73122 (125–500 nM) but not by U73343 as an inactive analog of U73122 (Fig. 3B and C), indicating that the activation of PI3K or PLC was required for cell migration by clusterin. The phosphorylation of Akt, a PI3K substrate, was further examined by a Wes-

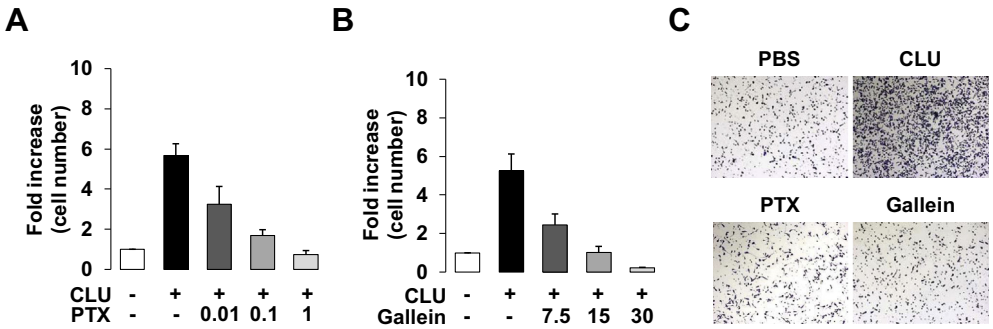

Fig. 2. Involvement of the GPCR in clusterin-induced macrophage chemotaxis. J774A.1 cells (4×10^5 cells/ml) were preincubated with indicated doses of pertussis toxin (PTX) (A) and gallein (B) for 30 min, and clusterin (3 μg/ml) was then added to lower wells for 4 h. Migration was assessed by counting the number of migrated cells in three microscopic fields per well. (C) Representative photomicrographs are shown at 100× magnification. Data shown are the mean ± S.D. of three independent experiments (each performed in triplicate).

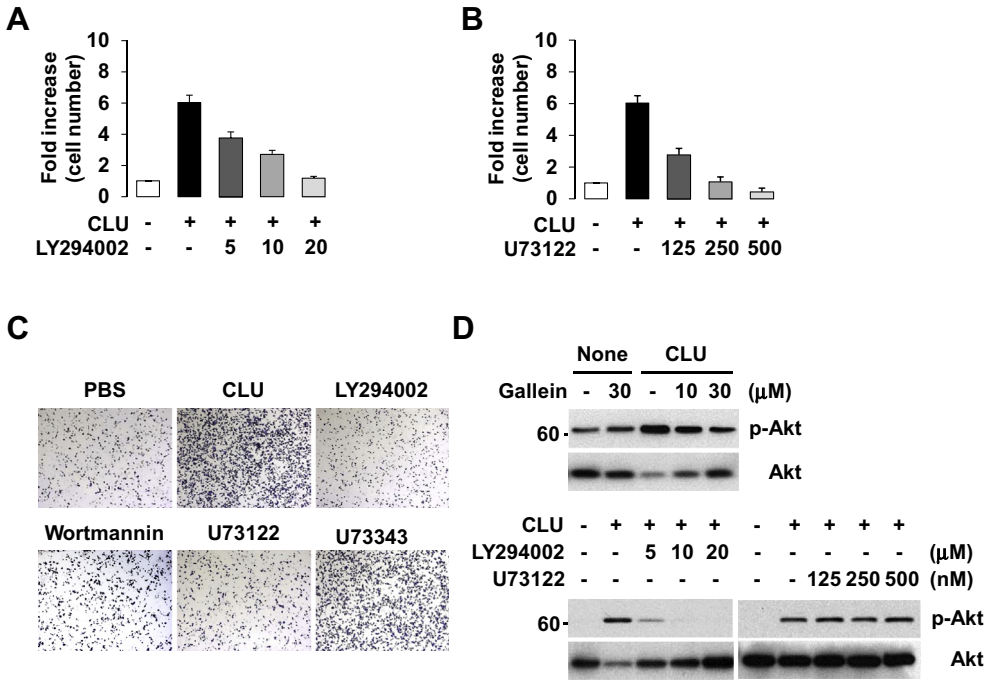

Fig. 3. Effect of inhibitors of PI3K (LY294002, wortmannin) and PLC (U73122) on clusterin-induced macrophage chemotaxis. J774A.1 cells (4×10^5 cells/ml) were preincubated with indicated doses of LY294002 (A) and U73122 (B) for 30 min, and clusterin (3 μg/ml) was then added to lower wells for 4 h. Migration was assessed by counting the number of migrated cells in three microscopic fields per well. (C) According to representative photomicrographs, LY294002 (20 μM), wortmannin (4 nM), and U73122 (250 nM) blocked clusterin-induced cell migration (100× magnification). (D) A Western blot analysis of Akt phosphorylation after clusterin treatment in the presence or absence of gallein, LY294002, and U73122 as indicated. Data shown are the mean ± S.D. of three independent experiments (each performed in triplicate).

tern blot analysis using a phospho-specific antibody against Serine 473 of Akt. Clusterin-induced Akt phosphorylation was suppressed by pretreatment with gallein and LY294002 but not with U73122 (Fig. 3D), indicating Akt is a downstream signaling molecule of $G_{\beta\gamma}$ and PI3K, but not of PLC. These results imply that clusterin stimulated J774A.1 cells through a PTX-sensitive GPCR for chemotactic migration and that $G_{\beta\gamma}$ released from the G_i-protein complex activated PLC and PI3K/Akt signaling pathways.

3.4. Activation of cellular MAP kinases was required for the clusterin-induced chemotactic migration of J774A.1 cells

The activation of mitogen-activated protein (MAP) kinases has been implicated in inflammatory responses and is well known to be involved in chemokine secretion and chemotaxis [14,24]. Therefore, the activation of MAP kinases was examined to identify the intracellular signaling network mediating chemotactic migration by clusterin in J774A.1 cells. Clusterin-stimulated chemotaxis experiments were conducted using several kinase inhibitors specific to MEK (PD98059), p38 (SB203580), and JNK (SP600125). The chemotactic response of J774A.1 cells to clusterin was significantly blocked in the presence of these inhibitors (Fig. 4A). Then, a Western blot analysis using their phospho-specific antibodies was conducted to analyze the phosphorylation of MAP kinases in response to clusterin in J774A.1 cells. Cells were exposed to clusterin for 30 min with or without pretreatment with gallein. Clusterin induced the phosphorylation of ERK, p38, and JNK MAP kinases within 30 min. Gallein blocked in a dose-dependent manner their phosphorylation induced by clusterin, whereas the inhibitor alone had no effect on the phosphorylation of MAP kinases (Fig. 4B). Clusterin stimulated J774A.1 cells for chemotactic migration through the activation of cellular MAP kinases, and $G_{\beta\gamma}$ released from the G_i-protein complex was required for the activation of these MAP kinases.

4. Discussion

Previous studies have demonstrated that clusterin induces the expression of various chemokines (TNF-α, MCP-1, MIP-1β) and that TNF-α secreted by clusterin treatment for 24–36 h plays a critical role in the migration of Raw264.7 macrophages [14], suggesting that the chemotactic response of clusterin is at least partially due to chemokinesis through clusterin-elicited cell activation. To rule out enhanced movements in random directions from the activation of macrophages, a checkerboard analysis was conducted at 4 h after clusterin stimulation by using J774A.1 cells. This study presents a novel chemotactic activity of a secreted isoform of clusterin as a chemokine for macrophages because it induced the directional migration of macrophages to the bottom side of the filter only when higher concentrations of clusterin were present in the lower compartment in the Transwell filter assay. Cell migration to the lower side was not enhanced when higher concentrations of clusterin were placed in upper wells during treatment with clusterin for 4 h (Fig. 1). Taken together, previous findings [14] and this study's results suggest that clusterin stimulates the migration of macrophages through both chemotaxis in early stage and chemokinesis in latter stage. Noteworthy is that no migration of HepG2 hepatocarcinoma cells to the lower side of the filter were observed when they were treated with clusterin for 24 h. Three days after clusterin treatment, however, cell migration to the lower side was sharply enhanced even when clusterin was present in the upper compartment as well as in the lower compartment (Supplementary Fig. 1). This result for a clusterin-induced chemokinetic response is consistent with the findings of previous studies showing that clusterin promotes the cell migration of several cancer cells such as hepatocarcinoma [4], and breast cancer [25] through presumably an epithelial-to-mesenchymal transition. Consistently, the chemokinetic property of clusterin was further confirmed. More specifically, it induced CXCR-4 in cardiac progenitor cells

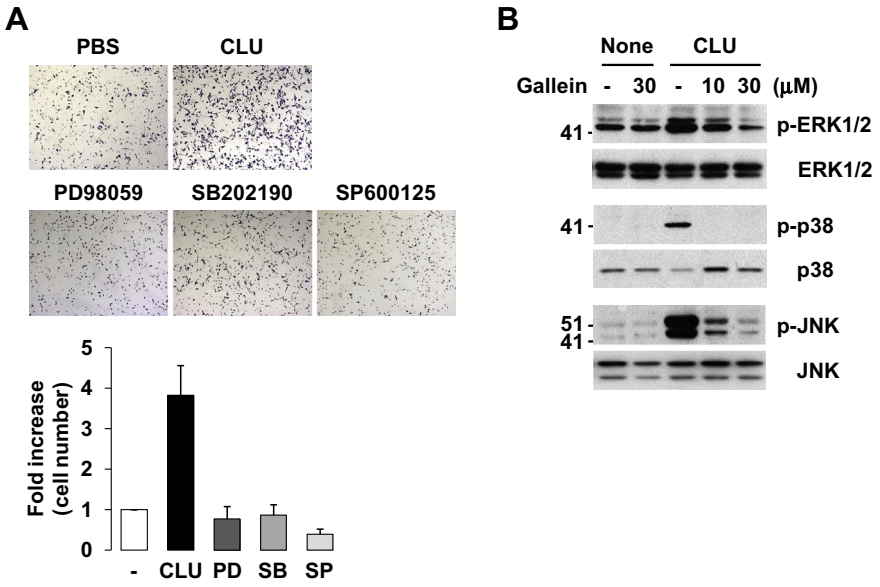

Fig. 4. MAP kinases were required for the chemotactic migration of J774A.1 cells by clusterin. (A) Cells (4×10^5 cells/ml) were preincubated with PD98059 (20 μM), SB203580 (10 μM), and SP600125 (10 μM) for 30 min and clusterin (3 μg/ml) was then added to lower wells 4 h. Migration was assessed by counting the number of migrated cells in three microscopic fields per well. Representative photomicrographs are shown at 100× magnification. (B) The activation of ERK, p38, and JNK by clusterin stimulation for 30 min in the presence or absence of gallein. Data shown are the mean ± S.D. of three independent experiments (each performed in triplicate).

and enhanced their migration stimulated by SDF-1 [26]. Besides the chemokinetic property of clusterin in macrophages, cancer cells, and cardiac progenitor cells [4,14,25,26], a new finding of this study is that clusterin promoted the recruitment of monocytes/macrophages through chemotaxis only to the gradient of clusterin. By contrast, clusterin inhibited the migration of vascular smooth muscle and endothelial cells [27,28]. This suggests that the role of clusterin varies according to the type of cell, the stimulus, and/or the tissue microenvironment.

The putative receptor(s) used in chemotaxis by clusterin was characterized. According to the results, the chemotactic activity of clusterin was completely inhibited by PTX, which uncoupled G_i from the GPCR. In addition, clusterin-stimulated chemotaxis was abrogated by pretreatment with gallein, indicating that it stimulated the chemotactic migration of macrophages through a PTX-sensitive GPCR and $G_{\beta\gamma}$-dependent pathways (Fig. 2). This is consistent with the findings that the activation of $G_{\alpha i}$-coupled receptors and subsequent release of $G_{\beta\gamma}$ dimers was required to initiate signal transduction leading to directed cell migration [19,20]. Further, PLC, PI3K/Akt, MAPK signaling pathways, as downstream target molecules of $G_{\beta\gamma}$, were essential for clusterin-induced chemotaxis because their specific inhibitors blocked chemotaxis and pretreatment with gallein reduced their phosphorylation induced by clusterin (Figs. 3 and 4). As mentioned earlier, TNF-α secreted by clusterin was involved in the migration of Raw264.7 macrophages [14]. Recently, unexpectedly Toll-like receptor 4 (TLR4) has been identified as a ligand for clusterin in upregulating the expression of TNF-α (unpublished data). In addition, TLR4 signaling augment monocyte chemotaxis [29] and GPCR agonists have been found to induce the transactivation of TLRs and cell signaling [30]. Therefore, to test the involvement of TLR4 in clusterin functions, peritoneal macrophages from TLR4-deficient and wild-type C57BL/6 mice were examined for chemotaxis and TNF-α expression. As shown in Supplementary Fig. 2A, clusterin stimulated the secretion of TNF-α in TLR4(+/+) mice but not in TLR4(−/−) mice, as in the case of the LPS as a positive control. By contrast, clusterin promoted the migration of peritoneal macrophages from both TLR4(+/+, −/−) mice relative to the PBS-treated control (Supplementary Fig. 2B), indicating no requirement for TLR4 in clusterin-induced chemotaxis. However, the identity of the clusterin receptor on the membrane surface of macrophages or the detailed mechanism of the stimulating membrane receptor remains unclear, although a PTX-sensitive GPCR and $G_{\beta\gamma}$-dependent pathways were involved in clusterin-induced chemotaxis, as noted earlier.

The level of clusterin increased sharply under pathologic conditions along with increased oxidative stress and chronic inflammation [1]. Consistently, clusterin was barely detected in the peritoneal fluid of mice, whereas it increased dramatically in the peritoneal fluid of mice with thioglycollate-induced peritonitis (Supplementary Fig. 3B). Noteworthy is that clusterin-deficient mice exhibited significantly decreased numbers of thioglycollate-induced peritoneal macrophages in comparison to wild-type C57BL/6 mice (Supplementary Fig. 3A). By contrast, the recruitment of macrophages into the peritoneal fluid increased significantly in normal C57BL/6 mice, as previously reported [31]. These results suggest that clusterin is upregulated in areas of inflammation and may function as a chemoattractant in the recruitment of macrophages into these sites. Consistent with these results, recent data show that clusterin increases in several cardiovascular diseases, including myocardial infarction, and its administration significantly reduces the size of infarction induced by the ligation of a coronary vessel, and while increasing the number of macrophages [32].

In conclusion, the results demonstrate that clusterin can recruit monocytes/macrophages in areas of inflammation through its chemotactic response (in addition to its chemokinetic response, chemokine secretion, and epithelial–mesenchymal transition). Clusterin-induced chemotaxis may depend on a PTX-sensitive GPCR and $G_{\beta\gamma}$-dependent pathways, and as downstream target molecules of $G_{\beta\gamma}$, PLC, PI3K/Akt, and MAPK pathways may also be involved. According to the results, the recruitment of macrophages decreased in the experimental model of thioglycollate-induced peritonitis in clusterin-deficient mice. Collectively, these results describe a novel role of clusterin as a chemoattractant of monocytes/macrophages and suggest its important roles in inflammation and tissue reorganization by recruiting immune cells.

Acknowledgment

This work was supported by the National Research Foundation of Korea (NRF-2012R1A1A2000904) and a Korea University grant (K1220371).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.071.

References

[1] I.P. Trougakos, The molecular chaperone apolipoprotein J/clusterin as a sensor of oxidative stress: implications in therapeutic approaches – a mini-review, Gerontology 59 (2013) 514–523.
[2] H.V. de Silva, W.D. Stuart, Y.B. Park, S.J. Mao, C.M. Gil, J.R. Wetterau, S.J. Busch, J.A. Harmony, Purification and characterization of apolipoprotein J, J. Biol. Chem. 265 (1990) 14292–14297.
[3] N. Hamada, M. Miyata, H. Eto, Y. Ikeda, T. Shirasawa, Y. Akasaki, T. Miyauchi, Y. Furusho, A. Nagaki, B.J. Aronow, C. Tei, Loss of clusterin limits atherosclerosis in apolipoprotein E-deficient mice via reduced expression of Egr-1 and TNF-alpha, J. Atheroscler. Thromb. 18 (2011) 209–216.
[4] C. Wang, K. Jiang, X. Kang, D. Gao, C. Sun, Y. Li, L. Sun, S. Zhang, X. Liu, W. Wu, P. Yang, K. Guo, Y. Liu, Tumor-derived secretory clusterin induces epithelial–mesenchymal transition and facilitates hepatocellular carcinoma metastasis, Int. J. Biochem. Cell Biol. 44 (2012) 2308–2320.
[5] P. Schedin, T. Mitrenga, S. McDaniel, M. Kaeck, Mammary ECM composition and function are altered by reproductive state, Mol. Carcinog. 41 (2004) 207–220.
[6] S. Astancolle, G. Guidetti, C. Pinna, A. Corti, S. Bettuzzi, Increased levels of clusterin (SGP-2) mRNA and protein accompany rat ventral prostate involution following finasteride treatment, J. Endocrinol. 167 (2000) 197–204.
[7] D.K. Swertfeger, D.P. Witte, W.D. Stuart, H.A. Rockman, J.A. Harmony, Apolipoprotein J/clusterin induction in myocarditis: a localized response gene to myocardial injury, Am. J. Pathol. 148 (1996) 1971–1983.
[8] B. Shannan, M. Seifert, K. Leskov, J. Willis, D. Boothman, W. Tilgen, J. Reichrath, Challenge and promise: roles for clusterin in pathogenesis, progression and therapy of cancer, Cell Death Differ. 13 (2006) 12–19.
[9] E.M. Stewart, J.A. Aquilina, S.B. Easterbrook-Smith, D. Murphy-Durland, C. Jacobsen, S. Moestrup, M.R. Wilson, Effects of glycosylation on the structure and function of the extracellular chaperone clusterin, Biochemistry 46 (2007) 1412–1422.
[10] S. Herbst, U.E. Schaible, B.E. Schneider, Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis, PLoS ONE 6 (2011) e19105.
[11] J.A. Joyce, J.W. Pollard, Microenvironmental regulation of metastasis, Nat. Rev. Cancer 9 (2009) 239–252.
[12] C.E. Lewis, J.W. Pollard, Distinct role of macrophages in different tumor microenvironments, Cancer Res. 66 (2006) 605–612.
[13] Y.J. Shim, B.H. Kang, H.S. Jeon, I.S. Park, K.U. Lee, I.K. Lee, G.H. Park, K.M. Lee, P. Schedin, B.H. Min, Clusterin induces matrix metalloproteinase-9 expression via ERK1/2 and PI3K/Akt/NF-kappaB pathways in monocytes/macrophages, J. Leukoc. Biol. 90 (2011) 761–769.
[14] Y.J. Shim, B.H. Kang, B.K. Choi, I.S. Park, B.H. Min, Clusterin induces the secretion of TNF-alpha and the chemotactic migration of macrophages, Biochem. Biophys. Res. Commun. 422 (2012) 200–205.
[15] H. Fujimoto, T. Sangai, G. Ishii, A. Ikehara, T. Nagashima, M. Miyazaki, A. Ochiai, Stromal MCP-1 in mammary tumors induces tumor-associated macrophage infiltration and contributes to tumor progression, Int. J. Cancer 125 (2009) 1276–1284.
[16] R. Cheung, M. Malik, V. Ravyn, B. Tomkowicz, A. Ptasznik, R.G. Collman, An arrestin-dependent multi-kinase signaling complex mediates MIP-1beta/CCL4 signaling and chemotaxis of primary human macrophages, J. Leukoc. Biol. 86 (2009) 833–845.
[17] G.M. Bokoch, Chemoattractant signaling and leukocyte activation, Blood 86 (1995) 1649–1660.
[18] E.R. Neptune, T. Iiri, H.R. Bourne, Galphai is not required for chemotaxis mediated by Gi-coupled receptors, J. Biol. Chem. 274 (1999) 2824–2828.
[19] H. Arai, C.L. Tsou, I.F. Charo, Chemotaxis in a lymphocyte cell line transfected with C–C chemokine receptor 2B: evidence that directed migration is mediated by betagamma dimers released by activation of Galphai-coupled receptors, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 14495–14499.
[20] E.R. Neptune, H.R. Bourne, Receptors induce chemotaxis by releasing the betagamma subunit of Gi, not by activating Gq or Gs, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 14489–14494.
[21] D.M. Lehmann, A.M. Seneviratne, A.V. Smrcka, Small molecule disruption of G protein beta gamma subunit signaling inhibits neutrophil chemotaxis and inflammation, Mol. Pharmacol. 73 (2008) 410–418.
[22] H. Kurosu, T. Maehama, T. Okada, T. Yamamoto, S. Hoshino, Y. Fukui, M. Ui, O. Hazeki, T. Katada, Heterodimeric phosphoinositide 3-kinase consisting of p85 and p110beta is synergistically activated by the betagamma subunits of G proteins and phosphotyrosyl peptide, J. Biol. Chem. 272 (1997) 24252–24256.
[23] A. Katz, D. Wu, M.I. Simon, Subunits beta gamma of heterotrimeric G protein activate beta 2 isoform of phospholipase C, Nature 360 (1992) 686–689.
[24] Y.L. Zu, J. Qi, A. Gilchrist, G.A. Fernandez, D. Vazquez-Abad, D.L. Kreutzer, C.K. Huang, R.I. Sha'afi, P38 mitogen-activated protein kinase activation is required for human neutrophil function triggered by TNF-alpha or FMLP stimulation, J. Immunol. 160 (1998) 1982–1989.
[25] A.E. Lenferink, C. Cantin, A. Nantel, E. Wang, Y. Durocher, M. Banville, B. Paul-Roc, A. Marcil, M.R. Wilson, M.D. O'Connor-McCourt, Transcriptome profiling of a TGF-beta-induced epithelial-to-mesenchymal transition reveals extracellular clusterin as a target for therapeutic antibodies, Oncogene 29 (2010) 831–844.
[26] Y. Li, J. Qu, H. Shelat, S. Gao, M. Wassler, Y.J. Geng, Clusterin induces CXCR4 expression and migration of cardiac progenitor cells, Exp. Cell Res. 316 (2010) 3435–3442.
[27] H.J. Kim, E.K. Yoo, J.Y. Kim, Y.K. Choi, H.J. Lee, J.K. Kim, N.H. Jeoung, K.U. Lee, I.S. Park, B.H. Min, K.G. Park, C.H. Lee, B.J. Aronow, M. Sata, I.K. Lee, Protective role of clusterin/apolipoprotein J against neointimal hyperplasia via antiproliferative effect on vascular smooth muscle cells and cytoprotective effect on endothelial cells, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1558–1564.
[28] N. Sivamurthy, D.H. Stone, F.W. LoGerfo, W.C. Quist, Apolipoprotein J inhibits the migration and adhesion of endothelial cells, Surgery 130 (2001) 204–209.
[29] Z. Liu, Y. Jiang, Y. Li, J. Wang, L. Fan, M.J. Scott, G. Xiao, S. Li, T.R. Billiar, M.A. Wilson, J. Fan, TLR4 signaling augments monocyte chemotaxis by regulating G protein-coupled receptor kinase 2 translocation, J. Immunol. 191 (2013) 857–864.
[30] S. Abdulkhalek, M. Guo, S.R. Amith, P. Jayanth, M.R. Szewczuk, G-protein coupled receptor agonists mediate Neu1 sialidase and matrix metalloproteinase-9 cross-talk to induce transactivation of TOLL-like receptors and cellular signaling, Cell. Signal. 24 (2012) 2035–2042.
[31] P.C. Leijh, T.L. van Zwet, M.N. ter Kuile, R. van Furth, Effect of thioglycolate on phagocytic and microbicidal activities of peritoneal macrophages, Infect. Immun. 46 (1984) 448–452.
[32] A. van Dijk, R.A. Vermond, P.A. Krijnen, L.J. Juffermans, N.E. Hahn, S.P. Makker, L.A. Aarden, E. Hack, M. Spreeuwenberg, B.C. Rossum, C. Meischl, W.J. Paulus, F.J. van Milligen, H.W. Niessen, Intravenous clusterin administration reduces myocardial infarct size in rats, Eur. J. Clin. Invest. 40 (2010) 893–902.